

## Monitoring of genetic diversity in autochthonous Czech poultry breeds assessed by genealogical data

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**Abstract:** Czech local poultry breeds face high risks of extinction. Because these populations are closed, they are more likely to lose genetic diversity. The aim of this analysis was to determine the loss of genetic diversity in three Czech autochthonous poultry breeds. Pedigree data from a total of 1 932 Czech Gold Speckled Hens, 325 Czech White Geese and 111 Czech Crested Geese registered in studbooks between 2000 and 2018 were evaluated. Data were analysed to determine the major factors that affect the genetic variability of these breeds. The average numbers of equivalent complete generations ranged from 2.53 to 4.82. The effective numbers of founders were from 29 to 59, representing from 43% to 62% of the total number of founders. The effective number of ancestors was estimated in the range of 21 to 41. The average inbreeding coefficient and relatedness coefficient (in parentheses) for the reference populations were 2.0% (6.5%), 1.9% (4.9%) and 2.1% (9.3%), respectively. The results showed that the effective population size derived from the rate of inbreeding ranged from 46 to 108 and if derived from the rate of coancestry it ranged from 35 to 74. With regard to these results, the analysed breeds showed a high probability of allele loss and consequent loss of genetic diversity.

**Keywords:** inbreeding; structure of population; chicken; goose

About one-fifth of livestock and poultry breeds became extinct during the second half of the twentieth century, and a large part of these breeds are endangered. This decrease in genetic variability is due to the replacement of original livestock and poultry breeds with highly productive ones (Hodges 2006). The genetic diversity of the commercial breed is

often quite small despite a large number of animals, due to the small number of sires used in reproduction with the large number of progeny. For these reasons, it is necessary to maintain a high level of genetic diversity by local breeds. In addition, the local breeds can complement specific breeding requirements associated with their better ad-

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adaptation to the local environmental conditions and the regional foodstuff production. Unfortunately, native local breeds are often kept only in small closed populations.

For this reason, the local breeds can be used in future breeding with commercial breeds, especially when future breeding goals differ from those of today (Woelders et al. 2006).

As a result of the small and closed population, original indigenous breeds may be affected by inbreeding and loss of genetic diversity. Inbreeding leads to the change of genotype frequencies by increasing homozygosity at the expense of heterozygosity. This change may subsequently increase the frequency of lethal and detrimental malformations and abnormalities as well as it may cause the appearance of inbreeding depression. For this reason, it is essential to monitor and conserve genetic diversity in farm animals (Curik et al. 2017).

In livestock species, microsatellites were historically dominant molecular markers for the study of genetic diversity and level of gene flow between populations (Vostra-Vydrova et al. 2018; Cortes et al. 2019; Landi et al. 2019). The development of high-density genotyping platforms brings new possibilities to estimate genetic diversity parameters or population structure (Grilz-Seger et al. 2019; Moravcikova et al. 2019; Nandolo et al. 2019). Despite the advantages of these methods, the genealogical records remain commonly used to determine the factors which affected total genetic diversity loss (Figueredo et al. 2019; Goleman et al. 2019; Hofmannova et al. 2019). In poultry, there were only a few studies based on pedigree data published (Marquez et al. 2010; Graczyk et al. 2015; Pham et al. 2016; Gholizadeh 2017).

There are three local poultry breeds, Czech Gold Speckled Hen (CGSH), Czech White Goose (CG) and Czech Crested Goose (CCG), which have been included in the government project for genetic resources conservation in the Czech Republic. The Czech Gold Speckled Hen is an ancient Czech breed which is defined as a light dual-purpose bird. The first documentation of this breed dates back to 1205. The breed was popular amongst the people around the mid-19<sup>th</sup> century when new breeds were imported.

The Czech White Goose belongs to the native breeds of geese originated from the domestication of wild goose in the Bohemian and Moravian region. The gene pool of the Czech White Goose remained

homogeneous until the 1870s. However, after that, the original Czech White Goose breed was crossed with various imported breeds. The original Czech White Goose breed was successfully regenerated in the 1930s. In the year 1992, the Czech White Goose breed was included in the genetic resources of the Czech Republic.

The Czech Crested Goose was derived from the Czech White Goose breed, in which the crest occasionally occurred. Between the 1970s and 1980s the gene pool of the Czech Crested Goose was stabilized and the population was recognized as a separate breed in 1988.

Due to that, all presented poultry breeds are closed and endangered by the loss of genetic diversity. The objective of the present study was to estimate the level of genetic diversity and to find factors which affected total genetic diversity loss.

## MATERIAL AND METHOD

### Data

The pedigree information on Czech Gold Speckled Hen, Czech White Goose and Czech Crested Goose animals was provided by the National Reference Centre for Animal Genetic Resources of the Czech Republic. The numbers of animals in analysed populations are given in Table 1. The analysed reference populations consisted of breeding animals within the years 2018–2019. The genealogical information was collected to maximise the number of the ancestral generations used in the analysis. The quality level of the pedigree information was characterized by computing the index of completeness and the equivalent complete generations (MacCluer et al. 1983). The generation interval comprising four real ways of gene transfer: from sire to son, from sire to daughter, from dam to son and from dam to daughter was also analysed.

### Genetic diversity

The pedigree data were used to estimate the genetic variability of the analysed populations. The genetic variability was estimated based on the probability of gene origin and probability of identity by descent. For all analysed populations, the following parameters were estimated: total number

Table 1. Description of the analysed Czech Gold Speckled Hen (CGSH), Czech White Goose (CWG) and Czech Crested Goose (CCG) breeds

		CGSH		CWG		CCG	
	<i>n</i>	276		182		72	
RP	sex	M	F	M	F	M	F
	<i>n</i>	31	245	71	111	27	45
	<i>n</i>	1 932		325		111	
WP	sex	M	F	M	F	M	F
	<i>n</i>	206	1 726	120	205	46	65

F = female; M = male; *n* = number of animals; RP = reference population; sex = sex of the analysed animal; WP = whole population

of founders ( $f$ ), effective number of founders ( $f_e$ ) (Lacy 1995), effective number of ancestors ( $f_a$ ) (Boichard et al. 1997) and founder genome equivalent ( $f_{ge}$ ) (Lacy 1995); the classical inbreeding coefficient ( $F_X$  - Malecot 1948; Wright 1922) and the new inbreeding coefficient ( $F_{new}$  - Kalinowski et al. 2000) by Grain v2.2 software (Doekes et al. 2020); the average relatedness coefficient of each individual (AR); and the realized effective population size based on the rate of inbreeding ( $N_{eF}$ ) (Gutierrez et al. 2008) or the rate of coancestry ( $N_{eC}$ ) (Cervantes et al. 2011). The loss of genetic diversity in the reference population due to genetic drift or bottleneck ( $GD$  - Lacy 1995) and unequal founder contributions ( $GD^*$  - Caballero and Toro 2000) were also estimated. When considering that the Czech Crested Goose was derived from the Czech White Goose, other components of total genetic diversity between these two breeds such as genetic diversity within individuals ( $GD_{WI}$ ), genetic diversity between individuals ( $GD_{BI}$ ), genetic diversity within subpopulations ( $GD_{WS}$ ) and genetic diversity between subpopulations ( $GD_{BS}$ ) were determined according to Caballero and Toro (2000). The genetic diversity between two breeds of analysed geese was evaluated using  $F$ -statistics (Wright 1949) according to Caballero and Toro (2000) and by the average genetic distance (Nei 1987).

## RESULTS AND DISCUSSION

### Pedigree completeness

The population size of analysed breeds was highly variable between 2000 and 2018 [Figure S1 in electronic supplementary material (ESM)]. The sample

size of the Czech Gold Speckled Hen was larger compared to the analysed breeds of geese. In the Czech Gold Speckled Hen, the number of registered animals first increased to a peak in 2008 (186 animals). Then it decreased to 109 animals in 2010. From 2010 to 2018, the number of registered Czech Gold Speckled Hens fluctuated between approximately 116 and 176 registered animals per year. In the Czech White Goose, the number of registered animals slowly increased until 2011, when the maximum number of individuals was registered in 2017 ( $n = 50$ ). In the Czech Crested Goose, the number of registered animals per year fluctuated around ten registered animals per year during the entire analysed period.

Figure S2 in ESM shows the pedigree completeness. The pedigree completeness dropped to less than 50% from the third generation in both goose breeds. The highest pedigree completeness was found in the Czech Gold Speckled Hen. The average equivalent complete generations in the analysed breeds were 4.82 (ranging from 1.0 to 7.02) for the Czech Gold Speckled Hen, 2.53 (ranging from 1.00 to 4.92) for the Czech White Goose and 2.63 (ranging from 1.00 to 4.70) for the Czech Crested Goose. Information about the completeness of pedigrees has a significant influence on the correctness of population parameters estimated from pedigree data (e.g. the level of inbreeding or effective population size). It has been shown that inbreeding levels are decreased, and  $N_e$  is underestimated when pedigree information is incomplete (Lutaaya et al. 1999; Cassell et al. 2003). The equivalent numbers of completely known generations in the analysed breeds were significantly higher than in other goose populations, e.g. in the Zatorska goose (3.76) (Graczyk et al. 2015). Generation intervals

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for poultry are shorter than for livestock species. The estimated generation intervals reached the values of 1.35 years for Czech Gold Speckled Hen, 4.10 years for Czech White Goose and 3.81 years for Czech Crested Goose. The estimated generation interval was longer than that of 2.32 years published by Graczyk et al. (2015) for the Zatorska goose. In fact, individuals of the analysed breeds remain in the reproductive population for a longer time, especially in the dam-daughter pathway of the Czech Gold Speckled Hen and sire-son pathway of both analysed goose breeds. In the Czech White Goose, also animals 20 years old were recorded in the reference population.

The longer generation interval is suitable in relation to the conservation of the highest genetic diversity in the population. However, the long-term keeping of individuals in breeding can also increase the probability of mating among related individuals.

## Genetic diversity

The averages  $F_X$  values by year are shown in Figure S3A–B in ESM. The highest increase in the inbreeding coefficient of the analysed breeds was recorded in 2013 for the Czech Crested Goose. The average values were estimated in the range from 1.9% (Czech White Goose) to 2.1% (Czech Gold Speckled Hen) for classical inbreeding coefficients and in the range from 1.6% (Czech White Goose) to 1.9% (Czech Crested Goose) for the new inbreeding coefficients (Table 2). The low difference between  $F_X$  and  $F_{new}$  indicated that the average classical inbreeding coefficient was mainly affected by mating between related individuals in the last generation. In all analysed breeds, the average values of  $F_X$  were twice lower than the average relatedness coefficient ( $AR$ ). Inbreeding increase can be expected in the next generation. The population of Czech Gold Speckled Hen showed the lowest

Table 2. Parameters describing the probability of gene origin, effective population size, parameters of inbreeding and genetic diversity loss of Czech Gold Speckled Hen (CGSH), Czech White Goose (CWG) and Czech Crested Goose (CCG) breeds

	CGSH	CWG	CCG
Number of animals	276	182	72
Total number of founders ( $f$ )	127	97	47
Effective number of founders ( $f_e$ )	55.66	58.45	29.03
Effective number of ancestors ( $f_a$ )	30.88	41.37	21.37
Founder genome equivalent ( $f_{ge}$ )	15.344	20.49	10.797
Effective number of founders/effective number of ancestors ratio ( $f_a/f_e$ )	0.555	0.708	0.736
Founder genome equivalent/effective number of founders ratio ( $f_{ge}/f_e$ )	0.276	0.351	0.372
Realized effective population size ( $N_{eF}$ ) <sup>1</sup>	107.623	46.099	64.473
Realized effective population size ( $N_{eC}$ ) <sup>2</sup>	74.093	60.140	34.949
$N_{eC}/N_{eF}$	0.688	1.305	0.542
Average inbreeding coefficient ( $F_X$ )	0.021	0.019	0.020
Range of inbreeding coefficient ( $F_X$ )	0.00–0.19	0.00–0.25	0.00–0.25
Average inbreeding coefficient ( $F_{new}$ )	0.017	0.016	0.019
Range of inbreeding coefficient ( $F_{new}$ )	0.00–0.14	0.00–0.25	0.00–0.25
Average relatedness coefficient ( $AR$ )	0.065	0.049	0.093
$\Delta F_i$ per generation	0.005	0.010	0.008
Average generations interval	1.355	4.096	3.813
Loss of genetic diversity due to:			
Unequal founder contribution and random genetic drift	0.033	0.024	0.046
Unequal founder contribution	0.009	0.012	0.017
Random genetic drift	0.024	0.012	0.029

<sup>1</sup>Gutierrez et al. (2008); <sup>2</sup>Cervantes et al. (2011)

average rate of inbreeding ( $\Delta F_i = 0.50\%$ ). The highest average rate of inbreeding was estimated in the population of Czech White Goose. All these coefficients are strongly affected by the quality and depth of pedigree. The estimated value of the equivalent numbers of completely known generations did not reach the value equal to 5. Therefore it can be assumed that  $F_X$  values and other parameters derived from this parameter may be underestimated in all analysed populations.  $\Delta F$  may be considered a more efficient tool for the study of genetic diversity (Falconer and Mackay 2009). The Food and Agriculture Organization of the UN (FAO 1998) stated that the value of  $\Delta F$  should not exceed 1%. This value was exceeded only in the Czech White Goose. This value means that on average less than 1% of heterozygosity was lost per generation in the analysed breeds. Higher values of  $\Delta F_i$  were presented by Pham et al. (2016) for the Taiwan chicken population (Ju-Chi, Hua-Tung and Shek-Ki). The average inbreeding coefficients of both Czech goose breeds reached higher values. Czech Gold Speckled Hen reached the lower average  $F_X$  value than the average  $F_X$  estimated from other Czech local livestock populations (Figure 1).

The effective population sizes (Table 2) reached the values of  $N_{eF} = 107.62$ , 46.10 and 64.47 and  $N_{eC} = 74.09$ , 60.14 and 34.95 in the reference population for the Czech Gold Speckled Hen, Czech

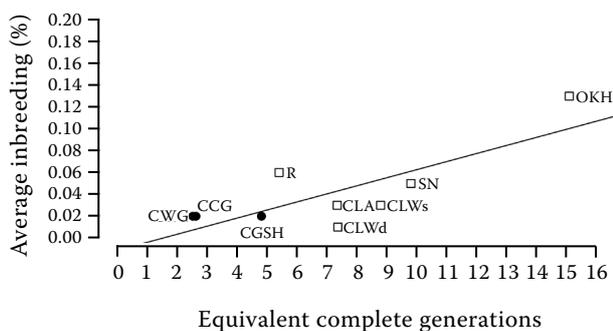


Figure 1. Comparison between mean equivalent complete generations and mean inbreeding coefficients for Czech Golden Spotted Hen (CGSH), Czech White Goose (CWG) and Czech Crested Goose (CCG) breeds analysed as well as for other Czech local livestock populations [Old Kladruber horse (OKH) – Vostra-Vydrova et al. (2016b), Silesian Noriker (SN – horse) – Vostra-Vydrova et al. (2016a), Romanov sheep (R) – Vostry et al. (2018), CLA, Czech Landrace (CLA – pig), Czech Large White dam line (CLWd – pig), Czech Large White sire line (CLWs – pig) – Krupa et al. (2015)]

White Goose and Czech Crested Goose, respectively. The estimated values of  $N_{eF}$  and  $N_{eC}$  of analysed breeds were higher than 50, the recommended minimum  $N_e$  for the conservation of genetic diversity (FAO 1998). The only exceptions were the Czech White Goose for  $N_{eF}$  and the Czech Crested Goose for  $N_{eC}$ . Additionally, the equivalent subpopulations ( $N_{eC}/N_{eF}$ ) (Cervantes et al. 2011) calculated in this study showed values lower than 1 in the Czech Gold Speckled Hen and Czech Crested Goose. Information on the occurrence of non-random mating between individuals from different subpopulations (farms) can be obtained from the difference between  $N_{eC}$  and  $N_{eF}$ . However, this type of mating increases the value of coancestry in the population. This mating is associated with a decrease in the level of genetic diversity in future. The estimated values of  $N_{eF}$  and  $N_{eC}$  in this study were lower than  $N_e$  values estimated in other poultry populations: Mazandaran native fowls ( $N_{eF} = 156$  and 97) according to Gholizadeh (2017) and Zatorska goose ( $N_e = 111$ ) according to Graczyk et al. (2015). The higher values of  $N_{eF}$  and  $N_{eC}$  were estimated than for two divergently selected lines of chickens ( $N_{eF} = 38$  and 32) according to Marquez et al. (2010) and eight Taiwan chicken populations ( $N_{eC}$  in range 21 to 35) according to Pham et al. (2016).

The parameters describing the genetic variability of analysed populations derived from the probability of gene origin are presented in Table 2. The reference populations were based on 127, 97 and 47 founders for the Czech Gold Speckled Hen, Czech White Goose and Czech Crested Goose, respectively. The effective number of founders ( $f_e$ ), the effective number of ancestors ( $f_a$ ) and the founder genome equivalent ( $f_{ge}$ ) showed similar values among the breeds. The measure of the probability of gene origin, including the  $f_e$  parameter, is essential for the assessment of whether the founder contribution is balanced (Lacy 1989).

If the founders contributed equally, the effective number of founders would be equal to the number of ancestors. The estimated values of these coefficients indicate the loss of genetic diversity caused by unbalanced contributions of founders and ancestors in the reference populations of analysed breeds. The genetic variability of the Czech Gold Speckled Hen, Czech White Goose and Czech Crested Goose indicates the unbalanced contributions of 31, 41 and 21 ancestors, representing the effective number of ancestors. The  $f_{ge}$  presented twice lower values than

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the  $f_a$  in the analysed breeds. The ratios of  $f_e/f_a$  and  $f_{ge}/f_e$  illustrated that the analysed populations were affected by bottlenecks and genetic drift. If the  $f_e/f_a$  ratio were close to 1, there is no bottleneck effect in the analysed population. The value of  $f_{ge}/f_e$  and  $f_e/f_a$  ratios was different from 1 and ranged from 0.55 to 0.74. When a bottleneck affects the genetic variability of a population, the  $f_a$  parameter should be preferred (Lacy 1989). The  $f_e$  parameter ignores the possible bottleneck effect. The bottleneck effect detected in this study was promoted by the overuse of a few sires or dams in the mating process. The  $f_a$  value suggests that the bottleneck effect identified in this study was promoted by the unbalanced contribution of breeding animals. The  $f_e/f_a$  ratios calculated in the present study were similar to values from other reports involving poultry populations [e.g., Marquez et al. (2010); Pham et al. (2016)].

Table S1 in ESM shows the cumulative marginal contributions of the most important ancestors. In the Czech Gold Speckled Hen, the most important ancestor explained 10% of the total genetic variability. The first ten ancestors were necessary for the explanation of 47% of the genetic variability. Among the first ten most important ancestors of the Czech Gold Speckled Hen all were born after 2009. Similar results were observed in both breeds of geese. Unlike the hens, females predominated in the ten most important ancestors of geese.

The total amount of genetic diversity ( $GD$ ) loss in the studied populations since 2002 is presented in Figure S4 in ESM. The average relative  $GD$  losses in the reference populations were 3.3%, 2.4% and 4.6% for the Czech Gold Speckled Hen, Czech White Goose and Czech Crested Goose, respectively. The loss of  $GD$  due to genetic drift ( $GD^* - GD$ ) was more important than the loss resulting from the unequal contribution of founders in both breeds. A decline in genetic diversity has been observed over the past 17 years (Figure S4 in ESM). This genetic drift was mostly influenced by the small number of mating animals, especially in the goose populations during the entire breeding period.

The overall difference between two analysed goose breeds expressed using a fixation coefficient ( $F_{ST}$ ) indicated low differentiation between them (Table 3). These low differentiations are caused by the fact that the Czech Crested Goose was derived from the Czech White Goose. Moreover, the Czech White Goose was still regularly used in the breeding of the Czech Crested Goose. The  $F_{ST}$  values

Table 3. The value of  $F_{IS}$  on diagonal, the value of  $F_{ST}$  above diagonal, genetic distances between breeds ( $D_{ij}$ ; Nei 1987) below diagonal of Czech White Goose (CWG) and Czech Crested Goose (CCG) breeds

	CWG	CCG
CWG	-0.025	0.014
CCG	0.032	-0.007

showed that 1.4% of genetic diversity was lost due to the subdivision of the total metapopulation into two breeds (Czech White Goose and Czech Crested Goose). It follows that 98.6% of the total genetic variability resulted from differences between individuals, regardless of the breed. The remaining 1.4% of the total genetic variability was expressed in genetic differences between the populations of the studied breeds. The average coancestry value between the Czech White Goose and the Czech Crested Goose also confirmed the gene flow between the breeds in the past (Table 4). According to this measure, the individuals of Czech White Goose and Czech Crested Goose breeds share 3% of their genes. The  $F_{ST}$  value obtained in this analysis was lower than the total  $F_{ST}$  values published by Zanetti et al. (2010) for seven Italian local chicken breeds based on microsatellite analysis ( $F_{ST}$  in the range of 0.035 to 0.142).

Table 4. Parameters describing the metapopulation of Czech White Goose and Czech Crested Goose breeds

Self-coancestry	0.510
Inbreeding	0.019
Average coancestry	0.031
Average genetic distance	0.013
Average coancestry over metapopulation	0.017
$F_{IS}$	-0.012
$F_{ST}$	0.013
$F_{IT}$	0.002
$GD_T$	0.983
$GD_{WI}$	0.491
$GD_{WS}$	0.970
$GD_{BI}$	0.479
$GD_{BS}$	0.013

$F_{IS}$ ,  $F_{ST}$  and  $F_{IT}$  = Wright's fixation coefficients (Wright 1949);  $GD_{BI}$  = genetic diversity between individuals;  $GD_{BS}$  = genetic diversity between subpopulations;  $GD_T$  = total genetic diversity;  $GD_{WI}$  = genetic diversity within individuals;  $GD_{WS}$  = genetic diversity within subpopulations

The  $GD_{WS}$  and  $GD_{BS}$  values were consistent with the  $F_{ST}$  values, while the values of  $GD_{BS}$  were significantly lower than those of  $GD_{WS}$  (Table 4). The contribution of genetic diversity within individuals ( $GD_{WI}$ ) was higher than that between individuals ( $GD_{BI}$ ). This value suggested the low genetic differentiation between the breeds. Standard Nei's genetic distance values also indicated low genetic differentiation overall (Table 3). The degree of heterozygosity within subpopulations due to non-random mating is described by the parameter  $F_{IS}$ . Negative values of  $F_{IS}$  were estimated for the metapopulation and both breeds of geese (Tables 3 and 4). These negative values indicated a higher ratio of observed heterozygosity to expected heterozygosity. The  $F_{IS}$  values estimated for both analysed breeds were very close to zero.

## CONCLUSION

The pedigree information on the three autochthonous Czech poultry breeds was used to estimate the level of genetic diversity. The results of the analyses showed a loss of genetic diversity in all analysed breeds resulting mainly from low effective numbers and unequal contributions of founders and ancestors. The low values of the effective population size were observed in particular based on the average coancestry coefficient. The average relatedness coefficients were more than twice higher than  $F_X$ , especially in the Czech Gold Speckled Hen, so the increase of inbreeding coefficient in the next generations can be expected. Thus, based on these results, the Czech poultry breeds showed a high probability of allele loss and consequent loss of genetic diversity.

## Conflict of interest

The authors declare no conflict of interest.

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