Computing effective population size from molecular data: The case of three rare Spanish ruminant populations

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A B S T R A C T
The aim of this study was to compare different methodologies to compute the effective size when the genealogies are not available (or are shallow) in three rare Spanish ruminant populations. For this purpose the authors used molecular information from three Spanish local ruminant populations (the Pajuna cattle, Payoya goat and Merino de Grazalema sheep populations). Several methods based on molecular or pedigree data were applied to estimate the effective population size in the three studied populations. Estimates based on increase in molecular coancestry ($N_{efm}$) in Pajuna (8.5) and Payoya (16.7) populations were 2- and 3-fold lower than those obtained using the linkage disequilibrium method. However, $N_{efm}$ in Merino de Grazalema population reached a higher value (110.5 vs 86.2). Regarding the effective size using temporal methods ($F$ statistics and coalescence theory), the results for Pajuna were very similar across methodologies with values ranging from 6.0 to 7.8. In the Payoya goat, the results obtained ranged from 15.0 to 33.4. For Merino de Grazalema was not possible to estimate the $N_e$ using temporal methods. Regarding the genealogical methods, pedigrees highly compatible with molecular information were generated from the genotypes of the individuals, the correlations between the molecular and the genealogical coancestry matrix were high from 0.82 to 0.94. The effective population sizes based on individual increase in inbreeding were similar for Pajuna (17.0) and Payoya (18.1) and for Merino de Grazalema was 24.2. The $N_e$ based on an increase in coancestry ($N_{ec}$) was higher in all cases ranging from 20.2 for Pajuna to 38.3 for Merino de Grazalema. The $N_{ec}$ for Payoya was 27.1. We can conclude that there is no single value of molecular-based $N_e$ for each population, because high ranges for effective size where found across methodologies. However, the assessed ranking was steady: the Pajuna is the most endangered population, followed by Payoya and Merino de Grazalema. When the priority for conservation is of concern, all methods seem to be useful, but it is not possible to combine them.

1. Introduction

The conservation of genetic diversity is a priority factor in rare small populations (Meuwissen, 2009). The effective size ($N_e$) is 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 characterise the risk status of livestock breeds (Duchev et al., 2006; FAO, 1998). Recently, Villanueva et al. (2010) have proposed to assess the genetic diversity in livestock using as indicator the average of effective size across populations. \( N_e \) is also necessary in the application of genomic selection of populations, since the accuracy of the breeding values depends on the linkage disequilibrium between the QTL and SNP, and a higher \( N_e \) means a higher number of markers is needed (Meuwissen et al., 2001). However, estimates of \( N_e \) vary with the methodology used to assess it, thus limiting its ability to characterise the risk status of a population or to predict the precision of the genomic selection. Therefore, assessing the reliability of the estimates of \( N_e \) is a very important challenge.

Classical expressions developed to obtain \( N_e \) rarely work well in real populations. When genealogies are available, and the mating policy is stable over time, the effective size can be computed using increases in inbreeding between two generations (Falconer and Mackay, 1996), but the reliability of the results depends on the pedigree depth. Other methodologies based on family variance size overestimated the parameter (Gutiérrez et al., 2008; Cervantes et al., 2008). The use of individual increase in inbreeding has showed that a pedigree with at least 2 generations can be enough to attain reliable results (Gutiérrez et al., 2009). However, pedigree records are usually missing in endangered populations and only molecular marker information is available. In this situation, the use of methodologies based on linkage disequilibrium (Hill, 1981; Waples, 1991) and temporal methods, based on variation on allelic frequencies (Waples, 1989; Jorde and Ryman, 2007) or based on coalescence theory (Berthier et al., 2002) are advisable. But, the size and the number of markers of the samples that cause variation in the final value should be taken into account. All of this, plus some specific requirements of livestock populations (sanitary movement restriction, breeding policy) increase the difficulty of assessing a reliable and useful effective size.

There is not a "gold method" to obtain molecular-based estimates of \( N_e \) (Luihart et al., 2010). Even some approaches have been proposed to combine different molecular-based estimates in order to increase their precision (Waples and Do, 2010), pedigree-based methodologies are already considered the best option. However, in rare populations genealogies are usually shallow or unknown (Álvarez et al., 2008). In such scenarios, demographic parameters do not reflect the history of the population such as bottlenecks, preferential matings or subdivision (Harris and Allendorf, 1989; Frankham, 1995). Therefore, molecular-based methods to estimate \( N_e \) are a nice alternative and microsatellites are in this case an advisable source of information.

In Spain there are more than 80 local livestock ruminant populations that are classified as endangered, following the Official Catalogue of Livestock breeds. In this study we used one example of cattle, sheep and goat populations to check the different methodologies. The Pajuna cattle population is located in Andalucía and Castilla-La Mancha regions, its census is about 797 individuals and is used for meat production. The Merino de Grazalema sheep, kept in Southern Andalucía region, is a double aptitude population (milk and meat production) with a census of 4912 individuals. The Payoya is a goat population bred in Andalucía for milk production with a census of 6971 individuals.

The aim of this study was to compare different methodologies to compute the effective size when the genealogies are not available (or are shallow) in three rare Spanish ruminant populations.

## 2. Material and methods

The average depth of the available pedigrees of the Pajuna cattle, Payoya goat and Merino de Grazalema sheep populations ranged from 0.2 to 1.2 equivalent complete generations, therefore being non-informative (Gutiérrez et al., 2009). We used molecular information from three Spanish local ruminant populations; the Pajuna cattle, Payoya goat and Merino de Grazalema sheep populations. The samples collected for this study numbered 60 for the Pajuna cattle population, 262 for the Merino de Grazalema sheep population, and 551 for the Payoya goat population. Samples were obtained at random and represented between 5 and 10% of the population census. The number of loci analysed for each population ranged from 11 for the Pajuna (BM1824, BM2113, ETH10, ETH3, TGLA122, TGLA227, ETH225, SPS115, TGLA126, INRA23, TGLA53) to 19 for the Merino de Grazalema (AE1129, CP49, CSDR247, FCB20, HSC, ILSTS005, ILSTS008, ILSTS011, INRA06, INRA023, INRA049, INRA063, INRA132, INRA172, MAF214, MAF65, Mcm042, SPS113, SPS115). For the Payoya goat population 14 markers were used (BM1258, CSDR247, ILSTS19, FCB20, HSC, ILSTS30, ILSTS87, INRA005, INRA023, INRA063, MAF65, SRCS5P, SRCS8P, TGLA53). All of these markers made part of the biodiversity panel recommended by ISAG/FAO. Samples were classified into two different groups emulating two consecutive discrete generations according to the year of birth of the animals and the generation interval for each population (5 years for Pajuna and 4 for Merino de Grazalema and Payoya populations). Therefore, generation 0 and generation 1 were composed by the following number of samples: 33 and 27 for Pajuna, 49 and 213 for Merino de Grazalema, and 133 and 418 for Payoya population (Table 1).

A pedigree of 5 discrete generations based on the molecular coancestry matrix was inferred using the program MOLCOAN (Fernández and Toro, 2006). This program is based on an annealing algorithm to maximize the correlation between the coancestry molecular matrix, given the data,

### Table 1

<table>
<thead>
<tr>
<th>Population</th>
<th>Generation</th>
<th>N</th>
<th>( N_{\text{eff}} )</th>
<th>( \text{Neff} )</th>
<th>( \text{Neff} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pajuna</td>
<td>0</td>
<td>33</td>
<td>28.1</td>
<td>[24.6;32.5]</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>27</td>
<td>14.7</td>
<td>[14.7;19.3]</td>
<td>157.1</td>
</tr>
<tr>
<td>Merino de Grazalema</td>
<td>0</td>
<td>49</td>
<td>122.8</td>
<td>[122.8;214.8]</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>213</td>
<td>82.1</td>
<td>[82.1;90.6]</td>
<td>108.7</td>
</tr>
<tr>
<td>Payoya</td>
<td>0</td>
<td>133</td>
<td>61.6</td>
<td>[58.2;65.1]</td>
<td>92.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>418</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and the genealogical coancestry matrix built from the fictitious created pedigree. Genealogies highly compatible with molecular information were generated from the genotypes of the individuals assigned to each generation 1.

Seven methodologies based on molecular or pedigree data were applied to estimate the effective population size in the three studied populations.

2.1. Molecular methods

2.1.1. Based on linkage disequilibrium (Hill, 1981; Waples, 1991)

This method is potentially an extremely useful estimator because, unlike most other genetic methods it requires only a single sample population. The method relies on the fact that in a system where gametes are distributed at random among a small number of zygotes there will be departures from expected genotype frequencies, and departures from expected gametic frequencies, both of which can be used to estimate $N_e$. Assumptions of the methods include that the population is stable, panmictic and there is no selection, migration or mutation. Effective population size based on linkage disequilibrium can be computed using this expression

$$N_e_{LD} = \frac{1}{3r(1-r)}.$$

where $r$ is the correlation among alleles, and $s$ is the sample size (Hill, 1981; Waples, 1991). The value for $r$ using:

$$r = \frac{D}{\sqrt{(p^r(1-p)^q + (1-q))}}$$

where $p$ and $q$ are the frequencies of allele A at locus 1 and allele B at locus 2, respectively and $D$ is Burrow’s composite measure of disequilibrium (Campton, 1987). The $N_e_{LD}$ effective size was computed for each generation using the NeEstimator v1.3 Program (Peel et al., 2004).

2.1.2. Increase in molecular coancestry

The effective size was also computed using the increase in molecular coancestry between samples from generation 0 to 1 using this expression,

$$\Delta f_m = \frac{f_{(m)1} - f_{(m)0}}{1 - f_{(m)0}},$$

where $f_{(m)1}$ is the coancestry in generation 1 and $f_{(m)0}$ is the coancestry in generation 0. Coancestry was adjusted by the sample sizes in the two generations. The aim of such adjustment was to avoid overestimation of selfcoancestries because, unlike most other genetic methods it requires only an approximation to the genealogy of the Wright-Fisher model, which assumes discrete generations. This method tries to find common ancestors between the individuals, the probability of the meeting of two ancestral lineages depending on the effective size ($N_e$). It outputs estimates of possible effective population sizes (harmonic mean over this time

where $T$ is the number of generations between the two samples ($T$ equals 1, in this case) and $S_0$ and $S_t$ the sample sizes. These analyses were performed with the NeEstimator v1.3 Program (Peel et al., 2004).

The Jorde and Ryman (2007) $F$-statistics method was also used. This method is unbiased for small samples and highly skewed allele frequencies even though at the cost of a large standard deviations. The parameter $F$ is computed with this expression

$$F_5 = \frac{\sum_{i=1}^{m} (x_i - y_i)^2}{\sum_{i=1}^{m} z_i(1-z_i)}.$$ 

This measure differs from those considered previously in that the numerators are estimated separately by summing over all alleles before carrying out the division. And $z_i$ is the average frequency of the $i_{th}$ allele over samples. The $N_e$ is computed with this expression

$$N_{e_{[W]}} = \frac{T}{2A_5 - 1} - \frac{1}{2k_t}.$$

where $T$ is the number of generations between the two samples (T equals 1, in this case) and $S_0$ and $S_t$ the sample sizes. These analyses were performed with the NeEstimator v1.3 Program (Peel et al., 2004).

2.1.3. Coalescence theory methods (Berthier et al., 2002)

As a temporal method, this approach assumes that gene frequency data are obtained from a closed population sampled at two points in time separated by a given number of generations. This period is sufficiently short to allow considering the effect of mutation on the observed gene frequencies to be negligible over the interval. However, it is only an approximation to the genealogy of the Wright–Fisher model, which assumes discrete generations. This method tries to find common ancestors between the individuals, the probability of the meeting of two ancestral lineages depending on the effective size ($N_e$). It outputs estimates of possible effective population sizes (harmonic mean over this time.

2.1.3.1. $F$-statistics methods

These methods are conducted by the sampling of individuals at two or more different moments and the estimation of the amount of genetic drift in the interrum. The Waples expression (Waples, 1989) was used to estimate the $F$ parameter,

$$F_k = \frac{1}{A-1} \sum_{i=1}^{A} \frac{(x_i - y_i)^2}{(x_i + y_i)/2},$$

where $A$ is the number of alleles for one locus and $x_i$ and $y_i$, the estimated frequencies for the $i_{th}$ allele in the two samples. The effective size can be computed with this expression

$$N_{e_{[F]}} = \frac{4}{T} \left(1 + \frac{F_5}{4} \right) \left(1 - \frac{1}{2n_5} \right).$$

where $n_5$ is the harmonic mean of the two samples, $n_5$ is the size of sample of last generation and $N$ the census when the first sample was collected. Computations were performed using the program TempoF5 (Jorde and Ryman, 2007).

2.1.3.2. Coalescence theory methods (Berthier et al., 2002)

As a temporal method, this approach assumes that gene frequency data are obtained from a closed population sampled at two points in time separated by a given number of generations. This period is sufficiently short to allow considering the effect of mutation on the observed gene frequencies to be negligible over the interval. However, it is only an approximation to the genealogy of the Wright–Fisher model, which assumes discrete generations. This method tries to find common ancestors between the individuals, the probability of the meeting of two ancestral lineages depending on the effective size ($N_e$). It outputs estimates of possible effective population sizes (harmonic mean over this time.
interval). These estimates can be regarded as (sequentially correlated) random variates from the posterior distribution of \( N_e \), which is the probability distribution of \( N_e \), given the data. Different priors were tested to check the consistency of the obtained estimates. The analyses were performed with the program tm3.1 (Berthier et al., 2002).

2.2. Genealogical data

The effective population size was computed in each generation for the simulated scenarios using both the individual increase in inbreeding (Gutiérrez et al., 2008, Cervantes et al., 2008, Gutiérrez et al., 2009) and increase in pairwise coancestry (Cervantes et al., 2011).

The individual increase in inbreeding is defined as

\[
\Delta F_i = 1 - \frac{1}{1 - F_i},
\]

with \( g_i \) the number of generations and \( F_i \) the inbreeding coefficient of an individual \( i \) (Gutiérrez et al., 2009).

The increase in coancestry between any pair of individuals \( j \) and \( k \) can be computed as,

\[
\Delta c_{jk} = 1 - \sqrt{1 - c_{jk}},
\]

where \( c_{jk} \) is the inbreeding of a descendent from both, and \( g_j \) and \( g_k \) are the generation numbers for the parents (Cervantes et al., 2011). Both parameters take into account the exclusion of self-fertilization.

By averaging the individual increase in inbreeding and the increase in pairwise coancestry for all pairs of individuals in a reference subpopulation we can estimate a effective population size based on inbreeding \( N_{ef} = \frac{1}{2\Delta F} \) or in coancestries \( N_{ec} = \frac{1}{2\Delta c} \).

The effective size analyses were performed using the ENDOG program (version v4.8) (Gutiérrez and Goyache, 2009).

3. Results

Table 1 gives the estimates for the effective size based on linkage disequilibrium per generation and population. For generation 1, estimates were lower than those computed for generation 0. The values decreased by 40.2% for Pajuna, 45.1% for Merino de Grazalema, and 43.3% for Payoya.

The results obtained using differences between both generations are given in Table 2. Estimates based on increase in molecular coancestry in Pajuna (8.5) and Payoya (16.7) populations were 2- and 3-fold lower than those obtained for generation 1 using the \( N_{LD} \) method. However, \( N_{eff} \) in the Merino de Grazalema population reached a value of 110.5. Regarding the effective size using temporal methods, the results for Pajuna were very similar across methodologies with values ranging from 6.0 for \( N_{efm} \) to 7.8 for \( N_{fam} \). In the Payoya goat, the results obtained for \( N_{efm} \) and \( N_{fam} \) were very similar (33.4 and 34.1, respectively) while the \( N_{efm} \) value was the lowest (15.0). The estimate of \( N_{efm} \) in the Pajuna cattle was also the lowest one. In the case of Merino de Grazalema, it was not possible to estimate reliable results using \( N_{efm} \), \( N_{efm} \) nor \( N_{fam} \).

The results for methodologies using genealogical information are in Table 3. The correlations between the molecular and the genealogical coancestry matrix, using the program MOLCOAN (Fernández and Toro, 2006) to create the pedigree, ranged from 0.82 to 0.94. This ensures the reliability of the reconstructed pedigree. The effective population sizes based on individual increase in inbreeding were similar for Pajuna (17.0) and Payoya (18.1). For the Merino de Grazalema was 24.2. The \( N_e \) based on increase in coancestry was higher in all cases ranging from 20.2 for Pajuna to 38.3 for Merino de Grazalema. The \( N_{ec} \) for Payoya was 27.1. The ratio \( \frac{N_{ec}}{N_e} \) was higher than 1 in all cases, indicating that the increase in inbreeding was faster than increase in coancestries.

4. Discussion

The methods based on linkage disequilibrium have the advantage that requires only one sample to estimate the value. Loci may be linked (Hill, 1981), and this increases the precision. Waples (1991) modified the method to give precise estimates of \( N_e \) using unlinked loci, but this method is biased when the sample size is small and below the true \( N_e \) (England et al., 2005). Here the estimations of \( N_{LD} \) for the three populations were below the size of the sample, but despite being a guarantee that the size of the sample is sufficient, the theoretical assumption of the method (no subpopulation structure, no migration) could not reflect the real situation of the population. The increase in molecular coancestry \( (N_{efm}) \) method gave different results despite the highest value was for Merino de Grazalema and the smallest value for Payuja as obtained with \( N_{LD} \). Regarding temporal methods, the values for the Pajuna population were very similar to those obtained with the increase in molecular coancestry and for the Payoya population it was an intermediate value between the \( N_{LD} \) and the \( N_{efm} \). For the Merino de Grazalema it was not possible to estimate the \( N_e \) using these methods. The methods based on F statistics measure the amount of genetic drift between the two samples, therefore the absence of genetic drift in Merino de Grazalema or the presence of related individuals.

Table 2

<table>
<thead>
<tr>
<th>Population</th>
<th>( N_{efm} )</th>
<th>( N_{fam} )</th>
<th>( N_{ec} )</th>
<th>( N_{ec} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pajuna</td>
<td>8.5</td>
<td>7.8</td>
<td>6.0</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>[5.2;11.7]</td>
<td>[4;10]</td>
<td>[5.8;10.1]</td>
<td></td>
</tr>
<tr>
<td>Merino de Grazalema</td>
<td>110.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Payoya</td>
<td>16.7</td>
<td>33.4</td>
<td>15.0</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>[22.7;48.5]</td>
<td>[11.25]</td>
<td>[25.3;45.3]</td>
<td></td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Population</th>
<th>( N_F )</th>
<th>( N_{ec} )</th>
<th>( \frac{N_{ec}}{N_F} )</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pajuna</td>
<td>17.0</td>
<td>20.2</td>
<td>1.2</td>
<td>0.94</td>
</tr>
<tr>
<td>Merino de Grazalema</td>
<td>24.2</td>
<td>23.8</td>
<td>1.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Payoya</td>
<td>18.1</td>
<td>27.1</td>
<td>1.5</td>
<td>0.89</td>
</tr>
</tbody>
</table>
between both samples could explain why it is impossible to estimate \( N_e \) (Waples and Yokota 2007).

On the other hand, the estimates of \( N_e \) based on 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 due to abusive use of reproductive individuals for the period in which the genealogies are known (under these conditions 5 discrete generations). All these phenomena influence the pedigree of the individual and are therefore reflected in the individual increase in inbreeding (Gutiérrez et al., 2008, 2009; Cervantes et al., 2008). Moreover another effective size based on increase in coancestry complement the information given by the first one in order that would provide information on the effective size of a population under random mating. Furthermore, it has been shown that the comparison between this \( N_{me} \) parameter and the individual increase in inbreeding gives information on the degree of population structure (Cervantes et al., 2011). The use of the molecular data to create a pedigree to use this genealogical methods seem to be a good solution, despite in this case the generations were assumed to be discrete. Both effective sizes gave values below 50–100, the recommended values for this parameter to maintain a viable population in a long-term (Mevissen, 2009). Since the \( N_{me} \) and \( N_e \) are equal under random mating, the ratio \( N_{me}/N_e \) showed a certain degree of subdivision mainly in Merino de Grazalema (1.6) and Payoya (1.5). In Merino de Grazalema population the presence of a double aptitude, and its recent creation (approximately 100 years ago) influenced by Merino and Churra populations make it a heterogeneous population. However, the Payoya population seems to be a very homogeneous population: actually, it is the population where the \( N_e \) based on linkage disequilibrium and genealogical estimations are more similar due the absence of population structure. Particularly under such conditions, the reconstruction of genealogies and the estimation of realised effective sizes could be a good alternative.

We can conclude that there is no single value of molecular-based \( N_e \) for each population, because high ranges for effective size where found across methodologies. However, the assessed ranking was steady: the Payoya is the most endangered population, followed by Payoya and Merino de Grazalema. When the priority for conservation is of concern, all methods seem to be useful, but it is not possible to combine them. It is recommendable to use the same method across populations to define the risk status of the list of populations. Moreover, if a precise value of \( N_e \) is needed, as for example to define the size of sampled animals to be genotyped under a genomic selection scenario, different methodologies would lead to different conclusions. There is room for further testing of molecular-based methodologies in populations in which pedigrees are sufficiently depth to obtain sound estimates of \( N_e \).

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References


