



Traditional maintenance breeding of landraces: 2. Practical and theoretical considerations on maintenance of variation of landraces by farmers and gardeners

Anton C. Zeven

c/o Laboratory of Plant Breeding, Wageningen University, POB 386, 6700 AJ Wageningen, the Netherlands; (e-mail: Anton.Zeven@users.pv.wau.nl)

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Thou shalt not let thy cattle mate with a different breed, thou shalt not sow thy field with impure seed

(Leviticus, XIX, 19, cited by White, 1970).

Summary

Maintenance breeding of landraces is discussed. Various practical and theoretical advices, presented in the literature to collectors of genebanks have been used to compare these with the methods applied by farmers. Although most farmers realize the utmost importance of yield stability, they and their ancestors experienced that maintenance breeding could better be replaced by seed replacement. A few crops such as rootstock rose and especially maize, form exceptions. In these crops the farmers actually collect 'hips', or ears for the next generation. However, they also apply seed replacement from time to time. More research is needed to obtain better knowledge about the level of variation of a landrace grown for various generations before and after seed replacement.

Introduction

The conclusion by Hawkes (1983) that domestication reduces the variation of the domesticated crop in comparison with its wild ancestral population, implies that more samples should be collected of the wild ancestral population as in that case a higher variation is to be expected. However, one can easily observe in the field the incorrectness of this conclusion. Indeed, due to domestication the variation for some characters will decrease but, on the other hand, the variation for other characters may have greatly increased (Zeven, 1975). During the domestication process man often will set apart interesting 'off-types'. These may have arisen by recombination and mutation, whereas 'contamination' may play a role as well. Promising off-types are maintained, multiplied and distributed. The many types of *Brassica oleracea* L. have often served as the

school example. During the domestication process of this species it 'segregated into' many types grown mostly as vegetables. However, no 'seed types' and 'true root types' were selected for, because other species or crops, probably, provided already the desired products, such as oil in seeds, and starch in seeds as well as in roots. As said newly arisen types will have been multiplied, and with time they developed into landraces.

In earlier publications related to the present topic, a definition of a landrace (Zeven, 1998), the inexplicable seed replacement (Zeven, 1999) and the (absence of) maintenance breeding of landraces of various crops (Zeven, 2000) were discussed. One of the conclusions was that the commonly occurring seed replacement is not included in any *in situ* conservation program, and that maintenance breeding of many crops by farmers is generally not applied because generations of farm-

ers apparently, have experienced that the work did not result in a higher income and that seed replacement was a better method to maintain high harvest stability (Zeven, 1999). This conclusion is supported by Soleri et al. (1999), who wrote about selection in maize (*Zea mays* L.) fields ‘–, *this is a habit that persists despite widespread recognition that it has no consequences in terms of changing populations traits.*’ (See further below.)

Within a (sublot of a) landrace, with time, the variation of each character is reduced, maintained as such, or increased under the influence of nature and by action, mostly unintentional, of man. For instance, ‘founder population effect’ may play a role when a small amount of the seed of a crop is conserved, or introduced into a region yet free of that crop or of that landrace. The resulting variation strongly depends on the variation ‘surviving’ in the seeds produced during the first generations.

Survival, thus, depends on nature and on man. ‘Nature’ may change the level of variation by favouring the development of ‘fit’ phenotypes. The ‘unfit’ ones have a reduced level of reproduction or do not reproduce at all.

Earlier, I concluded that *in situ* conservation will fail as on the long run all genetic variation will be lost, whereas only a part will be lost when applying *ex situ* conservation (Zeven, 1996).

Maintenance breeding versus ‘formal’ breeding

In general, farmers¹ rarely select (Zeven, 2000). If they select for material ‘true-to-type’, i.e. ‘true-to-the-(mental)-description of the landrace or cultivar’, they apply maintenance breeding. If they select with the intention to improve the landrace, for instance in a programme of so-called Collaborative or Participatory Plant Breeding (PPB), by multiplying interesting off-types, they apply classic or ‘formal’ breeding with the intention to produce cultivars (Sthapit et al., 1996). Such off-types could result from unobserved cross-fertilization, mutation or contamination. These new cultivars may replace their parental landraces as did happen all over the world after ‘classic’ breeding was started. It should be remembered that most so-called ‘formal’ breeding companies evolved from activities developed by farmers, market gardeners and seed traders who began (often as a hobby) to select in their

¹ The term ‘farmers’ in the text includes gardeners and their wives and relatives.

(landrace) material. So, PPB units and ‘formal’ breeding companies have the same aim: breeding of better cultivars. Such new cultivars may not only be selected for higher yield, but also for other purposes.

Maintenance breeding of landraces, if applied, means that ‘all true-to-type’ genetic variation is transferred to the next generation. Or in the words of Cleveland et al. (2000): V_g (in the equation $V_p = V_g + V_e + V_{g*e}$) should remain unaltered (V_p = phenotypic variance, V_g = genotypic variance, V_e = environmental variance, and V_{g*e} = genotype*environment interaction variance).

A major force of natural maintenance of landraces is stabilising selection by which off-types or their capacity to reproduce are suppressed.

Maintaining landraces

For crops such as wheat (*Triticum aestivum* L.) Thell.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and rye (*Secale cereale* L.), ears were, to some extent, bulk-harvested since the domestication of these crops. Farmers often cut the upper part of the culms to bundle these. Later, the lower part (together with the weeds) was harvested and used, for instance, as cattle feed. This method resulted in smaller amounts of weed seeds in the harvested seed and, consequently, in food and sowing seed.

As said, rarely any selection was carried out. After harvest and threshing the farmer would (and still does) take any amount of harvest seed needed to serve as sowing seed. The only ‘selection criterions’ are grain size and grain weight in case farmers ‘clean’ the seed by winnowing, removing the small and light seeds. The latter may result from plants with the genotype for small seeds, but more likely from plants under stress conditions. Consequently, the farmer unwittingly selects for genotypes for big seeds and for stress resistance and tolerance.

On the other hand, a grower of a landrace of a crop such as rootstock rose (*Rosa canina* L.) may apply maintenance breeding by going through his nursery to obtain a sufficient number of ‘rose hips’, selected from plants satisfying the ‘mental’ description of the landrace of this crop (de Vries, 1993; Zeven, 2000).

Selection in landraces for certain desired characters may result in new cultivars. With lack of maintenance selection, these (and other) cultivars may change into landraces. The examples, given in this context by Bray (1984) for several crops in China, however, are

questionable. He described that the farmers had ‘selected many varieties’ within the crops foxtail millet (*Setaria italica* L.), proso millet (*Panicum miliaceum* L.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), and rice (*Oryza sativa* L.) which are suitable for (i.e. adapted to) any soil and climate condition. These ‘varieties’ may have derived from an actual choice by a farmer, which means that he created a new cultivar, or by natural selective effects in a particular region. After the introduction of a landrace directional selection, followed by stabilising selection, may have resulted in a new landrace. These selection forces according to Bray (1984) lead to varieties suitable for certain periods (‘fifty day varieties’), and alcoholic beverages or gruel, and for saline soils, marshy areas and mountain slopes.

Bray (1984) continued that for wheat and barley only a few varieties were observed. Apparently, farmers felt no need for other types, or the landraces were, and are, cultivated in areas with more or less the same environment; or the areas under these two crops were small. Unfortunately, the origin of the large variation, observed for instance for a crop as foxtail millet, is not described.

How did the hundreds of landraces of glutinous foxtail millet originate and how were they maintained? For instance, in the Shantung Province some 600 varieties (i.e. landraces, ACZ) were maintained around 1950 as suggested by (Bray, 1984). Due to the presence of male sterility, quite some genetic recombination will have taken place, resulting in new genotypes that may or may not be favoured by nature and farmers. Similarly, but earlier, in the sixth century more than 100 forms of millet existed (Bray, 1984).

Many studies have been made to investigate the level of variation within landraces of a crop, or, irrespective of landraces, within a particular crop grown in a certain region. A few examples – white clover (*Trifolium repens* L.) and maize (*Zea mays* L.) – may serve to illustrate the importance of variation of certain characters in a landrace.

Annicchiarico & Piano (1997), investigating in Italy landraces of the cross-fertilizing forage crop, (Ladino) white clover, observed the occurrence of severe intraspecific competition. Under cultivation, up to 90% of the individuals could be eliminated after one year of crop cycle, resulting in a severe reduction of variation.

On the other hand, Aasmo Finne et al. (1999a, 1999b) observed in Norway little natural selection for seed production of the same crop in the past. Adapt-

ation has mainly operated in the vegetative phase, as has been shown to be the case with many long-lived perennials selected for persistence under grazing.

In maize landraces in West and Central Africa in the fifties and early sixties of the 20th century, an epidemic of maize rust (*Puccinia polysora* Underw.) occurred widely (van Eynatten, 1965). After some 20–30 years (identical to 20–30 plant generations) of natural selection for resistance genes the ‘new’ landraces possessed a high degree of resistance for this rust.

Interesting research results are presented by Soleri et al. (1999), who compared the characters of maize ears selected by farmers in Oaxaca, Mexico with a random sample of the same field. Ear length is polygenetically inherited and greatly influenced by environment. Consequently, its heritability is low. This low heritability must have been observed by the farmers, who realized that (continuous) selection for long ears would not result in a landrace with long ears, but in the maintenance of the character ‘highly variable ear length’.

Soleri et al. (1999) compared again the above characters in two types of populations, one derived from the farmers’ selected ears, and one derived from ears taken at random. No population derived from small ears was included, but it could be expected that this population would also produce plants with ears of variable length. Both types of populations did not differ significantly for various characters. This response could be expected as the landrace, which is already grown and maintained for many years, still is very variable for these characters. Although it was realized that the seed size had no effect, farmers still preferred to obtain large seeded sowing seed, even when buying sowing seed by the weight, because, apparently, it is against their nature not to use big seeds (Soleri et al., 1999).

The ICTA² Farm Corn (i.e. maize) may also serve as an example. This already improved selection derived from the landrace ‘Tuson’ of Trinidad, was grown for many generations on Antigua. The number of generations and level of selection, if any, are not given (Spaner et al., 1997). This material was grown on Trinidad as ICTA-0. Selection for the 10 biggest ears per plot of 10 neighbouring plants (a selection criterion applied by farmers (Zeven, 2000)) resulted in some 240 ears and, by mixing 25 gram seed of each ear, sowing seed of the ICTA-1 was ob-

² ICTA = Imperial College of Tropical Agriculture at Trinidad.

Table 1. Physiological and morphological characters of various selected farm-saved population of possible improved landrace ICTA Farm Corn (Spaner et al., 1997)

Population	Characters					
	No of days to silk	Plant height cm	Ear length mm	Ear width cm	Ear weight g	Grain yield t/ha
ICTA-0	60.0	254	192	40	196	4.90
ICTA-1	60.9	253	186	41	198	4.99
ICTA-2	61.0	245	195	42	205	5.64
ICTA-3	61.0	257	201	43	203	5.39

tained. Similarly, by repeating this procedure ICTA-2 and ICTA-3 were developed. Other farmer's selection criteria: early ripening and yellow kernel were not included in the trial. These four populations were studied for number of days to silk, plant height, ear length, ear width, ear weight and grain yield per area unit. The results for the ICTA-populations are presented in Table 1 (Spaner et al., 1997).

These four populations were compared with each other, and, unfortunately, also with two unrelated control populations. This explains the absence of any indication of significance of differences between the four ICTA-populations in Table 1. Furthermore, no correlation coefficients between traits were presented. Consequently, any difference could be caused by 'year effect'. However, the changes of data presented for the three ear characters and for yield may result from selecting for 'big ear'. This may point to presence of sufficient variation in the ICTA-0 population for these characters and probably also to the absence of selection on Antigua. Very likely, the Antiguan farmers did not carry out any selection, or, if they did, similarly to the experiment of Soleri et al. (1999) with the same crop, it had no effect. But, the ICTA-trial showed that, under experimental conditions, selection for 'big ear' had certainly a positive effect on this character and consequently on yield.

Only a few examples are available because farmers do not apply maintenance selection, but use the system of seed replacement (Zeven, 1999).

Number of seeds saved for the next generations

Except for a few crops, hardly any information is available about the actual number of seeds, put aside by the farmer to grow his next generation. If such

figures were available, we could use these in the equations presented below, to calculate the magnitude of the probability that a certain degree of genetic variation is included in the next generation. The example presented by Louette (1995) is an exception. She concluded that maize farmers, taking only some 40 ears per field for the next crop, would loose genetic variation. The other example is the maintenance of the common bean (*Phaseolus vulgaris* L.) in Dutch gardens, described below.

Many authors write about (genetic) variation, but often no mention is made about the character(s) involved. In this aspect, it should be remembered that variation differs for each character. For some characters it may be very high, for others it is low (Hennink & Zeven, 1990), giving the landrace its own recognizable appearance, and consequently its reputation to be suitable for cultivation in a certain environment and for a certain purpose.

No information is available on the maintenance of the variation of a landrace. For some crops we may assume that the variation was sufficiently maintained because of the high ratio: sowing seed versus harvest seed. For instance, because of the low yield of landraces of crops such as wheat and barley in NW Europe before 1800 or so, the farmers had to use about 10% of the harvest seed as sowing seed. In China, formerly 35 to 50% of the harvest seed was used as sowing seed (Bray, 1984). These high percentages will have guaranteed that all genetic variation present in the landrace would be maintained and transferred to the next generations. However, reduction of variation may occur for other crops when a small amount of sowing seed of the harvest seed as mixture is required, or when sufficient sowing seed is obtained from one or a few plants. Examples of the latter are maize, com-

mon bean, tomato, bell pepper (*Capsicum annuum* L.) or tobacco (*Nicotiana tabacum* L. and *N. rustica* L.). Each individually well developed plant will provide a large number of sowing seeds for a next crop. On the contrary, when establishing a coconut palm (*Cocos nucifera* L.) plot only a few nuts per palm are available pressing the farmer to obtain nuts of several palms.

Another example is the conservation of sowing seed of the mainly self-fertilizing bush dry-seed common bean grown as a garden crop in The Netherlands of which only a very few plants with their pods are stored in the shed (Zeven, 1979; Zeven et al., 1999). The method of sowing seed 'production' would lead, in a few generations, to a crop consisting of one genotype.

The same applies for vegetatively propagated crops. For instance, using stem cuttings of a few plants of cassava (*Manihot esculenta* Crantz) plants for the next crop, will certainly lead to a reduced number of clones per field, or even to a field consisting of one genotype only. A similar example is the perennial kale (*Brassica oleracea* L. var. *ramosa* DC), a crop which lost its ability to produce seeds, and, consequently, has to be maintained by tearing of (rooted) branches (Zeven et al., 1989). These are used for annual replanting and in this way in a few years only one clone will be left in a field. In Tanzania for maize, sorghum, finger millet, sunflower (*Helianthus annuus* L.) and common bean, some farmers, during the growing season, observe certain plants as possible seed source, while others do not (Friis-Hansen, 1999).

Mixing of propagules, mutation and cross-fertilization increase genetic variation, and may counteract any reduction of genetic variation. In most self-fertilizers some cross-fertilization also takes place, and the resulting hybrid plants may be positive-heterotic for yield and its components. Consequently, such plants may add more seeds to the next generation than the 'true' (homozygous) plants.

The low level of a variation of a 'founder population' has already been mentioned before, and will not be discussed again.

Variation in field and garden

Maintenance of a high level of genetic variation may result in an increase of yield stability, i.e. lowering the chance of a misharvest. Yield stability is of utmost importance in areas with a poorly developed infra-structure. In such areas it is of more importance

than yielding capacity as it may prevent famines. The higher the genetic variation of characters associated with reproduction the higher the level of yield stability. For instance, the higher the number of genes (loci) and that of alleles per locus for resistance and tolerance, the higher the frequencies of healthy and little affected plants, and the higher the chance that the so-called multiline effect is effective. And, consequently, the higher the chance of a good yield (per plant). Furthermore, some variation of harvestability of the fruits or the plants of a crop gives a farmer and his family the opportunity to spread out labour need over the season, and, as often is the case, in the absence of good storage facilities, the possibility to 'conserve' or to sell the produce over a longer period.

Brush et al. (1992) suggested the following equation to calculate the level of phenotypic variation on a farm (during a certain short period, ACZ):

$$D_i = \alpha_0 + \alpha_1 IMP_i + \alpha_2 S_i + \alpha_3 F_i + \alpha_4 SES_i + \alpha_5 OC_i + \Sigma_{1i}$$

in which D_i = phenotypic variation or diversity on farm i ; farmed by farmer i (i.e. number of landraces/cultivars/phenotypes on farm i); IMP_i = area of farm i under improved cultivars; S_i size of farm i ; F_i = farm fragmentation (= number of parcels divided by total land area); SES_i = socio-economic status of the farmer; OC_i = farmer's off-farm income opportunity. Of course, more variables such as RI_i = richness/poorness of the farm household, and A_i = age of household head of farm i could be included as suggested by Brush et al. (1992).

This equation falls short for several variables. For instance, fragmentation of the land does not mean that on each parcel another landrace or cultivar is grown. Furthermore, the socio-economic position of the farmer's wife may be higher than that of her husband and she may advise her husband to grow one or more cultivars instead of his landrace. Or there are frequent contacts with other regions from where new material is easily introduced. Therefore, the equation should include SES_{farmer} as well as $SES_{farmer's\ wife}$. And when an adult child is or adult children are assisting, $SES_{child(ren)}$ should be added. Another point concerns the difference between landrace and cultivar. As said above, when farmers grow crops under stress situations, they are more interested in high yield stability than in high yield. Therefore, they will mix 'foreign' material, including cultivars with autochthonous landraces (Zeven, 1998) to try out the 'foreign' material. Furthermore, landraces and cul-

tivars may become contaminated due to open flowering and accidental seed mixing. Where to include in the equation such a 'contaminated' landrace, or a 'contaminated' cultivar and its degree of contamination? Consequently, it must be concluded that as too many unknowns are included, an equation as suggested by Brush et al. (1992) cannot be used to calculate a meaningful D_i . It would be better to replace all the + signs in the Brush's equation by 'associated with' to understand which factors may affect D_i .

Another important variable, also not included in Brush's formula, is time span as all of a sudden a household may change. So with time traditions may change, and consequently landraces may be replaced by other landraces, or cultivars (see for instance Almekinders et al., 1994). Maybe, for one crop, one landrace is replaced by another, whereas for another crop the landrace is replaced by a cultivar. Further, there is the possibility that a new crop is adopted, whereas an old crop is discarded.

Practical advices offered to genebank collectors

As no information is available on the number of propagules of a landrace to be collected by a farmer for maintaining variation of a landrace, use can be made of the theoretical and practical methods suggested by scientists for maintenance work in genebanks. In addition, one could wonder whether it really is necessary to collect all genotypes of a population. And should we only include all alleles (each allele one time), or all genotypes (Yonezawa & Ichihashi, 1989) (each allele in various genetic backgrounds)?

Several authors have published suggestions and mathematical equations (see below) indicating the number of plants or plant parts to be collected in a field by the genebank collector in order to obtain as much genetic variation as possible at a certain level of probability. I will therefore start with a short overview of the suggestions made. It will be followed by a comparison with the little information available from practical research based on data for farmers' fields. Collected material could be seeds, pods, ears, panicles, other fruiting bodies, or any vegetative parts (including tissue samples).

Allard (1970), studying the variation of wild oat (*Avena strigosa*) of various localities in California, USA, suggested to collect 10 seeds of 200 plants each i.e. 2000 seeds per site to obtain a representative sample. Qualset (1975) reduced this number and

advised to collect 500 propagules per site to be sure to include at least one plant of each very rare genotype. This advice would certainly lead to impracticable big collections, and does it guarantee that indeed the very rare genotype is collected?

Marshall & Brown's (1975) question *What should be the minimum representative sample*, cannot be answered as long as no information is available on the variation within the population of each field before collecting is started. The help of a farmer with good knowledge of his material, would be very useful in this respect. However, it is imperative that this farmer does realize that the collector wants to make a random sample. This is necessary because farmers rarely select in his material (Zeven, 1999) and when he assists the collector, he will at that occasion certainly select those propagules which he considers the best ones. As a result of this it may be clear that selection should imply making a non-random collection, the farmers' samples will differ from those of the genebank collector.

In literature various advices based on a theoretical approach have been given about collecting 'all' genetic variation. They deal with the number of plants (or propagules) of a certain field, the number of plants of a certain field, and the number of fields or populations to be visited. An at first hand sound-looking advice, given by several authors, is to collect as many (or as much) as possible, so increasing the probability to collect all alleles. Other authors conversely advise to collect as many different phenotypes as possible. The rationale of this advice is, that the observed differences may have a genetic background and by collecting different phenotypes one may collect many different genotypes. However, the characters observed should be 'environmentally stable' (Erskine & Williams, 1980). It is also suggested that when a desirable allele has been observed in earlier collected material, more material should be collected, hoping to gather the same allele in other genetic linkage groups. Such a desirable allele may have a better expression in the other genetic backgrounds.

Often, simple advices (see below) are given to genebank collectors. One is that a representative, and therefore a random sample, could be obtained by deciding first how many accessions of a field (N) in a certain region should be collected. As field size as well as within-field variation will vary, and consequently N per field, a simple equation $T = N \times F \times O$ (T = the total number of accessions collected (in a certain region, F = number of fields, O = the number of plant propagules/plant) will not hold. If

no pre-collecting information of a field is available it is suggested to collect a random sample by sampling along a pre-arranged pattern. The experience of the collectors would help him in deciding the size of F and O. Ideal would be that the total number of collected propagules represents the variation of the region. However, as financial funds are limited, T can never be as large as one wishes it to be.

So, collectors wonder how little to collect to obtain much (i.e. not all) variation. Farmers, unwittingly, maintain sufficient variation in landraces of bulk-harvested cereals such as wheat, barley, oats and rye as during harvest, threshing and winnowing, the harvested seed is thoroughly mixed. This means that in the relatively large amount of sowing seed required 'all' variation is present and transferred to the next generation. However, during periods of famine and cash shortness sowing seed is eaten or sold respectively resulting in a small amount of sowing seed with probably not 'all' variation left.

Oka (1975) suggested that for wild populations 5 to 30 plants per population and 20 populations in total should be sampled. For semi-wild plants (i.e. landraces) these figures should be increased to 30 to 40 plants of 30 to 40 populations (i.e. fields with the same or different landraces) each. On the average for wild plants some 350 (range 100–600) plants should be maintained and for the semi-domesticated some 1225 (range 900–1600) plants. So, the more domesticated the crop, the more plants have to be collected.

In the same year, Marshall & Brown (1975) suggested to collect 50–100 plants, with which numbers they believed that all alleles would be included under most circumstances. These figures are quite a reduction compared with Oka's figures. But Marshall & Brown's advice, apparently, was not followed by many collectors. For instance, a very high number of plants to be collected is suggested by Hawkes (1980). He proposed that for a highly heterogeneous population 50 seeds per plant of some 100 plants, i.e. 5000 seeds, and for a fairly homogeneous population 2500 seeds should be collected.

For maize, Crossa et al. (1993) suggested as strategy for genebank collections the division of the accessions into the 'non-overlapping groups based on racial samples and/or ecogeographical criteria.' After having grouped the accessions in racial complexes they suggested to collect from each complex 25–100 accessions. These authors mentioned that doing so all alleles with a frequency of 0.03 or higher will be collected (at a probability of $p = 0.90-0.95$). This range

of 25 to 100 accessions probably often may refer also to the number of accessions collected of a field as due to cross-fertilization the maize population on a field or on a number of neighbouring fields may belong to one racial complex. These numbers contrast sharply with the one or few ears collected by a farmer himself for his next crop. Apparently, due to the sowing of his own seed or seed obtained from other farmers, and due to cross-fertilization with pollen grains of own and other maize fields, the variation of the farmer's maize population is maintained. The same holds for crops with a high sowing seed/harvest seed ratio (wheat, barley, see below), and for crops which produce only a few seeds per plant (e.g. coconut palm). For self-fertilizing crops producing many seeds per fruit or per plant and with easily recognizable individual plants, variation will be reduced within a few generations as has been described above for common bean. The same holds for tomato (*Lycopersicon esculentum* Mill.).

Theoretical equations developed for genebank collectors

For genebank collectors, equations have been developed to indicate the relationship between number of plants collected and the number of these plants being 'represented' in the next generation, or to calculate the probability that a certain allele will be present in a collected seed sample. Hammond & Gardner (1974) generated for maize the equation:

$$N = n - n\{(n-1)/n\}^r$$

in which N = expected number of plants represented in the sample, n = number of parent plants, and r = number of gametes.

Applying this equation for a maize farmer maintaining his landrace, r = 300 seeds (=egg cells) could be obtained of one ear of three plants each \times 100 kernels/ear. The logic outcome would be $n = N = 3$. However, although we do assume that the plants of the next generation are all hybrids between the egg cell donor ('mother') plants and an unknown number of pollen grain donor ('father') plants, some plants derived from self-fertilization may also be present. It is further quite possible that the 'father' plants nearer to the 'mother' plants have a greater part in the progeny than 'father' plants further away.

Another equation was generated by Sedcole (1977):

$$S = (r + 1.645\sqrt{r + 0.5})/p$$

in which S = sample size to be 95% certain that a minimum number r of plants with a trait that occurs in a population with frequency p is recovered. Again, if we apply the above figures in which $S = 300$ seeds and $r = 3$, $p = 0.02$. r should be reduced or S increased to further reduce p . Hence, more rare alleles will be collected.

This equation also indicates that, if one wants to ensure that at least one plant ($r = 1$) with a certain character with a frequency in the original population of 5% ($p = 0.05$) is present, at least 63 ($= S$) plants should be collected.

A third and a fourth equation has been generated by Brown (1978), and Brown & Munday (1982). For self-fertilizers ($s = 1$) they generated the equation:

$$P(p,n) = 1 - (1-p)^r$$

and for cross-fertilizers ($s = 0$) the equation is:

$$P(p,n) = 1 - (1-p)^{2r}$$

in both equations r = probability that an allele is included, r = frequency of the allele, and n = number of plants collected.

These two equations indicate that, what is already known for a long time, farmers of cross-fertilizing crops have a higher chance (P) to obtain an identical level of p with less propagules than farmers of self-fertilizing crops.

Comments on these equations

These equations refer to a single randomly taken allele or to a single randomly taken character. But genebank collectors and farmers are interested in many alleles and in many characters; each character occurring with a yet unknown frequency and being controlled by an unknown number of alleles. Consequently, only these alleles (not being linked to selected ones) and these characters will follow the above equations. This means that with the low number of plants collected for the next generation of a maize crop the probability that, in general, rare alleles and characters will be present in the next generation(s) is very small. Hence, the selection for one or a few characters by taking a few maize plants results in a (morphologically) quite uniform population. Furthermore, within a region, for instance a valley, (neighbouring) maize farmers will select within the same landrace for the same characters, and therefore, n (number of plants collected) should not refer to one field (or farmer), but to the whole valley. Due to seed replacement of sowing seed

by sowing seed of the same landrace obtained from a neighbouring farmer, any difference between field sublandraces, caused by natural selection, will be reduced. Consequently, per valley with some 20 maize farmers who collect each three ears and obtain some 300 kernels per ear, the probability that each rare allele is maintained by one of the farmers approximates 1. But as maize farmers maintain the landrace, its characteristics remain, which means little variation for these characteristics and, consequently, for the alleles conditioning them.

Lawrence et al. (1995a, 1995b), applying the equation developed by Brown (1978) and Brown & Munday (1982), also calculated the number of plants to be collected at random. They concluded that ca 172 plants would suffice to reach a very high probability of including all or very nearly all of the 20 000 polymorphic loci, provided that the frequency of each allele is higher than or equal to 0.05, irrespective of the magnitude of s for each individual of the species [or the landrace, ACZ]. When samples are taken from a number of populations [e.g. fields], the size of the sample per population, may be 172 divided by the number of populations. So, a maize farmer picking some 10 to 20 seeds per ear for the next crop should collect these of some 18 or 9 ears respectively, harvested from different plants. So some 150–180 kernels would be sufficient to maintain variation. These seeds would allow for a new maize field of 100–150 m².

This conclusion supposes a uniform geographic distribution of all alleles, which might be true for (almost) all alleles within the landrace, as due to seed replacement all alleles are continuously taken to other fields.

An example of distribution of alleles is presented by Brown & Munday (1982), who studied 25 individual plants of 12 either two- or six-rowed barley landraces each, collected from various sites in Iran. They observed 56 alleles conditioning for 25 isozyme loci, of which 47 alleles were common and nine rare. Of the common alleles 31 occurred widespread, 8 sporadically and 8 locally, whereas of the rare alleles one occurred widespread and 8 locally. So, the smaller the number of populations (= fields) and the smaller the number of plants per populations sampled, the higher the probability that the rare alleles are not included. But as suggested by Witcombe and Gilani (1979, quoted by Brown & Munday, 1982) '*isozymes bear little if any relation to agronomic characters and do not reflect adaptive variation*'. Furthermore, we should bear in mind that the Irani farmers, except for

maintaining the number of rows within the ear, apparently did not apply any selection in their landraces. This character number of rows per ear is conditioned by one locus only, and therefore, easily selectable.

Farmers of landraces of cereals such as wheat and barley, obtained, in general, low yields. For instance, the yield of landraces of winter wheat (mainly a self-fertilizer) in The Netherlands before 1850 was about 750 kg/ha (Zeven, 1990). Ten percent of the harvested seed had to be used for sowing seed. When assuming that some 100 000 plants are grown in a field of 1000 m², and a production of 30 seeds per plant some 3 000 000 grains (1000 grain weight 25 g = 75 kg/1000 m²) are harvested. Further assuming bulk harvesting and mixing of the grains (during transport, threshing, winnowing and storage), an equal contribution of each plant to the harvest seed, 7% of the harvest seed used as sowing seed, and an emergence of 50%, 20 000 grains would be needed for the next sowing of a field of the same size. From these grains again some 10 000 plants per field of 1000 m² would fully develop. Although it is possible that there is a high linkage between alleles promoting non-emergence and some other alleles, the high score for n shows that in the above equation P approaches 1, i.e. all genetic variation will be included in the next crop.

The same holds when smaller fields are employed, or when the harvest seed is obtained from 1% of the harvest, P would still approximate 1. With landraces of cross-fertilizers like rye P approximates 1 with less plants used for the next crop. Thus, rye fields of some 20 m², as observed in Galicia, Spain and Northern Portugal are still large enough to maintain variation. In that area, variation is further maintained by the influx of pollen grains of neighbouring rye fields. In fact, as said above for maize, all small fields together should be considered as a large field.

Our reflections agree with the conclusion of Yonezawa and Ichihashi (1989) for landraces of most agricultural crops that, due to the commonly occurring large sowing seed/harvest seed ratio, almost all variants will be present in the next generation(s). These authors calculated that a few hundreds seeds per population would be adequate for crops with $s < 0.8$ ($s = 0.8$ indicates quite some cross-fertilization), whereas with higher s values the number of plants, in general, should be accentuated.

A potato landrace shows conformity with a self-fertilizer. In its native area the potato landrace may continually be enriched with genetic variation from tubers, introduced from elsewhere, purpose-

fully or not, and from tubers either derived from volunteer plants grown from intra-landrace or from landrace*wild hybrid plants. Recombination may also occur within intra-clone hybrids. However, any geographical partitioning of allelic variation will be blurred, in part, by the high rates of seed tuber replacement (Zimmer & Douches, 1991).

For self-fertilizers Yonezawa and Ichihashi (1989) argued that, if $s < 0.8$ (some cross fertilization), it makes not much difference when attention is paid to number of plants per site (i.e. population) as well as number of seeds. This holds if the total number of seed (n) is not too small, and if one does not wish to collect rare alleles. The higher the number of seeds or plants the higher the probability that the rarest alleles are also collected. However, the collector should, for each landrace to be sampled, be certain that $s < 0.8$.

Yonezawa & Ichihashi (1989) summarized their paper: '*It is concluded that in predominantly selfing populations [i.e. populations existing of mainly self-fertilizing plants, ACZ] the success of sampling is primarily determined by the number of plants rather than seeds per plant. The number of plants plays an essential part in keeping the allelic multiplicity of highly selfing populations. If for some reasons the number of plants is limited the number of seeds per plant plays a role. [-] The number of plants does not need to be large. The drawback of a shortage in the number of plants can be avoided by collecting sufficient seeds from each plant.*'

Zoro Bi et al. (1998), using a more complicated equation, calculated for the, predominantly self-fertilizing, limabean (*Phaseolus lunatus* L.) the number of seeds to be collected. If one wishes to collect all alleles at a probability of $p = 0.05$, 1–2 healthy seeds per plant and 10–80 plants per field should be collected. In this case at least one seed of each plant should develop into a seed-producing plant in the next generation. This is often not the case resulting in a loss of variation. Loss is further increased by the low probability level agreed. Moreover, the mentioned range of 10 to 80 plants per field is quite large, and as mostly financial sources are limited, a collector might be inclined to take the lowest number of plants.

When comparing the above with the earlier mentioned practice of the dry bean gardeners in The Netherlands, one could easily conclude that within a few generations only one allele per (homozygous) locus is left. Some variation may be promoted in case the gardener grows French beans in addition to his dry-bean crop. Overlapping flowering periods often

result in cross-fertilization. Deviating characters that are easily observable by eye, such as plant habit, and (after harvest) seed shape, size and colour (pattern), will be discarded during seed cleaning, but this is not the case for the many characters which are not easily detectable. However, for the Netherlands the present dry-bean land- and gardenraces all belong to one group: the Dutch bush bean land/gardenrace group (Zeven et al., 1999; Zeven, 2000), despite their differences for characters such as plant habit, pod, seed, and despite the geographically fragmented distribution of various genes for these characters (Zeven, 1979).

With respect to the maintenance of landraces no general conclusion is yet published and therefore we adapted Bogyo et al.'s (1980) conclusion which was made for the maintenance of genetic variation of genebank collections: *it is [almost]³ impossible to make general recommendations for optimum sampling strategy. This strategy depends on the genetics of the character observed.* (The rare occurrence of) *visual selection* (by maize farmers) *will reduce quantitative genetic variation and thus will reduce the primary purpose of [collecting and] maintaining genetic variation. Further, the purpose of collecting may determine the success of the [collecting mission]* (activities by farmers) *[, maintaining variation]. For instance, the [mission] (farmer) could be highly successful if [a collector] (he) restricts himself to healthy plants, or short plants. However, collectors (i.e. farmers) will certainly observe, visually, new or rare types and include them, (if the farmer wishes to grow them), but they (the farmers) will miss the rare types which cannot be observed by eye. Farmers, being specialists, will know which characters are not or little influenced by environment and hence the degree of genetic variation* (for these characters). (End of modified citation).

A final point to be mentioned in this section is that due to random genetic drift, small sample sizes may also result in the loss of alleles, present at low frequencies (Cossa et al. (1993). So, this problem is similar to that of the 'founder population effect'.

Discussion

The most important agronomic characteristic of a landrace is its yield stability. Farmers have (unconsciously) related the level of variation with the importance of this character, and they have, consciously,

³ [] = deleted by me, () = added by me.

promoted or created variation, or allowed its presence. An increase of variation was obtained, for instance, by mixing various phenotypes, as was described by Whiteman (1980) for Nepal where wheat farmers added some 10% of the landrace 'Doma' (earlier-maturing, amber-grained) to the landrace 'Trogka' (later-maturing, white-grained, hairy glumes, sweeter tasting). More examples are presented by Zeven (2000).

Above we distinguished two groups of crops: those crops which are bulk-harvested (no selection), and crops which are hand-harvested (enabling selection during and after harvest). Examples of the first are the cereals wheat, barley and rye, whereas maize, sorghum and tomato are examples of the second group. One could wonder whether crops exist which are in the transition zone between these two groups? In other words: which crop (or maybe, landrace within a crop), is, by the same farmer, bulk-harvested in one year and hand-harvested in some other year? In this latter year the crop may or may not be subject to some selection.⁴ I have not heard of examples of such crops. What will be the threshold value i.e. number of plants of a crop per area and the number of propagules produced by a single plant for such a crop? Such a threshold value will also depend the type of farmer or gardener. The fewer the seeds produced per plant the more care will be taken to obtain sufficient sowing seed of the desired type. But when only a small number of seeds is required for the next crop, probably, less care will be taken.

Which number of plants per area makes a crop either a garden crop or a field crop? And which number of plants per grower makes dry-bean a garden crop or a field crop? I have not found any information on these subjects.

It goes beyond saying that sufficient sowing seed should be saved and here too, the number of seeds preserved depends on the crop and, again, on the farmer. Some farmers preserve twice the amount of sowing seed required, in order to be safe in case of bad emergence or misharvest (Linnemann & de Bruyn, 1987). Bean farmers in Malawi will know that less seeds are needed of the small-seeded genotypes than of the bigger-seeded types in their mixed fields. In such mixtures more white-skinned seeds are needed, as they often are more susceptible to diseases during germination than other colour types (Adams

⁴ I do not refer to rice, which is bulk-harvested in some countries, and hand-harvested in some other countries.

and Martin, 1988). In Cuba, farmers have experienced that black-seeded cultivars grow less vigorously than plants grown of other seed colours, because of their reduced nodulation with local *Rhizobium* strains (Hernandez et al., 1993). In these cases more material of the type with the lower reproduction rate should be added to the mixture of sowing seed.

On first thoughts, the practical and theoretical advices for genebank collectors about the numbers of propagules to be collected to safeguard (genetic) variation in the next generation, could be applied to determine the variation maintained by farmers. However, these advices suggest either collection of impracticable numbers, or give unexplicable wide ranges as 25 to 100 or 10 to 80 propagules or accessions per field. Furthermore, most theoretical advices are based on equations dealing with one allele or one character only, and assume pre-collecting knowledge about level of variation of such an allele or character. But farmers have to deal with many alleles and characters, each with other frequencies.

The equation to calculate the diversity on a farm by Brush et al. (1992) is also difficult to apply in practice, because most vectors cannot be calculated, and other, not-included vectors may have an influence on diversity too.

More research work, such as published by Soleri et al. (1999) has to be carried out to obtain more information about variation within a maize landrace, and similar investigations should be done with many other crops in which no maintenance selection is applied. Such projects should cover a number of generations, before and after seed replacement.

Final conclusion

Wilkes (1995) concluded that when the self-reliant South-American farmers were faced with potential hunger or starvation, they devised an ingenious system of crop growing to maximize their chance for survival. This system was based on the genetic variation of their staple subsistence crop maize. This conclusion, which means that they relied on yield stability, holds not only for the South-American farmers, but for all the farmers in the world. Earlier I (Zeven, 1999) already concluded that variation (and hence yield stability) was successfully maintained by seed replacement.

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