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Original study

Sister chromatid exchange analysis in cats (*Felis catus*)

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Abstract

Sister chromatid exchange (SCE) is one of the cytogenetic methods which diagnoses damage to chromosomes and allows evaluation of the mutagenic influence of a given factor on a cell's DNA. The purpose of the experiment was to determine the level of spontaneous and inductive SCE in the domestic cat. The research was carried out on 23 domestic cats *Felis catus*. Chromosome preparations were prepared from lymphocytes of peripheral blood after 72 h of in vitro breeding with the addition of bromodeoxyuridine (BrdU) in five different concentrations: 0.25, 0.5, 1.0, 2.5, 5.0 µg/ml. Chromosomes were stained by means of the fluorescence plus Giemsa (FPG) technique in order to carry out microscopic analysis. It was stated that the level of spontaneous SCE in the domestic cat occurs at a concentration 0.5 µg/ml on the basis of research previously carried out. Higher concentrations of this substance have a genotoxic action and damage DNA of chromosomes and induct additional SCEs in chromosomes of this species. Moreover, it was stated that the number of SCEs is higher in males than females. Our research also proved that the number of exchanges increases along with age in cats of both sexes.

Keywords: sister chromatid exchange, mitotic chromosomes, cat

Abbreviations: BrdU: bromodeoxyuridine, CA: Chromosome aberrations, DNA: deoxyribonucleic acid, DSB: double-strand break, FPG: fluorescence plus Giemsa, SCE: sister chromatid exchange, SSB: single-strand break, UV: ultraviolet

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Introduction

The domestic cat (*Felis catus*) is characterized by skills of quick adaptation to different environmental conditions, which is why they can be found on nearly every continent. It is thought that cats were domesticated for the first time about 6000 years ago in ancient Egypt (Driscoll *et al.* 2009). They probably come from the Nubian cat or maybe the domestic cats crossed to some extent with the jungle cat (*Felis chaus*), the European wildcat (*Felis silvestris*, group *silvestris*) and even with the manul (*Otocolobus manul*). Some researchers think that the domestic cat, European wildcat and Nubian cat are subspecies of one species *Felis*: *Felis s. catus, Felis s. silvestris* and *Felis s. libyca*). General morphological and genetic similarities among these three forms make it difficult to unambiguous determine which of the wild cats was the original form of the domestic cat. About 100 breeds of domestic cat including many colourful ones developed by way of mutations and selection (O'Brien *et al.* 2002).

The number of chromosomes of *Felis catus* is 2n=38. Karyotype consists of 16 pairs of meta- and submetacentric chromosomes classified in six morphological groups (A-F). Chromosome of the X is of the metacentric type and belongs to the proper type, whereas the Y chromosome is the smallest metacentric. The pattern of G stripes for *Felis catus* was first described and presented in 1971. Later, Q and R patterns of stripes were also determined (Shibasaki *et al.* 1987, Cho *et al.* 1997).

The cat genome was mapped in 2007. Its size is estimated at 2.7 billion base pairs (Pontius *et al.* 2007). The closeness of cat and human life leads to situations in which these organisms are exposed to the action of the same environmental pollutants (pesticides, herbicides, fungicides, and antibiotics, as well as ionizing radiation, gases and pollens). Moreover, the domestic cat is used as an animal model in research on the aetiology of chosen diseases affecting man, both contagious and hereditary, including cardiomyopathy, dominating polycystic kidney disease, leukaemia, sarcoma, severe acute respiratory syndrome and acquired immune deficiency syndrome (Murphy *et al.* 2000).

Correct functioning of a cell depends on the correct structure and organization of the chromosome DNA, which is very sensitive to environmental pollution (Ossowska *et al.* 2008). One of the factors which determines harmful influence of chemical factors of various origins on a genome is an increase in the number of sister chromatid exchanges (SCE). The core of the SCE test is to use the process of embedding of analogues of thymidine (BrdC, 5-IdU, OA) in its place in DNA during replication (Wolff 1977). The phenomenon of chromatid exchange itself consists of a mutual exchange of homological parts of chromatids in the same chromosome due to single-strand (SSB) or double-strand (DSB) breaks in the DNA strand (Wójcik & Smalec 2010). SCE can occur spontaneously during the cellular cycle and it can also be induced by various factors of mutagenic character. Examinations with use of SCE have so far only been conducted both in humans and chosen species of animals (Lambert *et al.* 1976, Leibenguth & Thiel 1986, Wójcik & Smalec 2010, Murali & Panneerselvam 2011). It was proved that SCE is characterized by substantial species conservatism. It concerns foremost locations on chromosomes and concentrations of bromodeoxyuridine (BrdU) in SCE tests (Di Berardino *et al.* 1995, Murali & Panneerselvam 2011, Wójcik *et al.* 2011, Wójcik & Smalec 2012a, Wojcik et al. 2012, Azimi Dezfouli 2012). Observation of the phenomenon of SCE spontaneity in particular species by means of SCE testing requires individual choice of BrdU concentrations in methodology, because BrdU is a strong inductor of DNA (Di Berardino *et al.* 1995).

The aim of the research was to determine the BrdU concentration in which SCE occurs spontaneously in the domestic cat and characteristics of exchange of sister chromatids in chromosomes of this species with particular consideration to number, place of occurrence and inter-subject variables.

Material and methods

Research was carried out on a group of 23 domestic cats *Felis catus* (13 male and 10 female). They were animals from cities (11) and the countryside (12), in two age groups of young individuals (12) and old ones (11). Young individuals were at or under two years of age, while the older ones were over two. Research material constituted full peripheral blood taken from *V. cephalica antebrachi*. A group of five individuals of the same sex (female), which come from the same environment (rural) were chosen in order to determine BrdU concentrations at which SCE occurs spontaneously (index of SCE test). Cytogenetic analysis was made on chromosomes obtained from in vitro breeding of lymphocytes made over 72 h at 38 °C. In the 36th hour of the breeding process BrdU in different concentrations was added: 0.25 µg/ml - Group I; 0.5 µg/ml - II; 1.0 µg/ml - III; 2.5 µg/ml - IV; 5.0 µg/ml - V according to binding procedure (Di Berardino *et al.* 1995). To the other cats only a set index of BrdU was applied.

A harlequin structure of chromosomes was generated on the basis of the fluorescence plus Giemsa (FPG) technique according to Kihlman & Kronborg (1975). Procedure of FPG staining consisted of two stages: preliminary digestion and staining with Giemsa. In the first one chromosomes were digested by the RNAse solution (10 µg/ml) for 1h; incubated in Hoechst solution 33258 (0.5 µg/ml) for 1h; and exposed to ultraviolet (UV) radiation for 1 h. The next incubation was carried out at 4 °C in darkness for 24 h. On the next day the procedure of exposure to UV radiation for 0.5h was repeated; preparations were incubated in 0.5×SSC (0.75M sodium chloride + 0.075 sodium citrate, pH=7.0) for 2 h, at 58 °C; and in the end preparations were stained with a 3 % solution of Giemsa (pH=6.8) for 45 min.

Analysis of preparations was conducted on a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan), and estimation of SCE was conducted using the NIS Elements F2.30 programme (Nikon Corporation, Tokyo, Japan) for computer analysis of pictures. 50 metaphases were analysed in every individual. SCEs were identified in all chromosomes in the karyotype. The total SCE was determined as the amount of three different forms of SCE (interstitial, terminal and centromeric). Counted SCEs on chromosomes of cats were characterized by means of arithmetic mean and standard deviation.

The effects of the environment and the sex and age of the cats on SCE distribution were studied using the analysis of variance (PROC GLM, SAS Institute Inc., Cary, NC, USA, 2004). The SCEs were analysed using the following linear model.

$$Y_{ijkl} = \mu + SEX_i + AGE_j + ENV_k + (SEX \times AGE)_{ij} + (SEX \times ENV)_{ik} + (AGE \times ENV)_{jk} + E_{ijkl} \quad (1)$$

where Y_{ijkl} is the SCE interstitial, SCE terminal, SCE centromeric or total SCE, μ is the overall mean, SEX_i is the effect of i-th sex of cats (male or female), AGE_j is the effect of j-th age of cats (≤ 2 years or > 2 years), ENV_k is the effect of k-th environment (town or village), $(SEX \times AGE)_{ij}$ is the effect of interaction between sex and age of cats, $(SEX \times ENV)_{ik}$ is the effect of interaction

between sex of cats and environment, $(AGE \times ENV)_{jk}$ is the effect of interaction between age of cats and environment, E_{ijkl} is the error term.

Results

250 complete and well-spread methaphase plates obtained from five female individuals were analysed in order to determine the BrdU concentration in which the SCE phenomenon occurs spontaneously. In the case of a concentration of 0.25 µg/ml neither SCEs nor harlequin structures were observed and sister chromatids were stained uniformly (Figure 1a). Therefore it was decided that this concentration is too low to carry out such analyses. In case of higher concentrations of BrdU: 0.5, 1.0, 2.5 and 5.0 µg/ml, chromatids were stained differentially with visible places where SCE was present (Figure 1b, 1c, 1d, 1e). It was also observed that among analysed plates 30 % was in the third cellular cycle, whereas the remaining registered SCEs (70 %) came from earlier cellular divisions (Figure 1f).

It was stated that there is increasing tendency towards growth of SCE depending on the applied dose of BrdU in cultures. This trait was observed in every analysed individual. The lowest number of SCE exchanges amounting to 3.38±1.86 (from 2.00±1.41 to 5.00±2.45) was noted in the breeding of lymphocytes with the addition of 0.5 µg/ml of BrdU. The higher the concentration used in cultures of lymphocytes, the higher the level of SCEs. Partial results are given in Table 1. Based on the obtained results it was stated that in a concentration of BrdU amounting to 0.5 µg/chromatids in *F. catus* is spontaneous. Such a dose of BrdU was used in further research as an index in SCE testing for analysis of chromosomes in all 23 examined individuals.

Table 1
Number of SCEs depending on the BrdU concentration (mean±SD)

Cat's name	BrdU concentration, µg/ml				
	Group I 0.25	Group II 0.5	Group III 1.0	Group IV 2.5	Group V 5.0
CAT 1	0	3.20±2.64	4.00±1.64	4.20±2.17	5.80±1.41
CAT 2	0	2.00±1.41	3.80±2.07	6.20±0.84	6.50±0.84
CAT 3	0	5.00±2.45	6.00±2.83	6.20±1.64	6.50±2.18
CAT 4	0	3.10±2.45	3.60±0.89	4.80±1.30	5.70±2.28
CAT 5	0	3.40±1.52	4.00±1.58	4.60±2.07	5.90±2.92
Total	0	3.38±1.86	4.50±2.16	5.43±1.71	6.15±1.29

Analysing chromosomes in the domestic cat's karyotype SCEs occurring in different locations of the chromosome were observed, including terminal, interstitial and centromeric ones. On the basis of microscopic observation and statistic analysis it was proved that the most frequently occurring form of SCE in experimental cats is terminal SCE (65.1 %) of observed exchanges in examined material) and centromeric SCE (26.9 %), whereas interstitial SCE (7.9 %) occurred rarely. Under microscopic analysis a higher rate of exchanges on arm q (54 %), than on p (46 %) of chromosomes was observed. The highest number of cracks of chromatids was observed on the border between euchromatin and heterochromatin (79 %).

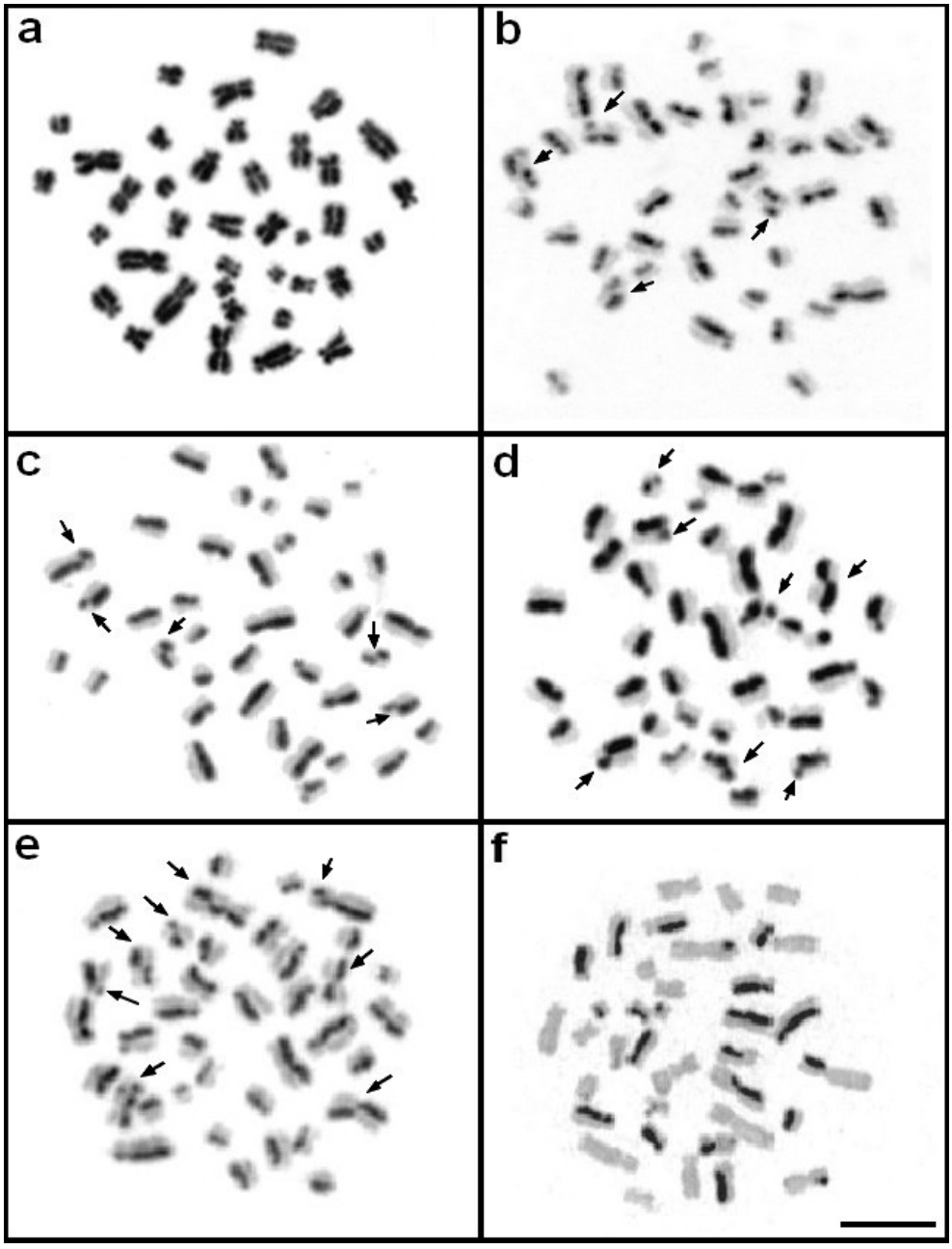

Figure 1
Influence of different concentrations of BrdU on the number of SCEs in domestic cats: a) concentration 0.25 µg/ml; b) 0.5 µg/ml; c) 1.0 µg/ml; d) 2.5 µg/ml; e) 5.0 µg/ml; f) metaphase plate in third cycle. Scale 10 µm

An example of chromosome B1 of karyotype of domestic cats with visible SCEs occurring in the terminal part of the chromosome between stripes of the 5th and 6th q3 regions (Figure 2b), in the interstitial area of the chromosome between regions 1q and 2q (Figure 2c) and the centromere (Figure 2d) is presented in the picture.

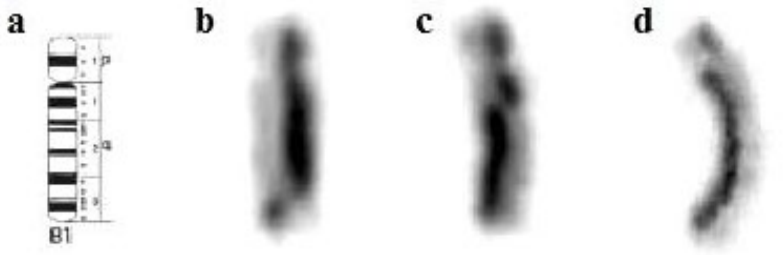

Figure 2
Places of occurrence of SCE in chromosomes of the domestic cat: a) ideogram of chromosome B1 (O'Brien *et al.* 2006); b) terminal SCE; c) interstitial SCE; d) centromeric SCE.

The average frequency of SCE in the examined population of cats amounted to 5.03±3.13. A higher SCE frequency was observed in the male cats than in the female ones (Table 2). A significant difference in the frequency of total SCEs ($P<0.05$) between sexes was found. However, no significant difference between sexes in the frequency of interstitial, terminal and centromeric SCEs was observed.

An increase of occurrence of SCE was observed with age in the examined population of domestic cats (Table 3). A higher frequency of SCE in the case of two forms, terminal and centromeric, was observed in older cats. Highly significant differences ($P<0.01$) between older and younger cats in the frequency of terminal, centromeric and total SCEs were found.

The average number of SCEs/Cell for cats coming from an urban area was 4.99, and from rural areas, 5.07. However, observed differences were not significant (Total SCE). In analysing this trait in detail regarding location of occurrence of SCE in the chromosome highly significant differences ($P<0.01$) were only observed in the case of interstitial SCE (Table 4).

A highly significant interaction ($P<0.01$) between sex and environment in the case of total SCEs was observed.

Table 2
Number of studied animals, examined cells and SCE distribution (mean±SD) in the cat chromosomes depending on sex

Sex	Animals	Examined cells	Interstitial SCE	Terminal SCE	Centromeric SCE	Total SCE
Female	10	99	1.64±0.79	3.54±2.05	1.87±1.10	4.20[a]±2.84
Male	13	134	1.56±0.83	3.64±2.29	2.42±1.55	5.64[a]±3.18
Total	23	233	1.60±0.81	3.60±2.21	2.19±1.41	5.03±3.13

[a]significant difference ($P<0.05$)

Table 3
Number of studied animals, examined cells and SCE distribution (mean±SD) in the cat chromosomes depending on age

Age, years	Animals	Examined cells	Interstitial SCE	Terminal SCE	Centromeric SCE	Total SCE
≤ 2	12	123	1.42±0.64	3.16^{A}±1.67	1.72^{B} ±1.04	3.89^{C}±2.10
> 2	11	110	1.65±0.85	4.11^{A}±2.61	2.61^{B}±1.55	6.29^{C}±3.56
Total	23	223	1.60±0.81	3.60 ±2.21	2.19±1.41	5.03±3.13

^{ABC}The mean values marked with different letters are statistically different (P<0.01).

Table 4
Number of studied animals, examined cells and SCE distribution (mean±SD) in cat chromosomes depending on the environment

Environment	Animals	Examined cells	Interstitial SCE	Terminal SCE	Centromeric SCE	Total SCE
City	11	116	1.06^{A}±0.24	3.80±2.22	2.06±1.11	4.99±2.97
Village	12	117	1.83^{A}±0.87	3.38±2.18	2.31±1.62	5.07±3.28
Total	23	233	1.60±0.81	3.60±2.21	2.19±1.41	5.03±3.13

^{A}highly significant difference (P<0.01)

Discussion

An SCE test had not yet been used on domestic cats, therefore it was necessary to work out appropriate working conditions of the test including foremost the choice of appropriate BrdU concentration. BrdU concentration as an index in the SCE test is different in respective species. Among the research on primates doses of BrdU oscillated from 10 to 500 µM BrdU/ml (Lambert *et al.* 1976). Leibenguth & Thiel (1986) carrying out research in domestic cattle used a concentration equal to 14-120 µg BrdU/ml breeding. Murali & Panneerselvam (2011) applying SCE test SCE in crossing of *Bos taurus* × *Bos indicus* stated that a concentration of 10 µg BrdU/ml is the best for analysis of spontaneous SCE and chose it from among five different BrdU concentrations (5, 10, 15 and 20 µg BrdU/ml). This value is considered by most scientists to be standard, although it is worth noticing that these are not the lowest levels of BrdU which allow observation of SCE (Peretti *et al.* 2007, Nicolae *et al.* 2009, Murali & Panneerselvam 2011, Wójcik *et al.* 2011, Wójcik & Smalec 2012a, b). Wilson & Thompson (2007) emphasize that spontaneous exchanges are these which occur only in very small concentrations of BrdU, which should be applied as an index in SCE testing. Among the research on the spontaneity of exchanges the lowest concentrations were used by Di Berardino *et al.* (1997). Using concentrations of 0.1, 0.25, 0.5, 1.0, 5.0 µg BrdU/ml, they proved that at a BrdU concentration equal to 0.1-0.25 µg/ml of breeding one can obtain a positive result of SCE testing in cattle. Five different BrdU concentrations including 0.25, 0.5, 1.0, 2.5, 5.0 µg/ml were used in the research on spontaneity of SCE in cats on the basis of the literature data. The lowest BrdU level in the species *Felis catus* which allows observation of SCE amounted to 0.5 µg/ml of breeding and thus it was determined as spontaneous. Too low concentrations make detection of SCE in chromosomes impossible. Di Berardino *et al.* (1995, 1997) claim that the amount of BrdU below 0.1 µg/ml of breeding in cattle and 0.25 µg BrdU/ml of breeding in goats does not induce SCE. Similarly, in cats this number amounted to 0.25 µg BrdU/ml of breeding.

Depending on the species the average noted number of spontaneous SCE/cell in respective species oscillated from 2.3 in the Chinese hamster (Kato 1974), to 2.48 in cattle (Di Berardino *et al.* 1995), 3.2 in the goat (Di Berardino *et al.* 1997), and 5.15 in the horse (Wójcik *et al.* 2011). The level of spontaneous reactions which we observed in the domestic cat was 3.38 SCE/cell.

The molecular mechanism of SCE is not entirely well known and differences in spontaneous and induced exchanges can be evidence that there is more than one way of their arising (Wójcik & Smalec 2010). It is claimed that places of exchanges in chromosomes are not totally accidental and there are more exchanges on the border between eu-and heterochromatin segments or in heterochromatin segments themselves (Hsu & Pathak 1976). A higher rate of SCE was found in parts of chromosomes with large heterochromatin segments in *Microtus agrestis* (Hübner & Pojda 1980, Rønne 1995), in comparison to other places. Also, centromeric regions of *Mus musculus* are susceptible to cracks of chromosomes and SCE induction due to a high rate of repetition of DNA (Lin & Alfi 1976). In examined *Felis catus* SCE mainly found on the border between euchromatin and heterochromatin in stripes, proximal part of chromosomes and in centromeres. A similar dependency was observed in goats (Wojcik & Smalec 2012a), and geese (Wójcik & Smalec 2012b). The mechanism of SCE creation is also bound with composition of alkalis in DNA. However, the latest research proves that places of occurrence of SCE are different and irregular in respective species. In humans (Homo sapiens) the smallest number of exchanges is observed in the centromere whereas these exchanges dominate in this region in the Chinese hamster, the rat (*Rattus norvegicus*), the musky-rat kangaroo (*Hypsiprymnodon moschatus*), and the mouse (*Mus musculus*) (Marin & Prescott 1964, Gibson & Prescott 1972, Lin & Alfi 1976). Lindahl (1993) and Di Meo *et al.* (2000), stated that SCE placement in chromosomes of different animal species is different and irregular because of differences in the location of heterochromatin in the chromosome suit. It was also proved that SCEs are proportional to the length of chromosomes and also to the DNA content (Vijh *et al.* 1991, Wójcik & Smalec 2012a, b). It concerns all examined species (including humans, cattle, goats, and geese). Rudd *et al.* (2007) proved that single strand parts of telomeres and subtelomeres in terminal parts of arms are more sensitive to occurrence of instability in the form of deletion (form of chromosome aberrations, CA) and SCE from other parts of every chromosome. Similarly in the examined cats SCE occurred mainly in telomeric areas of the chromosomes mainly in the q arm.

Sex, age and race are other factors which have influence on SCE frequency. Higher rates of SCE were observed in karyotypes of female species than in male ones of goats, cattle, sheep and pigs, although these differences were not statistically binding (Ianuzzi *et al.* 1991, Di Meo *et al.* 2000, Peretti *et al.* 2006, Wójcik & Smalec 2012a, b, Wójcik *et al.* 2013). In the case of the domestic cat the average number of SCEs in cells of male individuals was higher (5.64 SCE/cell) than in female ones (4.20 SCE/cell).

Sparse research concerning the influence of age on number of SCEs indicates that age has a substantial influence on frequency of occurrence of SCE and the differences were statistically significant. In older individuals SCE frequency is substantially higher than in younger ones (Hedner *et al.* 1982, Peretti *et al.* 2006, Husum *et al.* 2008, Wójcik *et al.* 2011). Also, in examined cats there was a difference in the number of SCEs between old and young individuals and it exceeded 2 (2.4 SCE/cell).

The SCE test used in the research is a helpful cytogenetic tool which allows detection of DNA damage resulting from contact with a mutagen. This test can be used not only for estimation of influence of genotoxic factors on chromosomes, but also can have more global applications, allowing for assessment of such factors as sex, age, and race, as well as for assessment of genetic immunity of animals.

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