

Zymogram for phosphoglucosaminidase from skeletal muscle homogenates of 5 rainbow trout showing the (bb) homozygote, (bc) heterozygote (3), and (cc) homozygote.

Finally, a very faint non-variable band, designated (d) migrated still farther to the anode. Thus all specimens were identical for (a) and (d). Because of the wide separation of (a) and (d) with the insertion of (bc) bands it is assumed that (a) and (d) represent products of 2 different homozygous loci. Consequently, a total of 3 loci are postulated for phosphoglucosaminidase in rainbow trout.

The possibility exists that these 3 loci are homologous to the PGM<sub>1</sub>, PGM<sub>2</sub> and PGM<sub>3</sub> loci in man, an idea supported by the similar nature of the fastest band. HOPKINSON and HARRIS<sup>5</sup> description of the PGM<sub>3</sub> banding as barely detectable in human muscle extracts would apply to the trout muscle zymograms. In the present study, this band has not been studied in extracts of other tissues which might show it more clearly. Despite the apparent similarity of trout and human phosphoglucosaminidase in being under the control of 3 loci, the total number of isozymes in man is about twice the number in trout.

**Zusammenfassung.** Es wird aufgrund von Stärke-Gel-Zymogrammanalysen der Phosphoglucosaminidase in 72 Forellen (*Salmo gairdneri*) die Existenz eines polymorphen und zweier nicht variierender Gene ermittelt. Der Polymorphismus beruht auf 2 Allelen mit übereinstimmenden Frequenzen im Hardy-Weinberg-Gleichgewicht.

F. L. ROBERTS<sup>8</sup>, J. F. WOHNUS<sup>9</sup>  
and S. OHNO

Department of Biology, City of Hope Medical Center,  
Duarte (California 91010, USA), 13 June 1969

<sup>8</sup> Present address: Dept. of Zoology, University of Maine, Orono (Maine 04473, USA).

<sup>9</sup> Present address: Dept. of Biology, Bennington College, Bennington (Vermont 05201, USA).

### Purothionins in *Aegilops-Triticum* spp.

Purothionin was first obtained from the endosperm of hexaploid wheat (*Triticum aestivum* L.) and crystallized by BALLS et al.<sup>1</sup> This high sulphur protein moiety of a proteolipid has bactericidal and fungicidal activity<sup>2</sup>. Recent work<sup>3-6</sup> has established that the crystallized material is a mixture of approximately equal amounts of 2 forms: purothionins  $\alpha$  and  $\beta$ . Molecular weight determinations, amino acid composition and other properties indicate that the 2 forms are very closely related<sup>5</sup>. We have found that both the allohexaploid *T. aestivum* L. (genomes ABD) and the allotetraploid *T. durum* Desf. (genomes AB) synthesize the  $\alpha$  and  $\beta$  forms<sup>6</sup>. This note is to report some phylogenetic implications of purothionins.

The diploid species *T. monococcum* (A) synthesizes only the  $\beta$  form, suggesting that the A genome of *T. durum* is responsible for the genetic control of  $\beta$  form synthesis and the B genome for that of the  $\alpha$  form. Analysis of the potential B genome donor, namely, the diploid species *Aegilops speltoides* (S = B), which does synthesize the  $\alpha$  form, substantiates the hypothesis. This indicates that  $\alpha$  and  $\beta$  purothionins are the result of divergent evolution at the diploid level and have come to coexist by the convergent process of allopolyploid formation.

We have further investigated the occurrence of  $\alpha$  and  $\beta$  forms in the remaining species of the *Aegilops-Triticum*

group. A micromethod was used because only small amounts of material were available. The samples, 200 to 400 mg of ground kernels were macerated for 2 h with twice the amount (v/w) of petroleum ether (b.p. 35–60°C). The supernatant was transferred with the aid of a capillary tube to a piece of paper (Whatman No. 3, 2 × 8 mm) and evaporated in the process. Lipid was dissociated from purothionin by treating the paper with 1N HCl in ethanol: petroleum ether (3:1) with the aid of a capillary and then was extracted by immersion in petroleum ether for 1 h. The dried paper was wet with buffer and the purothionins fractionated by starch-gel electrophoresis.

The results are summarized in the Figure. The occurrence of the previously described<sup>7</sup> linoleate (L) and palmitate-

<sup>1</sup> A. K. BALLS, W. S. HALE and T. H. HARRIS, Cereal Chem. 19, 279 (1942).

<sup>2</sup> L. S. STUART and T. H. HARRIS, Cereal Chem. 19, 288 (1942).

<sup>3</sup> C. C. NIMMO, M. T. O'SULLIVAN and J. E. BERNARDIN, Cereal Chem. 45, 28 (1968).

<sup>4</sup> N. FISHER, D. G. REDMAN and G. A. R. ELTON, Cereal Chem. 45, 48 (1968).

<sup>5</sup> D. G. REDMAN and N. FISHER, J. Sci. Fd Agric. 19, 651 (1968).

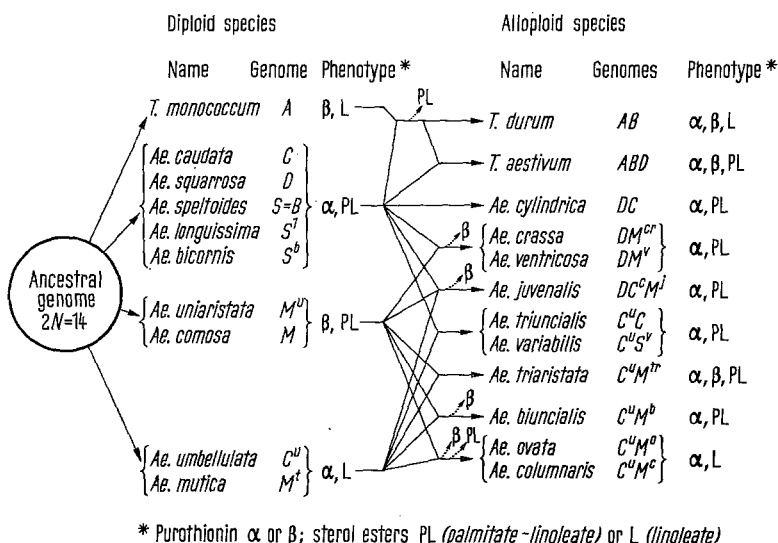
<sup>6</sup> F. GARCÍA-OLMEDO, I. SOTELO and R. GARCÍA-FAURE, An. Inst. nac. Invest. agron. 17, 433 (1968).

<sup>7</sup> F. GARCÍA-OLMEDO, Nature 220, 1144 (1968).

linoleate (PL) systems for  $\beta$ -sitosterol esters synthesis has been also recorded.

In diploid species, all 4 possible combinations of purothionin and sterol esters phenotypes are present. This points to heterogeneity within the  $\alpha$  and  $\beta$  puro-

genomes and not the so-called pivotal genomes. This is consistent with the cytogenetical observation that pivotal genomes are completely homologous with known diploids, while the additional genomes are extensively modified and only partially homologous with diploid analyzers.



Cytogenetical relationships in *Aegilops-Triticum* species and distribution of purothionins and  $\beta$ -sitosterol esters systems.

thionins, but further characterization of purothionins from these species must wait until enough material is grown.

In allopolyploid species where the parental genomes have genetic information for electrophoretically different purothionins, the coexistence of the  $\alpha$  and  $\beta$  forms is not always observed. A similar observation can be made with the  $\beta$ -sitosterol ester systems. It seems that duplicate genetic activity for similar systems represents an adaptive advantage but not necessarily a physiological one. Consequently redundant systems might be lost in the course of evolution following allopolyploid formation. It is to be noted that all observed losses affect the additional

*Resumen.* En *Triticum durum* Desf. (genomios AB), el genotipo A controla la síntesis de purotionina  $\beta$  y el genotipo B la de purotionina  $\alpha$ . Las especies diploides del grupo *Aegilops-Triticum* sintetizan  $\alpha$  ó  $\beta$ , pero no las dos. En numerosos alopoloides de este grupo se observa la pérdida de la actividad sintética para la purotionina correspondiente a uno de los genotipos.

P. CARBONERO and F. GARCÍA-OLMEDO

Instituto Nacional de Investigaciones Agronómicas,  
Madrid-3 (Spain), 2 June 1969

## Complement with 38 Chromosomes in Two South American Populations of *Rattus rattus*

Since the appearance of technical improvements for chromosomal study, most papers dealing with the complement of the rat have been devoted to *Rattus norvegicus*<sup>1-12</sup>. On the other hand, only the recent report from YOSIDA<sup>13</sup> has analyzed the number and chromosomal morphology in *Rattus rattus*. From those papers it can be concluded that, although both varieties of rats exhibit chromosomal polymorphism, 42 seems to be the diploid chromosome number for *R. norvegicus* and *R. rattus*.

The present paper deals with 2 populations of *R. rattus*, having chromosome morphology and a diploid number different from those described in *R. norvegicus* and *R. rattus*.

**Material and methods.** A total of 16 animals (3 ♂ and 13 ♀) collected in Punta Lara, Province of Buenos Aires (Argentina) and the environs of São Leopoldo, State of Rio Grande do Sul (Brasil) were studied.

The animals were injected with 1 ml of a 0.04% colchicine solution and 3 h later were sacrificed. Chromosome spreads from bone marrow, spleen and testes were prepared as described elsewhere<sup>10-12</sup>. In each animal no fewer than 10 metaphases from each one of the tissues processed were analyzed.

**Results and discussion.** The 16 specimens of *R. rattus* studied had a diploid number of 38 chromosomes. The analysis of the complement showed the existence of 9 pairs of metacentric, 3 pairs of subterminal, and 7 pairs of acrocentric chromosomes. Although X-chromosomes were difficult to identify with accuracy, it could be determined that they were second or third in size among the acrocentric elements. The Y-chromosome was the smallest acrocentric chromosome of the set (Figure 1).

<sup>1</sup> S. MAKINO and T. C. HSU, *Cytologia* 19, 23 (1954).

<sup>2</sup> J. H. TJIO and A. LEVAN, *Hereditas* 42, 218 (1956).

<sup>3</sup> P. H. FITZGERALD, *Expl. Cell Res.* 25, 191 (1961).

<sup>4</sup> P. C. NOWELL, S. FERRY and D. H. HUNGERFORD, *J. natn. Cancer Inst.* 30, 687 (1963).

<sup>5</sup> M. VRBA, *Folia biol.* 10, 75 (1964).

<sup>6</sup> G. DOWD, K. DUNN and W. C. MOLONEY, *Blood* 23, 564 (1964).

<sup>7</sup> W. O. RIEKE and M. R. SCHWARTZ, *Anat. Rec.* 150, 383 (1964).

<sup>8</sup> T. H. YOSIDA and K. AMANO, *Chromosoma* 16, 658 (1965).

<sup>9</sup> N. TAKAGI and S. MAKINO, *Chromosoma* 18, 359 (1966).

<sup>10</sup> N. O. BIANCHI and O. MOLINA, *Naturwissenschaften* 53, 590 (1966).

<sup>11</sup> N. O. BIANCHI and O. MOLINA, *J. Hered.* 57, 231 (1966).

<sup>12</sup> N. O. BIANCHI, *Cytologia* 31, 276 (1966).

<sup>13</sup> T. H. YOSIDA, A. NAKAMURA and T. FUKUYAMA, *Chromosoma* 16, 70 (1965).