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GENETIC STRUCTURE OF A SMALL CLOSED POPULATION OF THE NEW ZEALAND WHITE RABBIT THROUGH PEDIGREE ANALYSES

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Abstract: The genetic structure of a small population of New Zealand White rabbits maintained at the Sheep Breeding and Research Station, Sandynallah, The Nilgiris, India, was evaluated through pedigree analyses. Data on pedigree information (n=2503) for 18 yr (1995-2012) were used for the study. Pedigree analysis and the estimates of population genetic parameters based on the gene origin probabilities were performed. The analysis revealed that the mean values of generation interval, coefficients of inbreeding and equivalent inbreeding were 1.49 yr, 13.23 and 17.59%, respectively. The proportion of population inbred was 100%. The estimated mean values of average relatedness and individual increase in inbreeding were 22.73 and 3.00%, respectively. The percentage increase in inbreeding over generations was 1.94, 3.06 and 3.98 estimated through maximum generations, equivalent generations and complete generations, respectively. The number of ancestors contributing the majority of 50% genes (f_{a50}) to the gene pool of reference population was only 4, which might have led to reduction in genetic variability and increased the amount of inbreeding. The extent of genetic bottleneck assessed by calculating the effective number of founders (f_e) and the effective number of ancestors (f_a), as expressed by the f_e/f_a ratio was 1.1, which is indicative of the absence of stringent bottlenecks. Up to 5th generation, 71.29% pedigree was complete, reflecting the well maintained pedigree records. The maximum known generations were 15, with an average of 7.9, and the average equivalent generations traced were 5.6, indicating a fairly good depth in pedigree. The realized effective population size was 14.93, which is very critical, and with the increasing trend of inbreeding the situation has been assessed as likely to become worse in future. The proportion of animals with the genetic conservation index (GCI) greater than 9 was 39.10%, which can be used as a scale to use such animals with higher GCI to maintain balanced contribution from the founders. From the study, it was evident that the herd was completely inbred, with a very high inbreeding coefficient, and the effective population size was critical. Recommendations were made to reduce the probability of deleterious effects of inbreeding and to improve genetic variability in the herd. The present study can help in carrying out similar studies to meet the demand for animal protein in developing countries.

Key Words: effective population size, genetic structure, inbreeding, pedigree analysis, rabbit

INTRODUCTION

In animal breeding, implementation of an effective selection programme is dependent on the population structure and within population genetic variability. Pedigree analysis is an important tool to describe the population structure and genetic variability. Studies utilizing historical pedigree records have the potential to identify the factors that have influenced the genetic history of a population (Valera *et al.,* 2005). A complete pedigree is essential for evaluating

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inbreeding, effective population size, generation interval, genetic diversity and several other important population parameters (Martínez *et al.,* 2008). Computation of effective population size is a key to understanding the genetic diversity (Falconer and Mackay, 1996).

A number of concepts and methods based on the probability of gene origin have been proposed to monitor the amount of genetic diversity in a population. Lacy (1989) introduced the concept of the effective number of founders (f_e), which measures the overall founder representation in a population accounting for the loss of genetic variability from unequal founder contributions. Boichard *et al.,* (1997) proposed the effective number of ancestors (f_a) as a metric to assess bottlenecks in the population, a major cause of gene loss in domestic populations. Analysis of inbreeding rates and related parameters has traditionally been used to analyse the evolution of genetic diversity in populations (Sorensen *et al.,* 2005). Genetic drift, loss of heterozygosity and decrease in genetic variability are the consequences of high rates of inbreeding over generations (Falconer and Mackay, 1996).

Accurate rabbit flock management that takes into consideration the genetics of rabbit breeding should focus on controlling the level of inbreeding in future generations in order to prevent a fall in the performance or a threat to the sustainability of selection programmes. This point is especially important in schemes where genetic variability cannot be increased by introducing unrelated individuals. In the present study, the New Zealand White rabbit population has been closed for more than 15 yr, with practically no introduction of animals from outside. Closed populations are subject to inbreeding accumulation and increasing of inbreeding rate is even faster if the population is small. Inbreeding reduces the mean of fitness traits such as survival and reproduction, and consequently worsens general vigour and fertility i.e., inbreeding depression (Planinc *et al.,* 2012). Preservation of maximum genetic diversity is one of the main objectives in small populations (Martín de la Rosa *et al.,* 2016).

The main objective of the present study was to evaluate the population genetic structure of a nucleus flock of the New Zealand White rabbit through pedigree analyses. The generation interval (GI), pedigree completeness level, probability of gene origin in terms of effective number of founders (f_e) and effective number of ancestors (f_a), inbreeding coefficient (F), average relatedness (AR), effective population size (N_e) and GCI were calculated from pedigree information to assess the genetic structure of the population.

MATERIALS AND METHODS

Data and rabbit management

Data on the New Zealand White rabbits were collected from the breeding flock maintained at the Sheep Breeding and Research Station (SBRS), Sandynallah, The Nilgiris, Ooty, India (11°25' N latitude and 76°46' E longitude), at an altitude of 2200 m above mean sea level. Data on 2503 animals spread over 18 yr (1995-2012) were used for the pedigree analysis. Reference population, a subset of the main population, was considered as the cohort born from 2009 to 2011, for which different population demographic parameters were estimated. The herd was a closed type where 40 to 60 breeding females were maintained every year with a 1:5 male to female ratio. After weaning at 42 d of age, animals were kept individually in wire cages of standard dimensions under similar housing and management conditions. Animals were fed concentrate (16% crude protein; 2500 kcal digestible energy), seasonal grasses (*Pennisetum clandestinum and Phalaris aquatica*), tree Lucerne and carrots *ad libitum*. Concentrates were used in graded quantity from 75 to 200 g according to age, body weight and lactation. Animals were weighed exactly on the target ages *viz*., weaning (42nd day), post-weaning (70th day) and marketing (135th day). The bucks start breeding at 5 mo of age and the does at 6 mo of age. Mating of close relatives was avoided as far as possible to keep the inbreeding at its lowest level. Selection pressure (proportion of population selected) applied was 2 to 5% and 10 to 20% for males and females, respectively. The bucks were culled after 2 yr and the does after 3 yr of age. A standard prophylactic schedule along with symptomatic treatment was adopted in disease management. Based on the climatic conditions prevailing in the region, seasons were distinctly classified as winter (December to February), summer (March to May), south-west monsoon (June to August) and north-east monsoon (September to November).

Statistical analysis

The pedigree information was obtained for the whole pedigree of the research station. The GI was estimated based on the average age of the parents at the birth of their selected offspring. GI estimates for the four transmission pathways (sires of buck, sires of doe, dams of buck, and dams of doe) were obtained for the animals born in years 2009 and 2010, as this population group was the most recent one that could complete at least one generation in the flock. For analyses, the reference population was defined as those animals which were alive.

The completeness of pedigree was assessed by computing the equivalent number of generations and studied by analysing the account of the completeness of each ancestor in the pedigree several generations back. The following parameters were calculated for each individual in order to avoid the bias introduced by animals with limited pedigree records: (1) the maximum number of generations traced, defined as the number of generations separating the individual from its furthest ancestor; (2) the number of equivalent complete generations, computed as the sum over all known ancestors of the terms computed as the sum of $(½)^n$, where n is the number of generations separating the individual to each known ancestor (Maignel *et al.* 1996) and (3) the number of fully traced (complete) generations, defined as those separating the offspring of the furthest generation where the 2^g ancestors of the individual are known, where *g* is the number of generations back. Ancestors with no known parent were considered as founders (generation 0).

The genetic history in terms of probability of gene origin was assessed by calculating the effective number of founders (f_e) and the effective number of ancestors (f_a). The effective number of founders is defined as the number of equally contributing founders that would be expected to produce the same genetic diversity as in the population under study (Lacy, 1989). This is computed as:

$$f_e = 1 \Big/ \sum_{k=1}^{f} q_k^2$$

where q_k is the expected proportional genetic contribution of founder *k,* calculated by the average relationship of the founder to each animal in the current population, and f is the total number of founders. The effective number of ancestors, as proposed by Boichard *et al.* (1997), represents the minimum number of animals (founders or non-founders) that are necessary to explain the complete genetic diversity of the study population and as a metric to assess bottlenecks in the population, a major cause of gene loss in captive and domestic populations. This parameter complements the information offered by the effective number of founders by accounting for the losses of genetic variability produced by the unbalanced use of reproductive individuals producing bottlenecks. It is calculated as:

$$f_a = 1 \Big/ \sum_{j=1}^{a} q_j^2$$

where q_j is the marginal contribution of ancestor *j*, which represents the genetic contribution made by an ancestor that is not already explained by a previously chosen ancestor, and *a* is the total number of ancestors. The sum of marginal contributions of all ancestors is 1. The genetic bottleneck occurring in the population was assessed by computing the values of the number of ancestors contributing the majority of 50% genes (f_{a50}) and f_e/f_a ratio. The former explains the effective number of ancestors contributing to 50% of the population i.e., the number of equally contributing founders that would be expected to produce half the genetic diversity, as in the population under study. The effective number of ancestors is expected to be smaller than the effective number of founders if there is a bottleneck, which can be indicated by the f_e/f_a ratio.

The inbreeding coefficient (F) of each individual, defined as the proportion of loci carrying alleles that are identical by descent from a common ancestor (Wright, 1931), was calculated following Meuwissen and Luo (1992). The individual change in inbreeding or increase in inbreeding (ΔF_i) was calculated using the standard formula as modified by González-Recio *et al.* (2007), Gutiérrez *et al.* (2008) and Gutiérrez *et al.* (2009):

$$\Delta F_i = 1 - \sqrt[t_i - 1]{1 - F_i}$$

where F_i is the inbreeding coefficient for the individual *i* and t_i is the equivalent complete generations for this individual. The number of equivalent complete (EqG) generations for each individual *i* was calculated as:

$$EqGi = \sum (1/2)^n$$

where 'n' is the number of generations separating each known ancestor (1= parents, 2 = grandparents, and so on), and the sum being computed across all known ancestors of 'i' (Maignel *et al.,* 1996).

The equivalent inbreeding coefficients (EF) were obtained as a product of the increase in inbreeding for each individual and average equivalent number of generations for this pedigree (Panetto *et al.,* 2010).

The AR coefficient could be defined as twice the probability that 2 random alleles, one from the animal and the other from the population in the pedigree (including the animal), are identical by descent and can then be interpreted as the representation of the animal in the whole pedigree, regardless of the knowledge of its own pedigree. This was computed as described in Dunner *et al.* (1998). It is the average of the coefficients in the row corresponding to the individual in the numerator relationship matrix.

The estimate of effective population size (N_e) is defined as the number of breeding animals that would lead to the actual increase in inbreeding (ΔF) if they contributed equally to the next generation. The realized effective size ($\overline{N_e}$) by Cervantes *et al.* (2008) was computed from $\overline{\Delta F}$ by averaging ΔF of the *n* individuals included in the reference subpopulation, as $\overline{N_e} = 1/2\,\overline{\Delta F}$. The effective population size (N_e) as $N_e(t)=1/2\Delta F_t$ was also computed for each generation having $F_t > F_{t-1}$ to characterize the effect of remote and close inbreeding, where $\Delta F_t = (F_t - F_{t-1})/(1 - F_{t-1})$ and F_t and F_{t-1} are the average inbreeding at t_{th} generation. In small populations with shallow pedigrees, whatever method is used to compute N_e, this parameter fits poorly with real populations, giving an overestimate of the actual effective population size (Goyache *et al.,* 2003). To better characterize this, 3 additional values of N_e were derived by computing the regression coefficient (*b*) of the individual inbreeding coefficient over: i) the maximum number of generations traced; ii) the equivalent complete generations and iii) the number of complete generations traced, and considering the corresponding regression coefficient as the increase in inbreeding between 2 generations ($F_t - F_{t-1} = b$), and consequently $N_e = 1/2b$. When available information is scarce, these estimations can be useful to inform on the upper (i), real (ii) and lower (using iii) limits of N_e in the analysed population. The increase in inbreeding (ΔF) for the reference subpopulation was also computed using different regression based approaches. First, ΔF as $\Delta F = F_t - F_{t-1} / 1 - F_{t-1} \approx b / 1 - (F_t - b)$ being F_t the average F of the reference population and *b* the regression coefficient of the individual inbreeding coefficients over the equivalent complete generations. Additionally, the effective size is estimated, following Gutiérrez *et al.* (2003) from the regression coefficient (*b*) of the inbreeding coefficients over the year of birth in the reference population and computing the increase in inbreeding between 2 generations as $F_t - F_{t-1} = I \times b$, where I is the average generation interval and F_t is the mean inbreeding in the reference subpopulation. Finally, N_e via a log regression of $(1-F_t)$ on generation number where F_t is obtained from $F_t = 1-(1-\Delta F)t$ as $1-F_t = [1-(1/2N_e)]t$ was estimated following Pérez-Enciso (1995). When datasets with no discrete generations are analysed, N_e was estimated by a log regression of $(1-F_t)$ on the date of birth and then divided by the generation interval.

Genetic conservation index (GCI; Alderson, 1992) was calculated for all the individuals in the population under study. The index was computed from the genetic contributions of all the identified founders as $GCI = 1/\sum p_i^2$ where p_i is the proportion of genes of founder *i* in the pedigree of an animal. The founders have unknown parentages and are assumed to have independent ancestries. The founder contributions to each descendant are simply the genetic relatedness between the founders and the descendants. Each founder's contribution to the living, descendant population is then the sum of that founder's relatedness with the descendants. The proportional contributions (p_i) are obtained by dividing each founder's contribution by the number of living descendants. The Alderson's index was based on the assumption that the objective of a conservation programme is to retain the full range of alleles possessed by the base population. In this respect, the ideal individual would receive equal contributions from all the founder ancestors in the population and, consequently, the higher the GCI value, the higher the values of an animal for conservation.

All analyses were carried out using the ENDOG program (version 4.8) (Gutiérrez and Goyache, 2005).

Table 1: Generation intervals (in years) for the 4 gametic pathways of the New Zealand White rabbit.

Pathway	Number	Mean	Standard Deviation	SEM
Sires of buck	125	1.362	0.928	0.083
Sires of doe	147	1.531	0.887	0.073
Dams of buck	125	1.444	1.039	0.093
Dams of doe	147	1.594	0.925	0.076
Overall mean	544	1.489	0.945	0.041

SEM: standard error of the mean.

RESULTS

Generation interval

The descriptive details of generation intervals calculated across all pathways are presented in Table 1. The mean generation interval for the population was 1.49 yr. It was found that the pathway of buck to son was lowest (1.36) and doe to daughter was highest (1.59).

Pedigree completeness

Concerning the pedigree depth for the reference population, the completeness level was 98.51% for the parent generation, 94.18% for the grandparent generation, 82.58% for the great-grandparent generation, and 71.29% for the great great-grandparent generation. The per cent ancestral knowledge over generations is depicted in Figure 1. The mean values of maximum number of generations, equivalent number of generation and complete generations traced from the pedigree were 7.910, 5.609 and 4.310 respectively (Table 2). The maximum values for these parameters were 15.0, 10.2 and 8.0, respectively. The mean maximum generations, equivalent generations and complete generations traced by year for the whole pedigree are depicted in Figure 2.

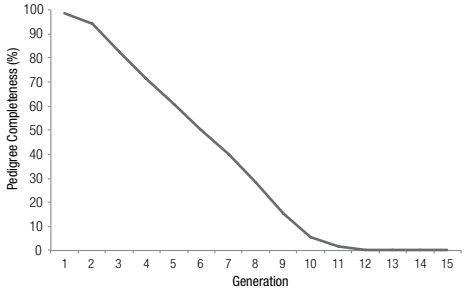

Figure 1: Mean percentage of known ancestors per generation (pedigree completeness) for the whole population of the New Zealand White rabbit (generation 1=parents, generation 2=grandparents, generation 3=great grandparents, and so on).

Probability of gene origin

The number of founders and ancestors contributed for the whole population were 40 and 29, respectively. The effective number of founders and effective number of ancestors for the reference population were 11 and 10, respectively. The f_{a50} of the population was only 4 and the f_e/f_a ratio was 1.1 (Table 3).

Inbreeding and average relatedness

The average coefficients of inbreeding and equivalent inbreeding in the population were 13.23 and 17.59%, respectively (Table 2). About 11.17% of total matings were highly inbred, in which full-sib, half-sib and parent-offspring matings were 1.20, 6.30 and 3.67%, respectively. The mean values of inbreeding, average relatedness and percentage of inbred animals with their inbreeding coefficients over complete generations are presented in Table 4. After the third generation, the per cent inbred population becomes 100% where F and mean inbreeding of inbred population becomes the same. About

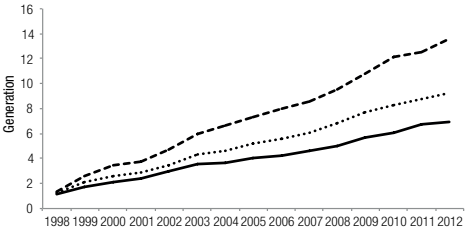

Figure 2: Average maximum generations (▬ ▬ ▬), equivalent generations (••••) and complete generations (▬▬) traced by year for the whole pedigree of the New Zealand White rabbit.

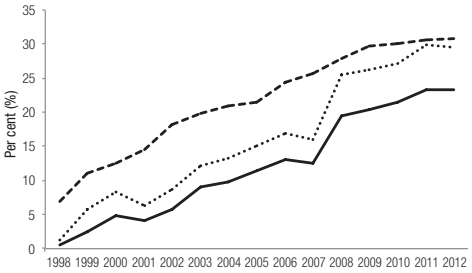

Figure 3: Trends for inbreeding (— F), the average relatedness (--- AR*)* and the equivalent inbreeding coefficient (····· EF) by year of birth for the whole pedigree of the New Zealand White rabbit.

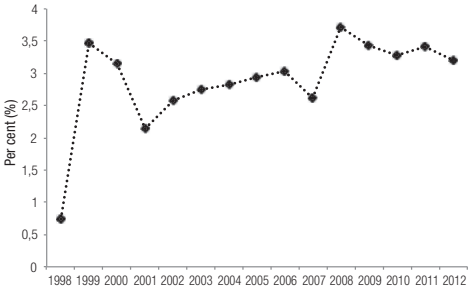

Figure 4: Trend for the mean individual increase in inbreeding (ΔF) by year for the whole pedigree in the New Zealand White rabbit.

one third of animals in the population had the inbreeding coefficient of 10 to 20% and another one third was within the range of 20 to 35%. The trends for the coefficients of inbreeding, average relatedness and equivalent inbreeding by year of birth for the whole pedigree are depicted in Figure 3.

The estimated mean average relatedness and individual increase in inbreeding were 22.73 and 3.00%, respectively (Table 2). The trend for the average individual increase in inbreeding over the years is depicted in Figure 4. The per cent increase in inbreeding over generations was 1.94, 3.06 and 3.98 estimated through maximum generations, equivalent generations and complete generations, respectively (Table 5).

Effective population size

The estimates of effective population size measured from the pedigree information are given in Table 5. The N_e estimates were 25.71, 16.32 and 12.56 estimated from maximum generations, equivalent generations and complete generations, respectively. The effective sizes estimated from regression and log regression analysis on birth date were 12.19 and 11.26 and those on equivalent generations were 18.50 and 19.66, respectively. The historical genetic bottlenecks in the population were assessed based on the effective size and are depicted in Figure 5 and 6. The trends of effective population size over generations estimated by the variances of family sizes and by the rate of inbreeding are depicted in Figure 5 and the trend of effective population size over the years estimated by the variances of family sizes is depicted in Figure 6. The major bottlenecks that occurred during the study period were during the years 1999 and 2005.

Genetic Conservation Index (GCI)

The frequency distribution of animals with their GCI is given in the Table 6. The maximum GCI value of 11.554 was estimated in 4 animals. The proportion of animals with GCI greater than 9 was 39.10%.

Table 2: Estimates of inbreeding, average relatedness and the number of generations traced in the whole population of the New Zealand White rabbit.

Parameter	Mean	Minimum	Maximum
Inbreeding Coefficient (%)	13.233±0.002	0.000	44.827
Average Relatedness (%)	22.727± 0.002	0.530	34.634
Individual increase in Inbreeding (%)	3.004±0.001	0.000	20.558
Equivalent inbreeding coefficient (%)	17.585±0.003	0.000	64.493
Maximum generations	7.910±0.076	1.000	15.000
Equivalent generations	5.609±0.050	1.000	10.188
Complete generations	4.310±0.036	1.000	8.000

Table 3: Estimates of probability of gene origin in the New Zealand White rabbit.

Population detail	Parameters	Value
Whole population	Total number of animals	2503
	Number of founders	40
	Number of founders actually contributed	38
	Expected inbreeding	4.46
	Computed inbreeding	13.23
Reference population	Proportion of animals with known pedigree (2468)	98.6
	Number of ancestors contributing	29
	Effective number of founders (f_e)	11
	Effective number of ancestors (f_a)	10
	Contribution of the main ancestor (%)	17.34
	Number of ancestors explaining 50% (f_{a50})	4
	f_e/f_a ratio	1.1

DISCUSSION

An efficient breeding programme of any population requires the constant monitoring of its performance for selection criteria traits. The New Zealand White rabbit population has been maintained in the farm as a closed population by pure breeding without intense selection. In India, there has been no study so far on the pedigree analysis of rabbit populations. In general, the studies on genetic structure of rabbit population in developing countries are very few.

Generation interval

Average generation interval is an essential population parameter in selection programs, as it directly affects the yearly genetic trend for the selected traits. The mean generation interval presently studied for the whole population of New Zealand White rabbit was 1.49 yr (543 d), which is comparable with earlier reports on rabbit populations. Rafat *et al.* (2009) reported the average generation interval of 1.54 yr (562 d) and 1.64 yr (601 d) for 2 different lines (LL and HL) of Angora rabbits in France. Nagy *et al.* (2010) studied the generation interval on Pannon White rabbits in Hungary and reported the overall mean as 1.211 yr for the whole population. Similar study was carried out on Sika rabbits in Slovenia by Planinc *et al.* (2012) and Ibicenco rabbits in Spain by Martín de la Rosa *et al.,* (2016) who reported the mean generation intervals as 1.16 and 1.0 yr, respectively.

The estimates obtained for all the 4 gametic pathways of generation interval were 1.36, 1.53, 1.44 and 1.59 yr for buck-son, buck-daughter, doe-son and doe-daughter, respectively. The pathway of father to son was least (1.36 yr) and mother to daughter was highest (1.59 yr).

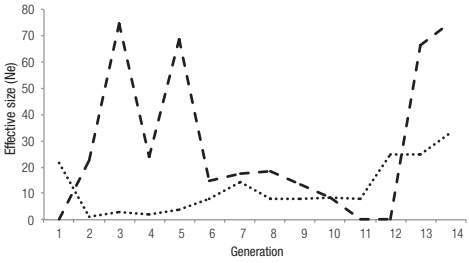

Figure 5: Trends of the effective population size over generations estimated by variances of family sizes and by rate of inbreeding in the New Zealand White rabbit. •••• N_e family variance, ▬▬ N_e inbreeding.

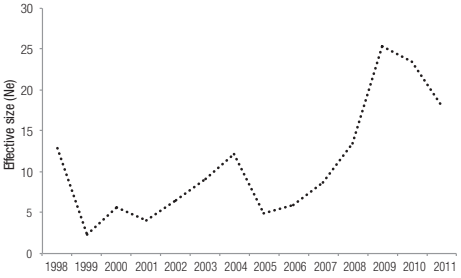

Figure 6: Trend of the effective population size over the years estimated by variances of family sizes in the New Zealand White rabbit.

Table 4: Mean inbreeding (F), rate of inbreeding (ΔF), proportion inbred, mean inbreeding of inbred population, average relatedness and effective population size (N_e) based on complete generations) in the small population of New Zealand White rabbit.

Generation	Number	F (%)	ΔF	Proportion inbred (%)	F of inbred (%)	AR (%)	N_e
0	40	0	0	0	0	4.20	
1	163	2.45	2.45	11.04	22.22	7.84	20.30
2	366	2.98	0.52	22.95	12.97	12.26	93.10
3	343	5.95	2.97	79.88	7.45	17.95	16.30
4	447	11.84	5.89	100.00	11.84	22.92	7.90
5	342	18.37	6.53	100.00	18.37	27.37	6.70
6	524	20.66	2.29	100.00	20.66	29.80	17.80
7	274	22.68	2.03	100.00	22.68	30.49	19.50
8	9	23.59	0.90	100.00	23.59	31.03	42.70

These results are in contrary to the reports of Nagy *et al.* (2010) and Planinc *et al.* (2012), where the paternal pathway showed maximum generation interval in Pannon White and Sika rabbits, respectively.

Pedigree completeness

The more complete the pedigree, the more accurate and reliable the estimates will be. The knowledge on pedigree completeness is important, as the inferences drawn on inbreeding and effective population size depend on it. The pedigree depth is important because it plays a significant part in the rest of the parameters that require genealogical data for their calculation (Martín de la Rosa *et al.,* 2016). Up to 5th generation, 71.29% pedigree was complete (Figure 1). This reflects the well maintained pedigree records at the farm database of the research station. The maximum known generations were 15 with an average of 7.9 in the present study. The average equivalent generations traced for the population were 5.609 (Table 3) which is indicative of a fairly good depth in pedigree. However, this value is much lower than the value reported (11.36 generations) for Pannon White rabbits by Nagy *et al.* (2010). The pedigree completeness up to best known generations is very essential to have accurate estimates of inbreeding, gene flow, *etc.*

Probability of gene origin

The analyses regarding probability of gene origin are of great concern when analysing small populations, as all the genetic stock, except for migration and mutation, comes from the founder animals. Management of this initial stock is crucial for the future of the population (Martín de la Rosa *et al.,* 2016). The number of founders and ancestors for the whole population were 40 and 29, respectively, whereas the f_e and f_a for the reference population were 11 and 10, respectively. The effective number of founders accounted for quite a large proportion (27.5%) out of the total number of animals belonging to the founder population. Moreover, a 1:5 buck to doe ratio was followed throughout in

Table 5: Estimates of the increase in inbreeding and effective population size in the New Zealand White rabbit.

Parameter	Method of estimation	Value
Inbreeding increase (%)	Maximum generations	1.94
	Equivalent generations	3.06
	Complete generations	3.98
Effective population size	Maximum generations	25.71
	Equivalent generations	16.32
	Complete generations	12.56
	Individual increase in inbreeding	14.93
	Regression on equivalent generations	18.50
	Log regression on equivalent generations	19.66
	Regression on birth date	12.19
	Log regression on birth date	11.26

the animals maintained for breeding. Therefore, including effective number of founders in a pedigree report may provide valuable information that could be used for more effective management of inbreeding.

The f_{a50} to the gene pool of the population was only 4, which might have led to reduction in genetic variability and increased amount of inbreeding (13.23%). This reduced genetic variability in the population has possibly influenced the genetic variance of a number of different traits and, as a consequence, the potential for selective breeding. In the present study, the most important ancestors were a single buck and a single doe responsible for 17.34 and 15.79% of the genetic variability of the population, respectively.

Table 6: Frequency distribution of animals on their Genetic Conservation Index (GCI) in the small population of New Zealand White rabbit.

GCI	N	%N
≤1	35	1.4
1.1-3.0	170	6.8
3.1-5.0	253	10.1
5.1-7.0	285	11.4
7.1-9.0	782	31.2
9.1-11.0	936	37.4
>11.0	42	1.7

The ratio between f_e and f_a allows an evaluation of the extent that the genetic variability available in the founders has been reduced because of bottlenecks between the base population and the reference population. The effective number of founders is expected to be higher than effective number of ancestors because ancestors with many progenies have a higher probability of contribution of their genes to the gene pool of the population (Boichard *et al.,* 1997). In the present study, the difference between f_e and f_a in the New Zealand White rabbit population was not very high. According to Pedrosa *et al.* (2010), the marginal contribution of all ancestors must add up to one, and the f_a should always be lesser than or equal to the f_e. The ideal ratio would be one. However, in the present population, the extent of genetic bottleneck as expressed by the ratio was 1.1, which is indicative of absence of stringent bottlenecks. The bottleneck effect is related to the intensive use of a small number of animals for breeding in recent generations. Logically, bottlenecks are more frequent in populations with a long historical pedigree (Santana *et al.,* 2012).

These values of effective number of founders and ancestors for the reference population are very low if we compare them with values attained with other studies such as the Pannon White in Hungary (48 and 26) (Nagy *et al.,* 2010), Sika rabbit in Slovenia (36 and 24) (Planinc *et al.,* 2012) and Ibicenco rabbit in Spain (10 and 11) (Martín de la Rosa *et al.,* 2016) for f_e and f_a, respectively. The f_{a50} value in Sika rabbit was 9 (Planinc *et al.,* 2012) and in Ibicenco rabbit was 4 (Martín de la Rosa *et al.,* 2016). But these comparisons are to be made with caution, due to the differences in the size of the population and pedigree depth.

Inbreeding and average relatedness

The estimated coefficients of inbreeding and equivalent inbreeding were 13.23 and 17.59%, with a maximum of 44.8 and 64.5%, respectively. These values are quite high in magnitude when compared to the reports of inbreeding in other rabbit populations. Nagy *et al.* (2010, 2011 and 2012) reported the inbreeding values of 3 different populations of Pannon White rabbits as 5.54, 6.30 and 7.69%, respectively. Similar values were also reported in Botucatu (7%) and Sika (6.5%) rabbit populations as reported by Moura *et al.* (2000) and Planinc *et al.* (2012), respectively. Martín de la Rosa *et al.* (2016) reported a mean inbreeding coefficient of 10.8% in Ibicenco rabbit.

The fact that the average inbreeding coefficient increased over the years (Figure 3) and over the generations (Table 4) clearly indicates the occurrence of mating among closely related individuals as evidenced by full-sib, half-sib and parent-offspring mating in the present study. In every closed population, whatever its size, inbreeding will increase. Moreover, in a small population, even an efficient mating monitoring cannot prevent inbreeding, but only delay its onset. The average relatedness coefficient for the present study was 22.73% with a maximum of 34.63%, which is very high compared with the reports of Nagy *et al.* (2010) in Pannon White (5.4%) and Planinc *et al.* (2012) in Sika (7.8%) rabbit populations. Values of average relatedness show an increasing trend over the years (Figure 3) similar to that of the inbreeding coefficient. Average relatedness was 7.84 for first generation and crossed 20% in fourth generation (Table 4). The reason for the high values of inbreeding and average relatedness can be attributed to the smaller size of the population in the present study, and hence implementation of proper strategies to avoid inbreeding is required. According to Goyache *et al.* (2003), when AR reaches high relative values, mating should be

carefully planned, otherwise matings between individuals showing a certain degree of relatedness will occur at an unacceptably high frequency.

The mean value of individual increase in inbreeding was found to be 3% with a maximum of 20.6% (Table 3), which is quite high in the present study. An increased rate of inbreeding means an increased risk to a breeding programme in terms of the variance of genetic gain, and a reduction in the additive genetic variance (Meuwissen, 1991). Analysis for whole pedigree revealed that the increase in inbreeding by maximum generation was 1.94%, whereas by complete generation it was 3.98%. This indicates that the incomplete pedigrees tend to underestimate the level of inbreeding. This is because the probability of finding common ancestors increases along with the pedigree completeness level. The best strategy to manage genetic diversity when the pedigree of the population is available is to optimize the contributions of parents by minimizing their coancestry weighted by their contributions (Fernández *et al.,* 2003).

One possible strategy would be to use the average relatedness coefficient to guide selection of animals (Gowane *et al.,* 2014). A very high AR coefficient indicates that the parents of an individual have close common ancestors, while a low average relatedness coefficient indicates that the animal shares alleles by common descent with only a relatively small percentage of the population. Hence, it is advisable to use individuals for breeding with the lowest possible AR coefficients. If relatively high AR values are observed in a herd, it should be opened up to new, underrepresented animals (Goyache *et al.,* 2003).

Effective population size

The effective population size has become one of the most important issues in population genetics, given its usefulness as a measure of the long-term performance of the population regarding both diversity and inbreeding and, therefore, to characterize the risk status of livestock breeds (Duchev *et al.,* 2006). It is characterized by the number of animals that mate in an ideal population and generate the same inbreeding increment of the population under study (Hill, 1979). At present, one of the methods of choice to compute N_e is the realized effective population size (Leroy *et al.,* 2013). The estimates of N_e based on the individual increase in inbreeding would accurately reflect the genetic history of the populations, namely the size of their founder population, their mating policy or bottlenecks. All these phenomena influence the pedigree of the individual and are therefore reflected in the individual increase in inbreeding (Cervantes *et al.,* 2008; Gutiérrez *et al.,* 2008, 2009).

The mean effective size varied between 11.26 and 25.71 depending on the chosen calculation method (Table 5). The realized effective population size computed via the individual increase in inbreeding for the reference population was 14.93. When compared with other commercial breeds of rabbits, the effective size in the present study is found to be much smaller, which can be attributed to the higher rate of inbreeding in the population. The effective size reported in 2 different lines of Angora rabbits ranged from 29 to 47 (Rafat *et al.,* 2009) and in Pannon White rabbits from 37.19 to 91.08 (Nagy *et al.,* 2010). However, this effective size is higher than the value (9.6) reported by Martín de la Rosa *et al.* (2016) in Ibicenco rabbit population. The trend of N_e estimated over generations by different methods revealed similar pattern of effective population size. In most cases, the logarithmic regression estimation gave similar results to those for the family variance based effective population size. Boichard *et al.* (1997) showed that when the pedigree information is incomplete, the computed inbreeding is biased downward and the realized effective size is overestimated. Given the low degree of pedigree knowledge for the populations studied, the true effective size would be even less, a fact worsening the problem of maintenance of genetic variability (Gutiérrez *et al.,* 2003).

The effective size values in the present study are far below 50 to 100, which is the recommended value for this parameter to maintain a viable population in the long-term (Meuwissen, 2009). However, Leroy *et al.* (2013) reported that these threshold values of 50 to 100 had been criticized and should be revised. It is important to note that the present estimate is critical, however, estimates of average N_e is not constant and do change with time according to the level of inbreeding in the herd. With the increasing trend of inbreeding, the situation is going to be worse.

Genetic conservation index

The genetic conservation index is based on the assumption that the aim of a conservation programme is to retain the full range of alleles possessed by the base population. The ideal individuals would receive equal contributions from all the founder ancestors in the population and, consequently, the higher values of animal for conservation. The

higher the GCI value, the higher the values of an animal for conservation. In the present study, the maximum GCI value of more than 11.0 was estimated in 1.7% animals and the proportion of animals with GCI greater than 9 was 39.10% (Table 6). This could be used as a scale to use such living animals with higher GCI to maintain balanced contribution from the founders (Venkataramanan *et al.,* 2013). Thus, the GCI can be used as an aid to the selection of breeding individuals. However, the index has limitations such as not accounting for any concentration of breeding to non-founder animals in subsequent generations in a pedigree and is inapplicable without pedigree records (Alderson, 1992).

CONCLUSIONS

The population structure of the New Zealand White rabbit was addressed here from the pedigree information perspective, which revealed the genetic structure of the small population of this breed of rabbit. From the study, it is evident that the herd was completely inbred, with a very high inbreeding coefficient, and the effective population size was critical. These findings support the need for continuous monitoring of the accumulated inbreeding and resulting loss in genetic variability. It is essential to keep track of inbreeding and the average relatedness periodically to avoid adverse effects on fitness and production traits. Looking into the high levels of average relatedness, it is important that the mating should be carefully planned; otherwise, matings between individuals showing a certain degree of relatedness will occur at an unacceptably high frequency, leading to unfavourable effects in the population. Hence, before introducing new individuals in order to reduce the probability of deleterious effects of inbreeding in the herd, it is necessary to check the real effect of inbreeding on genetic progress of production and fitness traits. Moreover, if this population of purebred New Zealand White rabbits is crossed with other rabbit populations, the inbreeding will not be a nuisance, as it will be dissipated. The present study highlights the importance of maintaining the pedigree and can help in carrying out similar studies on rabbits in the developing countries where small populations are inevitable.

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