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## **Rapid Communication**

# Genetic variability of Lizard canary breed inferred from pedigree analysis

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#### **Abstract**

The genealogical data of 471 (whole population:  $\overline{\text{WP}}$ ) Lizard canaries of an Italian breeder were analyzed to evaluate the genetic variability of the breed. The reference population ( $\overline{\text{RP}}$ ) comprised 346 living reproductive birds. Average generation interval was  $1.61 \pm 0.718$  for males and  $1.72 \pm 0.863$  for females. The average value of inbreeding ( $\overline{\text{FP}}$ ) and relatedness ( $\overline{\text{AR}}$ ) in the  $\overline{\text{RP}}$  were 15.83% and 22.63%, while the average increase in inbreeding was estimated to be 6.71% per generation (Effective population size,  $\overline{\text{NP}} = 7.49$ ). The results showed the need to reduce the level of inbreeding which would result in significant loss of genetic variation and in significant inbreeding depression.

**Key words:** inbreeding, Lizard canaries, pedigree analysis.

# Introduction

The Lizard canary, one of the oldest canary breeds, was first developed in the 1700s. This type of canary is named for its beautiful markings: black crescent-shaped spots running down its back and breast that resembles the scales of a lizard. Its genetic basis was laid in the northwest of France and from there exported to England by Huguenot refugees. The Lizard canary almost became extinct in the early 1900s due to the ravages of both World Wars and disease epidemics. The Lizard canary Association of Great Britain started a breeding program to save the breed. Even in Italy a Club of Lizard was founded in 1989, more precisely in Florence (Tuscany). Often, to produce animals with typical features, breeders employed inevitable inbreeding practices without introducing new genes which could alter the typicality of offspring. Inbreeding can have adverse effects for population health, increasing genetic diseases, mortality of young and inbreeding depression. For these reasons genetic typifying is an important preliminary step in any safeguard biodiversity program. The genetic variability of a population can be estimated using Short Tandem Repeat (STR) molecular markers (Chbel et al. 2002; Saladin et al. 2007) and from genealogical data. These are available for the livestock species (Cecchi et al. 2006; Cervantes et al. 2008; Gutiérrez et al. 2008) and for pet animals (Cecchi et al. 2009, 2013; Ciampolini et al. 2013) but in birds it is more difficult to have genealogical data (Daniell & Murray 1986; Korrida et al. 2012). The objective of this study was to analyze the genealogical information of the Lizard canaries to ascertain their generation intervals, genetic diversity, inbreeding and relatedness, and to compute a number of genealogical parameters that could identify strategies to conserve or restore the genetic variability of the breed.

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### **Materials and Methods**

This research was carried out in 2012 in an Italian Lizard canary herd. Genealogical data of 471 birds were acquired. Canaries were born between 2000 and 2011 and all canaries constitute the whole population (WP) containing all founders, ancestors and their offspring. The population is divided into: the base population (BP) defined as individuals with one or both unknown parents; and the reference population (RP) defined as the population of interest that includes the living animals. The effective population size of the RP was performed using the methodology described by Gutiérrez et al. (2009) and using the regression-based estimates. The following demographic and genetic parameters have been calculated, using the program ENDOG v4.8 (Gutiérrez & Goyache 2005): (i) the effective number of founders (fe), the absolute number of founders (ft) and the effective number of ancestors (fa); (ii) the inbreeding coefficients (F), the number of inbred animals and average inbreeding coefficient for each traced generation; (iii) the average relatedness coefficient (AR); (iv) the pedigree completeness, that is, the number of full traced generations, the maximum number of generations traced and the equivalent complete generations; and (v) the generation intervals. Popular sires (defined here as > 20 recorded offspring) and popular dams (> 20 offspring) were identified. The number of inbred and the average inbreeding coefficient per year were performed using CFC software (Sargolzaei et al. 2006). The distribution of inbreeding level in the whole population was analyzed and eight different class levels of inbreeding were considered:  $0 < F \le 0.05$ ;  $0.05 < F \le 0.10$ ;  $0.10 < F \le 0.15$ ;  $0.15 < F \le 0.20$ ;  $0.20 < F \le 0.25$ ;  $0.25 < F \le 0.30$ ;  $0.30 < F \le 0.35$ ; and  $0.35 < F \le 0.40$  (Sargolzaei *et al.* 2006).

#### **Results and Discussion**

Genealogical data of the 471 animals included 191 males, 199 females and 81 undefined sex. In Figure 1, the details of the pedigree quality in Lizard canaries are shown.

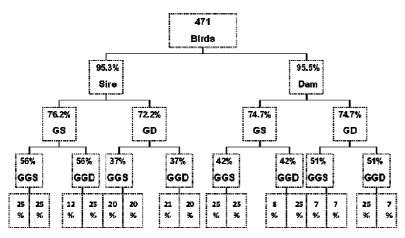


Figure 1 Pedigree completeness level in the whole pedigree data files (GS and GD = grandparents; GGS and GGD = grandparents).

The percentage of completeness was computed as about 95% for the parental generation, from 72.2% to 76.2% for the grandparent generation and from 37% to 56% for the third generation of the great-grandparents. The pedigree completeness level was similar both in dam and sire pathways. Pedigree completeness was also assessed by calculating the mean maximum generations, the mean complete and the number of equivalent generations traced. The mean maximum generations and the mean complete generations were 2.97 and 1.90, respectively, while 2.40 was the number of equivalent generations traced (sum, on all the generations of the ancestors, of the ancestors' proportion known to every generation). The generation interval is an important parameter to derive various demographic indexes of the populations. It has been calculated for the four selection paths (parents/offspring) in the RP. The mean generation interval was  $1.61 \pm 0.718$  for males and  $1.72 \pm 0.863$  for females; no significant differences were found between the two pathways within each parent. Sires and dams in total were 43 and 58, respectively, with a ratio of dams-to-sires of 1.35. Popular sires were nine (21% of the sires) while popular dams were six (10% of the dams). The number of full-sib groups was 69 with an average

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family size of  $6.33 \pm 2.163$ . Table 1 summarizes the main genealogical parameters computed by ENDOG. The number of animals in the reference population was 346 (131 males, 134 females and 81 undefined sex), 33.92% of which were inbred. The number of animals in the base population (one or both unknown parents: absolute founders = [5] was 21. The number of ancestors was 18 in RP, while the effective number of ancestor [6] was five. To explain 50% of the genetic variability only two ancestors were enough in the RP; the first (a male) explained 30.94% and the second (a female) explained 26.86% of the genetic variability within the breed. In terms of number of progeny, the two ancestors had 46 offspring. Average value of inbreeding (F) and average relatedness coefficient (AR) in the whole population (WP) were 14.25% and 21.81%, respectively, while the average value of F and AR in the reference population (RP) were 15.83% and 22.63%. The values of the inbreeding coefficients were higher, more than double 6.25%, that is, the value resulting from the mating of two animals sharing two grandparents (cousin mating). The total number of inbred animals was of 309. The coefficient of inbreeding is less than 10% in 19 birds, whereas it is more than 40% in 19 birds. Values higher than 40% corresponds to the closest inbreeding, when breeding of brothers with sisters or parents with descendants takes places in several successive generations.

Table 1 Main genealogical parameters

Whole population (WP)	471	
- Males	191	
- Females	199	
- Not sexed	81	
Inbreeding in the WP	309	
Reference population (RP)	346	
- Males	131	
- Females	134	
- Not sexed	81	
Inbreeding in the RP		
Base population (one or two unknown parents = $f_t$ ) (BP)		

Table 2 shows, in the whole database, the evolution of the average coefficient of inbreeding and average relatedness coefficient within the different generations. As we can see, the depth of the pedigree was equal to six generations. The inbreeding for each traced generation was high in all generations, with peaks around 38% in birds belonging to the sixth generation, while the average relatedness coefficient was higher than 25% starting from the birds with three traced generations. The percentage of inbred has an increasing trend with values greater than 80% from birds with three generations traced. The birds of the last three traced generation were all inbred

Table 2 Population statistics on average inbreeding

Traced generation	No. animals	Average F (%)	% Inbred	Average F for inbred (%)	Mean AR (%)
0	21				
1	56	0.00	0.00	0.00	7.31
2	102	8.22	35.29	25.00	18.36
3	122	12.04	84.43	14.26	24.92
4	96	20.83	100.00	20.83	28.14
5	57	29.78	100.00	29.78	28.88
6	17	37.89	100.00	37.89	29.44

F, average value of inbreeding; AR, average relatedness coefficient

Figure 2 shows the trend of inbreeding computed by the year of birth of individuals. Due to scarcity of data, average inbreeding is equal to zero for 5 years; starting from 2006 inbreeding increases consistently and reaches a maximum peak in 2011 (F = 0.31). AR values had the same trend with the maximum value in 2011 (F = 0.31). All results show that inbreeding is too high and indicate the importance of its reduction, avoiding mating between too many related animals and introducing new animals from other breeders as strategies to restore the genetic variability of the breed.

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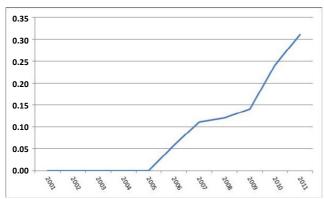


Figure 2 Inbreeding trend for birth year.

Data prove that the over-representation of some individuals in the whole pedigree can be highly detrimental to maintain the genetic diversity of the breed and that only two ancestors explained 50% of total genetic variability of the breed. In fact the trend of inbreeding was increasing with rather high values in comparison to that reported in literature on Maroccan Houbara Bustard (Korrida *et al.* 2012) and it is over the range of the acceptable level of 0.5-1.0% recommended by the FAO (1998) guidelines for livestock populations. Furthermore the short generation length and the small  $N_e$  are factors that can decrease genetic diversity. The effective population size reveals losses in fitness as well as in genetic variability and it is considered one of the most fundamental parameters that strongly influence the nature and the rate of genetic information transmission across generations, population variability and conservation strategies. It is well known a population, which reduces its size, loses genetic variability, resulting in a reduction of genetic fitness and increasing of inbreeding depression.

## References

Cecchi F, Bramante A, Mazzanti E, Ciampolini R. 2009. A colony of dog guides: analysis of the genetic variability assessed by pedigree data. *Italian Journal of Animal Science* 8, 48–50.

Cecchi F, Ciampolini R, Ciani E, Matteoli B, Mazzanti E, Tancredi M, Presciuttini S. 2006. Demographic genetics of the endangered Amiata donkey breed. *Italian Journal of Animal Science* 5, 387–391.

Cecchi F, Paci G, Spaterna A, Ciampolini R. 2013. Genetic variability in Bracco Italiano dog breed assessed by pedigree data. *Italian Journal of Animal Science* 12, 348–352.

Cervantes I, Goyache F, Molina A, Valera M, Gutiérrez JP. 2008. Application of individual increase in inbreeding to estimate realised effective sizes from real pedigrees. *Journal of Animal Breeding and Genetics* 125, 301–310.

Chbel F, Broderick D, Idaghdour Y, Korrida A, McCormick P. 2002. Characterization of 22 microsatellites loci from the endangered Houbarabustard (Chlamydotis undulata undulata). *Molecular Ecology Notes* 2, 484–487.

Ciampolini R, Cecchi F, Paci G, Policardo C, Spaterna A. 2013. Investigation on the Genetic Variability of the American Pit Bull Terrier dogs belonging to an Italian breeder using Microsatellite Markers and genealogical data. *Cytology and Genetics* 47, 217–221.

Daniell A, Murray ND. 1986. Effects of inbreeding in the budgerigar Melopsittacus undulatus (Aves: Psittacidae). *Zoo Biology* 5, 233–238.

FAO. 1998. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans: Management of Small Populations at Risk. FAO, Rome, Italy.

Gutiérrez JP, Cervantes I, Goyache F. 2009. Improving the estimation of realized effective population sizes in farm animals. *Journal of Animal Breeding and Genetics* 126, 327–332.

Gutiérrez JP, Cervantes I, Molina A, Valera M, Goyache F. 2008. Individual increase in inbreedingallows estimating realized effective sizes from pedigrees. *Genetics Selection Evolution* 40, 359–378.

Gutiérrez JP, Goyache F. 2005. A note on ENDOG: a computer program for analysing pedigree information. *Journal of Animal Breeding and Genetics* 122, 172–176.

Korrida A, Gutiérrez JP, Aggrey SE, Amin-Alami A. 2012. Genetic variability characterization of the moroccan houbara bustard (Chlamydotis undulata undulata) inferred from pedigree analysis. *Zoo Biology* 00, 1–14.

Saladin V, Ritschard M, Roulin A, Bize P, Heinz H. 2007. Analysis of genetic parentage in the tawny owl (*Strix aluco*) reveals extra-pair paternity is low. *Journal of Ornithology* 148, 113–116.

Sargolzaei M, Iwaisaki H, Colleau JJ. 2006. CFC (Contribution, Inbreeding (F), Coancestry, Release 1.0. A software package for pedigree analysis and monitoring genetic diversity. Proc. 8th World Cong. on Genetics Applied on Livestock Production, Belo Horizonte, Brazil, CD-ROM comm. N 27-28.