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Sister Chromatid Exchanges in Coniferous Forest Trees

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Summary

A significant increase of the sister chromatid exchange (SCE) frequency is typical for mutagen-treated cells and widely considered as an indicator of genotoxic environmental impacts. SCEs were studied in 3 widespread gymnosperms: the spruce *Picea abies,* the pine *Pinus sylvestris,* and the larch *Larix decidua,* each with 2n=24 chromosomes.

Unifilarly BrdUrd-substituted chromosomes revealed, by means of the fluorescent plus Giemsa (FPG) technique, a basic SCE frequency of 36.9/cell in *Picea abies,* 36.2/cell in *Pinus sylvestris,* and 27. 6/cell in *Larix decidua.*

Seedlings from damaged *P. abies* trees revealed significantly more SCEs/cell than seedlings from healthy trees of the same area.

Key words: Picea abies, Pinus sylvestris, Larix decidua, sister chromatid exchanges, genotoxicity testing, forest damage, environment.

FDC: 165.3; 181.45; 425.1; 425.3; 174.7 *Picea abies;* 174.7 *Pinus sylvestris;* 174.7 *Larix decidua.*

Zusammenfassung

Die Häufigkeit von Schwesterchromatidenaustauschen (SCEs) kann durch Mutagenbehandlung drastisch erhöht werden. Eine erhöhte SCE-Frequenz gilt auch als Indikator für genotoxische Umwelteinflüsse.

Die SCE-Frequenzen für 3 weitverbreitete Gymnospermenarten (*Picea abies, Pinus sylvestris, Larix decidua*) mit je 2n=24 Chromosomen wurden untersucht.

Einsträngig mit dem Basenanalogon BrdUrd substituierte Chromosomen ergaben nach 'Fluoreszenz-plus-Giemsa'-Färbung eine SCE-Frequenz pro Zelle von 36,9 für die Fichte, 36,2 für die Kiefer und 27,6 für die Lärche.

Sämlinge von deutlich geschädigten Fichten wiesen durchgängig signifikant mehr SCEs/Zelle auf als Sämlinge benachbarter gesunder Bäume.

Introduction

Sister chromatid exchanges result from recombination events between the identical DNA double strands of sister chromatids detectable by methods recording a bias of incorporated base analogues. The mechanism(s) of origin and the biological significance are still a matter of controversial discussion (for review see SCHUBERT, 1990). Nearly all genotoxic influences clearly enhance the basic frequency of SCEs in all systems investigated (TAKEHISA, 1982). Though SCEs are usually no mutagenic events themselves, they represent a sensitive, reliable and simple means to study experimental/environmental genotoxic influences.

The conifers *Picea abies* (L.) KARSTEN, *Pinus sylvestris* L., and *Larix decidua* MILLER are forest trees with similar chromosome complements (2n=24). They are widespread throughout the Northern hemisphere and endangered by anthropogeneous environmental impacts in several areas. Here, the basic frequency of SCEs is reported for these three species and compared for seedlings of closely neighbouring healthy and damaged individuals of *P. abies* trees from the same area.

Material and Methods

Seeds were harvested in spring and germinated on wet filter paper for 7 days at 24 °C. The detection of SCEs was as described by SCHUBERT and RIEGER (1994). Briefly, root tips (1 cm in length) were incubated for 17 h in BrdUrd (100 µM) + FdUrd (0.1 µM) + Urd (5 µM) and for 19 h in dThd (100 µM) + Urd (5 µM), submersed in 1% colchicin for 15 h, fixed in ethanol:acetic acid (3:1 at 4 °C over night), softened in 1% pectinase +1% cellulase at 37 °C for 0.5 h, squashed in 45% acetic acid, and differentially stained by the FPG technique (SCHUBERT et al., 1979).

(This procedure is also suitable for root tips of older seedlings grown on perlit, provided possible changes of cell cycle duration are being considered).

Results and Discussion

The base-line value in unifilarly BrdUrd-substituted chromosomes (TB/TT) was 36.9 SCEs/metaphase for *Picea abies,* 36.2 SCEs/metaphase for *Pinus sylvestris,* and 27.6 SCEs/metaphase for *Larix decidua* (*Tab.1, Fig. 1*). This corresponds to ~1.5 SCEs/chromosome for *Picea abies* and *Pinus*

sylvestris and 1.15 SCEs/chromosome for *Larix decidua* and paralleles the DNA content which is similar for many *Picea* and *Pinus* species and usually lower for species of the genus *Larix* (OHRI and KHOSHOO, 1986).

Previously it was shown that additional mutagen treatments increase SCE frequency in spruce in a similar way as reported for other plant species and that it is much easier and more reliable to detect low genotoxic effects by counting SCEs instead of chromosomal aberrations (SCHUBERT and RIEGER, 1994).

Table 1. – Sister chromatid exchanges in forest conifers.

subject	cells/chromosomes scored*[1]	SCEs/cell
Larix decidua*[2]	40/960 (8)	27.6
Pinus sylvestris*[3]	50/1200 (10)	36.2
Picea abies	40/960 (8)	36.9
①	40/1549 (8)	38.7
②	40/2645 (8)	66.1
②	40/3337 (8)	83.4

*[1]) in brackets: number of seedlings from which SCEs were scored.

*[2]) seeds from a cultivated tree of a forest near Flechtingen.

*[3]) seeds, kindly provided by Dr. WERNER HELLER, GSF Neuherberg, descend from an autochtonuous population of scots pine of mountain Wank near Garmisch-Partenkirchen.

① Seedlings of a healthy tree; ② Seedlings of damaged trees.

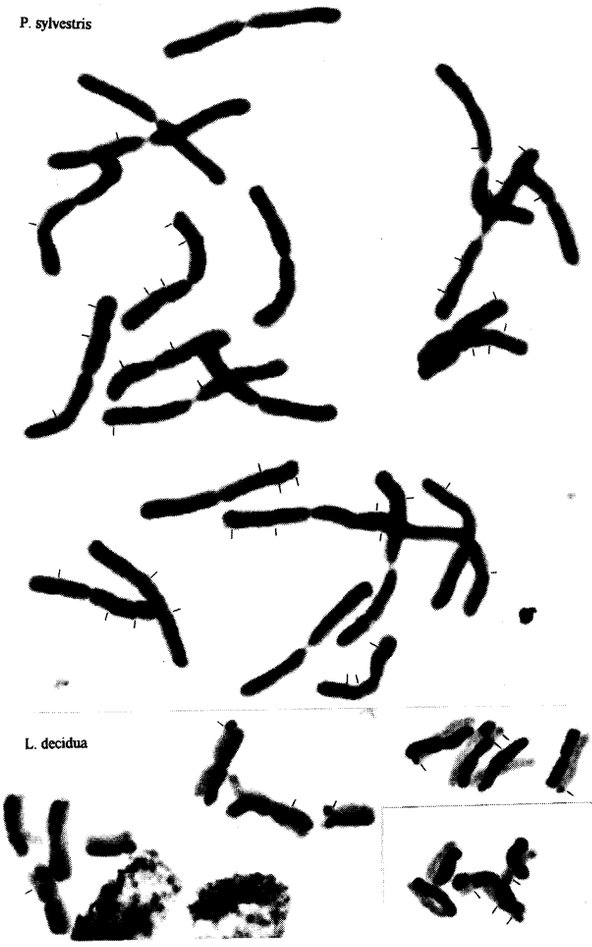

Figure 1. – Sister chromatid exchanges in *Pinus sylvestris* and *Larix decidua* chromosomes.

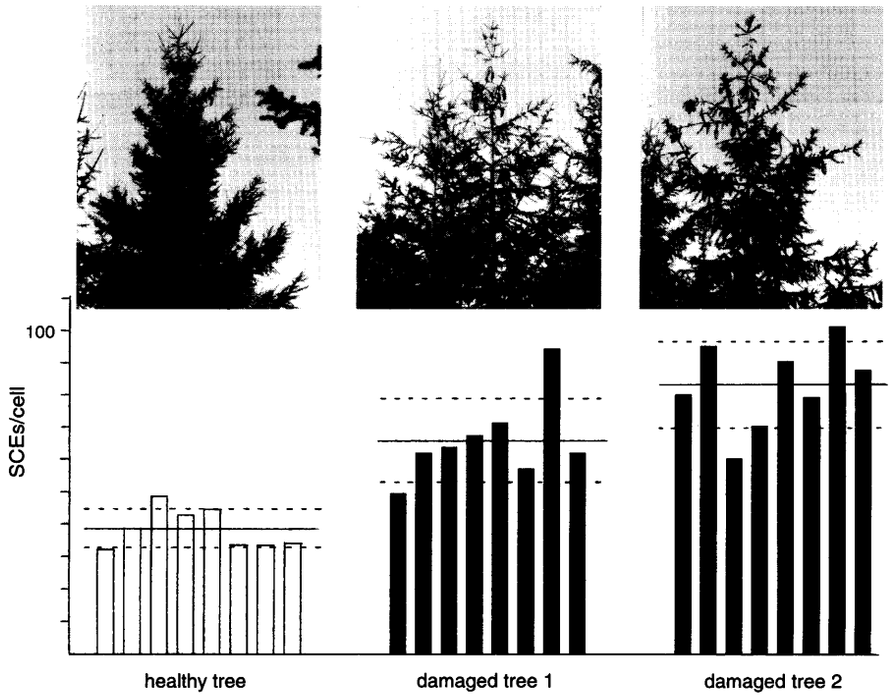

Figure 2. – Sister chromatid exchanges/cell corresponding to 5 metaphases ≙ 120 chromosomes for each of 8 seedlings from a healthy and two damaged *P. abies* trees (average ± SD).

For 24 seedlings, 8 from a healthy and 16 from 2 damaged *P. abies* trees (*Fig. 2*) cultivated in the same area within a distance of about 100 m (Selketal of the Harz mountains), the SCE frequency was compared. 960 chromosomes corresponding to 40 metaphases in each variant were evaluated. On average, 38.7±6.1 SCEs/metaphase in seedlings from the healthy and 66.1±13.1 or 83.2±13.4 SCEs/metaphase in seedlings from the damaged trees were scored (*Tab. 1* and *Fig. 2*). The differences between healthy and damaged trees were significant ($P < 0.001$) according to the MANN-WHITNEY test. This may indicate either more DNA lesions or a less efficient removal of lesions during the first postgermination cell cycles in seedlings of damaged as compared to seedlings of healthy spruce trees.

All seedlings descending from one individual showed the same tendency although many of these should be genetically heterogeneous due to cross-pollination. It therefore seems to be probable that the SCE frequency is maternally determined in these cases. For example, limited supply of cations (such as Mg^{2+}) to the mother tree necessary for normal functioning of enzymes responsible for correct repair of DNA damage could result in higher amounts of DNA lesions not removed before DNA replication and thus giving rise to an increased SCE frequency. Experiments are under way to test the validity of this hypothetic explanation.

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Outcrossing Rate of Teak (*Tectona grandis* (L.))

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Summary

The outcrossing rate of (*Tectona grandis* (L.)) was estimated from allozyme segregation in progenies from 15 teak trees collected near Ngao, Thailand. The average single-locus outcrossing rate was found be 0.89, with a standard error based on the variation between loci of 0.08. The multilocus outcrossing rate was estimated to be 0.95 with a standard error based on re-sampling between progenies within families (bootstrapping) of 0.07. The results suggest that teak is mainly a outcrossing species. This result is in agreement with experiences from controlled pollinations.

Key words: Tectona grandis, mating system, allozymes.

FDC: 165.4; 176.1 *Tectona grandis.*

Introduction

Tectona grandis (L.) is a mainly insect pollinated tropical tree species with a large natural distribution in South East Asia (KAOSA-ARD, 1981). It has a long history as a plantation species due to its valuable timber and today it is of major importance in many plantation programs throughout the tropical world. Tree improvement activities were initiated in Thailand (KAOSA-ARD, 1993) and India (KUMERAVELU, 1993) in the 1960s. Today, tree improvement is recognized as an important part of plantation programs in many countries (see KJÆR and FOSTER, 1995, for references).

The flower biology and mating system attracted early attention (GRAM and LARSEN, 1958; BRYNDUM and HEDEGART, 1969; HEDEGART, 1973) because low fruit and seed yield were found to be serious obstacles to large scale propagation in seed orchards. One hectare of clonal seed orchard can for example only produce seed sufficient for a 16 ha teak plantation with the prevailing nursery technique (WELLENDORF and KAOSA-ARD, 1988).

The pollination studies by BRYNDUM and HEDEGART (1969) found that many apparent pollen vectors operated only within the crown (e.g. ants). Controlled crosses showed, however, that the investigated trees were almost self-incompatible. In this study the outcrossing rate is estimated by isozyme markers on trees of the same origin as were used in the BRYNDUM and HEDEGART studies.

Material and Methods

Seeds were collected in January, 1994, by climbing 20 selected trees in the seed stands (seed production areas) close to the Teak Improvement Center, Ngao, Lampang. The stands were planted in the 1940s. They are located close to natural stands and are within the natural distribution area of teak.

The seeds were germinated after pre-treatment (80 °C for 48 hours followed by 6 hours soaking in water) at 34 °C with alternating 12 hours of light and dark. Five families were excluded from the study due to insufficient germination.

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