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Review article

Measuring and managing genetic variability in small populations

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Abstract — Genetic variability in small populations is affected by specific phenomena. The joint effects of genetic drift and selection, in addition to the decrease in genetic variance due to the mere selection (Bulmer effect), enhance the risk of losing alleles at selected or unselected genes and increase the inbreeding in the population by changing the family structure. Criteria for measuring this change in genetic variability are derived from the three approaches to describe the genetic variability. At the genealogical level, the kinship and inbreeding coefficients, or the effective population size, can be used. At the trait level, the estimation of its heritability is a good measure of remaining genetic variance. At the genome level, studying the polymorphism of known genetic markers can inform on the degree of genetic diversity. These criteria are to be integrated in specific tools for the management of the genetic variability. After a short introduction on the basic concepts needed for the study of genetic variability in small populations, the main criteria available to measure its change in populations is exposed and their relative efficiencies discussed. The strategies for monitoring genetic variability, deriving from the previous criteria, are illustrated through different examples.

small population / genetic variability / genetic drift / genetic management / conservation programme

Résumé — **Mesure et gestion de la variabilité génétique dans les petites populations.** Plusieurs phénomènes spécifiques modifient la variabilité génétique dans les petites populations. Les effets combinés de la dérive génétique et de la sélection, auxquels s'ajoutent la réduction de la variance génétique due spécifiquement à la sélection (Effet Bulmer), renforcent le risque de perdre des allèles à des loci sélectionnés et non sélectionnés et augmentent la consanguinité de la population du fait de la modification de la structure familiale. Les critères de mesure de la variabilité génétique dérivent des 3 approches utilisées pour la décrire. Les coefficients de consanguinité et de parenté ou l'effectif génétique résument l'information généalogique. L'estimation de l'héritabilité d'un caractère synthétise la variabilité génétique restante. L'étude du polymorphisme pour des marqueurs génétiques

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décrit la variabilité existante au niveau du génome. Ces critères servent à construire des outils de gestion de la variabilité. Après une brève introduction qui présente les concepts utiles à l'étude de la variabilité génétique, les principaux critères utilisés pour suivre son évolution sont décrits, et leur efficacité est comparée. Les stratégies de gestion qui dérivent de ces critères sont ensuite illustrées à partir de l'étude de quelques exemples.

petites populations / variabilité génétique / dérive génétique / gestion génétique / programme de conservation

1. INTRODUCTION

Genetic variability may be defined as the "genetic ability to vary", and therefore the capacity to respond to environmental variations or changes in the selection objectives. Genetic variability is also the basis of any genetic progress, when a population is undergoing selection. Its maintenance at a consistent level is then of great concern in any population, selected or not, and whatever its size. However, the smaller the population, the higher the need for conservation, as there are less individuals so less "containers" for genetic variability.

But how can we decide that a population is "small"? What may be called "small population" is a population where the number of individuals really contributing to the next generation is restricted, whether the total population size is really small (up to several hundreds of individuals) or the use of techniques allowing a large diffusion of progress (artificial insemination, multiple ovulation and embryo transfer) reduces the number of reproducers in one sex or both, or provokes a disequilibrium in the reproducers' contributions to subsequent generations. Some domestic populations may then be considered as "small populations" and be concerned by the following.

This paper aims to present the basic concepts and the main tools for the management of genetic variability in a small population, with or without selection. After a description of the phenomena acting on genetic variability in such a population, the criteria derived from the different definitions of genetic variability used to measure its evolution will be compared. A presentation of more or less complex rules for monitoring small populations will conclude this paper. The concepts developed in the first part will concern any kind of small population, but the last part of the paper will focus on populations under conservation programmes.

2. BASIC PHENOMENA AND CONCEPTS

2.1. Genetic drift and inbreeding

A restricted number of individuals contributing to the next generation in a small population will have two consequences: genetic drift and inbreeding.

Genetic drift has been defined by Wright [51] for a neutral, (i.e. non selected) bi-allelic locus, as random fluctuations of allelic frequencies around their initial value, due to the sampling of alleles from one generation to the next, finally leading to the fixation of one of the alleles (and the loss of the other). The higher the number of generations considered and the smaller the population, the greater the fluctuations. This can be extended to more than one locus, providing a progressive increase of homozygosity over all the genes in the population, due to the successive samplings of alleles over time and the consecutive random fixations of some alleles and losses of others.

The probabilistic approach of inbreeding was derived by Malecot [29]. In small

populations, the number of founder ancestors is restricted ("founder" means an individual whose parents are unknown). Over successive generations, even if matings are panmictic, individuals are more likely to be related, due to one or more common ancestors, and thereafter, matings between relatives produce inbred individuals. As a consequence, two homologous genes could be "identical by descent", i.e. they are both deriving by copy from the same gene in a common ancestor.

2.2. Consequences on genetic variability

Genetic drift and inbreeding were two aspects of a phenomenon which increases the rate of homozygous genes in the population. As the genetic variability of the trait under study can be characterised by the number of different alleles available at the loci controlling the trait in the whole population, the loss of alleles due to genetic drift or inbreeding consecutively decreases the genetic variability.

The previous concepts were developed for one single neutral locus. Most of the time, geneticists are interested in "quantitative" traits, i.e. traits with continuous variation, which they suppose controlled by a very large number of independent genes of small and identical effect [14]. The performance P of an individual is then split in two parts, the genetic effect G and the environmental effect E:

$$P = G + E$$

where G and E are assumed to be independent and normally distributed with mean zero and variances σ^2_G and σ^2_E respectively. The genetic effect itself is generally considered as the sum of:

– an additive genetic effect A, which is the sum of individual gene effects at each locus and which constitutes the genetic part expected to be transmitted from parents to offspring;

– a dominance effect D, resulting from interactions between the paternal and maternal alleles at a given locus;

– an epistatic effect I, concerning interactions between alleles at different loci.

In most cases, only the additive genetic part of the performance is considered and the genetic variability of a quantitative trait is approached by its additive genetic variance. Several models, either analytic [6, 49] or stochastic [15], differing by the hypotheses they rely on, are available to describe and predict the evolution of additive genetic variance over generations. The more complex the model, the more accurate the prediction of genetic variance over time. This is illustrated in Figure 1, where the predictions provided by three analytical models based on Gaussian theory are compared. Wright model [51] and Bulmer model [3] consider only one effect at a time on genetic variance, either genetic drift (Wright model) or selection (Bulmer model). The Verrier et al. model [49] accounts for genetic drift, selection and interactions between the two factors. The Wright model highlights the effect of genetic drift on genetic variance: the remaining variance after 30 generations is 1.4 times higher when the population effective size is 4 times higher. The Bulmer model evidences the influence of selection, detailed in the next part, on the evolution of genetic variance.

2.3. Selection in small populations

Selection has a direct effect on genetic variance:

– Frequencies of "favourable" genes for the selected trait are increased by selection, modifying the genic variance (i.e. the variance of gene effects), which is one component of genetic variance. The pattern of evolution of the genic variance depends on initial frequencies of the favourable alleles, but in any case this variance will tend to zero due to the fixation of favourable alleles.

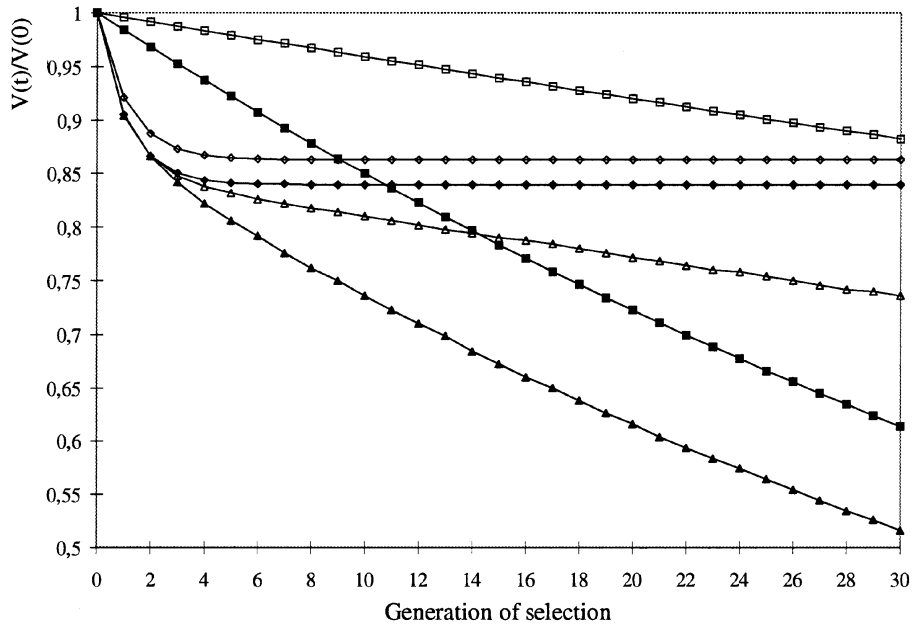

Figure 1. Evolution of genetic variance in three models, depending on effective population size Ne, with a proportion of selected males *p* (the proportion of females being 50%), for a trait with heritability 0.25 – W for Wright [53], with *Ne* = 120 (□) or *Ne* = 31 (■), B for Bulmer [3], with *p* = 50% (◇) or *p* = 6.25% (◆), and V for Verrier et al. [49], with *Ne* = 120 and *p* = 6.25% (△) or *Ne* = 31 and *p* = 6.25% (▲).

This effect of selection on the genic variance is related to the magnitude of gene effects on the selected trait and decreases as the number of loci increases [10]. Changes of gene frequencies can be neglected under the infinitesimal hypothesis (i.e. an infinite number of independent genes of small and identical effects controlling the selected trait) but may be significant for other kinds of modelling where the number of loci is assumed to be finite.

– Genetic disequilibrium between the selected loci is induced by selection. Genetic disequilibrium consists in an excess of intermediate combinations of genes (i.e. as many genes with favourable effect on the selected trait than genes with unfavourable effect) when selection is directional. This leads to negative covariances between gene effects and reduces genetic variance in the selected parents [3, 27]. The genetic variance among breeding individuals in a selected population will then also depend on the selection intensity and accuracy, decreasing when selection is more intense and accurate.

Selection has also an indirect effect on genetic variance:

– Selection modifies the family structure of the population whatever its size. This effect is enhanced when the population is small because it increases inbreeding over time. The chance of two related individuals being selected or rejected together is higher than for two unrelated ones. The relationship between selected individuals then becomes closer and closer over generations of selection. This effect can be partly considered in the computation of the inbreeding coefficient [49]. This effect will be enhanced

in a small population as the inbreeding increases to the population size, to the selection and to interactions between selection and genetic drift.

– Moreover, when the number of candidates is finite, the expected selection differential is smaller compared with an infinite population. For normally distributed traits, the selection intensity must be calculated or approximated using order statistics theory [4, 21].

The response to selection in a small population will then differ from the classical expected response in an infinite population, due to the decrease in genetic variance. The ratio between the observed response in a selection experiment and the expected response provides an estimate of the realised heritability and therefore of the remaining genetic variance in the population.

2.4. Conclusion

Various phenomena influence the evolution of genetic variability in a small population, selected or not. Several approaches are available to study this evolution and to manage the population in order to obtain the optimum compromise between actual breeding objectives and conservation of genetic variability. The effectiveness of the different criteria derived from these approaches are compared and the main rules for monitoring small populations are developed.

3. CRITERIA FOR ASSESSING GENETIC VARIABILITY: A COMPARATIVE APPROACH

3.1. Criteria based on pedigree information

Analysis of genetic variability of a population is frequently based on genealogical data. Coefficients of inbreeding and kinship, as well as genetic contributions of founders, have been largely used for a long time (see Vu Tien Khang, [50] for a review; see also [13, 17, 32]). More recently, various criteria derived from probabilities of gene origin have been proposed. Boichard et al. [2] presented an overview of these methods and developed an original one. Many of the following considerations, as well as notations, originate from their work.

3.1.1. Coefficients of kinship and inbreeding [29, 52, 53]

Two related figures are used to measure inbreeding in a population: the coefficients of kinship and inbreeding. The coefficient of kinship Φ_{XY} of two individuals X and Y is defined as the probability that two homologous genes, one chosen at random from each of these individuals, are "identical by descent" [29]. The inbreeding coefficient F_I of an individual I is defined as the probability that the two genes present at one autosomal locus are identical by descent: it is equal to the coefficient of kinship of its parents. Inbreeding will then also increase the total homozygosity in the population by the appearance of these identical genes in the individuals. The mean kinship coefficient, defined as the mean of the $N(N-1)/2$ coefficients of kinship in a population of N individuals, is an alternative way to characterise the level of inbreeding.

Coefficients of kinship and inbreeding result from pathways connecting two individuals through common ancestors. Therefore, these criteria depend strongly on the extent and quality of pedigree information: missing or unreliable data may lead to large biases in their calculation. Several authors presented methods to compute them quickly, even in large populations [30, 47]. The main drawback of the average coefficient of inbreeding is its inability to reflect recent changes, such as bottlenecks in the number of parents. Another drawback is its sensitivity to the mating system used to procreate animals included in the set under study. A way to take this effect into account is to split

total inbreeding into 'close inbreeding' (which result from matings between close relatives) and 'remote inbreeding' (which follows mainly from cumulative effects of genetic drift). The mean coefficient of kinship is less affected by these drawbacks than the mean coefficient of inbreeding, but it requires more calculation: $N(N\text{-}1)/2$ coefficients of kinship instead of N coefficients of inbreeding in a sample of N animals. Moreover, the average coefficient of kinship between animals kept for mating gives an indication about future trends of inbreeding under random mating.

3.1.2. Realised effective population size N_e

To illustrate the evolution of mean inbreeding over generations, Crow and Kimura [10] consider an idealised population in the sense of Wright [53], i.e. a closed monoecious population of N diploïd individuals mating randomly (self-fertilisation included), with non-overlapping generations and all individuals contributing equally to a large pool of gametes. The probability of drawing the same parental gene twice when producing an offspring is $1/2N$ and the probability of drawing two different genes is $1 - 1/2N$. However, the probability that these two different genes are in fact identical by descent is the mean kinship coefficient at the parental generation (or the inbreeding coefficient of an individual in the parental generation, as mating is at random). $F^{(t)}$ is the coefficient of inbreeding at generation (t). Then the coefficient of inbreeding in the population can be written as:

$$F^{[t]} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F^{[t-1]}$$

$$\Leftrightarrow 1 - F^{[t]} = \left(1 - \frac{1}{2N}\right)\left(1 - F^{[t-1]}\right) \quad (1)$$

$$\Leftrightarrow 1 - F^{[t]} = \left(1 - \frac{1}{2N}\right)^{t}. \quad (2)$$

Recalling that $(1 - F^{[t]})$ is proportional to the rate of heterozygosity, the formula (1) allows expressing the decrease in heterozygosity H from generation t-1 to generation t as:

$$H^{[t]} = \left(1 - \frac{1}{2N}\right) H^{[t-1]}.$$

The increase in inbreeding coefficient and the consecutive decrease in heterozygosity is then higher as the population size is smaller.

These formulae were obtained for an idealised population, in which each parent is expected to contribute equally to the pool of gametes. The rate of increase in inbreeding, ΔF, is:

$$\Delta F = \frac{1}{2N}. \quad (3)$$

Most populations depart from this ideal, as they are dioecious and as parents produce more or less offspring depending on their sex and even in the same sex, according to their fertility or their genetic value. To study the evolution of inbreeding in these populations, N is replaced by N_e, called the "effective population size", and defined as the number of individuals in an idealised population in the sense of Wright [53] characterised by the same increase of inbreeding rate or the same decrease in genetic variance as observed in the studied bisexual population. The effective population size N_e can be calculated from the formula (3) as function of the rate of increase in inbreeding:

$$N_e = \frac{1}{2\Delta F}.$$

The effective population size N_e can also be calculated from the variance of change of gene frequency observed in the actual population under consideration [10, 42].

The effective population size N_e of a population can be estimated from the rate of increase in inbreeding (calculated from pedigrees) during a given lapse of time. In a population with stable size and breeding

characteristics, N_e is constant and presents a predictive value as long as conditions do not change. Like the mean inbreeding rate from which it is derived, the realised effective size is very sensitive to pedigree information: N_e may be overestimated when genealogical data are missing, particularly when a long time period is considered [2].

3.1.3. Probability of gene origin [12, 23, 26, 40, 41]

A complementary approach to measure the level of genetic drift in a population is derived from the probabilities of gene origin. This concept relies on the principle that a gene drawn randomly in an individual at an autosomal locus has a 1/2 probability of coming from each of its parents, a 1/4 probability of originating from each of its 4 grandparents, and so on. Applying this rule to the complete pedigree allows calculating the probability for one gene randomly drawn to originate from any of the known founders of the population [23]. Each founder k can then be characterised by its expected contribution q_k to the genetic pool of the population under study. By definition, the genetic contributions of all founders sum up to 1. The concept of "effective number of founders" f_e then corresponds to the number of equally contributing founders, and allows to measure the balance of genetic contributions among real founders. If f is the real number of founders, the effective number of founders is calculated as:

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

f_e is equal to the actual number of founders if they contribute equally. If not, it is smaller: the more unbalanced their contributions, the smaller the effective number of founders. As shown by Boichard et al. [2], f_e is very stable across generations as long as conditions do not change. Unlike the effective population size, the effective number of founders is more descriptive than predictive, and is efficient for describing recent evolutions in a population structure.

As this concept did not account for the possible bottlenecks in the population, another criterion was proposed by Boichard et al. [2], the effective number of ancestors f_a, i.e. the minimum number of individuals (founders or not) required to explain the complete genetic diversity in the studied population. It is defined by analogy with the effective number of founders but using the marginal contributions of the individuals p_k, i.e. the contributions not yet explained by the other ancestors:

$$f_a = \frac{1}{\sum_{k=1}^{f} p_k^2}.$$

Ancestors (founders or not) are successively designated, according to an iterative procedure, on the basis of their marginal contributions. The number of ancestors with a non-zero marginal contribution is less than or equal to the number of founders and the sum of their marginal contributions is equal to 1. Consequently, f_a is always less than or equal to f_e. In large populations, identification of every contributing ancestor may require very long computations, so the iterative procedure could be stopped according to a predetermined rule. Upper and lower bounds of the true value of f_a are then calculated.

Under steady conditions, the effective number of ancestors decreases slightly with the number of generations. This parameter, which reflects shorter ascent lines than the others, shows a noteworthy robustness to partial lack of genealogical data [2].

A third concept derived from the probabilities of gene origin is the effective number of founder genomes [8, 26, 28]. It consists in calculating the probability x_k for a given autosomal gene among the $2f$ present in the founders to be drawn at random in the population under study. The effective number of founder genes is then:

$$N_a = \frac{1}{\sum_{k=1}^{2f} x_k^2}.$$

As each individual is carrying two genes, the effective number of founder genomes is defined as:

$$N_g = \frac{1}{2} N_a = \frac{1}{2\sum_{k=1}^{2f} x_k^2}.$$

The concept of effective number of founder genomes N_g is based on the probabilities that $2f$ genes carried by f founders at a given autosomal locus are still present in the population under investigation. These probabilities can be calculated by an analytical derivation (not feasible in large pedigrees), or estimated by Monte-Carlo simulation.

The main property of N_g is to account, not only for unbalanced contributions of parents to the next generation (as f_a and f_e) and for bottlenecks in pedigrees (as f_a), but also for random loss of genes from parents to their offspring: therefore, N_g is always smaller than f_a and f_e, and decreases more quickly over time. However, it should be kept in mind that the number of alleles carried by f founders is lower than $2f$ 'founder genes': as a consequence, loss of alleles is usually much slower than loss of founder genes.

While coefficients of kinship and inbreeding reflect pathways connecting two individuals through common ancestors, probabilities of gene origin depend only on ascent lines up to the founders. Therefore, probabilities of gene origin are easier to calculate. Moreover, they are less affected by missing data in pedigrees, as well as the various criteria originating from them.

3.2. Criteria derived from demographic analysis

Genetic variability of a population reflects the fate of its genetic stock, which is strongly dependent on the history of the individuals carrying the genes. It is therefore useful to carry out a demographic description of the population under investigation. As a matter of fact, genetic analyses are often accompanied by a demographic approach (see examples reviewed by Vu Tien Khang [50]). Describing the structure and dynamics of a population considered as a set of individuals gives keys to interpret genetic criteria (see [18]). For instance, demographic analysis provides information on crucial aspects such as functional structure of the population of herds (or flocks), circulation of breeding material among them, numbers of male and female parents, distribution of the size of their progeny, generation length... Demographic parameters can be used to infer evolution of genetic variability, either by simulation [7] or by estimating the effective population size N_e.

Assume that the number of sires (N_m) is different from that of dams (N_f) and that these are constant over generations. Therefore without other deviation from the idealised population [51]:

$$\frac{1}{N_e} = \frac{1}{4N_m} + \frac{1}{4N_f}.$$

Assume now that the number of sires is smaller than that of dams and each sire is mated to N_f/N_m dams. Afterwards, one choose as parents one male and N_f/N_m females from each sire's progeny and one female and N_m/N_f males from each dame's progeny. In this situation, we obtain [35, 42]:

$$\frac{1}{N_e} = \frac{3}{16N_m} + \frac{1}{16N_f}.$$

A more general formula was derived for the effective size of random mating populations of constant size and sex ratio with overlapping generations [20, 22]. The effective size is equal to the effective size of a population with discrete generations which have the same number of individuals entering the population at each generation and the same variance of lifetime family number. Each year, M sires and F dams are taken for breeding. V_{mm} is the variance of the number of male progeny of one sire and V_{mf} is the variance of the number of female

progeny of one sire. V_{fm} and V_{ff} are the corresponding variances for one dam. Let the covariance of the number of male and female progeny from each sire be C_{mmmf} and from each dam be C_{mmmf}. Hill [20] has shown that:

$$\frac{1}{N_e} = \frac{1}{16ML}\left[2+V_{mm}+2\left(\frac{M}{F}\right)C_{mmmf}+\left(\frac{M}{F}\right)^2 V_{mf}\right]$$

$$+\frac{1}{16FL}\left[2+\left(\frac{F}{M}\right)^2 V_{fm}+2\left(\frac{F}{M}\right)C_{fmff}+V_{ff}\right]$$

where L is the generation interval.

3.3. Criteria based on observed genetic polymorphisms

Tests of departure from Hardy-Weinberg proportions are frequently made to check on random mating in a population (see [32]), and excess of homozygotes above expectations may be used to estimate the inbreeding coefficient, defined here in terms of correlation between uniting gametes relative to the gamete pool of the present population. Robertson and Hill [36] analysed distribution of the deviations from Hardy-Weinberg proportions and of the estimates of inbreeding coefficient obtained from these deviations, according to the structure of the population under study.

Allelic diversity of a population at a given autosomal locus may be measured by the effective number of alleles [10]:

$$n_a = \frac{1}{\sum_i p_i^2}$$

where p_i is the estimated frequency of the allele i.

This parameter is related to the Hardy-Weinberg heterozygosity H observed at this locus:

$$H = 1 - \sum_i p_i^2 = 1 - \frac{1}{n_a}.$$

Nei and Roychoudhury [34] gave sampling variance of average heterozygosity according to the number of loci and the number of individuals per locus. They concluded that a large number of loci rather than a large number of individuals should be used. Nei [33] presented statistical methods to obtain unbiased estimates of this parameter.

Loci currently used are among those coding for visible features, enzymes or antigenic factors (e.g. blood groups, Major Complex of Histocompatibility). In farm animals, blood typing, which has achieved widespread application in detecting wrong parentages, constitutes the main source of data. In the future, molecular genetics tools will provide a rising amount of information. Statistical methods intended to assess genetic variability of populations on the basis of DNA polymorphisms (e.g. microsatellites) will have to be improved to take into account the specificities of both DNA polymorphisms and structure of farm animals populations. An important issue is how many markers should be used and how they should be distributed along the chromosomes.

3.4. Criteria derived from quantitative genetics

A classical approach is based on the estimation of realised genetic parameters by regression of selection responses on selection differentials. On the other hand, Restricted Maximum Likelihood fitting an 'animal' model is being increasingly used: under the 'infinitesimal model', it provides estimates of parameters (heritabilities, additive genetic variances) in the base population, before it is submitted to drift and selection [44]. In order to assess changes in additive genetic variance over time, Meyer and Hill [31] applied this method to various segments of data and relationship information corresponding to a small number of consecutive generations: parents of the oldest generation considered in each segment are treated as unrelated base animals, omitting data available about earlier generations.

Although one of the major reasons for preserving the genetic diversity of the populations is to maintain their ability to respond to artificial selection, the quantitative genetics approach has not been fully used, until now, to measure genetic variability. Unlike criteria based on pedigree information (which refer to any neutral autosomal locus) or criteria based on observed genetic polymorphisms (which refer to genes often considered as neutral or to non-coding regions), criteria derived from quantitative genetics mirror phenomena affecting the only genes involved in genetic variation of traits under consideration. Consequently, a critical aspect of this approach lies in the choice of these traits. In addition to the classical ones related to production, traits associated to adaptation (e.g. behaviour, stress resistance, disease resistance) should draw particular attention on breeds considered as adapted to rigorous and changing environments: further studies are needed to find reliable methods for measuring such characters.

4. GENERAL RULES FOR MANAGING GENETIC VARIABILITY

4.1. Simple rules

The effective population size N_e highlights a first rule: the distribution of the family size should be as uniform as possible. Table I (from [35]) analyses fluctuations of N_e depending on the variance of the family size along the 4 paths (male-male, male-female, female-male, female-female). Solution 3 is unrealistic; in practice it is not possible to completely fix the number of offspring for each parent. Solution 2 is often a good compromise; each sire has a son and only one, the number of offspring is at random for each other path.

Furthermore, Table I points out the consequences of an increase in numbers of males (N_m) and females (N_f). In most animal domestic populations the sex ratio is unbalanced; N_f is greater than N_m. The second rule is to increase the number of males in order to reduce the sex ratio imbalance. Nevertheless, from an economical point of view, it is difficult to increase too much the number of males used for breeding. Conservation programmes generally lead to an extra-cost related to the rearing and the use of a greater number of reproducing males.

4.2. More complex methods

4.2.1. Rotational schemes

Since the famous paper of Wright [51], systems of mating to avoid inbreeding were studied in detail (see for example [9, 10,

Table I. Effective size according to the rule applied on the various parent-offspring path (from [35]). (Solution 1: choice at random for each path; Solution 2: each sire has one and only one son, choice at random for each other path; Solution 3: the numbers of offspring are completely fixed).

Number of parents		Effective size			Coefficient of inbreeding (%) after 10 generations		
		Solution			Solution		
Nm	Nf	1	2	3	1	2	3
8	500	31	42	63	15	11	8
16	500	62	82	124	8	6	4
32	500	120	157	241	4	3	2
8	250	31	41	62	15	12	8
8	1 000	32	42	63	15	11	8

25]). Inbreeding would be kept at a minimum if the least related individuals are mated. A system involving the creation of separate groups which exchange individuals, allows to minimise inbreeding.

Rochambeau and Chevalet [39] have proposed a method taking account of usual breeding constraints (generations overlap, demographic parameters change among farm and among year, founders animals are related, the distribution of the population between various herds avoids random mating...). Table II refers to the French Poitevine goat population [39]. It deals with the research of an optimal strategy to minimise the drift over a period of 15 years. Three numbers of groups (5, 11 or 23) and 2 mating schemes are compared. The population is split in various reproduction groups. Male and female offspring are assigned to the group of their dam. Males of a given group never mate to the group of their dam. In the fixed scheme, males of group (*i*) are always mated with the females of group (*j*). In the circular one, a periodic function gives the correspondence between (*i*) and (*j*). Changing the number of reproduction groups or the mating scheme turn out to have very small effects, if we consider the effective number of founder genomes (N_g) or the mean kinship coefficient (Φ). Regarding the mean inbreeding coefficient (F), the best solution is a circular mating scheme with 23 groups. Moreover, circular mating schemes lead to a reduction in the variance of inbreeding coefficients, and induce a genetic structure independent of the initial relationships between founders animals.

Hall [19] points out that the success of genetic conservation can be assessed by pedigree analysis. Djellali et al. [13] evaluate the conservation programmes of two sheep breeds, managed with circular mating systems. Demographic analysis indicates that both the number of males and their replacement rate are high in accordance with the management rules. Although progeny sizes are not always balanced, the various founder animals, as well as the reproduction groups from which they originate, contribute to the gene pool in a balanced way (Fig. 2). The genetic conservation programmes prevent close inbreeding and restrict total inbreeding.

The genetic conservation programmes are well implemented and effective. One practical problem deserves some comments. The splitting up of the population may be made on the basis of the observed kinship coefficients. Groups are then called families, i.e. groups of animals more related between them than with other animals. Ascending hierarchical classification, factorial analysis of a distance table or clustering analysis are used to split up a set of animals using information from a table of kinship coefficient. Probabilities of gene origin in relation to founders or to major ancestors provide another description of the sample [37, 38]. Unfortunately when the

Table II. Effective number of founder genomes (N_g), mean individual inbreeding coefficient (F) and mean kinship coefficient (Φ) in relation with the number of reproduction groups and the mating scheme after 15 years in a model goat population (from [40]).

Criteria	5 Groups		11 Groups		23 Groups	
	Fixed	Circular	Fixed	Circular	Fixed	Circular
Ng	38.5	37.5	43.5	44	52	51.5
F	0.63	1.18	1.49	0.64	1.70	0.44
Φ	1.24	1.24	1.06	1.07	0.88	0.89

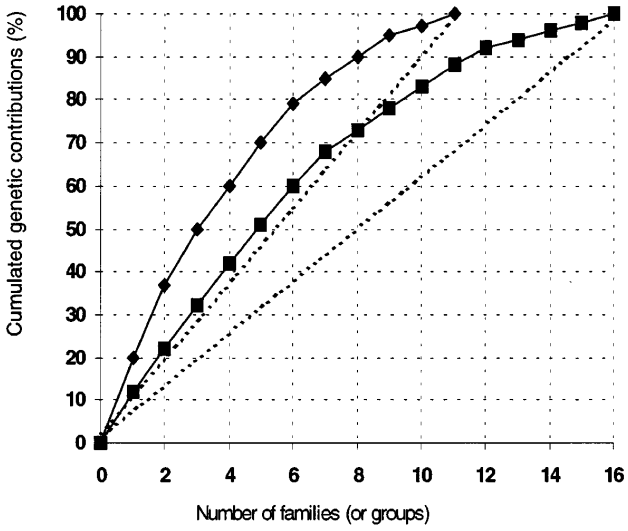

Figure 2. Cumulated genetic contributions of the families or the reproduction groups in two sheep breeds. Total number of groups is 11 for Solognote (◆) and 16 for Mérinos précoce (■).

reproducing females are distributed into different farms, splitting up the population on the basis of pedigree may lead to management difficulties [48]. Therefore, the splitting up of the population is now made on the basis of the distribution of females into farms: each group includes the whole or a part of the females from a given farm only.

Artificial insemination with frozen semen appears to be a useful aid in various domestic species like cattle. It can be used to improve the management of the males. Mating rules can be more easily applied: physical exchanges of males are not needed, and their number per breeding group can be kept to a minimum. Chevalet and Rochambeau [7] discuss the conservation programme of the French Bretonne Pie Noire dairy cattle population. The programme was initiated several years ago according to a scheme that lengthens the generation interval and makes use of artificial insemination with frozen semen. Table III summarises the main results. The population is split into 8 groups of about 40 cows. Only cows more than 5-year-old are used for the renewal of the breed. In Scheme 1, 8 apparently unrelated bulls were chosen among offspring of old cows. Each bull, whose semen is frozen, is used to inseminate females from other groups. It is mated during 2 consecutive years with cows of one group, and then transferred to another group. When the bull has been used over all groups it is replaced by one son. In Scheme 2, old females are mated to 8 chosen males at the beginning of the programme. Male offspring are kept, and their semen frozen. This provides for 8 "replacement males", whose use is deferred until the first bulls are withdrawn. The circulation of males over females remains the same, but at the time a bull is replaced, one of his sons is kept as a new "replacement male". In the last scheme, bulls are used during 2 years, instead of 16 in Schemes 1 and 2. Scheme 3 is a basis for comparison with the methods developed for populations reared under natural mating. As expected, Scheme 2 is generally better than Scheme 1, and Scheme 3 is the best. The percentage of genes originating from the 8 initial bulls provides a clear separation between the third scheme and the first two. The rapid renewal of bulls enables the population to keep genes from the females founders, their contribution being 80% instead of about 40%, after 40 years.

Table III. Mean individual inbreeding coefficient (*F*), mean kinship coefficient (Φ), percentage of genes originating from the initial males (*M*), and percentage of original genes still present (*S*) in relation to the management rule. (Scheme 1: frozen semen from 8 old bulls; Scheme 2: frozen semen from 8 offspring; Scheme 3: natural mating. See text for more details).

Criteria	After 20 years			After 40 years		
	Scheme 1	Scheme 2	Scheme 3	Scheme 1	Scheme 2	Scheme 3
F	1.4	0.78	0.26	5.5	2.3	1.8
Φ	4.0	3.0	1.2	7.4	4.0	2.5
M	59	52	20	62	56	20
S	13	15	21	5	7	11

Reduction of inbreeding levels between the first scheme and the second, is primarily due to the longer generation interval, rather than to an enlarged genetic background. However artificial insemination with frozen semen is still a useful tool, but it is necessary to keep the second rule in mind: the number of males should be as high as possible in order to reduce the sex ratio imbalance.

Storage of frozen semen and embryos are suggested also for conservation of genetic variability of endangered livestock populations, as an alternative to living animals. In that case, sample size must be considered to minimise genetic drift in sampling [43]. Gandini et al. [16] analyse the probability distribution of founder genes in a semen storage of a small cattle population. In both cases, we have to manage a population made up of a small number of animals before and after obtaining frozen material.

4.2.2. Schemes based on probabilities of gene origin

Circular mating schemes are effective to maintain genetic variability. However, it is not possible to use them in many situations (for example when the population size is too large to manage the reproduction groups, or when the number of females in each farm is too small to make reproduction groups). Hall [18, 19] proposes the following definition of successful genetic conservation: "the continuing representation of a high proportion of animals registered as foundations stocks, in pedigrees of recent generations". Alderson [1] develops a similar idea: "the ideal animal would receive equal contributions from all the founder ancestors in the breed. This is likely to represent the best opportunity to maximise the retention of founder alleles". Then Alderson measures the value of an animal by calculating f_e, the effective numbers of founders in its pedigree.

For such a purpose, Hall [18] points out that the gene flow among farms is the statistics of most value for monitoring breeds. One practical conservation method, with great opportunities for development of public relations, is the organisation of sales which facilitate gene flow within breeds. The structure of the population should show no hierarchy between farms, and the gene flow between farms should be as large as possible. Criteria like percentage of farms which supply males and percentage of breeding males born in the same farm are useful to characterise the population. One can also draw a matrix describing exchange of males between farms. Kennedy and Trus [24] develop a method that measures the exchange of genes between herds.

Giraudeau et al. [17] provide a description of an example of the ideas discussed by Alderson and Hall. The Parthenaise breed is a multiple-purpose breed of the west of France. Giraudeau et al. compute the matrix of coefficients of kinship between the 135 natural service bulls used in 1988 / 1989. Then a procedure of automatic classification is used for pooling these bulls into families. Later, they choose 10 famous Artificial Insemination (AI) bulls, which have large numbers of offspring; these bulls are similar to the major ancestors defined by Boichard et al. [2]. A given family of natural service bull is characterised by the average values of coefficients of kinship between the members of this family and the 10 famous AI bulls (Fig. 3). In this example, kinship coefficients and probabilities of gene origin are similar.

Figure 3 underlines the distinction of three kinds of families: families much related to the AI bulls, showing a pronounced kinship with one or two famous AI bulls, as family "*B*"; families relatively related to the AI bulls, with more balanced coefficients of kinship with the famous AI bulls, as family "*E*"; families slightly related to the AI bulls, as family "*K*". For Alderson and Hall, family "*E*" has the best profile. However, family "*B*" deserves some consideration: the strategy could be to look at a balanced contribution not at an individual level but at a higher level like the sample of renewal bulls chosen on year. Finally, if family "*K*" has a genetic information as good as the other two families, family "*K*" deserves also some consideration because it enlarges the available genetic variability. Further work is needed to define management strategies on the basis of gene origin probabilities related to major ancestors.

4.2.3. "Marker Assisted Conservation"

The genetic variability of a population may be defined by lists of alleles and their frequencies at many loci in the various subsets of the population (herd, age classes, sex...). In the former paragraphs, pedigree information was used to infer the change in genetic variability. This probabilistic approach will be supplemented by a more biological approach. Molecular genetics techniques make it possible to consider the allelic frequencies for many loci in domestic animals species. It will be possible to provide a better description of the genetic variability. One will be able to control the efficiency of a conservation programme. The choice of genotypes to conserve will

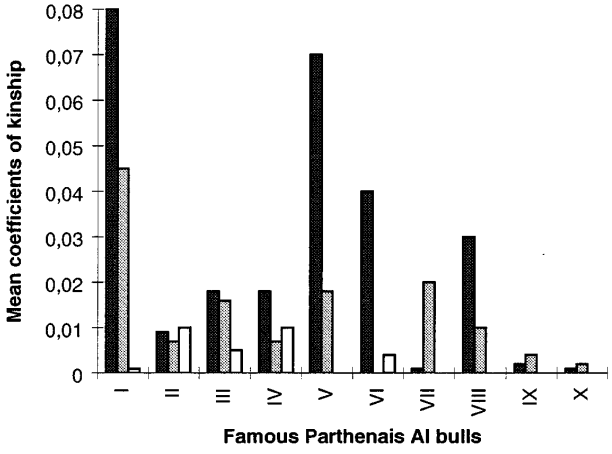

Figure 3. Average coefficient of kinship (Φ) between 3 families of natural service bulls and 10 famous Parthenais AI bulls. (■ for family "*B*", ▨ for family "*E*" and □ for family "*K*").

be based on marker alleles linked to specific traits. Moreover methods of conservation will have to be re-evaluated. Chevalet [5] makes a first step to investigate the Marker Assisted Conservation (MAC) by analogy to Marker Assisted Selection (MAS). This paper discusses the possibility to use polymorphic markers to control genetic drift of allelic frequencies in small populations. The selection criterion is an index based on the heterozygosity at the marker loci. In some conditions (less than 100 breeding animals, 3 markers for 100 centimorgans) MAC is effective. In this area further work is needed. Later, Toro et al. [45] conclude that a conventional tactic, such as the restriction of the variance of family sizes, is the most important tool for maintaining genetic variability. Frequency-dependent selection seems to be a more efficient criterion than selection for heterozygocity. Nevertheless, an expensive strategy with respect to the number of genotyped candidates and markers is required in order to obtain substantial benefits. At last, these authors [46] introduce a new selection criterion: the overall expected heterozygosity of the group of selected individuals. When a limited number of markers and alleles per marker are considered, the optimal criterion is the average group coancestry based on markers.

A realistic modelling of the problem of the management of genetic variability should take into account a completely integrated genome structure, including the effects of recombination, mutations and natural selection. In other respects, Dempfle [11] underlines that nature might be able to increase genetic variability by some factors. If the role of mutations in creating genetic variability is well known, it is also considered to be of importance only for changes in an evolutionary time span. But it is at present not known if transposable elements can create useful genetic variation in livestock species. Other similar mechanisms could be described in the next years.

5. CONCLUSION

To assess genetic variability, various complementary criteria are available, deriving from demographic analysis, pedigree information, genetic polymorphisms and quantitative genetics. Some of them, like inbreeding and kinship coefficients or effective population size, are concepts originating from the beginning of population genetics. They remain operational and are widely used. The improvement of computers (memory capacity and computing speed) even extended their scope of application. Therefore, their limit is related to their definition, with respect to a “neutral autosomal locus”, which is quite an abstract concept, independent from a specified trait. The improvement in genome analysis of domestic livestock might allow to identify the chromosomic areas involved in genetic variability of traits (“classical” traits in animal production or “adaptation” traits). This evolution of knowledge might induce, in a near future, a change in the methods of description and management of the genetic variability, by focusing more specifically on genes (or chromosomic areas) involved in the genetic variability of the populations considered.

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