Suppression of 'dot-like' exchanges in C-bands and late replicating DNA-rich regions of chromosomes

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Summary. An apparent suppression of 'dot-like' exchanges in C-bands and late replicating DNA-rich regions of chromosomes has been observed in *Allium cepa*. This result suggests that the occurrence of SCE very near each other could be avoided in these chromosomal regions.

The 5-bromodeoxyuridine (BrdU) dye techniques have proved useful for studying sister chromatid exchanges (SCE) both in animal and plant metaphase chromosomes. These techniques are based on the incorporation of the base analogue BrdU into DNA in place of thymidine, and subsequent cytological detection of BrdU-substituted DNA by a reduction in the fluorescence of certain dyes[2-5] or by a differential Giemsa staining[6,7]. The analyses performed have revealed that SCE seems to occur spontaneously[4,8] but the frequency can be highly increased experimentally by a variety of physical[9,10] and chemical agents[11,12].

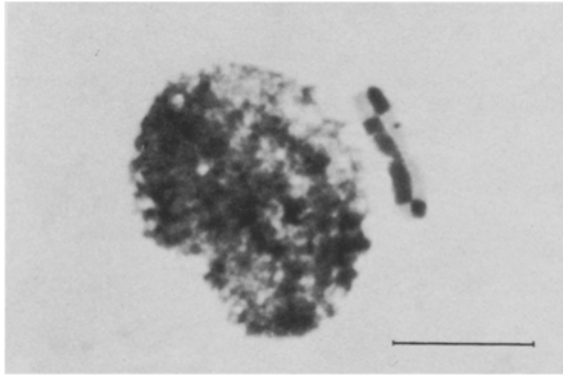
Kihlman[13] was the first who described for *Vicia faba* chromosomes that some of the reciprocal SCE involved material of smaller dimensions than the width (diameter) of the chromatid. These so called 'dot-like' exchanges were subsequently observed by Schvartzman and Cortés[14] in *Allium cepa* L. chromosomes (fig.) and in order to test Kihlman's view that they represented exchanges between short stretches of chromatin fibres, a statistical analysis was performed with positive results[15].

The incidence of SCE have proved to be different in euchromatic and heterochromatic regions of metaphase chromosomes, although the results reached by different authors are contradictory. While in some cases a preferential occurrence of SCE in heterochromatin have been reported[16,17] in others a lower than expected frequency has been observed in such chromosomal regions[14,18,19]. In the present study, we tried to determine the frequency of 'dot-like' exchanges in euchromatin and heterochromatin of *Allium cepa* L. chromosomes. In this species it has been shown that C-bands are localized at the telomeres, whereas late replicating DNA-rich regions correspond to the telomeres as well as the pericentromeric regions[20]. The result obtained by us was an apparent suppression of the occurrence of 'dot-like' exchanges in these chromosomal regions.

Distribution of 'dot-like' exchanges along chromosomes. The expected[1]-values were calculated assuming that 'dot-like' exchanges may occur randomly all along chromosomes according to the length of each region, while the expected[2]-values were calculated on the basis of the differential SCE frequency observed at each chromosome region

Chromosome region	'Dot-like' exchanges[a]		
	Expected[1]	Expected[2]	Observed
C-band regions	25.56	21.22	0
Late replicating DNA-rich regions	51.12	36.60	0
Other chromosome regions	90.88	105.4	142

[a] These data were obtained after studying the localization of 142 'dot-like' exchanges.



Allium cepa Giemsa-stained metaphase chromosome showing a 'dot-like' exchanged segment between sister chromatids. The bar represents 10 µm.

Material and methods. Meristem roots of *Allium cepa* L. (onion) bulbs were grown in the dark at a constant temperature of 25 °C ± 0.5 in tap water renewed every 24 h and aerated continuously (by bubbling air) at a rate of 10–20 ml/min. The bulbs were placed in such a manner that only their bases remained submerged in the water.

The experiments began when roots reached 15–20 mm. In order to analyze the occurrence of SCE, bulbs were treated with 10^{-4} M BrdU and 10^{-7} M fluorodeoxyuridine (FdU) for a total time of 25 h to ensure BrdU incorporation into DNA throughout 2 consecutive rounds of replication. During the BrdU treatment, the culture receptacles and the bulbs were preserved from light by wrapping them with aluminium foil. Finally roots, still attached to the bulbs, were treated with 0.05% colchicine for 3 h and root tips were fixed overnight in ethanol-acetic acid (3:1) at 4 °C. After fixation, roots were treated with pectinase (Sigma, from *Aspergillus niger*) dissolved in citrate buffer adjusted to pH 4.2 at 37 °C for 1 h and then squashed.

Coverslips were removed by the dry ice method and preparations were hydrated by passing them through ethanol solutions (absolute, 96, 70, 50, 30%) and distilled water. Then, the preparations were submitted to a incubation with RNase (Sigma, Ribonuclease-A from bovine pancreas) at 28 °C for 1 h and the slides were treated with 33258 Hoechst at room temperature for 30 min and subsequently, exposed to a fluorescent sun lamp radiating in the 280–380 nm band (Philips HP 3202) at a distance of 10 cm. Such exposure was performed in a moist chamber with 0.5 × SSC for 1 h. Slides were treated at 55 °C in the same solution again for 1 h and finally, stained during 9 min with Giemsa (3%) in phosphate buffer adjusted to pH 6.8, briefly washed in the same buffer, air dried and mounted with Euparal.

Results and discussion. The distribution of 'dot-like' exchanges in euchromatin and heterochromatin proved not to be random (table), but to be exclusively in the former chromosomal regions. As can be seen both in C-bands (telomeres) and late replicating DNA-rich regions (telomeres and pericentromeric regions of chromosomes) the occurrence of 'dot-like' exchanges seemed to be suppressed. Although a less than expected frequency of SCE in heterochromatin has been reported in *Allium cepa* L. metaphase chromosomes[14] this drastic suppression of 'dot-like' exchanges may be considered as a new result of special significance in order to gain an understanding of the relation existing between chromatin structure and SCE occurrence.

The general view is that the minor incidence of SCE in heterochromatic regions could be a result of the high degree of coiling of chromatin fibres in such regions throughout the cell cycle except for a brief replication period. This supercoiling is thought to be responsible for making DNA more difficult of access to breakage, as a 1st step for the occurrence of a SCE, although factors other than chromatin condensation, e.g. base composition and base sequence of DNA may also enter the SCE picture[18]. On the other hand it is now clear that BrdU concentration has an effect on the incidence of SCE[21,22] and it can be postulated that fractions of DNA rich in AT-pairs might incorporate more BrdU than DNA rich in GC-pairs, resulting in a higher sensitivity to SCE occurrence.

If we consider that SCE occur in heterochromatic regions in *Allium cepa* L. chromosomes at a lower frequency than expected, we certainly cannot establish any conclusion about the intrinsic cause of this phenomenon, e.g. chromatin condensation or the base sequence of the DNA, but the case of the apparent suppression of 'dot-like' exchanges in heterochromatin seems different.

If we assume that 'dot-like' exchanges may be a consequence of 2 exchanges occurring in close proximity it is difficult to imagine that the base composition or base sequence of DNA in these chromosomal regions coult prevent the occurrence of such neighboring exchanges. This statement allow us to postulate that the special structural characteristics of heterochromatin rather than the base-composition of DNA could be the cause of the suppression of 'dot-like' exchanges. With respect to pericentromeric regions of chromosomes (late replicating DNA-rich but not C-banded) an explanation is more difficult to reach, mainly because there is not yet complete knowledge about its inclusion or not in constitutive heterochromatin. In this sense, it seems that the pericentromeric banding in *Allium cepa* L. chromosomes can be obtained under certain conditions[23].

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Action des températures élevées sur la reproduction du planorbe *Biomphalaria glabrata* (gastéropode pulmoné)

Effects of high temperature on reproduction of the freshwater snail *Biomphalaria glabrata* (Gastropoda: Pulmonata)

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Summary. A temperature of 33 °C increases growth of young snails but not that of adults. Adult fecundity is reduced and young snails do not reach sexual maturity. Oogenesis remains normal but late stages of spermatogenesis are scarce. The weight of the albumen gland in adult snails is increased, as is that of the albumen gland and the female part in young specimens. High temperatures, however, do not prevent differentiation of the genital apparatus but disturb its functioning.

Chez les mollusques vecteurs, il existe une température optimum permettant d'observer une fécondité importante: au delà de cette température, on constate le plus souvent que la croissance s'accélère et que la fécondité diminue[1]. Chez *Biomphalaria glabrata,* c'est vers 25 °C que la fécondité est maximum. A 30 °C la croissance corporelle est plus forte qu'à 25 °C mais la fécondité diminue ou s'annule[2–7]. Michelson[3] a étudié l'appareil génital d'animaux élevés à 30 °C. Les individus primitivement immatures de 7 mm de diamètre s'accroissent plus vite qu'à 25 °C mais leur fécondité est très faible; la glande de l'albumine est réduite et l'ovotestis contient peu d'ovocytes. Si les animaux ont 4,8 mm de diamètre, ils sont totalement stérilisés; leur glande de l'albumine est réduite ou absente, il y a peu d'ovocytes dans la gonade mais la spermatogenèse est normale. Chez les adultes, on a des résultats similaires mais la réduction de la glande de l'albumine est plus limitée. Souvent, l'été, j'ai observé une diminution de la fécondité des planorbes en élevage, sans que leur dissection ne revèle de modification spectaculaire de l'appareil génital. J'ai donc entrepris une étude de l'action des températures élevées sur le fonctionnement de l'appareil génital.

Matériel et méthodes. Les expériences sont effectuées à partir de 3 lots de *Biomphalaria glabrata* de souche brésilienne de taille et poids comparables, élevés à une température constante de 25, 30 ou 33 °C. Les animaux placés à 25 °C constituent le groupe témoin.

La 1re série d'expériences porte sur des individus adultes fonctionnels (diamètre supérieur à 13 mm), et dure au moins 4 semaines.