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Health Status in an Antarctic Top Predator: Micronuclei Frequency and White Blood Cell Differentials in the South Polar Skua *(Catharacta maccormicki)*

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Abstract: The estimation of the level of genome instability and immune status of *Catharacta maccormicki* (Aves, Charadriiformes) was performed. The studied colony of skua situated on Galindez Island (65°15' S, 64°15' W; Argentine Archipelago, Antarctica); the field work was carried out in February, 2002. The level of genome instability was evaluated by frequency of micronuclei (MN) and nuclear anomalies (NA) in mature erythrocytes. The measured parameters of immune status were leukocyte number (in relation to erythrocytes number), white blood cell (WBC) differential, and the ratio of heterophils to lymphocytes (H:L, a reliable index of avian chronic stress). The average frequencies of MN and total NA were 0.07 ±0.02 and 0.71 ±0.08 per 10,000 mature erythrocytes, respectively. Mean total leukocyte number was 41.8 ±1.9 per 10,000 erythrocytes. Mean WBC differential was 51.9 ±1.3 % heterophils, 0.14 ±0.04 % eosinophils, 2.3 ±0.2 % basophils, 0.71 ±0.1 % monocytes and 45.0 ±1.2 % lymphocytes. The average H:L ratio was 1.52 ±0.09. The presented results may be useful for further studies of Antarctic birds' health status.

Zusammenfassung: Gegenstand der Arbeit ist die Bestimmung des Grades der Genom-Instabilität und des Immunstatus von *Catharacta maccormicki* (Aves, Charadriiformes). Die im Februar 2002 untersuchte Skuakolonie befindet sich auf Galindez Island (65°15' S, 64°15' W, Antarktis). Der Grad der Genom-Instabilität wurde anhand der Häufigkeit von Micronuclei (MN) und von Kernanomalien in reifen Erythrozyten bestimmt. Die gemessenen Parameter für den Immunstatus waren die Leukozyten-Zahl (im Verhältnis zur Erythrozyten-Zahl), das Differentialblutbild der weißen Blutkörperchen und das Verhältnis der heterophilen Blutzellen zu den Lymphozyten (H:L; ein verlässlicher Index für chronischen Stress bei Vögeln). Die durchschnittliche Häufigkeit von MN und der Kernanomalien betrug 0,07 ±0,02 bzw. 0,71 ±0,08 per 10.000 reifer Erythrozyten. Die mittlere Gesamt-Leukozyten-Zahl war 41,8 ±1,9 pro 10.000 Erythrozyten. Das mittlere Differentialblutbild betrug 51,9 ±1,3 % Heterophile, 0,14 ±0,04 % Eosinophile, 2,3 ±0,2 % Basophile, 0,71 ±0,1 % Monozyten und 45,0 ±1,2 % Lymphozyten. Das durchschnittliche H:L-Verhältnis war 1,52 ±0,09. Die dargestellten Ergebnisse können gegebenenfalls genutzt werden, um weitere Untersuchungen zum Gesundheitsstatus antarktischer Vögel durchzuführen.

INTRODUCTION

Health status of Antarctic birds is one of many directions of the International Polar Year (IPY) initiatives (Loonen 2006). Important information about birds' health may be obtained from blood smears made in field studies. Among them are the level of genome instability and blood cell counts which reflects the level of stress and immune status. Blood is the convenient material for the above-mentioned health manifestations; birds' erythrocytes are nucleated and allow using blood smears analysis for these health important traits.

Genome stability, one of basic genome properties, is influenced by two main factors: individual genetic information and environmental factors. Thus, genome stability can be used as index for an individual's reaction on environmental impact and stress. The genome is organized so that it has a certain level of stability. Among the adequate methods for the estimation of genome instability are the micronuclei test and the nuclear anomalies test (Tolbert et al. 1992, Zuniga-Gonzales et al. 2001), which may be useful in field research of birds (Kursa et al. 2005). These methods enable scoring of visible manifestations of genome instability.

Another index of an organism's response to the environmental impact that can be checked with blood smears is leukogramma. Differential white blood cell (WBC) count is a good method for the evaluation of a bird's general health (Bolotnikov & Soloviov 1980, Bolotnikov et al. 1983, Campbell 1995). Pathological leukocytosis and leucopoenia usually develops as a consequence of an increase or decrease of different kinds of leukocytes. WBC differential is a relative proportion of five types of leukocytes based on a cell count in blood smears. Each cell type plays a different role in the body's first line of defence. Changes in the WBC differential can reflect responses of an organism to stressful agents. The WBC count is thus useful in the assessment of the course of an avian disease (Bolotnikov & Soloviov 1980, Bolotnikov et al. 1983, Campbell 1995). The ratio of heterophils to lymphocytes (H:L ratio) has been shown to be a reliable physiological index of avian chronic (long-term) stress (Gross & Siegel 1983, Maxwell 1993). The H:L ratio increasing can be caused by various stressors, such as infectious disease, parasite infection, food deprivation, temperature extremes, light conditions, social disruption, etc. (Gross 1992, Altan et al. 2000, Vleck et al. 2000, Edler et al. 2004).

The traits studied in our work can not be treated as mutually independent and one may expect a certain correlation between parameters of genome instability and WBC count data. A high level of stress and a weak immune status may increase the level of genome instability (Tolbert 1992, Zuniga-Gonzalez et al 2001, Fenech & Crott 2002). At the same time, a high level of genome instability may affect the immune system (Ilinskih et al. 1986, Howell 1992, Charames 2003). Thus, blood smear analysis produces important information on the general health of birds.

Here we present first data on cytogenetic manifestations of genome instability (NA, MN) and measures of the immune status (H:L ratio, WBC count) for the South Polar Skua *(Catharacta maccormicki)*.

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MATERIALS AND METHODS

We studied breeding South Polar Skuas *Catharacta maccormicki* (Aves, Charadriiformes) located on Galindez Island (65°15' S, 64°15' W) and two other nearby islands – Winter Is. and Skua Is. (Argentine Archipelago, Antarctica). There are no other skua species in this area (Naveen 2003, Ritz et al. 2006). The field-work was carried out in February, 2002. 162 adult birds of unknown sex were captured at the place of their feeding using a clap-net.

A drop of peripheral blood was spread on a microscope slide, air-dried and fixed in 96 % ethanol for 30 min. The slides were stained according to the following protocol: 3 min – 2 % May-Grunwald stain, 1 min – dist. H_2O, 10 min – 2 % Giemsa stain (Bolotnikov & Soloviov 1980). Slides were scored with light microscope (magnification 90 x 12, oil immersion).

The genome instability was estimated with micronucleus (MN) test in mature erythrocytes (Tolbert et al. 1992). The MN in skua erythrocytes appeared very rarely and we used the frequency of nuclear anomalies (NA) in mature erythrocytes as an additional parameter of genome instability. NA were scored as a sum of the three most frequent nuclear anomalies – "budding nucleus", "two-lobe nucleus" and "tailed nucleus" (Kursa et al. 2005). Approximately 10,000 mature erythrocytes were examined per blood smear for each bird.

The WBC differential, the ratio of H:L, and the number of different leukocyte types per 10,000 mature erythrocytes were selected as manifestations of birds' immune status. The leukocytes were classified according to standard criteria for birds (Bolotnikov & Soloviov 1980, Campbell 1995, Fudge 2005). We considered 100 leucocytes on smears of each bird for evaluation of WBC differential and the total number of leukocytes per 10,000 mature erythrocytes. These data were used for calculation of heterophil:lymphocyte ratio (H:L) and the number of leukocytes of different types per 10,000 erythrocytes.

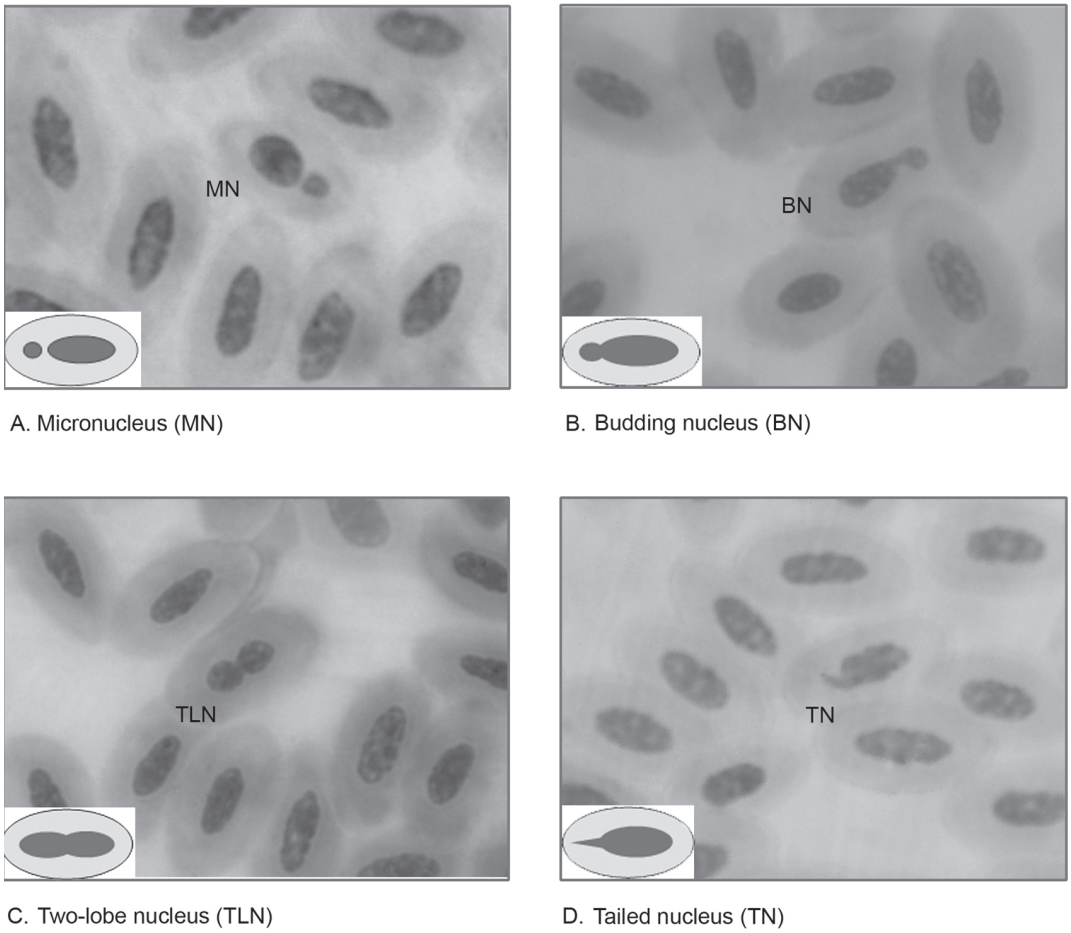
Statistical analyses were performed according to the general accepted procedures. Mean, standard error (SE), range (min ÷ max), confidence interval (CI), variance (σ^2) and Pearson's correlation coefficient with $df = N-2$ were calculated using the "STATISTIKA 6.0" software (StatSoft Inc.).

According to individual values of H:L and NA, the birds were arranged into classes. The number of classes and the length of class interval were calculated according to Stergess formula $K = 1 + 3.32 \lg N$ (N = 162 – the number of birds in the sample) (Lakin 1990). The obtained distributions are shown in the "Results".

RESULTS

A. Micronuclei and nuclear anomalies

Figure 1 shows the most frequent deviations from normal morphology of nuclei: micronuclei (MN), budding nuclei



A. Micronucleus (MN)

B. Budding nucleus (BN)

C. Two-lobe nucleus (TLN)

D. Tailed nucleus (TN)

Fig. 1: Manifestations of genome instability in mature erythrocytes of *Catharacta maccormicki.* A = micronucleus, B = budding nucleus, C = two-lobe nucleus, D = tailed nucleus (Kursa et al. 2005).

Abb. 1: Manifestation von Genom-Instabilität in reifen Erythrozyten von *Catharacta maccormicki.* A = Micronucleus, B = budding nucleus, C = two-lobe nucleus, D = tailed nucleus (Kursa et al. 2005).

(BN), two-lobe nuclei (TLN), and tailed nuclei (TN). These deviations were quite rare and the last three kinds of anomalies were considered together as "nuclear anomalies" (NA). The micronuclei was analyzed separately because it is a generally used criterion of chromosome instability (TOLBERT et al. 1992, ZUNIGA et al. 1996, ZUNIGA-GONZALEZ et al. 2001, FENECH 2003).

The rates of MN and NA varied among individuals in the population. Among all 162 inspected birds (10,000 erythrocytes for each bird) we found only eleven mature erythrocytes with micronuclei. Mean frequency of micronucleated erythrocytes was 0.07 ±0.02 per 10,000 erythrocytes. The frequency of the combined other NA classes was ten times higher (Tab. 1).

Parameter	Mean ± SE	Range	CI (95%)	Variance (σ^2)
MN	0.07 ± 0.02	0÷2	0.05	0.09
NA	0.71 ± 0.08	0÷6	0.16	1.06

Tab. 1: Number of micronuclei (MN) and nuclear anomalies (NA) per 10,000 mature erythrocytes of *Catharacta maccormicki* (N = 162). SE = standard error; CI = confidence interval.

Tab. 1: Zahl der Micronuclei (MN) und der Kernanomalien (NA) pro 10.000 reifer Erythrozyten von *Catharacta maccormicki* (N = 162) SE = Standardfehler, CI = Konfidenz-Intervall.

B. White Blood Cell count and H:L ratio

Mean WBC differential (per 100 leukocytes) is as following: 51.9 ±1.3 % heterophils, 45.0 ±1.2 % lymphocytes, 0.71 ±0.1 % monocytes, 2.3 ±0.2 % basophils, 0.14 ±0.04 % eosinophils. Variability between individuals of *C. maccormicki* in WBC differential is considerably high. Percentage of heterophils ranged from 15 % to 86 %, of lymphocytes from 12 % to 80 %, of monocytes from 0 % to 12 %, of basophils from 0 % to 12 % and eosinophil percentage varied between 0 % and 3 %. The total leukocyte count and ratio of heterophils to lymphocytes per 10,000 erythrocytes are shown in Table 2.

Parameter	Mean ± SE	CI (0.95%)	CV	Variance (σ^2)
WBC (total)	41.08 ± 1.91	3.77	3.56	590.50
Heterophils	21.05 ± 1.07	2.12	32.75	186.90
Lymphocytes	18.80 ± 1.29	2.55	34.79	269.58
Monocytes	0.31 ± 0.06	0.11	194.66	0.49
Basophils	0.87 ± 0.09	0.18	110.20	1.30
Eosinophils	0.06 ± 0.02	0.04	358.85	0.06
H:L	1.52 ± 0.09	0.19	79.10	1.44

Tab. 2: Numbers of leukocyte types per 10,000 erythrocytes and H:L ratio of *Catharacta maccormicki* (N = 162). SE = standard error; CI = confidence interval, CV = coefficient of variation.

Tab. 2: Zahl der Leukozyten-Typen pro 10.000 Erythrozyten und das H:L-Verhältnis bei *Catharacta maccormicki* (N = 162). SE = Standardfehler, CI = Konfidenz-Intervall, CV = Variations-Koeffizient.

C. Relation between genome instability and immune status

NA frequency and H:L ratio were not correlated (r = -0.03, df = 160, p >0.05). Heterophil and lymphocyte numbers correlated with H:L ratio (r = 0.55, p <0.01 and r = -0.38, p <0.01, respectively) as well as did basophil number with H:L ratio (r = -0.33; p <0.01). NA and any leukocytes types were not correlated.

Values of WBC differential and NA frequency varied highly between individuals of *C. maccormicki* and were considerably higher than the mean in some birds. We assumed that the most frequent values of H:L and NA are "normal" for studied group of skuas while the deviating values of H:L or NA may indicate some level of health disorder: increased level of genome instability (NA) or certain problems with immune statuses (H:L). To check this hypothesis we compared two groups of birds – with "modal" and "marginal" values of H:L or NA respectively.

The distributions of birds for both H:L and NA are presented in Table 3. According to distribution of birds for H:L, the two most frequent classes 1 and 2 (72 and 52 individuals respectively, totally 124 birds) were defined as the modal group. The rest of birds (38) were distributed among classes 3-8 and this part of sample was defined as marginal group. Similarly, the modal group for NA was identified as the two first classes (1 and 2) with 136 birds (92 and 44 respectively). The rest of birds – 26 individuals – were distributed among classes 3-7 with low frequency. These modal and marginal groups were compared for different parameters with Student's t-test with N-2 degrees of freedom (N = 1).

Classes	H:L ratio		NA	
	Class interval	Birds number	Class value	Birds number
1	0.00-1	72	0	92
2	1.01-2	52	1	44
3	2.01-3	19	2	19
4	3.01-4	11	3	2
5	4.01-5	4	4	3
6	5.016	3	5	1
7	6.01-7	0	6	1
8	7.01-8	1		
Total N		162		162

Tab. 3: Distribution of H:L ratio and nuclear anomalies (NA) of *Catharacta maccormicki* (N = 162).

Tab. 3: Verteilung des H:L-Verhältnisses und der Kernanomalien (NA) bei *Catharacta maccormicki* (N = 162).

The modal and marginal groups for H:L ratio were significantly different for heterophil frequency (t = 7.37, $d.f.$ = 160, p <0.001), lymphocytes (t = 5.95, p <0.001), monocytes (t = 2.88, p <0.01) and basophils (t = 7.2, p <0.001). The difference was not significant for eosinophil frequency (t = 0.26, p >0.05). Analysis on percentages of leukocytes types (WBC differential) showed similar differences between groups. No differences were found for NA rates between modal and marginal H:L-ratio groups (t = 0.22, p >0.05). In NA distribution the modal group consisted of NA frequency lying in 0-1 ranges (N = 136), while the marginal group consisted of NA frequency lying in 2-6 ranges (N = 26). The two groups

differed for basophils (t = 2.22, p <0.05) and eosinophil (t = 2.54, p <0.05) numbers but not for H:L ratio (t = 0.4, p >0.05), heterophils (t = 0.27, p >0.05), lymphocyte (t = 1.66, p >0.05) and monocytes (t = 0.65, p >0.05).

DISCUSSION

The link between genome instability and sickness, as infertility, embryonic growth, cancer and neurodegenerative disease is well established (Ilinskih et al. 1986, Tolbert et al. 1992, Fenech & Crott 2002, Thompson & Schild 2002, Charames & Bapat 2003, Fenech 2003, Wiseman et al. 2003). The MN assay is useful as a biomarker for diagnosing genome damage (chromosome breakage, chromosome rearrangement, gene amplification and aneuploidy) (Tolbert et al. 1992, Fenech 2003) and one of the standard cytogenetic tests for biomonitoring genotoxic agents for various classes of animals (Zuniga et al. 1996, Zuniga-Gonzalez et al. 2001). MN rate depends on genotype, immune status, sex and age of individual (Zuniga-Gonzalez et al. 2001, Fenech & Crott 2002, Fenech 2003). Other nuclear anomalies analyzed in our work were described for different species in vivo and in vitro and are considered as concerned with disorder of normal structure and function of genome (Tolbert et al. 1992, Fenech & Crott 2002, Fenech 2003).

South Polar Skuas in our study had a very low rate of MN (0.007 ±0.002 ‰) and NA (0.071 ±0.008 ‰) indicating low levels of genome instability. Although MN ratio varies among bird species, the MN level in South Polar Skuas is very low. For some species micronuclei were not found at all *(Cassiculus melanicterus, Polyborus plancus),* while an owl (*Otus* sp.) had a rather high rate of 15.8 ‰ (Zuniga-Gonzalez et al. 2001). For Gentoo penguin *Pygoscelis papua* the average rate of MN was 0.03 ±0.01 ‰ and NA was 0.20 ±0.02 ‰ (Afanasieva et. al. 2006). In most species the frequency of MN is between 0.4 - 4.3 ‰ (Zuniga-Gonzalez et al. 2001).

The WBC count is a sensitive indicator of the immune status and general health of birds and its response to the environment (Bolotnikov & Soloviov 1980, Bolotnikov et al. 1983, Campbell 1995). For *C. maccormicki* leukocytes were 0.41 % of the total erythrocytes number what is within the normal range for birds. The WBC differential of South Polar Skuas is also within the normal range for birds. The WBC differential described in our work is similar to counts for the closely related Great Skua C. skua (Bearhop et al. 1999): the mean values for the percentages of heterophil were 51.88 ±1.32 % and 44.9 ±9.34 %; lymphocyte ratio was 45.01 ±1.22 % and 34.4 ±8.34 %; basophil ratio was 2.26 ±0.20 % and 2.81 ±1.97 and monocyte ratio was 0.71 ±0.11 % and 5.45 ±1.97 % for *C. maccormicki* and *C. skua* respectively. The monocyte percentage of *C. maccormicki* was less than that of the *C. skua* but both values are in the normal range (0-6 %). The reason for this discrepancy could be in speciesspecific values. The mean value for the eosinophil percentage of *C. maccormicki* of 0.14 ±0.04 % was much less than that of *C. skua* (12.3 ±5.86). It also could be explained by species specificity. However, peripheral eosinophils are quite rare in the normal haemogram of many avian species (0-5 %) and eosinophilia can usually be observed in cases of parasite infections and allergic reaction (Bolotnikov et al. 1983, Edler et al. 2004).

The H:L ratio is a reliable physiological index of chronic stress in birds (Gross & Siegel 1983, Maxwell 1993, Vleck 2000). The average H:L ratio in the South Polar Skua equaled 1.52 ±0.09 and is relatively high compared to other birds. For instance, the H:L ratio for gulls *Larus marinus* and *Larus argentatus* was about 0.60 (Averbeck 1992), it was 1.65 for white leghorn chickens (Gross 1992), 0.25 ±0.02 for broiler chickens (Altan et al. 2000), 0.71 for Adelie penguins *Pygoscelis adelie* (Vleck 2000) and 1.35 ±0.08 (Rushkovsky et al. 2005) and 2.3 (Hawkey et al. 1985) for Gentoo penguins *P. papua.* Various stressors can increase H:L ratio as a result of decreasing lymphocyte number and simultaneously increasing heterophil number (Bolotnikov et al. 1983, Gross & Siegel 1983, Maxwell 1993).

The H:L ratio has been shown as a physiological index of chronic stress in birds that may be more useful in assessing response to chronic stressor than a measure of corticosterone concentration that measure short-term change (Gross & Siegel 1983, Maxwell 1993, Vleck 2000). Various stresssors can increase H:L ratio as a result of decreasing lymphocyte number and simultaneously increasing heterophil number (Bolotnikov et al. 1983; Gross & Siegel 1983, Gross 1992, Maxwell 1993, Vleck et al. 2000). But little is known about this index of health status in free-living populations, in particular, in Antarctic ones. The H:L ratio in adult reproducing South Polar Skuas equaled 1.52 ±0.09 (mean ±SE) and ranged from 0.19 to 7.17. For instance, the H:L ratio for gulls *Larus marinus* and *Larus argentatus* was about 0.60 (Averbeck 1992), it was 1.65 for white leghorn chickens (Gross 1992), 0.25 ±0.02 for broiler chickens (Altan et al. 2000), about 0.71 for Adelie penguins *Pygoscelis adelie* (Vleck 2000), 1.35 ±0.08 (Rushkovsky et al. 2005) and 2.3 (Hawkey et al. 1985) for Gentoo penguins *P. papua* and 1.19 ±0.72 (male) and 1.13 ±0.84 (female) for Magellanic penguins *Spheniscus magellanicus* (Moreno et al. 2002).

Genome instability (NA frequency) and immune status (H:L ratio) were not correlated in individuals (r = -0.03, p <0.05). Another analytical approach of the studied traits is based on the assumption that the optimal values of a trait are those in the modal class of the trait's distribution. In contrast, the tails of the distribution may reflect significant deviations from the normal status of the bird's health (infection, food deprivation, diseases, etc). Actually, comparing studied parameters for modal and marginal groups revealed certain differences between the groups: for H:L – heterophils, lymphocytes, monocytes and basophils frequencies; for NA – only basophiles and eosinophils frequencies. No differences between groups were found for NA (H:L distribution) and for H:L (NA distribution).

The obtained results let us suppose that H:L ratio and the studied parameters of chromosomal instability are different manifestations of the bird's health status and reflect the different components of an organism's response to environmental impact. These parameters may be a valuable tool in future conservation efforts.

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