National Rural Development Programme 2014-2022

Measure 10.2 – Biodiversity

Project: TuBAvI-2 (2021-2024)

REPORT ON THE ACTIVITIES PERFORMED DURING THE SECOND YEAR

UniTO

The present report describes the activities performed from May 1st, 2022 to April 30, 2023. The activities are described by Action, according to the original programme.

University of Turin performed activities within Actions 1, 2, and 4. Activities within Action 1 were performed as a consultant for Partner UniPI, and activities within Actions 2 and 4 were perfomed as a consultant for Partner UniMI.

Action 1 – Phenotypical characterisation of autochtonous breeds and species

Task 1.1 - Phenotypic Characterization of Bianca di Saluzzo and Bionda Piemontese chickens breeds

The breeding roosters of Bianca di Saluzzo (BS) and Bionda Piemontese (BP) chicken breeds, raised at the Center for Conservation of Local Avian Genetic Resources (Interdepartmental Center PRISAn DSV-DISAFA) during 2022, were in their first reproductive cycle. In 2022, the BP breeders (n=80) were organized into 6 family groups, while the BS breeders (n=70) were organized into 5 family lines. Egg production and their weight were recorded daily. The data were processed on a weekly basis and presented in Figures 1.1-1.3.

In this report, it is possible to present the complete deposition curves of both breeds, integrating the previously presented data with those collected in the remaining part of 2022. For both breeds, the peak of egg laying occurred between weeks 33 and 36 of life, reaching a maximum of 77.4% for BS (Figure 1.1) and 74.3% for BP (Figure 1.2). The comparison between the deposition curves of the two breeds is jointly presented in Figure 1.3.

All the chickens were raised on the floor with permanent litter in a controlled environment, following standard guidelines for poultry farming. The recording of egg production and their weight for the second year of production is ongoing and will be the subject of the next report, which will present the complete data for the 2023 laying season.

In the spring of 2022 and 2023, a fertility and hatchability test was carried out for the two established breeds (BP and BS). Additionally, in 2023, 134 eggs were provided from the *Millefiori Piemontese* (MP) farms, which had been identified and genetically characterized in 2022, to initiate the reconstruction of the conservation nucleus.

Regarding BS, a total of 700 eggs were incubated in 2022 and 594 in 2023. As for BP, a total of 750 eggs were incubated in 2022 and 569 in 2023. Both in 2022 and 2023, incubation was conducted simultaneously to obtain contemporaneous subjects. The hatched chicks in 2022 were used for other purposes, while those hatched in 2023 were used for the internal breeding of BP and BS and the creation of the conservation nucleus for MP.

In Figure 1.4, the comparison of the trend of fertility and hatchability % in the *Bianca di Saluzzo* breed (during years 2022 and 2023), *Bionda Piemontese* breed (during years 2022 and 2023), and *Millefiori Piemontese* breed (during year 2023) is shown. Currently, phenotypic observations and growth measurements are being conducted for the three Piedmontese poultry breeds. The technical and scientific value of the 2023 data is significant, as it represents the first rigorously collected data for the MP breed. Figure 1.5 shows one-day-old chicks of *Millefiori Piemontese* breed.

Action 2 - Genetic characterization of breeds and species bred in Italy also through the use of genomic information (analysis service UNITO)

Task 2.1 Genetic characterization using microsatellite markers

Genetic characterization with microsatellite markers is designed to provide useful tools for biodiversity conservation in nucleus populations managed by the PA and in the semen cryobank. In particular, the following objectives are considered: 1) identifying candidate males for recovery and semen production; 2) characterizing new genetic lines in breeds under conservation; 3) characterizing new chicken breeds.

Task 2.1.1 Characterization of male candidates.

For recovery and management plans, the candidates present in the UNIPG conservation nuclei belonging to 5 populations (Ancona, Livorno in golden, silver, white, and black colorations) have been characterized. The candidates present in the UNIFI conservation nuclei and the offspring of the UNIMOL conservation nucleus are currently being characterized.

Task 2.1.3 Identification of new genetic lines in chicken breeds LI, AN, MB and SI

As part of the identification of new genetic lines, 26 individuals belonging to the Mericanel della Brianza breed, 17 females and 9 males, from local farms were genetically characterized.

The observed average heterozygosity (Ho=0.48) was analyzed as an estimate of inbreeding, as shown in the Figure 2.1, along with the median, standard deviation (SD), standard error (SE), maximum, and minimum values. The Hardy-Weinberg equilibrium test shows a significant deviation from equilibrium only at certain loci.

The overall distribution is discontinuous, although the mean and median values are very similar; individuals with high homozygosity (Ho<0.30) and low values of individual variability, indicating inbreeding, can be observed.

Principal component analysis highlights the existing genetic variability in the sampled group (Figure 2.2). The graph illustrates the distribution of subjects in terms of genetic diversity (subjects with higher similarity are closer in the graph, while genetically distant ones are farther apart). Based on genetic distance evaluations, females can be grouped into 2 families. (Figure 2).

The genetic variability of the analyzed subjects was compared with that of the subjects present in the conservation nucleus (Figure 2.3). The analysis of allele patterns (Figure 2.4) also reveals the presence of private alleles in the sampled family (27 private alleles).

Action 4 - Estimation of genetic and genomic indices and reproductive management

Task 4.1 Selection of males for reproduction and production of seminal material

The typing data were used to calculate the parentage matrix by assessing the number of shared alleles for each pair of individuals belonging to the same breed. The average kinship between all breeders (P)

and the average kinship with family lines (Pf) were calculated. Individual heterozygosity (H-ind) was calculated for each subject.

Task 4.2 Reproductive Management

For the Mericanel breed, new genetic lines identified in the territory were introduced in reproductive management. The individual variability index (H-ind) and the average kinship index (P) were estimated for the mating plans with all the analyzed subjects. Males with higher H-ind values allow for the preservation of greater genetic variability, while those with lower P values help contain inbreeding. Based on the results of the genetic analyses, females have formed the new families 5 and 6 of the conservation nucleus. Mating was carried out by calculating the average kinship of the genotyped males with the subjects of each family present in the nucleus (1 to 4) and with the 2 identified families (5-6) (Figure 4.1).

Furthermore, the kinship indices of the two new families with the males present in the conservation nucleus were also calculated (Figure 4.2).

The selection of breeders was made to cover the maximum existing variability while considering individual heterozygosity. Mating was planned between subjects belonging to different bloodlines to minimize the relationship between the males and the female groups. The average kinship between the female groups and the selected male was calculated for each male to minimize the rate of inbreeding (ΔF). The results of the analysis were reported in the genetic evaluation document sent to the UniMI coordinator and the relevant PA.

For the populations present in the UniPG conservation nucleus, the 5 populations (Ancona, Livorno in golden, silver, white, and black colorations) were analyzed. For these populations, the bloodlines were identified by also typing the females, and the subjects belonging to each genetic line were defined. To identify genetically similar subjects from the parentage matrix, the distance matrix between individuals was calculated. To maintain maximum genetic variability, females from each family belonging to the same genetic line were grouped together in the same family group and bred with genetically distant males. The indices will be published on the project website.

FIGURES AND TABLES

Figura 1.1 – Trend of egg laying % and evolution of egg weight for Bianca di Saluzzo hen

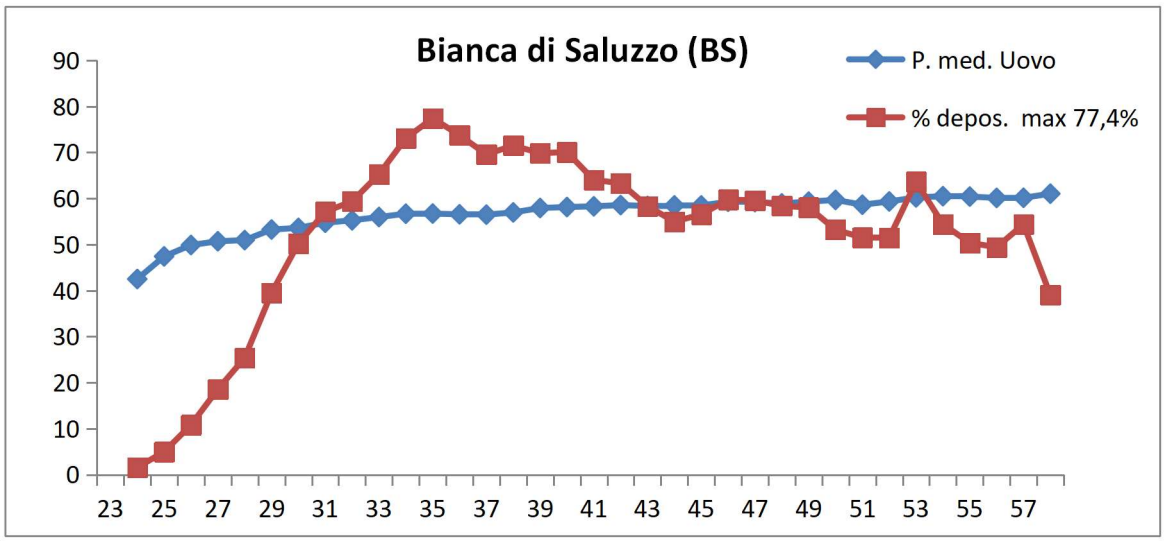

Figura 1.2 – Trend of egg laying % and evolution of egg weight for Bionda Piemontese hen

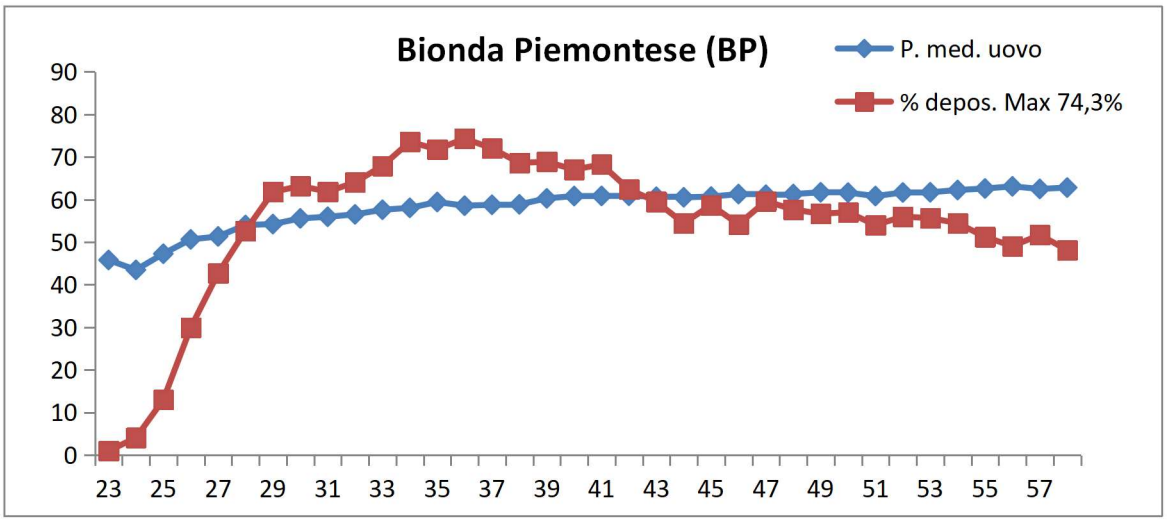

Figura 1.3 – Comparison of the trend of egg laying % and evolution of egg weight between Bianca di Saluzzo (BS) and Bionda Piemontese (BP) hens

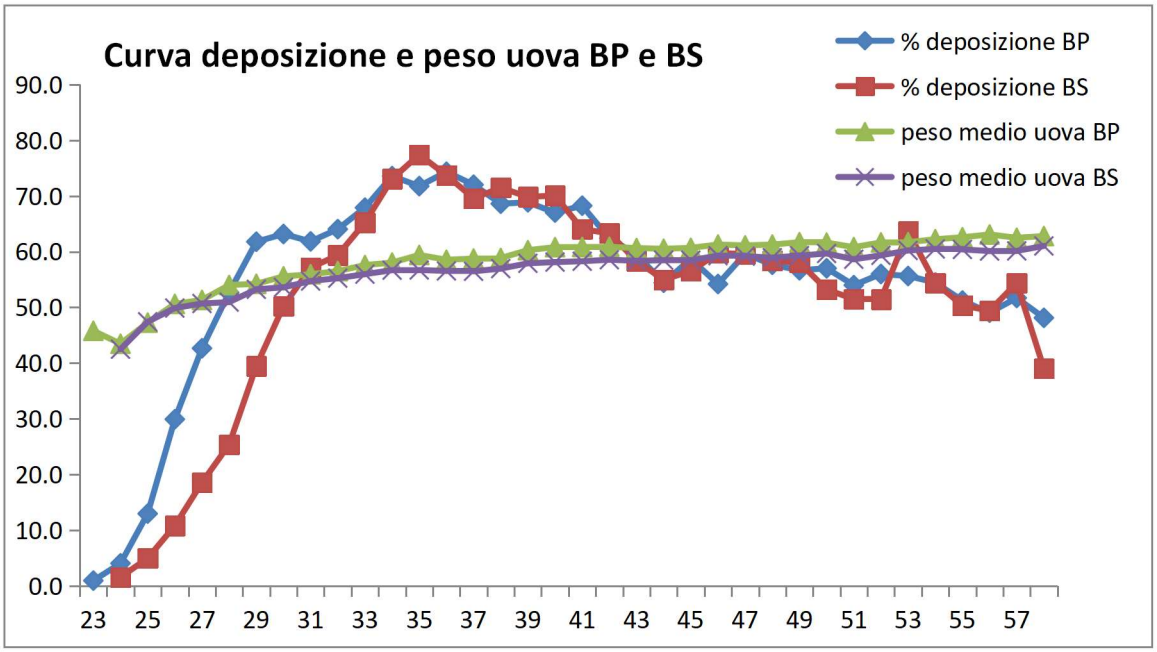

Figura 1.4 – Comparison of the trend of fertility and hatchability % in the Bianca di Saluzzo breed (2022 and 2023), Bionda Piemontese breed (2022-2023), and Millefiori Piemontese breed (2023)

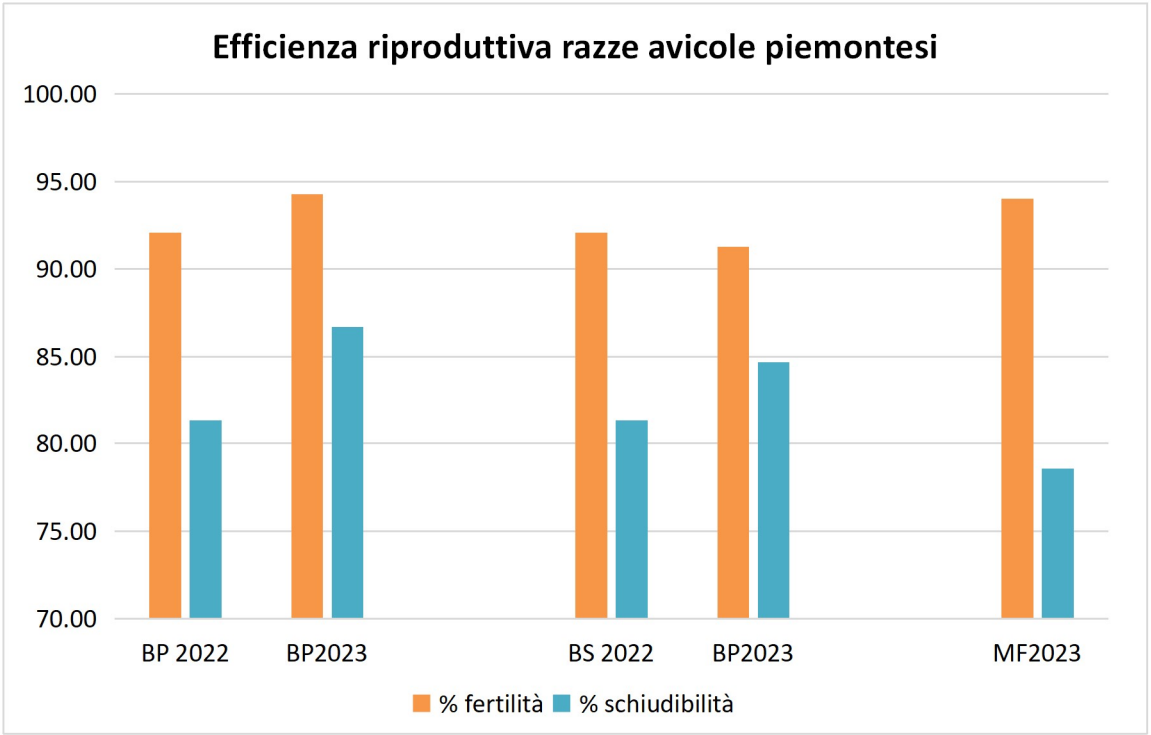

Figura 1.5 – One-day-old chicks of Millefiori Piemontese breed

Figure 2.1 – Distribution of individual molecular inbreeding (Hind) in the analysed Mericanel della Brianza population (MB23)

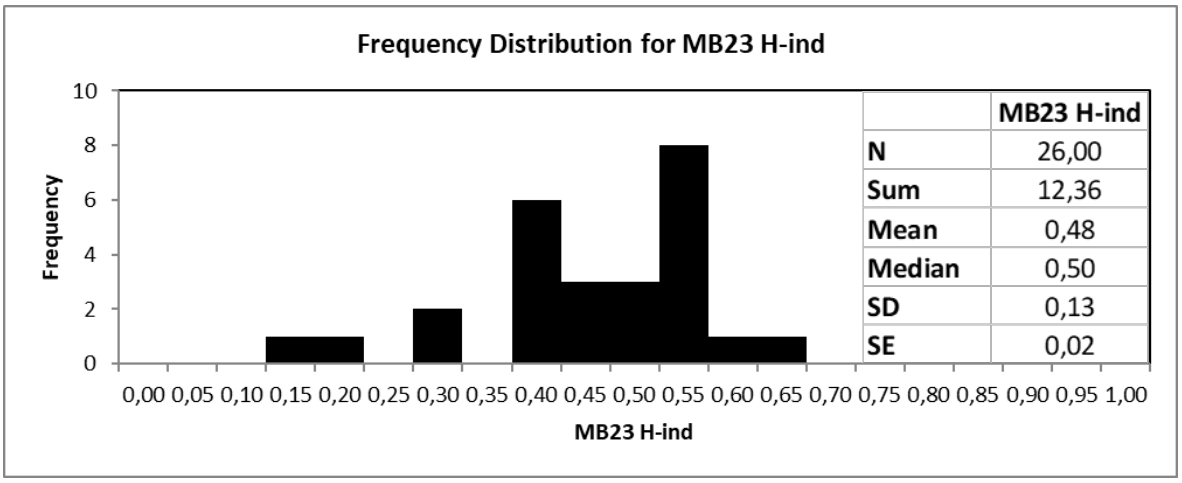

Figura 2.2 – Principal component analysis in the analysed Mericanel della Brianza population (MB23)

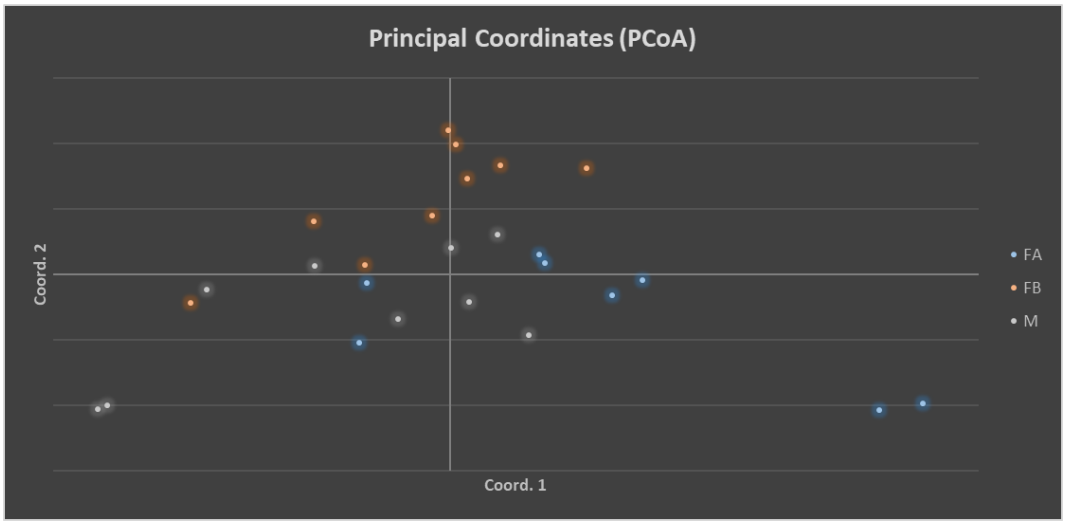

Figura 2.3 – Principal component analysis: comparison between Mericanel della Brianza sampled subjects (MB23, in green) and those present in the nucleus

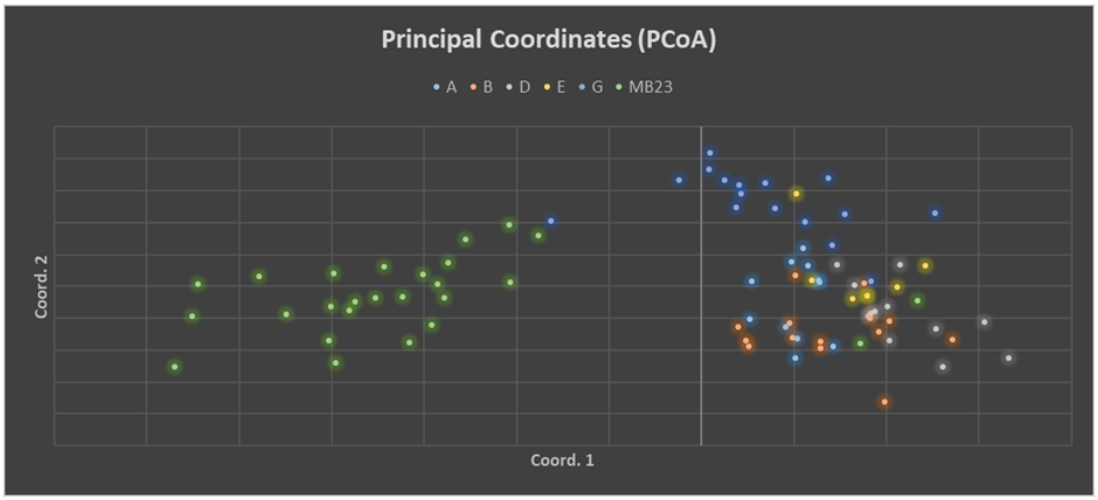

Figura 2.4 – Analysis of allele patterns in Mericanel della Brianza sampled subjects (MB23) and in those present in the nucleus

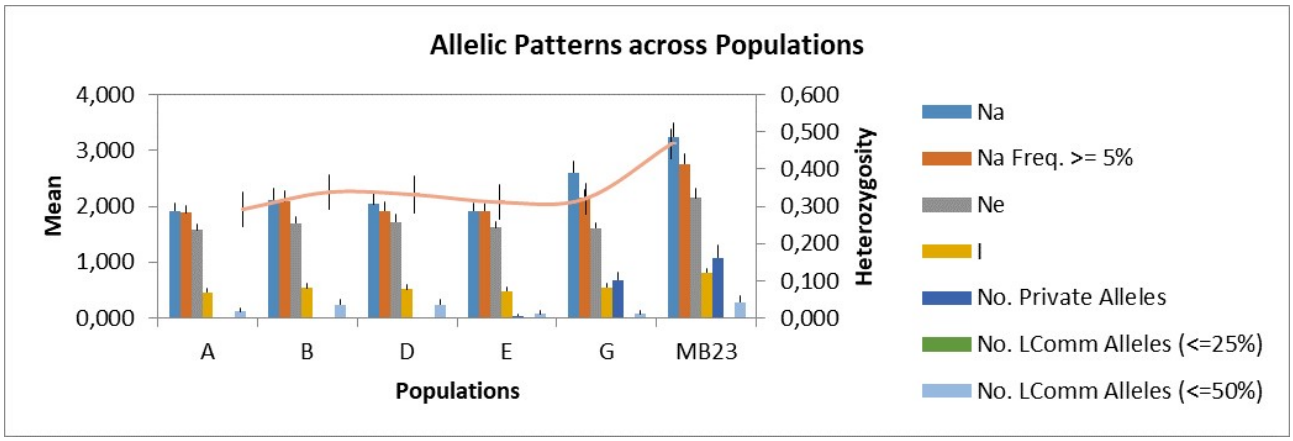

Figura 4.1 – Average kinship indexes of new genotyped males with subjects in the nucleus and in the new families

Maschi nuovi		1	2	3	4	5	6		1=A	
ME903	M	**0,62**	**0,61**	**0,62**	**0,65**	**0,58**	**0,65**		2=B+C	
ME907	M	**0,57**	**0,54**	**0,58**	**0,51**	**0,58**	**0,65**		3= D, E ,F	
ME955	M	**0,48**	**0,47**	**0,50**	**0,45**	**0,51**	**0,58**		4=G	
ME971	M	**0,59**	**0,55**	**0,58**	**0,56**	**0,59**	**0,65**		5= FAMIGLIA NUOVA	
ME981	M	**0,64**	**0,61**	**0,62**	**0,62**	**0,62**	**0,66**		6=FAMIGLIA NUOVA	
ME982	M	**0,58**	**0,56**	**0,60**	**0,63**	**0,61**	**0,62**			
MEA834	M	**0,55**	**0,58**	**0,56**	**0,54**	**0,53**	**0,59**			
MEB184	M	**0,57**	**0,56**	**0,59**	**0,53**	**0,60**	**0,63**			
MEB198	M	**0,47**	**0,45**	0,49	**0,41**	**0,50**	**0,57**			

Figura 4.2 – Kinship indices of males in the conservation nucleus with new genotyped females

maschi nucleo		femmine nuove	
POP		5	6
ME156	D	0,52	0,45
ME112	D	0,54	0,51
ME122	B	0,54	0,54
ME139	D	0,55	0,52
ME150		0,55	0,49
ME141	D	0,55	0,53
ME113	G	0,56	0,53
ME137	E	0,56	0,56
ME119	D	0,56	0,54
ME140	E	0,57	0,50
ME129	A	0,57	0,56
ME147B	E	0,57	0,53
ME125	B	0,57	0,56
ME121	D	0,57	0,56
ME138	B	0,57	0,59
ME150B	A	0,57	0,57
ME131	A	0,57	0,56
ME146	G	0,58	0,53
ME148B	B	0,60	0,61
ME155		0,61	0,59