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A note on cytogenetic monitoring of the Polish hare population *(Lepus europaeus)**

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A comparative cytogenetic analysis of the Polish hare (*Lepus europaeus* Pallas, 1778) population was carried out using chromosome aberration (CA) and sister-chromatid exchange (SCE) tests. The frequency of CA and SCE in blood lymphocytes was determined in 73 individuals from five hunting areas (ranges): Grodno (GR), Rozniaty (RZ), Rogów (RG), Wieluń (WL), and Wierzchowiska (WK) differing in hare population density. The lowest content of cells with chromosome aberrations, among which were counted chromatid and chromosome breaks, as well as acentric fragments, was recorded for hares from the WL range (1.44%), while the highest in hares from the WK (2.6%) and RG (2.71%) ranges, the two latter differing ($P<0.05$) from that found in WL range hares. The mean SCE frequency/cell ranged from 5.51 in the WL range through 6.05 in WK and RG, up to 6.40 in the RZ range hares ($P<0.05$). The results obtained showed a marked differences between the hares from different hunting areas (ranges). The lowest frequencies of CA and SCE were found in the WL hunting area with the highest density of hares.

KEY WORDS: chromosome aberrations / cytogenetic monitoring / hare / sister-chromatid exchange

Animals living at large are directly subjected to an unfavourable impact of different environmental pollutions, caused by the use of considerable quantities of pesticides in agriculture, and by industrial emission. Most of those pollutants, beside the direct

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toxic effect on the organism resulting in, among much else, metabolic disturbances and a lowered resistance to diseases, may also have a genotoxic effect, *i.e*. damage the genetic material [Rubeš *et al.* 1992, Parada and Jaszczak 1993, Lioi *et al.* 1998]. DNA damage can be repaired, but some other damages, if not repaired, may lead to gene mutations or chromosome aberrations [Kimball 1987, De Jong *et al.* 1988].

Monitoring the genotoxic effect of environmental pollutants is nowadays commonly included in investigations on both the animal and human health. One of the most effective and most often used methods for determining the mutagenic properties of various chemical and physical factors is an analysis of the frequency of chromosome aberrations (CA) in the lymphocytes of peripheral blood, even though not all aberrations may be identified with this test and though their absence does not exclude other defects of the genetic material [Carrano and Natarajan 1988, Ishidate *et al.* 1988, Kirkland 1998].

Another method often used for evaluation of mutagenic effect is determining the frequency of sister-chromatid exchanges (SCEs) in metaphase cells. The test is considered to be one of the most precise indicators of the effect of genotoxic agents, even appearing at low concentrations and with a short period of exposure [Perry 1980, Rudek 1985, Hadnagy *et al.* 1989].

During the recent years the population of hares has declined distinctly throughout Europe [Hartl *et al*. 1992, Tapper 1992, Kunst *et al.* 2001]. In Poland, comprehensive programme of relevant investigations has been arranged to determine the reasons for this tendency [Dziedzic and Dzięciołowski 2001] among which cytogenetic monitoring was carried out.

The present paper is concerned with the assessment of the frequency of chromosomal aberrations (CA) and sister-chromatid exchanges (SCEs) in lymphocytes of brown hares from five hunting areas (ranges) differing in animal population density.

Material and methods

The material for cytogenetic tests consisted of 73 hares from five hunting areas (ranges): Grodno (GR, n=13), Rozniaty (RZ, n=15), Rogów (RG, n=14), Wieluń (WL, n=16, and Wierzchowiska (WK, n=15), located in the following districts of Poland: Toruń, Bydgoszcz, Skierniewice, Sieradz and Lublin, respectively (Fig 1). The hunting areas (ranges) and their estimated hare population density are presented in Table 1.

Blood for testing was collected from hares during two hunting seasons (1999/2000 and 2000/2001). Directly after shooting, the animal's chest was opened and the blood taken into test tubes containing heparin. That same day, or the next, a lymphocyte culture was established. One ml of whole blood was added to 9 ml of the RPMI 1640 medium supplement with 10% foetal calf serum and pokweed mitogen (GIBCO) at a final concentration of 10 μl/ml. When analysing chromosome aberrations (CA) the cultures were incubated at 37.6°C for 48 h, while for the sister-chromatid exchange (SCE) – for 72 h in the presence of BrdU (10 μg/ml). One hour before harvest the

Fig. 1. Location of hunting areas (ranges) troughout the country (○).

Table 1. Characteristic of areas (hunting ranges)

Area (hunting range)	Estimated hare population density (n/100 ha)	Habitat
GR	low: 5-15	large-scale arable land and intensive agriculture
RZ	low: 5-15	large-scale arable land and intensive agriculture
RG	mean: 15-30	mixed small-scale arable land and forest
WL	high: 30-40	mixed small-scale arable land and grassland
WK	mean: 15-30	arable land, intensive agriculture and industrial works in vicinity

GR – Grodno, RZ – Rozniaty, RG – Rogow, WL – Wielun, WK – Wierzchowiska.

colchicine was added – 10 μl/ml of medium. Preparations for the analysis of CA were obtained traditionally by air drying and staining in a 5% Giemsa solution. A differentiated staining of SCEs determinations was performed according to the FPG technique, described by Perry and Wolf [1974]. The preparations were made for each animal, and a 100 metaphase plates on several preparations were analysed for CA.

The SCEs were counted in 30 cells. The following types of CA were identified: chromosome breaks, chromatid breaks, and acentric fragments. Moreover, the number of chromatid and chromosome gaps was recorded as well as polyploidal cells, but those were not included in the CA index. Chromatid gaps were described as an achromatic region, smaller than the chromatid width, while the chromatid breaks were counted when the interval exceeded the width of the chromatid or else when the chromatid was translocated. In turn, chromosome breaks and gaps referred to both chromatids at the same location [Savage 1976, Carrano and Natarajan 1988].

The CA rate was expressed as the per cent of mitoses examined per group. Statistical analysis of results was performed using chi-square test to evaluate CA and t-test to compare SCEs frequencies between groups.

Results and discussion

Table 2 presents results of an analysis of the CA frequency in blood lymphocytes of hares from five hunting areas (ranges), differing as regards structure of farming and density of the hares population. The CA test demonstrated differences in the frequency of cells with particular chromosomal aberrations between hares from different ranges. A lower per cent of cells with CA, which included chromatid and chromosome breaks and acentric fragments, was observed in hares from the hunting ranges WL (1.44±1.30), RZ (1.60±1.02) and GR (1.76±1.56), while higher in those from RG (2.71±1.42) and WK 2.67±1.70) ranges, both latter differing, however, only from WL (1.44±1.30) hares (P<0.05).

The WL hunting range is marked by a

Table 2. Mean frequency of chromosome aberrations (CA) in the blood lymphocytes of hares from different hunting areas (hunting range:

Hunting range	Number of hares	Number of cells scored	Aberrations/100 cells										t
			chromatid breaks	chromosome breaks	fragments	chromatid gaps	chromosome gaps	polyploid cells		total breaks			
								mean	SD	mean	SD		m
GR	13	1300	0.53	0.61	0.53	0.53	0.69	0.92	0.86	1.76	1.56		1.
RZ	15	1500	0.73	0.46	0.33	0.53	0.40	0.80	0.80	1.60	1.02		0.
RG	14	1400	1.00	1.21	0.50	0.78	1.00	1.35	1.28	2.71^{a}	1.42		1.
WL	16	1600	0.31	0.75	0.37	0.37	0.68	0.93	0.82	1.44^{ab}	1.30		1.
WK	15	1500	0.73	1.13	0.80	0.66	0.93	1.73	1.30	2.67^{b}	1.70		1.

GR – Grodno, RZ – Rozniaty, RG – Rogow, WL – Wielun, WK – Wierzchowiska.
[ab]Within column "total breaks" means bearing the same superscripts differ significantly at P<0.05.

specific habitat of hares: domination of small farms, considerable variation in grain and root production, the presence of grassland and orchards. The WL range is also characterized by the greatest density of the hare population. In turn, the WK range, due to rich soils and high level of fertilization, is dominated by medium size farms, with intensive wheat and sugar beet production. Moreover, large industrial plants are located in the vicinity. The RG hunting range is covered by a mosaic of forests, but the principal biotope consists of arable land on light soils.

Other chromosome defects, of the gap or polyploidal types, observed during a microscope analysis, demonstrated a similar tendency (Tab. 2). On the other hand, some authors consider those chromosome and chromatid gaps not be counted among chromosome aberrations (or should even be omitted in analyses) as they are difficult to identify [Savage 1976, Preston *et al.* 1987]. However, in some publications the relations have been demonstrated between the frequency of chromosome or chromatid gaps and factors of a genotoxic character [Brogger 1982, Paz-y-Mino *et al*. 2002]. A similar situation exists in the case of the frequency of polyploidal cells, where certain chemical compounds were found to have a negative effect on the spindle and thus on the increase in the number of induced polyploidal cells [Sbrana *et al.* 1993]. In the case of the hares examined in the present study, tetraploidal cells (4N) were the principal polyploidal cells observed. Dicentric chromosome and rings were found in very few cases only.

Evaluating the whole group of 73 hares examined cytogentically one may state that the mean frequency of aberrations amounted to about 2% and was lower than that reported for other species. A similar value (2.25%) was observed only in red deer [Rubeš *et al*. 1992]. In pigs, cattle and horses cytogenetically examined under different environmental conditions, the frequency of CA was much higher and reached 4-6% [Rubeš 1987, Parada and Jaszczak 1993]. The differences in the number of CAs may have been affected by various factors. It is known that among other factors, genotoxic may be pesticides used in agriculture, as well as various types of industrial pollutions [Ishidate *et al.* 1988, Hadnagy *et al.* 1989, Bagchi *et al.* 1995, Bolognesi 2003].

Table 3 presents the mean SCEs frequency in *in vitro* cultured blood lymphocytes. The mean SCE frequency per cell ranged from 5.51±1.14 in the WL, through 6.05±12 in WK and RG, up to 6.40±1.10 in the RZ hunting range ($P<0.05$ and 0.01).

Similarly as in the case of CA the lowest SCE frequency was observed in the blood lymphocytes of hares from the WL, while the highest from the RZ range. In the latter the density of the hare population was the lowest and the environmental conditions the least favourable (Tab. 1). The intensive production is conducted on large areas and the principal crops are grains, industrial plants and vegetables. Intensive production means a considerable rate of the use of machines and chemical preparations.

In individual hare cells the number of SCEs ranged from 4 to 11 (Tab. 3). The mean SCE frequency (5.72 per cell) is similar to the values reported for other animal species [Rubeš 1987, Parada and Jaszczak 1993]. In numerous studies a relation was

Table 3. Frequencies of SCEs in the blood lymphocytes of hares from different hunting areas (hunting ranges)

Hunting range	Number of hares	Cells scored	SCE per cell		
			mean	SD	min/max
GR	10	300	5.74	1.28	4-8
RZ	10	300	6.40^{A}	1.10	4-11
RG	10	300	$6,05^{b}$	1.13	4-9
WL	10	300	5.51^{Abc}	1.14	4-8
WK	10	300	6.05^{c}	1.12	4-9
Total	50	1500	5.72	1.18	4-11

GR – Grodno, RZ – Rozniaty, RG – Rogow, WL – Wielun, WK – Wierzchowiska.
$^{aA...}$ Within columns means bearing the same superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

demonstrated between the SCE frequency and the concentration and time of exposition to mutagens [Byrnes and Bloom 1980, Yager 1987, Quero *et al.* 1997]. It does however happen that certain mutagens, strongly inducing chromosome aberrations, do not lead to an increase in the SCE frequency. For this reason, when monitoring factors of a genotoxic character, the SCE test is treated as supplementary to the CA index.

The results obtained point to a marked differentiation between the groups of hares examined. The lowest frequencies of CA and SCE were found in the WL hunting area with the highest density of hares.

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Wstępny monitoring cytogenetyczny populacji zajęcy w Polsce

S t r e s z c z e n i e

Częstość aberracji chromosomowych (*chromosomal aberrations* – CA) i wymian chromatyd siostrzanych (*sister-chromatid exchange* – SCE) badano w limfocytach krwi 73 zajęcy z pięciu obwodów łowieckich: Grodno (GR), Rozniaty (RZ), Rogów (RG), Wieluń (WL) i Wierzchowiska (WK), o różnej liczebności populacji. Najniższy udział komórek z CA, do których zaliczono złamania chromatydowe i chromosomowe oraz fragmenty acentryczne stwierdzono u zajęcy z obwodu WL (1,44%). Najwyższy udział komórek z CA stwierdzono u zajęcy z obwodu WK (2,67%) i RG (2,71%), które pod tym względem różniły się istotnie tylko od zajęcy z obwodu WL (1,44%). Średnia częstość SCE na komórkę w badanych grupach zajęcy wahała się od 5,51 (z obwodu WL) do 6,40 (z obwodu RZ). Istotne okazały się różnice między zającami z badanych obwodów w liczbie SCE. Uzyskane wyniki wskazują na znaczne zróżnicowanie między badanymi grupami zajęcy. Najniższa częstość CA i SCE wystąpiła u zajęcy z obwodu WL, charakteryzującego się najliczniejszą populacją tych zwierząt.