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

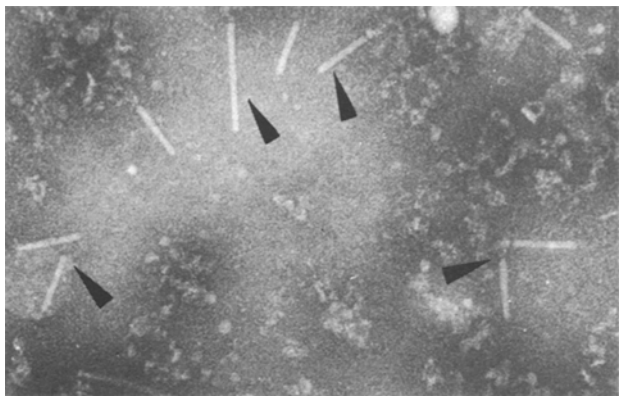

Fig. 1. Electron micrograph of washings from clear plaque showing rodshaped particles (arrows). Negatively stained with 2% phosphotungstic acid. × 128,000.

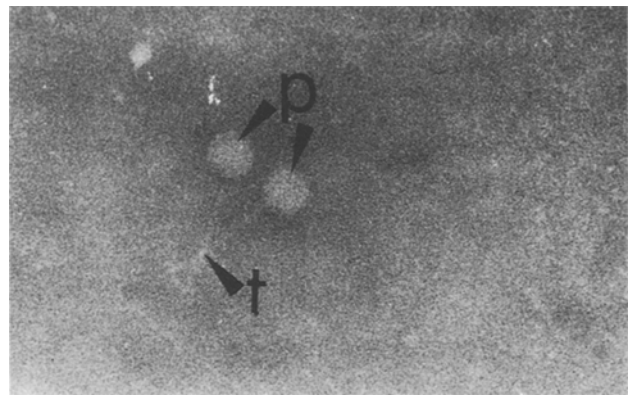

Fig. 2. Electron micrograph of filterate prepared from lawns of Negroni-52 showing phage-like particles with polyhedral head (p) and long tail (t). Negatively stained with 2% phosphotungstic acid. × 128,000.

(0.5 ml/plate). The plates were incubated at 37 °C till heavy growth was visible. Each plate was then layered with 3 ml of phosphate buffered saline (PBS; pH 7.3) for 18 h at room temperature, the washings harvested and filtered through Millipore filters with a pore size of 0.22 µm. The filterates were examined for the presence of viruses by placing a drop of each filterate on agar plates seeded 6 h earlier with strains Ash or Negroni-52 organisms as well as on lawns of *A. laidlawii* strain JA1. After incubation for 24 h at 37 °C, large turbid plaques were observed in lawns of *A. laidlawii* strain JA1 in which filterate from *M. pulmonis* strain Negroni-52 was spotted. Filterates prepared in identical manner from *M. pulmonis* strain Ash, *A. laidlawii* strains JA1 and 1305/68, and those obtained from 2 *M. pneumoniae* strains, showed no evidence of plaque-forming activity in lawns of JA1. PPLO broth inoculated with killed organisms of Negroni-52 also failed to induce plaques in lawns of JA1. Top surface of agar containing the turbid plaque was removed from 1 of the dishes and transferred in 1 ml of PBS and serial dilutions were spread evenly on lawns of *M. pulmonis* strains Ash or Negroni-52 and *A. laidlawii* strains JA1 and 1305/68. Interestingly, clear plaques (indicating lysis of organisms) instead of turbid were observed in lawns of *A. laidlawii* strains JA1 and 1305/68. The test samples had high plating efficency (titer of 10^9 PFU/ml).

Electron microscopic examination of negatively stained preparations from these clear plaques revealed that morphologically the plaque-forming agent belonged to mycoplasmavirus group 1, i.e. nonenveloped rod-shaped particles (figure 1). The reason for the observed variability in the appearance of plaques from turbid in primary isolation to clear in subsequent passages is unclear. Till now we have been unable to obtain any evidence of virus activity when filterates from Negroni-52 were tested on lawns prepared with Negroni-52 or Ashi strain organisms. On the contrary, in at least 4 independent experiments carried out at different time intervals, reproduciability of turbid plaque formation in lawns of JA1 following spotting with filterates from Negroni-52 was observed. Thus, our present observation is in marked contrast to the situation reported by Gourlay et al.[9] where a strain of *M. pulmonis* possessed plaque-forming activity in lawns of certain *M. pulmonis* strains and a strain of *M. gallisepticum*. Since the authors were unable to propagate the plaques serially and could not find virus particles in them, it was concluded that the observed plaques were not due to the presence of virus in *M. pulmonis*.

The principal question raised by our finding concerns the origin of rod-shaped virus particles which we observed, since it has been reported that *A. laidlawii* strains JA1 and 1305/68 are carriers of latent viruses and may show rarely spontaneous plaques[2,4]. This important question was approached in several ways. Active filterates obtained from lawns of Negroni-52 were kept at 80 °C for 30 min and then assayed for virus activity on lawns of JA1. Plaque forming activity could not be retrieved from heat-treated samples. This observation, however, by itself does not prove that the activity responsible for plaque formation is a virus, because some heat-labile substance (s) in filterates of Negroni-52 could induce latent virus from lawns of indicator *A. laidlawii* strain. Next, we carried out neutralization tests using specific antiserum against a known isolate of group 1 mycoplasmavirus which was prepared in rabbits according to described protocol[10]. To 0.5 ml of active filterate prepared from Negroni-52 was added 25 µl of undiluted antiserum. After incubation at 37 °C for 1 h, the reaction mixture was diluted 1:5 before assaying for plaque-forming activity in lawns of JA1. In 2 separate experiments, the antiserum could effectively neutralize the plaque-forming activity in filterates from Negroni-52, whereas normal rabbit serum included to control the experiments had no measurable effect. In limited attempts, we have been unable to demonstrate electron microscopically the presence of rod-shaped particles characterizing mycoplasmavirus group 1 in Negroni filterates. Surprisingly, however, we have observed in these filterates small numbers of classical bacteriophage-like particles with polyhederal head and long tails (figure 2). To our knowledge, such particles have not been observed so far in any mycoplasma or acholeplasma species. Any participation of these particles in the production of plaques observed in lawns of JA1 with Negroni-52 filterates remains unknown. Attempts are being made to grow and characterize these particles.

1 R.N. Gourlay, Nature (London) *225,* 1165 (1970).
2 R.N. Gourlay, CRC Critical Rev. Microbiol. *3,* 315 (1974).
3 J. Maniloff, J. Das and J.R. Christensen, Adv. Virus Res. *21,* 343 (1977).
4 A. Liss and J. Maniloff, Science *173,* 725 (1971).
5 R.J. Phillpotts, K.K.T. Patel and D.G. Edward, J. gen. Virol. *36,* 211 (1977).
6 R.N. Gourlay, Pathogenic Mycoplasmas, Ciba Foundation Symposium, p. 145. ASP, Amsterdam 1972.
7 W.A. Clyde, Ann. N.Y. Acad. Sci. *225,* 159 (1973).
8 L. Hayflick, Tex. Rep. Biol. Med. *23,* 285 (1965).
9 R.N. Gourlay, S.G. Wyld and D. Taylor-Robinson, Nature (London) *259,* 120 (1976).
10 W.A. Clyde, Colloques int. Cent. natn. Rech. Scient. *33,* 109 (1974).