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Analysis of DNA Polymorphism (RAPD-PCR) and Reciprocal Effects of Geese Crossbreeds

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Commercial geese breeding in Poland is based on two strains of White Italian geese (W11 and W33). The crossbreeds W33 (paternal line) and W11 (maternal line) are distributed in Poland under the commercial brand of White Kołuda® goose. However, there are several breeds which are covered by the animal genetic resources conservation program and kept as conservative flocks. These breeds proved invaluable to commercial geese breeding to stabilize body weight, improve muscling and decrease the amount of fat in the carcass of the crossbreeds. Therefore, this study analyzed the reciprocal crossbreeds of White Kołuda® geese with the individuals from conservative flocks. DNA polymorphism (RAPD-PCR) of the crossbreeds as well as the phenotypic effect of crossbreeding was evaluated. PCR amplification of five RAPD markers resulted in obtaining 14.25 band/crossbreed group. The genetic similarity of the crossbreeds expressed as band sharing frequency (BS) ranged from 0.44 to 0.97. The direction of crossing of the W33 goose with one of the individuals from the conservative flock strongly affected the genetic similarity estimates. The body weight in the 17^{th} or 24^{th} week of life and the percentage of leg muscle weight in the 24^{th} week of life differed significantly depending on the crossbreed genotype. A similar relationship was demonstrated for egg fertilization and number of nestlings per goose. As the lines were differentiated only by origin of the Z chromosome, the background of the differences in genetic polymorphism and the phenotypic records is hypothesized as (i) the linkage of some production traits with sex chromosomes; (ii) the impact of selection on W33 individuals resulting in lower performance of geese with a W33-derived Z chromosome; (iii) genetic imprinting displayed as the effect of either maternal or paternal origin of the Z chromosome.

Key words: Geese, White Kołuda®, conservative flock, reciprocal cross, RAPD-PCR, genetic imprinting.

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Geese breeding is one the few examples of breeding processes implemented in Poland with the use of indigenous breeds only. The preliminary effect of the breeding was the differentiation of genetic parameters and production performance of the input breed – White Italian – into two strains: W11 and W33. Further improvement of these strains was conducted by the application of different levels of selection pressure which caused divergence in body weight of young individuals from W11 and W33 strains as well as in their reproductive traits. The W33 population is characterized by increased body and egg weight, and also by delayed age of sexual maturity, lower number of eggs laid, and lower egg fertilization and hatchability (ROSINSKI 2000). The crossbreeds of W33 (paternal line) and W11 (maternal line) are distributed in Poland under the commercial brand of White Kołuda® Goose.

Apart from the above-mentioned geese strains routinely exploited in commercial breeding programs, there are several breeds covered by the animal genetic resources conservation program and kept as conservative flocks (11 groups) in the National Centre of Animal Production in Poland. Production performance of these breeds was analyzed on the basis of crossbreeding effects and the performance of crossbreds (SMALEC & MAZANOWSKI

1995; MAZANOWSKI 1999abc). Interesting results were obtained through reciprocal crossing of White Kołuda® Geese with the individuals from conservative flocks (MAZANOWSKI & BEDNARCZYK 2001; MAZANOWSKI & SZUKALSKI 2000ab).

Research on genetic differentiation and effects of crossbreeding was usually based on phenotypic effects analysis, whereas only a few studies were performed with the application of molecular markers. In many reports, randomly amplified polymorphic DNA (RAPD) markers have proven to be valuable in estimation of genetic differentiation, detection of polymorphism among different species and other population studies as reviewed recently by SALEM *et al.* (2005).

Among indigenous geese populations, the genetic evaluation of the Zatorska goose was performed with the use of minisatellite polymorphism and the linkage of DNA fingerprints with quantitative traits loci was consecutively analyzed (ZAWADZKA 1999; ZAWADZKA *et al.* 2001). Previous reports (BEDNARCZYK *et al.* 2002; MACIUSZONEK *et al.* 2005) have indicated that ten generations of selection with different emphasis on meat and reproductive traits resulted in the genetic differentiation of the goose strains, identified by the RAPD-PCR method. It was also shown that the selection on meat traits contributed to higher genetic differentiation in comparison to the selection on reproductive traits. Finally, the application of the RAPD-PCR method made it possible to assess the genetic differences between geese breeds from conservative flocks. Some of the geese breeds included in the conservation program proved to be invaluable in commercial geese breeding to stabilize body weight, improve muscling and decrease the amount of fat in the carcass of the crossbreeds.

The goal of this study was to estimate the phenotypic effect and analyze the DNA polymorphism (RAPD-PCR) in crossbred geese. Application of reciprocal crosses in these analyses accounted for a unique research model.

Material and Methods

Research was performed on crossbred geese obtained as a result of two types of crosses. The first was a reciprocal cross between ganders or goose from conservative flocks of regional varieties (Kartuska – Ka, Kielecka – Ki, Suwalska – Su and Podkarpacka – Pd) with heavy White Kołuda® ganders or goose (strain W33), whereas in the second type of reciprocal cross White Kołuda® goose (strain W11) and Cuban ganders (C) were used. Finally two crossbred strains were mated, which resulted in obtaining four-way crossbred geese. An example of the cross scheme including Suwalska geese from the conservative flock and remaining geese strains evaluated in the experiment is depicted in Figure 1.

According to the scheme, further crossbreeds were obtained with the use of the individuals from consecutive conservative flocks (Ka, Ki and Pd).

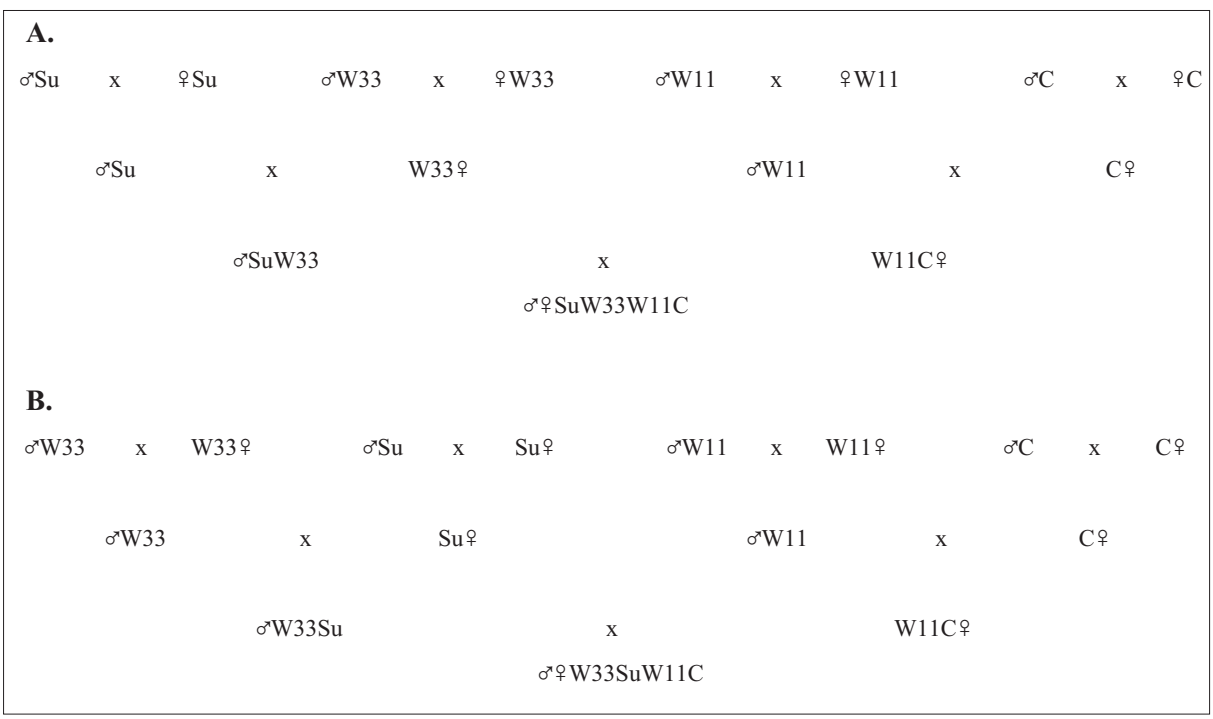

Fig. 1. Experimental cross scheme of different geese lines. A, B – examples of the crosses with a share of Suwalska geese. Su – Suwalska geese (conservative flock), W11 and W33 – White Kołuda® geese (commercial strains), C – Cuban geese (genetic reserve).

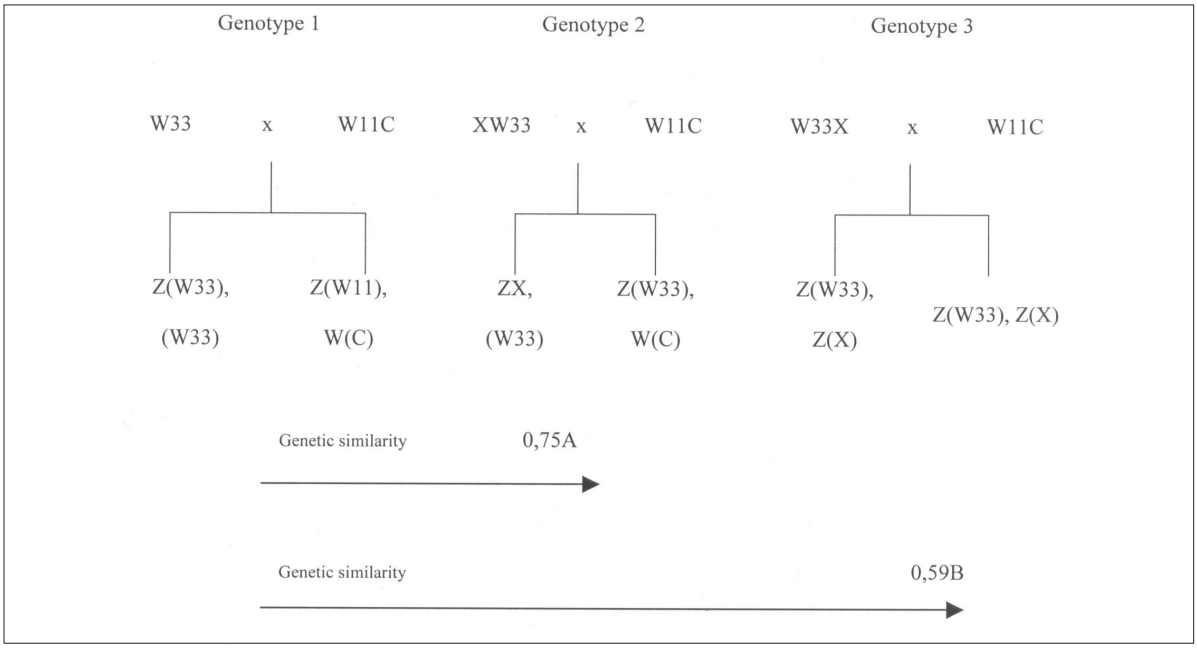

Fig. 2. Relationship between the parental genotype and genetic similarity by RAPD-PCR of the offspring.

All birds were kept in the Waterfowl Genetic Resources Station in Dworzyska under identical environmental conditions. The feeding system and feed composition were the same in all groups, as described earlier (MAZANOWSKI & BEDNARCZYK 2001; MAZANOWSKI & SZUKALSKI 2000). Egg number was noted daily with division on hatching and non-hatching eggs (too small, too large, damaged, improperly structured). These records were used for the calculation of the mean number of eggs laid by a single goose. Egg laying as well as hatching was performed in Petersime incubators. The results of fertilization and hatching were registered weekly during the entire reproductive period.

Meat traits were evaluated on 1536 crossbreeds (8 crossbreeds groups, 32 birds of each sex, 3 replications), that had been fed *ad libitum* and kept up to the 17th or 24th week of life (2 replications). Body weight was determined individually, whereas feed use was assessed within the groups. After reaching the 17th and 24th week of life five ganders and five goose of typical body weight for the group and sex were chosen out of each group. The animals were slaughtered and afterwards the dissection of the cooled and plucked carcass was performed according to HAHN & SPINDLER (2002).

Table 1

Sequences of the random primers used for genetic similarity assessment

RAPD marker	Sequence (5' - 3')
AB1 - 02	TGATCCCTGG
AB1 - 03	CATCCCCCTG
AB1 - 10	CTGCTGGGAC
AB1 - 26	TTTGCCCGGA
AB1 - 28	AGGGAACGAG

DNA polymorphism was analyzed on 20 individuals (10 geese and 10 ganders) from each crossbreed group, including geese from conservative flocks and W33 x W11C crossbreeds in order to determine the relationships between genotype, origin of the Z chromosome in ganders as well as genetic similarity.

Blood was taken from the wing vein and used for DNA isolation, according to the procedure of SMITH *et al.* (1996). Five primers of random nucleotide sequence were tested (Advanced Biotechnologies Inc., USA).

PCR reactions were performed in a total volume of 50 μl. The mastermix included 50 ng genomic DNA template, 40 pmol primer, 10 nmol dNTP, 2U Taq polymerase (Terpol, Poland), 1x buffer A (100 mM Tris-HCL pH 8.3, 500 mM KCL, 1 mM $MgCl_2$, 0,1% gelatine) and nuclease-free water. Amplification was carried out in MJ Research PTC 100 thermocycler, according to the following thermal conditions: 2 min of initial denaturation (94°C) followed by 46 cycles (denaturation – 1 min in 94°C, annealing – 2 min in 36°C, DNA elongation – 1 min in 72°C) and final elongation (10 min, 72°C). PCR products were electrophoresed in 1.8

% agarose gels stained with ethiudium bromide. As a molecular weight, pUC 19 (BTL, Poland) was used. A volume of 25 μl of the PCR product was suspended in 2 μl of the loading solution (0.25 % bromophenol blue, 0.25 % cyanol xylene, 15 % ficoll). Gel electrophoresis was performed in TBE buffer for 80 min under 100V with the use of APPELEX ST 606 T apparatus. Resolved agarose gels were visualized in UV light emitted by a transilluminator (Spectroline TC-312A). The gel pictures were archived with Grab-it software, and consecutively analyzed in the GelScan program.

Genetic identity among crossbreeds expressed as band sharing frequency (BS) was estimated based on RAPD fingerprints analysis. JEFFEREY'S *et al.* (1986) formula was used for BS calculation:

$$BS = 2\ N_{ab} / (N_a + N_b)$$

Where: N_{ab} – number of shared bands between individuals a and b; N_a – number of bands in individual a; N_b – number of bands in individual b.

Results

The overall band sharing frequency (BS) of the crossbreeds estimated with all 5 RAPD markers (Table 2) ranged from 0.44 (W33Ki'WllK vs. W33Pd'WllK) to 0.97 (PdW33'WllK vs. W33Su'WllK). Group 1 (W33'W11K) was characterized by the lowest BS (0.67) in relation to other groups, whereas the crossbreed groups (with share of breeds from conservative flocks) were genetically similar from 0.69 to 0.82 on average.

The results of the genetic similarity of W33'W11K crossbred geese to other crossbreeds (Fig. 1) were significantly ($P<0.01$) different depending on the direction of crossing W33 geese with individuals from conservative flocks (denoted with X symbol) during the process of reproducing ganders. The presence of geese from the conservative group in the maternal position in crosses with individuals from the W33 strain influenced the genetic diversity of the crossbreeds (0.59) more than using ganders from conservative groups in the paternal position (0.75).

The average number of amplified PCR products for each RAPD marker and the geese genotype is presented in Table 3. Briefly, 16 PCR products in total were obtained for geese W33'W11K, whereas for other genotypes 13.5 (XW33'W11K) and 13.25 (W33X'W11K) different bands were amplified. The latter values are calculated as the mean band number for each of four genotypes that include individuals from conservative flocks (X equals to Su, Ka, Ki or Pd geese).

Table 2

Genetic similarity between crossbred geese, based on RAPD – DNA polymorphism

Group	Group								
	1	2	3	4	5	6	7	8	9
1		0.76	0.80	0.77	0.70	0.71	0.51	0.59	0.53
2			0.80	0.67	0.81	0.79	0.58	0.64	0.67
3				0.75	0.88	0.83	0.80	0.91	0.80
4					0.63	0.62	0.86	0.67	0.86
5						0.97	0.71	0.86	0.73
6							0.74	0.80	0.81
7								0.72	0.81
8									0.44
Mean	0.67	0.72	0.82	0.72	0.79	0.80	0.70	0.70	0.69

Table 3

Average number of amplified RAPD-PCR products

Genotype	RAPD marker					Sum
	AB1-02	AB1-03	AB1-10	AB1-26	AB1-28	
W33 x WllC	2.00	3.00	1.00	6.00	4.00	16.00
XW33 x WllC*	1.25	3.50	2.50	3.00	3.25	13.50
W33X x WMC**	3.25	2.75	2.75	3.00	1.50	13.25

* X – Gander from conservative flock (Ka; Ki; Pd or Su); ** X - goose from conservative flock (Ka; Ki; Pd or Su).

Table 4

Impact of gander genotype on meat traits of offspring in the 17th week of life

Genotype	Body weight [g]	Feed conversion [g]	Carcass proportion [%]	Percentage proportion in carcass [%]		
				Breast muscle	Leg muscle	Skin with back fat
XW33 x WK11	5448 ±26a	5210	63.9 ± 0.2A	18.9 ± 0.2A	16.1 ± 0.1 A	23.8 ± 0.3
W33X x WK11	5304 ±25b	5202	62.1 ± 0.2B	18.3 ± 0. IB	15.6 ± 0.IB	23.8 ± 0.2

Table 5

Impact of gander genotype on meat traits of offspring in the 24th week of life

Genotype	Body weight [g]	Feed conversion [g]	Carcass proportion (%)	Percentage proportion in carcass [%]		
				Breast muscle	Leg muscle	Skin with back fat
XW33 x WK11	6073 ±31A	6815	64.2 ±0.3	19.6 ±0.1	15.1 ±0.la	24.3 ±0.3
W33X x WK11	5835 ±29B	6767	63.9 ±0.3	19.3 ±0.2	15.5±0.2b	24.2 ±0.3

Table 6

Impact of gander genotype on reproductive traits of geese

Genotype	Egg fertilization [%]	Hatching of fertilized eggs [%]	Number of nestling from 1 goose
XW33 x WKll	50.3	78.6	32.3
W33X x WKll	62.9	77.4	41.7

Among meat traits evaluated, two assessments (body weight in the 17th or 24th week of life and percentage of leg muscle weight in the 24th week of life) were significantly different ($P<0.01$ or $P<0.05$) depending on gander genotype (Table 4 & 5). Offspring of males XW33 was heavier and characterized by a higher proportion of leg muscle percentage in comparison to the offspring of males W33X.

A similar relationship was demonstrated for egg fertilization and number of nestlings per goose (Table 6). A share of W33 blood in the maternal position (XW33) during reproduction of ganders resulted in a decrease of egg fertilization ($P<0.01$) from 62.9% to 50.3% and a lower number of crossbred nestlings from 41.7 to 32.3.

Discussion

The impact of cross direction on crossbreed phenotype is well known, although its mechanism are not thoroughly explored. Perhaps this phenomenon can be partly explained by the plausible effect of linkage of some production traits with sex chromosomes. The female sex in avian species is heterogametic, i.e. it is determined by two different sex chromosomes, Z and W. Thus, the chromosome W is passed on to another generation of female offspring.

Many authors point out the higher heritability estimation of avian reproductive traits based on maternal rather than paternal variability (BEDNARCZYK *et al.* 2000; SZWACZKOWSKI 1995). The heritability coefficient of fertilization, assessed on the basis of the parental variability, equaled 0.09 (fathers) and 0.31 (mothers) (BEAUMONT *et al.* 1997). At the same time, no significant impact of cytoplasmatic variation on the discussed traits has been demonstrated so far (SZWACZKOWSKI *et al.* 1999). This suggests that the localization of the genes responsible for reproductive traits lies on the avian W chromosome.

Another explanation, strongly supported by our results, can also be hypothesized. The goose genotypes evaluated were differentiated only with the origin of the Z chromosome in ganders, as it was passed on either from the W33 goose or from individuals belonging to one of the local breeds, denoted as X. Geese from the W33 strain were selected for many generation, the selection criteria being meat traits. An effect correlated with this selection is a low level of reproductive traits, which is common in all avian species that had been subjected to selection on meat traits (HUEY *et al.* 1982; BARBATO 1994).

However, the results also suggest that the widely investigated phenomenon of imprinting (DE KONING *et al.* 2000), which is explained as differing expression of the same allele depending on maternal or paternal origin, can strongly affect the phenotypes of the birds. Chromosome Z of W33 ganders used in crosses can be found in each of the genotypes under study, but in one genotype it is of paternal (W33X) and in the other of maternal (XW33) origin. The latter case is associated with a significantly lower level of egg fertilization in the present study. These results are in concordance with current knowledge about imprinting in avian species, reviewed by TUISKULA-HAAVISTO and VILKKI (2007). The parent-of-origin effects have not only been confirmed in reciprocal crosses, as in this study, but also in the QTL-mapping study of the chicken autosomes (TUISKULA-HAAVISTO *et al.* 2004) and through comparative genomics characterizing some fragments of the avian macrochromosomes as the typically imprinted regions (DUNZINGER *et al.* 2005).

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