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Thesis Abstracts

Evaluation of the mutagenic or antimutagenic effects of Mushroom-of-Sun (*Agaricus blazei*) extracts in cultured cells

(Avaliação dos efeitos mutagênicos ou antimutagênicos de extratos de Cogumelo-do-Sol (*Agaricus blazei*) em culturas celulares)

*Regina Carla Rodrigues Notoya Menoli**

The Basidiomycete *Agaricus blazei* Murril, native of Brazil and popularly known as Cogumelo-do-Sol, because its medicinal properties, is often consumed as food and as teas, and in some countries, is utilized to combat diverse diseases. This mushroom has recently been studied for the purpose of isolating and identifying the active substances in its fruiting body, and to test their biological properties. In some cases, it has demonstrated anticarcinogenic or antibacterial capacity.

In the present work, the mushroom teas of ancestries AB96/07, AB96/09 and AB97/11 were chemically prepared in 2.5% aqueous solution, at three different temperatures (room temperature, ice-cold and warm), and its possible mutagenic and/or antimutagenic potential was evaluated in lung cells of Chinese hamsters (line V79), *in vitro*, at three different concentrations (0.05, 0.1 and 0.15%). The evaluation was based on two parameters: cytogenetic (micronucleus test) and molecular (Comet assay).

Agaricus blazei did not prove to be mutagenic in any of the types of teas and at none of the three concentrations tested in V79 cells submitted to the Comet alkaline assay or micronucleus test. In fact, it demonstrated to be antimutagenic with regard to the induction of micronuclei by the mutagenic agent methylmethanosulfonate in all types and concentrations of teas, and when applied in any form of these treatments: simultaneous, pre-treatment and post-treatment. Through the Comet assay, the mushroom demonstrated antimutagenic activity relative to the effect of methylmethanosulfonate, when the cells were pre-treated with the following types and concentrations of teas: warm 0.1% and 0.15%, room temperature 0.05% and ice-cold 0.1%.

On the basis of the data obtained, we can conclude that, in the conditions studied and the cells evaluated, Cogumelo-do-Sol, in the form of tea, is not mutagenic and exhibits effective protective action against the induction of micronuclei by methylmethanosulfonate and a partial cellular protection against the production of DNA breaks, as detected by the Comet alkaline assay.

Research supported by CAPES.

**2000. Pós-Graduação em Genética e Melhoramento, Departamento de Biologia Geral, Universidade Estadual de Londrina, Londrina, PR, Brasil. Master's thesis. Orienting Professor: Dr. Berenice Quinzani Jordão.*

Evaluation of the toxic, cytotoxic and genotoxic potentials of environmental waters of Corumbá in *Allium cepa* roots

(Avaliação dos potenciais tóxico, citotóxico e genotóxico de águas ambientais de Corumbá-MS em raízes de *Allium cepa*)

*Danielle Serra de Lima Moraes**

The toxic, cytotoxic and genotoxic effects of the different substances can be evaluated in *Allium*, through the following parameters: a) degree of inhibition of root growth; b) reduction of the proliferation activity of the root tip meristems; c) frequencies of cells with aberrations, and d) types of aberrations in meristematic cells. These are rather rapid and reliable tools for detection of harmful substances in the environment, for bio-monitoring the natural environment and measuring the extent of water pollution. The purpose of the present study was to evaluate the surface water quality of the Paraguay River in the city of Corumbá, Mato Grosso do Sul (MS), in Brazil, as well as the municipal waste waters that discharge into the Paraguay River. The quality of these waters was evaluated from their influence on the roots and root cells of *Allium cepa*, during the annual seasons of high and low river levels. Water samples were collected in the months of July/98, October/98, January/99 and March/99. Growing roots were exposed to samples of municipal effluent and the river water for 20 and 72 h, after an initial growth up to 1.5-2.0 cm in non-chlorinated water. This non-chlorinated water was used as the negative control of the system. The alkylating agent mitomycin C was used as a positive control. The mean values of the root growth were obtained by measuring the lengths of the longer and shorter roots of each bulb. The measures were taken at the start of the treatments (hour 0) with the test substances and at the end of 20 h and 72 h. A growth curve was obtained from the percentages of the attained lengths in the environmental waters relative to the negative control value. The analysis of the mitotic index, mitotic stages index, frequency of cells with aberrations and types of aberrations in meristematic cells was made 20 h and 72 h after the treatments started. The results obtained from the treated groups and the controls were compared statistically by F test, at

probability level of 1 and 5%, and the Tukey test, at the level of 5% probability. Physical and chemical analyses of the environmental samples were performed and their results were analyzed concurrently with the results obtained from the biological tests. The levels of the following metals were analyzed: Mg^{2+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ca^{2+}, Fe^{3+} and Pb^{2+}. The high Ca^{2+} content and the pollution elements (organic matter, NH_3^-, NO_2^-, NO_3^-, pH, PO_4^- and SO_4^-) were suggested as the main sources of the toxic and cytotoxic effects of the waters examined. Probably, the high frequency of cells with aberrations observed in the present study were caused by the high Pb^{2+} levels. Although the municipal wastewater showed significant levels of toxic, cytotoxic and genotoxic effects, thus representing a potential risk to organisms, it exerted little influence on the waters of the Paraguay River, mainly in seasons of high river levels. These results suggest that: a) the municipal wastewater of Corumbá (MS) shows toxic, cytotoxic and genotoxic effects to exposed organisms; b) the waters of the Paraguay River were not clearly affected by the municipal wastewater, during the analyzed seasons, but the elements discharged into the Paraguay River during its course could exert a pronounced influence on the river water quality; c) the *Allium* test was useful in discriminating the quality differences between the environmental surface waters as well as the waste waters discharged into them, proving to be a sensitive system for bio-monitoring environmental waters, where the quickness of results is necessary for decision-taking.

Research supported by CAPES.

**2000. Departamento de Biologia Geral, Universidade Estadual de Londrina, Londrina, PR, Brasil. Master's thesis. Orienting Professor: Dr. Berenice Quinzani Jordão.*

Fungistatic responses of *Lentinula edodes* (Berkeley) Pegler against *Candida albicans* (Robin) Berkhout and analysis of intraspecific variability

(Respostas fungistáticas de *Lentinula edodes* (Berkeley) Pegler sobre *Candida albicans* (Robin) Berkhout e análise da variabilidade intraespecífica)

*Cristina Sayuri Maki**

The purposes of the present study were to evaluate the fungistatic action of 34 *Lentinula edodes* (Berkeley) Pegler strains against the 577 strain of *Candida albicans* (Robin) Berkhout *in vitro*, the developmental responses of the strains in different conditions of pH and temperature, the influence of both factors (pH and temperature) in the antagonistic interaction *L. edodes* x *C. albicans*, and the use of RAPD markers technique to assess the genetic diversity of the *L. edodes* strains. In general terms, we observed that the fungistatic characteristic of *L. edodes* against *C. albicans* is well widespread among the strains, although it appeared to be dependent on the duration of the mycelial growth period and on the developmental conditions. The K2 strain of *L. edodes* was the most effective in the production of the active compound against *C. albicans* development, at least under the experimental conditions here employed. Twenty primers (*Operon Technologies*) were used for the analysis of intraspecific variability by the RAPD method, which detected sufficient polymorphism to allow differentiation of the strains. However, three of the tested primers (OPA06, OPA15 and OPA16) appeared to be innefficient for the amplification of *L. edodes* DNA. Characterization of the 34 *L. edodes* strains was achieved after the analysis of 93 polymorphic bands, which was able to separate these strains into two groups: the first was comprised of the strains obtained from the Instituto Botânico de São Paulo (Culture Collection of Basidiomycetes), and the second, the shiitake strains cultivated on Paraná. The second group of strains showed a more efficient effect on *C. albicans* inhibition, suggesting further investigation on the chemical nature and isolation of the active compound(s) against *C. albicans*. This second group of strains is, nowadays, cultivated by shiitake commercial farmers.

**1999. Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, PR, Brasil. Master's thesis. Orienting Professor: Dr. Luzia Doretto Paccola-Meirelles.*

Investigation of the mutagenic and antimutagenic activity of the chlorophyll A, B and chlorophyllin molecules in cell culture V79

(Investigação da atividade mutagênica e antimutagênica das moléculas de clorofilas *A*, *B* e clorofilina, em cultura de células V79)

*Girlene de Cássia Bez**

A wide variety of mutagenic or genotoxic substances has been identified by genetic toxicology using different test systems. Presently great efforts are being made to identify substances of plant origin which have antimutagenic or antigenotoxic properties by using the same test systems used in mutagenesis. Chlorophyll and its derivatives are an example of plant compounds (purified and/or extracted) which protect DNA from damage caused by chemical or physical agents, but some studies have identified clastogenic activity by these molecules. It has been suggested that the main action mechanisms of these molecules are desmutagenesis. This study was carried out to assess the mutagenic activity of chlorophyll molecules -*a* (Chl-*a*), -*b* (Chl-*b*) and chlorophyllin (Chl) and the antimutagenic activity of their molecules compared with DNA damage-inducing methyl methanesulfonate (MMS) under

simultaneous treatment conditions, pre-treatment, post-treatment and simultaneous treatment after complexing. The micronucleus test (MN) was used on binucleated cells (induced with cytochalasin-B) in a mammal cell culture (V79), on mutagenic and antimutagenic activity. The genotoxicity of the Chl-*a*, Chl-*b* and Chl molecules and the antigenotoxic activity in MMS under simultaneous treatment conditions were also assessed with Comet test (SCGT - single-cell gel test).

Chlorophyll was tested in the experimental protocols at three different concentrations (0.1375; 0.275; 0.55 μM) alone or associated with MMS (4×10^2 μM - MN; 1.6×10^2 μM - Comet). The treatments were kept for two hours in the MN test and for one hour in the Comet test. Two thousand binucleate cells were analyzed in each culture to verify the micronucleoles and 50 cells were analyzed for the Comet test.

The three concentrations of Chl-*a*, Chl-*b* and Chl concentrations were not mutagenic and the MMS mutagenic action decreased (74 to 117%) under all treatment conditions. The results showed that there was no significant difference among the treatment types, the concentration or the types of chlorophyll molecules used. The Comet test showed that the three Chl-*a* concentrations were not genotoxic, but Chl-*b* and Chl were genotoxic at all the concentrations tested. No decrease in the action of MMS was observed by the Chl-*a*, Chl-*b* and Chl molecules in any of the concentrations tested.

The data obtained suggest that Chl-*a*, Chl-*b* and Chl when associated with the DNA damage-inducing agent MMS may protect the DNA by desmutagenic action and/or action by a bio-antimutagenic mechanism, with the same efficiency.

**2000. Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, PR, Brasil. Master's thesis. Orienting Professor: Dr. Mário Sérgio Mantovani.*

Genetic variability for regenerated protoplasts and sensitivity to fungicides in *Metarhizium flavoviride*

(Variabilidade genética por regeneração de protoplastos e sensibilidade a fungicidas em *Metarhizium flavoviride*)

*Júlia Kuklinsky Sobral**

Two wild strains of the entomopathogenic fungus *Metarhizium flavoviride*, CG423 and CG366, were studied for sensitivity and mutant induction by ultraviolet light to investigate the genetic variability. Survival curves were obtained and the mutants were induced to provide 1 to 5% survival. Morphologic mutants were isolated by conidia color, grouped into three classes: yellow conidia, violet conidia and white conidia, and compact mutants with reduced diameter were isolated for the colony phenotype. Both strains presented all the types of mentioned morphologic mutants. Only the strain CG432 presented an auxotrophic mutant for biotin. The protoplast formation and regeneration of these two wild strains and two morphologic mutants, 423ylo and 366vio, were also analyzed. The protoplasts were obtained after treating the mycelium with Novozym 234 and with the osmotic stabilizer KCl 0.7 M, resulting in over 5×10^6 protoplasts/ml formation. The regeneration frequency varied from 6.65 to 27.92%. The stable mutants obtained from regenerated protoplasts of the strain CG423 were morphologically analyzed and tested for sensitivity to the fungicides Benomyl and Captan. The sensitivity of the four wild strains CG366, CG423, CG429 and CG430 to fungicides Benomyl, Captan, Iprodione and Procymidone was also analyzed. Difference in sensitivity to fungicides was observed in the strains. These data demonstrate the genetic variability of the species.

**1999. Pós-Graduação em Genética, Departamento de Genética, Centro de Ciências Biológicas, Universidade Federal de Pernambuco Recife, PE, Brasil. Master's thesis. Orienting Professor: Dr. Elza Áurea de Luna Alves Lima.*

Agrobacterium tumefaciens-mediated transformation of two processing tomato cultivars and incorporation of the gene *Sw*-5

(Transformação genética de dois cultivares de tomateiro industrial mediada por *Agrobacterium tumefaciens* e incorporação do gene *Sw*-5)

*Marcio Gilberto Cardoso Costa**

Tomato spotted wilt virus, caused by species of the genus *Tospovirus*, is one of the main viral diseases affecting the tomato crop in Brazil. Control is difficult for several reasons: the viruses have a wide host range, estimated in more than 550 plant species; chemical control of the insect vectors is inefficient, and the difficulties associated with the incorporation of varietal resistance in commercial cultivars. The recent cloning of the gene *Sw*-5 from *Lycopersicon peruvianum* should allow the fast incorporation of this gene in elite tomato cultivars using plant genetic transformation techniques. The first objective of this study was to develop an *Agrobacterium tumefaciens*-mediated genetic transformation system for 'IPA-5' and 'IPA-6' processing tomato cultivars, aiming at posterior transformation with a cosmid clone that contains *Sw*-5. Cotyledon segments of tomato cultivars 'IPA-5' and 'IPA-6' were used as source of explants and several shoot induction media were evaluated. The best regeneration frequencies were achieved onto an MS-based medium supplemented either with 1 mg/l of zeatin plus 0.1 mg/l of IAA or with 2.5 mg/l of BAP plus 0.2 mg/l of IAA. Aiming the optimization of the transformation protocol available, some factors which

affect transformation efficiency were examined, including: genotype, vector, temperature of co-cultivation and antibiotics. The cultivar 'IPA-5', the binary vector pBI121, temperature of co-cultivation of 22 or 24ºC, and the selective medium supplemented with 75 mg/l kanamycin and 300 mg/l timentin gave the best transformation efficiencies. The *Sw*-5 gene was transferred to cultivars 'IPA-5' and 'IPA-6' using the optimized protocol generated by this study. By means of transgene segregation analysis, it was verified that at least two copies of *Sw*-5 were inserted into the 'IPA-5' and 'IPA-6' genomes.

**1999. Departamento de Biologia Vegetal (Genética e Melhoramento), Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil. Master's thesis. Orienting Professor: Wagner Campos Otoni.*

Morphogenesis *in vitro* and genetic transformation of chrysanthemum (*Dendranthema grandiflora* Tzvelev 'Repin Rosa')

(Morfogênese *in vitro* e transformação genética de crisântemo (*Dendranthema grandiflora* Tzvelev 'Repin Rosa'))

*Maria Rita de Cássia Campos**

Aiming the optimization of a regeneration protocol of chrysanthemum plants (*Dendranthema grandiflora* 'Repin Rosa'), experiments of morphogenesis *in vitro* were accomplished, being tested stem and pedicel explants concerning the regeneration ability. The explants were inoculated in several media of shoot induction. The cultivation medium consisted of MS basic salts, supplemented with different concentrations and combinations of growth regulators BAP and AIA. At the end of 15-30 days of cultivation, significant differences were observed in the regeneration frequency, number of shoots per explant and in the length of the shoots among the appraised explants: pedicel explants presented a morphogenetic potential higher than the stem explants. The pedicel explants presented the best regeneration frequences in medium of cultivation that contained MS basic salts supplemented with 0.5 mg/l of BAP and 2.0 mg/l of AIA. It was also tested the effect of the sectioned position of the explant in contact with the medium of cultivation. Significant differences were observed among the positions and the best answers were obtained in the position where the surface, that is not sectioned, stays in contact with the medium. All the regenerated plants had the same morphologic characteristics when compared to the control plants. Flowers 45-60 days after the transfer to the glasshouse were obtained. The presence of the antibiotic timentin in the medium of cultivation caused increasing in the organogenesis *in vitro* of this cultivation when compared to the antibiotic cefotaxime. Pedicel explants presented drastic reduction in regeneration frequency and number of shoots per explant in the presence of 50 mg/l when cultivated in medium of shoot regeneration supplemented with kanamycin. In a similar process, the antibiotic hygromycin inhibited the morphogenesis *in vitro* of chrysanthemum in the presence of 2.5 mg/l of the antibiotic. Transgenic chrysanthemum plants, 'Repin Rosa', were obtained and confirmed by amplification (PCR) and by the histochemistry test for enzyme β-glucuronidase 45 days after the inoculation with *Agrobacterium.* The obtained results confirmed reports concerning the regeneration potential *in vitro* and possibility of *Agrobacterium tumefaciens*-mediated genetic transformation of chrysanthemum.

**1999. Curso de Genética e Melhoramento, Universidade Federal de Viçosa, Viçosa, MG, Brasil. Master's thesis. Orienting Professor: Wagner Campos Otoni.*

Genetics, pollen-pistil interaction and protein expression in the self-incompatibility of the passion fruit (*Passiflora edulis* Sims.)

(Genética, interação pólen-pistilo e expressão de proteínas da auto-incompatibilidade do maracujazeiro (*Passiflora edulis* Sims.))

*Mailson Monteiro do Rêgo**

With the objective of studying the self-incompatibility in the passion fruit (*Passiflora edilus* Sims.), six progenies selfed in the flower-bud stage were obtained. The evaluations were done based on the fruit set. Four hundred and fifty-five self-pollinations were carried out, in the anthesis, in 73 plants not set fruit, while plants from the purple passion fruit showed self-compatibility. In 23 self-pollinations carried out in the flower-bud stage, in 10 plants, an average of 29.6% of fruit set was obtained, showing the possibility of its use for self-fertilization. In the six progenies originated from self-fertilization, the plants were studied in reciprocal crosses and gathered in self-incompatible groups. Crossing plants from the incompatibility groups, with known phenotypes, made possible to gather them in six phenotypes: S_1, S_2, S_3, S_4, S_5 and S_6, being S_5 and S_6 new phenotypes. Studies of the pollinic tube development in selfing and compatible and incompatible hybridations were conducted, using the bleached blue aniline method. In self-pollinations, the pollen grain germinated and penetrated the cuticule and the cell papillar wall, however, it was inhibited 30 min after pollination in the papilla cell interior. In most of the incompatible crosses, the pollen behaved as in the previous situation; however, in some cases, the inhibition of the pollinic tube growth occurred in the style transmission tissue, whereas in the compatible cross-breeding, the pollinic tubes grew basically achieving the eggs. Pistil protein, associated to

six different incompatibility phenotypes, expressed themselves in a tissue-specific way, and their synthesis began from the 40-mm length flower-buds to the anthesis. The passion fruit self-incompatibility is controlled, probably by two genes: the S gene, sporophytic, and a second locus, which action must be studied.

**1997. Departamento de Fitotecnia (Genética e Melhoramento), Universidade Federal de Viçosa, Viçosa, MG, Brasil. Master's thesis. Orienting Professor: Cláudio Horst Bruckner.*

Cytogenetic and molecular evaluation of autism and other pervasive developmental disorders

(Avaliação citogenética e molecular em autismo e outros transtornos invasivos do desenvolvimento)

*Marcos Roberto Higino Estécio**

The pervasive developmental disorders (PDDs) constitute a group of neurobiological conditions characterized by specific delays and deviation in social, communicative, and cognitive development, and the onset of these conditions occurs in the first years of life. This class of disorders includes autism, Asperger's disorder, Rett's disorder, childhood disintegrative disorders and pervasive developmental disorder, not otherwise specified (PDD-NOS). Because genetic factors have been implicated in the etiology of these disordes, the purpose of this work was to investigate the occurrence of genetic abnormalities in patients with PDDs. The patients of the School of the Autist, São José do Rio Preto, SP, diagnosed by a multidisciplinary professional staff in accordance with criteria of CID 10 and DSM-IV were evaluated. This group was composed of 30 individuals, of which 19 patients were autists, eight had PDD-NOS, two had Asperger's disorder, and one had Rett's disorder.

All patients were evaluated for chromosome abnormalities. Cytogenetic techniques were performed, using cultures with folate-deficient medium and folate antagonists such as trimethoprim and fluordeoxyuridine (FudR), which allow expression of fragile X chromosome (FRAXA) and other fragile sites. The male patients were also evaluated by means of a molecular test for FRAXA using polymerase chain reaction (PCR).

Two patients showed fragile X syndrome. Two other individuals showed constitutional chromosomal abnormalities: one case of mosaicism 46,XX/46,XX,inv(7)(p15q36) and one case of a supernumerary chromosome der(15). One of the individuals with fragile X syndrome and the patient that showed inv(7) were autists. The other patients had PDD-NOS. Another autist patient showed chromosome breaks at Xq27-q28 in 3% of the cells, but FRAXA was excluded by DNA analysis. Some common fragile sites were observed during cytogenetic analysis, in bands 1q21, 1q32, 3p14, 6q26 and Xq22.

The inv(7)(p15q36) was observed in only 2% of the analyzed cells of an autistic patient. Chromosome regions involved in this rearrangement contain important developmental genes (homeogenes) related to the development of the central nervous system, such as homeogenes *EN-2* and *HOXA*.

The patient with extranumerary chromosome der(15) showed autistic disorder associated with mental retardation, epilepsy and behavioral disorder, like other cases reported with similar cytogenetic anomaly. The fluorescence *in situ* hybridization (FISH) technique identified the der(15) with a copy of the 15q11-q13 region. The preparations hybridized with probes for D15Z1 (centromeric region), D15S10 and SNRPN (15q11-q13 region) and PML (15q22 region), allowing to propose the karyotype 47,XX,+mar.ish der(15)(D15Z1+,D15S10+,SNRPN+,PML-). In the region 15q11-q13 some genes were mapped, including the *ITO*, *UBE3A*, *CHRNA7* and the genes for three GABA receptor subunits, *GABRB3*, *GABRA5* and *GABRG3*, all related to the nervous system.

Our findings reinforce the need of genetic evaluation in PDDs. The results of cytogenetic and molecular analysis suggest the participation of candidate genes localized in the regions 7p15, 7q36, 15q11-q13 and Xq27.3 related to susceptibility for autism and other PDDs. These findings also support the detailed molecular analysis of these regions as a strategy to clarify the etiology of these disorders and to contribute to the establishment of therapies.

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**1999. Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas de São José do Rio Preto, UNESP, São José do Rio Preto, SP, Brasil. Master's thesis. Orienting Professor: Prof. Dr. Ana Elizabete Silva. Co-orienting Professor: Prof. Dr. Agnes Cristina Fett-Conte.*

Cytogenetics and embryology of *Eupatorium polystachium*, *E. purpurascens* and *E. laevigatum* (Compositae)

(Citogenética e embriologia de *Eupatorium polystachium*, *E. purpurascens* e *E. laevigatum* (Compositae))

*Maristela Sanches Bertasso-Borges**

The present study investigated the mode of reproduction and the cytogenetics of three species of *Eupatorium* (Compositae): *E. polystachium* Sprengel, *E. purpurascens* Schultz-Bip and *E. laevigatum* Lam., and included the following stages: megasporogenesis and female gametophyte development, microsporogenesis and pollen grain formation, pollen viability, pollination frequency, karyotype and seed production and germinability. The material of *E. polystachium* used in this investigation was collected from natural populations of Mirassol, SP. The embryological studies used the method of ovule clarifi-

cation, from buds fixed in FAA. Embryo sac formation in different floral stages revealed dyads and tetrads of megaspores, which indicates regular occurrence of reductive meiosis. The mature 8-nucleate embryo sac is of the Polygonum type. Embryo and endosperm were present only in ovules from open florets, which indicates that pollination and fertilization of the egg and central nucleus are essential. Microsporogenesis in *E. polystachium* was studied using the smear technique of buds fixed in Carnoy's solution and stained in aceto-carmine. During diakinesis bivalent formation was normal as were all subsequent aspects of microsporogenesis and pollen formation. Pollen viability was determined by staining with cotton blue-lactophenol and revealed that 99.68% of 2,500 grains stained. Counts of pollen grains occurring on stigmas revealed that of 173 pistils examined, 91.33% had pollen, with a mean of 20.06 grains. An analysis of 826 germinated pollen grains demonstrated that all were devoid of nuclei, which indicates that the male nuclei were released into the pollen tube. Karyotype studies in *E. polystachium* were based on analysis of eight cells from root tips pretreated with oxyquinoline (0.002 M), fixed in Carnoy's solution, hydrolyzed in 1 N HCl and stained in aceto-carmine. Measurements of total chromosome length, long arm and short arm length and arm ratio permitted the recognition of two morphologically identical genomes in the complement, which characterizes the species as diploid (2n = 2x = 20). Metaphase chromosomes were determined to measure from 3.59 μm to 4.93 μm. The complement showed seven pairs of median chromosomes, one submedian pair and two subterminal pairs. Seed production in 2,265 ovaries from 7 plants showed that 33.02% produced seeds with embryos. Germination tests revealed that 48.25% germinated. The material of *E. purpurascens* was collected from natural populations of Mirassol and São José do Rio Preto, SP, while the material of *E. laevigatum* was collected only in Mirassol, SP. Embryological studies revealed the complete absence of dyads and tetrads of megaspores in both species and, therefore, of reductive meiosis. The megasporocyte functions directly as the megaspore. The mature embryo sac is of the Polygonum type. Precocious embryo and endosperm formation in ovules of unopened florets is strong evidence of apomixis. Embryo sac formation directly from the megasporocyte indicates the type of apomixis to be diplospory. Endosperm development is autonomous. The analysis of microsporogenesis, in both species, demonstrated abnormalities in chromosome pairing, which result in the formation of univalents, bivalents, trivalents and higher polyvalents, with consequent production of lagging chromosomes, unbalanced nuclei, micronuclei and sterile pollen. In *E. laevigatum* viable pollen grain frequency was low (4.0% of 3,000 pollen grains), and of 181 pistils examined 58% showed a mean of 1.04 pollen grains, which shows ineffective pollination. However, in *E. purpurascens*, although meiotic disturbances indicated a high degree of male sterility, viable pollen grain production was high (83.7% of 3,000 pollen grains). The unexpectedly high pollen viability demonstrates a high tolerance to genetic imbalance. Pollination studies revealed that, of 147 pistils examined, 99.3% presented a mean of 34.16 pollen grains. Nevertheless, 497 germinated pollen grains, with clearly visible tube, remained in the trinucleated condition, showing that, although viable, the pollen grains were nonfunctional as a consequence of the male nuclei failing to enter the pollen tube. Karyotype studies were based on analysis of three metaphases in *E. laevigatum* and four in *E. purpurascens*, and followed the methods used in *E. polystachium*. The results indicate the presence of six morphologically similar genomes in the complement of each species, which characterizes both as being autohexaploid (2n = 6x = 60). In *E. purpurascens*, total chromosome length ranged from 1.39 μm to 2.18 μm, all chromosomes being median. In *E. laevigatum*, total chromosome length ranged from 1.33 μm to 2.08 μm, eight being median and two submedian. Seed production was determined in 3 plants of *E. purpurascens*, and a total of 2,935 ovaries showed that 56.86% produced seeds with embryos. Germination tests revealed that 44% germinated. In *E. laevigatum*, 3,632 ovaries from 7 plants were examined, 28.2% producing seeds with embryos. Germination tests revealed that 33.42% germinated. On the basis of these studies it is concluded that *E. polystachium* is a sexual, diploid species while *E. purpurascens* and *E. laevigatum* are autohexaploid and apomictic.

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**2000. Instituto de Biociências, Letras e Ciências Exatas de São José do Rio Preto, UNESP, São José do Rio Preto, SP, Brasil. Doctoral thesis. Orienting Professor: Dr. James Robert Coleman.*

Study of the spermatogenesis in triatomines of genus *Rhodnius* (Heteroptera, Triatominae) with emphasis on nucleolar activity

(Estudo da espermatogênese em triatomíneos do gênero *Rhodnius* (Heteroptera, Triatominae) com ênfase à atividade nucleolar)

*Alessandra Morielle**

The objective of this work was a comparative study on six species of triatomines which belong to the genus *Rhodnius* (*R. domesticus, R. neglectus, R. neivai, R. pallescens, R. prolixus* and *R. robustus*), through the analysis of the different phases of spermatogenesis, the accompaniment of the nuclear cycle, the localization of necleolar-organizing regions (NORs), with the aim of verifying the occurrence of interespecific variation and to increase the understanding of chromosome evolution in these species.

The cytological preparations of the testicular tubes

were obtained by the conventional method of cell squash and posterior application of the techniques of cytogenetic staining with lacto-acetic orcein and impregnation with silver ions.

Rhodnius domesticus, R. neglectus, R. neivai, R. pallescens, R. prolixus and *R. robustus* present the karyotype 2n = 20 A + XY, which agrees with the modal number suggested for the subfamily Triatominae. These six species do not show primary constrictions on the chromosomes, which characterizes them, therefore, as being holocentric.

In triatomines, during the first meiotic division, the sex chromosomes present themselves arranged in two ways on the equatorial plate. The first is characterized by a ring formed on the perifery of the metaphase plate with the autosomes in the center of the ring. In the other arrangement, the chromosomes assume a periferal position on the spindle. In the present study both forms of arrangement of the sex chromosomes were verified. In metaphase II, the sex chromosomes are always found in the center of the ring of autosomes, which reinforces the post-reductional behavior of these chromosomes.

Silver impregnation was clear in the sex chromosomes of *Rhodnius domesticus, R. pallescens* and *R. prolixus*, which therefore suggests them to be the carriers of the nuclear organizing regions. In the other species (*R. neglectus* and *R. robustus*) there is evidence for the participation of autosomes in the organization of the nucleolus.

The phenomenon of chromatid lagging is frequent in meiosis and functions as a genetically controlled mechanism of chromosome elimination. This phenomenon occurs at anaphase of all species of *Rhodnius* included in this study. Anaphase chromatin bridges are also present in *R. neivai.* These bridges can occur spontaneously or as consequence of irradiation or chemical treatment, in somatic tissue or in germinative lines. The anaphase chromatin bridges are of interest since they have evolutionary significance and can have several origins.

At metaphase of the first meiotic division silver impregnation in the species *Rhodnius domesticus, R. neglectus, R. neivai, R. pallescens, R. prolixus* and *R. robustus* permitted the observation of locally dispersed nuclear bodies and fragments. At metaphase of second meiotic division these fragments were observed only in the species *R. neglectus, R. neivai, R. pallescens* and *R. prolixus.* The fragments and/or corpuscles persisted during anaphase in all the species and at telophase II of the species *R. domesticus.*

These discoveries reveal the occurrence of the phenomenon of persistence of nuclear material, which would be the result of the fragmentation of the nucleus during the first meiotic prophase. Thus, it can be inferred that the nucleus possibly does not disappear entirely in triatomines, but remains in the form of small pre-nuclear corpuscles which will unite *de novo* to form the next nuclear cycle which, in the case of meiosis, will only be completed if fertilization occurs and a zygote is formed. In these Heteroptera, therefore, the reactivation of synthesis of rRNA is post-meiotic, probably related to cell differentiation.

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Genomic distribution of *P* transposable element in *Drosophila sturtevanti* populations

(Distribuição genômica do elemento transponível *P* em populações de *Drosophila sturtevanti*)

*Luciane Madureira de Almeida**

The occurrence, number of copies, integrity and degree of activity of element *P* in nine strains of *Drosophila sturtevanti* was investigated. The strains are from Mexico (APA and MAT), Colombia (COL), and Brazil (I27, Minas Gerais; BRA, RP_1, RP_2, NHO, from São Paulo State, and MAQ, from Rio Grande do Sul State). The COL strain was derived from stocks brought from University of Texas (Austin, USA), the BRA strain was collected in 1971, and the others were collected between 1995 and 1998.

To evaluate the degree of activity of *P* sequences intracrosses and reciprocal intercrosses between the Colombia strain and the others, at 27°C, were done. The morphology of ovaries and testis and productivity of offspring were analyzed. The gonadal dysgenesis indexes (DG) were low, between zero and 8.56. Considering rudimentary ovaries as dysgenic, these indexes became larger, mainly in the offspring of the crosses between COL females and males I_{27} (DG: 25.71) and its reciprocals (DG: 12.86). The productivity of the intracrosses, mainly of COL, I_{27} and MAQ (12.37; 7.27 and 8.84 flies), were inferior to the respective intercrosses (35.99; 35.07 and 22.79 flies). The low DG indexes and its absence of asymmetry and the nonreduction of productivity among the offspring of the intercrosses suggest that transposition of *P* elements did not happen in hybrids of these strains.

PCR and dot blot analysis confirmed the presence of *P* sequences in all strains. Southern blots with genomic DNA of the strains digested with *Sal*I restriction enzyme and probed with the first 764 pb of *P* element (amplified from the DNA of the strains or from the *D. melanogaster P* element pπ 25.1) showed that the medium number of *P* element copies varied between 9 and 11. Common bands were observed in all strains, independent of geographical origin, as well as were observed bands common just to geographically more related strains.

Multivariate analysis was done to compare the band patterns resulting from the analysis of copy number. The Mexican and Colombian strains were grouped into a cluster with similarity levels close to 90% and the Brazilian strains were grouped into another, with similarity varying between almost 50% to nearly 95%. These data suggest that the fragments common to all the strains are sequences that were present, and already inactive, since the beginning of the dispersion of *D. sturtevanti* in the Americas, and they did not suffer mutation in the restriction sites of enzymes used to detect them. The sequences common to the Colombian and Mexican strains, on one hand, and to the Brazilians, on the other, should have more recent origin. Possibly they descend from an ancestral population that still possessed active elements and, after the dispersion, originated populations in different geographical areas, in which some *P* sequences evolved differently.

To investigate the integrity of the *P* sequences, the DNA was digested with the enzymes *Acc*I and *Ava*II and probed with the *P* element pπ 25.1 sequences (the fragment *Puv*II, the PCR product of the initials 764 pb or the entire pπ 25.1). The *Acc*I digests liberated fragments with sizes similar to 2.36 kb in all the strains, which suggests the occurrence of complete *P* sequences. The *Ava*II digests probed with pπ 25.1 generated two of the three expected fragments (544 pb and 1883 pb). The fragment corresponding to the initials 480 pb of the *P* sequence was not observed; it was also not observed when the same blot was probed with the 764 pb of the *P*. Additional bands, bigger than 1.8 kb, were observed in this blot. These bands were also observed when the same blot was hybridized with the *Puv*II fragment.

PCR amplification showed the existence of the 764-pb initial sequence of the *P* element that contains the 0.48 kb produced by *Ava*II. The absence of the corresponding band suggests the absence of the first *Ava*II site, possibly due to a point mutation, which would explain the occurrence of bands bigger than 1.8 kb. The 0.54 kb and 1.8 kb fragments indicate that the rest of the element is complete. The occurrence of alterations, point mutations or deletions, also in the second site of *Ava*II, in elements from other *P* subfamilies, would explain the maintenance of these bigger than 1.8 kb bands in membranes probed with *Puv*II. These results are in agreement with literature that shows the existence of two subfamilies of *P* elements at least in *D. sturtevanti* and allows to make inferences about some of the modifications that these elements present. Significant differences among the *P* sequences of old and recently collected strains were not found, as demonstrated by the very similar banding patterns of the BRA strain and RP_1, NHO and RP_2 strains.

The phenotypic and molecular analyses showed that the *P* element is present in all strains of *D. sturtevanti*, even though in its inactive state. This inactivity can be due to the absence of complete copies of the *P* element, or also to mechanisms of transposition regulation. Additional studies are necessary to determine the structure of these elements and to identify the different subfamilies, as well as for understanding the dispersion dynamics of the *P* element family in populations of *Drosophila sturtevanti*.

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Analysis of metanucleated cells from alcoholics bearing oral carcinomas

(Análise de células metanucleadas de alcoólicos portadores de carcinomas orais)

*Andréa Ramirez**

Micronuclei (MN) test has been used as an indicator of genotoxic exposition since it is associated with the occurrence of chromosomal aberrations. A comprehensive revision of data on this subject published in the pertinent literature revealed the evolution of concept, the difficulties as a screening test as well as the cytogenetic and statistical biases involved in its application in current investigation.

The frequency of MN among 30 subjects with oral and oropharyngeal carcinomas, whose alcohol consumption varied from four to 59 years, was compared to that of 30 healthy control individuals, abstinent for alcohol and matched for social-economic status. Difference (14.5 years) between average age of patients (52.9 ± 1.6) and that of controls (38.4 ± 1.5) was statistically significant ($P < 0.0001$).

The investigation included the examination of 2,000 cells per individual from each of three distinct areas in the mouth of patients and controls: around the lesion (B), opposite to the lesion (A) and in the upper gengivo-labial gutter (C) taken as control site because of its low tumor occurrence.

The cells were fixed, dried and analyzed under "blind test", according to the technique of Sarto *et al.* (*Mutagenesis, 2(1): 11-7, 1987*), modified and fitted strictly to the requirements of the research. The number of MN per 2,000 cells per individual among patients as well as controls showed a Poisson distribution with a positively asymmetric dispersion and an increased variance among patients. Distribution of metanucleated cells also departed significantly from normal dispersion.

The frequency of MN in the three oral regions of patients, evaluated through the Kruskal-Wallis test, revealed a highly significant heterogeneity ($P = 0.005$) and pairwise comparison B vs. C was statistically significant ($P < 0.01$) but not comparisons between A vs. B or A vs. C ($P > 0.05$), through Dunn's multiple comparison test. Comparisons of pairwise inter-regional oral differences of MN frequencies (A-B, A-C, B-C) increased the significance levels of the

results for regional heterogeneity (P = 0.0003), becoming also the comparison A vs. C statistically significant. Otherwise, nonparametric analysis of variance of the MN distribution in the three oral regions of the controls indicated great statistical homogeneity (P = 0.943).

Frequencies of MN and metanucleated cells in the oral regions of patients were also compared to those of controls, through the Mann-Whitney test. Differences were highly significant (P < 0.001) for tumoral region and significant for the region opposite to the lesion (P = 0.03) but not for the upper gengivo-labial gutter (P = 0.44), in comparison to those of controls. These results indicated a seven-time increase in the frequency of MN in the region around the lesion, a three-time increase in the opposite region and a two-time but nonsignificant increase in the upper gengivo-labial gutter, revealing a gradient frequency in the way C $\rightarrow$ A $\rightarrow$ B. Comparisons of frequencies of metanucleated cells (binucleated (BI), cariorrhexis (CR), cariolisis (CL) and broken egg (BE)) in the three oral regions, between patients and controls, showed highly significant differences, except for BE frequencies in all oral regions and for CR frequency in the upper gengivo-labial gutter.

Dichotomous comparisons of nonparametric independent variables, with MN frequencies, through contingency and Mann-Whitney tests, were not significant at 5% level of probability, except for CAGE diagnosis of alcoholism, which confirmed the alcohol effect.

Contrary to the expected results, sistematically frequencies of MN and metanucleated cells were not significantly correlated to age among patients as well as controls. Moreover, stepwise multiple regression analysis of MN and metanucleated cells in the patients revealed small negative, but significant, regression coefficients upon intervening factors such as age, end and time of alcohol consumption and time of tobacco usage, but regression coefficients of CL, upon alcohol consumption, were significantly small, but positive, before or after square root transformation of dependent variables.

However, the apparently contradictory results from analysis of regression among patients could be explained by the assumption that frequencies of MN, under alcohol exposure, had an early strong increase, but decreased, afterwards, to a level significantly greater than that before alcohol consumption, while CL frequency conversely increased significantly as a result from MN transformation, during the repair process.

It could be stated from the results of the present research that examination of cells with MN and metanucleated anomalies should follow critical and strict cytological criteria of standardization. The number of cell counts should be fixed and above 2000 in order to include normal (spontaneous) variability in the distribution of MN and, therefore, to prevent biases in the estimation of its frequencies. Also, sample size should be above 30 individuals, so that the statistical representativity be assured and significance of intergroup differences could be estimated through nonparametric tests. Moreover, intra-individual (or intra-regional) examinations and specific inter-individual controls matched for intervening factors (sex, age, socio-economic level, etc.) should be an usual methodological practice.

As a conclusion, it must to be considered that MN test is a simple, practical, non-expensive and non-invasive screening technique of diagnosis for clinical prevention and management of subjects under carcinogenic risks, after exposition to genotoxical agents or situations, such as abusive and chronical consumption of alcohol, tobacco and/or other mutagenic drugs or professional manipulation of derivatives of petroleum and other toxical industrial substances. In the context of the present investigation, the MN test must particularly be indicated for monitoring the clinical evolution of subjects with healed or surgically removed tumors or leukoplasic lesions, after chemo or radiotherapic treatments, by means of intra- and inter-individual cellular comparisons.

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Genetical and clinical study of the Waardenburg syndrome

(Estudo genético-clínico da síndrome de Waardenburg)

*Eliete Pardono**

We present the results of the study of 59 patients affected by the Waardenburg's syndrome (WS), 30 presenting the I variant, 21 the type II, and 8 of them being isolated cases without telecanthus. These patients belong to 37 families. All patients were examined as to the presence of eight cardinal signs important for the diagnosis of the condition; from each patient, from many of his/her normal relatives and from a control sample of 300 normal individuals - stratified by age and sex - 23 different craniofacial measurements were obtained. The data on the latter group were used for the construction of growth curves indicating the limits of the 2.5, 25, 50, 75 and 97.5th percentiles of the Gaussian distribution. In order to originate discriminant functions to separate individuals affected by one of the two variants, both metric (from craniofacial measurements) as well as categoric data (based on the frequencies of the cardinal signs or symptoms) were used. Discriminant analysis based on the frequency of the eight cardinal signs can improve the separation of WSI patients without telecanthus from those presenting the variant II. We present also a table with the conditional probabilities favoring the hypothesis of WSI for suspect subjects without telecanthus and any combination of the other seven signs/symptoms. The discriminant func-

tion based on the four ocular measurements (inner and outer intercanthal, interpupillary and inferior lacrymal distances), on the other side, perfectly classifies patients affected by one of the variants of WS, the same taking place when the average values of the W index of all affected individuals per family are used. However, the discriminant function based solely on the individual W index values of patients correctly classifies 93% of WSII subjects, but only 60% of the patients with the I variant of WS. The use of metrical measurements for separating affected from non-affected individuals must be regarded with caution, since it is weakened by many factors, such as a) the order of magnitude of the craniofacial measurements; b) the degree of heterogeneity among measurements taken by different observers; c) the virtual impossibility of their taking in identical conditions of facial expression; d) the difficulties arising from the imprecise delimitation of certain anatomical reference points, and e) the fact that most researchers dealing with this kind of material make no use of double-blind tests.

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Diversity, abundance and temporal variation of the *Drosophila* species (Diptera, Drosophilidae) in two woods in the northwestern region of the São Paulo State

(Diversidade, abundância e variação temporal de espécies de *Drosophila* (Diptera, Drosophilidae) em duas matas da região noroeste do estado de São Paulo)

*Felipe Rafael Torres**

Despite the great amount of studies on the genetics and evolution of the genus *Drosophila*, Brazilian species still need to be deeplier explored in relation to many aspects, mainly those involving systematics and ecological data.

In this study, data on the *Drosophila* fauna including diversity, abundance and temporal variation of species in three wood areas in the northwestern region of São Paulo State are presented.

The flies were collected monthly using traps placed at a height of 1.7 m from the ground. Baits were prepared with banana and baker's yeast (*Saccharomyces cerevisiae*). In each area species fluctuations were followed during 12 months and correlated to variations in temperature and rainfall. The three areas were compared by Morisita and Jaccard similarity indices.

A total of 24,595 flies were captured, 10,800 being from São José of Rio Preto (RP), 4,779 from Novo Horizonte 1 (NH1) and 9,016 from Novo Horizonte 2 (NH2). Among the 25 species and a subgroup collected, *Drosophila sturtevanti* was the most frequent and numerous. No other species competed with it in absolute number.

The greatest amount of flies was captured in hot and wet season (October to March). High correlation values between the abundance and rainfall were obtained, but they were not significant in both RP and NH1 areas ($P > 0.05$). However, this correlation was significantly positive along the year and in relation to the hot and wet season in NH2 area.

The curves of the diversity dominance component were very inclined, reflecting the lower diversity degree and the higher dominance of few species. These results characterize the structure of populations in the septentrional latitudes and in the tropics where the seasons are well defined exhibiting humid and dry periods.

Drosophila sturtevanti and *D. simulans* were the dominant species. Some species showed intermediate abundance but 100% of frequency in the collections, among them *D. polymorpha* and *D. paranaensis* in RP area. On the opposite to data in literature, *D. paranaensis* abundance and frequency were greater than those from *D. mercatorum*. *Drosophila buzzatii* was rare in RP and NH1 and was classified as a species of intermediary abundance but of high frequency in NH2. This can be explained by the presence of cactaceous in this area.

Another observation in contrast to the literature was the positive correlation between abundance and rainfall for *D. nebulosa* ($P \leq 0.05$).

Species richness varied in the three areas studied. In RP the distribution was uniform over the period of collections, suggesting a stable and less disturbed environment. In contrast, the richness in NH1 was very irregular; this was the area with the smallest number of species. The NH2 area showed the greatest richness, with 21 collected species. The species richness was correlated with temperature in RP and humidity in NH2. None of these correlations was observed in NH1. The differences are indicative of different support capacity among the studied areas.

The two similarity indices used showed different results. According to the Morisita index, RP and NH2 areas were more similar to each other than to NH1. However, the Jaccard index indicated a greater similarity between RP and NH1.

The three areas shared 13 species and the *willistoni* subgroup. Exclusive species were not observed in RP or NH1, but they showed 4 species in common: *D. austrosaltans*, *D. ananassae*, *D. guaru* and *D. mediopunctata*. *Drosophila busckii*, *D. canalinea*, *D. fuscolineata*, *D. nigricruria* and *D. serido* were exclusive from NH2. *Drosophila pallidipennis* and *D. hydei* were found both in NH2 and RP. Only *D. coroica* was exclusive from NH1 and NH2.

The greatest diversity index was in RP (0.73), followed by NH2 (0.71) and NH1 (0.62). Despite the great proximity of the RP to the urban region, this area exhibited the greatest species diversity in comparison with the other two areas that were 23 km far from urban regions. It seems that NH1 and NH2 are under strong effect of human and cattle activity, mainly in the river margins.

The observation on the population explosion of *D. sturtevanti* in the three studied areas, the greater abundance of *D. paranaensis* in comparison with *D. mercatorum*, and the positive correlation between the *D. nebulosa* abundance and rainfall are indicative of changes in the population structure and/or new adaptive strategies arised in answer to environment modifications. These findings deserve a more detailed study.

The present work showed that the *Drosophila* populations were submitted to clear fluctuations in fly numbers during the 12 months, some species being abundant or almost absent in the traps in different times. Those that were equally frequent during the entire year, most of the time were present in low numbers.

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