FACTORS AFFECTING FERTILITY IN SUCCESSIVE GENERATIONS OF BARLEY AUTOTETRAPLOIDS

by

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ABSTRACT

The fertility differences, the effect of selection for fertility and the relation of cytological observations to fertility in several artificially induced autotetraploid barley varieties were determined in a study carried out at the Ontario Agricultural College, Guelph and the University of Manitoba, Winnipeg, from 1954 to 1957.

Four of the autotetraploid varieties used in this study were obtained by soaking germinating seeds of diploid O.A.C. 21, Brant, G.B. 61 and Montcalm barley in 0.1 per cent aqueous colchicine solutions. A new visual method was successfully used to screen the colchicine treated material for tetraploidy in the C_1 generation. The varieties tested varied greatly in mean per cent fertility and each variety had a wide fertility range. Continuous selection for fertility from C_1 to C_4 generations did not change the fertility level in the O.A.C. 21 tetraploid.

A considerable number of dwarf plants was found in the autotetraploid populations and most of these did not produce seed. The number of the dwarf plants within a tetraploid variety was not indicative of the mean per cent fertility of the variety.

Most of the dwarf plants were aneuploids with 26, 27, 29, 30 and 31 chromosomes. The aneuploids with a particular chromosome number could not be identified by their morphological characteristics.

The chromosomes at metaphase I of the four barley varieties were associated mainly as bivalents and quadrivalents. The mean number of quadrivalents, ranging from 4.6 to 5.1 per cell, was higher than generally reported.

The mean number of multivalents per cell was not indicative of the mean per cent fertility in the varieties studied. However, the chromosome distribution at anaphase I was found to be partly associated with the mean per cent fertility. The differences in fertility between the four autotetraploid barley varieties could not be explained by meiotic irregularities only. Therefore it was concluded that genetically controlled physiological sterility, suggested by other workers, is partly responsible for low fertility in autotetraploid barley.

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I N T R O D U C T I O N

The discovery of colchicine as a polyploidizing agent has given plant breeders a useful tool for developing new and improved strains or species. Besides its use in doubling the chromosome number of sterile interspecies hybrids, colchicine has been widely used to produce autotetraploids in economic crop species. The induced autotetraploids very often have larger fruits or seeds, broader leaves and sturdier stems than the diploids. However, autotetraploidy is usually accompanied by partial sterility. This is a great disadvantage in crops where seed is the important end product.

The fertility of induced tetraploids of some species is only slightly reduced. In such species the tetraploid strains compare favourably with the diploids in yield, because larger seed size compensates for lower fertility. In other species some success in overcoming sterility has been obtained by repeated selection of the more fertile plants in the generations following induced chromosome doubling.

Some field crops in which the fertility of the tetraploids has been improved by selection are: rye, buckwheat, oil-seed rape, red clover and alsike clover. Several varieties of tetraploid rye are now in commercial production. On the other hand all attempts to obtain tetraploid barley varieties with fertility sufficiently close to that of the diploids to give increased yields have been unsuccessful to date.

The cause for lower fertility in autotetraploids has not as yet been explained satisfactorily. Some authors attribute it to high frequency of multivalent chromosome associations, others to genetic or some obscure physiological factors. Whatever the cause may be, the artificially

induced autotetraploids are not finished products. They merely give a new base from which the actual breeding work begins. Consequently any information that would throw light on the causes of sterility would be helpful to the plant breeder.

The purpose of this study was to compare fertility in several colchicine induced autotetraploid barley varieties; to determine the effect of selection on fertility, and to relate cytological observations with fertility in autotetraploid barley.

LITERATURE REVIEW

Production and characteristics of artificial autotetraploids

According to Müntzing (46) more than half of all angiosperms are natural polyploids. The association of desirable characteristics with polyploidy is so common a phenomenon (22) that the use of chemical agents to produce polyploidy artificially has aroused a profound interest the whole world over.

According to Randolph (72) polyploid plants were first produced artificially by regeneration techniques with mosses in 1908 by Marchals. Jorgensen (36) reported that among the higher plants tetraploid strains using the cross-grafting method were first induced in 1916 in Solanum by Winkler. Since 1930 a number of workers (23, 46, 66, 71) have induced tetrapoloidy in plants propagated by seeds by applying high temperature shocks. The heat treatment, which gave relatively limited results (22), was followed by the colchicine method.

Eigsti et al. (27) reported that the first study of the action of colchicine on mitosis was published in 1889 by Pernice. According to Dermen (22), Dustin and his co-workers in Belgium in 1934 were the first to report on the use of colchicine as a mitotic poison.

In the United States successful plant polyploidy by colchicine was first obtained by Blakeslee and Avery (7) in 1937. Since then a large number of artificially induced autotetraploids have been produced in many crop species (5, 25, 26, 48).

In horticultural crops several of the induced autopolyploids show economically desirable characteristics (54). In field crops promising autotetraploid strains have been obtained in rye, buckwheat, annual-rape,

red clover and alsike clover (1, 11, 45, 49). In 1951, in Sweden and Germany tetraploid rye varieties were found to be superior to the corresponding diploid varieties and were released for commercial use (11).

The effect of colchicine on plant tissues has been described by several workers (6, 22, 54, 58, 63). The extensive literature on colchicine as a polyplodizing agent has been summarized by Eigsti (25) and Eigsti and Dustin (26, 27). Randolph (72) has summarized the information on desirable and undesirable effects of induced polyploidy in various plant species. Noggle (56) gave a literature review on the physiology of polyploidy in plants.

In cultivated barley some autotetraploids occurred spontaneously (24, 81). Artificially the first autotetraploid barley strain was produced with heat shock by Müntzing in 1936 (49, 81). A considerable number of autotetraploid barley lines have since been obtained with colchicine (13, 24, 33, 48, 60, 73, 87).

The methods of colchicine application to induce tetraploidy in barley are given by several workers (7, 8, 12, 13, 22, 24, 33, 43, 50, 73). A wide variation between concentrations and duration of the treatment exist. Most often, however, germinating seeds are immersed in a 0.1 to 0.2 per cent aqueous colchicine solution for two to six hours at room temperature.

The extensive literature on morphological, chemical and physiological changes in autotetraploid barley has been summarized by Smith (82). Methods of studying these changes were described by Chen et al. (14) and Chen and Tang (15).

The economic value of new tetraploids in barley has not been as

striking as in the case of rye. It has been found that the barley tetraploids are more resistant to lodging (48), with shorter, thicker culms (13, 30, 42, 61), producing higher yield of straw than the diploids (33). Several workers (13, 30, 48, 50, 60, 61, 73, 74, 81) have found that the seeds produced by tetraploid barley plants are usually larger but the number of seeds per spike is lower than that in the diploids. It has been indicated (33, 73) that some of the seeds are under-developed and a lower percentage of them germinate. The lower number of seeds per spike in tetraploid barley is mainly due to sterility and results in considerably lower yields than the diploids from which they were derived. Müntzing (48) found that the average yields of 10 tetraploid strains of barley were from 60 to 79 per cent lower than the yields of their corresponding diploids. Several other authors (60, 61, 73, 74) have reported low fertility in induced tetraploids of barley. however, they indicated that the fertility varies with the variety and environment.

Cytological observations

Meiosis in autotetraploid barley has been found to be irregular with more or less random pairing among the four homologous chromosomes (13, 66, 73, 74). Other irregularities observed were: autosyndetic associations involving more than four chromosomes, univalents, trivalents, bridges, and there has been some evidence of structural changes (13, 30, 73, 74).

According to Müntzing (46) the proportion of chromosomes forming multivalents in autotetraploids varies with the organism. The tendency to form multivalents in tetraploid barley is not very high (74). The usual number of quadrivalents at first metaphase is one to three, but a maximum

of seven has been observed (13, 18, 74). Chen et al. (13) found some autotetraploid barley plants that seemed regularly to have 14 pairs of chromosomes at meiosis.

Berg (3) and Chin (16, 17) studied meiosis in the tetraploid form of Hordeum bulbosum L. and decided that it was an autotetraploid. Maximum associations of seven quadrivalents were observed. Of the 734 quadrivalents studied nearly 90 per cent of them were arranged so that adjacent chromosome would disjoin at the first metaphase causing a normal anaphase distribution of chromosomes. This was attributed in most cases to the terminal chiasmata formation.

Chin (18) compared the frequency of multivalents in tetraploid rye and H. bulbosum. It was found that H. bulbosum with 25.6 per cent chiasma frequency had 4.2 multivalents per cell, while in rye, which had longer chromosomes and 31.1 per cent chiasma frequency, only 1.1 multivalents were formed. He concluded that the per cent of chromosomes forming multivalents was determined by the size of the possible chiasma frequency of the multivalent components but not by the total chiasma frequency per cell. He also indicated that probably the length of the chromosome arms may be determining factors for chiasma frequency.

Peto (66) observed in tetraploid barley that, on the average, 50 per cent of the chromosomes formed quadrivalents, and concluded that random attraction of homologous regions at zygotene would account for the proportions obtained. Contrary to other authors he did not observe any univalents or trivalents.

Konzak et al. (39) crossed self-fertile tetraploid H. bulbosum with diploid H. vulgare and found that in the F_1 triploids a large number

of multivalents were formed. They indicated that apparently a high degree of homology exists between at least some of the chromosomes in H. bulbosum and H. vulgare. However, the number of multivalents was found to be lower when the variety Moore was used than when Bolivia or Wong were used as parents in crosses with H. bulbosum.

Morrison (45) indicated that in tetraploid rye unequal distribution of chromosomes to egg cells and pollen grains, followed by the chance union of gametes, will result in production of aneuploids. He believed that the percentage of tetrads with micronuclei can be taken as an indication of the number of aneuploids expected.

Müntzing (49) observed variations in chromosome number in tetraploid rye. He found that in tetraploid Steel rye 22.7 per cent of plants were aneuploids with 27, 29 or 30 chromosomes. The fertility of the aneuploids, if seed set occurred at all, was greatly reduced. The aneuploid seeds were underdeveloped and could be removed by sieves.

Armstrong (1), working with tetraploid alsike clover, reported that no aneuploids were found in this species. He concluded that the unbalanced zygotes apparently fail to function in this crop.

Randolph (71) observed variations in chromosome number in tetraploid maize. He found that the addition or deletion of a few chromosomes had very little effect on the appearance of the plants.

In tetraploid barley Chen et al. (13) found 24 to 30 chromosomes in pollen mother cells and 12 to 16 chromosomes in microspores. Dorsey (24) in the second tetraploid generation obtained a barley plant with 31 chromosomes. This plant was dwarfish and tillered profusely.

Smith (81) found that in each of three tetraploid barley lines studied occasional small plants appeared forming rosette-like clumps. Some of these small plants were found to possess 26 or 27 chromosomes, others 29 or 30, and one plant had 28 chromosomes. The rosette-like plant with 28 chromosomes was explained by an unbalanced constitution, i.e., more than four homologues of one kind balanced by less than four homologues of another.

Müntzing (48) found that in tetraploid Opal-B the frequency of aneuploid plants in space-planted plots was as high as 40 to 50 per cent. Most of the aneuploids showed very poor growth and did not form heads. At higher seeding rates the aneuploids were eliminated. There was found to be an obvious difference in frequency of aberrants in different strains.

Aneuploids in tetraploid barley have been reported also by Rosendahl (74). He studied 18 crosses among autotetraploid races and observed F_2 plants ranging from 25 to 31 chromosomes. The aneuploid plants usually did not develop. However, in F_3 two plants which produced seeds were observed with 24 and 30 chromosomes respectively. In general, among the tetraploids studied, the disjunction of chromosomes was normal and there was no difference in the number of tetravalents formed in the different crosses.

Sterility in autotetraploids

Darlington (19) considered that the sterility of autotetraploids is due to the formation of multivalents, which divide irregularly at meiotic anaphase and produce gametes with abnormal chromosome combinations. Kostoff (40) therefore, postulated that plants with small chromosomes and

low chiasma frequency would be less sterile than species with large ones. He cited a few examples which seemed to follow this pattern. Müntzing (46), however, did not accept this view. He showed that some autotetraploids with small chromosomes and bivalent pairing, like in Solanum, were still quite sterile, while many spontaneous autotetraploid species and races were highly fertile despite a number of multivalent associations. He attributed the sterility of autotetraploids mainly to the physiological disturbances of the tetraploid mother plant which may kill the gametes.

The effect of chromosome pairing on the fertility in tetraploids has been studied intensively by other workers. Chen et al. (13) found that the amount of sterility in 149 tetraploid barley lines was correlated with the formation of multivalents and other irregularities. A fertility level comparable to that of the diploids apparently was obtained only in lines having 14 bivalents at metaphase.

Bremer et al. (11) claimed that selection of plants with the most regular meiosis decreased the number of aneuploids and apparently was the cause for increased fertility in tetraploid fall rye. Selection of lines with regular meiosis resulting in increased fertility has been reported also in tetraploid spring rye (89). Morrison (45) postulated that in cross-pollinated species like rye regular meiosis, resulting in increased fertility, could be obtained by altered chromosomes causing more frequent bivalent pairing or by selection for pairing-controlling genes.

Ramanujam et al. (70) stated that any increase in fertility in Tetra-Petkus rye over the original diploid must have a genetical basis or some obscure physiological cause. This viewpoint was shared by Plarre (67).

Myers and Hill (53) working with Dactylis glomerata concluded that

the reduced fertility in the tetraploids of this species is due largely to meiotic irregularities but that these irregularities do not depend on irregular segregation of multivalents. The frequency of univalents was positively correlated with frequency of tetrads containing micronuclei but not with the frequency of quadrivalents. Sparrow et al. (83) obtained similar results in Anthirrhinum majus L. and Myers (52) in autotetraploid Lolium perenne. Myers concluded that genetic differences as well as meiotic irregularities condition variations in fertility in the species studied.

Berg (3) found that meiosis in the completely fertile tetraploid form of H. bulbosum was quite similar to that of induced autotetraploids of H. vulgare. This species, however, is normally cross-pollinated.

Rommel (74) found that the number of bivalents in different tetraploid barley varieties was in no way correllated with the sterility in the same plant. Abnormalities in tetrad formation and the occurrence of micronuclei also could not be correllated with sterility.

Armstrong and Robertson (1) working with tetraploid alsike clover and Morrison (45) working with Tetra-Petkus rye, concluded that fertility is mainly gene controlled and that the increase in fertility after selection is not accompanied by changes in meiotic behaviour.

Randolph (72), working with maize, concluded that only about 5 to 10 per cent of the sterility was caused by meiotic irregularities and that segregating sterility factors are responsible for differences in self-fertility. Fisher (39) obtained similar results to Randolph. The presence of genes for self- and cross-sterility in tetraploids, when such factors had not been found in the diploids, suggested that doubling of chromosome number results in a change of genic balance due to cumulative action of some

genes and non-cumulative action of others. Melchers (44) also indicated that a change of genic balance could take place after chromosome doubling. He concluded that the genetically controlled physiological processes adjusted to the diploid level would be disturbed in tetraploids.

Schlösser (78) demonstrated in diploid and tetraploid crosses of Solanum racemigerum that a quantitative change in chromosome number primarily caused a physiological change, which was shown to be altered osmotic pressure. This change of the osmotic pressure created a barrier of incompatibility between the diploid and tetraploid strains.

Schwanitz (79) believed that the sterility of autotetraploids is mainly caused by physiological disturbances in the plant. He indicated that the increased cell size would slow down nutrient transport leaving larger amount of the nutrients in the vegetative parts of the plant. As a result the fertility may be reduced.

Darmer (21) and Kukuck and Levan (41) postulated that the disturbed volume relationships between the nucleus and cytoplasm may be one of the causes that upset the physiological functions of the tetraploids resulting in sterility.

Rosendahl (74) found that in some tetraploids, despite a high percentage of fertile pollen, the seed set was low. Within 200 tetraploid barley plants examined the maximum pollen fertility was 90 to 95 per cent but maximum seed set reached only 40 to 60 per cent. Similar results were obtained by Rommel (73).

Hakanson (34) observed normal fertilization in both tetraploid and diploid barley. A few days after fertilization, however, the tetraploids differed in having a large number of embryos with considerably slower

development. This indicated that disturbed embryo and endosperm development may be the cause of sterility. Poorly developed embryos in tetraploids have been observed also by Katterman (38) and Rommel (73).

Müntzing (48) observed that in many tetraploid two-rowed barley varieties part of the heads often remained in the sheath and were sterile in this part of the plant.

From the literature reviewed it can be seen that there are apparently at least three different causes for sterility in autotetraploids. Stebbins (84) has summarized them as follows:

"1. Irregular chromosomal distribution caused by unequal separation of multivalents.

2. Irregular distribution caused by meiotic abnormalities of a physiological nature, presumably controlled genetically.

3. Genetic-physiological sterility of an unexplained nature but not associated with meiotic irregularity."

Stebbins (84) indicated that the relative importance of these causes varies with the tetraploid in question but in most examples the first is less important than the last two.

Despite the fact that the factors causing sterility in autotetraploids are not completely understood as yet, it should be possible for the plant breeder to make definite progress in producing more fertile tetraploids if selection for fertility can be done. Increased fertility by continuous selection has been demonstrated by various workers. Müntzing (46) indicated that the natural autotetraploids are more fertile than the artificially induced ones because natural selection has increased fertility. Randolph (72) successfully selected more fertile tetraploid

inbred lines in maize. He indicated, however, that complete fertility could not be obtained because of inherent chromosome irregularities but this percentage was not high and could be compensated by favourable agronomic characteristics. In tetraploid rye and red clover a considerable increase in yield has been obtained by selection (1, 9, 11). Bremer et al. (11) after seven years of selection from 49 seed-bearing plants with an average fertility of 60 per cent obtained a population with an average fertility of 70 per cent.

Armstrong and Robertson (1) have summarized the data on other induced tetraploids in which the fertility was increased by selection. In several cases in buckwheat, annual rape and alsike clover tetraploid lines were obtained with seed yield equal to that of the corresponding diploids.

Several authors (48, 60, 61, 73, 74) have found that in tetraploid barley the fertility varies with the variety and environment. The belief has been expressed also that in this species the yield difference between tetraploids and diploids can be decreased by selection (48). Ono (60, 61, 62) observed fertility for seven generations in 19 artificially induced tetraploid barley strains. In the two-rowed types the fertility progressively increased from 59.4 per cent in the F_1 to 86.1 per cent in the F_7. In the six-rowed types, however, no increase of the fertility (which was 64.5 per cent in the F_1) was obtained. The cause of the difference between the two-rowed and the six-rowed types was not known but Ono expressed optimism that successive selection may increase the fertility in certain tetraploid barley varieties.

Müntzing (48) showed that tetraploids obtained from hybrids were superior to tetraploids obtained from standard varieties. The yield of

the hybrid tetraploids was considerably higher. Similar results were obtained by Rommel (73). The seed set in the tetraploids obtained by doubling the chromosome number in the F_1 generation was 65 per cent as compared with 46 per cent seed set in the tetraploids obtained from the parents. This occurred despite the fact that the number of quadrivalents at metaphase I was higher in F_1 tetraploids than in the parental lines.

The data obtained from the F_1 tetraploids indicated that the fertility in tetraploid barley could be increased by changed gene combinations. Consequently, Müntzing (48) postulated that it should be possible to do further selection for fertility in the offspring of the F_1 tetraploids in order to obtain highly fertile tetraploid lines.

MATERIALS AND METHODS

Preliminary work on the development of tetraploids in barley was started in September, 1954 at the Ontario Agricultural College. In the review of literature many different colchicine treatment schedules for the induction of tetraploidy in barley were noted. Because of this a large scale preliminary test was conducted in order to develop a method of treatment applicable to a wide range of barley varieties. Three varieties differing considerably in morphology and source of origin were used, namely, O.A.C. 21, Hannchen and Jet.

The preliminary test confirmed the reports in literature that varieties differ considerably in their response to colchicine. However, a treatment schedule was worked out that gave good average results on each of the three varieties tested. The schedule, which was adapted to induce tetraploidy in the barley varieties used in this investigation, was as follows:

Barley seeds were germinated in Petri dishes on moist filter paper at room temperature to the point where the acrospires started to emerge at the apical end of the kernels and the rootlets were approximately one half to one inch long (36 to 48 hours). The germinating seeds were then placed in a shallow layer of 0.1 per cent aqueous colchicine solution for three hours. The treatment was prolonged if the acrospires were longer. After the treatment the material was washed in tap water and transplanted directly in soil in the greenhouse.

By this method tetraploidy was induced in four six-rowed barley varieties, namely, O.A.C. 21, Montcalm, Brant and G. B. 61. O.A.C. 21 and Montcalm are malting barley varieties and are used as standards for malting quality in Canada. Brant is a new, high-yielding feed barley

well adapted to Ontario conditions. G. B. 61 is a promising strain of barley selected at the Ontario Agricultural College from the cross (Stephan x Galore) x (O.A.C. 21 x Peatland).

One hundred seeds of each variety were treated with colchicine. The tetraploid population of O.A.C. 21 from this treatment was increased by adding the material obtained in the preliminary tests. The colchicine induced tetraploid material of Hannchen and Jet from the preliminary test was unfortunately lost in the field during the summer of 1955 because of extremely dry weather.

Several morphological characteristics such as the length of stomatal openings, size of pollen grains and some visual characteristics of the seedlings, were examined in order to find tetraploid plants in the treated generation. None of these methods proved satisfactory. Therefore the actual screening for tetraploidy, at least initially, was carried out in the C_1 generation.

In the spring of 1955 the seed of one head from each of the C_0 plants, i.e., plants produced from the colchicine treated seed, was planted in a five-foot row in the experimental field at the Ontario Agricultural College. The tetraploid plant rows and rows containing both tetraploid and diploid plants were readily distinguishable. The tetraploids were characterized by long kernels (especially laterals), incomplete emergence of the head, low fertility and a low rate of tillering. The height of the plants seemed to be a reliable indicator under field conditions but later was found to be unreliable under greenhouse conditions.

Each of the visually determined tetraploid plants was checked for chromosome number. For this purpose two or three seeds of each plant

were germinated in Petri dishes on moistened filter paper. The root tips were fixed for 2 to 24 hours in a combined stain-fixative (3:2:1) as described by Peters (65). After fixation the root tips were boiled in a little glass vial with aceto-carmine over a flame and squashed in a fresh drop of acetocarmine on the slide. As the main aim in this generation was to distinguish the diploids from tetraploids and not to determine the exact number of chromosomes present, no pretreatments to shorten the chromosomes were used. By this method up to 50 plants could be checked for tetraploidy in one day.

The cytological examination did not reveal a single diploid plant among the visually determined tetraploids. It is possible that not all of the tetraploids were found by the visual inspection in C_1, especially where they were mixed in a dense diploid population. It is believed, however, that this percentage would not be high in a six-row barley.

As a result of becoming familiar with the identification of tetraploids in the C_1 generation it was found that visual selection for tetraploidy was effective also in the treated generation. Thus the obviously diploid plants could be eliminated and the C_1 progeny could be grown only from the plants which appeared to be completely or partially tetraploid. This method of visual selection in the C_0 generation was successfully tested while inducing tetraploidy in 65 barley strains not included in this study.

In the C_1 generation spikes of the main tillers of tetraploid plants were harvested in separate envelopes and used for fertility determinations. Per cent fertility in each spike was determined by the following procedure:

The 15 spikelets attached to the five lower internodes of the spike

were cut off by means of small scissors. The next 30 spikelets were counted and the part of the spike above them was removed. The remaining 30 central spikelets were threshed by hand and the number of seeds were counted. The fertility of the plant was recorded as 100 per cent if 30 seeds were found in this part of the spike.

The spikes of tetraploid barley plants are rather short and the top and bottom spikelets usually do not develop. This is particularly true under greenhouse conditions. Therefore the method described above was considered suitable for fertility comparisons. This method was used in all the tetraploid barley generations studied as well as in the diploid controls.

In order to show the range of fertility within each tetraploid population, ten different classes based on the per cent fertility were established and the frequency of plants in each class was recorded. A pedigree system was adopted by which every plant in the advanced generations could be traced back to its C_1 parent.

The fertility in the C_2 generation of the four tetraploid barley varieties was determined in greenhouse grown material at Winnipeg in May, 1956. In January two to eight seeds from each C_1 plant were sown in a seven inch pot. After emergence seedlings were thinned to not more than five per pot. Because of the lack of greenhouse space at the University of Manitoba, remnant seed of the C_1 generation of O.A.C. 21 tetraploids was sown at Guelph for additional data on fertility. Five seeds, where available, from each C_1 plant were space planted (4" x 6") in greenhouse beds.

The fertility in the C_3 generation of all four of the tetraploid

varieties was determined in field grown material at Guelph during the summer of 1956. From each C_2 plant grown in the greenhouse at Winnipeg 20 seeds or less were sown in a five foot row. The rows were spaced nine inches apart.

Four other tetraploid varieties, namely, Breuns Visa, Strangs Franken, Goldfoil and Firlbeck III, supplied by Dr. M. Rommel*, also were grown in this year for fertility comparisons.

The fertility in the C_4 generation of the four tetraploid varieties was determined in greenhouse grown material at Winnipeg in January 1957. In the earlier generations the populations were relatively small and seed from all plants was utilized. Because of greenhouse space limitations sampling in the C_3 was necessary. For the tetraploid O.A.C. 21, 200 seeds from the most fertile plants in C_3 with high fertility pedigrees in C_2 and C_1 and 200 seeds from the most sterile C_3 plants having low fertility pedigrees in C_2 and in C_1 were planted. For the G. B. 61 and Brant tetraploids 40 seeds, picked at random, were planted in the C_4 generation. As the seed set in Montcalm was very low in each generation every seed available of this tetraploid variety was planted.

In all generations plants of the corresponding diploid varieties were included as checks. The diploid varieties did not differ in fertility, all being almost completely fertile. Therefore the data on fertility of the tetraploids required no correction, and the varieties could be compared directly.

Cytological studies were carried out in the C_4 generation. In order

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to obtain meiotic as well as root tip material from every plant, each seed in this generation was planted separately in a four-inch pot. Root tips were collected when the coleoptiles started to emerge from the soil. Usually by this time the roots had reached the bottom or the sides of the pot.

Cold water pretreatment suggested by Dr. A. Mochizuki*, was used to shorten the chromosomes. The excised root tips were placed in glass vials filled with tap water and kept in a refrigerator at 2° C. for 24 hours. They were then fixed in Farmer's solution for at least 24 hours, stained with aceto-carmine, boiled and squashed in aceto-carmine as described for the C_1 material.

In the C_2 and C_3 generations the plants were checked for tetraploidy by visual inspection only. In C_4 however, root tips of every plant grown were tested for exact chromosome number. No diploid plants were found, indicating the reliability of the visual checking method.

The material for meiotic studies was collected from the secondary tillers of the C_4 generation plants. All the meiotic observations were done in PMC's. For this purpose whole spikes were fixed in Carnoy's (6:3:1) and preserved in 70 per cent alcohol. The general cytological procedure was carried out as outlined by Darlington and La Cour (20) and Boyle (10).

Meiotic configurations were observed during metaphase I and the number of different types of multivalent associations was determined in

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all four of the tetraploid varieties. Chromosome disjunction was studied at anaphase I. In O.A.C. 21 barley the chromosome configurations and disjunction were determined separately for different fertility groups of the normal tetraploids as well as in some of the aneuploid plants. In the other three varieties only a random sample from the plants with 28 chromosomes was studied.

In the C_2 generation, when the tetraploid varieties were grown in pots in the greenhouse, a number of dwarf plants were observed. A rough check of the chromosome number of the dwarf plants indicated that they were aneuploids. The percentage of dwarf plants differed from variety to variety. Therefore an attempt was made to correlate the percentage of dwarfs with fertility of the different populations. For this purpose the number of the dwarf plants was counted in each variety and also in the different fertility groups of the O.A.C. 21 tetraploids.

In all generations, except C_1, the number of dwarf and completely sterile plants was determined. The figures obtained are recorded in separate tables and were not used in the calculations of the average seed set of the populations. The main reason for this was that in dense stands the dwarf plants did not develop to maturity and often disintegrated at an early stage of growth. Early disintegration of dwarf plants was found even in space-planted rows under field conditions. Therefore, unless each seed planted is marked, as was done in C_4 generation, the number of dwarf plants obtained at the end of the season may not be accurate.

In the C_4 generation the sterile and dwarf plants were classified according to their morphological characteristics into six groups (see Figure 1) as follows:

D-1 - very small, dwarf plants. Single tiller. No heads produced.

D-2 - very small, dwarf plants. Profuse tillering. No heads produced. Normal leaf width.

D-3 - similar to D-2 but with very narrow leaves.

D-4 - very small, bushy, dwarf plants which die off early in the growing season.

D-5 - normal looking plants but short. Very few tillers. No heads produced.

D-6 - similar to D-5 but with better tillering. Heads always produced. No seed set in main tillers. Later-developing tillers sometimes may set seed.

Statistical analyses were carried out according to the methods described by Goulden (32).

The term "tetraploid" was used for varieties and populations which basically consisted of tetraploid plants but could contain aneuploids or plants with unbalanced chromosome complement.

Figure 1.---A normal autotetraploid barley plant (N) and six morphologically different types of dwarf plants (D-1 - D-6) found in the C_4 generation of four autotetraploid barley varieties.

EXPERIMENTAL RESULTS AND DISCUSSION

Fertility comparisons of autotetraploid barley varieties

The fertility means of four tetraploid barley varieties in four successive generations are presented in Table I. In the C_1 generation all of the varieties tested, except O.A.C. 21, had lower fertility than in later generations. The mean per cent fertility fluctuated within each variety from generation to generation with no apparent trend for increased or decreased fertility with advancing generations.

Environment appeared to affect the fertility of the four varieties tested. In general the generations grown in the greenhouse showed better fertility than those grown in the field. There was a highly significant difference in the fertility of the O.A.C. 21 tetraploids grown in two different greenhouses, i.e., at Guelph and at Winnipeg.

The inter-generation differences in fertility for each variety could not be analysed statistically because each generation was grown in a different place under different environmental conditions.

Within each generation the varieties differed considerably in mean per cent fertility. A statistical comparison of the variety means was made and the "t" values are presented in Table II.

Except in the C_1 wherein O.A.C. 21 was significantly more fertile than all the other three varieties, there were no significant differences in fertility between O.A.C. 21 and Brant. These two tetraploids consistently had the highest average fertility. In all generations the Montcalm tetraploid was significantly lower in fertility than the other three tetraploids. In fact, it was very difficult to maintain the Montcalm tetraploid under ordinary growing conditions because, in addition

Table I:---Mean per cent fertility of four tetraploid barley varieties in successive generations.

Generation	Place Where Grown	O.A.C. 21		Brant		G. B. 61		Montcalm	
		No. of Plants Tested	Fertility in Per Cent	No. of Plants Tested	Fertility in Per Cent	No. of Plants Tested	Fertility in Per Cent	No. of Plants Tested	Fertility in Per Cent
C_1	Field Guelph	238	41.3±1.0	9	23.3±4.76	8	20.4±3.23	17	6.5±1.08
C_2	Greenhouse Winnipeg	704	40.0±0.81	17	43.9±5.80	20	39.2±1.81	6	7.8±1.63
C_2	Greenhouse Guelph	617	50.2±0.94	-	-	-	-	-	-
C_3	Field Guelph	3688	45.5±0.99	62	41.6±2.80	101	32.0±1.60	3	11.1±4.80
C_4	Greenhouse Winnipeg	212	48.8±1.80	25	48.3±5.20	21	40.0±4.60	2	6.7±3.92

Table II:---The "t" values for the comparison of the mean per cent fertility of four tetraploid barley varieties in C_1 to C_4 generations.

	Generation							
	C_1		C_2		C_3		C_4	
Varieties Compared	D.F.	"t"	D.F.	"t"	D.F.	"t"	D.F.	"t"
O.A.C. 21 - Brant	245	3.74**	719	0.67	3926	1.33	235	0.09
O.A.C. 21 - G. B. 61	244	6.10**	722	0.40	3965	7.48**	231	1.79
O.A.C. 21 - Montcalm	253	25.5**	708	17.9**	3867	7.02**	212	4.30**
Brant - G. B. 61	15	0.51	35	0.80	161	2.99**	44	1.20
Brant - Montcalm	24	3.43**	21	6.00**	63	5.55**	25	6.9**
G. B. 61 - Montcalm	23	4.09**	24	13.10**	102	8.63**	21	5.1**

* significant at the 5% level

** significant at the 1% level

to its low fertility the germination percentage of the seed was also low.

The C_3 generations of the four tetraploid varieties obtained during this study were grown side by side with the tetraploid varieties introduced from Germany. Consequently the fertility of the eight tetraploids could be compared directly.

The frequency distribution of plants in ten fertility classes of the eight tetraploids is presented in Table III. As can be seen from the data in Table III fertility varied greatly from plant to plant within each variety. In the varieties where the populations were large highly fertile plants were found. In general, however, the percentage of plants with high fertility was low.

The eight tetraploids ranged in mean fertility from 11.1 to 54.5 per cent. The fertility means were compared by "t" test and the "t" values are presented in Table IV. From the data given in Table IV it can be seen that Breuns Visa had significantly higher fertility than any one of the other seven varieties. O.A.C. 21 and Brant did not differ significally in mean fertility. Montcalm had the lowest mean fertility among the eight varieties included in the test.

Table III.---Frequency distribution of plants at various fertility levels in eight tetraploid barley varieties grown at Guelph in 1956.

Variety	Number and Per Cent	Median Fertility in Per Cent										Total	Mean ± S. E.
		5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5		
Breuns Visa	No.	-	-	-	2	5	1	3	-	-	-	11	54.5±3.4
	%	-	-	-	18.2	45.4	9.1	27.3	-	-	-	100	
O.A.C. 21	No.	140	279	491	677	691	659	501	324	90	14	3866	45.5±1.0
	%	3.6	7.2	12.7	17.5	17.9	17.0	13.0	8.4	2.3	0.4	100	
Brant	No.	4	9	10	9	5	10	9	4	2	-	62	41.6±2.8
	%	6.5	14.5	16.1	14.5	8.1	16.1	14.5	6.5	3.2	-	100	
Strangs Franken	No.	1	2	3	4	2	2	1	-	-	-	15	39.3±4.3
	%	6.7	13.3	20.0	26.7	13.3	13.3	6.7	-	-	-	100	
Goldfoil	No.	1	3	1	7	2	2	-	-	-	-	16	37.5±3.6
	%	6.2	18.8	6.2	43.8	12.5	12.5	-	-	-	-	100	
Firlbeck III	No.	1	-	2	2	-	1	-	-	-	-	6	35.0±6.7
	%	16.7	-	33.3	33.3	-	16.7	-	-	-	-	100	
G. B. 61	No.	11	13	21	32	10	10	1	3	-	-	101	32.0±1.6
	%	10.9	12.8	20.8	31.7	9.9	9.9	1.0	3.0	-	-	100	
Montcalm	No.	1	1	1	-	-	-	-	-	-	-	3	11.1±4.8
	%	33.3	33.3	33.4	-	-	-	-	-	-	-	100	

Table IV:---The "t" values for the comparison of the mean per cent fertility of eight tetraploid barley varieties grown at Guelph in 1956.

Tetraploid	Mean Per Cent Fertility	Compared Tetraploid	D.F.	"t"
Breuns Visa	54.5	O.A.C. 21	3875	3.58*
		Brant	71	2.94**
		Strangs Franken	24	2.77*
		Goldfoil	25	3.14**
		Firlbeck	15	2.61*
		G. B. 61	110	5.92**
		Montcalm	12	7.36**
O.A.C. 21	45.5	Brant	3926	1.33
		Strangs Franken	3879	1.40
		Goldfoil	3880	2.16*
		Firlbeck	3870	1.56
		G. B. 61	3965	7.48**
		Montcalm	3867	7.02**
Brant	41.6	Strangs Franken	75	0.45
		Goldfoil	76	0.92
		Firlbeck	66	0.92
		G. B. 61	161	2.99**
		Montcalm	63	5.55**
Strangs Franken	39.3	Goldfoil	29	0.33
		Firlbeck	19	0.55
		G. B. 61	114	1.59
		Montcalm	16	4.41**
Goldfoil	37.5	Firlbeck	20	0.33
		G. B. 61	115	1.40
		Montcalm	17	4.40**
Firlbeck	35.0	G. B. 61	105	0.61
		Montcalm	7	2.91*
G. B. 61	32.0	Montcalm	102	8.63**
Montcalm	11.1	-	-	-

* significant beyond the 5% level
** significant beyond the 1% level

Fertility distribution and the effect of selection on fertility in four generations (C_1 to C_4) of autotetraploid O.A.C. 21 barley.

Data of the frequency distribution of plants at various fertility levels in the C_1, C_2, C_3 and C_4 generations of tetraploid O.A.C. 21 barley are presented in Table V and illustrated in Figure 2.

As can be seen in Figure 2, the distribution patterns in C_1, C_2 Winnipeg and C_3 were very similar and approached normality. The C_1 generation had a narrower fertility range than the C_2 and C_3 generations, but as the population in this generation was rather low, this could be expected. Similarly the presence of some plants in the 95.5 per cent fertility group in the C_3 generation can be explained by a greatly increased population. The histogram representing the fertility in the C_4, however, indicates that the percentage of plants with good fertility was higher in this than in the three previous generations.

From the C_4 data it may appear that the fertility increased in this generation. On the other hand it can be seen that the frequency distribution for fertility in the C_2 generation, grown at Guelph, had the same tendency and the mean fertility in this generation was even higher than that obtained in C_4 (Table V). Therefore it is believed that the increased fertility in the C_4 generation and in the Guelph C_2 generation could be environmental responses.

A possible explanation for the wide range in fertility in each generation is that the progeny of plants with low fertility would likewise be low in fertility. Similarly, plants with high fertility would produce highly fertile progeny. If this hypothesis was true, it would serve as an excellent explanation for the increase in the number of fertile plants

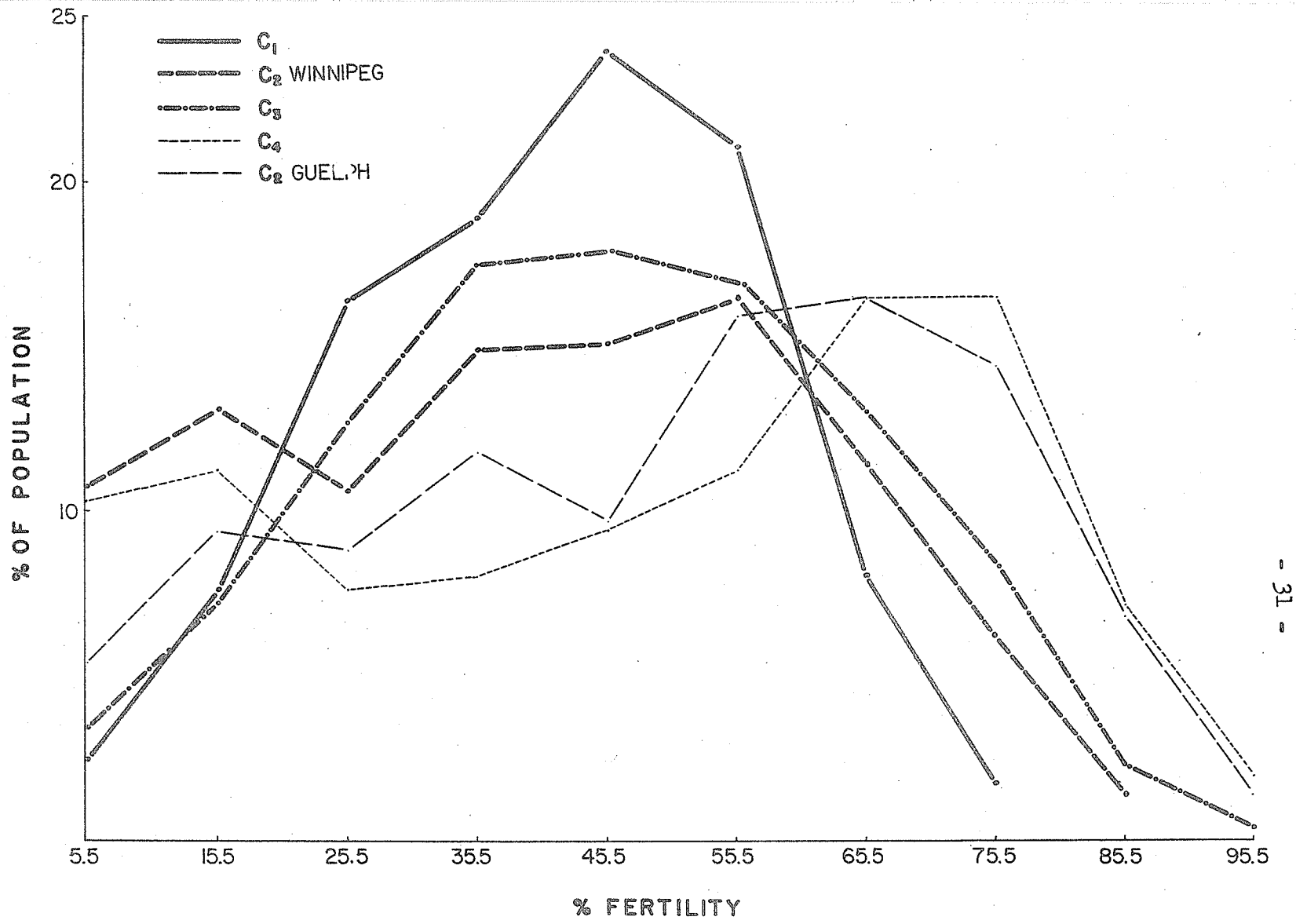


Figure 2.---Frequency distribution of plants at various fertility levels in four generations, C_1 to C_4, of tetraploid O.A.C. 21 barley.

Table V:---Frequency distribution of plants at various fertility levels in four generations (C_1 to C_4) of tetraploid O.A.C. 21 barley.

Generation	Number and Per Cent	Median Fertility in Per Cent										Total	Mean ± S. E.
		5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5		
C_1	No.	6	18	39	45	57	50	19	4	-	-	238	41.3±1.00
	%	2.5	7.6	16.4	18.9	23.9	21.0	8.0	1.7	-	-	100	
C_2 Guelph	No.	33	58	54	73	60	98	102	89	42	8	617	50.2±0.94
	%	5.4	9.4	8.8	11.8	9.7	15.9	16.5	14.4	6.8	1.3	100	
C_2 Winnipeg	No.	76	92	75	105	106	116	80	44	10	-	704	40.0±0.81
	%	10.8	13.1	10.6	14.9	15.1	16.5	11.4	6.2	1.4	-	100	
C_3	No.	140	279	491	677	691	659	501	324	90	14	3866	45.5±0.99
	%	3.6	7.2	12.7	17.5	17.9	17.0	13.0	8.4	2.3	0.4	100	
C_4	No.	22	24	16	17	20	24	35	35	15	4	212	48.8±1.80
	%	10.4	11.3	7.6	8.0	9.4	11.3	16.5	16.5	7.1	1.9	100	

in C_4 and also would mean that selection for fertility is possible.

The data were arranged to test this hypothesis and are presented in Tables VI to XII inclusive.

In Tables V and VI the offspring of plants from each of the fertility classes established in the C_1 generation were rated for fertility in the two C_2 generations. Likewise in Table VII the offspring from plants of each of the fertility classes established in C_2 were rated for fertility in the C_3 generation.

From the data presented in Tables VI, VII and VIII it can be seen that in general the plants with low fertility parents did not differ in fertility from plants which had highly fertile parents. In the C_3 generation (Table VIII), in which each fertility class was represented by a considerable number of plants, even the offspring of the 5.5 per cent fertility class did not differ in fertility from the offspring of the 85.5 per cent fertility class.

For graphical presentation and for statistical comparisons the fertility data in the two C_2 generations and in the C_3 generation were arranged in two groups (Tables IX, X and XI). All plants from parents with fertility below 40 per cent were included in one group and plants from parents with fertility above 40 per cent were included in another group.

The C_4 generation data (Table XII) were also arranged in two groups. however in this generation one group represented only plants with pedigrees that never exceeded the 40 per cent fertility level in any one of the previous generations. The other group included plants having pedigrees with fertility higher than 40 per cent in all the previous generations.

Table VI:---Frequency distribution of plants at various fertility levels in the C_2 generation (grown at Winnipeg) derived from seven fertility classes in the C_1 generation of tetraploid O.A.C. 21 barley.

Median Fertility Percentages in C_1	Median Fertility Percentages in C_2									Total Number of Plants
	5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	
5.5	-	1	1	1	2	-	-	-	-	5
15.5	7	7	4	4	6	6	4	2	-	40
25.5	16	16	9	10	10	13	15	5	1	95
35.5	15	16	15	24	24	28	15	8	1	146
45.5	17	26	27	28	27	30	26	16	5	202
55.5	14	17	14	28	20	28	17	11	3	152
65.5) 75.5)	7	9	5	10	17	11	3	2	-	64
Total	76	92	75	105	106	116	80	44	10	704

Table VII:---Frequency distribution of plants at various fertility levels in the C_2 generation (grown at Guelph) derived from seven fertility classes in the C_1 generation of tetraploid O.A.C. 21 barley.

Median Fertility Percentages in C_1	Median Fertility Percentages in C_2										Total Number of Plants
	5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5	
15.5	-	-	2	-	1	2	4	4	-	-	13
25.5	1	1	2	3	5	10	8	8	7	3	48
35.5	3	8	9	11	5	20	28	21	15	-	120
45.5	9	22	19	19	21	35	31	38	15	3	212
55.5	16	20	13	31	20	19	8	5	-	2	134
65.5) 75.5)	4	7	9	9	8	12	23	13	5	-	90
Total	33	58	54	73	60	98	102	89	42	8	617

Table VIII:---Frequency distribution of plants at different fertility levels in the C_3 generation derived from nine fertility classes in the C_2 generation of tetraploid O.A.C. 21 barley.

Median Fertility Percentages in C_2	Median Fertility Percentages in C_3										Total Number of Plants
	5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5	
5.5	5	14	32	34	38	40	20	15	4	-	202
15.5	16	21	43	53	49	48	30	17	9	2	288
25.5	11	33	37	79	49	60	33	17	7	1	327
35.5	28	44	68	99	95	93	71	47	15	1	561
45.5	31	50	87	128	122	110	91	66	14	3	702
55.5	25	62	106	121	161	128	111	62	17	3	796
65.5	13	39	63	99	115	110	77	57	17	4	594
75.5	9	14	45	58	56	56	50	34	4	-	326
85.5	2	2	10	6	6	14	18	9	3	-	70
Total	140	279	491	677	691	659	501	324	90	14	3866

Table IX:---Frequency distribution of plants at various fertility levels in the C_2 generation (grown at Winnipeg) derived from two fertility classes (below and above 40 per cent) in the C_1 generation of tetraploid O.A.C. 21 barley.

Fertility Class in C_1	Number and Per Cent	Median Fertility Percentages in C_2									Total	Mean ± S. E.
		5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5		
Below 40%	No.	38	40	29	39	42	47	34	15	2	286	38.5±1.3
	%	13.3	14.0	10.1	13.6	14.7	16.4	11.9	5.3	0.7	100	
Above 40%	No.	38	52	46	66	64	69	46	29	8	418	41.1±1.0
	%	9.1	12.5	11.0	15.8	15.3	16.5	11.0	6.9	1.9	100	

Table X:---Frequency distribution of plants at various fertility levels in the C_2 generation (grown at Guelph) derived from two fertility classes (below and above 40 per cent) in the C_1 generation of tetraploid O.A.C. 21 barley.

Fertility Class in C_1	Number and Per Cent	Median Fertility Percentages in C_2										Total	Mean ± S. E.
		5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5		
Below 40%	No.	4	9	13	14	11	32	40	33	22	3	181	58.26 ± 1.60
	%	2.2	5.0	7.2	7.7	6.1	17.7	22.1	18.2	12.1	1.7	100	
Above 40%	No.	29	49	41	59	49	66	62	56	20	5	436	46.85 ± 1.12
	%	6.7	11.3	9.4	13.5	11.2	15.2	14.2	12.8	4.6	1.1	100	

Table XI:---Frequency distribution of plants at different fertility levels in the C_3 generation derived from two fertility classes (below and above 40 per cent) from C_2 generation of tetraploid O.A.C. 21 barley.

Fertility Class in C_1	Number and Per Cent	Median Fertility Percentages in C_2										Total	Mean ± S. E.
		5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5		
Below 40%	No.	60	112	180	265	231	241	154	96	35	4	1378	44.02±0.46
	%	4.3	8.1	13.1	19.2	16.8	17.5	11.2	7.0	2.5	0.3	100	
Above 40%	No.	80	167	311	412	460	418	347	228	55	10	2488	46.35±0.39
	%	3.2	6.7	12.5	16.6	18.5	16.8	13.9	9.2	2.2	0.4	100	

Table XII:---Frequency distribution of plants at different fertility levels in the C_4 generation derived from two fertility classes (below and above 40 per cent) of C_1 to C_3 generations of tetraploid O.A.C. 21 barley.

Fertility of the C_1 to C_3 Generations	Number and Per Cent	Median Fertility Percentages in C_4											Mean ± S. E.
		5.5	15.5	25.5	35.5	45.5	55.5	65.5	75.5	85.5	95.5	Total	
Below 40%	No.	10	7	6	7	10	11	12	18	8	1	90	50.61±2.74
	%	11.1	7.8	6.7	7.8	11.1	12.2	13.3	20.0	8.9	1.1	100	
Above 40%	No.	12	17	10	10	10	13	23	17	7	3	122	47.47±2.38
	%	9.8	13.9	8.2	8.2	8.2	10.7	18.9	13.9	5.7	2.5	100	

The fertility data of the two groups in each generation are represented by histograms in Figure 3. From the histograms it can be seen that selection of plants with high or low fertility in any of the three generations did not change the fertility distribution in the subsequent generation.

The "t" test applied to the fertility means of the two groups of the C_2 generation grown at Winnipeg (Table IX) indicated that they were not significantly different at the five per cent point ("t" = 1.56). The correlation coefficient (r = 0.054) calculated for the C_1 and C_2 (Winnipeg) generations also indicated that there was no correlation between the fertility of plants in these two generations.

In the C_2 generation grown at Guelph (Table X) the offspring of the low-fertility C_1 class, however, had better mean fertility than that of the high-fertility C_1 class. The "t" test applied to the population means indicated that this difference was significant beyond the 1% level (t = 6.92). The correlation coefficient (r = -0.198) calculated for the C_1 and C_2 generations also indicated significant negative correlation for fertility. From these data it would seem that in Guelph the poorest C_1 plants gave the best C_2 offspring. However, at Guelph the plants were grown in fixed beds in the greenhouse and natural light conditions were more favourable for the C_2 offspring obtained from the low fertility C_1 group. Therefore it is believed that the differences in fertility obtained in these two C_2 populations were mainly the result of environmental conditions.

In the C_3 generation (Table XI) the mean fertility in the first group was only 2.33 per cent lower than that in the second group. However, the

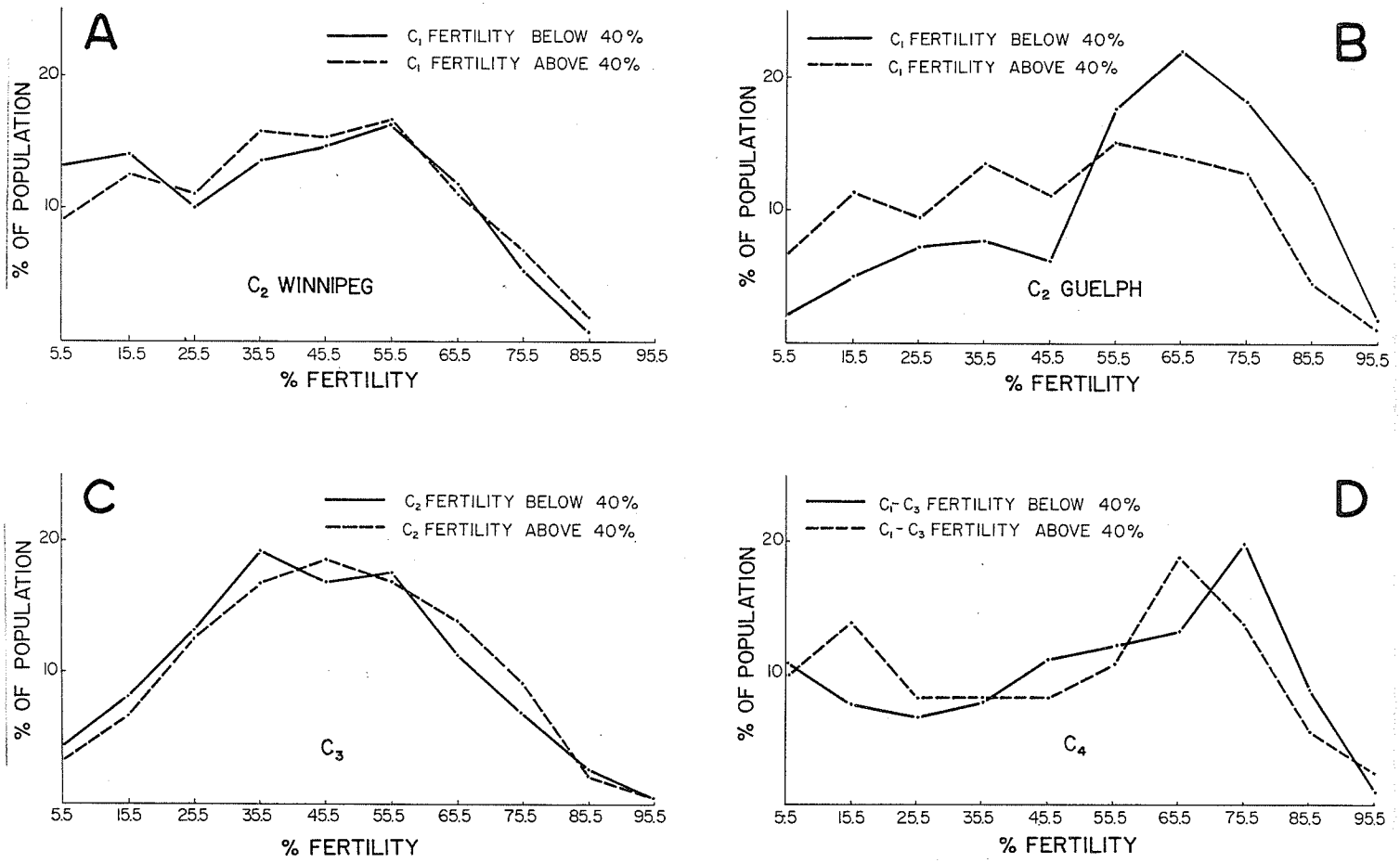


Figure 3.---Frequency distribution of plants at various fertility levels in C_2 (A - grown at Winnipeg, B - grown at Guelph), C_3 (C), and C_4 (D) generations of tetraploid O.A.C. 21 barley derived from two fertility classes in previous generations.

"t" test applied to the means indicated that this difference was significant ("t" = 3.5) at the five per cent level and correlation coefficient (r = 0.219) calculated for the fertility of the C_2 and C_3 generation plants (Table X) also showed significance at the five per cent level.

On the other hand the histogram shown in Figure 3-C clearly indicates that there was very little difference in fertility between the two groups in the C_3 generation. The C_3 generation consisted of a large population of plants covering considerable area in the field. Therefore even small fertility differences caused by environment could give statistically significant results.

In the C_4 generation (Table XII) the difference between the fertility means of the two groups was only 3.14 per cent and in favour of the group with the low fertility pedigree. The "t" value ("t" = 0.86) indicated that this difference was non significant.

The statistical data for the four generations of the tetraploid O.A.C. 21 barley support the conclusions drawn from the histogram, i.e., that selection for low or high fertility apparently did not have any effect on fertility in future generations. Consequently it can be concluded that the increased number of fertile plants in the C_2 and C_4 generations of the O.A.C. 21 tetraploids (Figure 2) was a response to the environment.

The occurrence of dwarfed and aneuploid plants and their relationship to fertility in autotetraploid barley varieties.

The number and percentage of dwarf plants obtained in eight autotetraploid barley varieties in 1956 are presented in Table XIII. The per cent of dwarfs ranged from 20.0 for Goldfoil to 66.7 for Montcalm.

The Montcalm tetraploid which was significantly the lowest in mean fertility, had by far the highest percentage of dwarfs. However, considering all eight of the tetraploids, there was no apparent correlation between the frequency of dwarfs and the mean per cent fertility. Breuns Visa had the highest fertility but the percentage of dwarf plants in this variety was higher than in some of the other varieties. O.A.C. 21 and Brant did not differ significantly in fertility but Brant produced twice as high a frequency of dwarf plants as O.A.C. 21. Similarly Firlbeck III and Goldfoil did not differ significantly in fertility but differed greatly in the frequency of dwarfs.

The number and percentage of dwarf plants in three successive generations of four varieties is given in Table XIV. The percentage of dwarf plants varied from 19 to 85.7 in different populations. There did not seem to be any relationship between the percentages of dwarf plants and fertility within a variety. As an example, in the C_2 generation grown at Guelph, O.A.C. 21 had the highest mean fertility obtained in this variety but the lowest percentage of dwarfs. In C_4, however, O.A.C. 21 had almost as good a fertility as in C_2 at Guelph but the percentage of dwarfs was twice as high.

The number and percentage of dwarf plants in the two fertility groups of the C_1 to C_4 generations of tetraploid O.A.C. 21 barley are given in

Table XIII:---The number and percentage of dwarf plants in eight autotetraploid barley varieties grown at Guelph in 1956.

Variety	Mean Per Cent Fertility	Size of Population*	Dwarf Plants	
			No.	Per Cent
Breuns Visa	54.5	19	8	42.10
O.A.C. 21	45.5	4987	1121	22.48
Brant	41.6	111	49	44.10
Strangs Franken	39.3	24	9	37.50
Goldfoil	37.5	20	4	20.00
Firlbeck III	35.0	11	5	45.45
G. B. 61	32.0	145	44	30.34
Montcalm	11.1	9	6	66.67

* The size of population includes dwarfs in addition to the normal plants previously reported.

Table XIV:---The number and percentage of dwarf plants in three successive generations of four tetraploid barley varieties.

Generation	Place Where Grown	O.A.C. 21			Brant			G. B. 61			Montcalm		
		Size of Population	Dwarfs		Size of Population	Dwarfs		Size of Population	Dwarfs		Size of Population	Dwarfs	
			No.	%		No.	%		No.	%		No.	%
C_2	Greenhouse Winnipeg	995	291	29.3	21	4	19.0	24	4	20.0	36	30	83.3
C_2	Greenhouse Guelph	746	129	17.3	-	-	-	-	-	-	-	-	-
C_3	Field Guelph	4987	1121	22.5	101	49	44.1	145	44	30.3	9	6	66.6
C_4	Greenhouse Winnipeg	312	100	32.1	32	7	21.9	31	10	32.3	14	12	85.7

Table XV.

In each of the generations the percentage of dwarf plants was lower in the group which had the best fertility in previous generations. The percentage of dwarfs in both groups, however, did not change from generation to generation, despite continuous selection for low and high fertility each year. The difference between the two fertility groups was more noticeable in the C_4 than in the previous generations. In this generation each plant was planted in a separate pot and practically no competition took place, therefore the C_4 figures may represent more closely the exact number of dwarfs.

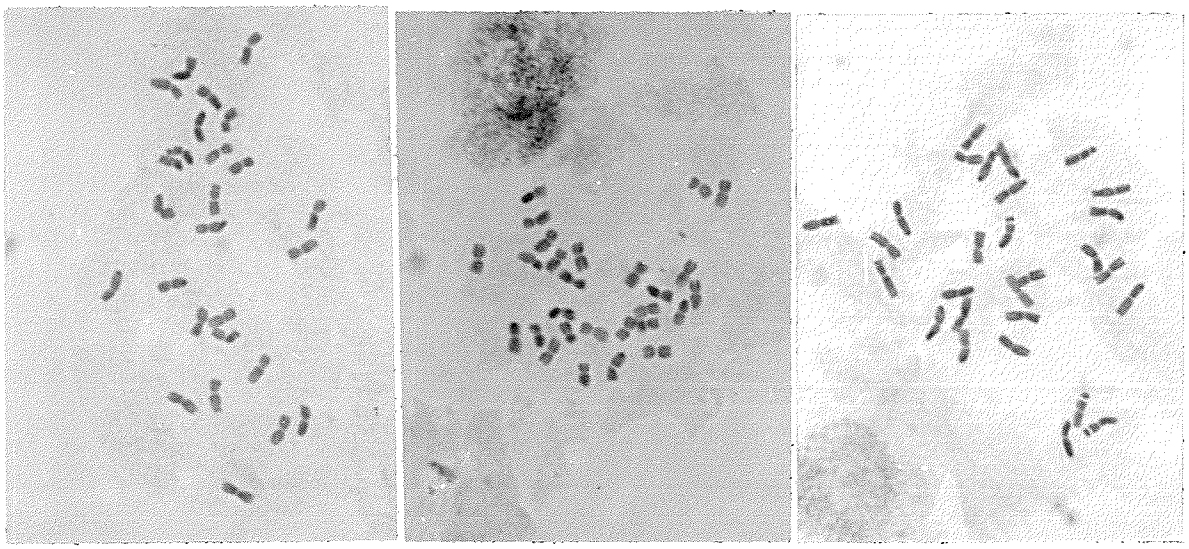
The data indicate that the occurrence of dwarfs in autotetraploid O.A.C. 21 barley could not be reduced by continuous selection of plants with high fertility.

Reports in the literature and preliminary data in the C_2 generation indicated that some of the dwarf plants were aneuploids. Therefore in the C_4 generation the exact chromosome number was determined in every plant of the four tetraploid barley varieties tested. It was found that aneuploid plants with 26, 27, 29, 30 and 31 chromosomes were present. Mitotic metaphases in root tip cells of the aneuploids as well as of a 28 chromosome plant are shown in Figure 4.

The number and percentage of plants deviating in chromosome number in the C_4 generation are presented in Table XVI. The data indicate that the percentage of aneuploid plants fluctuated from 21.9 in Brant to 38.7 in G. B. 61. In general, however, the differences between the varieties were relatively small. By comparing the percentages of plants with different chromosome numbers it can be seen that the 29 chromosome plants

Table XV:---The number and percentage of dwarf plants in C_2 and C_4 generations of tetraploid O.A.C. 21 barley divided in two groups according to the fertility of previous generations.

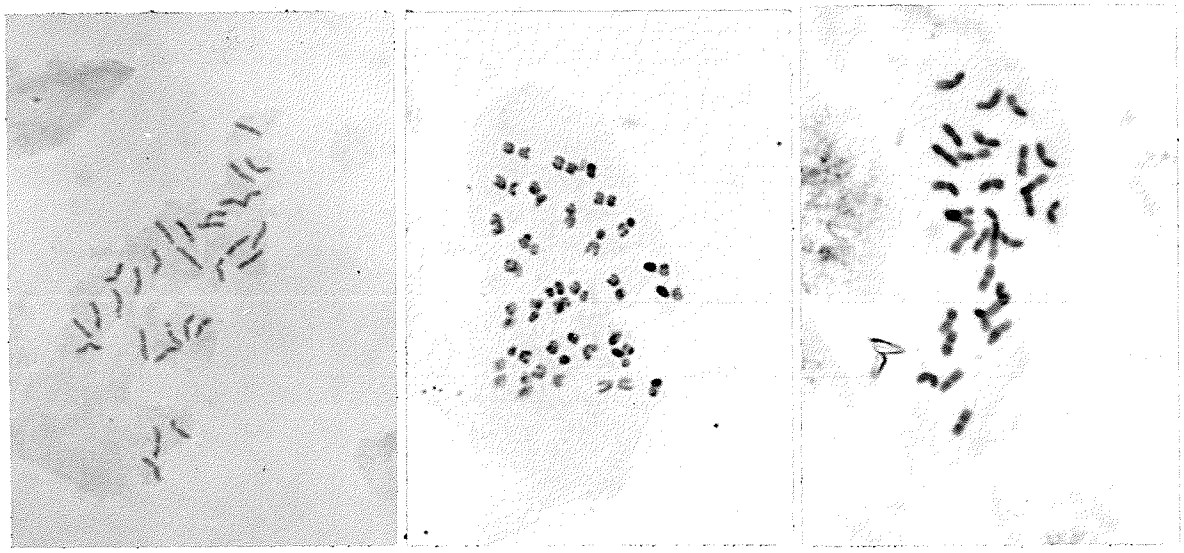
Fertility Group of Previous Generations	C_2 Winnipeg			C_2 Guelph			C_3			C_4		
	Size of Population	Dwarfs No.	Dwarfs %	Size of Population	Dwarfs No.	Dwarfs %	Size of Population	Dwarfs No.	Dwarfs %	Size of Population	Dwarfs No.	Dwarfs %
Below 40%	412	126	30.6	225	44	19.6	1870	492	26.3	142	52	36.6
Over 40%	583	165	28.3	521	85	16.0	3117	629	20.2	170	48	28.2



A (Ca. 2500x)
26 chromosomes

B (Ca. 2500x)
27 chromosomes

C (Ca. 2500x)
28 chromosomes



D (Ca. 900x)
29 chromosomes

E (Ca. 2500x)
30 chromosomes

F (Ca. 2500x)
31 chromosomes

Figure 4.---Mitotic metaphases in root tips of barley plants representing the various chromosome numbers found.

Table XVI:---The number and percentage of plants with different chromosome numbers in the C_4 generation of four tetraploid barley varieties.

Variety	Number of Seeds Planted	Number of Plants Obtained	Number and Per Cent	Chromosome Number 26	27	28	29	30	31	Total Aneu-ploids	Total Tetra-ploids
O.A.C. 21, C_1 to C_3 Fertility Above 40%	200	170	No.	2	20	114	30	4	-	56	114
			%	1.2	11.8	67.1	17.6	2.3	-	32.9	67.1
O.A.C. 21, C_1 to C_3 Fertility Below 40%	200	142	No.	2	17	78	35	10	-	64	78
			%	1.4	12.0	55.0	24.6	7.0	-	45.1	54.9
O.A.C. 21, Total Mean	400	312	No.	4	37	192	65	14	-	120	192
			%	1.3	11.9	61.5	20.8	4.5	-	38.5	61.5
Brant	40	32	No.	-	3	25	2	1	1	7	25
			%	-	9.4	78.1	6.3	3.1	3.1	21.9	78.1
G. B. 61	40	31	No.	-	4	19	7	1	-	12	19
			%	-	12.9	61.3	22.6	3.2	-	38.7	61.3
Montcalm	20	14	No.	-	-	9	3	2	-	5	9
			%	-	-	64.3	21.4	14.3	-	35.7	64.3
Total	500	389	No.	4	44	245	77	18	1	144	245
Mean			%	1.0	11.3	63.0	19.8	4.6	0.3	37.0	63.0

were the most frequent of the aneuploids found. The 27 chromosome aneuploids constituted the next largest group. The number of 26 and 30 chromosome plants was relatively low and only one 31 chromosome plant was found.

The two groups of O.A.C. 21 barley were classified separately and the data also presented in Table XVI. The group arising from pedigrees with low fertility produced more aneuploid plants than the high fertility pedigree group. The increase was mainly in the number of 29 and 30 chromosome plants.

The number of different types of dwarfs in populations of plants with chromosome numbers ranging from 26 to 31 of four tetraploid barley varieties is presented in Table XVII. The D-6 type of dwarf was the most common. Types D-1 and D-4 were least often found, but, where the populations were large, most of the six types described were present. Normal appearing plants were found in the 27, 29 and 31 chromosome groups. The highest percentage of plants classified as normal in the overall aneuploid population was in the 29 chromosome group. The second largest percentage appeared in the 27 chromosome group. Eight to 77.8 per cent of the plants with 28 chromosomes in the four auto-tetraploid barley varieties showed dwarf growth habit. The same types of dwarfs were observed in the 28 chromosome group as in the different groups of aneuploids.

The data presented indicate that a given dwarf type was not associated with a particular chromosome number and that the changes in chromosome number in a basically autotetraploid barley population were not always reflected in the morphological appearance of the plants.

Table XVII:---The distribution of different types of dwarfs in varying chromosome number groups in the C_4 generation of four tetraploid barley varieties.

Chromosome Number	Variety	Total Population	Types of Dwarfs						Total Dwarfs	
			D-1	D-2	D-3	D-4	D-5	D-6	No.	Per Cent
26	O.A.C. 21	4	-	-	1	-	-	3	4	100.0
27	O.A.C. 21	37	2	7	3	1	3	15	31	83.8
	Brant	3	-	-	-	-	-	2	2	66.7
	G. B. 61	4	-	1	-	-	1	1	3	75.0
	Total	44	2	8	3	1	4	18	36	81.8
28	O.A.C. 21	192	2	-	5	-	5	10	22	11.6
	Brant	25	1	-	-	-	1	-	2	8.0
	G. B. 61	19	-	1	-	-	2	1	4	21.1
	Montcalm	9	-	-	-	1	2	4	7	77.8
	Total	245	3	1	5	1	10	15	35	14.3
29	O.A.C. 21	65	2	5	2	3	4	16	32	47.8
	Brant	2	-	-	-	-	-	1	1	50.0
	G. B. 61	7	-	-	-	-	-	2	2	28.6
	Montcalm	3	-	1	-	-	1	1	3	100.0
	Total	77	2	6	2	3	5	20	38	49.4
30	O.A.C. 21	14	1	3	2	-	2	3	11	78.6
	Brant	1	-	-	-	-	-	1	1	100.0
	G. B. 61	1	-	-	-	-	-	1	1	100.0
	Montcalm	2	-	1	1	-	-	-	2	100.0
	Total	18	1	4	3	-	2	5	15	83.3
31	Brant	1	-	-	-	-	-	1	1	100.0
	Grand Total	389	8	19	14	5	21	62	129	33.2

Most of the aneuploid plants were dwarfs and did not produce any seed. The fertile ones, however, could have had some effect on the mean fertility of the tetraploid barley populations.

The mean per cent fertility of the plants with different chromosome numbers in the C_4 generation of four autotetraploid barley varieties is given in Table XVIII. The 28 chromosome plants had slightly higher fertility than the mean fertility of the total populations reported in Table III. The differences, however, were not great, apparently because only a low percentage of aneuploids produced any seed. The 26 and 31 chromosome plants were completely sterile. Also most of the 30 chromosome plants did not produce seed. Only a few of the 27 chromosome plants set seed and would influence but slightly the mean per cent fertility.

The effect of the 29 chromosome aneuploids was more noticeable. In O.A.C. 21, 15.6 per cent of the population of fertile plants had 29 chromosomes. The fertility range of these plants approached the range obtained in the 28 chromosome group. The mean fertility, however, was considerably lower. Therefore in this variety the 29 chromosome group was mainly responsible for the small differences in fertility in the total population as compared with the 28 chromosome plant group.

From the data presented it can be seen that the 28 chromosome plants mainly determined the fertility level in the four autotetraploid barley varieties.

Table XVIII:---The number and fertility of plants with different chromosome numbers in the C_4 generation of four tetraploid barley varieties.

Chromosome Number	Variety	Total Population	Fertile Plants		Per Cent Fertility	
			No.	Per Cent of the Total Population of Fertile Plants	Mean	Range
26	O.A.C. 21	4	0	-	0.0	3 -
27	O.A.C. 21	37	6	2.8	18.9	3.3-50.0
	Brant	3	1	4.0	10.0	-
	G. B. 61	4	1	4.8	3.3	-
28	O.A.C. 21	192	170	80.2	54.9	3.3-100.0
	Brant	25	23	92.0	51.7	3.3- 83.3
	G. B. 61	19	15	71.4	50.0	20.0- 70.0
	Montcalm	9	2	100.0	6.7	3.3- 10.0
29	O.A.C. 21	65	33	15.6	27.4	3.3- 83.3
	Brant	2	1	4.0	6.7	-
	G. B. 61	7	5	23.0	17.3	10.0- 30.0
	Montcalm	3	0	-	-	-
30	O.A.C. 21	14	3	1.4	11.1	10.0-13.3
	Brant	1	0	-	-	-
	G. B. 61	1	0	-	-	-
	Montcalm	2	0	-	-	-
31	Brant	1	0	-	-	-

The meiotic behaviour in relationship to fertility of four autotetraploid barley varieties.

The distribution of quadrivalent associations per cell observed at metaphase I in the 28-chromosome group of plants of O.A.C. 21 barley is presented in Table XIX. Sample configurations are illustrated in Figure 5.

The plants were divided in three groups according to the fertility of the main tillers. The number of quadrivalents per cell ranged from zero to seven. Cells with four to six quadrivalents were most often found. Only one of the 337 cells tested had complete bivalent pairing of chromosomes. The mean number of quadrivalents per cell in the three fertility groups varied from 4.82 to 4.95 and these differences were not significant when compared by a "t" test.

Very few univalents and even fewer trivalents were observed, averaging 0.04 and 0.02 per cell respectively. Only one other configuration was observed. This was an octovalent which was found in only one cell during the entire study.

Assuming that the quadrivalent associations in the secondary tillers are representative of the main tillers the data indicate that the number of quadrivalents per cell was not associated with fertility.

The meiotic configurations at metaphase I in the 28 chromosome plants of Brant, G. B. 61 and Montcalm are recorded in Table XX, XXI and XXII. In these three varieties no cells with one quadrivalent or with bivalents only were found. In G. B. 61 the lowest number of quadrivalents was three per cell. In all three varieties most of the cells had four to six quadrivalents but several cells with seven quadrivalents were noticed.

Table XIX:---The distribution of quadrivalent associations per cell at metaphase I in the 28-chromosome group of plants of O.A.C. 21 barley in C_4 generation.

Fertility Groups	Per Cent Fertility		Number Examined		Quadrivalents								
					Number per Cell								Mean per Cell
	Mean	Range	Plants	Cells	0	1	2	3	4	5	6	7	
I	74.0	66.7-83.3	10	102	1	0	3	7	21	31	34	5	4.95±0.12
II	55.6	50.0-60.0	3	65	0	2	4	4	11	22	17	5	4.82±0.18
III	16.0	0.0-33.3	10	170	0	2	7	18	27	54	48	14	4.91±0.10
Total	-	0.0-83.3	23	337	1	4	14	29	59	107	99	24	-
Mean	46.4	-	-	-	-	-	-	-	-	-	-	-	4.90±0.07

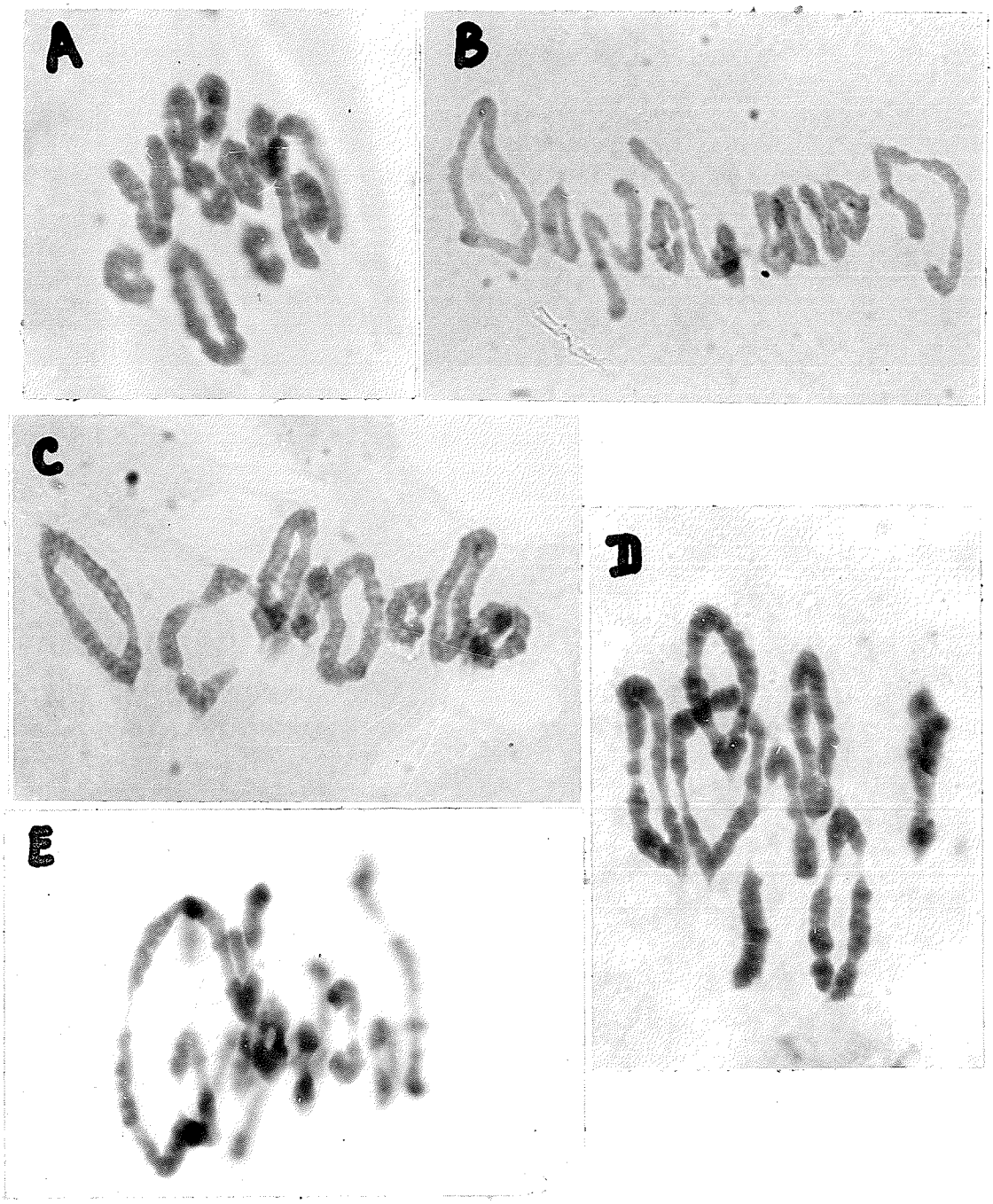


Figure 5.---P.M.C.'s with three (A), four (B), five (C), and seven (D) quadrivalents and one octovalent (E) found at metaphase I of four autotetraploid barley varieties in C_1 generation (Ca 4500 x)

Table XX:---Meiotic configurations at metaphase I in 28-chromosome plants of autotetraploid Brant barley in C_4 generation.

Plant No.	Per Cent Fertility	No. of Cells Examined	Quadrivalents: Number per cell 0	1	2	3	4	5	6	7	Quadrivalents: Mean per Cell	Univalents: Mean per Cell	Trivalents: Mean per Cell
B-7	60.0	9	-	-	-	1	-	6	-	2	5.2	-	-
B-39	60.0	1	-	-	-	-	-	1	-	-	5.0	-	-
B-31	56.7	10	-	-	-	2	2	3	2	1	4.8	0.10	0.10
B-2	16.7	47	-	-	6	6	12	13	9	1	4.3	0.21	0.09
B-14	13.3	29	-	-	-	6	6	9	7	1	4.7	0.17	0.03
B-37	3.3	4	-	-	-	1	1	1	1	-	4.5	0.25	0.25
Total	-	100	-	-	6	16	21	33	19	5	-	-	-
Mean	35.0	-	-	-	-	-	-	-	-	-	4.6±0.13	0.17	0.07

Table XXI:---Meiotic configurations at metaphase I in 28-chromosome plants of autotetraploid G. B. 61 barley in C_4 generation.

Plant No.	Per Cent Fertility	No. of Cells Examined	Quadrivalents: Number per Cell 0	1	2	3	4	5	6	7	Quadrivalents: Mean per Cell	Univalents: Mean per Cell	Trivalents: Mean per Cell
61-4	66.7	49	-	-	-	5	9	16	14	5	5.1	0.08	-
61-37	66.7	3	-	-	-	-	-	1	1	1	6.0	-	-
61-5	50.0	12	-	-	-	1	-	4	7	-	5.4	-	-
61-10	43.3	4	-	-	-	1	1	1	-	1	4.8	-	-
61-32	33.3	9	-	-	-	1	2	2	4	-	5.0	0.11	0.11
61-3	30.0	18	-	-	-	3	4	8	3	-	4.6	0.11	-
Total	-	95	-	-	-	11	16	32	29	7	-	-	-
Mean	48.3	-	-	-	-	-	-	-	-	-	5.1±0.11	0.07	0.01

Table XXII:---Meiotic configurations at metaphase I in 28-chromosome plants of autotetraploid Montcalm barley in C_4 generation.

Plant No.	Per Cent Fertility	No. of Cells Examined	Quadrivalents: Number per Cell 0	1	2	3	4	5	6	7	Quadrivalents: Mean per Cell	Univalents: Mean per Cell	Trivalents: Mean per Cell
M-15	10.0	19	-	-	1	3	4	5	5	1	4.7	0.05	0.05
M-7	3.3	20	-	-	-	-	4	5	10	1	5.4	-	-
M-2	0.0(Dwarf)	26	-	-	2	3	6	6	6	3	4.8	0.08	-
M-10	0.0(Dwarf)	7	-	-	-	1	1	-	4	1	5.4	-	-
M-11	0.0(Dwarf)	16	-	-	-	-	4	7	2	3	5.3	0.06	0.06
Total	-	88	-	-	3	7	19	23	27	9	-	-	-
Mean	2.7	-	-	-	-	-	-	-	-	-	5.0±0.13	0.04	0.02

There was very little variation in the mean number of quadrivalents per cell from plant to plant. The variations observed between plants within the three varieties did not seem to be associated with the fertility in the main tillers of the same plants. In Montcalm even the dwarfs, which were completely sterile, had approximately the same number of quadrivalents per cell as the plants with ten per cent fertility.

The mean number of quadrivalents per cell of the four varieties (Tables XIX, XX, XXI, XXII) ranged from 4.6 for Brant to 5.1 for G. B. 61. The "t" values calculated for the comparisons of the variety means (Table XXIII) show that Brant had a significantly lower number of quadrivalents per cell than G. B. 61 or Montcalm. There were no other significant differences.

There was no relationship between the average number of quadrivalents per cell and the mean fertility of the same plants in the varieties tested. For example, the mean fertility of Montcalm was only 2.7 per cent and the mean number of quadrivalents per cell was 5.0, whereas G. B. 61 with approximately the same number of quadrivalents per cell (5.1) had a mean fertility of 48.3 per cent.

The number of trivalents and univalents per cell was very low in all four varieties and could not be associated with fertility in the same plants.

The formation of complex configurations at meiosis may be influenced by environment. The lateral tillers develop later than the main tillers and therefore the environmental conditions could be different at the time of meiosis. In such cases the fertility data and the meiotic configurations would not be comparable within the same plant tested. However, the

Table XXIII:---The "t" values for comparison of the mean number of quadrivalents per cell in four autotetraploid barley varieties.

Varieties Compared	D.F.	"t"
O.A.C. 21 - Brant	436	0.22
O.A.C. 21 - G. B. 61	431	1.15
O.A.C. 21 - Montcalm	424	0.88
Brant - G. B. 61	194	2.76**
Brant - Montcalm	187	2.45*
G. B. 61 - Montcalm	182	1.12

** significant at 1% level

* significant at 5% level

differences in fertility of each of the two varieties Montcalm and O.A.C. 21 were considerable (Table I), and remained relatively constant for four generations grown under different environmental conditions. It was observed that the lateral tillers of Montcalm and O.A.C. 21 were similar in fertility to their main tiller counterparts. The meiotic configurations changed very little from plant to plant within each variety. Therefore, if the mean number of quadrivalents and trivalents per cell has any effect on the fertility, it should be in some way associated with the mean fertility of the two varieties. Such associations were not found.

The data so far presented apply to the 28 chromosome plants only. It was found previously (Table XVIII) that this group of plants mainly determined the mean per cent fertility in the varieties compared. Therefore the conclusion from these results can be applied to the whole population, i.e., that the mean number of quadrivalents and trivalents per cell were not the factors which determined the fertility differences between the four autotetraploid barley varieties.

The mean number of multivalents and univalents per cell at metaphase I in the 27-chromosome plants were also studied and are recorded in Table XXIV. In the 62 cells examined the number of quadrivalents varied from one to six per cell. The mean number of quadrivalents per cell was 4.3 which was lower than the number found in the 28-chromosome plants. Since a chromosome was missing, it could be expected that trivalents would replace some of the quadrivalents. The number of trivalents per cell was actually greater in the 27- than in the 28- chromosome plants and when these were combined with the quadrivalents in the 27-chromosome

Table XXIV:---Meiotic configurations at metaphase I in the 27-chromosome plants of O.A.C. 21 barley in the C_4 generation.

Plant No.	Per Cent Fertility	No. of Cells	Quadrivalents									Univalents	Trivalents	Total Multivalents
			0	1	2	3	4	5	6	7	Mean per Cell	Mean per Cell	Mean per Cell	Mean per Cell
162	3.3	31	-	-	1	2	13	11	4	-	4.5	0.25	0.81	5.3
142	6.7	18	-	-	-	4	6	6	2	-	4.3	0.22	0.78	5.1
199	0.0 (dwarf)	13	-	1	-	5	4	2	1	-	3.7	0.46	1.00	4.7
Total	-	62	-	1	1	11	23	19	7	-	-	-	-	-
Mean	3.3	-	-	-	-	-	-	-	-	-	4.3±0.13	0.29	0.84	5.1±0.15

group the sum total was almost identical with the total number of quadrivalents plus trivalents in the 28-chromosome group, i.e., 5.1 and 4.9 respectively.

The trivalents were distributed quite uniformly in the cells tested. Usually only one trivalent per cell was found. Very seldom were two and never three or more trivalents noticed in the same cell. The occurrence of a trivalent and univalent in the same cell was very rare indicating that the number of trivalents arising from a loose association of four homologous chromosomes was infrequent and could not be the reason for low fertility.

The mean number of multivalents and univalents per cell at metaphase I in the 29 chromosome plants are presented in Table XXV. The number of quadrivalents in the 29 chromosome plants varied from two to seven per cell. The mean number of quadrivalents per cell was 4.4, i.e., very close to that obtained in the 27 chromosome plants but lower than in the 28 chromosome plants.

This could be expected because the five homologous chromosomes in the 29-chromosome plants could form trivalents and bivalents, or pentavalents, instead of quadrivalents found in the 28-chromosome counterpart.

The mean number of multivalents per cell in the 29-chromosome group did not differ significantly from the number of multivalents in the 28-chromosome or the 27-chromosome groups ("t" = 1.36 and 0.31 respectively).

The relationship between chromosome configurations in the C_4 generation and the fertility in previous generations was studied in the O.A.C. 21 tetraploid, and the results are presented in Table XXVI. The average number of quadrivalents per cell in the plants of the low

Table XXV:---Meiotic configurations at metaphase I in the 29-chromosome plants of three barley varieties in the C_4 generation.

Variety and Plant Number	Fertility	No. of Cells	Quadrivalents: Number per cell 0	1	2	3	4	5	6	7	Mean per Cell	Univalents Mean per Cell	Trivalents Mean per Cell	Pentavalents Mean per Cell	Total Multivalents Mean per Cell
O.A.C. 21-33	0 (dwarf)	11	-	-	1	1	1	4	3	1	4.9	0.4	0.5	0.2	5.5
Brant-B-16	6.7	11	-	-	-	3	2	4	2	-	4.5	0.3	0.6	0.4	5.4
Montcalm-14-4	0 (dwarf)	8	-	-	1	3	2	2	-	-	3.6	0.4	0.6	0.3	4.5
Total	-	30	-	-	2	7	5	10	5	1	-	-	-	-	-
Mean	2.2	-	-	-	-	-	-	-	-	-	4.4±0.24	0.3	0.5	0.3	5.2±0.24

Table XXVI:---The relationship between chromosome configurations in the C_4 generation and the fertility ratings in previous generations of the O.A.C. 21 tetraploid.

Fertility Rating in the C_1, C_2 and C_3.	No. of Plants	No. of Cells	Quadrivalents: Number per Cell 0	1	2	3	4	5	6	7	Mean per Cell	Univalents: Mean per Cell	Trivalents: Mean per Cell	Others
Below 40%	7	116	-	1	2	6	16	41	45	5	5.1±0.13	0.06	0.02	-
Above 40%	16	221	1	3	12	23	43	66	54	19	4.8±0.88	0.03	0.02	1 VIII
Total	23	337	1	4	14	29	59	107	99	24	4.9±0.07	0.04	0.02	1 VIII

pedigree group did not differ significantly from the average number of quadrivalents per cell in the plants of the high fertility pedigree group ("t" = 0.37) indicating that the fertility in previous generations had no effect on the meiotic configurations in the C_4 generation of O.A.C. 21 barley.

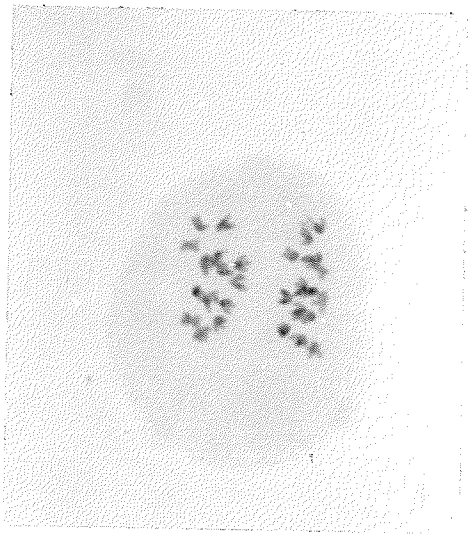
Although the mean number of multivalent associations at metaphase I remained relatively constant in all of the fertility groups, the distribution of chromosomes at anaphase I could have an important bearing on fertility. Therefore the distribution of chromosomes at anaphase I in three fertility groups of the 28-chromosome plants in the C_4 generation was studied. The data are presented in Table XXVII and some of the distribution patterns are illustrated in Figure 6.

The data presented in Table XXVII indicate that cells with normal chromosome distribution (14-14) constituted the largest group. Of the abnormal types, the 13-15 distribution pattern was the most common. The 12-16 distribution pattern was found in only 3.5 per cent of cells. In 11.2 per cent of cells one or more chromosomes remained at the metaphase plate after the others had started to move to the poles (Figure 6-D).

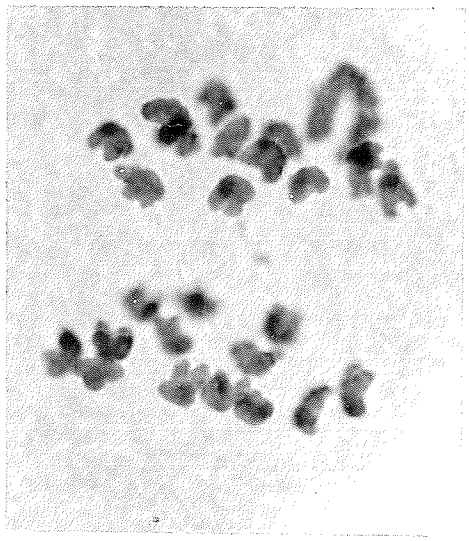
Since the normal tetraploid distribution pattern of 14-14 was by the far most frequent in the three fertility groups, the means of the normal distribution patterns were compared. Although there was no significant difference in the means of the normal distributions between the highest two fertility groups, group I had a highly significant greater mean number of normal distributions than group III. The "t" values were 4.35 and 2.38 respectively.

Table XXVII:--- Distribution of chromosomes at anaphase I in three fertility groups of the 28-chromosome O.A.C. 21 barley plants in the C_4 generation.

Fertility			Number of		No. and Per Cent	Distribution Patterns								
Group	Mean	Range	Plants	Cells		14-14	13-15	12-16	11-1-16	12-1-15	12-2-14	12-4-12	13-1-14	13-2-13
I	74.6	70.0-83.3	8	118	No.	76	23	3	-	2	-	-	14	-
					%	64.4±0.85	19.5	2.5	-	1.7	-	-	11.9	-
II	53.4	50.0-56.7	2	109	No.	68	32	5	1	-	-	-	3	-
					%	62.4±1.05	29.3	4.6	0.9	-	-	-	2.8	-
III	17.4	0.0-33.3	9	235	No.	139	56	8	-	3	3	1	20	5
					%	59.2±0.86	23.8	3.4	-	1.3	1.3	0.4	8.5	2.1
Total	45.3	0.0-83.3	19	462	No.	283	111	16	1	5	3	1	37	5
					%	61.3±0.50	24.0	3.5	0.2	1.1	0.6	0.2	8.0	1.1

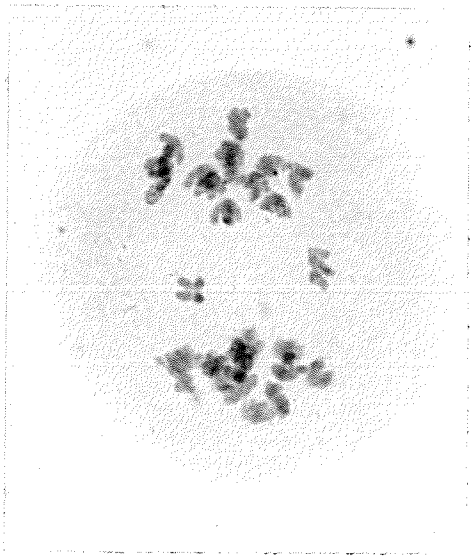


A 14-14
(Ca. 1700 x)



B 13-15
(Ca. 4400 x)

C 16-12
(Ca. 2500 x)



D 14-2-14
(Ca. 2500 x)

Figure 6.---Chromosome distributions at anaphase I in PMC's of 28-chromosome barley plants in the C_4 generation.

To determine the relationship of fertility in previous generations to the anaphase chromosome distribution in PMC's the C_4 data on chromosome distribution at anaphase I in the 28-chromosome plants of O.A.C. 21 barley were arranged in two fertility groups. The results are presented in Table XXVIII.

In the group which had pedigrees with fertility below 40 per cent, 64.6 per cent of the PMC's had normal tetraploid chromosome distribution. In the group with the higher fertility pedigrees (over 40 per cent) only 59.3 per cent of cells had normal tetraploid chromosome distribution. Therefore, normal anaphase distribution of chromosomes was not improved by selecting for fertility in previous generations.

The chromosome distributions at anaphase I in Brant, G.B. 61 and Montcalm are given in Tables XXIX, XXX and XXXI. In Brant 61.6 per cent and in G.B. 61, 50.0 per cent of the cells had normal chromosome distribution. As with O.A.C. 21, the 13-15 distribution pattern was the second largest one in these two varieties. In Brant one cell was found in which 17 chromosomes moved to one pole and 11 to the other pole. This was the most unbalanced distribution found in the material tested.

In Montcalm (Table XXXI) the percentage of PMC's with abnormal distribution was larger than that of normal distribution. In 43.3 per cent of the cells 13 chromosomes moved to one pole and 15 to the other. Only 42.3 per cent of the cells had a normal 14-14 chromosome distribution.

There seemed to be some association between the variety means for fertility and the mean percentage of normal chromosome distribution in the PMC's of each variety. In the C_4 generation (Table I) G.B. 61 had

Table XXVIII:---Distribution of chromosomes at anaphase I in two groups (with low and high fertility pedigrees) of the 28-chromosome O.A.C. 21 barley plants in the C_4 generation.

Fertility Rating in the C_1, C_2 and C_3	Number of Plants	Number of Cells	No. and Per Cent	Distribution Patterns								
				14-14	13-15	12-16	11-1-16	12-1-15	12-2-14	12-4-12	13-1-14	13-2-13
Below 40%	7	167	No.	108	40	4	1	1	-	-	13	-
			%	64.6±0.49	24.0	2.4	0.6	0.6	-	-	7.8	-
Over 40%	12	295	No.	175	71	12	-	4	3	1	24	5
			%	59.3±0.71	24.1	4.1	-	1.4	1.0	0.3	8.1	1.7
Total	19	462	No.	283	111	16	1	5	3	1	37	5
			%	61.3±0.50	24.0	3.5	0.2	1.1	0.6	0.2	8.0	1.1

Table XXIX:---Distribution of chromosomes at anaphase I in the 28-chromosome plants of Brant barley in the C_4 generation.

Plant No.	Per Cent Fertility	Total No. of Cells	Distribution Patterns								
			14-14	13-15	12-16	11-17	11-2-15	12-1-15	12-2-14	13-1-14	13-2-13
B-7	60.0	22	16	4	-	-	-	1	-	1	-
B-39	60.0	2	2	-	-	-	-	-	-	-	-
B-31	56.7	32	18	10	1	-	-	2	-	1	-
B-2	16.7	85	51	14	1	-	-	1	2	13	3
B-14	13.3	89	53	20	-	1	1	2	1	19	2
B-37	3.3	28	19	5	-	-	-	-	-	4	-
Total	-	258	159	53	2	1	1	6	3	28	5
Mean %	35.0	-	61.6±0.35	20.5	0.8	0.4	0.4	2.3	1.2	10.9	1.9

Table XXX:---Distribution of chromosomes at anaphase I in the 28-chromosome plants of G.B. 61 barley in the C_4 generation.

Plant No.	Per Cent Fertility	Total No. of Cells	Distribution Patterns								
			14-14	13-15	12-16	11-1-16	12-1-15	12-2-14	12-3-13	13-1-14	13-2-13
61-4	66.7	85	45	12	5	-	4	3	-	16	-
61-37	66.7	5	2	-	-	-	-	-	1	1	1
61-5	50.0	34	11	4	1	1	1	-	1	13	2
61-10	43.3	15	4	5	1	-	2	1	-	1	1
61-32	33.3	6	3	2	-	-	-	-	-	1	-
61-3	30.0	23	19	2	1	-	-	-	-	1	-
Total	-	168	84	25	8	1	7	4	2	33	4
Mean %	48.3	-	50.0±1.25	14.9	4.7	0.6	4.2	2.4	1.2	19.6	2.4

Table XXXI:---Distribution of chromosomes at anaphase I in the 28-chromosome plants of Montcalm barley in the C_4 generation.

Plant No.	Per Cent Fertility	Total No. of Cells	Distribution Patterns						
			14-14	13-15	12-16	12-1-15	12-2-14	13-1-14	13-2-14
M-15	10.0	30	14	14	-	1	-	1	-
M-7	3.3	13	5	7	-	-	-	1	-
M-2	0 (Dwarf)	107	42	44	11	1	1	7	1
M-10	0 (Dwarf)	3	-	1	-	-	1	1	-
M-11	0 (Dwarf)	55	27	24	3	-	-	1	-
Total	-	208	88	90	14	2	2	11	1
Mean %	2.7	-	42.3±0.47	43.3	6.7	1.0	0.9	5.3	0.5

about eight per cent lower fertility than O.A.C. 21 and Brant. From the "t" values presented in Table XXXII it can be seen that G. B. 61 had a significantly lower percentage of PMC's with normal chromosome distribution as compared with O.A.C. 21 and Brant. Montcalm had much lower fertility in C_4 (Table I) than the other varieties. The percentage of PMC's with normal chromosome distribution in Montcalm was also significantly lower than in the other three varieties tested. This indicates that at least in the Montcalm tetraploids, the abnormal distribution of chromosomes at anaphase I may be a partial cause for sterility.

However, the fertility differences between the four varieties as well as between the three fertility groups of O.A.C. 21 cannot be explained on the basis of abnormal anaphase distribution of chromosomes alone.

With 40 to 60 per cent of PMC's dividing normally there should be enough viable pollen to result in complete pollination. If we assume that the anaphase distribution in the megaspore mother cells was similar to that obtained in the PMC's the number of normal anaphases should be associated with the fertility of the varieties tested. Assuming that the chromosome distribution at anaphase I was the main factor determining fertility in autotetraploid barley, Montcalm should have close to 40 and the other varieties approximately 50 to 60 per cent fertility. However the mean fertility of Montcalm was only 6.7 per cent and the fertility of the other three varieties ranged from 40.0 to 48.8 per cent. Similarly plants in group III of O.A.C. 21 (Table XXVII) with 59.2 per cent normal chromosome distribution at anaphase, had a

Table XXXII:---The "t" values for comparison of the mean per cent of cells with normal chromosome distribution at anaphase I in the 28-chromosome plants of four barley varieties in the C_4 generation.

Varieties Compared	D.F.	"t"
O.A.C. 21 - Brant	719	0.62
O.A.C. 21 - G.B. 61	629	8.33**
O.A.C. 21 - Montcalm	669	27.90**
Brant - G.B. 61	425	8.95**
Brant - Montcalm	465	32.80**
G.B. 61 - Montcalm	345	5.76**

** significant at 1% level

* significant at 5% level

mean fertility of only 17.4 per cent. Plants in group III with 64.4 per cent normal distribution at anaphase had a mean fertility of 74.6 per cent.

The distribution of chromosomes at anaphase I in the 27- and 29-chromosome plants in the C_4 generation is given in Tables XXXIII and XXXIV. In the 27-chromosome plants the 13-14 distribution pattern was the most common one. It was found in 59.3 per cent of the 81 cells tested. The other patterns which occurred frequently were 12-15 and 13-1-13.

In the 29 chromosome plants 56.2 per cent of the PMC's had the 14-15 distribution pattern. The 14-1-14 pattern made up the second largest group.

In the plants with 27 and 29 chromosomes the percentage of cells with the 13-14 and the 14-15 patterns respectively was similar to the percentage of cells with the 14-14 pattern in the 28-chromosome plants. In the aneuploids, even assuming the most ideal distribution possible, only one half of the pollen and egg cells may have 14 chromosomes. Therefore the 27- and 29-chromosome plants can be expected to be 50 per cent less fertile than the normal tetraploids of the same variety.

Table XXXIII:---Distribution of chromosomes at anaphase I in three 27-chromosome plants of O.A.C. 21 barley in the C_4 generation.

Plant No.	Per Cent Fertility	Total No. of Cells	Distribution Pattern						
			13-14	12-15	11-1-16	13-1-13	12-1-14	12-2-13	14-2-11
162	3.3	45	23	7	-	10	4	1	-
142	6.7	21	14	3	1	2	-	-	1
199	0.0 (dwarf)	15	11	3	-	1	-	-	-
Total No.	-	81	48	13	1	13	4	1	1
Mean %	3.3	-	59.3	16.1	1.2	16.1	4.9	1.2	1.2

Table XXXIV:---Distribution of chromosomes at anaphase I in three 29-chromosome plants of O.A.C. 21, Brant and Montcalm barley in C_4 generation.

Variety and Plant No.	Per Cent Fertility	Total No. of Cells	Distribution Pattern							
			14-15	13-16	12-17	14-1-14	12-1-16	13-1-15	11-1-17	13-2-14
O.A.C. 21-33	0.0 (dwarf)	24	13	1	1	5	2	2	-	-
Brant-B-16	6.7	25	14	2	1	3	-	3	1	1
Montcalm-M-14	0.0 (dwarf)	15	9	3	-	3	-	-	-	-
Total No.	-	64	36	6	2	11	2	5	1	1
Mean %	2.2	-	56.2	9.4	3.1	17.2	3.1	7.8	1.6	1.6

GENERAL DISCUSSION AND CONCLUSIONS

One of the observations in this study was that each autotetraploid barley variety had a specific mean fertility which could be influenced to a certain extent by the environment. This is in agreement with several reports (48, 60, 61, 73, 74) which indicate that the fertility in autotetraploid barley varies with the variety and environment.

A wide range in the mean fertility of the barley tetraploids developed and tested in this study was found. It is highly improbable that the entire possible range in mean fertility would have been obtained in so small a sample. Therefore, it can be concluded that if tetraploidy was induced in a large number of diploid varieties, more fertile tetraploids could be found than herein reported. As the fertility differences were determined by the C_2 generation the screening work could be done in a relatively short time.

The extent to which fertility could be improved by this method may not be large. According to the literature no autotetraploid barley varieties have been found to date with fertility close to the corresponding diploid varieties (60, 61, 73, 74). The fertility levels in the tetraploid varieties usually have not exceeded 60 per cent.

Müntzing (46) indicated that because of selection autotetraploids occurring in nature are more fertile than artificially induced ones. The results obtained in this study showed no change in fertility in the four autotetraploid barley varieties tested from C_1 to C_4. This agrees with the findings on six-row barley varieties reported by Ono (62). In the two-row types, however, Ono obtained an increase in fertility from 59.4 to 86.1 per cent in seven generations. He could not explain

the difference in fertility between the two-row and six-row types.

A close examination of Ono's data revealed that some of the two-row varieties like H.E.S.I. did not increase in fertility from F_1 to F_7 even though on the average the two-row varieties did. Similarily some of the six-row varieties, such as Mochimugi I, Barbless I and Barbless II, showed considerable increase in fertility from F_1 to F_6. Therefore, the progressive increase in fertility from generation to generation was not necessarily associated with the two-row or six-row head types but rather with the particular strains used.

The variations in fertility from C_1 to C_4 generations in the O.A.C. 21 tetraploids in this study were unquestionably due to environmental responses. It is possible that in Ono's material part of the seeming increase in fertility reported was due to different environmental conditions in the later generations.

It could be that natural selection for increased fertility in autotetraploid barley is rather slow and thus was not detected in the four generations compared. Ono (62) concluded that it should be possible to breed a tetraploid barley variety with high fertility through successive selection.

The results obtained during this study with O.A.C. 21 barley indicate that the selection of the plants with best fertility in previous generations was not successful in increasing the fertility in the subsequent generation. Ono's figures (62) show that not all of the 19 varieties studied had an increase in fertility from generation to generation. The data obtained in O.A.C. 21 barley therefore may not be representative of other autotetraploid barley varieties .

It may be concluded that continuous selection within a variety is not an universal tool for increasing fertility.

The diploid barley varieties chosen for this study were highly homozygous. Therefore, the autotetraploids obtained by chromosome doubling could also be homozygous. The possibility of selecting for higher fertility in such populations could be based only on the assumption that the plants in early generations after doubling would have an unstable chromosome complement which can be stabilized later.

It has been reported that artificially induced polyploids are "raw" polyploids and do not represent the yield potential which can be obtained later (26). The data from this study, however, indicate that the fertility differences between the varieties remained relatively constant from generation to generation. This indicates that the seed setting ability in autotetraploid barleys may be genetically controlled. Genetic differences conditioning variations in fertility of tetraploids have been reported in other crops such as Lolium perenne (52), corn (29, 72) and rye (67, 70). Müntzing (48) and Rommel (73) have found that the fertility in autotetraploid barleys obtained from hybrid material was better than in the autotetraploids of the parental material.

From the reports in the literature and the data obtained in this study it can be concluded that hybridization and subsequent selection may be one way to increase fertility in autotetraploid barley. Müntzing (48) has postulated that it should be possible to obtain a highly fertile autotetraploid barley variety by using this method.

In the autotetraploid barley varieties tested the percentage of

dwarf plants varied from 20 to 66.7 per cent. Most of the dwarf plants did not produce seed.

A large percentage of dwarfs would cut down considerably the number of productive plants in an autotetraploid barley population. As some of the dwarf types cannot compete with the normal plants the loss could be partly compensated by an increased seeding rate. However, it has been reported in the literature (33, 73) that autotetraploid barley varieties have a lower germination percentage than the diploid varieties. Taking in account this factor as well as the high percentage of dwarf plants the amount of seed necessary to obtain a solid stand of autotetraploid barley would be rather high. Therefore, even if the dwarf plants were not directly responsible for lower fertility, their elimination would be desirable.

In the variety O.A.C. 21 the percentage of dwarf plants was not influenced by continuous selection of plants with high and low fertility for four generations. This indicates that the number of dwarfs is rather constant in a specific tetraploid variety and that new dwarf plants arise from the seeds of normal plants in the previous generation.

The percentage of aneuploids found and the variations in chromosome number noticed in this study in general agree with those reported in the literature (24, 48, 74). However, contrary to Rosendahl (74) who reported some aneuploids with 25 and 24 chromosomes, the lowest number of chromosomes found was 26.

Aneuploidy was definitely associated with dwarfism and sterility. In addition to most of the aneuploids, some of the 28 chromosome plants

were dwarf in growth habit and were completely sterile.

Smith (81) reported a rosette like plant with 28 chromosomes in autotetraploid Everest barley. He explained it as being due to unbalanced constitution of chromosomes. A similar explanation could be applied to the 28-chromosome dwarf plants obtained in this study. In these plants apparently groups of three and five homologous chromosomes were present, but the additional chromosome could not compensate for the loss of the missing chromosome.

Some of the aneuploid plants set seed but were low in fertility. Therefore it could be expected that some of the non-dwarf 28-chromosome plants with unbalanced chromosome complement also produced seed and were low in fertility. These together with the 27- and 29-chromosome partially fertile aneuploids could be responsible for the low mean fertility in the autotetraploid varieties.

The seed producing 27- and 29-chromosome plants constituted a relatively low percentage of the total number of fertile plants in the basically autotetraploid populations. Therefore it could be expected that the percentage of seed producing 28-chromosome plants with unbalanced chromosome complement was also low. Consequently it can be concluded that the abnormalities in chromosome number and chromosome complement were not the main factors causing low fertility in the autotetraploid barley varieties tested.

The mean number of quadrivalents per cell at metaphase I in the four autotetraploid barley varieties varied from zero to seven. This corresponds to the figures reported by other workers. Contrary to the findings of Chen *et al.* (13) no autotetraploid barley plants were found

with completely bivalent pairing. In fact 14 bivalents were observed in only one cell of the 620 cells examined for meiotic configurations. Similarly no barley plants were found having only quadrivalents as reported by Peto (66).

There was very little difference between the four varieties tested in the mean number of quadrivalents per cell. The lowest mean number of quadrivalents per cell was higher (4.6) than the mean number of quadrivalents in autotetraploid barleys reported by other workers. Rosendahl (74) indicated that the tendency to form quadrivalents in autotetraploid barley is not high, and that usually one to three are observed. Chin (18) examining 21 cells found on the average 4.24 quadrivalents and Peto (66) 3.8 quadrivalents per cell. It was found that the mean number of multivalents per cell apparently had no affect on the differential in fertility of the four autotetraploid barley varieties tested. This is in agreement with the findings reported by Rommel (73) and Berg (3). The results do not support Darlington's (19) theory that the number of quadrivalents is responsible for low fertility in autotetraploids. The results are also contrary to those reported by Chen et al. (13) who found a correlation between sterility and the number of multivalents formed in 149 lines of autotetraploid barley.

The percentage of cells with normal anaphase distribution of chromosomes was partly associated with the mean per cent fertility of three groups of autotetraploid O.A.C. 21 barley. There was also some association between the fertility means of the varieties and the mean percentage of normal anaphases in the PMC's in each variety. It was quite obvious that at least in Montcalm the

abnormal distribution of chromosomes at anaphase I could be a partial cause for sterility in the variety.

By comparing the meiotic configurations and anaphase distribution of chromosomes it was found that the disjunction of chromosomes forming quadrivalents at metaphase I was more important in causing unbalanced gametes than the number of quadrivalents formed. The percentage of the normal distribution pattern was specific for each variety, indicating that it may be genetically controlled. Bremer et al. reported (11) that in rye, selection of plants with the most regular meiosis decreased the number of aneuploids.

Thompson (88) found that in rye the variation in disjunction frequency of the same chromosome interchanges is due in part to variation in the genotype. A heritable variation in chromosome behaviour is subject to the action of selection in the same way as more conventional morphological characters. Therefore it should be possible to select for more normal chromosome disjunction at anaphase in heterozygous autotetraploid barley populations, without increasing bivalent pairing.

That such arrangement theoretically is possible can be seen from Berg's work (3) on the tetraploid form of H. bulbosum. He found that in this species from the 734 quadrivalents studied nearly 90 per cent were arranged so that adjacent chromosome disjoined at first metaphase.

Randolf (72) and Fisher (29) working with corn concluded that only 5 to 10 per cent of the sterility in autotetraploids was caused by meiotic irregularities. Other authors (11, 52, 53, 83) also reported that meiotic irregularities alone could not explain the low fertility

level in autotetraploids.

The data obtained in this study indicate that fertility differences among the four autotetraploid barley varieties as well as among the three fertility groups in O.A.C. 21 could not be explained by meiotic irregularities alone. Therefore even in an autotetraploid barley in which complete bivalent pairing or quadrivalents with normal chromosome distribution are obtained the fertility may be far below the diploid level.

It is possible that the differences in fertility between the four autotetraploids tested were caused in part by genetically controlled physiological disturbances as has been reported by a number of authors (29, 44, 67, 70).

General conclusions arising from this work are:

1. A wide range in mean fertility exists in barley autotetraploids, indicating that more fertile tetraploids than those reported in the present study may be found.
2. Selection within a variety is not an effective method of improving fertility.
3. The percentage of aneuploids in basically autotetraploid barley is large and since most of these are dwarfs they contribute to reduced yields but do not explain lowered fertility in normal autotetraploids.
4. Since the fertility differences in the autotetraploid barley varieties tested could not be associated with the number of quadrivalents formed nor could they be ~~explained~~ more than partially accounted for by the abnormal distribution of chromosomes, it is possible that genetically controlled physiological sterility described by Stebbins (84) is partly responsible for low fertility in autotetraploid barley.

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