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**To cite this article:** Giuseppe Carcò, Bartosz Grajewski, Martino Cassandro, Mirosław Lisowski & Tomasz Szwaczkowski (2018): Genetic variability of some Italian and Polish duck breeds, Italian Journal of Animal Science, DOI: <u>10.1080/1828051X.2018.1436006</u>

To link to this article: https://doi.org/10.1080/1828051X.2018.1436006

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Published online: 09 Feb 2018.

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# Genetic variability of some Italian and Polish duck breeds

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#### ABSTRACT

This study is aimed to estimate and compare the inter- and within-breed variability of duck populations under genetic conservation programmes. The following four duck breeds were analysed: Germanata Veneta (AGV) and Mignon (AMG) from Italy, Pekin Krajowy (33P) and Pomniejszona (2K) from Poland. The characterisation of the four populations was carried out through a panel of 23 microsatellite markers. The analysis involved 180 individuals: 39 for AGV, 41 for AMG, 50 for 33P and 50 for 2K. An average of 11.36 alleles per locus was identified. Twenty-two loci showed high values of polymorphism information content from 0.575 to 0.912, while CAUD136 was monomorphic for the Italian breeds. The breeds showed relatively high heterozygosity: higher for the Polish populations (0.6920 for 33P and 0.6521 for 2K), and lower for the Italian (0.4497 and 0.3718 for AGV and AMG, respectively). The inbreeding coefficient was higher for the Italian breeds, AMG in particular (0.133, 0.097 and 0.121), as well as the differentiation index (0.253). The Nei's minimum distances  $(D_M)$  and Reynolds distances  $(D_R)$  were low between the Polish populations (0.131 and 0.088, respectively); these were associated to AGV ( $D_{\rm M}$  = 0.191 and  $D_{\rm R}$  = 0.259 for 33P;  $D_{\rm M}$  = 0.174 and  $D_{\rm R}$  = 0.226 for 2K). Finally, AGV was distant from AMG  $(D_{\rm M}=0.259$  and  $D_{\rm R}=0.317$ ). The molecular coancestry, or mean kinship was higher for the Italian breeds compared to Polish populations. The Italian populations showed intermediate values. The obtained results can be perceived as an important tool for the applied genetic conservation programmes.

### Introduction

Duck breeding for meat and egg production is a widespread activity in many developing countries, but, as in the poultry industry, is supported only by few genetically unified commercial lines. These strains have a restricted genetic variation, which results in their ability to produce only in specialised management and controlled environmental conditions (Delany 2003). Therefore, even in the duck, as well as other livestock species (Oldenbroek 1999), the selection of high productive genotypes has led to the loss of numerous local breeds. Indeed, today in the world there are 398 breeds from Anas platyrhynchos domesticus species, 48 from Cairina moschata and 15 hybrids from the two species, but the majority of them is still at risk of extinction (DAD-IS 2017, FAO). Among the endangered populations, only two breeds are registered in Italy, both reared in Veneto region, while twenty-five breeds are counted in Poland, where there is a long culinary tradition based on duck products.

In this context, numerous conservation programmes have involved local duck populations with the aim to preserve their genetic diversity. Indeed, their protection from the extinction allows conserving those traits of adaptability necessary to face future changes in the environmental and production conditions. In this case, local breeds could be perceived as good components for crossbreeding schemes to create more resistant commercial lines. Moreover, their productions have promoted a diversification of poultry product market around the world.

The conservation activities are today supported by the study of molecular analysis (Davoli 2011), and different classes of molecular markers have shown a

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#### **ARTICLE HISTORY**

Received 31 August 2017 Revised 5 December 2017 Accepted 11 December 2017



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great applicability in the study of genome, for the evaluation of genetic variability within and among the populations (Khan Ahmadi et al. 2007; Colli et al. 2011). By contrast to chicken, knowledge about the genome of the duck species is still scarce. In the recent literature, microsatellites were recommended as the best and easiest-to-use markers for the character-isation of duck genome, while only one study had identified a set of SNPs in the *Anas platyrhynchos domesticus* species (Kraus et al. 2011).

In our study, twenty-three microsatellite markers were used to estimate the intra genetic variability of some Italian and Polish populations with the aim to verify the effectiveness of their conservation programmes and to evaluate the genetic distances among the populations, from evolutionary perspective.

#### **Material and methods**

#### Birds

The experimental procedures were approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padua (Italy) and the Local Ethical Commission for Animal Experiments in Cracow (Poland).

The study involved 180 ducks of four Italian and Polish breeds. Thirty-nine Germanata Veneta ducks (AGV) and forty-one Mignon ducks (AMG) were sampled in two conservation centres located in Veneto region: I.I.S. 'Antonio Della Lucia', and Experimental Farm 'Sasse Rami'. Venous blood samples were conserved into Vacutainer tubes containing sodium citrate as anticoagulant agent. Instead, clavicle blood samples from 50 Pekin Krajowy ducks (33P) and 50 Pomniejszona ducks (2K) from Waterfowl Genetic Resource Station of National Research Institute of Animal Production in Dworzyska was stored into tubes with EDTA. A serial number identified all individuals of each population. The blood samples were refrigerated and stored at -20 °C until DNA extraction.

#### Molecular procedures

Molecular analysis was carried out by the Laboratory of Molecular Biology Techniques of the Faculty of Biology at Adam Mickiewicz University of Poznan (Poland).

The DNA extraction was performed using DNeasy Tissue from Quiagen. Twenty-three markers were selected from the literature for the DNA amplification (Buchholz et al. 1998; Maak et al. 2003; Huang et al. 2005; Huang et al. 2006). The loci were amplified using PCR Multiplex with fluorescently tagged primers, according to the procedure described by Mucha et al. (2014). Polymerase chain reactions were performed on a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA) using Type-it Microsatellite PCR Kit Qiagen (Cat No./ID: 206243). PCR reaction for each multiplex panel was set up in a 10  $\mu$ L volume which contained 5  $\mu$ L 2x concentrated reaction mix Type-it, 1 µL of DNA matrix (approximately 50 ng) and each of the primers with 0.25 µM concentration. The amplification conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at a specific temperature (Table 1) for 90 s, extension at 72 °C for 30 s, with a final extension at 60°C for 30 min. In addition, data from loci CAUD013 and CAUD019 were collected from previous studies (Cassandro et al. 2014; Mucha et al. 2014).

Products of amplification were diluted with 100  $\mu$ L of ddH<sub>2</sub>O and 1  $\mu$ L of this solution was added to 9  $\mu$ L of formamide containing 0.5  $\mu$ L of DNA GeneScan-600 LIZ Size Standard (Applied Biosystems). The solutions were arranged in a specific 96-well plate and denatured for 5 min at 95 °C. Capillary electrophoresis was performed in ABI Prism 3130 XL (Applied Biosystems), 36 cm long capillaries, polymer POP7 and G5 filter. The allele sizes were read in Peak Scanner version 1.0 (2006; Applied Biosystems, http://www.appliedbiosystems.com).

#### **Statistical methods**

Twenty samples showing data for less than 20 loci were excluded from the analysis. Therefore, the final dataset counted 160 individuals: 37 for AGV, 40 for AMG, 44 for 33P and 39 for 2K. The analysis of the genetic diversity within and between the populations considered the following parameters:

- The total allele number, the allele size for each locus, the allele frequencies, the expected and observed heterozygosity were computed by GENETIX software (Institut de Sciences de l'Evolucion, Montpellier, France) (Belkhir et al. 1996–2004).
- The polymorphism information content (PIC) was estimated by CERVUS 3.0.7 software (Field Genetics, London, UK) (Marshall et al. 1998; Kalinowski et al. 2007).
- The F-statistics (F<sub>ST</sub>, F<sub>IS</sub>) and the molecular coancestry (*f*<sub>ij</sub>) were computed by MOLKIN 3.0 (Gutiérrez et al. 2005), comparing each population in pair with another one. In this way, the results were obtained for the following six meta-populations: AGV vs. AMG; AGV vs. 33P; AGV vs. 2K; AMG vs. 33P; AMG vs. 2K; 33P vs. 2K.

Locus	GenBank accession	Primer sequences (5'-3')	TA, °C
CAUD038 AY493283		AY493283 GATAATGGCTGGCTCCTTGA	
		GACCACAACATCGTGCAGAG	
CAUD024	AY493269	TCGCATTAAGCTCTGATCT	55.5
		ATCAACAGAATCCAAAATATG	
CAUD050	AY493295	GGACAAGTGGCATGTGTCAT	66.0
		GGCTTCTGTGCTCCTCAGAT	
CAUD117	AY587036	GCCTTCATTCCTCTGCTAC	63.5
		GCTCATCCCTGCTGCTCA	
CAUD069	AY493314	CAGCATTATTATTTCAGAAGG	50.3
		CTCATTCCAATTCCTCTGTA	
CAUD070	AY493315	GTAACAACTCAGTGCTTTCAA	55.5
		GTAAGTATTGACAGAGACATC	
CAUD120	AY587039	AATATCCTGTCGCCGTGGT	60.8
		AATTCTTGCTGAGATTATAGAG	
CAUD126	AY587045	TTGCCACATAAACCCACTAC	50.3
		CAGAGAATTTTAGTAAGAGT	
CAUD111	AY587030	TGACATTACACACCCAAAC	53.2
	,	CAAGGGCAGGGGTAAGGAT	5512
CAUD013	AY493258	ACAATAGATTCCAGATGCTGAA	58.1
	/11.00200	ATGTCTGAGTCCTCGGAGC	5011
CAUD026	AY493271	ACGTCACATCACCCCACAG	60.8
0.000020	/	CTTTGCCTCTGGTGAGGTTC	0010
CAUD124	AY587043	CCAGCCAAGAACCTCCAGT	50.3
	,	CTTTGAATGTCCATGTAGCAG	5015
CAUD093	AY493338	AGAGCGGTGTGAGAGCAGAG	55.5
		GATATCGCTCGCAATTTTGG	5515
CAUD112	AY587031	CAACTGACAGAGAGGGGCACG	58.1
0.000.112	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	GACTGTGTTTCCAATGCTCC	5011
CAUD060	AY493305	AGAAAGCTCCTGTATGTGAT	58.1
		ATGCTGGTGTGAGATTTGAA	5011
CAUD040	AY493285	TGTGTAACCCTGATAGACTGA	50.3
		TCCCACCCCAAACCCTGC	
CAUD019	AY493264	CTTAGCCCAGTGAAGCATG	58.1
		GCAGACTTTTACTTATGACTC	
CAUD086	AY493331	AACACAGCTTCACCCCACAG	58.1
		GCAGAGCGGTGTGAGAGCA	
CAUD136	AY587055	GTTGCATGAAAAAGGAAAGG	63.5
	,	GGAAGATAGAAGATGGAATG	0010
CAUD036	AY493281	AAGTTGGGAGAGGAGTCAG	55.5
	/// ///201	CTAAGGCTTTTCCAGAATGC	5515
CAUD091	AY493336	GAAAAAGGCAGCACAGCAC	58.1
		GCAAAGTTGAGGCATGTAATC	55.1
CAUD039	AY493284	GGGACATCTCTTGGAGCAAA	60.8
		AGTGAAAGCTGCTGCTGGAT	00.0
CAUD082	AY493327	ATGTAAAGCAAGGAAGAGCC	60.8
		AAGAGTCTGAGCCAAGCAC	00.0

Table 1. Characteristics of duck microsatellite markers.

TA: annealing temperature.

Finally, genetic distances among the four populations were estimated by different approaches:

- Nei's minimum distance (D<sub>M</sub>) (Nei 1973);
- Reynolds distance (*D*<sub>B</sub>) (Reynolds 1983);
- Euclidean distance (*D*<sub>E</sub>) (Nei and Tajima 1981).

The three genetic distances computations were performed using GENETIX, MOLKIN 3.0 and SAS (2009) package programmes, respectively.

#### **Results and discussion**

#### Polymorphism of the microsatellites

The total number of alleles for the 23 microsatellites was 261, with an average of 11.36 (Table 2).

The highest number of alleles was 26 (CAUD024) and the lowest was four (CAUD136). The CAUD024 showed the longest fragments (maximum 485 bp), while the shortest fragments were observed for CAUD111 (minimum 68 bp). According to Barker (1994), microsatellite markers used in the estimation of genetic distance should have more than four alleles, in order to reduce the standard errors of distance estimation. Therefore, the microsatellites of this study were good indices for the analysis of genetic variability.

Furthermore, the populations showed 113 private alleles, namely, allelic forms specific for each breed: 11 for AGV, 9 for AMG, 51 for 33P and 42 for 2K. Among these, only 12 had a frequency higher than 15%. The different management practices, intended to avoid the crossbreeding, were probably the cause of this good

differentiation (Zanetti et al. 2010). Thus, the great presence of private alleles may help in the future development of genetic trace back protocols, aimed to distinguish the products of the four populations from others with different origin (Dalvit et al. 2007).

PIC measures the quantity of information of each microsatellite and depends on the number of alleles identified and the allele frequencies (Purwantini and

Table2. Numberofallelesandallelesizeof23microsatellites.

		Allele	size, bp
Locus	Number of alleles	Min	Max
CAUD050	19	265	427
CAUD024	26	237	485
CAUD117	12	264	436
CAUD038	17	212	364
CAUD070	18	228	296
CAUD126	16	221	407
CAUD120	5	271	279
CAUD069	13	173	249
CAUD112	5	208	308
CAUD040	18	229	321
CAUD093	7	202	220
CAUD060	22	169	325
CAUD086	6	170	194
CAUD124	5	138	148
CAUD136	4	167	192
CAUD036	7	136	148
CAUD039	8	196	210
CAUD091	6	170	186
CAUD026	5	142	170
CAUD111	11	68	170
CAUD013	10	83	113
CAUD019	15	131	209
CAUD082	6	130	170

Purwantini 2010). Normally, the diversity of a locus is low when PIC < 0.25 and high when PIC > 0.5 (Botstein et al. 1980). In this study, the average PIC of all sites and all populations was 0.753, with 22 microsatellites showing high diversity (Table 2). Only the CAUD136 had a low value (0.274), resulting monomorphic for the two Italian breeds. Since its large number of allelic forms, CAUD024 was the most polymorphic locus, with a PIC value of 0.912.

Our results did not differ from those of Huang et al. (2005), who included CAUD024, CAUD013 and CAUD019 for the characterisation of duck genome. In that study CAUD024 had the highest PIC (0.880), and CAUD019 and CAUD013 were high polymorphic (0.870 and 0.630, respectively). Moreover, in the analysis performed by Mucha et al. (2014) for crossbreed populations, some loci of the present panel (CAUD112, CAUD39, CAUD126, CAUD024, CAUD069 and CAUD117) showed good PIC values, often similar to our results.

#### Genetic diversity within the breeds

By definition, the expected heterozygosity represents the probability that an individual chosen randomly from a population in Hardy Weinberg equilibrium is heterozygote, while the observed heterozygosity indicates the effective proportion of heterozygotes in each locus. Table 3 shows these results. The different loci showed a wide variation among populations.

Table 3. Average PIC, expected and observed heterozygosity for each locus across populations.

			Populations						
			Expected heterozygosity			Observed heterozygosity			
Locus	PIC	AGV	AMG	33P	2K	AGV	AMG	33P	2K
CAUD050	0.815	0.104	0.265	0.845	0.832	0.108	0.050	0.932	0.744
CAUD024	0.912	0.815	0.508	0.904	0.899	0.838	0.475	0.955	0.897
CAUD117	0.833	0.545	0.372	0.830	0.799	0.514	0.250	0.818	0.744
CAUD038	0.863	0.659	0.489	0.721	0.822	0.784	0.375	0.682	0.795
CAUD070	0.852	0.579	0.229	0.867	0.802	0.541	0.050	0.796	0.744
CAUD126	0.817	0.641	0.502	0.817	0.859	0.703	0.425	0.818	0.872
CAUD120	0.649	0.505	0.359	0.680	0.734	0.405	0.350	0.773	0.692
CAUD069	0.847	0.693	0.511	0.878	0.857	0.757	0.450	0.886	0.897
CAUD112	0.575	0.533	0.583	0.665	0.549	0.432	0.375	0.705	0.513
CAUD040	0.830	0.321	0.451	0.844	0.709	0.361	0.300	0.864	0.718
CAUD093	0.753	0.494	0.690	0.670	0.701	0.568	0.575	0.796	0.615
CAUD060	0.806	0.471	0.415	0.915	0.814	0.514	0.325	0.837	0.923
CAUD086	0.735	0.400	0.675	0.469	0.550	0.000	0.250	0.364	0.308
CAUD124	0.706	0.636	0.545	0.723	0.784	0.649	0.450	0.705	0.795
CAUD136	0.274	0.000	0.000	0.404	0.551	0.000	0.000	0.302	0.211
CAUD036	0.737	0.419	0.477	0.635	0.576	0.028	0.050	0.182	0.282
CAUD039	0.792	0.548	0.643	0.668	0.774	0.487	0.575	0.682	0.697
CAUD091	0.742	0.493	0.653	0.632	0.711	0.444	0.700	0.546	0.455
CAUD026	0.619	0.471	0.162	0.665	0.374	0.460	0.125	0.419	0.385
CAUD111	0.801	0.564	0.503	0.530	0.746	0.595	0.500	0.568	0.744
CAUD013	0.823	0.561	0.650	0.754	0.783	0.571	0.649	0.841	0.821
CAUD019	0.857	0.185	0.643	0.799	0.770	0.028	0.778	0.659	0.790
CAUD082	0.689	0.540	0.503	0.816	0.356	0.559	0.475	0.791	0.359

PIC: polymorphism information content; AGV: Germanata veneta; AMG: Mignon veneta; 33P: Pekin Krajowy; 2K: Pomniejszona.

CAUD024 had high values for three populations (0.838, 0.955 and 0.897 for AGV, 33P and 2K, respectively). In this locus, 33P showed the highest value of observed heterozygosity. AMG had the lowest value in CAUD050 and CAUD036 (0.050). In addition, CAUD136 resulted monomorphic for the Italian populations, where heterozygote individuals were not present.

The average observed heterozygosity was lower than expected, proving that the four breeds were not in in Hardy–Weinberg equilibrium (Table 4). Hence, the values differed from the results obtained in the previous characterisation of the Italian duck breeds (Cassandro et al. 2014), where AGV was in balance. However, our panel showed only two loci in common with the previous work (CAUD013 and CAUD019).

The average observed heterozygosity showed a high genetic variation for the Polish population, 0.692 and 0.652, respectively, while the Italian breeds had lower values: 0.450 for AGV and 0.372 for AMG. Khan Ahmadi et al. (2007) found similar levels of genetic variation within the Peking and Muscovy populations, which showed values of heterozygosity of 0.530 and 0.440, respectively. The high levels of inbreeding observed in the two populations were the main cause of the lower heterozygosity observed. This could be also the situation of the Italian duck populations, where the mean kinship was hiah (0.530). Nevertheless, the average values of heterozygosity and PIC for the Italian breeds were higher in comparison with those found by Cassandro et al. (2014) (0.300 and 0.270 for AGV and AMG, respectively).

The mean PIC values showed high diversity of the Polish breeds (0.682 and 0.665 for 33P and 2K, respectively), and intermediate for the AGV and AMG (0.408 and 0.414, respectively).

These results are consistent with the findings of Wu et al. (2009), who found similar values of mean PIC and observed and expected heterozygosity, when

comparing Beijing (BJ) and Charrey Valley (CV) ducks: 0.570, 0.600 and 0.510 in BJ duck; and 0.590, 0.630 and 0.530 in CV duck, respectively.

Finally, the mean allele number showed considerable differences only between the Italian and the Polish groups, while the high values observed in 33P and 2K indicated the great variability within the two populations.

Barati et al. (2009) evaluated the genetic diversity between two urban populations of mallards living in Arno and Mugnone rivers, a captive-bred breed and the wild local population of Massaciuccoli lake in Italy. A panel of 11 microsatellites showed an overall allele number of 87, with an allele size ranging between 88 and 287 bp. The urban populations and captive-bred strain showed similar values of observed heterozygosity (0.520, 0.540 and 0.570, respectively), slightly higher than AGV and AMG, while, as expected, the wild population had the highest value of observed heterozygosity 0.680, similar to 33P and 2K. These results suggested a good management of the conservation activities for the four breeds of this study, which showed levels of diversity similar to those found in wild populations, where the natural processes of migration and random mating, as well as the absence of artificial selection might have positive effects on their genetic variability (Keller et al. 2001).

#### Genetic structure of the four populations

The genetic variation among the four populations was measured by the F-statistics. Table 5 shows the inbreeding coefficients ( $F_{IS}$ ) and the heterozygosity deficiencies ( $F_{ST}$ ) of the overall populations due to the inbreeding within each subpopulation. Basically,  $F_{ST}$  considers the differences between the individuals from different breeds. Hence, a high diversity between two populations can occur when the individuals from the

Table 4. Average expected and	observed	heterozygosity, average I	PIC and average num	ber of allele	s across the populations.
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Populations	H exp.	SD	H obs.	SD	PIC	SD	Number of alleles	SD
AGV	0.486	0.0391	0.450	0.017	0.408	0.168	3.430	1.670
AMG	0.471	0.037	0.372	0.016	0.414	0.154	3.870	1.520
33P	0.727	0.029	0.692	0.015	0.682	0.150	7.390	4.040
2K	0.711	0.031	0.652	0.016	0.665	0.156	7.090	2.920

H exp.: expected heterozygosity; SD: standard deviation; H obs.: observed heterozygosity; PIC: polymorphism information content; AGV: Germanata veneta; AMG: Mignon veneta; 33P: Pekin Krajowy; 2K: Pomniejszona.

 Table 5.
 F<sub>ST</sub> and F<sub>IS</sub> indices among six metapopulations.

			Metapop	ulations		
	AGV vs. AMG	AGV vs. 33P	AGV vs. 2K	AMG vs. 33P	AMG vs. 2K	33P vs. 2K
F <sub>ST</sub>	0.253	0.174	0.160	0.179	0.204	0.085
F <sub>IS</sub>	0.133	0.047	0.066	0.097	0.121	0.051

AGV: Germanata veneta; AMG: Mignon veneta; 33P: Pekin Krajowy; 2K: Pomniejszona.

F1

same breed show great uniformity. According to Wright (1978), the diversification among the populations is moderate when  $F_{ST} < 0.05$  and high when  $F_{ST} > 0.15$ . The results for the Polish breeds (33P vs. 2K), indicated that only 8.5% of the genetic variation was between the populations, while the 91.5% was within the breeds. This great similarity suggests the origin of the Polish breeds from common ancestors present in Central Poland. Wu et al. (2009) found similar results in the genetic comparison of two natural populations of Beijing duck and two varieties of Charrey Valley. In this case, the low value of  $F_{ST}$  (0.08) between the breeds was explained by the breeding history of Charrey Valley duck, which derives from the hybridisation between Beijing and Aylesbury ducks.

The Italian populations (AGV vs. AMG) showed a good differentiation, with the 25.3% of the total

**Table 6.** Nei's minimum distance (above the diagonal) and Reynolds distance (below the diagonal) among the four populations.

	AGV	AMG	33P	2K
AGV	0.000	0.317	0.259	0.226
AMG	0.291	0.000	0.261	0.296
33P	0.191	0.198	0.000	0.131
2K	0.174	0.229	0.088	0.000



genetic variation caused by the differences between the two breeds and the remaining 74.7% depending on the differences of individuals within breeds. In the study of Tadano et al. (2007) the high mean  $F_{ST}$  values among Japanese long-tailed chicken breeds (0.380) suggested a great diversity among these populations due to higher values of inbreeding within the breeds and an intensive selection to fix desirable traits. Thus, the great variability of  $F_{ST}$  values may reflect the effect of artificial selection, as Su and Chen (2009) explained in their study on genetic variability among four Chinese local laying-type ducks.

Furthermore, higher  $F_{ST}$  values between AMG and the Polish populations (0.179 and 0.204, respectively), indicated a good differentiation among these breeds, while AGV showed slightly smaller diversity compared

 Table 7. Average molecular co-ancestry within the metapopulation (MeanKin) and the breeds (MeanKinSubp).

		·
Populations	MeanKin	MeanKinSubp
AGV vs. AMG	0.371	0.530
AGV vs. 33P	0.420	0.390
AGV vs. 2K	0.293	0.407
AMG vs. 33P	0.273	0.404
AMG vs. 2K	0.271	0.420
33P vs. 2K	0.224	0.289

AGV: Germanata veneta; AMG: Mignon veneta; 33P: Pekin Krajowy; 2K: Pomniejszona



Figure 1. Euclidean distances among the four population. AGV: Germanata veneta; AMG: Mignon veneta; 33P: Pekin Krajowy; 2K: Pomniejszona.

The Nei's minimum distance  $(D_M)$ , the Reynolds distance  $(D_{\rm R})$  and the Euclidean distance  $(D_{\rm F})$  among the four populations were very similar each other: the genetic relationship between the Polish populations was very high; the Polish cluster was related to AGV, while AMG was the farthest population (Table 6). A dendrogram based on the Euclidean distance confirmed this structure (Figure 1). This result was consistent with the breeding history of the four populations. The low diversity between 33P and 2K may be attributed to geographical location, as sugaested their bv Purwantini and Purwantini (2010), who found a small genetic distance (0.170) in two Indonesian local breeds that lived in the same area. According to these authors, a large genetic divergence can occur in condition of low geographical isolation and abundant diversity within the breeds. As already mentioned, the Polish breeds probably derive from the same local ancestors. This condition and their current farming in the same area might have caused of crossing or gene flow phenomena and the consequent loss of genetic diversity between the two breeds. Instead, the large genetic distance between the Italian populations suggested a marked differentiation of the two populations. The high values of  $D_M$  and  $D_R$  confirmed the great variability found by Targhetta et al. (2005) during the characterisation of Germanata and Mignon ducks through 71 AFLP markers.

Finally, the molecular co-ancestry  $(f_{ij})$  is the average kinship between an individual and the other members of the same population (Ballou and Lacy 1995). The parameter can assume values from 0 to 1, and lower values correspond to higher genetic diversity. The values of molecular co-ancestry within six meta-populations (MeanKin) and within each breed (MeanKinSubp) are reported in Table 7. The Polish breeds (33P vs. 2K) showed a great genetic diversity and reduced molecular co-ancestry (MeanKin = 0.224 and MeanKinSubp = 0.289), compared to the Italian breeds (AGV vs. AMG), where higher values of co-ancestry (MeanKin = 0.371 and MeanKinSubp = 0.530) confirmed their lower heterozygosity. Lastly, the values obtained by comparing an Italian population with a Polish breed (AGV vs. 33P, AGV vs. 2K, AMG vs. 33P and AMG vs. 2K) were intermediate.

## Conclusions

From conservation genetic programme perspective, the characterisation of the four duck populations through a

panel of 23 microsatellites showed acceptable levels of genetic diversity. The analysed microsatellites have high polymorphism, with a good number of alleles specific for each population. The Italian populations showed relatively high heterozygosity, despite the higher values of FIS and molecular co-ancestry, in particular for Mignon duck. In addition, the genetic distances and the factorial analysis showed a marked differentiation between the Italian populations, due to a large genetic homogeneity of the individuals. Conversely, the Polish populations showed large number of allelic variants for each locus, high values of heterozygosity and low values of molecular co-ancestry. On the other hand, low values of  $F_{ST}$  and a small genetic distance between the Polish populations suggest directing the conservation programmes towards a more pronounced diversification of the breeds. However, for all the four populations, the results confirm the effectiveness of the applied conservation programmes, which have led each breed to show its own genetic identity.

#### **Disclosure statement**

The authors declare that they have no competing interests.

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