Genetic diversity in the wild progenitor of barley in Israel[1]

E. Nevo, A.H.D. Brown and D. Zohary

Institute of Evolution, Haifa University, Haifa (Israel), CSIRO Division of Plant Industry, P.O. Box 1600, Canberra (Australia), and Department of Genetics, Hebrew University of Jerusalem, Jerusalem (Israel), 11 December 1978

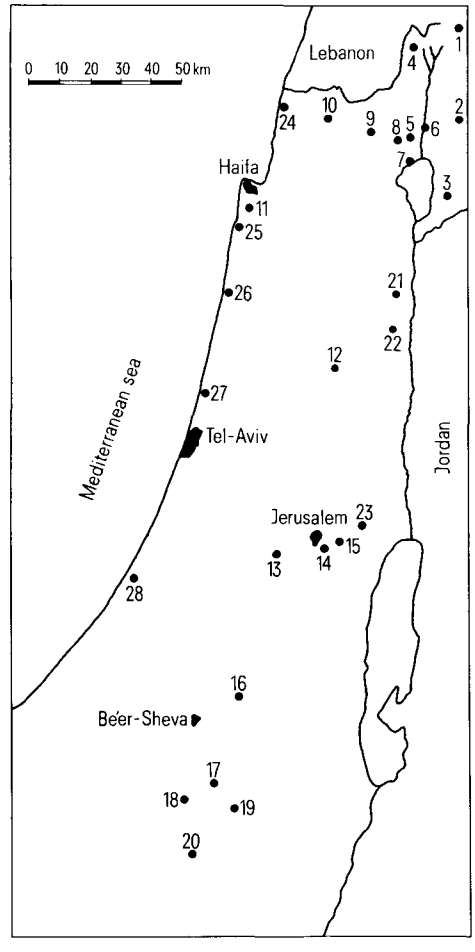
Summary. Genetic structure of populations of *Hordeum spontaneum*, the wild progenitor of barley, was studied electrophoretically in proteins encoded by 28 gene loci in 1179 individuals representing 28 populations covering the entire ecological range in Israel; for comparison, the same test was conducted in 100 random seeds of Composite Cross XXI (generation 17) of cultivated barley, *Hordeum vulgare*. The results indicate that: a) *H. spontaneum* in Israel is genetically highly polymorphic, b) clinal, regional and local genetic patterns are significantly correlated with and predictable by climatic and soil variables, suggesting the operation of natural selection, and c) natural populations are on the average more variable than the tested Composite Cross generation. This genetic variation awaits testing and exploitation in breeding programs.

Genetic variation stored in the wild progenitors of cultivated crops represents a major resource for future breeding[2]. Yet the amount and spatial patterns of genetic polymorphisms harbored in the wild relatives of cultivated crops are still largely unknown. A major challenge for crop plant evolutionists and plant breeders lies therefore in rapid and efficient screening and recording of the wild gene pools. Of considerable importance is the unveiling of that portion of genetic variation which is implicated in adaptations to various climates, soils, disease-resistance, etc. Recently the technique of gel electrophoresis has permitted the screening of allozymic variation in plant populations at both the single and multilocus levels[3]. The objectives of this study were to test the structuring of genetic variation in *Hordeum spontaneum*, the wild progenitor of barley, across its entire ecological range in Israel, to compare it with the variation present in cultivated barley, and to seek correlations with environmental variation. The results might shed light on a) the range and structure of genetic variation in wild *H. spontaneum* within a significant part of its distributional range, and b) correlations of these patterns with environmental diversity. These results might help to formulate optimal sampling strategies for the future collection and utilization of genetic resources in the wild gene pool of barley over its entire geographic range in the Near East.

It is now well established that the annual, predominantly self-pollinating diploid (n = 14) *Hordeum spontaneum* is the wild ancestor of cultivated barley[4], with which it forms fully fertile hybrids. Its main distribution lies in the Near East Fertile Crescent Belt where it occupies primary habitats as well as man-made, secondary formations. It constitutes an important component of open plant communities characteristic of the summer-dry hilly belt arcing the Euphrates Basin and the Jordan Rift Valley[5-7]. In Israel, *H. spontaneum* occupies an extraordinarily wide diversity of habitats, ranging from mesic Mediterranean to desert ones. It is common over north and central Israel where it frequently builds continuous massive stands. It is more locally and sporadically distributed in the drier Negev and Dead Sea regions. Also morphologically it is conspicuously variable. Robust plants with large spikes occur in mesic and warm environments whereas slender, smaller forms grow in arid zones and on cold mountain slopes.

Allozymic variation encoded by 28 gene loci[8] was studied in 1179 specimens representing 28 wild populations of *H. spontaneum* in Israel (figure). From each population seeds from 30–50 plants were germinated and tested by homogenizing fresh leaves and flooded roots of seedlings. For comparison, the same test was conducted on 100 random seeds of Composite Cross XXI[9] (generation 17) of cultivated barley *H. vulgare*. Details on electrophoretic procedures and on the localities from which wild barley was sampled are published elsewhere[10]. Following are the gene loci studied, their abbreviation, and, in parenthesis, first the number of alleles detected in *H. spontaneum* over its range in Israel, and second the number of alleles found in *H. vulgare* Composite Cross XXI: Malate dehydrogenase, *Mdh-1* (2,1); *Mdh-2* (4,1); 6-phosphogluconate dehydrogenase, *6-pdg-1* (3,1); *6-pgd-2* (3,2); phosphoglucomutase, *Pgm* (3,1); phosphoglucoisomerase, *Pgi* (3,1); glutamic oxaloacetic transaminase, *Got-1* (2,1); *Got-2* (4,1); peptidase, *Pept-1* (3,1); *Pept-2* (2,1); esterase, *Est-1* (6,3); *Est-2* (15,3); *Est-4* (7,2); *Est-5* (6,4); acid phosphatase, *Acph-1* (2,1); *Acph-2* (9,1); *Acph-3* (4,3); tetrazolium oxidase, *To-1* (1,1); *To-2* (2,1); alcohol dehydrogenase, *Adh-1* (3,1); *Adh-2* (3,1); NADH diaphorase, *Nadhd-1* (4,1); *Nadhd-2* (3,1); glutamate dehydrogenase, *Gdh* (3,1); aldolase, *Ald* (1,1); catalase, *Cat* (2,1); phosphoenol pyruvate carboxylase, *Pepc* (1,1); general protein, *Gp* (4,1).

H. spontaneum in Israel is extraordinarily variable geneti-



Geographic localities of 28 populations of *Hordeum spontaneum*.

cally. Of the 28 loci tested, 25 (89%) were polymorphic in 1 or more populations. 13 loci varied regionally and were strongly polymorphic *(Mdh-2, Pgm, Pgi, Pept-2, Est-1, 2, 4, 5, Acph-1,2, Adh-1, Nadhd-1, Gdh).* 12 were locally and weakly polymorphic *(Mdh-1, 6-pgd-1,2, Got-1,2, Pept-1, Acph-1, To-2, Adh-2, Nadhd-2, Cat, Gp).* Only 3 loci *(To-1, Ald, Pepc)* were found to be monomorphic in all 28 populations. The table gives the means for all 28 populations of the proportion of polymorphic loci per population, P, (mean 0.30, range, 0.00–0.43); number of alleles per locus, A, (mean 1.48, range 1.00–1.79); and proportion of heterozygosity per locus per individual, H, (mean 0.003, range 0.00–0.019). Pooled together, the number of alleles detected throughout Israel in the 28 loci is indeed impressive, totalling 105 (mean per locus 3.75, range 1–15). As in other selfers, genetic variation is carried primarily between homozygotes or inbred lines within the populations. The number of distinct lines, on a multilocus basis, ranged from 1 (at localities 1 and 9) to 33 (at locality 8). However H_e[11], the equivalent estimate of heterozygosity (H) assuming an outbreeding system, is surprizingly high, though widely varying geographically (mean $H_e = 0.098$, range 0.00–0.176). The observed inbreeding coefficient, or Wright's fixation index[12], is $F = 0.97$, approximately. The genetic identities (Nei's I[13]) between all pairs of Israeli populations had a mean of 0.89 and range 0.75–0.99. These estimates indicate that *H. spontaneum* is a) highly self pollinated (high F and low H values), b) genetically a highly polymorphic species (high P, A, H_e values), and c) genetic differentiation of populations includes clinal, regional and local patterns sometimes displaying sharp geographic differentiation over short distances (widely varying P, A, H_e values within short distances, and relatively low I values). The mean P and H_e values are typical of organisms which can be classified as habitat generalists (mean values for 117 animal and plant habitat-generalist species are $P = 0.35$, SD 0.20; mean $H = 0.106$, SD 0.06)[14]. The I values of *H. spontaneum* resemble those of *Avena barbata*[15]. Both are highly successful, self-pollinated, colonizing annuals. In both, different local populations may be practically similar genetically. However, they can be extremely different presumably when they grow in distinct habitats regardless of geographical distance; i.e., local ecotypic adaptation seems to predominate.

The pattern of variation suggests the operation of natural selection in *H. spontaneum* in Israel. 1. Levels of genetic diversity differ regionally and locally. 2. The population frequencies of some alleles at the polymorphic loci *Pgi, Acph-2, Acph-3, Est-2, Adh-1* show correlations with altitude or latitude. Likewise, the widely varying levels of A, P, and H_e reflect sharp local genetic differentiation. 3. The level of polymorphism is high in low, warm and dry environments (P, is negatively correlated with altitude and annual rainfall ($r = -0.68$; $p < 0.001$; -0.43; $p < 0.05$, respectively), and positively correlated with mean annual temperature ($r = 0.51$; $p < 0.01$). Moreover, the patterns of genetic and morphological variation in *H. spontaneum* are significantly correlated with the environment and are ecologically predictable chiefly by combination of temperature and humidity variables[16]. 4. Both the linkage disequilibria found in esterase polymorphism[17] and the high degree of multiple heterozygosity[18] can also be taken as evidence of selection.

Finally, it is instructive to compare and contrast the level of genetic diversity in the average population of wild barley in Israel with that found in cultivated barley Composite Cross XXI. The latter was synthesized from 6200 cultivated barley genotypes and is regarded by barley breeders as highly variable. In our test of generation 17 the average estimates of A, P, and H_e were 1.43, 0.25 and 0.068, respectively. Significantly, natural populations of wild barley in Israel

Genetic variation at 28 protein loci in 28 populations of *H. spontaneum*

Locality	Sample (N)	Alleles per locus (A)	Polymorphic loci (P)	Heterozygosity observed (H)	Diversity (H_e)
1. Hermon	30	1.00	0.00	0.000	0.000
2. Shifon	50	1.29	0.21	0.000	0.049
3. Afiq	32	1.64	0.25	0.000	0.129
4. Tel-Hay	49	1.32	0.21	0.001	0.109
5. Rosh Pinna	40	1.32	0.25	0.000	0.088
6. Gadot	49	1.75	0.39	0.001	0.176
7. Tabigha	45	1.68	0.39	0.001	0.145
8. Zefat	50	1.75	0.25	0.007	0.099
9. Mt. Meron	46	1.00	0.00	0.000	0.000
10. Ma'alot	50	1.46	0.29	0.003	0.053
11. Damon	50	1.39	0.25	0.004	0.083
12. Shechem	30	1.50	0.39	0.003	0.095
13. Bar Giyyora	50	1.46	0.39	0.006	0.108
14. Talpiyyot	30	1.54	0.39	0.019	0.147
15. Eizariya	30	1.82	0.36	0.003	0.165
16. Tel Shoqet	30	1.61	0.39	0.001	0.115
17. Bor Mashash	50	1.43	0.29	0.000	0.042
18. Revivim	31	1.32	0.25	0.000	0.054
19. Yeroham	33	1.46	0.25	0.000	0.085
20. Sede Boqer	32	1.46	0.36	0.005	0.127
21. Bet Shean	39	1.64	0.36	0.007	0.110
22. Mehola	47	1.64	0.36	0.006	0.089
23. Wadi Qilt	45	1.43	0.32	0.001	0.106
24. Akhziv	49	1.54	0.32	0.001	0.144
25. Atlit	49	1.71	0.43	0.001	0.156
26. Caesarea	48	1.46	0.36	0.001	0.059
27. Herzliyya	50	1.36	0.25	0.000	0.078
28. Ashqelon	45	1.68	0.39	0.006	0.141
Total	1179				
Mean		1.48	0.30	0.003	0.098
Range		1.00–1.82	0.00–0.43	0.00–0.019	0.00–0.176

are on average more variable than the tested Composite Cross XXI generation, based on the H_e value. Thus the gene pool of wild barley in Israel is indeed very rich and is at least in part adaptive in its nature. Furthermore, it seems that only a portion of this genetic variation is present in the cultivated gene pool. This variation represents a genetic resource awaiting the tests and future utilization of the plant breeder.

1 This research was supported by grants from the United States-Israel Binational Science Foundation (BSF) Jerusalem (Israel), the Gifrid Foundation and the Reuben and Edith Hecht Fund, University of Haifa, We thank M. Avrahami for help in field collections; Ora Dagan for laboratory assistance, Dr M. Haber for statistical assistance, Dr A.L. Kahler for seed of CC XXI, and E. Golenberg for commenting on the manuscript.
2 J.R. Harlan, in: Crops and Man. Am. Soc. Agron., Crop Sci. Soc. of America, Madison, Wisconsin 1975.
3 R.W. Allard, A.L. Kahler and M.T. Clegg, in: Isozymes IV. Genetics and Evolution. Ed. C.L. Markert. Academic Press, New York 1975.
4 D. Zohary, Proc. 1st int. Barley Genet. Symp. Wagenigen, p.27.
5 J.R. Harlan and D. Zohary, Science *153*, 1074 (1966).
6 D. Zohary, in: The domestication and exploitation of plants and animals, p.47. Ed. P.J. Ucko and G.W. Dimbleby. Duckworth & Co., London 1969.
7 D. Zohary, in: Plant Life in South West Asia, p.235. Ed. P.H. Davis. Bot. Soc. Edinburgh 1971.
8 The genetics of the isozyme variation is based on: 1. Our data which comes in family batches and thus directly tests mendelian segregation[18]. 2. Direct comparisons for some loci in cultivated barley, *H. vulgare* (A.L. Kahler and R.W. Allard, Crop Sci. *10*, 444 (1970), A.L. Kahler, R.W. Allard and S. Pagliuso, in preparation; G. Almgard and U. Landegren, Z. Pfl. Zücht. *72*, 63 (1974); S. Hvid and G. Nielsen, Hereditas *87*, 155 (1977)). 3. Many of these loci are well studied genetically in other grasses, and their patterns are analogous to those of barley.
9 Composite Cross XXI was developed by C.A. Suneson and G.A. Wiebe, Crop Sci. *2*, 347 (1962).
10 A.H.D. Brown, E. Nevo, D. Zohary and O. Dagan, Genetica *49*, 97 (1978).
11 M. Nei, in: Molecular population genetics and evolution, p.129. North-Holland Publ., Amsterdam 1975.
12 S. Wright, Evolution *19*, 395 (1965).
13 M. Nei, Am. Natur. *106*, 283 (1972).
14 E. Nevo, Theor. Pop. Biol. *13*, 131 (1978).
15 M.T. Clegg and R.W. Allard, Proc. natl Acad. Sci. USA *69*, 1820 (1972).
16 E. Nevo, D. Zohary, A.H.D. Brown and M. Haber, Evolution (June 1979).
17 A.H.D. Brown, E. Nevo and D. Zohary, Nature *268*, 430 (1977).
18 A.H.D. Brown, D. Zohary and E. Nevo, Heredity *41*, 49 (1978).

Presence of viruses in a strain of *Mycoplasma pulmonis*

A.A. Rahman and K.K. Sethi

Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, D-53 Bonn-Venusberg (Federal Republic of Germany), 23 October 1978

Summary. Filtrates prepared from heavily grown agar cultures of *M. pulmonis* strain Negroni-52 formed plaques on lawns of *A. laidlawii* strain JA1 but not on those of *M. pulmonis* strains Ash or Negroni-52. The plaque-forming agent proved to be rod-shaped particles morphologically identical with mycoplasmavirus group 1. Evidence supporting the conclusion that the virus originated from Negroni-52 has been obtained. Electron microscopy revelaed that Negroni-52 is also a carrier of long-tailed phage-like particles.

Since Gourlay's initial report[1] on the isolation of a virus infecting *Acholeplasma laidlawii,* a member of the order Mycoplasmatales, at least 3 morphologically and serologically distinct viruses have been isolated from *A. laidlawii* strains[2,3]. These viruses, were designated as Mycoplasmatales virus laidlawii 1 (MV-L1), 2 (MV-L2) and 3 (MV-L3). MV-L1 consists of non-enveloped, rod-shaped particles containing single stranded DNA, MV-L2 consists of spherical, enveloped particles containing double stranded DNA, and MV-L3 is characterized by non-enveloped, short-tailed polyhederal-shaped particles containing double-stranded DNA[2,3]. Owing to some degree of biological heterogeneity which has been observed amongst different isolates of MV-L1[4] and MV-L2[5], it has been proposed that these viruses be assigned in 3 different groups, namely mycoplasmavirus groups 1, 2 and 3 represented by the prototypes MV-L1, MV-L2 and MV-L3 respectively[3].

Besides, *A. laidlawii,* group 1 viruses have also been reportedly isolated when washings from agargrown cultures of *A. granularum*[6] or *Mycoplasma gallisepticum*[7] were layered on lawns of sensitive *A. laidlawii* strains. It has been argued, however, that the group 1 viruses claimed to have been recovered from mycoplasmas other than *A. laidlawii,* were not in factindigenous to these organisms but rather originated from latently infected indicator *A. laidlawii* cultures[2], presumably as a result of some sort of stimulation[7]. In the present note we report the isolation of group 1 virus from a *M. pulmonis* strain, which is a murine pathogen, using a sensitive *A. laidlawii* indicator strain. Evidence obtained suggests that the virus originated from the test strain of *M. pulmonis.* Additionally, electron microscopic evidence has been obtained for the presence of classical bacteriophage-like particles in the same strain of *M. pulmonis.*

Materials and methods. M. pulmonis strains Ash (obtained from FAO/WHO collaborative center for animal mycoplasmas, Denmark) and Negroni (originally provided by Dr Hayflick) were examined for the presence of viruses. The Negroni strain had undergone several mouse passages in our hands, and the isolate tested was a reisolation from the lungs of an experimentally infected mouse, henceforth referred to as Negroni-52. To confirm the identity of the 2 strains at the time of screening for viruses, fluorescent antibody tests were performed on cloned cultures. Both Ash and Negroni-52 strains gave a 4+ reaction only with fluorescein conjugated antiserum specific for *M. pulmonis,* but not with antisera directed against *M. gallisepticum* or *M. pneumoniae.* Also, included in the present study were *A. laidlawii* strains JA1 and 1305/68 which originated from the laboratories of Dr Maniloff and Dr Gourlay and have been used extensively as indicators for mycoplasma viruses[2,3]. *M. pulmonis* strains were grown in culture using Hayflick's PPLO broth or agar[8] and *A. laidlawii* strains were propagated in tryptose broth or agar medium[4].

Results and discussion. Broth cultures of *M. pulmonis* strains Ash and Negroni-52 were allowed to grow exponentially for 24 h at 37 °C and then seeded on PPLO agar plates