The adaptive role of the karyotype has been demonstrated in rodents as *Thomomys*[24] and *Spalax*[25]. Bickham and Baker[26], stressing this karyotypical adaptiveness, proposed the canalization model of chromosomal evolution in which 'the karyotype contributes significantly to the fitness of the individual, and that for a given set of biological parameters faced by an evolving lineage, there is an optimal karyotype. Thus, those lineages which have reached that optimum adaptive karyotype would show chromosomal stability; such is the case with these species of *Akodon* and, as they point out, also the situation of *Lasiurus* (i.e. virtually identical karyotypes and morphological distinctness). The 2 subspecies of *A. olivaceus* illustrate chromosomal adaptiveness since animals trapped in 4 localities covering 1400 km latitudinally showed an unvarying karyotype in spite of strong biotic and abiotic differences. In addition, consistent stability of banding patterns has been retained in species belonging to the different subgenera *Abrothrix* and *Akodon* and thus reveals a close genetic relationship between them, which strongly favors the interpretation of descent from a single common ancestor.

Based on morphological grounds, the systematic relationships of these species are clear. It follows that morphological diversification was not accompanied by a significant amount of chromosomal disruption which would markedly alter banding patterns. The biological significance of these morphological differences rests upon the maintenance of distrinctness when the species occur together. Consequently, areas of sympatry provide a test of species status supporting previous assignments.

1 The research was partially supported by Proyecto de Investigación I-78-86, Dirección de Investigación, Universidad Austral de Chile and Programa Especial de Citogenética, U. de Chile sede Norte, Santiago, Chile.
2 Present address: Department of Biology, Box 3AF, New Mexico State University, Las Cruces (New Mexico 88003, USA).
3 Bianchi, N.O., Reig, O.A., Molina, O.J., and Dulout, F.N., Evolution *25* (1971) 724.
4 Reig, O.A., Olivo, N., and Kiblisky, P., Cytogenetics *10* (1971) 99.
5 Yonenaga, Y., Cytogenetics *11* (1972) 488.
6 Yonenaga, Y., Kasahara, S., Almeida, E.J.C., and Peracchi, A.L., Cytogenet. Cell Genet. *15* (1975) 388.
7 Osgood, W., Publ. Field Mus. nat. Hist. (Zool. Series) *30* (1943) 1.
8 Ford, C.E., and Hamerton, J.L., Stain Technol. *31* (1956) 247.
9 Seabright, M., Lancet *2* (1971) 971.
10 Schnedl, W., Nature New Biol. *233* (1971) 93.
11 Spotorno, O.A., Mammal. Chrom. Newsl. *17* (1976) 13.
12 Miller, D.A., Science *198* (1977) 1116.
13 Arnason, U., Hereditas *77* (1974) 1.
14 Arnason, U., Hereditas *76* (1974) 179.
15 Wurster-Hill, D.H., and Gray, C.W., Cytogenet. Cell Gent. *15* (1975) 306.
16 Harada, M., and Yosida, T.H., Chromosoma *65* (1978) 283.
17 Mascarello, J.T., Warner, J.W., and Baker, R.J., J. Mammal. *55* (1977) 881.
18 Pathak, S., Hsu, T.C., and Shirley, L., Chromosoma *42* (1973) 215.
19 Yosida, T.H., and Sagai, T., Chromosoma *41* (1973) 93.
20 Hsu, T.C., Markvong, A., and Marshall, J.T., Cytogenet. Cell Genet. *20* (1978) 304.
21 Bianchi, N.O., Vidal-Rioja, L., and Bianchi, M.S., Cytologia *41* (1976) 139.
22 Pathak, S., and Wurster-Hill, D.M., Cytogenet. Cell Genet. *18* (1976) 245.
23 Bianchi, N.O., Vidal-Rioja, L., and Bianchi, M.S., Can. J. Genet. Cytol. *15* (1973) 855.
24 Patton, J.L., Chromosoma *31* (1970) 41.
25 Nevo, E., and Shkolnik, A., Experientia *30* (1974) 724.
26 Bickham, J.W., and Baker, R.J., Bull. Carnegie Mus. nat. Hist. *13* (1979) 70.

Dystrophic mutation (dy^{2J}) affecting regulation of lactate dehydrogenase (LDH) and pyruvate kinase (PK) in C57BL/6J mice[1]

Shiva M. Singh, Caroline H. Wang and Ann Phillips

Department of Zoology, University of Western Ontario, London (Ontario, Canada N6A 5B7), 7 June 1982

Summary. The genotype difference (dystrophic vs nondystrophic) in the LDH isozymes is observed in kidney. These differences are evident only at birth and at early developmental stages (before the expression of dystrophic symptoms). The tissue specific genotype differences for PK are limited to the thigh muscle (M form) and heart (L form), after the onset of the condition. These differences may reflect the pleiotropic effect of the dy^{2J} locus during the temporal regulation of these and other enzymes implicated in muscular dystrophy (MD).

The phenomenon of enzyme heterogeneity is a general aspect of metabolic structure and function of cells. In general it is characterized by tissue specificity and developmental profiles. The understanding of this general pattern of heterogeneity is particularly desirable for evaluating the role of enzyme(s) in an organ specific genetic defect. Animal models of muscular dystrophy (MD), which is genetically heterogeneous in humans, are a muscle specific genetic defect. In mice the dy^{2J} dystrophic mutation is expressed as the degeneration of thigh muscles, around 3 weeks after birth[2]. Although several enzymes, including lactate dehydrogenase (LDH, E.C. 1.1.1.27), and pyruvate kinase (PK, E.C. 2.7.1.40) have been suggested to be involved in MD[3–6], the basic defect and how it affects specific tissues is not understood[6].

LDH and PK are key enzymes of carbohydrate metabolism (L. lactate $\xrightarrow{\text{LDH}}$ pyruvate $\xrightarrow{\text{PK}}$ P enol pyruvate) with a wide distribution. The genetics, subunit structure and biochemistry of these enzymes are partly understood. Although attempts to utilize these enzymes in Duchenne MD have yielded encouraging results[3], their role in producing dystrophy remains unclear. Most enzyme studies in MD have concentrated on levels of enzyme activity and little attempt has been made in understanding the ontogenic pattern and tissue distribution of different subunits. Such studies are useful in providing insight into the genetic regulation of enzymes with altered activity, which may in fact form the basis for MD and other inborn errors of metabolism for which no basic enzyme defect has been demonstrated. This report deals with the tissue distribution

and developmental profiles of LDH and PK in the 3 genotypes of MD mutation dy^{2J} in C57BL/6J mice. Our results suggest the involvement of tissue specific regulatory mechanism(s) associated with LDH and PK during the expression of the dystrophic phenotypes.

Materials and methods. The source of genetic stocks, breeding system used to yield +/+, $+/dy^{2J}$ and dy^{2J}/dy^{2J} genotypes, tissue preparation, and electrophoretic procedures have been previously described[2]. For LDH, the gel (0.006 M NaH_2PO_4, 0.004 M citric acid) and electrode (0.245 M NaH_2PO_4, 0.15 M citric acid) buffers were at pH 5.9 with agar or electrostarch as the electrophoretic medium. Following electrophoresis the gel was incubated (37 °C) in 15 ml Tris-HCl (pH 8.5), 135 ml H_2O, 35 mg L-lactic acid, 20 mg NAD, 10 mg nitroblue tetrazolium (NBT) with 1 ml 10% phenazine methosulfate (PMS) added after 30 min. The gel and electrode buffers for PK included 0.01 M Tris, 0.01 M $MgSO_4$, 0.01 M KCl and citric acid at pH 7.5. The PK gels were stained using a staining mixture with 15 ml 2% boiling agar overlaid on the gel and incubated at 37 °C. The staining mixture consisted of 20 mg glucose, 20 units G.6.PDH, 4 mg NADP, 3 mg PMS and 2 mg MTT in 15 ml 0.1 M. Tris HCl, pH 8.0. Bands for both enzymes were visible within 30–40 min. A Gelman DCD-16 densitometer was used to evaluate relative proportions of isozyme bands on agar gels.

Results and discussion. The dy^{2J} mice mutation in the C57BL/6J background results in a milder form of dystrophy[7]. It is possible to use 9–14-week-old dy^{2J}/dy^{2J}, dystrophic genotypes in breeding to produce the young of the 3 dy^{2J} genotypes (+/+, $+/dy^{2J}$ and dy^{2J}/dy^{2J}) from appropriate crosses. Homozygote dy^{2J} genotypes express dystrophy around day 21, following which these animals lag behind the normals in their growth rate[2].

The genetic determinants[8,9], regulatory mechanism[10] and ontogenic pattern[11] of LDH are well understood in mice. The mechanism of the involvement of LDH in muscular dystrophy, however, remains to be clarified. Figure 1 shows the electrophoretic LDH isozyme pattern for the 3 genotypes in 6 tissues. Pattern for lung, spleen, heart, upper and lower thigh muscles did not change during development and they follow the representative tissue specific patterns as shown in figure 1A for adults. Such tissue differences may represent tissue specific differential rate of synthesis of A/B subunits and/or their differential degradation. The pattern in the 3 kidney genotypes however was age dependent. In the +/+ and $+/dy^{2J}$ genotypes, isozymes with the B subunit predominated with complete absence of A_4 isozyme reaction at all stages of development. In the dy^{2J}/dy^{2J} genotypes, however, the LDH pattern at birth was different from the pattern in animals old enough to express dystrophy. At birth the A subunit predominates with trace levels of B_4. This pattern gradually reverses with age and within 3 weeks, the dystrophic genotype is similar to the nondystrophic genotypes. At birth the A_4 and A_{3B1} isozymes represent approximately 50% of LDH activity in dy^{2J}/dy^{2J}, while in +/+ and $+/dy^{2J}$ the B_4 and B_3A_1 account for over 50% of this activity. By day 21, however, there are no genotype related LDH isozyme differences in kidney. It is interesting to note that these age dependent genotype differences correspond to the expression of dystrophy in dy^{2J}/dy^{2J} animals.

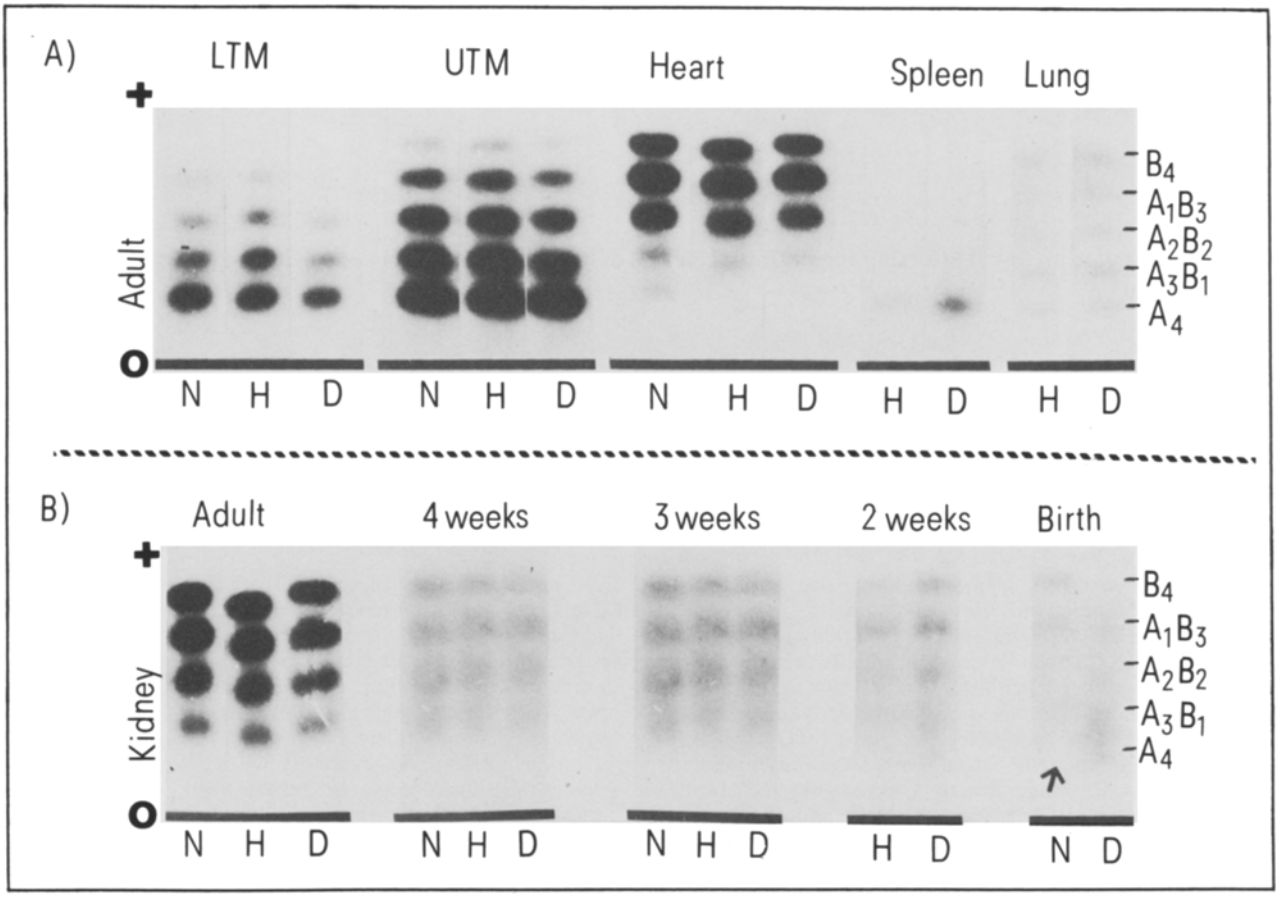

Figure 1. Electrophoretic pattern of LDH in 6 adult tissues (A) and at 5 developmental stages in kidney (B) of the 3 dy^{2J} genotypes (N = +/+, H = $+/dy^{2J}$ and D = dy^{2J}/dy^{2J}; UTM and LTM refer to upper and lower thigh muscles respectively).

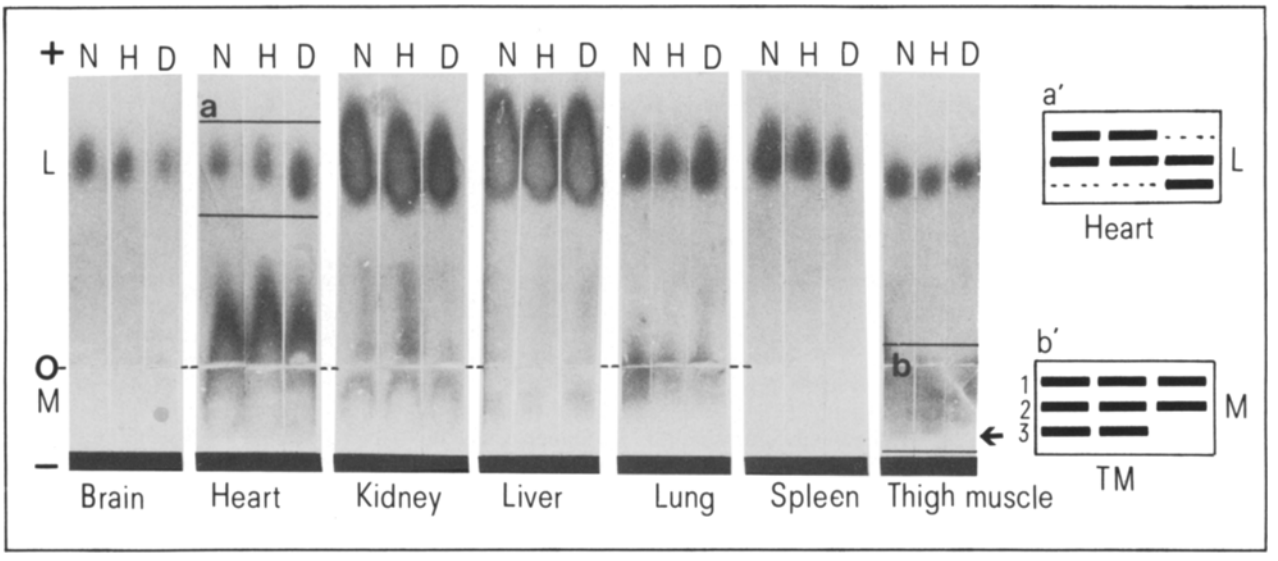

Figure 2. Electrophoretic pattern of PK in 7 adult tissues of the 3 dy^{2J} genotypes (N = +/+, H = +/dy^{2J}, D = dy^{2J}/dy^{2J}; L. liver form; M, muscle form; inserts a′ and b′ show diagrammatic representation of L and M isozymes in heart and thigh muscle respectively in the 3 genotypes).

PK is known to exist in 2 isozyme forms (L and M)[12] and their kinetic and immunological properties have been described[13]. The developmental profiles of a number of enzymes including PK in C57BL/6J mice have been evaluated[14]. An elevated PK activity has been implicated in different forms of MD[15], with some success in the detection of heterozygotes[16], when used in combination with other enzymes[2]. The underlying mechanism reflecting PK alterations in MD, however, are not understood. Figure 2 shows the electrophoretic pattern of PK in 6 adult tissues of the 3 dy^{2J} genotypes. The tissue specific PK patterns with the absence of the M isozyme in brain and spleen can bee seen. No genotype differences were observed in isozyme patterns of brain, kidney, liver, lung and spleen at different developmental stages. However, differences between dystrophic and nondystrophic genotypes were evident in heart and thigh muscle after the expression of dystrophy. In heart, the L isozyme is predominantly represented by a slower migrating subunit in dystrophic animals (insert a′ fig. 2). Furthermore, the enzyme activity associated with this form of isozyme is approximately 40% higher in dystrophics as compared to the nondystrophic genotypes. The L form of PK is known to be allosteric in nature and at least in the liver it has been shown to be under hormonal and dietary control[17]. The observed genotype differences for this isozyme, therefore, should be interpreted with caution. Figure 2 (with insert b′) also shows the genotype difference in the thigh muscle for the M type PK isozyme. The three forms of this isozyme (M_1, M_2 and M_3) are different in electrophoretic mobility with similar immunologic and kinetic properties[18] and the genotype differences are reflected in the absence of the M_3 band in the dy^{2J}/dy^{2J} genotypes.

It is evident that the expressions of LDH and PK are not only tissue specific, but are affected by the genotype at the dy^{2J} locus in specific tissues as well. Furthermore, the pattern may depend on the ontogeny, particularly when compared before and after the expression of the dystrophy caused by the dy^{2J} locus. The genotype differences for LDH in kidney associated with age may represent transcriptional, translational or post translational regulation, as this difference is observed in the relative proportions of A and B subunits. The LDH intersubunit interaction, which is known to be influenced by pH[8] and other epigenetic factors[19], may in fact represent the regulatory mechanism associated with these observations. Observed genotype dependent alterations in heart for the L form, and thigh muscle for the M form of PK indicate the effect of the dy^{2J} locus on the expression of these isozymes. It may be pointed out that neither LDH nor PK represent the basic defect associated with MD and these alterations should be viewed as the pleiotropic effects of the dy^{2J} mutation during the temporal regulation[20] of these enzymes.

1 This research was financed by a Natural Sciences and Engineering Research Council Canada grant to S.M.S.
2 Singh, S.M., Phillips, A., and Wang, C.H., Experientia *38* (1982) 917.
3 Percy, M.E., Chang, L., Murphy, E.G., Oss, I., Pitt, M.A., and Thompson, M.W., 11th int. Congr. Biochem. Toronto 1979, p. 654.
4 Emery, A.E.H., Nature, Lond. *201* (1964) 1044.
5 Ebashi, S., Joyakura, Y., Momoi, H., and Sugita, H., J. Biochem. *46* (1959) 103.
6 Strickland, K.P., Hudson, A.J., and Jhakar, J.H., Ann. N.Y. Acad. Sci. *317* (1979) 187.
7 Sidman, R.L., Cowan, J.S., and Eicher, E.M., Ann. N.Y. Acad. Sci. *317* (1979) 497.
8 Shakley, J.B., in: Isozymes, p. 1. Ed. C.L. Markert. Academic Press, New York 1975.
9 Nance, W.E., Claflin, A., and Smithies, O., Science *142* (1963) 1075.
10 Nadal-Ginard, B., J. biol. Chem. *253* (1978) 170.
11 Markert, C.L., and Ursprung, H., Devl Biol. *5* (1962) 363.
12 Tanaka, T., Harano, Y., Morimura, H., and Mori, R., Biochem. biophys. Res. Commun. *21* (1965) 55.
13 Tanaka, T., Harano, Y., Sne, F., and Morimura, H., J. Biochem. *67* (1967) 71.
14 Burkhart, J.G., and Mohrenweiser, H.W., Enzyme *23* (1978) 246.
15 Harano, Y., Adair, R., Vignos, Jr, P.J., Miller, M., and Korval, J., Metabolism *22* (1973) 493.
16 Alberts, M.C., and Samaha, F.J., Neurology *24* (1974) 462.
17 Harano, Y., Osaka Igaku Zashi *18* (1966) 593.
18 Tanaka, T., Imamura, K., Ann, T., and Taniuchi, K., in: 8th int. Congr. Biochem. Switzerland 1970, p. 227.
19 Yamamura, K., Ogita, Z., and Markert, C.L., J. exp. Zool. *208* (1979) 271.
20 Paigen, K., A. Rev. Genet. *13* (1979) 417.