down by phosphatase secreted into the medium; 2) utilization of phosphorylcholine requires the mediation of food vacuoles; it is possible that the enzyme concentrations required to break down these compounds are high enough only in food vacuoles, in view of findings that wild type DIII and food-vacuole-less NP1 secrete comparable amounts of enzyme activities (Silberstein[4] and confirmed by own unpublished results); and 3) 2-aminoethyl phosphonic acid cannot be hydrolyzed and therefore it is not utilized as a phosphate source by our cells, although they produce large amounts of this compound[5] and it has been reported that *Tetrahymena* homogenates cleave it[6,7].

We want to point out that the results of the figure correlate well with our unpublished results on extracellular phosphatase activities. We found values of 100, 4, and 2% against α-glycerophosphate, phosphorylethanolamine and phosphorylcholine, respectively, whereas no activity was observed against 2-aminoethyl phosphonic acid.

The synthetic growth medium for *T. thermophila* is easy to prepare and supports good growth (doubling times down to 1.6 h) under optimal conditions. It can be used to get insight into aspects of nutrient utilization and interactions between components of the medium[8,9] and, when combined with suitable mutants, the roles of various uptake systems, like cell surface and food vacuoles[10], can be separately analyzed.

Here we have shown that *T. thermophila* cannot use 2-aminoethyl phosphonic acid as a phosphate source for growth and cell multiplication; that it needs food-vacuolar functions to utilize phosphorylcholine; and that these functions are not required for the utilization of trimetaphosphate, α-glycerophosphate or phosphorylethanolamine. Our results also suggest that the activities of the external phosphatases suffice to satisfy the cells' need for phosphate for fast growth. The ability of *Tetrahymena* to use phosphate esters in the absence of food vacuole formation is the first reported evidence for the exploitation of extracellular digestion of nutrients by this type of cell.

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Tetrasomy and quadruple trisomy in pea

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Summary. Two off-type plants, morphologically distinguishable from each other and from their respective sister euploid, were isolated in the M_3 generation of pea interchange heterozygotes. Pollen sterility was very high, ranging from 63.0 to 90.0%. Cytologically one of them was tetrasomic (2n+2 = 16) and the other one was quadruple trisomic (2n+1+1+1+1 = 18). In the tetrasomic plant 1IV+6II was the most frequent (46.7%) chromosome configuration, while cells with 4III+3II were predominant (40.0% cells) in the quadruple trisomic plant.

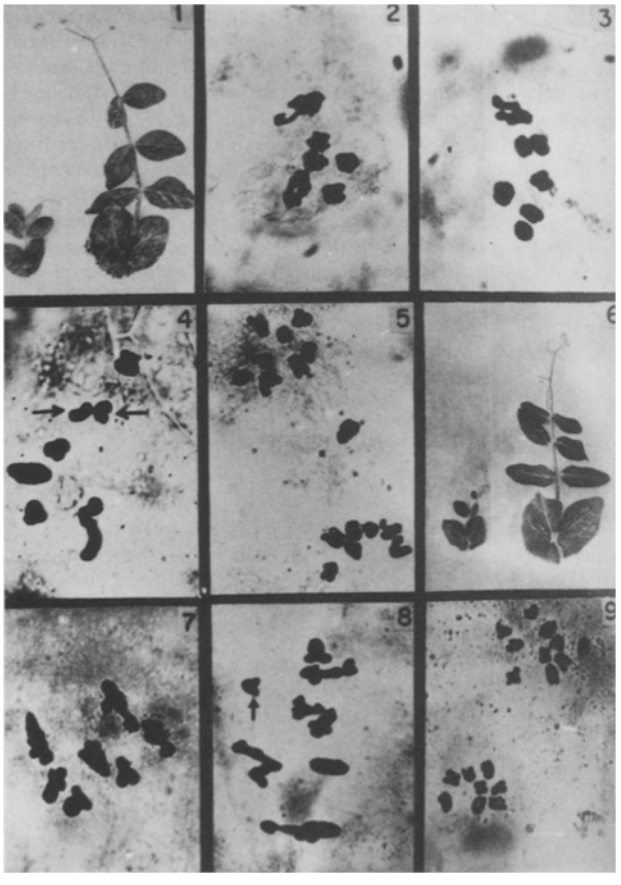
Key words. Pisum sativum; pea interchange heterozygotes; tetrasomics; quadruple trisomy; cytomorphology; off-type plants.

Tetrasomics have been reported in guava[3], datura[4] and other species. However, neither tetrasomy nor quadruple trisomy seem to have been reported in pea. In view of the importance of tetrasomics and multiple trisomics as a source of primary trisomics, and also in determining the homologous series[5-7], the present communication on the cytomorphological behavior of such mutants in pea is of considerable significance.

Two off-type plants were isolated in the M_3 generation of the selfed progeny of interchange heterozygotes induced through gamma-irradiation (10 krad) of the F_1 seeds from the diverse cultivars of pea (*Pisum sativum* L.); namely, T 163 (a local cultivar), 5806-S (a normal plant selection from the progeny of chlorophyll mutant, L-5806 of Dr S. Blix, Sweden), 68 C (Dr W. Gottschalk, West Germany) and PI210613 (Dr A. E. Slinkard, Canada).

The tetrasomic plant was (L613-2, T163 × 5806-S-1-11-13-2) dwarf (52.3 cm; ⅓ of the sister euploid) and characterized by the presence of a slender stem, profused branching with small and yellow green foliage, reduced leaf-length (fig. 1) and late flowering (by 10 days) as compared to its sister diploids. Chromosome configurations 1IV+6II (46.67% cells, fig. 3) and 2IV+4II (33.33% cells, fig. 2) were most frequent, which obviously indicated that the tetrasomy was in interchange heterozygote background. Moreover, presence of cells with III or V or both were not observed. At anaphase I (AI), normal disjunction was observed in 31.8% cells; 22.7% cells had laggards (fig. 5). Pollen grains were of variable sizes and were highly sterile (about 90%). This plant did not set any seed.

The quadruple trisomic plant (L421-5, 68 C × PI210613-11-5-11-5) was very dwarf (15.5 cm, compared to 55.5 cm of its sister euploid), and weak with a slender stem. Stipule and leaflets were yellow-green and very small. Leaf-length was highly reduced (fig. 6) and it was early in flowering by 18 days. At metaphase I, 40% cells showed 4III+3II configuration (fig. 7) indicating the presence of trisomy for four different chromosomes. The next most frequent configuration, 3III+4II+1I (fig. 8) was observed in 30% cells. Univalent frequency was high, ranging from 1 to 12 per cell. At AI, 10-8 separation was most frequent (50.0% cell, fig. 9). Laggards ranging from 1 to 4 per cell were observed. Pollen sterility was high (63.0%) and the plant did not set seed. Morphologically, the tetrasomic plant differed the quadruple trisomic with respect to shape and size of stipules and leaflets. Tetrasomics in guava were also shorter than diploids and male sterile[3]. Reduced vigor and viability of these mutants indicate



the detrimental effect of the extra chromosomes and the narrow tolerance limit. Studies[8] on pea aneuploids indicate that even the addition of one chromosome causes high imbalance leading to reduction in viability. The resultant effect of the extra chromosomes and their tolerance limits varies in different species, as is evident from the literature[7].

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Figure 1. Leaf; tetrasomy (left) and control (right) of pea. Figures 2–5. Meiotic stages of tetrasomy. Figure 2. Metaphase I, 1IV+4II (× 850). Figure 3. Metaphase I, 1IV+6II (× 850). Figure 4. Metaphase I, 2IV+3II+2I (univalents arrowed) (× 1050). Figure 5. Anaphase I, 8 and 7 disjunction with one laggard (× 950). Figure 6. Leaf, quadruple trisomy (left) and control (right). Figures 7–9. Meiotic stages of quadruple trisomy. Figure 7. Metaphase I, 4III+3II (× 1000). Figure 8. Metaphase I, 3III+4II+1I (univalent arrowed) (× 1000). Figure 9. Anaphase I, 10 and 8 disjunction (× 850).

Hyper-tetraploids in pea

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Summary. Cytomorphologically, five hypertetraploids; three hexasomic (4n+2 = 30), one double pentasomic (4n+1+1 = 30) and one multiple aneuploid tetraploid (4n+2+1+1+1 = 33), where isolated in the C_3 generation of pea autotetraploids. Plants with 30 chromosomes were morphologically very distinct from their euploids and characterized by variation in plant height and morphology of leaves. Hexasomic and double pentasomic tetraploids were characterized by the presence of a hexavalent and two pentavalents, respectively. The multiple aneuploid tetraploid showed very vigorous growth and varying frequencies of hexavalents and pentavalents. All the five aneuploids showed high Anaphase-I anomalies; pollen sterility ranged from 50% to 81%.
Key words. Pisum sativum; pea hypertetraploids; hexasomic tetraploids; double pentasomics; anaphase I-abnormalities.

Aneuploids constitute an apparently inescapable component of tetraploid populations and as such exert a negative influence on the productivity of tetraploids. Although the behavior of tetraploid aneuploids has been studied in a wide range of crops, like rye, barley, maize, etc.[3–7] such information for peas is very preliminary[8,9]. The present communication deals with the cytomorphological behavior of certain hypertetraploids in the pea (*Pisum sativum* L.) isolated in the self and F_2 progenies of C_3 generation autotetraploids which were induced through colchicine treatment of the diploid seeds of four cultivars[10], namely, T163 (a local cultivar), 68C (Dr W. Gottschalk, West Germany), 5064-S (a normal plant selection from the progeny of a chlorophyll mutant, L-5064 of Dr S. Blixt, Sweden) ad PI280064 (Dr A.E. Slinkard, Canada).

A total of 212 out of 3423 plants (6.19%) were morphologically distinct; this group consisted of plants of varying chromosome numbers, such as 26 and 27 (1.49%), 29 (3.62%), 30 (1.05%) and 33 (0.03%). None of the morphologically distinct plant was normal euploid (4n = 28). Four out of 36 plants (11.1%) which had 30 chromosomes (three hexasomics, one double pentasomic) and a lone multiple (4n = 33) aneuploid tetraploid were cytomorphologically analyzed (figs 1–9) as follows.

Hexasomic tetraploids (4n+2 = 30):

a) L282-1: It was a dwarf, thick and hardy in nature. The blades of the stipules and leaflets were wrinkled, with an irregular shape. It flowered earlier by 10 days.

b) L81-4: It was weak and a semi-dwarf, with a thin stem. Total leaf-length was reduced, with a compact arrangement of round stipule and leaflets.

c) L157-3: This plant was a dwarf with a thick and hardy stem.