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Original article

Cytogenetic study of diploid and spontaneous triploid oaks, *Quercus robur* L

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Summary — Data are presented on the cytogenetics of 2 unusually large oak trees found in the Voronezh region of Russia. In both trees, cells with $2n = 3x = 36$ chromosomes were predominant, with occasional observations of diploid hypoaneuploid and hyperaneuploid cells. Functionally, the trees can be considered triploids, although in a strict sense, they are mixoploids. Meiosis in microsporogenesis of these trees is very disturbed and, as a consequence, pollen with unbalanced chromosome numbers are produced. Correspondingly, the progeny from each tree were very different in morphological characteristics and cytogenetic constitution. These progeny can be used in gene mapping studies and for other basic research purposes. Studies on some diploid oaks reveal the presence of $2n$ pollen, formed by parallel spindles in the 2nd meiotic division. Methods for producing additional oak triploids that have a potential for heterosis are discussed.

oak / triploid / mixoploid / meiosis / meiotic mutant / progeny

Résumé — Cytogénétique de chênes diploïdes et triploïdes spontanés (*Quercus robur* L). *Des données cytogénétiques relatives à 2 chênes de très grande taille situés dans la région de Voronezh (Russie) sont présentées dans cet article. Des cellules comprenant $2n = 3x = 36$ chromosomes sont prédominantes dans chaque arbre. Des cellules hypoaneuploïdes et hyperaneuploïdes ont également été rencontrées. Au plan fonctionnel, les 2 arbres peuvent être considérés comme triploïdes, plus précisément mixoploïdes. La méiose durant la microsporogenèse est très perturbée et produit des grains de pollen au nombre de chromosomes déséquilibré. Les descendants de ces arbres manifestent des caractéristiques morphologiques et une constitution cytogénétique très variables. Ces familles peuvent être utilisées pour des études de cartographie génétique et d'autres recherches fondamentales. Des études similaires faites sur des chênes diploïdes mettent en évidence des grains de pollen à $2n$ chromosomes formés par des fuseaux parallèles lors de la seconde division méiotique. Des méthodes de production de chênes triploïdes en vue de générer de l'hétérosis sont discutées.*

chêne / triploïde / mixoploïde / méiose / mutant méiotique / descendance

INTRODUCTION

The Voronezh region in south central Russia is famous for oak stands producing excellent quality lumber. Rich Voronezh chernozem provide optimum edaphic conditions for oak species. Two triploid trees of *Quercus robur* L were discovered in the Voronezh region by the scientific researchers of the Central Research Institute of Forest Genetics and Breeding, VV Ievlev and TI Pletmintseva. The trees were more than 100 years old and differed from oaks of a similar age. They were of gigantic height, weak fertility and exhibited unusual morphological and anatomical features. These characteristics have often indicated a polyploid nature in many plant species.

In order to obtain objective information about the cytogenetic nature of these 2 trees, we analyzed various cytological characteristics, including chromosome number, meiosis in microsporogenesis and the development of the male gametophyte.

MATERIALS AND METHODS

For the cytological investigations, branches were taken from each putative triploid and a number of putative diploid trees at the appropriate sampling period for meiotic observations. The branches were placed into water vessels and kept in a cold room. Somatic chromosome counts were made using vegetative buds that were removed from the branches and placed in a damp penicillin bottle under freezing conditions for 1–2 h to inhibit spindle fiber formation. The young leaves were then fixed in aceto alcohol.

For the study of meiosis in microsporogenesis and the process of development of the male gametotype, the flower buds were fixed from the stage of green cone up to the flowering.

All materials were stained in acetohaemotacsilin. The squash technique was used to prepare slides for microscopical examination.

RESULTS

Cells containing $2n = 3x = 36$ chromosomes were prevalent in leaf meristematic tissues in both trees, confirming the suspicions of a polyploid nature (fig 1a,b). In ad-

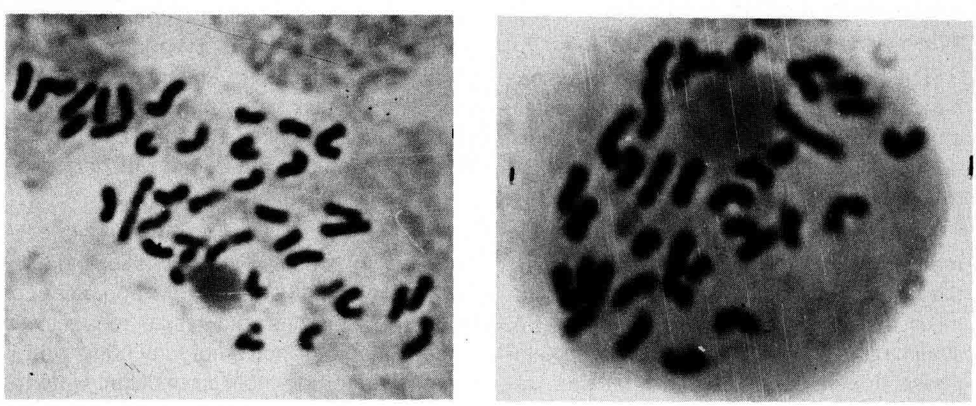


Fig 1. Typical prometaphase plates of triploid oak trees. **a.** $2n = 36$ in cell of the first tree. **b.** $2n = 36$ in cell of the second tree.

dition to the triploid cells, each tree had meristematic cells with other chromosome numbers. Diploid, hypoaneuploid and hyperaneuploids cells were found. Consequently, these trees could be classified as mixoploids in a strict sense.

Meiosis in both trees was abnormal, as typically found in plants with unbalanced chromosome numbers. At metaphase I, a broad spectrum of chromosomal configurations, from 36 univalents to 12 trivalents, were observed. Lagging chromosomes were frequent in anaphase I (fig 2a,b). Some chromosomes were delayed at the equatorial plate, while others were located outside the achromatic spindle (fig 2b). Occasionally, metaphase plates were formed and only one stage was observed: meta–anaphase (fig 2c). In such instances, the distribution of chromosomes in metaphase II was unequal (fig 2d,e). In some microsporocytes, aggregation of chromosomes into separate groups was observed (fig 2e).

The chromosomal disturbances in the second meiotic division were similar to abnormalities in the first division (fig 2f). Correspondingly, many unbalanced microspores were formed (fig 2g), that subsequently resulted in pollen grains with different chromosome numbers (fig 3a–c).

The number of cells with meiotic disturbances in both oak trees varied over different years. The maximum percentage of abnormal divisions was 98% of the total number of dividing microsporocytes.

Although the 2 triploid oak trees had many common meiotic characteristics, they also had specific peculiarities. Cytomixis was observed in one tree (fig 3a). Preliminary divisions of the nuclei in telophase II were found in the other tree, resulting in unbalanced pollen (fig 3b). The meiotic irregularities caused diversity in pollen chromosome number and disturbances in male gametophyte development (fig 3d-g). The same anomalies may be expected in the female gametophyte, since the progeny of these trees have variable morphological characters (Ievlev and Pletmintzeva, personal communication).

Meiosis in pollen mother cells of diploid oak trees was also investigated. This process was found to be essentially normal in these trees, with approximately 5% of the microsporocytes exhibiting abnormal division. However, among these trees, several individuals were found to form 2*n* pollen grains, comprising 5–10% of the microspores (fig 4a). The 2*n* pollen was found to be formed by parallel spindles (sensu, Mok and Peloquin, 1975) in the second meiotic division (fig 4b).

DISCUSSION

Chromosomal variation in progeny from the triploid trees can be the basis for further cytogenetic research. In particular, analyses of aneuploid offspring from the triploid trees would be an excellent method to genetically map oak chromosomes. Unfortunately, only these 2 triploid oaks are known to exist. Other mature triploids of oak have not been discovered. A possible cause may relate to dysgenic selection that was conducted in forests of this region for many years. It would be desirable to study additional trees in order to gain a better understanding of the mechanism(s) of origin for triploid oaks.

Other triploid *Q robur* have been observed in studies of twin seedlings by Johnsson (1946) and Burda and Schepotiev (1973). These scientists respectively speculated that polyembryony could be responsible for the triploid condition. Unfortunately, there have been no additional reports on the triploid seedlings identified in their studies. Our data suggests that triploid oaks may originate by participation of

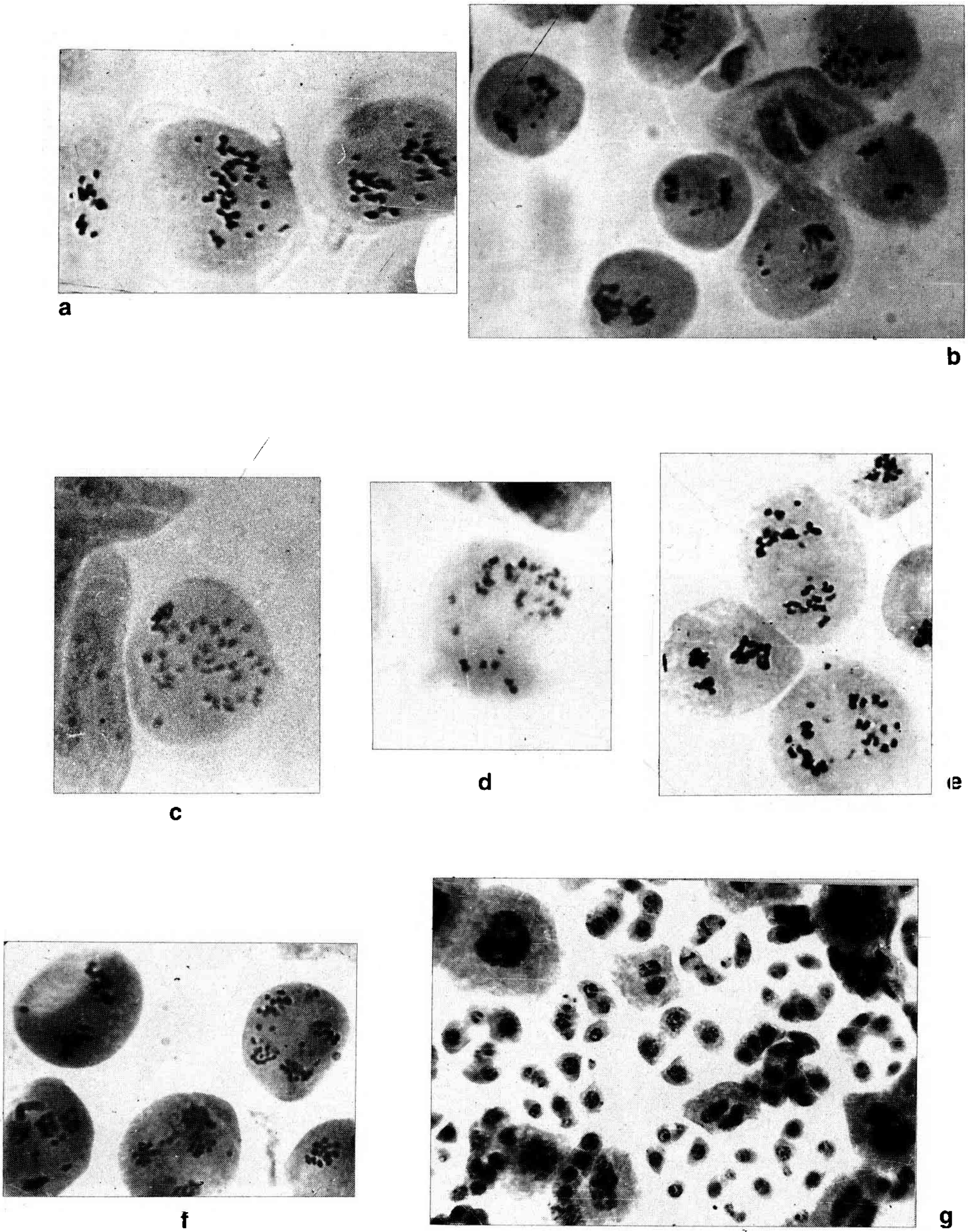


Fig 2. Meiosis and microsporogenesis in triploid oak trees. **a.** Premature movement of some chromosomes to pole in MI. **b.** Premature reaching of poles by some chromosomes in early AI and lagging univalents in late AI. **c.** Meta-anaphase. **d–e.** Unequal groups by chromosome number in M II; aggregations of some chromosome groups (microsporocyte on the right of e). **f.** Disturbances in MII and AII similar to those in the first division. **g.** Microspores.

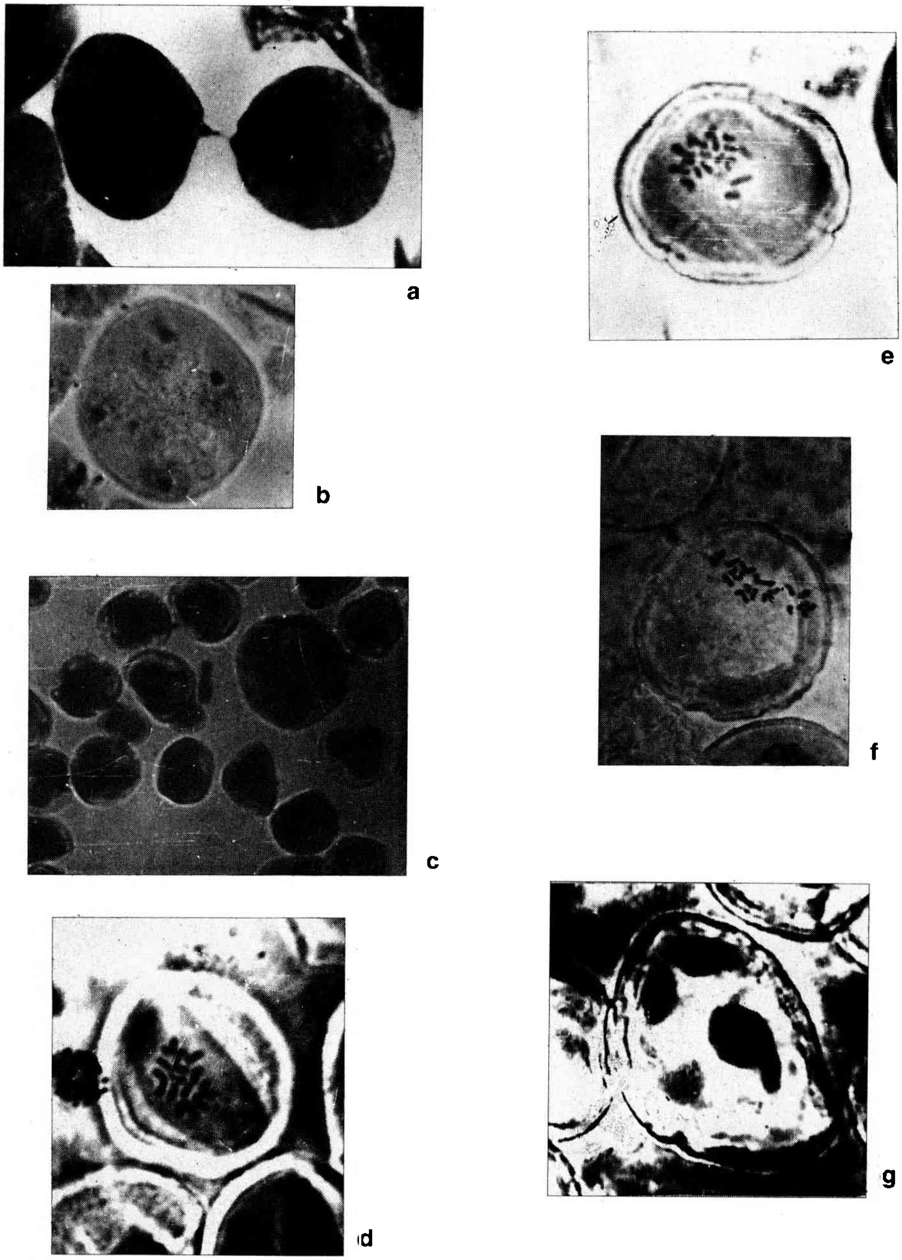
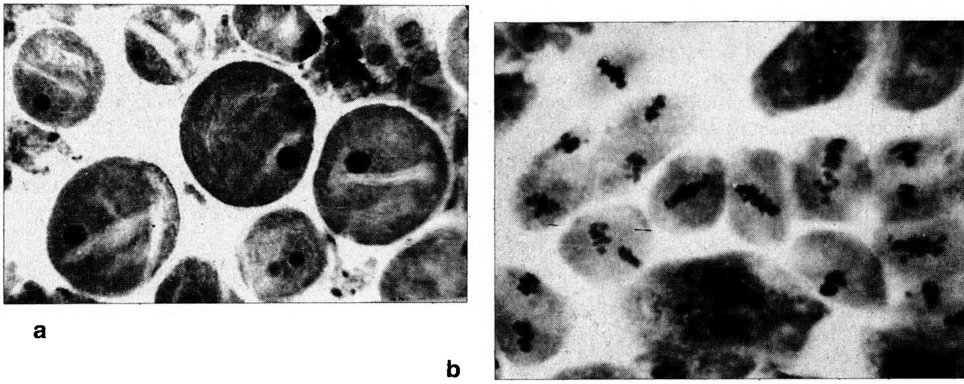


Fig 3. Meiotic disturbances in male gametophyte development in triploid oak trees. **a.** Cytomixis between two microsporocytes in the first triploid tree. **b.** Nuclear division in telophase-II. **c.** Pollen in the second triploid tree. **d–f.** Pollen grains with different chromosome numbers; **g.** Male gametophyte with 1 vegetative and 2 generative nuclei.



a

b

Fig 4. Meiotic mechanisms of 2*n* pollen formation. **a.** Haploid and diploid pollen grains in diploid oak tree. **b.** Parallel spindles in M II.

2*n* gametes as found in other plant species, *eg, Populus tremula.*

The observed mechanisms of 2*n* pollen formation by parallel spindles ensure high heterozygosity in the resulting progeny. This type of pollen formation could be the result of a meiotic mutant gene(s) as shown in *Solarum* (Mok and Peloquin, 1975) and *Medicago* (Vorsa and Bingham, 1979). In these 2 crop species, the triploids formed in this manner expressed heterosis due to the high heterozygous 2*n* gametes. Correspondingly, the meiotic mutant oaks detected in this study or the use of 2*n* pollen induced by thermoshock (*cf* Mashkina *et al,* 1989) could be used to produce additional triploids that have a potential for heterosis resulting in increased yield.

Triploid oaks appear to occur rarely, however, more thorough surveys need to be conducted. More observations are needed to determine the relative growth rates and development of triploid *versus* diploid trees. Triploid seedlings may be as competitive as diploid seedlings in early development, which is critical to establishment of oaks in forest setting. It is possible that triploid oak seedlings have some abnormalities in the development of their root system as in polyploid seedlings of pine (Isakov *et al,* 1977). When triploid seedlings are detected, morphological observations should be compared with those of diploid seedlings for possible identification of distinguishing features.

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