Spontaneous micronuclei in embryos of the Black-headed Gull (*Larus ridibundus* L.) populations

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Frequencies of spontaneous micronuclei have been established in the embryos of Black-headed Gull originating from two colonies in central and north-eastern Lithuania. In total, 141 blood samples collected from the same number of embryos were examined. At the blood sampling time the eggs were naturally incubated for 9–17 days. The mean cumulative frequency of micronucleated erythrocytes (MNE) of the colonies studied was 0.37‰ ± 0.44. The frequency of MNE correlated negatively with incubation lenght. In the both colonies the lower frequencies of MNE were observed in the peripheral blood of the first egg embryos in clutch comparing to the embryos of the second and third egg in clutch.

Key words: Black-headed Gull, embryo, micronucleus test

INTRODUCTION

Most chemical compounds of anthropogenic origin are influencing not only humans, but also all organisms. They induce mutations, neoplasm and degenerative statuses, diseases, disorganise the vital functions of individuals, decrease fertility (Lazutka, 1998). One of the outcomes of technogenic pollution is a genotoxicological impact on animal populations. The consequences of the effects of this very widely distributed threat might become especially pronounced in near future (Naruševičius, 2002). Therefore the significance of research on cytogenetical damages in natural populations is increasing. The results obtained can provide information on pollution levels of mutagenic substances in certain territories and indicate changes in pollution.

Pollution monitoring and the testing of environmental pollutants is limited by the sensitivity of the method. Avian embryo is a proper and important model in this type of research. The avian embryo in early stages of embryonal development provides a wide range of metabolic activities enabling activation of promutagens. Laying female birds excrete lipophilic persistent compounds into eggs via the yolk. Avian species are top predators in both aquatic and terrestrial environments and may therefore be highly exposed to environmental pollutants (Berg et al., 1999). Due to the above-mentioned reasons identification of impact of various pollutants on birds is less complicated.

The aim of this work is to establish the spontaneous frequency of micronucleated erythrocytes in the blood of embryos of selected populations of the Black-headed Gull (*Larus ridibundus*) and to test the relevance of the method of naturally incubated eggs for the monitoring system of cytogenetic damages in the populations of Black-headed Gull.

MATERIALS AND METHODS

Two colonies of the Black-headed Gull have been selected for the study. A short characteristic of the colonies is provided below:

– An island in the Krivėnai water reservoir, Kaunas district. Size of the colony ~400 breeding pairs (1999–2000). Geographical coordinates of the island: 55° 05' N 23° 48' E. Distance to the edge of the Kaunas–Klaipėda highway – 140 m. Distance to the Kaunas city (400,000 inhabitants) – 22.1 km. Intensively used agricultural land is dominating in the surroundings.

– An island in Lake Kretuonas, Švenčionys district. Size of the colony – ~200 breeding pairs (1999); ~600 breeding pairs (2001). Geographical coordinates of the island: 55° 15' N 26° 05' E. Distance to the nearest railway of regional importance – 1.46 km. Distance to a road of district importance – 5.25 km. Distance to the nearest town Švenčionėliai (7600 inhabitants) – 8.6 km. Forests and lakes are dominating in the surrounding land-

scape. Small scale extensive agriculture covers insignificant areas. Agricultural pollution has been decreasing in the region over the last decade.

The laying dates of the first egg in the observed nests were registered at the beginning of the breeding season. The eggs in these nests were marked with indelible ink according to their laying sequence. The clutches were collected in April–May in 1999–2001. They were collected on day 9–17 of incubation. All eggs were measured (length and width) with a calliper to the nearest 0.1 mm and weighed with a Pesola spring balance to the nearest 1 g before blood sampling.

The shell and the inner shell membrane of the egg was removed at the blunt end before blood sampling. Blood samples were collected with a standard 5 ml single-use syringe from the highest volume vessel of the peripheral circulatory system of the chorioallantoic membrane. One blood sample per embryo was collected. The obtained blood was spread out on slides immediately after sampling. Blood smears were air-dried and fixed for 10–15 minutes in 99.5% methanol (Merck, Darmstadt, Germany).

The fixed blood smears were stained for 10 min in standard 4% Giemsa solution (Merck, Darmstadt, Germany). After staining the slides were flushed out and intensively rinsed in demineralised water and air-dried. Examination of the stained slides was carried out using a Jenaval (Germany) microscope under bright field illumination (1250× with oil immersion). Ten thousand erythrocytes (polychromatic and normochromatic) were examined in each blood sample. Damaged erythrocytes were not examined. The frequency of micronucleated erythrocytes (erythrocytes with micronucelus (-ei)/1000 erythrocytes) were calculated on finishing sample examination.

The MNE frequencies established in blood samples of dead embryos were not used in the statistical analysis. Blood samples having ~70% of squashed or damaged cells were not examined.

According to Wolf, Luepke (1997), we used the following criteria for classifying the structure as a micronucleus:

– three-dimensionality of an object and its similarity to cell nucleus;

– similar staining reaction and texture;

– the size of the object does not exceed 2/3 of the size of cell nucleus;

– distinct border and round or oval shape.

RESULTS

In total, 79 blood samples from the colony in the Krivėnai water reservoir and 62 blood samples from the colony in Lake Kretuonas were examined. From 1 to 3 micronuclei were found in the aberrant erythrocytes of blood samples collected from the embryos of the both colonies studied.

The highest spontaneous MNE frequency was established in the blood of embryos from the Lake Kretuonas colony in 2001 and the lowest spontaneous MNE frequency was found in embryos from the Krivėnai water reservoir colony in 2000 (Fig. 1).

Spontaneous MNE frequency significantly differed in embryos of the Kretuonas colony in 1999 and 2001 (ANOVA, $F(1.60) = 13.64$, $p < 0.0005$). Spontaneous MNE frequency in embryos of the Krivėnai colony in 1999 and 2000 (ANOVA, $F(1.77) = 16.46$, $p < 0.0001$) was also significantly different. Statistically significant difference in spontaneous MNE frequency was determined among the embryos of the Kretuonas colony in 2001 and Krivėnai colony in 1999 (ANOVA, $F(1.100) = 5.13$, $p < 0.026$). Spontaneous MNE frequency was significantly higher in the Kretuonas colony in 2001 than in the Krivėnai colony in 2000 (ANOVA, $F(1.69) = 38.82$, $p < 0.00001$).

There was no statistical difference in spontaneous MNE frequency in embryos from the Kretuonas and Krivėnai colonies in 1999 (ANOVA, $F(1.68) = 3.49$, $p < 0.066$). The difference was insignificant between embryos of the Kretuonas colony in 1999 and the Krivėnai colony in 2000 (ANOVA, $F(1.37) = 3.19$, $p < 0.08$).

The influence of incubation length on spontaneous MNE frequency in the blood of Black-headed Gull embryos was also analysed. The mean incubation length of the collected eggs before blood sampling is shown in Table. The established spontaneous MNE frequency correlated negatively with incubation length in the embryos of the both studied colonies.

A significant negative correlation was found between cumulative spontaneous MNE frequency in embryos from the Krivėnai colony in 1999 and 2000 ($n = 40$) and the incubation length of the eggs (Fig. 2).

A similar relationship was established in embryos of the Kretuonas colony. Cumulative spontaneous MNE frequency of 1999 and 2001 in the Kre-

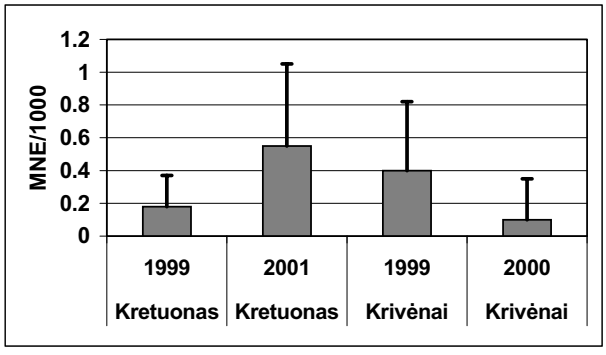

Fig. 1. Spontaneous MNE frequency in the blood of Black-headed Gull (*Larus ridibundus*) embryos in Kretuonas and Krivėnai colonies

Table. **Length of egg incubation before blood sampling in Kretuonas and Krivėnai colonies**

Colony name	Year	Number of examined blood samples	Mean incubation length, days (± s. d.)
Kretuonas	1999	15	13.8 ± 1.74
Kretuonas	2001	47	10.34 ± 0.6
Krivėnai	1999	55	12.46 ± 1.12
Krivėnai	2000	24	16.04 ± 1.12

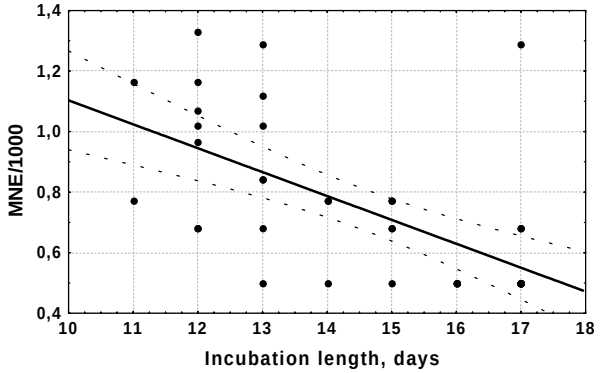

Fig. 2. Correlation between spontaneous MNE frequency in Black-headed Gull embryos and egg incubation length (r = –0.62, p < 0.05 (n = 40)) (Krivėnai colony in 1999 and 2000)

tuonas colony negatively correlated with the egg incubation length (Fig. 3).

Spontaneous MNE frequency was lowest in the embryos of the first egg in clutch in the Krivėnai colony in 1999 and 2000 (Fig. 4). These values did not differ markedly in the embryos of the second and third egg in clutch. Comparison of spontaneous MNE frequency values of embryos of the first, second and third egg in clutch revealed a significant difference between the embryos of the first and third egg in clutch in 2000 (ANOVA, F (1.14) = 6.11, p < 0.03). Other differences were not significant.

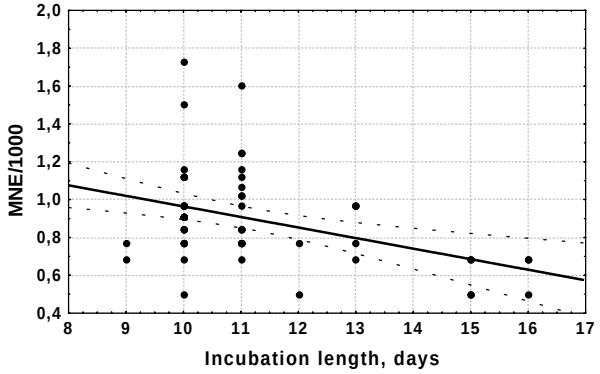

Fig. 3. Correlation between spontaneous MNE frequency in Black-headed Gull embryos and egg incubation length (r = –0.41, p < 0.05 (n = 62)) (Kretuonas colony in 1999 and 2001)

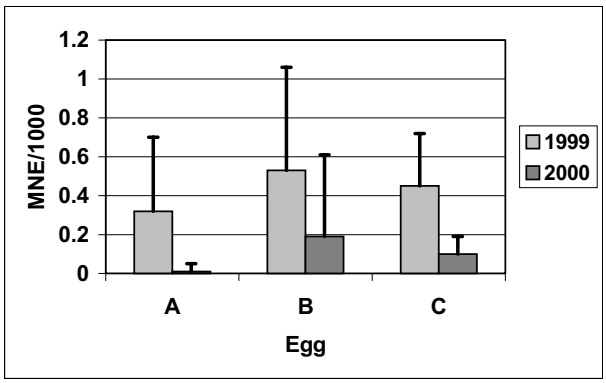

Fig. 4. Spontaneous MNE frequency in Black-headed Gull embryos in A, B and C eggs in Krivėnai colony (1999 (n = 55) and 2000 (n = 24))

Spontaneous MNE frequency was not correlated with egg laying sequence in the Krivėnai colony (1999 – r = 0.22; 2000 – r = 0.02).

Similar tendencies were found in blood samples from the Kretuonas colony. Both in 1999 and 2001 the lowest spontaneous MNE frequencies were found in the embryos of the first egg in clutch (Fig. 5). Spontaneous MNE frequency was similar in embryos of the second and third egg in clutch. A statistically significant difference was established between spontaneous MNE values in the embryos of the first and second egg in clutch in 2001 (ANOVA, F (1.31) = 6.0; p < 0.02).

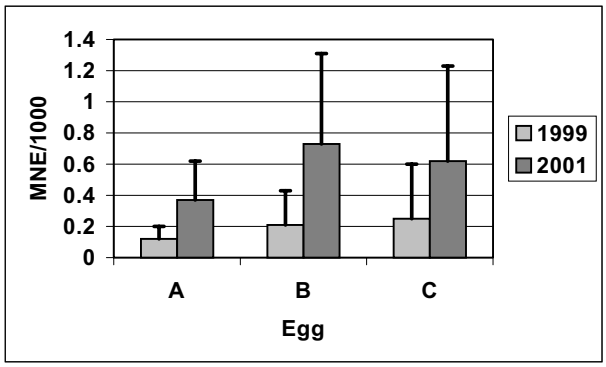

Fig. 5. Spontaneous MNE frequency in Black-headed Gull embryos in A, B and C eggs in Kretuonas colony (1999 (n = 15) and 2001 (n = 47))

Spontaneous MNE frequency in the Kretuonas colony did not correlate with the egg laying sequence as in the Krivėnai colony (1999 – r = 0.14; 2001 – r = 0.24).

DISCUSSION

Spontaneous MNE frequency correlated significantly negatively with incubation length in Black-headed Gull embryos from the Kretuonas and Krivėnai colonies. It could be explained by the start of spleen

function in later stages of embryonic development. The spleen eliminates aberrant erythrocytes from the blood in most mammals. Wolf, Luepke (1997) also stated that on day 11 of incubation micronucleated erythrocytes are accumulating in the circulating blood of hen (*Gallus domesticus*) embryos as a completely developed spleen is absent at that stage of incubation.

In both colonies, spontaneous MNE values were the lowest in the embryos of the first egg in clutch. This trend remained for a period of two years. Partial explanations could be obtained from the results of the studies on effects of egg size asymmetry on fledging success in gulls. To all larids including the Black-headed Gull with 3-egg clutches are characteristic the presence of asynchronous hatching and size reduction of the third (C) egg relative to the first 2 (A and B) eggs (Reid, 1987). Survival is generally lowest for youngest siblings in broods of terns and gulls (Bollinger, 1994). The third chicks suffer both from hatching from small eggs and from hatching last (Kilpi, 1995). Some authors argue that the smaller third egg of gulls is an adaptation to reduce the number of fledglings, others state that its size depends on feeding conditions during egg laying and is not adaptive (Reid, 1987). The experimental data support the second hypothesis (Reid, 1987). Though in the present study the lowest MNE frequency in the both colonies was characteristic of the embryos of the first egg in clutch, there were no marked differences in these values in the second and the third embryos. Perhaps spontaneous MNE frequency cannot be used as a direct index of juvenile survival probability.

Nevertheless, genetical damage and the number of micronucleated cells is determined by the ability of an organism to metabolise persistent, lipophilic compounds which are excreted to the bird eggs together with the yolk lipids. Therefore the survival probability of juveniles could be significantly influenced by the concentration of genotoxic substances in female's organism and the ability of the embryo to metabolise pollutants.

Cumulative spontaneous MNE frequency in Black-headed Gull embryos from the both colonies (0.37‰ ± 0.44) is significantly higher than the same values in hen embryos (*Gallus domesticus*) (0.25‰ ± 0.24) (Wolf, Luepke, 1997). A high variation of spontaneous MNE values was characteristic of Black-headed Gull embryos both from the Krivėnai and Kretuonas colonies, possibly because of a greater genetic diversity in wild birds than in domestic breeds (Naf et al., 1992).

The spontaneous MNE frequencies established in Black-headed Gull embryos in the Krivėnai and Kretuonas colonies were similar to the MNE values found in adult birds of the following species: *Tyto alba* (0.35‰, n = 3), *Meleagris gallipavo* (0.22‰, n = 3), *Scarstacella inca* (0.22‰, n = 6) (Zuniga-Gonzales et al., 2000).

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SPONTANINIAI MIKROBRANDUOLIAI RUDAGALVIO KIRO (*Larus ridibundus* L.) POPULIACIJŲ EMBRIONUOSE

Santrauka

Spontaninio eritrocitų su mikrobranduoliais (EMB) dažnio tyrimai atlikti dviejose rudagalvio kiro populiacijose, kurių veisimosi aplinka skyrėsi antropogenine apkrova. Iš viso ištirtas 141 embriono kraujo ėminys. Kraujo ėminiai surinkti iš 9–17 dienų natūraliai inkubuotų embrionų. Bendras abiejų kolonijų spontaninis EMB dažnis – 0,37‰ ± 0,44. Nustatyta statistiškai patikima neigiama EMB dažnio koreliacija su inkubacijos trukme. Tirtų dėčių pirmo kiaušinio embrionų kraujyje nustatyti mažesni EMB dažniai negu antro ir trečio kiaušinio.

Raktažodžiai: rudagalvis kiras, embrionas, mikrobranduolių testas