medium were subjected to horizontal starch-gel electrophoresis. The gels were prepared as described by Bush et al.[4]. Optimum resolution for esterases bands was obtained using borate 0.3 M electrolyte buffer, pH 8.2, and a gel buffer pH 8.7 containing 0.076 M tris (hydroxymethyl) aminomethane and 0.005 M citric acid. Each gel was prepared using 46.5 g of hydrolyzed starch (Sigma Chemical Company) and 300 ml of the gel buffer. The homogenate was absorbed into a small piece of Whatman No. 3 filter paper about 4×6 mm in size which was inserted in an incision made 3 cm from the cathode of the gels. Horizontal starch gel electrophoresis and enzyme assays were carried out using methods and solutions similar to those described by Toledo F° and Magalhães[5]. To prevent heat denaturation and loss of the enzyme activity, the runs were performed at 4 °C.

Each gel was incubated at 37 °C in a staining solution containing naphtyl acetate as substrate, Fast Garnet GBC salt as the dye Coupler and phosphate buffer in the mounts[5]. The stained gels were fixed in a solution of methanol, acetic acid and distilled water in the proportion of 5:1:5.

Also, in one strain (A 5a w), the pattern of electrophoretic isozymes of cultures from 1 to 7 days old was carried out.

Results and discussion. The esterase patterns of the wild-type and mutant strains are presented on figures 1 and 2. They differ both in mycelia and culture medium. In relation to the mutants, large variations were found in mycelia (figure 1) and only a slight variation on culture medium (figure 2).

The esterase pattern of A 5a w strain from 1 to 7 days development has shown that esterases are not produced during the first day and 3 bands are obtained in the 7th day (figure 3).

Esterases variations were found in all 3 strains of *A. flavus* used. Although only 3 strains were used, the strain with no aflatoxin production presented only 2 bands and 3 and 4 bands were found in strains with aflatoxin production in mycelia and medium respectively. A larger number of strains should be tested in order to see if the pattern of esterases production is related to aflatoxin production. Also, in relation to esterases in culture medium, the strain with no aflatoxin production, presented no bands, the strain which contained aflatoxin just in the mycelia, presented one band and the strain which synthetizes aflatoxin both in mycelia and culture medium, presented 2 bands. Mutants derived from A 6e wild-type strain gave the same pattern of esterase bands in relation to the original strain exception of A 6e y. Other mutants differ in relation to the wild-type in bands patterns. Several reasons can be given to explain such differences, such as: a) different rates of growth; b) the influence of the auxotrophic or morphological markers on esterase production; c) other mutation induced during the mutagenic treatment for the obtention of the auxotrophs mutants, which also interfere with esterase production. More extensive investigations, should be carried out making use of crosses between such strains. It has been shown[6] that *A. flavus* is ammenable to genetic studies through the parasexual cycle. The use of strains with different patterns in esterase bands can be useful in a genetic study with *A. flavus*. Also, if a correlation between esterases and aflatoxin production does occur, this can be of help in the study and detection of aflatoxin producing strains.

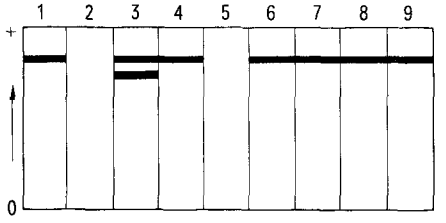

Fig. 2. Schematic representation of esterase zamograms from culture medium: 2, 3 and 8 are the culture medium of wild-type strains A5a, B2d and A6e, respectively. The 1, 4, 5, 6, 7 and 9 are the culture medium of mutants strains A6e y, A5a arg, A6e arg, A5a w, B2d lys and A6e pur, respectively.

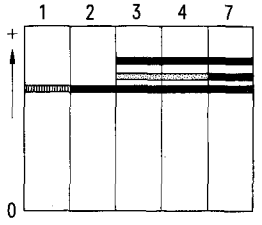

Fig. 3. Schematic representation of esterase zymograms from mycelia of A5a w-strain from 1 to 7 days development. Esterases are not produced during the first day and 3 bands are obtained in the 7th day.

4 E. L. Bush and R. N. Huettel, A manual of techniques – Project phase I. Int. Biol. Prog. *1972*.
5 S. Toledo F° and L. E. Magalhães, Ciênc. Cult. *25*, 1148 (1973).
6 K. E. Papa, Mycologia *65*, 1001 (1973).

Activity tests of alcohol dehydrogenases in wheat, rye and triticale[1]

F. Leibenguth[2]

Institut für Genetik der Universität des Saarlandes, D-6600 Saarbrücken (Federal Republic of Germany, BRD),
10 February 1977

Summary. The relative band staining intensities of ADH isoenzymes in wheat and triticale suggest alloploid genome interactions. Rye ADH is scarcely affected by anti-wheat-ADH. Despite the evolutionary divergence of their Adh genes, ADH monomers of wheat and rye form enzymatically active heterodimers in triticale.

A number of enzyme loci, found associated with homoeologous chromosomes in hexaploid wheat and diploid rye (*Secale cereale*), confirmed, at the biochemical level[3], the alloploid origin of wheat as well as of the wheat-rye alloploid triticale.

The data about the isoenzyme patterns of alcohol dehydrogenases of wheat, rye, and hexaploid triticale presented here, support the hypothesis of triplicate Adh genes in wheat and a 4th gene in rye[4]. However, minor differences in relative band staining intensities are revealed densitometrically, suggesting different activities of either the enzymes or the genes, which have hitherto been accepted to be equally expressed. Precipitation tests with antiserum against wheat ADHs should demonstrate

that the rye ADH is less closely related to wheat and triticale isoenzymes.

Methods. Whole mature grains of the winter wheat cultivars Altmärkischer, Mauerner Grannen, Perwi and Flamingo, and of hexaploid winter triticale[5], as well as of a rye cultivar of unknown origin, were homogenized with distilled water and the slurry used directly for horizontal starch gel electrophoresis. Electrophoretic and staining procedures, as well as densitometry, have already been described[6]. Rabbits were immunized with wheat ADH in incomplete Freund's adjuvant by 3 s.c. injections at weekly intervals[7]. The antigen was obtained by horizontal electrophoresis of wheat grain homogenates in cellogel blocks (Chemetron, Milano; 6×17 cm, 2,5 mm thick). After rendering visible by staining the block edge, the 3 zones of ADH activity were cut out and extracted by the Spuf ultrafilter syringe press (Chemetron).

Results and discussion. In Triticum aestivum (genome AABBDD) there are 3 homoeologous Adh genes Adh_A, Adh_B and Adh_D, coding for the subunits α, β and δ, respectively, which aggregate to form dimers[8] of the composition $\alpha\alpha$ (isoenzyme ADH-1), $\alpha\beta + \alpha\delta$ (ADH-2) and $\beta\beta + \delta\delta + \beta\delta$ (coincident electrophoretic mobility: ADH-3)[4]. Since all Adh genes are assumed to code for equal quantities of subunits, intensity distribution for the 3 ADH isoenzymes ADH-1,2,3 is expected to be 1:4:4.

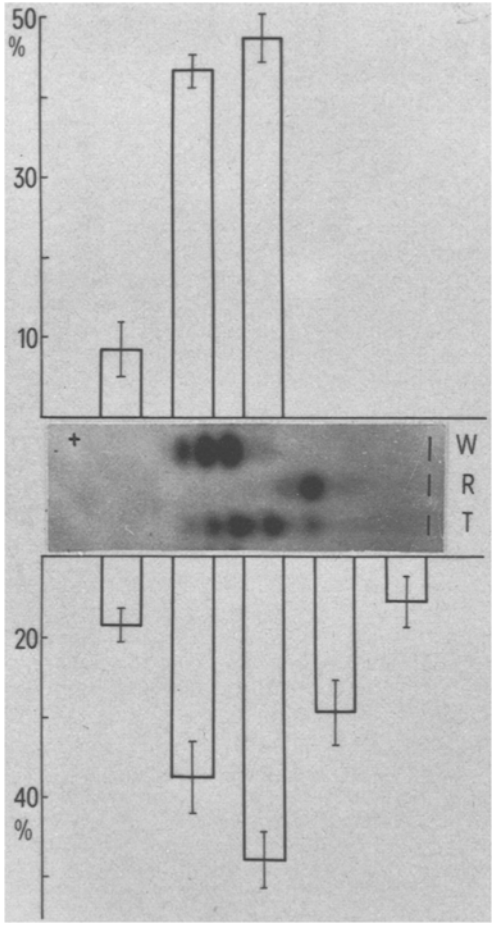

Fig. 1. ADH patterns of hexaploid wheat (W), rye (R) and triticale (T) from single mature grains and relative band staining intensities (%) for wheat ($\bar{X} \pm s$, n = 7, at the top) and triticale ADHs (n = 20, at the bottom). Exceptionally, the 3 fastest triticale bands show slower mobility than the corresponding wheat bands.

The data presented here do not fully agree with the intensity distribution expected: instead of 12:44:44%, relative staining intensities of 8.7:43.7:47.6% are found (figure 1) for ADHs of hexaploid winter wheat. A possible explanation could be a nonrandom dimerization of the subunits caused by unrelated structures. Results of the in vitro precipitation tests confirmed, for instance, great serological differences between proteins of T. speltoides and T. tauschii[9]. Further, the dimers may exhibit different enzymatic activity or unequal stability. Finally, unequal quantities of subunits may be synthesized in a manner that the sum of Adh_B and Adh_D activities exceeds the double of Adh_A gene activity. Modifier genes may alter Adh gene activities too, as is suggested by an intensity distribution of 4:36:60% in some summer wheat cultivars such as Tobari.

In hexaploid winter triticale [(AABBSS × AABBDD) × AABBSS, then selfpollination and selection[10]] the S genome is the source of a 4th gene, Adh_R, located on the 4R/7R translocation chromosome; it codes the ϱ subunit[3]. $\varrho\varrho$ homodimers compose the slowest moving band in the triticale pattern as well as the single ADH band of rye (figure 1). Intensity distribution in the 5-banded pattern of triticale should be 1:4:6:4:1 (= 6.25:25:37.5:25:6.25%) if equal quantities of subunits and equal dimer activities are presumed. But instead of the intensities expected, an asymmetrical distribution of 8.8:27.5:38:19.9:5.6% (from the anode) is found, which may be caused by any of the mechanisms already mentioned in the case of wheat pattern. But, if unequal quantities of subunits compete for random dimerization, the relative values for $\alpha:(\beta+\delta):\varrho$ of $2.9:(2\times2.4):2.3$ fit best with the intensity distribution found. This would imply an activity of $Adh_A > {}^1/_2(Adh_B + Adh_D)$, in contrast to hexaploid wheat where Adh_A is $< {}^1/_2(Adh_B + Adh_D)$. So, there appear to be different genome interactions in the alloploids wheat and triticale, whose nature remains unex-

1 Dedicated to Prof. Dr V. Schwartz, Tübingen, on the occasion of his 70th birthday.
2 I thank Miss Hiltrud Wambach and Miss Rita Lippert for technical assistance.
3 K. S. Tang and G. E. Hart, Genet. Res., Camb. *26*, 187 (1975).
4 G. E. Hart, Proc. nat. Acad. Sci. USA *66*, 1136 (1970).
5 Kindly supplied by F. von Lochow-Petkus, Bergen (Fed. Republic of Germany).
6 F. Leibenguth, Biochem. Genet. *14*, 299 (1966).
7 C. Wetter, Ann. Rev. Phytopath. *3*, 19 (1965).
8 G. E. Hart, Biochem. Genet. *3*, 617 (1969).
9 A. Aniol, Genet. Polon. *17*, 113 (1976).
10 K. D. Krolow, Berlin, personal communication.

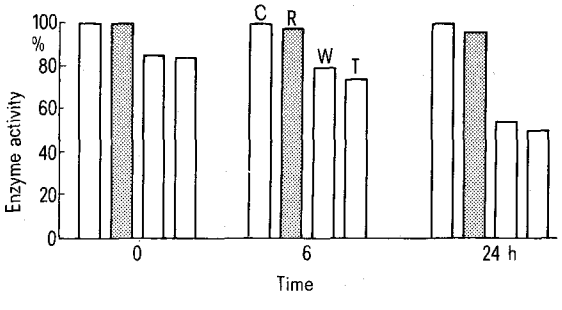

Fig. 2. Summarized remaining ADH activities of rye (R), wheat (W) and triticale (T) isoenzymes immediately (0 h), 6 h and 24 h after mixing single grain extracts with antiserum against wheat ADHs. Centrifugation at 3000 rpm for 30 min was followed by electrophoretic separation. Activities are given in percent of controls (C: aliquots of the same extracts incubated with control serum for 0, 6, 24 h, respectively).

plained. Also, cytoplasmic interference cannot be excluded since differences in albumin, globulin, gliadin and glutenin contents have been found to be the consequence of the source of cytoplasma in reciprocal triticale populations[11].

Among the 5 isoenzymes in triticale, besides $\varrho\varrho$ homodimers with the slowest mobility, there are 3 heterodimer types containing ϱ subunits: $\alpha\varrho$, $\beta\varrho$ and $\delta\varrho$. This ability to aggregate would first of all presuppose similar structures due to related genes on at least partially homoeologous chromosomes (beta-arm of chromosome 4 in wheat and chromosome 4R/7R in rye[3]). Minor evolutionary differences in the ADHs of wheat and rye should only be expressed immunologically. The results are consistent with the expectation. In figure 2 summarized recordings of all triticale bands (T), wheat bands (W) and the single rye band (R), incubated with antiserum against wheat-ADHs, are given in percent of the activities found in triticale, wheat and rye extracts after having been incubated with control serum (C). ADH activity of rye is scarcely affected by anti-wheat-ADH. Therefore, it seems to be proved that ADH subunits of wheat and rye can aggregate to form enzymatically active heterodimers in triticale in spite of the evolutionary divergence of their genes.

11 S. L. K. Hsam and E. N. Larter, Can. J. Genet. Cytol. *16*, 529 (1974).

Interorder transfer of mycoplasmalike microorganisms between *Drosophila paulistorum* and *Ephestia kühniella*. II. Numbers of MLO and sterility

F. J. Gottlieb, R. Goitein[1], L. Ehrman[2] and S. McAvoy[2]

Department of Life Sciences, University of Pittsburgh, Pittsburgh (Pennsylvania 15260, USA), and Division of Natural Sciences, State University of New York, Purchase (New York 10577, USA), 21 March 1977

Summary. A successful attempt was made to culture the mycoplasmalike microorganism causing semispecific hybrid male sterility in *Drosophila paulistorum* utilizing *Ephestia kühniella* as the intermediate host. Data gleaned from this passage indicates that the induction of sterility depends upon the quality not the quantity of infectious intracellular symbionts.

Genetic relationships between a host and its well established endosymbiont are difficult to study under normal conditions. Occasionally controls break down and the symbiont becomes a pathogen. When this happens we are able to gain insight into the modes of action of the symbiont in its 2 expressions. The involvement of mycoplasmalike organisms (MLO) in the male sterility of *Drosophila paulistorum* intersemispecific hybrids is known[3]. Each of the 6 semispecies possesses an MLO strain, possibly unique, which normally has no deleterious effect on its own host. The symbiont is present in the gonads of both sexes and is passed from generation to generation through the egg cytoplasm[4]. Male sterility results from the introduction of a strain of MLO into a host with a foreign genetic background, either by hybridization or by injection of testis-extracts[5,6]. Genetic incompatibility was thought to lead to unrestrained growth of the symbiont. Thus the sterility was assumed to be the direct result of rapid proliferation of MLO within sperm cysts. For this reason, and because MLO transfer is through the egg, it was concluded that the gonads were not only the target tissue in hybrid males but also the primary tissue of localization in both sexes[3]. Drosophila paulistorum MLO can be transferred to and proliferate in Ephestia kühniella Zeller (the Meditarranian meal moth)[7]. In this new host the MLO are pathogenic and can be further passaged in Ephestia or passed back to Drosophila without loss of host-strain recognition specificity[8]. The pathogenicity in *Ephestia*, as measured by the time and mode of dying of extract recipients, is dose dependent. It was expected that testis-extracts of pure strain Drosophlia would contain fewer MLO than those of hybrids because of the normal control of symbiont proliferation in its own host strain. This was not the case. Testis-extracts of Mesitas (M, Andean semispecific) strain or of Santa Marta (SM, Transitional semispecific) strain were as potent as those of the 2 reciprocal male hybrids: (SM♀ × M♂) and (M♀ × SM♂).

Heads of hybrids also proved to be highly pathogenic with an extract of 1 head/ml being equivalent to approximately 2–4 testes/ml. The assumption that MLO are mainly localized in the gonads is no longer tenable. The presence of MLO in heads provides an easy way to estimate the relative distribution and concentration of MLO in the pure strains of *Drosophila* as compared with one another and with hybrids. Extracts of heads of a representative pure strain from each of the 6 semispecies were injected into Ephestia larvae. All extracts were at least as higly pathogenic as the hybrid extracts. There appear to be as many MLO present in pure strain heads as in hybrid heads. Indeed, 2 of the extracts (Brazil, Amazonian semispecies, and Llanos, Interior semispecies) were more virulent than the hybrid extracts. We interpret this to indicate that larger numbers of MLO are present in these 2 pure strains, although we cannot as yet rule out the possibility that Brazil and Llanos MLO are more virulent than those of the other 4 semispecies (including Orinocan and Centroamerican ones).

Neither of these latter observations would be expected under the earlier assumptions. These results therefore raise serious questions about the relationship between the number of MLO and hybrid male sterility in *Drosophila paulistorum*. It can no longer be assumed that sterility in hybrids is due solely to the presence of larger numbers of MLO than is normal. Sterility is not just the product of mechanical problems caused by the massive numbers of MLO. Rather, semispecific male hybrid sterility must also involve both host-symbiont recognition factors and a tissue specific response in the testes to the presence of the 'wrong' MLO.

1 On leave from the Department of Genetics, The Hebrew University, Jerusalem (Israel).
2 Division of Natural Sciences, State University of New York, Purchase (NY 10577, USA). Reprint requests to L.E.
3 L. Ehrman and R. P. Kernaghan, in: Pathogenic Mycoplasmas, p. 227. Associated Scientific Publishers, Amsterdam 1972.
4 L. Ehrman and S. Daniels, J. Invert. Path. *24*, 14 (1974).
5 L. Ehrman, H. Levene and L. Ramos, Aust. J. biol. Sci., in press (1977).
6 D. L. Williamson and L. Ehrman, Genetics *55*, 131 (1967).
7 F. J. Gottlieb and L. Ehrman, Genetics *83*, 27 (1976).
8 F. J. Gottlieb, R. Goitein, L. Ehrman and B. Inocencio, J. Invert. Path. *30*, 140 (1977).