INTRODUCTION

Poultry production plays an important role worldwide as a source of eggs, meat, and processed food. Poland is a high-ranking producer and consumer of poultry meat and eggs. The intensification of poultry production and the introduction of new commercial hen hybrids in large commercial flocks have caused a rapid fall in the numerical strength of old local poultry breeds, almost leading to their extinction. The Greenleg Partridge and the Polbar hen are universal indigenous Polish chicken breeds. The Greenleg Partridge hen is chiefly known for laying eggs with a very low cholesterol content (Wójcik, 2003), as well as for its resistance to diseases, food foraging prowess, strong maternal instinct, and intensive broodiness (Calik, 2009; www.bioroznorodnosc.izoo.krakow.pl). The hens are perfect for the ecological production of eggs and meat (Krawczyk, 2008, 2009). The Polbar is an original autosexing breed developed for scientific purposes. The breed is popular with amateur breeders and farmers for its beautiful, downy plumage and autosexing (Gryzińska and Niespodziewański, 2009). Both chicken breeds are covered by the gene pool protection program for farm animals in Poland (Calik and Krawczyk, 2006; Calik, 2009; www.bioroznorodnosc.izoo.krakow.pl).

The genome of the domestic chicken (Gallus domesticus) is the best explored DNA sequence among all avian species (Schmid et al., 2000, 2005; Dodgson, 2003; Burt, 2004a,b; International Chicken Genome Sequencing Consortium 2004; www.animalgenome.org; www.ncbi.nlm.nih.gov/genome/guide/chicken; www.ensembl.org/Gallus_gallus; genome.wustl.edu/genomes/view/gallus_gallus). It is used in genetic studies as a standard for comparisons with other bird species (Schmid et al., 2000; Kasai et al., 2003; Zhang et al., 2011). The only standardized representation of avian chromosomes is the karyotype of Gallus domesticus (Ladjali-Mohammadi et al., 1999). It comprises the first 8 autosome pairs and the ZW chromosomes. It serves as a point of reference in studies of other bird species (Schmid et al., 2000; Guttenbach et al., 2003) and mammal species, including humans (Burt et al., 1999). The diploid number of

Sister chromatid exchange in Greenleg Partridge and Polbar hens covered by the gene-pool protection program for farm animals in Poland

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ABSTRACT A basic assay that detects genotoxic DNA damage disrupting DNA replication and repair mechanisms is the sister chromatid exchange test. The frequency of sister chromatid exchanges was analyzed in chromosomes of the following hen breeds: Greenleg Partridge and Polbar. Chromosome preparations were obtained from our in vitro culture of peripheral blood lymphocytes stained using the fluorescence plus Giemsa (FPG) technique. The sister chromatid exchange (SCE)/cell mean of the hens under analysis was 7.83 ± 1.76 (7.22 ± 1.70 in the Greenleg Partridge and 8.43 ± 1.61 in the Polbar population). Statistically significant differences were identified between the hen breeds. A higher mean number of SCE/cell was observed in the group of hens producing fewer eggs (8.55 ± 1.51) compared with the group with a better egg yield (7.10 ± 1.65). The differences were statistically significant. Additionally, SCE frequency in the first, second, and third chromosome was analyzed in detail. The highest number of SCE was observed in the first and the lowest in the third chromosome. The SCE distribution in the particular regions of the analyzed chromosomes was also studied. The most numerous exchanges were observed in the proximal region, followed by the interstitial and distal areas.

Key words: sister chromatid exchange, chromosome instability, mitotic chromosome, hen (Gallus domesticus)

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chicken chromosomes is $2n = 78$. A numerous group of small chromosomes, the so-called microchromosomes, and a group of large chromosomes, macrochromosomes, have been observed in the karyotype. Another important characteristic is the determination of the sex. It is determined by the presence of 2 identical ZZ sex chromosomes in the males, and 2 different ZW sex chromosomes in the females.

The knowledge of the karyotype makes it possible to analyze chromosomes and detect cases of chromosome instability, a latent cellular and organismal characteristic. The notion of instability denotes vulnerability to damage, incorrect chromosome behavior, and transformations connected with the impairment of repair mechanisms. The occurrence of the defects largely depends on contact with a mutagen. The SCE (sister chromatid exchange) test identifies chromosome damage resulting from incorrectly functioning DNA replication (Sonoda et al., 1999). The SCE occurs when the intermediary product of the Holliday connection is distributed in 1 of 2 directions; a change in the direction causes inconsistencies and errors (Wilson and Thompson, 2007). What is detected is DNA matrix damage that had not been repaired before the cell entered the S phase (Wójcik et al., 2004; Bayani and Squire, 2005). Ineffective or dysfunctional damage repair mechanisms in single-strand (break-induced replication (BIR)) and double-strand DNA [nonhomologous end-joining (NHEJ) and homologous recombination (HR)] contribute to SCE appearance (Sonoda et al., 1999; Wilson and Thompson, 2007). The BIR system generates SCE in cells with suppressed protein-encoding genes responsible for repairing single-strand breakages (Wilson and Thompson, 2007). The NHEJ repair consists in joining 2 damaged DNA ends through ligation (Poplawski et al., 2009). This process is inaccurate and often causes modifications in the DNA sequence that may be observed as SCE. The HR affects SCE appearance by cutting through the Holliday structure. The cleavage may have a standard course and produce another Holliday structure. This, in turn, leads to gene conversion and instability (Poplawski and Blasiak, 2006; Wilson and Thompson, 2007).

Observed with a light microscope, chromosomes with differentially stained sister chromatids look like harlequin chromosomes or asymmetrically stained chromatids in the chromosome locations where the DNA products had been exchanged in homologous loci.

The fact that little progress has been made in the cytogenetic research of sister chromatid exchanges in hens is the reason for performing the analyses presented in this paper. The study was aimed at analyzing sister chromatid exchanges in Greenleg Partridge and Polbar hens, relative to their egg laying performance.

**MATERIALS AND METHODS**

**Birds**

Chromosome instability was assessed by means of the SCE test in the following groups of laying hens: Greenleg Partridge (the Zk line) and Polbar (Pb), both covered by the gene pool protection program. The hens are kept at the University of Life Sciences in Lublin. In accordance with “Order 77/11, dated 30th November 2011, of the National Research Institute of Animal Production Directive on the Implementation of Updated Gene Pool Protection Programs for the Following Populations: Laying Hens, Geese and Ducks,” conservation flocks are tested for their performance. The following parameters are assessed: percentage index of rearing survivability; BW in 20 wk of age (g); sexual maturity index of the flock, defined as the number of days from hatching until the flock attains 30 and 50% laying capacity; egg weight at 33 and 53 wk of age (g); number of eggs laid during the exploitation period from 21 to 56 wk of age; hatching parameters, defined as percentage indices of egg fertilization and of the hatchability of set and fertilized eggs; percentage index of productive period survivability and qualitative characteristics of the contents of the egg and its shell assessed at 33 wk of age of the hens (www.bioroznorodnosc.izoo.krakow.pl). Table 1 shows the performance results of the Greenlegs and Polbars until 64 wk of age (Pałyszka, 2011).

**Greenleg Partridge**

The Greenleg Partridge hen is a universal chicken breed, developed toward the end of the 19th century on the basis of the so-called Galician chickens. It was first exhibited with the name of Greenleg at a show in Lvov in 1894. The breed standard was established in 1923 in Poland. Greenleg Partridge chickens have been maintained in a conservation flock since 1960. The breed has distinctive partridge-like plumage and light green legs.

**Polbar**

The Polbar is an autosexing breed of universal chickens. Its specificity lies in the fact that the sex of the chicks can be identified immediately after hatching. The breed was raised in 1946 by Professor Laura Kaufmann. It was derived from Greenleg Partridge hens crossed with a striped Plymouth Rock cock and subsequently improved with an admixture of Sussex blood. The traits of the latter breed were gradually suppressed. External admixtures were discontinued in 1953. In 1954–1962, nonstandard birds were eliminated from the population. Subsequently, the breeding concentrated on improving the purified variety. The Polbar was acknowledged as a conservation population in 1999. Young Polbar cocks are light gray (occasionally with a touch of regular gray), whereas young hens are dark, with a black supercilium in the extended line from the eye. The adult birds do not much differ in plumage. The cocks are light-colored with a touch of gray (silvery). The hens are clearly more partridge-like. The breed has only one color pattern: striped with gray and yellow legs.
Blood was sampled from the hens in the 58th wk of age. The analysis concerned the hens that had laid a low and a high number of eggs in the performance assessment (during one month) in the 33rd and 53rd wk of age. Each lineage was represented by 30 birds. Within a breed, the birds were divided into 2 groups. The first group was composed of poor layers (up to 15 eggs). The other one was composed of good layers (above 15 eggs). Thirty metaphase plates were analyzed for each bird. The chromosome preparations were obtained from our in vitro culture of peripheral blood lymphocytes. In h 24 of the culture, the medium was enriched with 10 µg/mL of BrdU (5-bromo-2-deoxyuridine). Differential sister chromatid staining was performed using the fluorescence plus Giemsa (FPG) technique described by Kihlman and Kronborg (1975). The staining procedure involved the following stages: 1-h 0.01% RNase treatment at 37°C, 1-h incubation in a 0.5 × SSC solution (0.75 M sodium chloride + 0.075 sodium citrate; pH = 7.0) including Hoechst’s solution (the basic solution: 0.5 mg of Hoechst 33258/1 mL of ethanol), 1-h UV exposure, 24-h incubation at 4°C in darkness, half-hour UV treatment, 2-h incubation at 58°C and 1-h Giemsa staining.

Analysis and Statistical Methods

The preparations were analyzed under an Olympus BX 50 light microscope. A detailed imaging analysis was carried out using the Multiscan image analyzing system, the Karyotype software, and graphic software compatible with the system (Computer Scanning Systems II, Warszawa, Poland).

The SCE/cell mean of the poor and good layers in both breeds (Zk and Pb) was identified with bivariate ANOVA on the basis of the following formula:

\[ y_{ijl} = m + a_i + b_j + ab_{ij} + e_{ijl}, \]

where \( y_{ijl} \) is the value of the parameter under analysis (SCE/cell mean), \( m \) is the population mean, \( a_i \) is the effect of the ith level of factor A (breed), \( b_j \) is the effect of the jth level of factor B (egg yield), \( ab_{ij} \) is the effect of the interaction between the breed and the egg yield, and \( e_{ijl} \) is the random error.

The significance of the main effects and interactions was determined with the Fischer-Snedecor test (F) at \( P < 0.05 \) and \( P < 0.01 \). Because the factors under analysis were considered at 2 levels: performance (poor and good layers) and breed (Zk and Pb), it was not necessary to apply post-hoc tests. The Statistica 9.1.PL software (StatSoft Polska, Kraków, Poland) was used for calculations.

Moreover, the first, second, and third chromosomes were examined in detail. The number of SCE in the particular chromosomes, the SCE sites and the number of single and multiple SCE were determined. The results were expressed as a percentage. The correlation between chromosome length and the number of

### Table 1. Performance results of the Greenleg Partridge and Polbar hens until the 64th week of age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Greenleg Partridge</th>
<th>Polbar</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of birds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>111</td>
<td>82</td>
</tr>
<tr>
<td>Female</td>
<td>918</td>
<td>780</td>
</tr>
<tr>
<td>Mortality (%) until 8 wk of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Female</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Mortality (%) until 20 wk of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Female</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Eggs/hen</td>
<td>169</td>
<td>150</td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>93.9</td>
<td>95.0</td>
</tr>
<tr>
<td>Hatchability (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set eggs (%)</td>
<td>84.6</td>
<td>84.1</td>
</tr>
<tr>
<td>Fertile eggs (%)</td>
<td>90.1</td>
<td>88.5</td>
</tr>
<tr>
<td>BW (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1</td>
<td>36.9</td>
<td>37.3</td>
</tr>
<tr>
<td>20 wk of age</td>
<td>1,655</td>
<td>1,834</td>
</tr>
<tr>
<td>Male</td>
<td>1,215</td>
<td>1,210</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>47.2</td>
<td>50.0</td>
</tr>
<tr>
<td>33 wk of age</td>
<td>53.5</td>
<td>56.0</td>
</tr>
<tr>
<td>53 wk of age</td>
<td>161</td>
<td>157</td>
</tr>
<tr>
<td>Sexual maturity (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qualitative egg characteristics in wk 33 of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>47.2</td>
<td>50.0</td>
</tr>
<tr>
<td>Yolk weight (g)</td>
<td>15.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Cholesterol (mg/g in yolk)</td>
<td>14.2</td>
<td>14.9</td>
</tr>
<tr>
<td>Yolk proportion per egg (%)</td>
<td>32.4</td>
<td>32.6</td>
</tr>
<tr>
<td>Shell weight (%)</td>
<td>6.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Shell color (%)</td>
<td>61.6</td>
<td>60.9</td>
</tr>
<tr>
<td>Shell thickness (µm)</td>
<td>308</td>
<td>304</td>
</tr>
<tr>
<td>Level of dense egg white (mm)</td>
<td>4.1</td>
<td>4.3</td>
</tr>
</tbody>
</table>
SCE identified in the chromosomes was determined by means of the Pearson correlation coefficient, at \( P < 0.01 \).

**RESULTS**

The frequency of SCE in chromosomes of the Greenleg Partridge and Polbar hen breeds was analyzed. Figure 1 depicts the metaphase plates of the Greenleg Partridge and Polbar chromosomes. The mean number of SCE/cell in the cells under analysis was 7.83 ± 1.76. The mean number of SCE/cell was 7.22 ± 1.70 in the case of the Greenleg Partridge hens and 8.43 ± 1.61 for the Polbars. The mean values were statistically significant \( (P < 0.01) \). A higher mean number of SCE/cell was observed in the group of hens producing fewer eggs \((8.55 ± 1.51)\) compared with the group of hens with a better egg yield \((7.10 ± 1.65)\). The mean values were statistically significant \( (P < 0.01) \). The results underwent bivariate ANOVA. Both parameters were found to have significant effect on SCE incidence. The breed-related \( F_{\text{emp}} \) was 16.71 at \( P = 0.00 \), whereas the \( F_{\text{emp}} \) for the other parameter was 76.55 at \( P = 0.00 \).

The paper analyses the SCE/cell distribution in the 3 largest autosome pairs. The highest number of sister chromatid exchanges was observed in the first chromosome (48%), followed by the second (32%). The fewest exchanges were identified in the third chromosome (20%). The number of identified SCE was proportional to chromosome length; the longer the chromosome, the more exchanges occurred there. A positive correlation was determined between the characteristics. The Pearson correlation coefficient was 0.98 \( (P < 0.01) \). Sister chromatid exchanges were observed in the proximal, interstitial, and distal regions of the chromosomes. The most exchanges were identified in the proximal part of the analyzed chromosomes (54%), followed by the interstitial (26%) and distal (20%) regions. Single, double, and triple SCE were detected in the chromosomes. Differences in the number of the particular types of SCE were observed between the hen groups. The chromosomes of the poorly performing layers were found to develop more double and triple exchanges compared with the other group (Figure 2).

![Figure 1. Metaphase plates of the chromosomes of the Greenleg Partridge (a) and Polbar hens, stained by means of the sister chromatid exchange (SCE) technique (arrows indicate SCE).](image)

**DISCUSSION**

Among numerous cytogenetic methods used for the assessment of the effect of endo- and exogenous genotoxic substances, the SCE test plays an important role in research. The test is a sensitive and relatively fast and easy assay of chromosome damage. The SCE test is usually carried out as part of routine in vitro monitoring of peripheral blood lymphocytes. Lymph cells raised in strictly defined aseptic conditions provide information on the degree of chromosome damage in the organism.

The SCE is induced by many factors. A significant factor that influences SCE incidence is the breed of the species (Iannuzzi et al., 1991b; Catalan et al., 1995; Wójcik et al., 2011). Differences in SCE frequency may stem from the origin of the animals, the autochthonous breeds having a more stable genome in contrast to other, genetically improved breeds of the same species. The Greenleg Partridge hen is an old Polish indigenous breed. On the other hand, the Polbar is a breed created through genetic improvement. Lower SCE frequency was observed by the present authors in the Greenleg Partridge hens in comparison with the Polbars.

Selection is not conducted in flocks of laying hens covered by gene pool protection programs. Hence, any variation in the values of the analyzed parameters is due to genetic and environmental factors (Calik, 2009). The egg laying performance is a low-heritable trait of hens, ranging from 0.25 to 0.35; thus, variability of the trait depends on environmental conditions in 65 to 75% of cases. The conditions created for the flocks being equal, the differences that were observed in the frequency of SCE between the hens defined as poor and good layers may have resulted from individually higher vulnerability to certain exo- and endogenous genotoxic factors of a mutagenic and carcinogenic nature. The SCE test makes it possible to detect preclinical or early pathological changes in an individual. It plays an im-
portant role in the etiology of many diseases in hu-
mans (Caggana and Kelsey, 1991; Emingil et al., 2002; 
Youssoufian and Pyeritz, 2002; Amor-Gueret, 2006; 
Karaman and Aliagaoglu, 2006). The phenomenon of 
elevated SCE incidence was observed by Peretti et al. 
(2008) in Mediterranean Italian buffaloes affected by 
limb malformation (transversal hemimelia). The pre-
sent authors observed a much higher frequency of double 
and triple SCE and a lower frequency of single exchang-
es in the poorly performing layers.

Sister chromatid exchanges were observed in the 
proximal, interstitial, and distal regions of the chromo-
somes. The most numerous exchanges were identified 
in the proximal region, followed by the interstitial and 
distal areas. A high SCE incidence was observed in the 
proximal (centromeric) region in rodents (Marin and 
Prescott, 1964; Gibson and Prescott, 1972; Lee, 1975; 
Lin and Alfi, 1976). The fewest SCE were identified in 
this region in humans (Latt, 1974). The centromeric 
region in rodents is characterized by numerous DNA 
repetitions. It is in this region that chromosome cracks 
and recombinations predominantly occur (Lee, 1975; 
Lin and Alfi, 1976), which result in a high number of 
SCE. The SCE observed in the present study in the 
centromeric and interstitial regions may be associated 
with a specific characteristic of the chicken genome – 
the presence of a considerable number of telomeric se-
quences (TTAGGG)n in the interstitial, centromeric, 
and subcentromeric areas of chromosome arms (Griffin 
et al., 2007). Highly repetitive telomeric sequences act 
as recombination hot spots and play a role in mitotic 
recombinations of somatic cells (Delany et al., 2000; 
Nanda et al., 2002). Ineffective repair of ongoing DNA 
damage leads to the generation of SCE (Baird, 2008). 

According to Latt (1974), Carrano and Wolff (1975), 
and Lindahl (1993), the reason for different SCE lo-
cation in particular animal species are differences in 
the configuration of heterochromatin and euchroma-
tin. Centromeric and telomeric regions of hen chromo-
somes contain blocks of constitutive heterochromatin 
(Carlenius et al., 1981). The present study identified 
the highest number of SCE in exactly these parts of 
the chromosomes. A high SCE frequency of 17% was 
observed in the telomeric and subtelomeric region by 
Rudd et al. (2007), who analyzed human chromosomes.

Chromosome length is an equally important factor 
that influences SCE frequency. The frequency of SCE 
is proportional to chromosome length. The longer the 
chromosome, the more SCE take place (Latt, 1974; 
Carrano and Wolff, 1975; Iannuzzi et al., 1991a; Arias, 
2000). According to Di Meo et al. (1993, 2000), the 
distribution of SCE in chromosomes is irregular. The 
authors identified higher or lower SCE frequency in cer-
tain chromosomes in relation to chromosome length.

Another important factor that affects SCE incidence 
is age (Sinha et al., 1985; Lazutka et al., 1994; Peretti 
et al., 2006; Husum et al., 2008; Wnuk et al., 2011; 
Wójcik et al., 2011). Higher SCE frequencies were 
observed in older animals. The present authors analyzed 
SCE frequency in old birds. Blood was sampled from 
the layers at 58 wk of age. The observed high frequency 
of SCE may have resulted from the age of the birds. 
The mean number of SCE/cell in the analyzed hens 
was 7.83. Arias (2000, 2003, 2007) observed much lower 
values of SCE frequency in hens. The experiments were, 
however, performed on embryos. The SCE/cell mean 
ranged from 1.22 in 4-d-old embryos to 2.23 in 10-d-old 
embryos. Differences between the mean values may be 
caused by ongoing epigenetic processes in the organism.

The DNA methylation level of embryos is much lower 
than that of mature individuals as the methylation lev-
el rises along with age (Vanyushin, 2005; Sulewska et 
al., 2007). This may account for the growing instability 
observed to accompany advancing age (Kaina, 2004; 
Peretti et al., 2006; Wnuk et al., 2011; Wójcik et al., 
2011).

The SCE test employed in the study is a very sensi-
tive method for detecting the effects of DNA and chro-
mosome damage caused by faulty replication and dys-
functional repair mechanisms. The results suggest that 
the SCE test may be useful as a cytogenetic biomarker 
for the identification of negative effects of endo- 
and exogenous genotoxic factors on the organism.

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