Heredity **84** (2000) 476–486 *Received 20 August 1999, accepted 18 January 2000*

Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. *vulgare*) conserved for different lengths of time in *ex situ* gene banks

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Large numbers of crop plant accessions from all over the world have been amassed in gene banks to secure a gene pool for future breeding programmes. Maintenance of accessions held as seed samples in cold stores involves frequent rejuvenation cycles to ensure the viability of seeds. The practice of rejuvenation by multiplication of a sample of each accession in small field plots has the potential to create population bottlenecks, leading to loss of genetic diversity and changes in gene frequencies every rejuvenation cycle. In order to determine whether these undesirable effects occur, genetic diversity levels were assessed for morphological and isozyme markers within gene bank accessions of two barley landraces from Syria that had been stored for 10, 40 and 72 years. These were compared with genetic diversity levels for the same markers in barley landraces collected recently at locations in Syria where they are still under cultivation. Average gene diversity (H), alleles per locus (A) and percentage polymorphic loci ($P(0.01)$) all showed very significant declines with length of time in storage, and genetic differentiation F_{ST} among accessions increased over time. If the observed differences in genetic diversity are caused by genetic drift in gene bank accessions rejuvenated every 5.3 years, it was estimated that the effective population size N_e of rejuvenation populations over their period in storage was only 4.7. Implications for gene bank management are discussed.

Keywords: barley landrace, gene bank, genetic conservation, genetic erosion, genetic markers, isozymes.

Introduction

Much of the effort expended in conservation of crop genetic resources has involved the establishment of *ex situ* gene banks. A large proportion of the material in these gene banks comprises accessions of traditional landraces of cultivated species. For instance in barley, *Hordeum vulgare* ssp. *vulgare* approximately one-half of the existing accessions in the BBSRC collection take the form of landraces (BBSRC, 1999). Landraces of inbreeding crops such as barley are genetically heterogeneous populations comprising inbreeding lines and hybrid segregates generated by a low level of outcrossing (Nevo, 1992). In the initial collections of landraces held in gene banks at least 50–60% of the total genetic variation captured resides within the landraces, the remainder being accounted for by differences between landraces (Brown & Munday, 1982; Jana & Pietrzak, 1988).

A great deal of thought has been put into devising sampling strategies to ensure that the accessions entering gene banks from the wild contain a high proportion of the 'within population' genetic variation that exists in traditional landraces (Brown & Briggs, 1991). Much less attention has been paid, however, to ensuring the maintenance of this genetic diversity throughout the lifetime of the gene bank (Sackville Hamilton & Chorlton, 1997). It has been common practice in gene banks to retain seed of many crops in airtight containers in cold stores (Clark *et al*., 1997). In these circumstances, frequent rejuvenation of seed is needed to maintain high seed viability. To accomplish this in a species like barley, samples are taken from the stored accessions and grown in plots that rarely exceed 3 m^2 in area. Rejuvenated seed is harvested from these plots for the next period of storage.

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If the number of parents contributing to the rejuvenated seed is low, i.e. the effective population size, N_e, of rejuvenation populations is small, genetic diversity could be lost rapidly each rejuvenation cycle from each landrace (Frankel, 1977; Marshall, 1990; Hamilton, 1994; Brown *et al*., 1997), and an accumulation of plants homozygous for deleterious mutations could occur (Bataillon *et al*., 1996; Schoen *et al*., 1998). Computer simulations suggest that effective population sizes of $N_e = 100$ (equivalent to 200 equally contributing parents in a self-fertilizing species) in the rejuvenation population are needed to avoid these deleterious effects. It is possible that such population sizes have not been achieved in practice, and that the problem of genetic erosion from accessions of landraces in gene banks is a real one. However there are no empirical data presently available to determine whether loss of variation has actually taken place within gene bank collections as a consequence of repeated rounds of rejuvenation.

The purpose of the present, preliminary study was to establish whether there is any evidence to support the prediction of loss of genetic variation from landrace populations that have been maintained *ex situ* within gene banks. Accessions of barley landraces from Syria were obtained from current *in situ* populations, and from a variety of *ex situ* gene banks where they had been held for different periods since initial collection. These periods ranged from 10 to 72 years.

Four seed morphological and eight isozyme markers were analysed to determine whether a significant reduction in genetic diversity could be detected in populations that had been maintained in gene banks, and whether this reduction in diversity was related to the number of generations spent in the gene bank collection. Making a number of assumptions about the origins of gene bank populations and their rate of rejuvenation in gene banks, the observed changes in genetic diversity were used to infer the genetically effective sizes of gene bank accessions during their time in storage.

Materials and methods

Sample material

All material used was of the two barley landraces, Arabi Aswad (black seeded) and Arabi Abiad (white seeded) from Syria. Barley production in Syria is entirely based on these two land races (Ceccarelli, 1996). Arabi Abiad is typically grown in more favourable environments than is Arabi Aswad. Extant populations were sampled *in situ* with the assistance of the International Centre for Agriculture in Dry Areas (ICARDA), Aleppo, Syria (Table 1). At least 100 seed heads were collected randomly from each of 11 populations located in different rainfall zones in the vicinity of Aleppo and elsewhere in northern Syria. Single seeds were taken from each seed head for analysis.

An exhaustive search of existing gene bank accessions of barley landraces derived from the same areas of Syria yielded a total of 11 accessions, ranging in storage time from 10 to 72 years, that were suitable for this study. Six samples of seed of the two Arabi landraces stored for 10 years were available (Table 1). Four were from ICARDA, Aleppo, and two from the BBSRC gene bank at the John Innes Institute in Norwich, UK, but originally collected by ICARDA. It is known that this material was derived from field collections of seed heads from at least 50 parents in each population. The populations have been through two rejuvenation cycles during their time in storage.

Two seed samples of the Arabi landraces stored for 40 years were supplied by the All-Union Institute of Plant Industry (VIR) gene bank located in St. Petersburg, Russia. Finally three seed samples that had been stored for 72 years were available, two from the VIR gene bank and one from the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany (Table 1). This sample had originally been collected by VIR. It can be assumed that the original accessions collected by VIR were large because subsamples were tested in extensive experiments at numerous locations (Vavilov, 1957).

Comprehensive documentation of rejuvenation practices was available from the IPK gene bank. An analysis of 20 different files, monitoring accessions collected from 11 to 51 years ago, showed that on average one rejuvenation cycle took place for every 5.3 years of storage time. This accords with the 5 years rejuvenation cycle for the 10-year-old material in the ICARDA gene bank.

Assessment of genetic diversity

Morphological markers Phenotype frequency was scored for four seed characters showing variation controlled by single genes (Nilan, 1964; Hockett & Nilan, 1985). Sample size ranged from 50 to 200 individuals (Table 3). The characters, phenotypes and corresponding genotypes were:
lemma colour — white (b/b), grey (B_g/B_g, B_g/b), brown (Bg/Bg, Bg/B_g, Bg/b) or black(B/-);
aleurone/pericarp colour — white (bl/bl), blue (Bl/Bl, Bl/bl), or black (B/-);
awns — rough (r/r) or smooth (R/-);
rachilla hairs — short (s/s) or long (S/-).

These simply inherited morphological characters have been used before to assess genetic diversity in barley and for testing the distinctiveness and uniformity of

Table 1 Details of 22 barley landrace accessions used in the study

Landrace	Collection site in Syria at	Location in Syria: longitude E/latitude N	Code	Source	Year of collection	No. of seed heads collected	Years stored
Arabi Aswad	Hassakeh	40.07/36.32	Aswad 3	Own collection	1997	100	0
Arabi Aswad	Quamishli	41.05/36.35	Aswad 4	Own collection	1997	200	0
Arabi Aswad	Aleppo, Zone A†	36.59/35.56	Aswad 5	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone A	36.58/35.59	Aswad 6	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone A-B	37.06/35.55	Aswad 7	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone A-B	37.08/35.55	Aswad 8	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone C	37.31/35.47	Aswad 9	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone C	37.33/35.45	Aswad 10	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone B	37.15/35.48	Aswad 11	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone B	37.17/35.47	Aswad 12	Own collection	1998	100	0
Arabi Abiad	Bural Sharqui	37.09/35.01	Abiad 3	Own collection	1997	200	0
Arabi Aswad	Palmyra	38.53/35.06	Aswad 1	ICARDA	1988	58	10
Arabi Aswad	Raqqa	39.13/36.23	Aswad 2	ICARDA	1988	82	10
Arabi Aswad	Syria	—	Aswad 20126	BBSRC/ICARDA	<1988		10
Arabi Abiad	Hama	36.43/35.08	Abiad 1	ICARDA	1988	62	10
Arabi Abiad	Suweida	36.44/33.02	Abiad 2	ICARDA	1988	68	10
Arabi Abiad	Syria	—	Abiad 20125	BBSRC/ICARDA	<1988		10
Arabi Aswad	Syria	—	H. vs. 18882	VIR	1959		40
Arabi Abiad	Damascus	36.31/33.25	Abiad 18881	VIR	1959		40
white BLR	Aleppo	37.13/36.11	White H.v. 7667	VIR	1926		72
white BLR	Homs	36.43/34.43	White H.v. 7659	VIR	1926		72
white BLR	Aleppo	37.13/36.11	White H.v.HOR7400	IPK/VIR	1926		72

† Zones refer to annual rainfall. Zone A, >350 mm; Zone B, 250–350 mm; Zone C, 200–250 mm.

cultivars (Brown & Munday, 1982; Allard, 1992). Allele frequencies were estimated on the assumption that all individuals were completely homozygous so that for these dominant markers, allele frequencies are equal to observed phenotype frequencies. This assumption is supported by the results of a parallel analysis of multilocus outcrossing rates in 10 of the populations used in this study (Arabi Aswad 3 to Arabi Aswad 12, Table 1) (Parzies, unpubl. data). Outcrossing rate was 1.7% and was not significantly different from the value of 1.6% found in wild barley, *H. vulgare* ssp. *spontaneum* (Brown *et al*., 1978), indicating a very low expected frequency of heterozygotes at inbreeding equilibrium.

Isozyme markers Variation at eight esterase (EST, E.C.3.1.1.1) loci was analysed in samples from the same 22 accessions scored for morphological markers (Table 1). The mean number of individuals scored per accession was 46 (range 17–55, Table 2). A modified protocol for starch gel electrophoresis based on the methods of Kahler & Allard (1970), Brown (1983), Kahler *et al*. (1981) and Cheliak & Pitel (1984), was used. Four to 10 day old coleoptiles were crushed in 40 μL of a 6-mM DTT solution and extracts absorbed into filter paper wicks, which were kept frozen until use. Gels were prepared using 11% hydrolysed potato starch in a solution of 0.01 M histidine HCl, 0.28 mM EDTA, pH 7.0 adjusted with 1 M Tris. The electrode buffer contained 0.125 M Tris (pH 7.0 adjusted with 1 M anhydrous citric acid). Samples were inserted into a slot of the gels and run on cooled flatbed electrophoresis units for 5 h at a current of 55 mA. Staining of samples was carried out at 37°C in the dark in a solution of 100 mg α-naphthyl acetate, 50 mg β-naphthyl acetate (both dissolved in 1 mL of acetone) and 100 mg Fast Blue RR salt in 50 mL of a 0.2-M phosphate buffer (pH 6.4 adjusted with 1 M NaH_2PO_4).

Methods described by Kahler & Allard (1970) and Brown (1983) were used to evaluate allele frequencies at eight different loci (*Est1* to *Est8*). The loci *Est1*, *Est2*, *Est4*, *Est5*, *Est6*, *Est7* and *Est8* are identical with the loci *A* to *G* described by Kahler & Allard (1970). A further locus between *Est2* and *Est4* with two alleles (single band or null allele) was found. This may be identical with *Est3* which has so far only been reported in embryo tissue (Brown, 1983).

Data analysis

Genetic diversity within accessions was quantified in terms of mean proportion of polymorphic loci using the 1% criterion ($P(0.01)$), mean number of alleles per

Table 2 Genetic diversity indices for 22 individual accessions of barley landraces from Syria calculated from variation at eight enzyme loci

Landrace code	Collection year	Years stored	Number of individuals (n)	Isoenzyme diversity				
				H	SD(H)	A	SD(A)	$P(0.01)$
Aswad 5	1998	0	50	0.2744	(±0.2033)	1.9	(±0.4)	0.88
Aswad 6	1998	0	50	0.2765	(±0.2895)	1.9	(±0.8)	0.63
Aswad 7	1998	0	50	0.2914	(±0.2043)	2.0	(±0.5)	0.88
Aswad 8	1998	0	50	0.3043	(±0.2515)	1.9	(±0.6)	0.75
Aswad 9	1998	0	50	0.2687	(±0.2498)	2.0	(±0.9)	0.75
Aswad 10	1998	0	50	0.3309	(±0.2193)	2.0	(±0.5)	0.88
Aswad 11	1998	0	50	0.2624	(±0.2325)	1.6	(±0.5)	0.63
Aswad 12	1998	0	50	0.3138	(±0.2560)	2.0	(±0.8)	0.75
Aswad 3	1997	0	50	0.2926	(±0.2217)	2.0	(±0.5)	0.88
Aswad 4	1997	0	50	0.2635	(±0.2159)	2.0	(±0.5)	0.88
Abiad 3	1997	0	55	0.2825	(±0.2165)	2.0	(±0.5)	0.88
Aswad 1	<1988	10	39	0.3166	(±0.2704)	2.1	(±1.0)	0.75
Aswad 2	<1988	10	42	0.2917	(±0.2444)	2.0	(±0.9)	0.75
Aswad 20126	<1988	10	50	0.2592	(±0.2431)	1.8	(±0.7)	0.63
Abiad 1	<1988	10	29	0.3480	(±0.2061)	2.1	(±0.6)	0.88
Abiad 2	<1988	10	17	0.2459	(±0.2197)	2.0	(±0.9)	0.75
Abiad 20125	<1988	10	50	0.1514	(±0.2176)	1.5	(±0.5)	0.50
Black BLR 18882	1959	40	50	0.1154	(±0.1696)	1.5	(±0.5)	0.50
Abiad 18881	1959	40	50	0.0147	(±0.0203)	1.4	(±0.5)	0.38
White BLR 7667	1926	72	50	0.0469	(±0.0434)	1.4	(±0.8)	0.38
White BLR 7659	1926	72	44	0.0312	(±0.0737)	1.5	(±0.5)	0.38

H, gene diversity; A, mean alleles per locus; $P(0.01)$ percentage polymorphic loci using 1% criterion.

locus (A) and average gene diversity (H) using the POPGENE programme (Yeh *et al.*, 1997). Morphological and isozyme genetic markers were initially analysed separately since they may differ in important characteristics such as mutation rate and degree of selective neutrality (Bataillon *et al.*, 1996).

In an isolated population of effective size N_e, gene diversity is expected to decline every generation as a consequence of genetic drift. Let the initial gene diversity be H_o. After t generations the remaining value of gene diversity H_t is given by:

$$H_t = H_o(1 \quad 1/2N_e)^t.$$

Then $\log(H_t) = \log(H_o) + t \cdot \log(1 \quad 1/2N_e)$.

In order to estimate the genetically effective size of gene bank populations during their time in storage that is compatible with the observed decline in gene diversity by genetic drift, a regression of $\log(H_t)$ on t was calculated. Because observed rates of decline for morphological and isozyme markers were very similar (Fig. 1a, and 1b) H_t for this analysis was calculated using all 12 loci. t is the number of cycles of regeneration passed through by accessions during their time in the gene bank, assuming a rejuvenation cycle time of 5.3 years. The slope of these regressions provides an estimate of $\log(1 - 1/2N_e)$ from which N_e can be calculated.

If genetic drift occurs independently in accessions over cycles of rejuvenation, an increase in genetic divergence between them is expected. To test for this effect all accessions held for the same length of time in gene banks were grouped and treated as a single subdivided population. Values of total genetic diversity H_T, for accessions in each age class (0, 10, 40 and 72 years), and of F_{ST} measuring the extent of genetic divergence among accessions in these age classes were estimated separately for morphological and isozyme markers using the POPGENE and FSTAT (Goudet, 1999) programs.

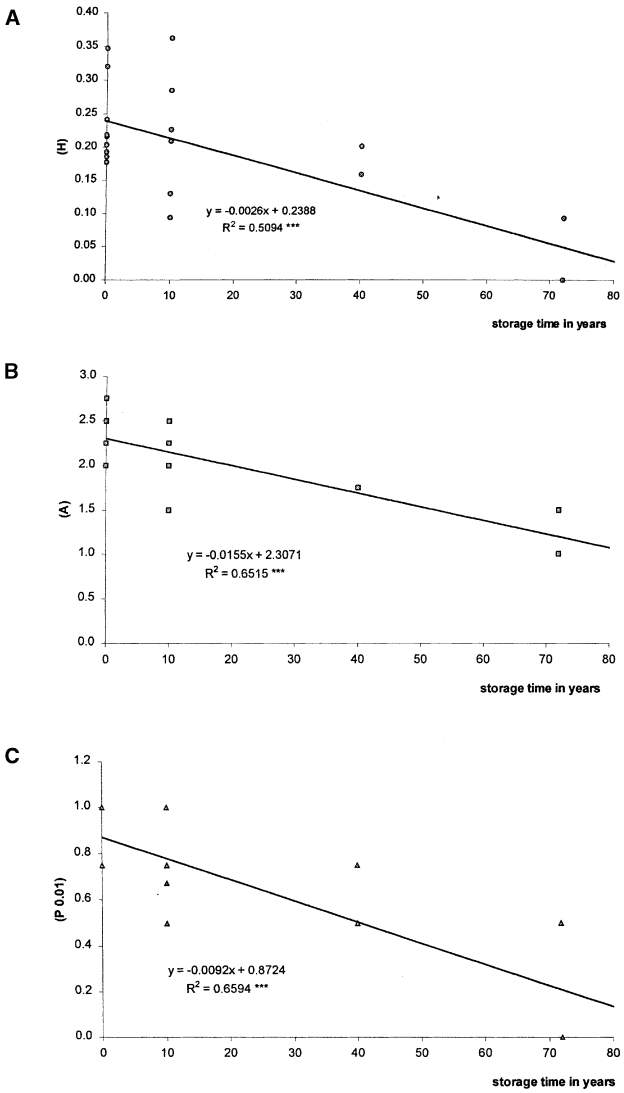

Fig. 1 Relationship between genetic diversity indices, measured using four morphological markers, and length of time for which barley landrace accessions have been held in gene banks. Indices of genetic diversity are gene diversity ((*H*), Fig. 1 A); mean alleles per locus (*A*), Fig. 1(B); and percentage polymorphic loci using 1% criterion ((*P*(0.01)), Fig. 1(C).

Results

Diversity levels within accessions

Table 4 summarizes the mean genetic diversity indices within accessions calculated from morphological and isozyme markers for groups of barley landrace accessions from Syria with identical storage periods. The individual results for all accessions are listed in Tables 2 and 3 and plotted against time in storage in Figs 1 and 2.

A decrease of genetic diversity with increase in storage period was observed for all three indices of diversity and for both marker types. Average maximum declines in diversity indices after 72 years storage were 60% for $P(0.01)$, 35% for A and 75% for H. For both the morphological and the isozyme markers, all three genetic diversity indices showed significant negative regressions ($P < 0.001$) on storage time (Figs 1 and 2). The only exceptional result was a slight increase in average gene diversity (H) of isoenzyme markers from the 40 to the 72 years accessions. This result was caused by the high H-value of one individual accession (HOR 7400) (Table 2).

Table 3 Genetic diversity indices for 22 individual accessions of barley landraces from Syria calculated from variation at four loci affecting seed morphology

Landrace code	Collection year	Years stored	Number of individuals (n)	Morphological diversity				
				H	SD(H)	A	SD(A)	P(0.01)
Aswad 5	1998	0	74	0.1771	(±0.2266)	2.3	(±0.5)	1.0
Aswad 6	1998	0	65	0.2157	(±0.2374)	2.0	(±0.8)	0.8
Aswad 7	1998	0	50	0.1918	(±0.2330)	2.3	(±0.5)	1.0
Aswad 8	1998	0	54	0.2172	(±0.2680)	2.0	(±0.8)	0.8
Aswad 9	1998	0	51	0.2186	(±0.2722)	2.5	(±1.3)	0.8
Aswad 10	1998	0	80	0.2413	(±0.2787)	2.8	(±1.0)	1.0
Aswad 11	1998	0	52	0.2025	(±0.2650)	2.3	(±1.3)	0.8
Aswad 12	1998	0	53	0.1855	(±0.2553)	2.0	(±0.8)	0.8
Aswad 3	1997	0	197	0.3469	(±0.3297)	2.8	(±1.0)	1.0
Aswad 4	1997	0	200	0.3207	(±0.2988)	2.5	(±1.3)	0.8
Abiad 3	1997	0	200	0.2422	(±0.2457)	2.0	(±0.0)	1.0
Aswad 1	<1988	10	58	0.2846	(±0.3096)	2.5	(±1.3)	0.8
Aswad 2	<1988	10	82	0.3630	(±0.3667)	2.5	(±1.3)	0.8
Aswad 20126	<1988	10	88	0.2259	(±0.2175)	2.0	(±1.0)	0.7
Abiad 1	<1988	10	62	0.0934	(±0.0624)	2.3	(±0.5)	1.0
Abiad 2	<1988	10	68	0.2086	(±0.2357)	2.3	(±0.5)	1.0
Abiad 20125	<1988	10	95	0.1308	(±0.2420)	1.5	(±0.6)	0.5
Black BLR 18882	1959	40	100	0.1588	(±0.2150)	1.8	(±1.0)	0.5
Abiad 18881	1959	40	100	0.2002	(±0.1840)	1.8	(±0.5)	0.8
White BLR 7667	1926	72	112	0.0000	(±0.0000)	1.0	(±0.0)	0.0
White BLR 7659	1926	72	100	0.0925	(±0.1082)	1.5	(±0.6)	0.5

H, gene diversity; A, mean alleles per locus; P(0.01) percentage polymorphic loci using 1% criterion.

Assuming a mean time between rejuvenation of 5.3 years, gene diversity (H) within accessions decreased on average by approximately 11% with every cycle of rejuvenation for both sets of markers. From the regression of $\log(H_t)$ on t, the genetically effective size of *ex situ* populations during their time in storage, estimated from the rate of decline of gene diversity, was $N_e = 4.7$ (Fig. 3).

Total diversity and its distribution among accessions

Total diversity levels (H_T) for combined accessions of a particular age held in gene banks show a decline with storage time (Table 5). Over 72 years storage H_T declined from 0.32 ± 0.08 to 0.12 ± 0.12 when measured with morphological markers and from 0.32 ± 0.07 to 0.11 ± 0.11 when isozyme markers were used. Measures of the proportion of genetic variation found among accessions relative to total genetic variation, F_{ST}, showed an increase in value with time of storage (Table 5). Thus total genetic variation was lower in older samples, and a greater proportion of this variation was distributed between rather than within samples. One exception to this trend was the group of accessions with a 72-year storage period scored for isoenzyme markers. The low F_{ST} value of this group is caused by a high H-value in one of the three accessions (HOR 7400, Table 2).

Discussion

The major finding of this study is that gene diversity within landrace accessions of barley in Syria is related to the time of collection of the accession. Genetic diversity is lower in early accessions than in accessions made more recently. An additional result is that a greater proportion of the total genetic variation is found between rather than within landrace accessions in older collections than in recent collections. These results are consistently found using both morphological and isozyme genetic markers.

A possible explanation for both these results is that the differences in genetic diversity and structure are due to genetic drift occurring during the rejuvenation cycles through which the *ex situ* collections have passed during their time in the gene bank. For this to be a convincing explanation a number of conditions must be met. The first is that the initial samples used to found the early gene bank accessions were large enough to include a high proportion of the genetic diversity present in the contemporary landrace populations. The second is that

Table 4 Diversity indices measuring mean genetic variation within 22 accessions of barley landraces stored in gene banks for identical time periods

Accessions with storage period of	Number of accessions	Morphological markers			Isozyme markers		
		H	*A*	*P*(0.01)	*H*	*A*	*P*(0.01)
0 years	11	0.2327 (±0.0544)	2.3 (±0.3)	0.89 (±0.10)	0.2874 (±0.0219)	1.9 (±0.1)	0.80 (±0.10)
10 years	6	0.2177 (±0.0988)	2.2 (±0.4)	0.80 (±0.19)	0.2688 (±0.0685)	1.9 (±0.23)	0.71 (±0.13)
40 years	2	0.1795 (±0.0292)	1.8 (±0.0)	0.65 (±0.21)	0.06505 (±0.0712)	1.5 (±0.07)	0.44 (±0.08)
72 years	3	0.0308 (±0.0534)	1.2 (±0.3)	0.17 (±0.29)	0.1015 (±0.1085)	1.5 (±0.1)	0.46 (±0.14)
All accessions	22	0.1962 (±0.0938)	1.6 (±0.9)	0.75 (±0.29)	0.2368 (±0.0988)	1.8 (±0.2)	0.70 (±0.18)

H, gene diversity; *A*, mean alleles per locus; *P*(0.01), percentage polymorphic loci using the 1% criterion. Standard errors are in parentheses. Values calculated from morphological markers and isozyme markers are shown separately.

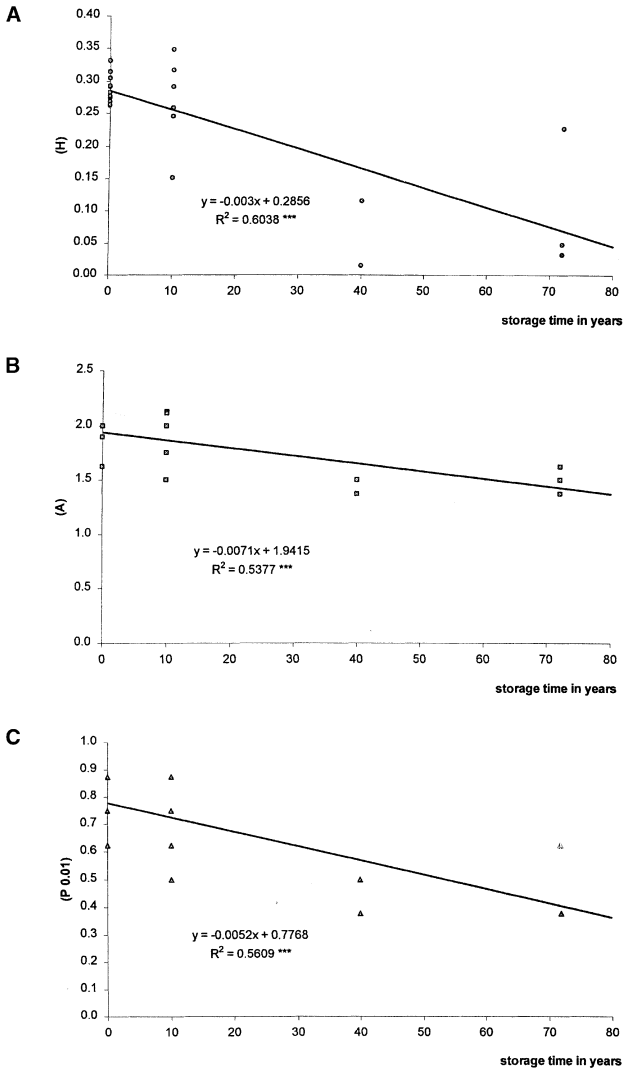

Fig. 2 Relationship between genetic diversity indices, measured using eight isozyme markers, and length of time for which barley landrace accessions have been held in gene banks. Indices of genetic diversity are gene diversity ((*H*), Fig. 2A); mean alleles per locus (*A*), Fig. 2(B); and percentage polymorphic loci using 1% criterion ((*P*(0.01)), Fig. 2(C).

there has been no systematic change in the gene diversity of the *in situ* landrace populations over time.

In the present study it is certain that the 10-year-old collections used were based on at least 50 seed heads and would have sampled a similar proportion of the genetic diversity available in landraces as present day collections (Marshall, 1990). Unfortunately details of the sampling procedures used to obtain the oldest accessions used in this study are not available. However the fact that such early collections were large enough to provide the material for extensive field trials demonstrating genetic heterogeneity within landraces,

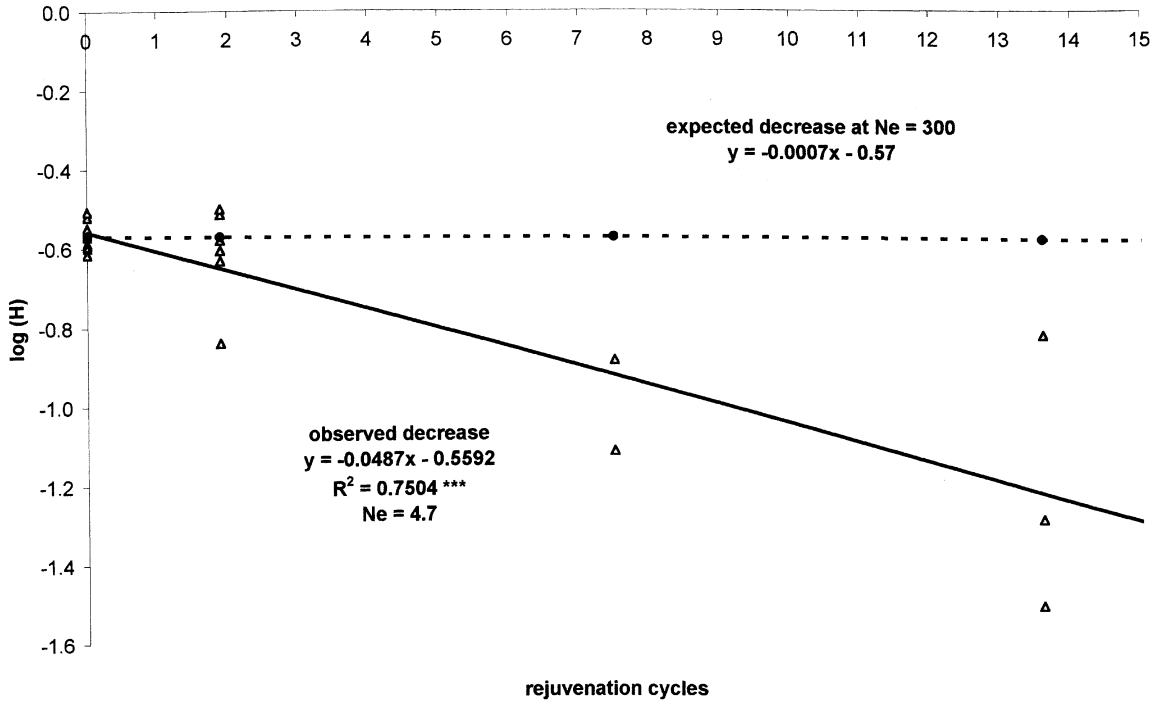

Fig. 3 Regression of log (gene diversity) (log(H)) on number of cycles of rejuvenation experienced by accessions of barley landraces in gene banks. The slope of the line is an estimate of log $(1 - 1/2N_e)$ where N_e is the effective size of rejuvenation populations.

Table 5 Total genetic variation H_T within collections of accessions held for particular time period, and genetic differentiation F_{ST} found among accessions within these collections. Results are shown separately for morphological and isozyme markers. Standard deviations are in parentheses

Accessions with storage period of	Morphological markers		Isoenzyme markers	
	(H_T)	(F_{ST})	(H_T)	(F_{ST})
0 years	0.3197 (±0.0823)	0.2722 (±0.0513)	0.3209 (±0.0670)	0.0929 (±0.042)
10 years	0.3204 (±0.1328)	0.3205 (±0.1258)	0.3266 (±0.1053)	0.2110 (±0.051)
40 years	0.2661 (±0.1850)	0.3254 (±0.0739)	0.2302 (±0.1856)	0.8477 (±0.078)
72 years	0.1242 (±0.1157)	0.7518 (±0.2482)	0.1127 (±0.0610)	0.2832 (±0.113)
All accessions	0.3549 (±0.0678)	0.4472 (±0.0564)	0.3594 (±0.0496)	0.3461 (±0.079)

suggests that they were not genetically depauperate at the time when they entered the gene banks (Vavilov, 1957). It therefore seems reasonable to conclude that observed differences in gene diversity among collections held for different lengths of time are unlikely to be accounted for by differences in initial sampling procedures.

The second assumption, that there has been no systematic change in the genetic diversity of *in situ* populations of barley landraces in Syria over the last 72 years, is supported by extensive fieldwork at ICARDA. This indicates the restricted exchange of barley landrace germplasm among climatic regions within Syria, and the minimal influence of modern barley cultivars on barley landraces in this region (Weltzien & Fischbeck, 1990; Ceccarelli, 1996). The continuity and stability of landrace cultivation in the area means that gene diversity levels in present day barley landraces are likely to be very similar to gene diversity levels in landrace populations grown throughout the last 72 years. If any changes have taken place over this time they are likely to have led to a reduction rather than an increase in gene diversity levels in contemporary landrace populations.

Comparison of gene diversity levels in accessions held for different lengths of time show decreasing levels of

genetic diversity with increasing length of storage, and this is consistent over diversity indices and genetic markers. If it is accepted that these differences were not caused by differences in initial sampling procedures or by a systematic increase in gene diversity over time within *in situ* populations, the observed decline of genetic diversity is most easily explained by the effects of genetic drift in rejuvenation populations used to maintain the accessions in the gene banks. The rate of decline of genetic diversity is consistent with a model of genetic drift in rejuvenation populations with an effective population size $N_e = 4.7$. In a predominantly inbreeding species where N_e is half the number of observed reproductive individuals, this is equivalent to a population of 9.4 unrelated adults contributing equally to the seed produced.

Such a low number of parents contributing to rejuvenated seed appears at odds with what is known of rejuvenation procedures in gene banks. Here accessions are grown on 3 m^2 field plots likely to accommodate 600 plants, and if all individuals contributed equally, effective population size would be $N_e = 300$. The expected decline in gene diversity every cycle of rejuvenation is negligible when the effective population size is as large as 300 (Fig. 3).

There are a number of factors that could account for this discrepancy between anticipated and inferred effective population size in the rejuvenation populations. Restricted sampling of the accession from the gene bank to establish the rejuvenation plot, and limited sampling of the seed produced from this plot could both contribute to a low value of N_e. In addition the differences in environmental conditions between the original site of collection and the rejuvenation plots may be so large that only a limited set of genotypes are able successfully to grow and produce seed. A large variance in the reproductive contribution of individuals will significantly reduce the effective size of a population (Lande & Barrowclough, 1987).

It should also be remembered that the value of N_e calculated relates to the genetic behaviour of accessions over a number of generations. The value of N_e in these circumstances is governed by the value of the smallest effective population size found over the time period in question (Lande & Barrowclough, 1987). Thus the low value of N_e could be accounted for by a single bottleneck event in the gene bank, rather than a chronically low effective population size at each round of rejuvenation.

If these estimated values of N_e in rejuvenation populations are reasonable, problems are likely to arise in gene bank collections not only as a consequence of loss of genetic variation, but also through fixation of deleterious mutations that are much more difficult to monitor (Schoen *et al.*, 1998). In this respect it is interesting to note that during growth of material for electrophoresis, chlorophyll deficient mutants were observed in accessions that had been stored for 10 years (Aswad 20126) and 72 years (white H.v. 7667) at frequencies of 1.1% and 3.8%, respectively, but not in any recent accessions. Computer simulations by Schoen *et al.* (1998) suggest that accumulation of plants homozygous for deleterious mutations will accompany recurrent rejuvenation of germplasm when effective population size is less than 75.

Another effect that is expected in gene banks, if genetic drift is occurring in rejuvenation populations, is that genetic differentiation among accessions with identical storage times should increase with time of storage. A trend of increasing values of F_{ST} with storage time is indeed seen for the barley landraces and this is clearest for the morphological markers. These show a greater than twofold increase in F_{ST} after 72 years in storage (Table 5). This change cannot be attributed to collection of a less diverse range of populations in more recent years because the present day collections cover the wide range of barley landrace sites within northern Syria.

The results are, however, as anticipated if genetic drift is occurring in gene bank accessions. The genetic variation originally present in the accessions collected at a particular time has not only declined overall (shown by a decrease in H_T, Table 5) but has become rearranged such that in older collections an increased proportion of the variation is distributed between accessions while less is found within accessions.

Simmonds (1962) has referred to gene banks as 'museum collections' because they contain a selection of ill-adapted genotypes (from the point of view of high input agriculture) which either cannot be preserved at all in the wild or can be preserved only with great difficulty and uncertainty. The present results suggest that gene banks face further problems because genetic drift during rejuvenation may lead to erosion of the original genetic diversity of seed accessions lodged in gene banks. The present study is only of a preliminary nature, relies on assumptions about early sampling procedures and continuity of landrace populations over time, and deals only with one crop species. However it suggests that more detailed empirical studies of genetic changes occurring during rejuvenation of gene bank accessions are justified. If genetic erosion proves to be a widespread phenomenon, changes in management are clearly required to obviate its undesirable effects and enable gene banks to serve as sustained reservoirs of crop genetic resources.

A rapid improvement of *ex situ* storage conditions and facilities (i.e. deep freezing of samples) would

facilitate maintenance of collections with fewer rejuvenation cycles. The use of larger rejuvenation plots and greater emphasis on adequate sample sizes for establishing and collecting from rejuvenation plots may also be important. To make the best use of old collections, in which genetic variation may now be found predominantly between rather than within accessions, it may be advisable to combine duplicate collections from a variety of different gene banks. Once a genetically variable collection had been amassed, this could be conserved *ex situ* with more modern techniques involving deep-freezing, possibly augmented by *in situ* conservation approaches.

The consistently high diversity indices of all recently collected barley landrace accessions found in this study is a clear justification for the use of *in situ* conservation strategies. However, given the inherent weaknesses of both systems it seems appropriate to promote crop conservation through a combination of *in* and *ex situ* conservation practices rather than favouring a single strategy.

Acknowledgements

This project was financed by the European Union (FAIR-BM-956373). The authors would like to thank the following persons for supplying seed samples and very valuable advice. Dr S. Ceccarelli, Dr S. Grando and Dr M. Turner (ICARDA, Syria); Prof. Viktor A. Dragavtsev (VIR, Russia); Dr H. Knueppfer (IPK, Germany); Dr Freese (Bundesanstalt für Züchtungsforschung an Kulturpflanzen (BAZ), Braunschweig, Germany); Dr M. Ambrose (BBSRC, U.K.); Dr T. van Hintum (Collections of the Centre for Genetic Resources (CPRO-DLO), Wageningen, The Netherlands).

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