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GENOME INSTABILITY OF *Catharacta maccormicki*

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Summary.

The comparative analysis of cytogenetic parameters of genome instability of population South Polar Skua *Catharacta maccormicki* was carried out. The blood samples of C. maccormicki (29 birds) were collected in austral summer 2001-2002 at Galindez Island (Antarctic Station Akademik Vernadsky). The rates of micronuclei (MN) and other nuclear anomalies (NA) in mature erythrocytes of peripheral blood were estimated. Blood smears were fixed in 96% ethanol, dried and stored about 30-40 days. The slides were stained with 2% Giemsa stain. The mature erythrocytes (10.000 cells for each bird) were scored with light microscope under magnification 100^{x}x20^{x}.

As parameters of genome instability of population we have choosed the level of MN and the most frequently registered nuclear anomalies (NA): ”budding nucleus” (**bn**), ”two-lobe nucleus” (**tln**), ”tailed nucleus” (**tn**) and ”nucleus with cavity” (**nc**). The average rates of these parameters of *C.maccormicki*:

MN = 0,014±0,007‰, **nc** = 0,297±0,032‰, **bn** = 0,048±0,013‰, **tln** = 0,048±0,013‰, **tn** = 0,017±0,008‰.

The average rate of MN and other five NA of C. Maccormicki was 0,424±0,038‰.

Introduction

The recent global climate changes, irrational exploitation of living resources and the periodical environment pollution influence on Antarctic seabird populations (range and distribution of species, the life cycles, reproduction, composition and interactions of communities, trophic connection, genetic structure, level of genome instability etc). It means that genetic features of the Antarctic organisms and their populations ought to become the main task of contemporary biology studies in Antarctica.

Skua *Catharacta maccormicki* (order Charadriiformes) is one of 3-4 skua species (taxonomy isn't defined clearly) which live in Antarctica. Skua is significant part of Antarctic ecosystem. It's (in the main) a predator which have adapted to extreme Antarctic conditions.

Genome instability, one of basic properties of genome, is caused by interaction of two-factors: genetic information of genome and the environmental factors influences. Thus, study of genome instability allows clarifying genetic features of reaction of species on environmental influences.

The conditions of field work in Antarctica (weather conditions, absence of the equipment, limits of time, *etc*) restrict the possible approaches for study genome instability by several methods. Among the most adequate methods for genome instability estimation are the micronuclei test and nuclear anomalies test. These methods enable revealing visible manifestations of genome instability.

Micronuclei test consists in calculation of micronuclei frequency in interphase cells of tissues with certain level of mitotic activity. Our previous data showed that micronuclei in Antarctic birds appeare quite rarely. For more comprehensive information we used also other nuclear anomalies (with higher frequency) as additional parameters of genome instability.

Both of above-mentioned tests are relatively cheap and fast. Blood is the convenient material for such tests; birds' erythrocytes are nucleated and allow using the both tests for the analysis.

The aim of our work was to estimate the level of spontaneous genome instability *C.maccormicki.*

Materials and methods.

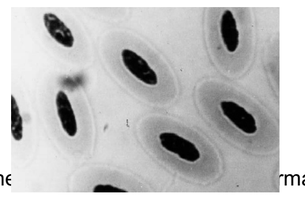
The blood samples of South Polar Skua Catharacta maccormicki were collected during austral summer 2001-2002 at Galindez Isl during the 7-th Ukrainian Antarctic expedition. Blood was obtained by incision of sceen of 5-th rudimentary finger of paw.

Slides preparation. Blood drop was spread onto the slide surface and dried. Then smears were fixed in 96% ethanol, air-dried and deliver to the laboratory (30-40 days of storage). Then they were stained with 2% Giemsa stain and scored.

Cytogenetic and statistical processing. The smears were scored under light microscope for micronuclei and nuclear anomalies under magnification 100^{x}x 20^{x}. We took into account only mature erythrocytes that were good painted, without overlapping and if cytoplasm and borders of a cell and a nucleus were non-destroyed. 10.000 cells for each bird were scored for the MN and NA frequency (per mille, ‰).

Results.

The normal mature erythrocytes of skua contain one nucleus located in the cell centre and occupied approximately j of total value. The nucleus is fusiform, not segmented, has non-uniformity of painting hetero- and euchromatine zones.



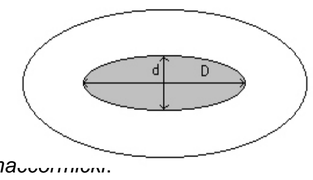
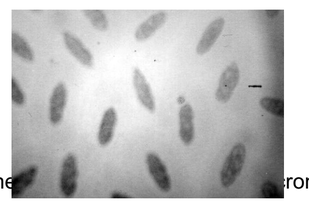


Figure 1.The rmal nucleus of *C.maccormicki*.

Micronuclei is an additional chromatin structure of almoust round shape, less than the basic nucleus in the size, located separately from the nucleus, with color and chromatine structure similar to the main nucleus, changing of preparate distance from microscope objective with microscrew results in the micronuclear clearness changing like the nuclear one [Tolbert P., C.Shy , J. Allen, 1992].



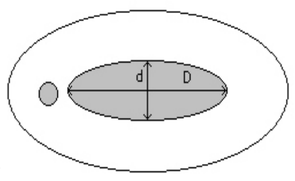
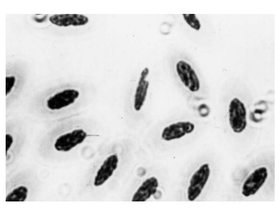


Fig ure 2. The ronuclei of *C.macc*

Besides of micronuclei, other anomalies of nucleus morphology were also fixed. More frequently registered NA were studied and classified as ”budding nucleus” (**bn**), ” two-lobe nucleus” (**tln**), ”tailed nucleus” (**tn**) and ”nucleus with cavity” (**nc**):

1.”Budding nucleus ” (**bn**) is a nucleus with constriction dividing it on two parts, one of which no more than 1/3 and not less 1/4 total amounts of a nucleus; the width of a nucleus in constriction area is equaled approximately 1/3 d.



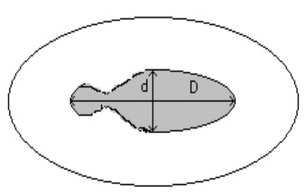
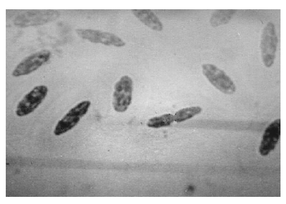


Figure 2. The mature erythrocytes with budding nucleus of *C.maccormicki*.

2. ”Two-lobe nucleus ” (**tln**) is a nucleus with constriction dividing it into two approximately equal parts; the width of a nucleus in constriction area is approximately 1/3 - 1/2 d.



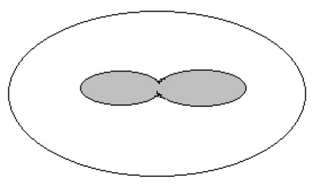
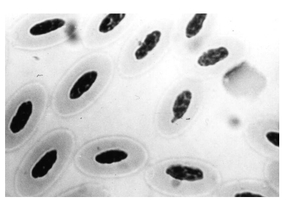


Figure 3. The mature erythrocytes with two-lobe nucleus of *C.maccormicki*.

3. ”Tailed nucleus” (**tn**) - one nucleus side is sharply narrowed and extend. The length of "tail" is within the limits of 1/4 - 1/3 D.



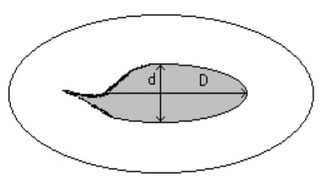
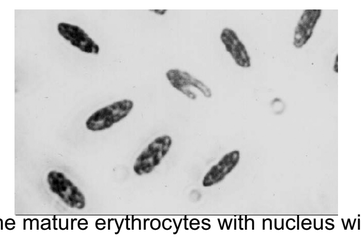


Figure 4. The mature erythrocytes with tailed nucleus of *C.maccormicki*.

4. ”Nucleus with cavity” (**nc**) is a nucleus with clear deep cavity, approximately up 1/2 *D* with non-adjoined edges.



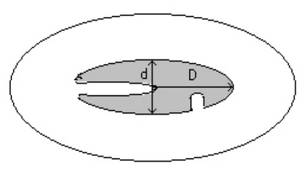


Figure 5.The mature erythrocytes with nucleus with cavity of *C.maccormicki*.

The results of the tests are shown in the Table 1 and at the diagram (Figure 6).

Table 1. The rate of micronuclei and nuclear anomalies in mature erythrocytes of *C.maccormicki*, ‰ *

Micronuclei **MN**		Budding nucleus **bn**		two-lobe nucleus **tln**		nucleus with cavity **nc**		tailed nucleus **tn**		Xtotal ± SE
lim	X±SE	lim	X±SE	lim	X±SE	lim	X±SE	lim	X±SE	
0-0,2	0,014±0,007	0-0,3	0,048±0,013	0-0,2	0,048±0,013	0-1,5	0,297±0,032	0-0,1	0,017±0,008	0,424±0,038

**lim* - accordingly the minimal and maximal figures;
X - average rate;
*X*total - average rate of the sum of MN and four NA;
SE - ctandart error.

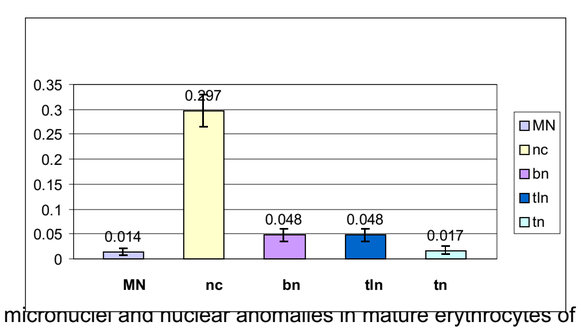


Figure 6. The rate of micronuclei and nuclear anomalies in mature erythrocytes of *C.maccormicki*, ‰

Table 1 and diagram 1 show that the most frequent indicator of genome instability is **nc** (Xtotal=0,017±0,008‰). The less frequent parameters are **bn** (Xtotal=0,048±0,013‰) and **tln** (Xtotal=0,048±0,013‰). **tn** (Xtotal=0,017±0,008‰); the MN frequency (Xtotal=0,014±0,007‰) is the lowest.

Table 2. Number of cells with MN and NA per 10 000 erythrocytes of individual birds.

N of smears	MN	**bn**	**tln**	**tn**	**nc**	Total
162					0	0
161					1	1
160		1			3	4
158			1		0	1
155					1	1
154	1	2			9	12
152					1	1
151		1			0	1
149			1	1	1	3
147				1	3	4
104					1	1
95			2		0	2
94				1	1	2
79			2	1	4	7
78					5	5
75					0	0
74			2		5	7
73					6	6
72		2			1	3
67	1	1			9	11
63			1		1	2
61	2		1		5	8
55			1		2	3
53		2			5	7
18		3		1	15	19
17					0	0
15	1	2	1		5	9
9			1		2	3
5			1		0	1
	4	14	14	5	86	123
Xtotal	0,014±0,007	0,048±0,013	0,048±0,013	0,017±0,008	0,297±0,032	0,424±0,038

The frequencies of MN and **tln** in smears of 29 individuals of *C.maccormicki* are ranged from 0 to 0,20, **bn** from 0 to 0,30, **tn** - from 0 to 0,10. The **nc** show the greatest variation of frequency between individuals from 0 up to 1,50‰. The MN occur in 10,4% of birds, **tn** - in 17,2%, **bn** - in 27,6%, **tln** - in 37,9%, and **nc** in 79,3% of inspected birds.

The rate of micronuclei and nuclear anomalies considerably varies among individuals in the population. Due to its genetic originality, each organism has its own level of genome instability. Some individual birds have high rate of studied parameters while other birds have very stable genome. For example, the bird N 18 have the greatest rate of NA at (1,9±0,43 ‰), but don't have MN, and the birds NN 162, 75, 17 have no NA per 10000 cells.

Discussion.

Genome has high stability and accuracy of the mechanisms that provide its functioning. However the level of stability is not absolute, in this case evolutionary changes will be impossible. Thus, the genome is organized so that it has certain level of stability and instability. A coarse estimate of the level of *Catharacta maccormicki* genome instability may be drawn up from results of micronuclear test in mature erythrocytes. Micronuclei appear during mitosis and are derivatives of the lost chromosomes or their fragments.

The spontaneous rate of MN in *C. maccormicki* varied from 0,02‰ to 0,20‰; average rate is 0,014±0,007‰, approximately 1,4 micronuclei per 100 000 cells (or ~1 MN per 71426 cells). But only 3 individuals (10,35 %) have one or more cells with micronuclei per 10 000 analyzed cells. According to literature, for some species micronuclei weren't find (*Cohounba flavostris*, *Cassiculus melanicterus*, *Forpus cyanopygius*, *Polyborus plancus*), the low rate of MN (0,1 ‰) was find in ostrich, while owl *Otus sp.* have rather high rate - 15,8 ‰. In most cases the frequency of MN is in limits 0,4 - 4,3 ‰ [G.Zuniga-Gonzalez at all, 2001]. In comparison with humans and other mammals the overwhelming majority of birds including Antarctic have lower rate of micronuclei.

Micronuclei are only one of many manifestations of genome instability. There are also other traits (nuclear morphology anomalies) that may be used as additional indicators of genome instability. In our work we took into account four nuclear anomalies that were the most frequent on the slides. Average rate of "nucleus with cavity" (**nc**) (0,297±0,032 ‰) was significantly higher than frequency MN (0,014±0,007‰). Traits “budding nucleus” (**bn**) and “two-lobe nucleus” (**tln**) were met with average frequency 0,048±0,013‰ which also exceeds MN rate. And only frequency of the cells with "tailed nucleus" (**tn**) was approximately equal to frequency of cells with MN.

Thus, *Catharacta maccormicki* have low spontaneous level of micronuclei and nuclear anomalies, that indicate high level of genome stability (or a low level of instability) individuals of this species.

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References

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2.Zuniga-Gonzales G., O. Torres-Bugarin, A. Zamora-Perez at all. (2001), Differences in the number of micronucleated erythrocytes among young and adult animals including humans spontaneous micronuclei in 43 species, Mutation research, 494, 161-167.