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# Pedigree analysis of seven small French sheep populations and implications for the management of rare breeds<sup>1</sup>

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**ABSTRACT:** Pedigree information was analyzed in 7 small populations of sheep raised in France (Bleu du Maine, Charmoise, Cotentin, on-farm Romanov, Romanov ex situ in vivo, Roussin de la Hague, Solognote) to estimate their genetic variability. The pedigree information for each breed, estimated by the number of equivalent generations traced, ranged from rather poor (4.6) to very good (10.5) when compared with other studies. On the basis of probabilities of gene origin, the effective number of ancestors ranged from 17 (on-farm Romanov breed) to 59 (Bleu du Maine). On the basis of the rate of inbreeding, the realized effective size was found to range from 65 (Romanov breed ex situ) to 231 (Bleu du Maine). The average kinship coefficients between rams from which semen doses are available in the

French National Cryobank and the active ram and ewe populations were also computed. Results found in each breed were analyzed by taking into consideration the demographic evolution of the breeds, their management practices, and the use of cryopreservation as a way to preserve genetic variability. It appeared quite clear that, in populations in which AI with frozen semen is seldom used, factors that mainly affect the genetic variability are the female-to-male ratio, which should be as small as possible, and the number of reproducing female offspring by males, which should be as balanced as possible. Finally, our work showed that all populations under study have fairly good genetic variability in comparison with other species, despite their scarce numbers.

**Key words:** cryoconservation, genetic variability, management of small populations, pedigree analysis, sheep

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## INTRODUCTION

There has been increased awareness of the need to preserve rare livestock breeds since the Food and Agriculture Organization alerted the public to the increased extinction of farm animal biodiversity (Scherf, 2000; Food and Agriculture Organization, 2007). Approximately one-half of the 60 sheep breeds raised in France are considered rare, according to the European Union definition (population size less than 10,000 ewes, Council Decision No. 2004/817), yet French sheep production is of great importance for land management and biodiversity.

Rare breeds are faced with the risk of 1) rapid extinction because of their scarce numbers, 2) a loss of genetic variability and an increase in inbreeding because of their small effective population size ( $N_e$ ), and 3) an effect of genetic drift on breed characteristics. Thus, developing or improving management methods for rare breeds is still an active research field (Goyache et al., 2003; Boettcher et al., 2005; Fernandez et al., 2005, 2008; Colleau and Avon, 2008).

However, managers of rare breeds are usually not aware of this research, and they generally do not wish to use elaborate processes; setting up these methods at the farm level is either too complicated or too constraining. There is a need to investigate the genetic consequences of the practices encountered in the field in addition to theoretical and simulation studies intended to assess the properties of some methods. The goal of such investigations is twofold: 1) to identify the main factors that affect the evolution of the genetic variability of real populations, and 2) to highlight the most efficient rules for management of rare breeds. The purpose of this paper was to conduct such an analysis

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for 7 small sheep populations raised in France. Pedigree information was used to assess the evolution of the within-population genetic variability. Results are discussed in view of the demographic evolution of each breed, management practices, and use of cryopreservation as a complementary tool to manage and preserve within-breed genetic variability.

## MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because data were obtained from an existing database (French national performance recording system, INRA CTIG, France).

### *Populations Studied*

The choice of populations to be studied was quite restricted because of the lack of pedigree information in most rare breeds. A total of 7 populations with sufficient genealogical data were chosen, including 6 breeds raised on the farm plus 1 experimental population. The 6 breeds, chosen to represent a varied panel of situations, were the following: the Bleu du Maine (**BDM**), Charmoise (**CHM**), Cotentin (**COT**), Romanov (**ROM**), Roussin de la Hague (**RLH**), and Solognote (**SOL**) breeds.

Unlike the other breeds under study, the ROM breed does not originate from France (it originated in Russia), but a selection program has been run in France without external influence for more than 30 yr. The last population under study was a ROM nucleus flock (**RIN**), fully disconnected from the selection program described above, that has been raised on an INRA experimental farm since the breed was imported and that has been managed with substantially different rules from the ROM breed on farm (Ricordeau et al., 1990). All these populations are used for meat production.

The main demographic characteristics of the 7 populations under study are shown in Table 1, and the evolution over time of the number of ewes (with both parents known) recorded per birth year is shown in Figure 1. The COT breed and the RIN nucleus always had a small population size, with decreases of  $-36$  and  $-93\%$ , respectively, over the period considered in Figure 1. The SOL breed also had a small population size, but with a substantial increase of  $+263\%$  since 1993. The RLH breed recently changed its status from a rare to a selected breed, with a  $+57\%$  increase in its population size since 1984. Both the BDM and ROM breeds, which used to be considered selected breeds, have had a severe downfall (approximately  $-80\%$  since 1984), which is why they have been considered rare breeds since the 1990s (BDM) and 2000 (ROM). Finally, the CHM has the largest population among the breeds under study, with a large selection nucleus compared with other meat breeds (Institut de l'Élevage, 2008). Approximately 100 CHM rams are evaluated for their

individual performance each year, and the best ones become available for AI.

Some analyses were performed for the ewes born between 2004 and 2007 with both parents known; this group was called the reference female population (Table 1). Reference populations were defined on a 4-yr time path because 4 yr represented approximately a generation interval (see Huby et al., 2003, and the present study).

### *Population Management*

The different methods by which the breeders from the nucleus flocks that are part of the breeding association purchase and use the breeding rams are summarized in Table 2. A ram breeding center has been implemented recently for all the breeds studied. The breeder associations are responsible for the definition and establishment of the breeding programs, which means that only rams from nucleus flocks are bought for the breeding centers, according to the criteria for each breeder association. In rare breeds, the goal of such a center is mainly to raise future natural-service rams under the same environmental and sanitary conditions. Their genetic evaluation, on the basis of individual performance, is therefore more accurate; however, in most cases, performance is not assessed by calculation of EBV, but rather by visual evaluation. Only 2 breeds select their rams according to their EBV, mostly based on maternal (CHM, ROM) and conformation traits (CHM; Table 3). Once the rams have been raised together for 3 to 4 mo, breeders, including those that are not part of the breeding program, are invited to the sale. Rams are dispatched, depending on the breed. The methods used for management of genetic variability vary greatly, from no organization at all in the BDM breed to the rather sophisticated mating system of the SOL breed (see below). The management of the SOL breed is generally considered exemplary. Since 1976, a genetic program has been implemented to avoid genetic drift. The breed is partitioned into 12 groups and the exchange of rams follows a rotational scheme, as described by Chevalet and Rochambeau (1985). It was interesting to study this breed to see the "in situ" effect of a management plan of a small population, because few examples exist of the actual use of these rules by breeder associations. A rotational mating scheme was also implemented within the RIN.

A successful selection program beginning in 2002 within the framework of a national program has been implemented to increase the frequency of the alleles of the *PrP* gene, which enhance resistance to scrapie (Brochard et al., 2006). This selection program is not mentioned in Table 2 because it is systematic.

### *Data Available*

All data came from the French national performance recording system. For each sheep, the file contains the

identification of the animal, sire and dam when known, birth date, sex, and breed. All the animals born from 1960 until 2007 were retrieved from the national system for all the breeds studied. The total number of animals in the file is given in Table 3.

### Demographic Variables

For each population, the evolution of the number of reproducing animals was assessed by comparing 3 different birth year periods: 1981 to 1984, 1991 to 1994, and 2001 to 2004. The simple variables calculated were the following: number of dam sires (for a sire born in the period considered), which is referred to in the text as “sires”; average number of ewe offspring per sire; number of paternal grand-sires (of sires born during the period considered); and average number of reproducing ewe by paternal grand-sire.

The generation lengths of the 4 pathways (sire-sire, and so on) were estimated as the average birth year difference between parents born in the period from 1999 to 2003 and their reproducing offspring (i.e., offspring having at least 1 known offspring). The average generation length for each population was defined as the average of the 4 pathways.

### Ex Situ Conservation

In France, a national cryobank for the long-term storage of animal genetic resources was created in 1999 (<http://www.cryobanque.org/>). In January 2009, the sheep species was represented in the cryobank by the semen doses of rams from a total of 23 different breeds, including the 6 breeds under study (see below). For rare breeds, the material stored represents long-term insurance. For selected populations, the aim of the cryobank is to preserve future possibilities for a given population to change its breeding goals by making available representative samples of the different populations at different periods (Verrier et al., 2003). In each breed, for the rams and ewes, respectively, we defined as active populations the animals used for the reproductive season beginning in July 2007. The average coefficient of kinship of each active population with the cryobank rams was computed.

### Analysis of Genetic Variability

The genetic variability of each population and its evolution over time were assessed through indicators derived from pedigree data. The PEDIG software ([http://dga.jouy.inra.fr/sgqa/article.php?id\\_article=110](http://dga.jouy.inra.fr/sgqa/article.php?id_article=110); Boichard, 2002) was used for that purpose. The principles of the methods and the corresponding equations are described in detail in Boichard et al. (1997), and a detailed overview of their application to animal populations is available in several publications (e.g., Baumung and Sölkner, 2002; Huby et al., 2003; Leroy et al., 2006).

**Table 1.** General variables for the populations studied

Item	Bleu du Maine (BDM)	Charmoise (CHM)	Cotentin (COT)	Romanov (ROM)	Romanov-INRA (RIN)	Roussin de la Hague (RLH)	Solognote (SOL)
Main characteristics							
Main color pattern	Blue faced	White	White	Black spotted	Black spotted	Brown faced	Red
Typical trait(s)	Prolific and maternal	Precocity, muscular development	Large size, grazing	Highly prolific	Highly prolific	Maternal	Hardy
General 2000 census, total No. of ewes	14,300	23,900	6,000	9,100	132	21,500	3,100
On-farm performance recording 2007							
No. of ewes	846	3,771	166	769	118	2,145	953
No. of flocks	24	34	13	8	1	44	18
Avg No. of ewes per flock	35	111	13	96	118	49	53
Female reference populations, No. of recorded ewes born from 2004 to 2007 with both parents known	2,482	6,556	533	1,105	138	5,095	1,891

**Table 2.** Management of the breeding rams from the nucleus flocks by the population studied

Population <sup>1</sup>	Supply and purchase from the breeding center	Selection on breeding value
BDM	No indicators of genetic variability provided. No rules for purchases (“whoever pays the most”).	None
CHM	Rams from the breeding center attributed to grand-sire families; all families have to be represented by at least 1 ram (when possible). No rules for purchases (“whoever pays the most”), but the buyers are invited to choose rams least related to their flocks.	Yes
COT	Existing breeding center is recent (2006). At the sale, a table presenting the average kinship between each ram and all the ewes from potential buyers is provided. No rules for purchases (“whoever pays the most”), but buyers are invited to choose rams least related to their flocks.	None
ROM	Rams from the breeding center attributed to grand-sire families. Since 2006, breeders have chosen rams least related to their flocks.	Yes
RIN	Strict following of a rotational mating system, in which all ewes from a given group ( $n$ ) are bred to a ram born in the previous group ( $n - 1$ ).	None
RLH	Existing breeding center is quite recent (2003). Rams from the breeding center attributed to grand-sire families; all families have to be represented by at least 1 ram (when possible). At the sale, a table presenting the average kinship between each ram and all the ewes from potential buyers is provided. No rules for purchases (“whoever pays the most”), but buyers are invited to choose rams least related to their flocks.	None
SOL	Rotational mating, breeding circle. All families have to be represented by at least 1 ram. Breeders can buy a ram only from the previous group in the rotational scheme.	None

<sup>1</sup>BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.

Pedigree completeness was assessed for the 2004 to 2007 reference ewe population by computing for each animal ( $i$ ) from this population its equivalent generations ( $EqG_i$ ), traced as follows:

$$EqG_i = \sum \left(\frac{1}{2}\right)^n, \quad [1]$$

with  $n$  being the generation rank of a given ancestor (1 = parents, 2 = grandparents, and so on), and the sum being computed across all known ancestors of  $i$ . The  $EqG$  for the whole population was computed as the mean of the  $EqG_i$ .

The average number of ancestors per ewe was computed by summing across all generations the percent-

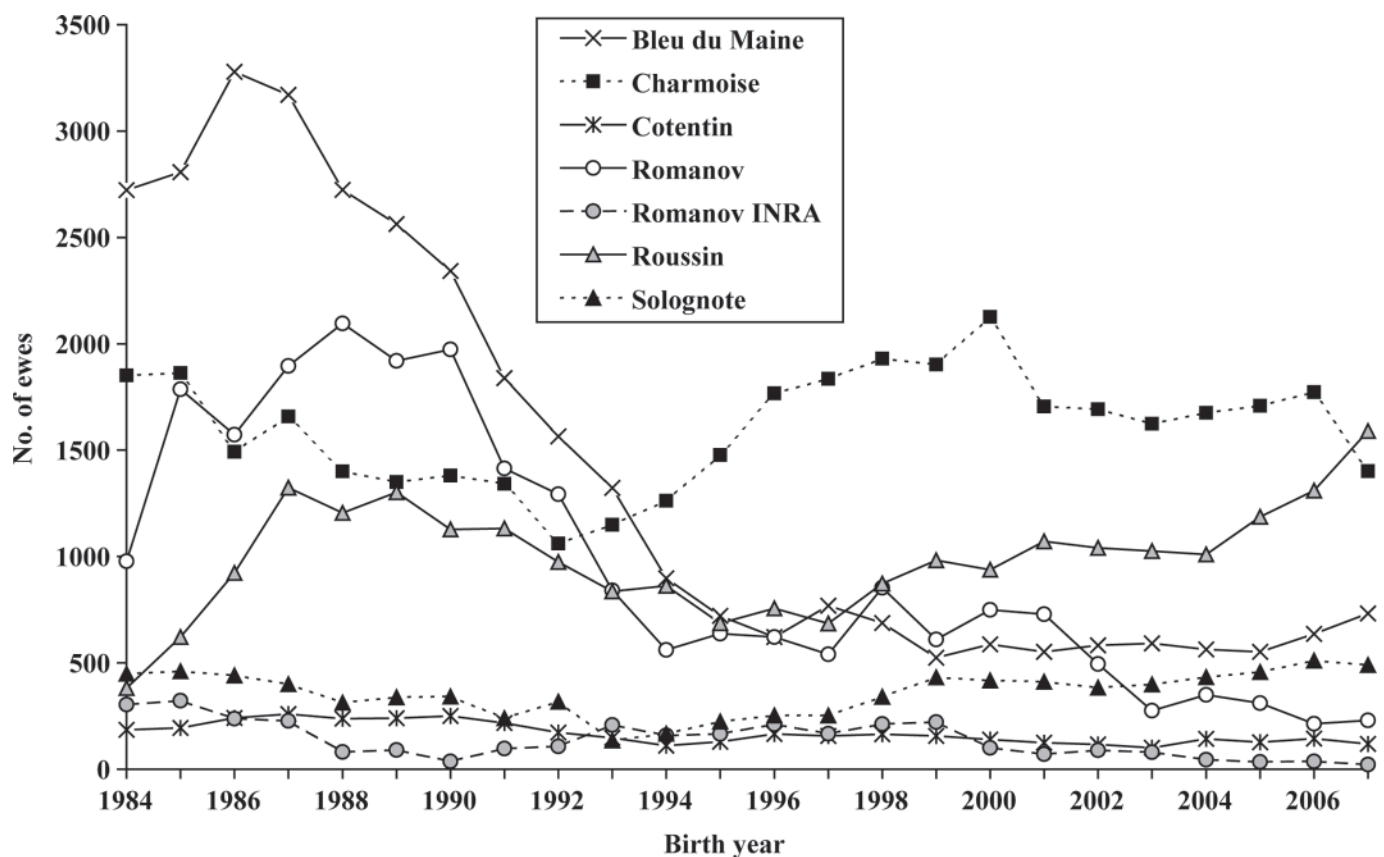


Figure 1. Evolution per birth year of the number of ewes recorded with both parents known for the 7 populations under study.

**Table 3.** Total number of animals in the data file and completeness of the pedigrees<sup>1</sup>

Item	BDM	CHM	COT	ROM	RIN	RLH	SOL
Whole file							
Total No. of animals	107,580	106,589	10,967	52,305	12,609	53,899	25,262
Proportion of animals with both parents known, %	88	85	90	83	98	86	88
Reference female cohort (born from 2004 to 2007)							
Equivalent generations traced	8.1	6.8	4.6	6.2	10.5	5.8	5.5
Avg No. of ancestors per ewe	6,732	784	1,581	853	9,528	448	820

<sup>1</sup>BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.

age of ancestors known for a total potential of ancestors ( $T$ ) of a given generation  $n$ , where  $T = 2n$ . The founders ( $j$ ) of the reference population (i.e., the ancestors of animals from this population with unknown parents) were detected by tracing the pedigrees up to the base population, and their expected contribution to the gene pool of the population ( $p_j$ ;  $\sum_j p_j = 1$ ) was computed (James, 1972). The effective number of founders ( $f_e$ ) of the population, defined as the reciprocal of the probability that 2 genes drawn at random in the population originate from the same founder, was computed as

$$f_e = 1 / \sum_j p_j^2. \quad [2]$$

The notion of major ancestor, founder or not, as defined by Boichard et al. (1997), was used in the present study. The major ancestors of the reference population were detected and their marginal expected contributions to the gene pool ( $q_k$ ;  $\sum_k q_k = 1$ ) were computed according to the iterative method of Boichard et al. (1997). The effective number of ancestors was defined and computed in a similar way as the effective number of founders:

$$f_a = 1 / \sum_k q_k^2. \quad [3]$$

The effective number of ancestors ( $f_a$ ) is less than the  $f_e$  and the comparison of both numbers can be used to find the bottlenecks that have occurred from the founders to the present population (Boichard et al., 1997): the greater the  $f_e/f_a$  ratio, the more stringent the bottlenecks.

The founder genome equivalents ( $N_g$ ), defined by Lacy (1989), were computed as one-half the effective number of founder genes:

$$N_g = 1 / 2 \sum_{m=1}^{2f} r_m^2, \quad [4]$$

where  $f$  is the total number of founders (see above) and  $r_m$  is the probability of founder gene  $m$ , at a given neutral locus, to still be present in the population under study. Probabilities  $r_m$  were estimated by gene-dropping simulation, as first proposed by MacCluer et al. (1986).

For a given replicate,  $2f$  founder genes were generated and labeled, segregations were simulated throughout the complete pedigree, and probabilities were determined by gene counting in the reference population. In the present study, for each population, a total of 100 replicates were run.

The coefficient of inbreeding of each animal in the file was computed using the method of Meuwissen and Luo (1992). The kinship coefficients of sires and dams of the reference population were computed. The average coefficient of kinship between both parental groups was compared with the average coefficient of inbreeding of their offspring (i.e., the reference population). Specific routines were developed to estimate the rate of inbreeding ( $\Delta F$ ) with the method first proposed by Gutiérrez et al. (2008) and modified by Gutiérrez et al. (2009). For each animal ( $i$ ) from the reference ewe population, the individual  $\Delta F$  from its founders ( $\Delta F_i$ ) was computed as follows:

$$\Delta F_i = 1 - \text{Eq}G_i^{-1} \sqrt{1 - F_i}, \quad [5]$$

where  $F_i$  is the coefficient of inbreeding of  $i$  and  $\text{Eq}G_i$  is its individual equivalent generations traced as computed with Eq. 1. The global  $\Delta F$  was simply computed as the average of all individual  $\Delta F$  ( $\Delta F_i$ ) of animals in the reference population. The realized effective population size ( $N_{e_r}$ ) was then computed according to the classical formula:

$$N_{e_r} = \frac{1}{2\Delta F}. \quad [6]$$

## RESULTS

### Pedigree Completeness

Table 3 shows the value of some indicators of pedigree completeness, for the whole population and for the reference ewe population. The COT breed showed the least  $\text{Eq}G$  value because of the frequent use of rams provided by breeders not in the selection scheme and somewhat disconnected from the selection nucleus. The  $\text{Eq}G$  of the ROM, RLH, and SOL breeds was between approximately 5 and 6. This smaller value, in compari-

**Table 4.** Generation lengths (years) by population for the animals born from 1999 to 2003 and their reproducing offspring

Population <sup>1</sup>	Sire-son	Sire-daughter	Dam-son	Dam-daughter	Avg.
BDM	2.9	4.0	3.0	3.6	3.4
CHM	3.6	4.5	3.4	4.2	3.9
COT	2.8	3.8	2.7	4.0	3.3
ROM	2.9	5.1	3.5	5.0	4.1
RIN	1.9	4.0	2.0	3.8	2.9
RLH	3.2	3.9	3.0	3.9	3.5
SOL	3.1	4.2	3.1	4.0	3.6

<sup>1</sup>BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.

son with the CHM and BDM breeds, was partly due to the registration in the flock book of a fairly large number of animals without pedigree data (SOL, RLH) from breeders who eventually decided to join the program or because of the lack of paternity control in some flocks (ROM). The value of  $EqG$  was greater in the CHM and BDM breeds because of a long tradition of selection and pedigree recording. The experimental RIN showed the greatest  $EqG$  value because it was raised in a single flock (“ex situ in vivo”), with a specific management plan and no external contributions for almost 40 yr.

The average number of known ancestors per ewe from the reference population ranged from less than 500 (RLH) to more than 9,000 (RIN). This number of known ancestors was not strictly linked to the  $EqG$ . For instance, the population with the least  $EqG$ , the COT breed, showed a substantially greater number of ancestors known per ewe than the other populations, except for the BDM breed and the RIN. In fact, when 2 populations with similar  $EqG$  values show 2 different values of ancestors known, the pedigree knowledge of the population with the greatest number of ancestors known is the most unbalanced; some pathways are informed on a large number of generations, whereas others show information after only 1 or 2 generations from the reference population.

### Demographic Variables

Regarding the number of parents and their use, for reasons of conciseness, only significant facts and facts relative to the genetic analysis are indicated in the text; others are available in an online-only supplemental table (<http://jas.fass.org/content/vol88/issue2>). During the period from 1981 to 1984, the number of sires used to produce newborn ewes ranged from 28 (RIN) to 383 (BDM). The number of paternal grand-sires of these newborn ewes ranged from approximately 25 (RIN and RLH breed) to 154 (BDM). In the period from 1991 to 1994, in all populations except the RIN and the RLH, the numbers of sires and grand-sires were substantially fewer than in the previous period; the decrease in the number of sires ranged from -30 (CHM) to -70% (BDM, ROM, and SOL) and the decrease in the number of grand-sires ranged from approximately -35 (CHM)

to -65% (BDM and SOL breeds). From 2001 to 2004, these numbers were greater than from 1991 to 1994 in the following populations: COT (+25 and +19%, respectively), RLH (+78% for both numbers), and SOL (+156 and +107%, respectively). In the other populations, the numbers of sires or grand-sires changed little (CHM and RIN) or decreased again, by approximately -40 (BDM) or -70% (on-farm ROM).

The generation lengths are shown in Table 4. In all populations, the generation lengths were shorter on the male offspring pathways than on the female offspring pathways. The shortest average generation length was found in the RIN, whereas the longest was found in the ROM breed on farm.

### Probabilities of Gene Origin

Results of the analysis of gene origin probabilities are shown in Table 5. Not surprisingly, the populations with the largest reference populations, namely, the CHM, BHM, and RLH breeds, showed the greatest values for the total number of founders. On the contrary, the RIN combined both the smallest reference populations and total number of founders. When comparing the ratio between the total and effective number of founders, the expected contributions of the founders appeared particularly unbalanced in the CHM, ROM, and RLH breeds, whereas the RIN showed the most balanced contributions.

The smallest values of the  $f_a$ , and consistently the greatest values of the expected contribution of the main ancestor, were found in the 2 ROM populations and the RLH, with the on-farm ROM population showing even worse values than the RIN. Ranking the major ancestors by decreasing expected contribution, the number of ancestors required to reach a cumulative expected contribution of 50% of the gene pool ranged from 6 (on-farm ROM population) to 21 (BDM and SOL breeds). The difference between the  $f_e$  and the  $f_a$  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 (Boichard et al., 1997). The greatest values for the  $f_e/f_a$  ratio were found in the CHM, SOL, and ROM breeds, indicating more stringent bottlenecks in these 3

**Table 5.** Criteria calculated from the probabilities of gene origin

Population <sup>1</sup>	Total No. of founders ( $f$ )	Effective No. of founders ( $f_e$ )	Effective No. of ancestors ( $f_a$ )	Contribution of the main ancestor, %	Founder genome equivalents ( $N_g$ )	$f/f_e$ ratio	$f_e/f_a$ ratio	$f/N_g$ ratio
BDM	698	120	59	4.9	22	5.8	2.0	31.6
CHM	1,347	134	50	6.1	23	10.1	2.7	58.1
COT	272	80	46	6.5	23	3.4	1.7	11.5
ROM	385	40	17	15.9	6	9.6	2.4	60.2
RIN	59	31	24	10.9	19	1.9	1.3	3.1
RLH	681	58	39	7.6	8	11.7	1.5	82.0
SOL	568	133	55	6.0	26	4.3	2.4	21.8

<sup>1</sup>BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.

breeds, whereas the least value of this ratio was found in the RIN, and then the RLH.

The average estimated value of the  $N_g$  ranged from 6 to 26, with an SE ranging from 1 to 4. The greatest estimated value of  $N_g$  was found in the SOL. This breed was followed by a group of 3 breeds, namely, the CHM, COT, and BDM breeds, and was followed next by the RIN. The other 2 populations, the RLH and on-farm ROM populations, showed substantially reduced values of  $N_g$ .

Finally, the  $f/N_g$  ratio accounts for all sources of loss of variability from the base population to the reference population: unbalanced contributions of founders, bottlenecks, and losses during segregations (Lacy, 1989; Boichard et al., 1997). The RIN showed a very small value for the  $f/N_g$  ratio (Table 5), highlighting the effectiveness of the management rules applied, despite a very narrow base population. Three other populations showed a relatively small value for the  $f/N_g$  ratio, namely, the COT, SOL, and BDM breeds. The other 3 populations (CHM, ROM, and RLH breeds) showed relatively large values for this ratio.

### Inbreeding and Relationship

Figure 2 shows the evolution of the average coefficient of inbreeding of ewes per birth year. In the SOL breeds, inbreeding decreased by 1.4% between 1994 and 1995 with the massive arrival in the performance recording system of animals without pedigree information. However, it is probable that these animals were indeed related to the animals recorded previously. In the last 5 yr, 2 breeds (the ROM and CHM breeds) showed a regular increase in their inbreeding level. The slope of the first breed is much steeper than that of the CHM, confirming some tendencies foreseen. To the contrary, since 2003, the level of inbreeding has declined, and has remained stable for the RLH. This reduction coincided with establishing selection for scrapie resistance and creating the breeding center.

The least values of the average coefficient of the reference population were found in the BLD, CHM, and SOL breeds (Table 6). However, it should be noted that, among these 3 breeds, the last one had the poorest pedigree information (see Table 3). To the contrary,

the 2 ROM populations were found to have the greatest average coefficients of inbreeding, with the RIN nucleus showing a value equal to almost twice the value of the breed raised on farm, but its pedigree knowledge was greater (see above). In general, the greater the average inbreeding value, the less the proportion of animals with a null individual value and the greater the proportion of animals with a large individual value, but there were some exceptions. For instance, in the COT breed, the proportion of inbred animals within the reference population was the least, but the proportion of high individual coefficients of inbreeding (i.e.,  $F_i > 6.25\%$ ) was one of the greatest, as was the proportion of inbred animals with a coefficient greater than 6.25% (45%). On the contrary, among the breeds with an average inbreeding value of approximately 2%, the CHM breed showed the smallest proportion of null values, as well as the smallest proportion of large values.

In all populations except one (the COT), the ratio of the average coefficient of inbreeding of the reference population to the average coefficient of kinship between all ram parents and ewe parents of this population was close to one (Table 6). Among these 6 populations, the SOL breed and the RIN showed values slightly greater than the other populations, and the BDM breed showed a value even less than one. In the COT breed, the average inbreeding of offspring was equal to almost twice the average parental kinship.

As shown in Figure 2, in most populations the evolution of inbreeding over the years was not regular. Under such conditions, estimation of the realized effective size from both the annual rate of inbreeding and the generation length (e.g., Huby et al., 2003; Leroy et al., 2006) would have led to negative values. Therefore, the method proposed by Gutiérrez et al. (2008, 2009) appeared to be an appealing method to estimate the realized  $Ne_r$ . The results are presented in Table 6. The 2 smallest values of  $Ne_r$  were found in the 2 ROM populations, with the value observed in the RIN being slightly less than the one observed in the on-farm population (ROM). For the other populations, the value of  $Ne_r$  was slightly (COT) or much (other breeds) greater than 100, which exceeds the minimum effective population sizes defined by the Food and Agriculture Organization and discussed by Meuwissen and Wooliams (1994).

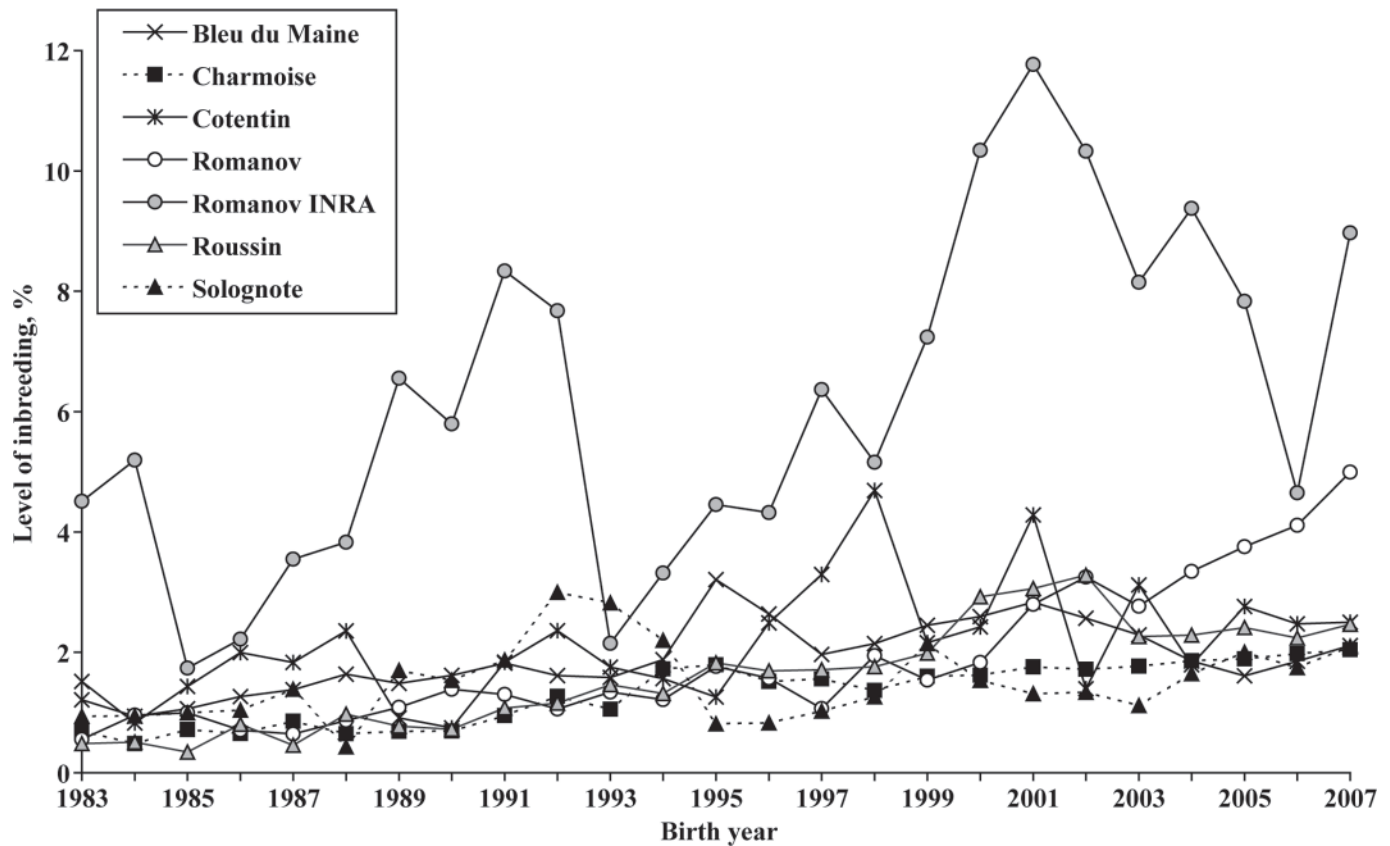


Figure 2. Evolution per birth year of the average inbreeding coefficient of ewes for the 7 populations under study.

### Ex Situ Conservation

We compared the average kinship of each ram cryopreserved (circles and triangles in Figure 3) with the active ram population (x-axis; Table 7), active ewe population (y-axis), and the average kinship of the active rams with themselves and the ewe population (cross on Figure 3). The populations under study can be categorized into 2 profiles. In the first category, the cryopreserved rams were found to be distributed between poorly and closely related to the active rams; this was the case for the CHM and SOL breeds (Figure

3a). In the second category, almost all the rams in the cryobank were found to be poorly related to the active population; this was the case for the RLH, COT, and ROM breeds (Figure 3b).

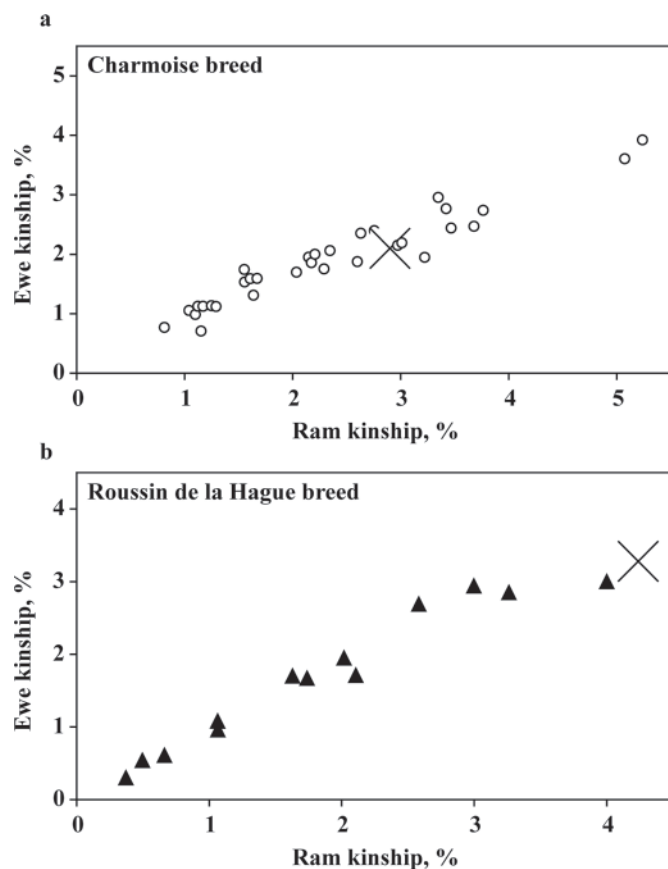
### DISCUSSION

When comparing the results presented in this paper with recent results obtained in other livestock species, the indicators of the genetic variability of the sheep populations under study and of their evolution over time seemed rather good. For instance, in comparison

**Table 6.** Inbreeding in the reference female population (born from 2004 to 2007), kinship between parents of these females, and realized effective population size

Population <sup>1</sup>	Coefficients of inbreeding ( $F_i$ ) in the reference population			Average coefficient of kinship ( $\Phi$ ) between parents of the reference population, %	$(F - \Phi)/\Phi$ , %	Realized effective population size
	Proportion of animals with $F_i = 0$ , %	Proportion of animals with $F_i > 6.25$ , %	Average value ( $F$ ), %			
BDM	20	5	1.9	2.2	-15	231
CHM	10	4	1.9	1.8	+8	182
COT	45	12	2.4	1.3	+82	108
ROM	9	13	4.0	4.1	-4	78
RIN	0	45	7.7	6.2	+24	65
RLH	15	7	2.4	2.1	+12	172
SOL	21	5	1.9	1.5	+24	154

<sup>1</sup>BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.



**Figure 3.** Average kinship of the rams cryopreserved in the French National Cryobank with the active ram population (x-axis) and ewe population (y-axis) for the Charmoise (a) and the Roussin de la Hague (b) breeds. The cross represents the average kinship of the active ram population with itself and the ewe population.

with populations with similar pedigree completeness, the values obtained here for the effective number of ancestors or for the effective population size were generally greater than in French (Verrier et al., 2005) or Swiss (Poncet et al., 2006) draft horse breeds; Austrian (Baumung and Sölkner, 2002), Spanish (Gutiérrez et al., 2003), Italian (Bozzi et al., 2006), or Irish (Mc Parland et al., 2007) beef cattle breeds; and Danish (Sørensen et al., 2005) or Irish (Mc Parland et al., 2007) dairy cattle breeds. It is especially noted that, except for the 2 ROM populations considered, the indicators were better for the rare or almost rare sheep breeds studied in this paper than for dairy cattle breeds with very large populations. In fact, the situation of many dairy cattle breeds has already been described by several authors (see above) and can be explained by the

severe bottlenecks these populations have suffered during the last 5 decades. The favorable situation of meat sheep breeds (Huby et al., 2003; the current report), from a genetic variability point of view, may be partly explained by the low potential to induce differences in male progeny sizes, despite the fact that little or no use of AI in these species could lead to a clear population subdivision with a negative impact on the evolution of genetic variability, as shown by Gutiérrez et al. (2003) in beef cattle.

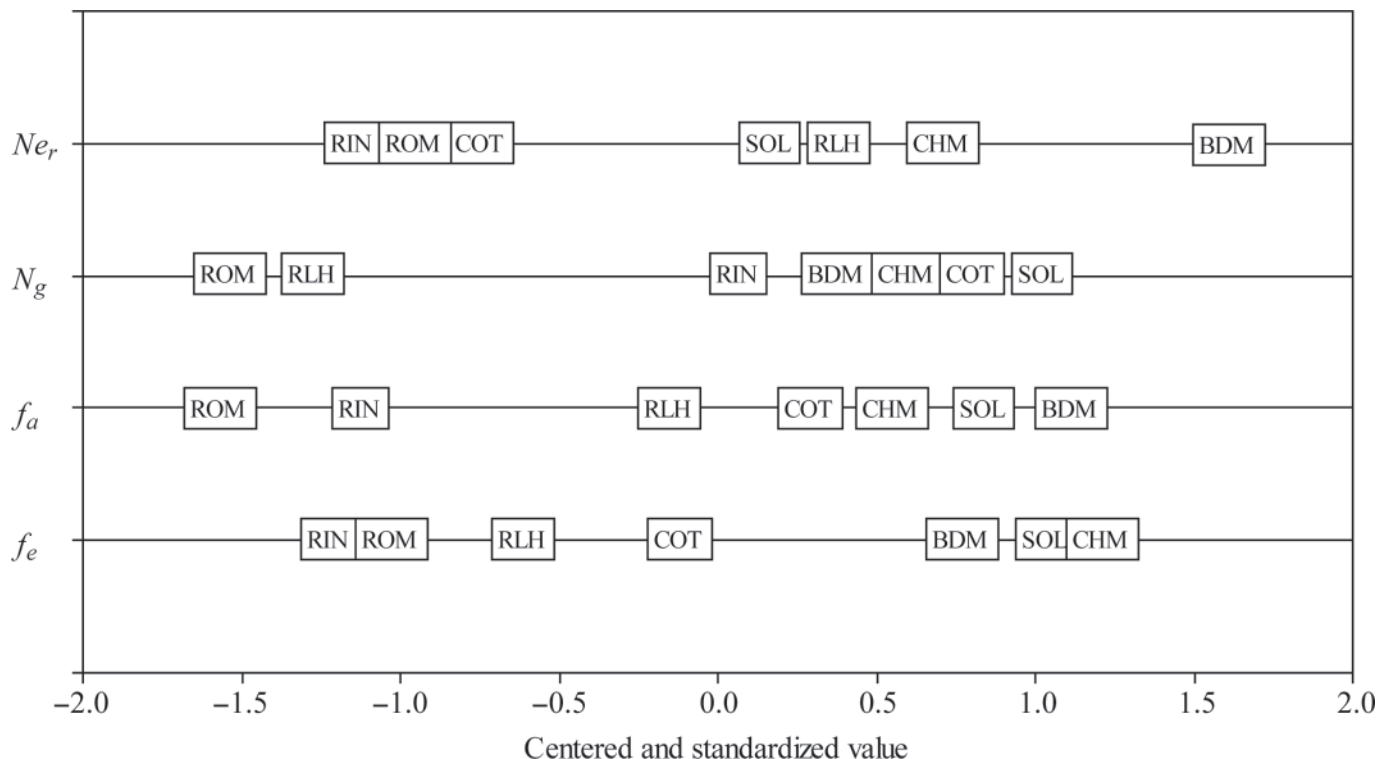
A synthetic view of the variability across populations of the results obtained in the present study is given in Figure 4, for 4 indicators of genetic variability and their evolution over time, namely the  $f_e$ , the effective  $f_a$ , the  $N_g$ , and the realized  $Ne_r$ . According to Figure 4, three groups of populations may be distinguished. First, the BDH, CHM, and SOL breeds showed the greatest values for almost all indicators. They do not have genetic diversity issues compared with other livestock populations. Second, the COT and RLH breeds showed more variable positions from one indicator to the other, generally in the middle part of the distribution, but sometimes on the left or on the right part of the distribution (see the  $N_g$  variable). Finally, the 2 ROM populations generally showed the least values for all indicators, with the exception of the  $N_g$  variable, for which the RIN was found to be at a central value. These results have to be interpreted in light of the recent history of the population, the differences in their pedigree completeness, and, in some cases, their demographics.

For the BDM, the results could be explained by the fact that, at the beginning of the 1980s, it was by far the breed with the greatest population numbers among all of the breeds studied. However, despite its tremendous demographic loss, almost no collective selection existed and the average number of animals per flock was always small, leading to a greater percentage of rams kept in comparison with other breeds. Last, its breeders were always attentive to inbreeding. The situation with the CHM is mainly due to ewe numbers, which remained quite large and stable over the years. However, its situation was not as good as that of the BDM because it suffered a genetic bottleneck during the 1994 to 1997 period because of a peak in selection pressure. In the following period (2004 to 2007), 2 actions restrained the pressure: the rather massive arrival of new ewes in the selection program because per-head payments were granted to the CHM (based on its being a rare breed), and the national scrapie breeding

**Table 7.** Number of rams in the French National Cryobank and in the active ram population<sup>1</sup>

Item	BDM	CHM	COT	ROM	RIN	RLH
No. of cryobank rams	3	32	11	6	13	14
No. of active rams	46	110	19	12	105	52

<sup>1</sup>BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.



**Figure 4.** Comparison of the 7 populations under study for their value for 3 indicators of genetic variability: effective number of founders ( $f_e$ ), effective number of ancestors ( $f_a$ ), founder genome equivalents ( $N_g$ ), and realized effective size ( $N_{e_r}$ ). For a given indicator and a given population, the value is centered and standardized according to the mean and SD across the 7 populations. BDM = Bleu du Maine; CHM = Charmoise; COT = Cotentin; ROM = Romanov; RIN = Romanov (INRA); RLH = Roussin de la Hague; SOL = Solognote.

program, whose impact was, somewhat curiously, to diversify the origin of the rams, as also observed in other breeds (Palhière et al., 2008). As for the SOL, its good results, despite rather small numbers, may be explained by a rather wide genetic basis when the program began, as indicated by the  $f_e$  value and by a strict management program intended to restrict the loss of genetic variability, as indicated by the small  $f/N_g$  ratio. However, the results of this breed were not as good as expected because of a bottleneck during the 1990s. Huby et al. (2003) reported the overuse of a single ram in the largest flock of the breed. Since this study, the situation seems to have improved because the contribution of the main ancestor decreased from 8.1 to 6.0%.

The results for the COT breed were remarkable because, despite being the smallest in situ breed in our study, its  $f_a$  and  $N_g$  values were large. The frequent importation of rams from small flocks disconnected from the nucleus flocks was probably the main explanation. However, the COT breed showed the largest discrepancy between inbreeding and kinship. The likely explanation was a lack of thoroughness by some farmers who had a tendency to keep rams related to their ewes. As for the RLH, it was the breed with the greatest  $f/N_g$  ratio and whose main ancestor had one of the greatest contributions of all of the breeds studied (7.6). Until 2002, 3 farmers were the main providers of the rams (Huby et al., 2003). With the implementation of the French national scrapie breeding program, the RLH was in an unfavorable situation, with a greater frequen-

cy of the most sensitive allele to scrapie, VRQ (Parot et al., 2004). Because the main providers of young rams for replacement also had a greater frequency of VRQ in their flocks, a breeding center was created to diversify the ram origins. Meanwhile, the breed numbers had been growing significantly since 2005. All this favorably affected the genetic variability of the breed. The inbreeding level and 2 criteria calculated from the probability of gene origin ( $f_e$  and  $f_a$ ) were very similar to those found by Huby et al. (2003) for a reference population (1996 to 2000) born almost 2 generation intervals before the sheep in the current study.

As for the results for the ROM, most of the indicators could be explained by its selection scheme. Only 25% of the purebred ewes were used for replacement; the rest were bred to create  $F_1$  crosses with a meat breed, which considerably restricted the genetic basis of a breed whose numbers were simultaneously dropping at a rapid speed. For the ex situ population, the rather narrow genetic basis of the breed, as well as its small numbers, would explain the situation. However, the case of this population and its result for the  $f/N_g$  ratio highlight the effectiveness of management rules, such as rotational mating, when they are rigorously applied in experimental conditions. A difference of almost +25% was observed between the reference population average coefficient of inbreeding and the average kinship coefficient between all the ram and ewe parents of this population. The same difference was found for the SOL breed. This illustrates the fact that, for popula-

tions under a rotational mating scheme, the average kinship between all parents is not the most relevant variable to describe their genetic variability. As shown by Rochambeau and Chevalet (1991), such a difference is expected if the group of origin of the rams to be mated with the ewes of a given group remains constant over time, which was the case for both populations considered here.

The results of the present study have several implications for the management of rare breeds. Vu Tien Khang and Rochambeau (1995) gave some simple rules that are necessary to maintain genetic variability: 1) use as many males and females as possible; 2) even the number of offspring per mate and replace each breeding animal swiftly; and 3) for each breeder, sell and buy animals to and from other farms. When comparing these rules with our analysis, it seems that for species in which AI is not commonly used, the most important factors are to keep as many males as possible, and to distribute the number of female offspring per male evenly. The sire-dam pathway is the one that needs the most attention.

From our results, both the creation of bottlenecks (as revealed by the evolution of some demographic variables and the  $fe/fa$  ratio) and gene losses during segregations largely affect the genetic variability of a breed. As has been widely assessed (e.g., Food and Agriculture Organization, 1998; Blackburn, 2004), an easy way to preserve breeds from such effects is the cryopreservation of biological material at regular time intervals. In our case, the current sheep collections allow preservation of either a mirror population (e.g., CHM) or a population related little to the current one (e.g., RLH). Rational rules have been defined to progressively establish collections that will allow preservation of both types of genetic variability (mirror and little related).

The results of both the SOL breed and the RIN show the efficiency of more complicated ways to manage a population, such as dividing it into groups and managing the exchange of reproducers between groups. However, as shown by the results in the SOL breed, human factors and practical constraints met on the farm will interfere with the necessary application of the very strict rules of rotational mating. Thus, such a mating plan should be recommended only in very specific situations (i.e., a rare breed raised in a limited number of flocks; 5 is probably a maximum).

For all these reasons, our recommendations are to place the most importance on helping the farmers 1) to keep a large number of males in their flock and 2) to keep changing them regularly. Additionally, use of cryopreservation is highly recommended, as our results showed. Emphasis should be placed on the choice of rams collected.

A pedigree analysis was performed using the pedigrees of 7 small sheep breed populations. Our work showed that, except in the case of the ROM breed raised on farm, all populations under study have good genetic variability, despite their scarce numbers.

Our goal was to determine which field factors have the greatest impact on the genetic variability of a breed. Our recommendations are validated only with species in which AI is not commonly used, and in which matings are managed at the flock level instead of individually. For these reasons, in France they are more suited to the case of the small ruminant species, but can be extrapolated to any species in which free-range farming is commonly used. However, although this advice is exactly the opposite of a selection scheme, it is not always easy to convince farmers to do so. This message is usually heard more easily in hardy or dairy breeds than in meat breeds because the male breeding value (when progeny testing is impossible to implement) is usually known with weak precision.

In the end, there still is an acute need to find a scientific and practical way to manage the genetic variability of breeds with poor pedigree information and in which several rams are used at the same time to service a large number of ewes, which is often the case in small ruminants under extensive farming systems. The implementation of the software SAUVAGE (Raoul et al., 2004) is currently under way, and it is anticipated that it will achieve both targets, simple management and scientific thoroughness.

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