strate that toluidine-blue staining can be a sensitive method, detecting pre-degranulation events; but that some batches of toluidine have too high an affinity for the basophil granules and will therefore only detect true exocytosis[20]. Lastly, the pH and the cell density in the reaction medium might have some influence. The effects of these conditions have not yet been determined.

Summarizing, we conclude that we have no evidence for an effect of antiserum in high dilutions, and we have some doubts about the reproducibility of the basophil degranulation model. This is not the first instance of an experiment with extreme dilutions proving to be difficult to reproduce[5]. We are of the opinion that if conclusive information about this subject is to be obtained, it is necessary that an experimental model be investigated by a number of independent laboratories.

* To whom correspondence should be addressed.

1 Acknowledgment. This study was partly funded by Homint.

2 Davenas, E., Beauvais, F., Amara, J., Oberbaum, M., Robinzon, B., Miadonna, A., Tedeschi, A., Pomeranz, B., Fortner, P., Belon, P., Sainte-Laudy, J., Poitevin, B., and Benveniste, J., Nature *333* (1988) 816.
3 Cazin, J. C., Cazin, M., Gaborit, J. L., Chaoui, A., Boiron, J., Belon, P., Cherruault, Y., and Papapanayotou, C., Human Toxic. *6* (1987) 315.
4 Daurat, V., Dorfman, P., and Bastide, M., Biomed. Pharmacother. *42* (1988) 197.
5 Fisher, P., House, I., Belon, P., and Turner, P., Human Toxic. *6* (1987) 321.
6 Reilly, D. T., Taylor, M. A., McSharry, C., and Aitchinson, T., Lancet (1986) 881.
7 Davenas, E., Poitevin, B., and Benveniste, J., Eur. J. Pharmac. *135* (1987) 313.
8 Mansvelt, van, J. D., and Amons, F., Z. Naturforsch. *30* (1975) 643.
9 Poitevin, B., Davenas, E., and Benveniste, J., Br. J. clin. Pharmac. *25* (1988) 439.
10 Wagner, H., Jurcic, K., Doenicke, A., Rosenhuber, E., and Behrens, N., Arzneim.-Forsch. Drug Res. *36* (1986) 1421.
11 Wagner, H., Kreher, B., and Jurcic, K., Arzneim.-Forsch. Drug Res. *38* (1988) 273.
12 Del Giudice, E., Preparata, G., and Vitiello, G., Phys. Rev. Letters *61*(9) (1988) 1085.
13 Beauvais, F., Hieblot, C., Burtin, C., and Benveniste, J., J. Immun. *144* (1990) 3881.
14 Maddox, J., Randi, J., and Stewart, W. W., Nature *334* (1988) 287.
15 Jaques, J., C.r. Acad Sci. Paris *310* (1990) 1437.
16 Benveniste, J., Davenas, E., Ducot, B., Cornillet, B., Poitevin, B., and Spira, A., C.r. Acad. Sci. Paris *312*(II) (1991) 461.
17 Snedecor, G. W., and Cochran, W. G., Statistical Methods, 6th edn, p. 325. The Iowa State University Press, Ames, Iowa, USA 1967.
18 Glantz, S. A., Primer of Biostatistics., 2nd edn, pp. 88–89. McGraw-Hill Book Company, New York 1987.
19 Scheepens, C. G. M., Smithuis, L. O. M. J., and Hovenkamp, J., Elkerliek Hospital, Helmond, The Netherlands (unpublished data).
20 Beauvais, F., Bidet, B., Descours, B., Hieblot, C., Burtin, C., and Benveniste, J., J. Allergy clin. Immunol. (1991) in press.

0014-4754/92/050504-03$1.50 + 0.20/0


## Sister chromatid exchanges in lymphocytes of normal and alcoholic subjects

J. R. Lazutka, V. Dedonytė and R. K. Lekevičius

*Ecological Genetics Laboratory, Vilnius University, 21 Čiurlionis St., 232009 Vilnius (Lithuania)*
*Received 15 August 1991; accepted 21 November 1991*

*Abstract.* The effects of alcohol consumption, cigarette smoking and age on sister chromatid exchange (SCE) frequency in human lymphocytes were assessed by means of multiple linear regression. An increase in SCE rates was associated with alcohol consumption ($p = 0.0001$), smoking ($p = 0.0231$), and, to a small extent ($p = 0.057$), age. These three confounding factors explain 48% of the inter-personal variation in SCE rates among subjects studied.
*Key words.* Sister chromatid exchange (SCE); human lymphocytes; alcohol consumption; smoking; aging.

A sister chromatid exchange (SCE) is the cytological manifestation of DNA breakage and rejoining at homologous sites of the two chromatids of a single chromosome. One application of SCE analysis is the monitoring of human populations exposed to chemical mutagens and carcinogens[2]. Such exposure may be associated not only with occupational factors but with common lifestyle factors as well.

Cigarette smoking is now generally agreed to be a SCE-inducing factor[3], but it is still controversial whether alcohol consumption also contributes to elevated SCE rates. Increased rates of chromosome aberrations[4] and SCEs[5] were found in peripheral blood lymphocytes of alcoholics as compared to a control population. However, moderate alcohol consumption ('binge drinking') has been reported to be a non-significant factor contributing to the inter-personal variation in SCE rates[6–8]. Similarly, alcoholism in mothers did not affect the SCE rate of their newborn infants[9]. Obe and Ristow[10] related the induction of SCEs in vitro to the action of acetaldehyde, a metabolic derivative of alcohol[11], but not to the action of ethanol. Another in vitro study[12] showed that induction of SCEs by alcoholic beverages could be explained not only by their content of ethanol, but also by the presence of the other SCE-inducing compounds. It has been suggested[13] that the agents responsible for the increase of chromosomal damage in alcoholics may be ingredients other than ethanol found in alcoholic beverages. However, no significant differences related to the kind of beverage consumed (wine, beer, vodka, whiskey)

were found in the number of SCEs in the lymphocytes of 11 alcoholics [5].

The present study sought to investigate the influence of alcohol consumption and of the type of alcoholic beverage consumed on the number of SCE in peripheral blood lymphocytes of alcoholic subjects.

*Study subjects.* We studied the frequency of SCEs in 48 male control subjects, 31 non-smokers (mean age 40.9 years, range 19–61) and 17 smokers (mean age 39.2 years, range 23–58), and in 26 male alcoholic subjects. Eleven of the subjects consumed exclusively vodka (mean age 38.3 years, range 30–47), and 15 consumed many other kinds of alcoholic beverages, including ersatz-drinks such as face lotion or eau-de-Cologne (mean age 44.2 years, range 32–57). The average daily intake of alcohol (recalculated in grams of absolute ethanol) was approximately 170 g/day in the first group, and 110 g/day in the second group of alcoholic subjects. There was also a difference between the two groups of alcoholic subjects in the average duration of the dependency on alcohol: 12.0 years (range 5–30) in the first one, and 18.5 years (range 9–32) in the second one. Among alcoholic subjects studied, three subjects were in the first, twenty in the second, and three in the third stage of alcoholism according to the three-stage classification [14]. Since alcoholics tend to be heavy smokers [15], it was very difficult to find non-smokers among alcoholic subjects. Only two alcoholic subjects in the present study were non-smokers.

## *Materials and methods*

The study was conducted with peripheral blood lymphocytes obtained by venipuncture from 48 control and 26 alcoholic male subjects. Heparinized whole blood was diluted in the ratio 1:15 with Eagle's MEM supplemented with 20% bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 µg/ml 5-bromo-2'-deoxyuridine (Serva, FRG) and 0.15% Bacto PHA 'P' (Difco, USA). Cells were cultured in sterile bottles made of amber-colored glass for 72 h at 37 °C in complete darkness, and colchicine (Merck, FRG) was present for the last 3 h at a final concentration of 1.5 µg/ml. After hypotonic treatment (0.075 M KCl for 30 min) and fixation (methanol: glacial acetic acid, 3:1, three times for 20 min), flame-dried slides were prepared.

Differential staining of sister chromatids was carried out by a modified fluorescence-plus-Giemsa technique [16]. Briefly, the slides were stained for 10 min with 10 µg/ml of Hoechst 33258 dye (Riedel–de Haen, FRG) dissolved in 0.07 M Soerensen's buffer (pH 6.8), rinsed and mounted with citrate buffer (pH 8.5). Then the slides were covered with cover slips and exposed to UV light (400 W mercury lamp at a distance of 15 cm) for 6–7 min. Slides were then rinsed and stained for 3 min with azure II – eosin K solution (0.025 and 0.01% respectively; Reachim, USSR) prepared in phosphate buffer (pH 6.8). All slides were coded, and second mitotic division cells with 46 chromosomes only were analysed. Between 15 and 65 cells (average 38) were analysed per each subject to evaluate SCE rates.

Associations between all variables and SCE rate were investigated using the method of general linear models which enabled an estimate to be made of the percentage of variation in SCE attributable to each of the variables in the model. A general linear model provides a predicted SCE level for every individual in the study group based upon his combination of predictor variables. The adequacy of the fit for the model has been checked by analysis of the correlation between the squared residual (the difference between the observed and the predicted SCE level for each individual) and the predicted SCE, and by the analysis of the distribution of the residuals. SCE data were analysed in a logarithmic ($\log_e$) scale as proposed by Soper et al. [17] using SAS procedures GLM, FACTOR MSA, CORR and UNIVARIATE [18].

## *Results and discussion*

Results of present investigation (table 1) show different SCE rates in the four groups studied. The lowest SCE rates were in the group of control non-smokers (10.36 ± 0.34 SCE/cell), and the highest ones were in the group of alcoholic subjects consuming any kind of alcoholic beverage (15.31 ± 0.62 SCE/cell). However, there were differences among the groups studied in smoking habits, the kind of alcoholic beverages consumed and the duration of alcohol consumption. Preliminary analysis (data not shown) suggested that there was a positive linear relationship between SCE frequency and smoking, age of donors and alcohol consumption (defined as an

Table 1. Sister chromatid exchange (SCE) frequency in various control and alcoholic subject groups

| Group | Number of individuals | Age (years) | | Smoking (packs/day) | | Duration of alcohol consumption (years) | SCE/cell | |
|---|---|---|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range | | Mean ± SE | Range |
| *Control subjects* | | | | | | | | |
| Non-smokers | 31 | 40.9 | 19–61 | 0 | - | - | 10.36 ± 0.34 | 7.56–17.17 |
| Smokers | 17 | 39.2 | 23–58 | 0.7 | 0.25–1.25 | - | 11.71 ± 0.44 | 8.09–14.75 |
| *Alcoholic subjects* | | | | | | | | |
| Vodka consumers | 11 | 38.3 | 30–47 | 0.6 | 0–2.0 | 12.0 | 12.65 ± 0.77 | 9.93–17.92 |
| Any beverage consumers | 15 | 44.2 | 32–57 | 1.2 | 0.5–2.0 | 18.5 | 15.31 ± 0.62 | 11.39–19.16 |

indicator variable 'alcoholic vs non-alcoholic subject'). However, there was no relationship between SCE values and average daily alcohol consumption (expressed in g of absolute ethanol) in the group of alcoholic subjects.

As was mentioned above, non-distilled alcoholic beverages may contain SCE-inducing compounds other than ethanol[12]. On the other hand, a clear dependency of chromosome aberration frequency on the duration of alcohol consumption has been demonstrated[4]. Thus, in order to identify a possible cause of the increased SCE frequency in alcoholics, 'alcohol consumption' was defined as two variables: a) as an indicator variable regarding the kind of alcoholic beverage consumed (0 = non-alcoholic subject, 1 = alcoholic subject consuming exclusively vodka, 2 = alcoholic subject consuming any other kinds of alcoholic beverages), and b) as a continuous variable regarding the duration of alcohol consumption in years. When each of the above variables was studied in a separate model, it appeared that the influence of the kind of alcoholic beverages consumed and of the duration of alcohol consumption was significant ($p = 0.0005$ and $p = 0.0034$ respectively, when adjusted to other variables used in the model). However, when both variables were studied in the same model, it appeared that the influence of none of them was significant ($p = 0.0563$ and $p = 0.6549$ respectively). Notwithstanding, the influence of the kind of alcoholic beverages consumed seemed to be more important in both cases.

In order to eliminate one of these two variables, partial correlation coefficients were calculated (table 2). This analysis demonstrated that there were positive statistically significant correlations between SCE frequency and smoking and the kind of alcoholic beverage consumed, and there was no correlation between SCE frequency and the duration of alcohol consumption. Multiple linear regression was then used to investigate the relationship between SCE frequency, the subject's age, smoking and the kind of alcoholic beverage consumed. Age was included in the model because in several large-scale studies[3, 17] it has been reported to be a significant confounding factor.

Table 2. Partial correlation matrix for the 5 variables used in the study

| Variables[a] | Age | Smoking | Kind of alcohol beverage consumed | Duration of alcohol consumption |
|---|---|---|---|---|
| SCE/cell | 0.22 | 0.26[b] | 0.23[b] | 0.05 |
| Age | | −0.01 | −0.03 | 0.03 |
| Smoking | | | 0.41[b] | −0.10 |
| Kind of alcoholic beverage consumed | | | | 0.74[b] |

[a] Age is a continuous variable expressed in years; smoking is a continuous variable defined as average number of packs of cigarettes smoked per day; kind of alcoholic beverage consumed is an indicator variable where 0 = non-alcoholic subject, 1 = alcoholic subject consuming vodka, 2 = alcoholic subject consuming any other kind of alcoholic beverage; alcohol consumption duration is a continuous variable expressed in decades of dependency on alcohol. SCE is analyzed in $\log_e$ scale.
[b] Statistically significant at $p < 0.05$ level.

Table 3. Results of regression analysis in logarithmic scale of SCE on the kind of alcoholic beverage consumed, smoking and age

| Variable[a] | Regression coefficient | Standard error | p |
|---|---|---|---|
| Intercept | 2.2008 | 0.0756 | 0.0001 |
| Kind of alcoholic beverage consumed | 0.1177 | 0.0323 | 0.0005 |
| Smoking | 0.1133 | 0.0506 | 0.0284 |
| Age | 0.0035 | 0.0018 | 0.0570 |

[a] Variables are the same as in table 2.

Table 4. Analysis of three confounders affecting SCE

| Source of variation[a] | Degrees of freedom | Percent of variance in SCE explained[b] | F statistics[c] | p |
|---|---|---|---|---|
| Kind of alcoholic beverage consumed | 2 | 41.1 | 27.24 | 0.0001 |
| Smoking | 1 | 4.1 | 5.40 | 0.0231 |
| Age | 1 | 2.8 | 3.76 | 0.0570 |
| Total variation explained | 4 | 48.0 | | |

[a] Variables are the same as in table 2.
[b] SCE is analyzed in logarithmic ($\log_e$) scale. Each variable is adjusted for the variables listed above it but not below it in this table.
[c] All F statistics have 73 degrees of freedom in the denominator.

The results of multiple linear regression analysis (table 3) suggested that $\log_e$ of SCE frequency per cell was associated with the kind of alcoholic beverage consumed, smoking, and, to a small extent, age. These three factors explain a total of 48% of the inter-subject variance in SCE frequency (table 4). The kind of alcoholic beverage consumed was the single most important predictor of SCE level in the model used, explaining 41.1% of the variance in SCE, which is highly statistically significant ($p = 0.0001$). After accounting for the variability explained by smoking and age, the kind of alcoholic beverages consumed still explained 9.9% of the total variability in SCE ($p = 0.0024$). Here, it is noteworthy that the indicator variable 'kind of alcoholic beverage consumed' included also the variable 'alcohol consumption' (since 0 is defined as 'non-alcoholic subject'), thus indicating that alcohol consumption per se is also an important predictor of SCE level. After adjusting for the effect of alcohol consumption, smoking was found to be a statistically significant ($p = 0.0231$) predictor of SCE level, which explains 4.1% of the variance in SCE. After accounting for the variability explained by alcohol consumption and age, smoking still explained 3.7% of the total variability in SCE ($p = 0.0295$). Finally, age was a predictor of SCE frequency at a significance level of $p = 0.057$, explaining, after adjustment for the effects of alcohol consumption and smoking, 2.8% of the variance in SCE.

Using parameters presented in table 3, the predicted SCE/cell values were calculated. It was found that predicted SCE values were 10.43 ± 0.08 SCE/cell for the control non-smokers, 11.25 ± 0.19 SCE/cell for the control smokers, 12.47 ± 0.17 SCE/cell for the alcoholic subjects consuming vodka, and 15.20 ± 0.20 SCE/cell for the alcoholic subjects consuming any other kind of alcoholic beverage. Evaluation of the SCE residuals (see above) gave no indication that this model was not adequate. Thus, the three-variable multiple linear regression model used in this study agrees exceedingly well with the observed data.

The main results of the present study are in agreement with previous observations about the significant influence of cigarette smoking[3,6,7], aging[3,17] and chronic alcohol consumption[5] on SCE frequency in human lymphocytes. The poor relationship between the subject's age and SCE frequency in our study might be because it was less representative than other studies[3,17]. We also found a significant correlation between the kind of alcoholic beverages consumed and SCE frequency. As was mentioned above, other authors[5] were not able to demonstrate a difference between the kind of beverage consumed and the number of SCEs. However, they studied only 11 subjects and divided them into 4 groups according to the beverage consumed (wine, beer, vodka, whiskey). Their study sample size was evidently too small to assess statistically significant differences[2].

There was no relationship between duration of alcohol consumption and SCE frequency in our study. Similar results were obtained by Butler et al.[5]. On the other hand, Obe et al.[4] observed correlation between the duration of alcohol consumption and the frequency of chromatid-type aberrations in the lymphocytes of alcoholics. However, they did not attempt to correlate the frequency of aberrations and the kind of alcoholic beverage consumed. It has been demonstrated recently that at the early stages of alcoholism, beverages with a high content of alcohol (vodka, whiskey, etc.) are preferred, whereas at the late stages wine, beer and other beverages with a low alcohol content are most frequently consumed[19]. Interestingly, the highly statistically significant correlation ($r = 0.74$) between the duration of alcohol consumption and the kinds of alcoholic beverages consumed was also found in the present study (table 2). Thus, the results of Obe et al.[4] may be biased by such correlation. Our results, however, may also be biased to some extent owing to the presence of correlation between the kind of alcoholic beverage consumed and smoking intensity (table 2).

It is noteworthy that it is still questionable whether the ethanol in alcoholic beverages is directly responsible for the cytogenetic effects observed in lymphocytes of alcoholic subjects. First of all, no relationship between the amount of alcohol consumed daily and elevated SCE frequencies could be demonstrated in the present study. Moreover, despite the fact that the alcoholic subjects consuming vodka had a higher daily alcohol intake (170 vs 110 g/day, see above), lower SCE rates were observed in their lymphocytes when compared with lymphocytes of consumers of other kinds of alcoholic beverages (table 1). Secondly, there was no increase in SCE frequency in the lymphocytes of 7 subjects under the acute influence of ethanol[20]. Thus, in our opinion, several possibilities exist to explain the elevated rates of cytogenetic damage in lymphocytes of chronic alcoholic subjects.

Increased SCE and/or chromosome aberration rates may be attributed to the action of the first ethanol metabolite, acetaldehyde. Indeed, acetaldehyde, but not ethanol, was demonstrated to induce SCEs in vitro[10]. Additionally, elevated SCE frequency was observed[20] in lymphocytes of patients under the influence of a minimum amount of alcohol and disulfiram, which is known to raise the blood acetaldehyde level. Interestingly, elevated blood acetaldehyde rates have been reported to be the rule in chronic alcohol addicts[21].

Increased rates of cytogenetic damage may also result from ingredients found in alcoholic beverages other than ethanol, as proposed by Obe et al.[13]. This idea is supported by our finding that SCE rates are dependent on the kind of alcoholic beverage consumed (table 4).

Alcohol abuse is clearly associated, all over the world, with an increased incidence of various cancers[11]. The mechanisms underlying increased cancer risk in alcoholics are clearly complicated. Cancer risk is likely to be affected both by the fact that alcoholic beverages are complex solutions containing low levels of various carcinogens[22], and by dietary deficiencies associated with alcohol abuse[23]. Thus, increased cancer risk in alcoholic subjects is more likely to be due to alcohol abuse than to direct carcinogenic action of ethanol[11]. Similarly, increased SCE rates may be attributed to alcoholism as a pathological condition of the human organism. Bearing in mind that increased SCE frequencies have been reported to be a very sensitive indicator of carcinogenic risk[24], such a parallelism seems to be quite reasonable. Indeed, it may be regarded as a general rule that increased SCE or chromosome aberration frequency is usually observed (as in the present study) in the case of chronic alcohol intake[5,25], but not in the case of 'binge drinking'[6–8].

Alcoholism is often associated with poor nutrition, including deficiencies of thiamine, folate and ascorbate[23]. Dietary ascorbate may protect the cells against the mutagenic action of a number of compounds[26,27]. Depressed activities of such repair enzymes as $O^6$-methylguanine transferase after chronic alcohol intake have also been found[28]. All these above-mentioned changes may play a significant role in mutagenic and carcinogenic processes occurring in the organism of alcoholics, which may be affected by various mutagenic occupational and lifestyle factors. Such interactions, however, seem to be very complex and difficult to identify. Thus, more extensive studies are needed for the elucidation of the actual reasons

underlying the increased SCE rates in lymphocytes of alcoholic subjects.

1 We are grateful to Dr D. Krapavickaitė for performing the statistical analysis, and to Mrs V. Navickienė for technical assistance. We also wish to thank Dr B. Lambert (Stockholm, Sweden) for his valuable suggestions.

2 Carrano, A. V., and Natarajan, A. T., Mutat. Res. *204* (1988) 379.
3 Husum, B., Wulf, H. C., and Niebuhr, E., Hereditas *105* (1986) 17.
4 Obe, G., Gobel, D., Engeln, H., Herha, J., and Natarajan, A. T., Mutat. Res. *73* (1980) 377.
5 Butler, M. G., Sanger, W. S., and Veomett, G. E., Mutat. Res. *85* (1981) 71.
6 Sarto, F., Faccioli, M. C., Cominato, J., and Levis, A. G., Mutat. Res. *144* (1985) 183.
7 Reidy, J. A., Annest, J. L., Chen, A. T. L., and Welty, T. K., Envir. molec. Mutagen. *12* (1988) 311.
8 Yardley-Jones, A., Anderson, D., Jenkinson, P. C., Lovell, D. P., Blowers, S. D., and Davies, M. J., Br. J. Ind. Med. *45* (1988) 694.
9 Seshadri, R., Baker, E., and Sutherland, G. R., Mutat. Res. *97* (1982) 139.
10 Obe, G., and Ristow, H., Mutat. Res. *56* (1977) 211.
11 Lieber, C. S., Baraona, E., Leo, M. A., and Garro, A., Mutat. Res. *186* (1987) 201.
12 Raat, W. K. de, Davis, P. B., and Bakker, G. L., Mutat. Res. *124* (1983) 85.
13 Obe, G., Ristow, H., and Herha, J., in: Alcohol Intoxication and Withdrawal, pp. 47–70. Ed. M. M. Gross. Plenum, New York 1977.
14 Ivanets, N. N., Alcohol Alcoholism *24* (1989) 377.
15 Mello, N. K., Mendelson, J. H., and Palmieri, S. L., Psychopharmacology *93* (1987) 8.
16 Perry, P., and Wolff, S., Nature *251* (1974) 156.
17 Soper, K. A., Stolley, P. D., Galloway, S. M., Smith, J. G., Nichols, W. W., and Wolman, S. R., Mutat. Res. *129* (1984) 77.
18 SAS User's guide: Statistics, Version 5. SAS Institute, NC 1985.
19 Schmidt, W., Suchtgefahren *34* (1988) 34.
20 Veghelyi, P. V., and Osztovics, M., Experientia *34* (1978) 195.
21 Korsten, M. A., Matsuzaki, S., Feinman, L., and Lieber, C. S., New Engl. J. Med. *292* (1985) 386.
22 Walker, E. A., Gastegnaro, M., Garren, L., Touissaint, G., and Kowalski, B., J. natl Cancer Inst. *63* (1979) 947.
23 Morgan, M. Y., and Levine, J. A., Proc. Nutr. Soc. *47* (1988) 85.
24 Brockman, H. E., and DeMarini, D. M., Envir. molec. Mutagen. *11* (1988) 421.
25 Obe, G., and Anderson, D., Mutat. Res. *186* (1987) 177.
26 Ginter, E., Chorvatovičova, D., and Košinova, A., Int. J. Vit. Nutr. Res. *59* (1989) 161.
27 Pohl, H., and Reidy, J. A., Mutat. Res. *224* (1989) 247.
28 Espina, N., Lima, V., Lieber, C. S., and Garro, J. A., Carcinogenesis *9* (1988) 761.

0014-4754/92/050508-05$1.50 + 0.20/0


## Selection for class II *Mhc* heterozygosity by parasites in subterranean mole rats

E. Nevo and A. Beiles

*Institute of Evolution, University of Haifa, Mt. Carmel, Haifa (Israel)*
*Received 20 March 1991; accepted 19 November 1991*

*Abstract. Mhc* organization and polymorphism have previously been studied[26] in the four chromosomal species of the *Spalax ehrenbergi* superspecies in Israel, serologically, and at the DNA, RFLP and sequence levels of class I and class II genes. Here we demonstrate that the observed heterozygosity of *Mhc* class II genes $P\alpha_1$ with 11 alleles, and $Q\beta$, with at least 14 alleles, is positively and significantly correlated with infectivities of ectoparasites (gamasid mites)[17] and endoparasites (helminths)[18]. *Mhc* heterozygosity is highest in the most infected area, which is in the most humid-warm region of the superspecies range, or where two zoogeographic regions overlap. We conclude that the evolutionary forces responsible for the *Mhc* class II two-gene polymorphisms include selection for increased heterozygosity as a defense strategy against ecto- and endoparasite infections.
*Key words. Mhc*; heterozygosity; parasites; natural selection; subterranean mole rats; *Spalax ehrenbergi.*

The major histocompatibility complex, *Mhc*[1], is among the most complex gene clusters so far known, reflecting a very long and involved evolutionary history. *Mhc* covers about one thousandth of the mammalian genome, 3800 kb in man[2], including two major gene classes (I and II) of cell surface glycoproteins with different but related functions of immunoregulation[1–4]. The function of the *Mhc* genes is to control the recognition of foreign and self proteins by T lymphocytes. Class II *Mhc* molecules present foreign peptides to helper T lymphocytes[5–8]. Helper T cell receptors recognize foreign protein-derived peptides only when these are associated with self class II *Mhc* molecules. A given T cell receptor is both peptide-specific and *Mhc*-restricted. The *Mhc* controls the specificity of the immune response against pathogens including viruses, bacteria and other parasites. It also contributes to the susceptibility to over 40 different autoimmune diseases[9], in which the body's immune system attacks self proteins.

Subterranean mole rats of the *S. ehrenbergi* superspecies in Israel represent an active case of ecological speciation[10–16]. The superspecies comprises four chromosomal species ($2n = 52$, 54, 58 and 60), displaying progressive stages of late chromosomal speciation. Their adaptive radiation in Israel from the Lower Pleistocene to Recent times is closely associated with fossoriality and increasing aridity, i.e., with distinct climatic diversity: $2n = 52$, cool-humid (north); $2n = 54$, cool-semidry (north-east); $2n = 58$, warm-humid (center); and finally, $2n = 60$, warm-dry, in the southern part of the range[13].