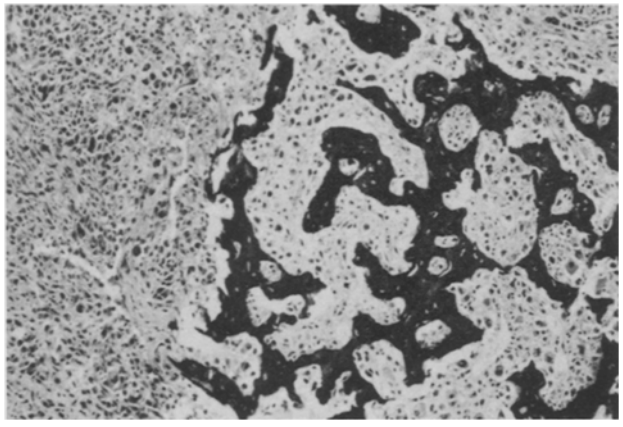

Figure 2. Histology of the tumor obtained by inoculating BMA1 cells into nude mice (six weeks after inoculation). Note the bone formation among tumor tissues (hematoxylin and eosin × 160).

and others, and it contains cells expressing adenoviral early antigens, which are known to immortalize and transform the cultured cells[10]. In the present study tumorigenicity of this cell line was established. Adenovirus DNA was demonstrated in DNA of the developed tumor (data not shown). Chondro-osteogenic gene activation is induced at the onset of the morphogenetic phase of bone development and is regulated by a combination of extra-and intracellular factors as well as intrinsic genetic and epigenetic factors[11]. The bone marrow stromal cells are considered to be more sensitive to bone morphogenic protein than almost any other known mesenchymal cell population in the body[12]. Although BMA1 cells have been considered to be derived from marrow stromal cells which have the capacity to produce GM-CSF[5], they also contain cells which may form bones or produce bone inducing substance(s). The identity of these two kinds of cells and the possible gene activation by adenovirus transformation in them are currently under investigation using cloned cells.

1 Dexter, T.M., J. Cell Physiol., suppl. *1* (1982) 87.
2 Dexter, T.M., Wright, E.G., Krisza, F., and Lajtha, L.G., Biomedicine *27* (1977) 344.
3 Pluznik, D.H., and Sachs, L., J. Cell Physiol. *66* (1965) 319.
4 Shadduck, R.K., Proc. Soc. exp. Biol. Med. *158* (1978) 542.
5 Fujita, J., Yoshida, O., Miyanomae, T., and Mori, K.J., Gann *74* (1983) 334.
6 Lan, S., Rettura, G., Levenson, S.M., and Seifter, E., J. natl Cancer Inst. *67* (1981) 1135.
7 Okabe, T., Nomura, H., and Ohsawa, N., J. natl Cancer Inst. *69* (1982) 1235.
8 Harigaya, K., Proc. natl Acad. Sci. USA *78* (1981) 6963.
9 Lanotte, M., Metcalf, D., and Dexter, T.M., J. Cell Physiol. *112* (1982) 123.
10 Flint, S.M., Biochim. biophys. Acta *65a* (1982) 175.
11 Urist, M.R., De Lange, R.J., and Finerman, A.M., Science *220* (1983) 680.
12 Takagi, K., and Urist, M.R., Clin. Orthop. *171* (1982) 224.

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Diploidy and triploidy in the hybrid minnow, *Phoxinus eos* × *Phoxinus neogaeus* (Pisces: Cyprinidae)[1]

G.R. Joswiak, R.H. Stasiak and B.F. Koop

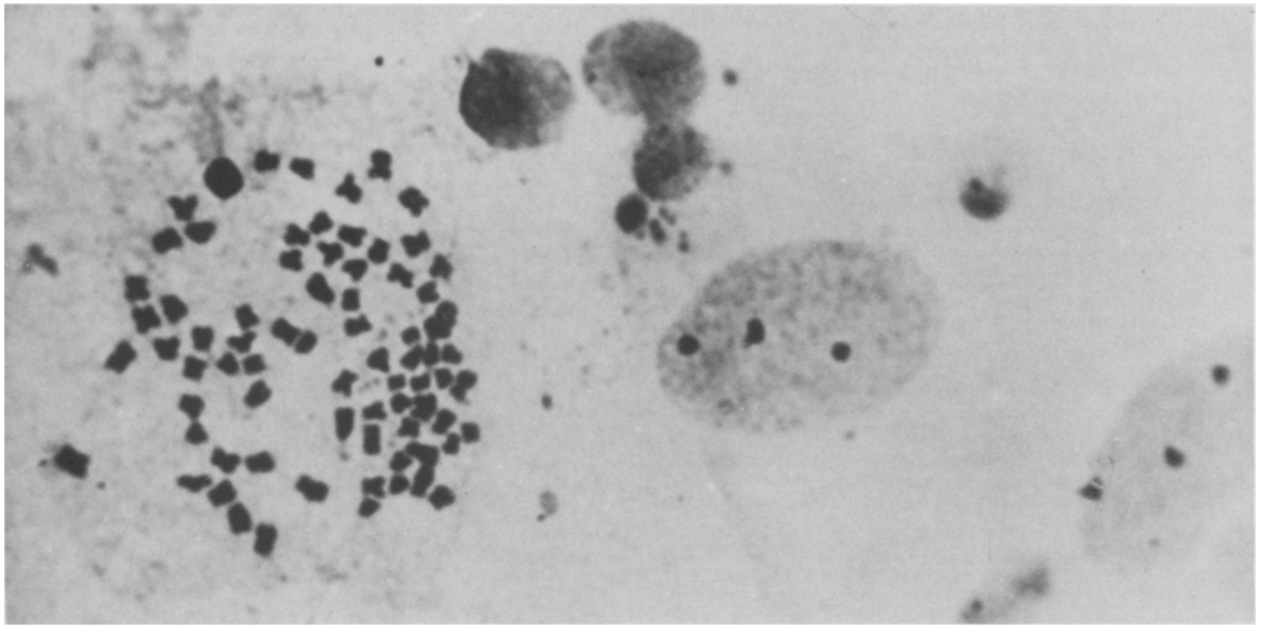
Office of Computer Services, Oakland University, Rochester (Michigan 48063, USA), Department of Biology, University of Nebraska at Omaha, Omaha (Nebraska 68182, USA), Department of Anatomy, Wayne State University, Detroit (Michigan 48202, USA), 5 April 1984

Summary. Two presumptive hybrid populations are examined. Nebraskan hybrids, all having allozymic F1 electromorphs, exist as both diploid (2n = 50) and triploid (3n = 75) forms. This supports the hypothesis of parthenogenesis as the mode of hybrid reproduction. The maximum number of nucleoli per cell is suggested as an indicator of ploidy level. In contrast, electrophoretic analysis of a postulated Mendelian hybrid population in Quebec failed to detect any allozymic heterozygotes or recombinants. A previous conclusion of introgressive hybridization in this population is not supported.
Key words. Cyprinid; triploidy; polyploidy; parthenogenesis; nucleoli.

The hybridization between the minnows *Phoxinus eos* and *Phoxinus neogaeus* is geographically widespread, although the occurrence and frequency of hybrids vary greatly among localities[2–4]. The question of hybrid fertility and mode of reproduction has been unanswered in previous studies. Two morphometric studies have led to varying conclusions, one suggest that the hybrids may be a all-female parthenogenetic species, the other suggesting introgressive hybridization. Based on samples from the United States, one researcher found that the hybrids were essentially all females, deviated from intermediacy, and where sometimes taken in the absence of one of the parental species[2]. Parthenogenesis was postulated as a method of maintaining all-female hybrids in the absence of one parent. Supporting this hypothesis are the facts that hybrids have been reported from Colorado and Montana where pure *P. neogaeus* has never been taken[5,6]. In contrast, an investigation based on discriminant analyses concluded that hybrids of both sexes exist in some Quebec lakes, with the hybrids forming a Mendelian population[7].

In an earlier allozymic investigation of two *Phoxinus* populations in Nebraska, we found data strongly supporting the parthenogenetic hypothesis[8]. We assayed specimens for three enzyme marker loci where fixed allelic differences occur between the species[8]. These loci encoded the following enzymes; phosphoglucomutase (E.C. 2.7.5.1), malate dehydrogenase (E.C. 1.1.1.37) and superoxide dismutase (E.C. 1.15.1.1). Hybrids, all females, comprised most of the *Phoxinus* at those localities, and were coexisting with only one parental species, *P. eos*. All the hybrids had patterns typical of F1 hybrids at each of the three marker loci: no recombinants classes were found. Despite their singular allozymic phenotype, the hybrids exhibited some morphological variability, with some resembling one parental species more then the other. These characteristics are strongly suggestive of hybrid parthenogenesis, possible of a diploid-triploid complex. Diploid-triploid unisexual complexes of hybrid origin are known to exist in a number of fish, amphibian and lizard species[9].

In this paper we present new data on both the Nebraskan and



Silver stained chromosome spread from intestinal epithelium in the triploid hybrid. Note two intact cell nuclei each containing three dark nucleoli.

Quebec populations. In an earlier study, the Quebec populations were found to be diploid, but none were examined for biochemical markers[7]. If the Quebec hybrids do form an introgressive Mendelian population, then it is reasonable to expect a number of phenotypes either heterozygous or recombinant for one or more of the three marker loci used in the Nebraskan investigation. If the Nebraskan hybrids are deviating from intermediacy because of gene dosage because of diploidy/triploidy then chromosomal triploids should exist.

We collected *Phoxinus* in Lac Triton, Quebec, during the summer of 1982. This locality was previously sampled and concluded to be a Mendelian population by the discriminant analysis study[7]. 200 individuals were used in electrophoresis for two marker loci, phosphoglucomutase and malate dehydrogenase, by previously described methods[8]. Of 200 fished assayed, 193 scored as *eos* at both loci while the remaining 7 scored as *neogaeus*. Unlike the Nebraskan populations, no heterozygotes were detected. Neither were any individuals found to be homozygous for one parent's alleles at one locus while homozygous for the other species alleles at the second locus. Indeed, we found no biochemical evidence of hybridization between these species in Lac Triton.

Five Nebraskan specimens, collected in Bone Creek, Brown County, Nebraska, were used for karyotypes, and their identities confirmed by subsequent electrophoresis. Slides were prepared from gill, intestine and kidney using previously described methods and were stained with a 50% aqueous solution of silver nitrate, then developed with a formic acid-colloid gelatin solution on a hot slide warmer[11]. The two *P. eos* and two of the hybrids possessed chromosome spreads of $2n = 50$, with a maximum number of two nucleoli observed a cell nucleus. In contrast the remaining hybrid possessed chromosome spreads of 75 chromosomes and had a maximum of three nucleoli within the nuclei of its cells. The figure is a photomicrograph of a chromosome spread from intestine.

The existence of both diploid and triploid hybrids in Nebraska supports the parthenogenesis hypothesis. In the eurasian cyprinid genus *Carassius*, unisexual polyploids are known with backcross hybrids in that genus sometimes being triploid[12–14]. However this is the first published report of a North American cyprinid hybrid that is triploid.

The assessment of the maximum nucleoli number per cell may provide an easy and simple method of triploid detection, even in the absence of chromosome spreads. The maximum number of nucleoli is known to correspond with the number of nucleolar organizer regions (NORs) which varies among taxa[15]. NORs are known to contain genes for the 18s and 28s ribosomal genes[15]. In *Phoxinus* it can be inferred that there is one NOR per genome, the diploids possess two while the triploid has three. Ploidy in specimens could be ascertained without sacrifice, thus allowing triploids to be used in other studies such as breeding experiments. A small number of cells could be sampled from a living specimen and then silver-stained for nucleoli number. Even historical hybrid collections have the potential to be assayed for nucleoli, as nucleoli have been successfully stained in preserved museum material of the coelacanth[16].

With respect to the Quebec population, several explanations can be put forth to explain the lack of biochemical evidence for hybridization. First allozymic hybrids might have existed in Lac Triton in the 1960s during the discriminant study but they are extinct at that locality now. Hybrids might exist there now but may have been backcrossed to *P. eos* to the point were alleles from *P. neogaeus* have been swamped. We consider this hypothesis unlikely, given the fact that some of the Quebec individuals score as pure *P. neogaeus* at the marker loci. We feel that the discrepancy lies within the discriminant analyses used to conclude that the population was hybrid. Discriminant analysis has been shown to have severe deficiencies in hybrid analysis for both theoretical and practical reasons[17]. The exaggerated variance of an unknown group classified on discriminant functions derived from reference samples can be an artifact of the analysis. Likewise mere intermediacy of discriminant scores is not proof of hybridization. In our work with discriminant analysis between the species *Phoxinus eos* and *P. erthrogaster*, specimens that were either misclassified or had intermediate scores on the discriminant function were found to be both small in size and from allopatric localities where hybridization is not possible[18]. Sources of variation, other than that of hybridization, probably account for the discriminant scores of the Lac Triton population.

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2 Greeley, J.R., and Greene, C.W., in: A biological survey of the 20th annual Rep. N.Y. State Conserv. Dep. (1931) 44.
3 Greene, C.W., The distribution of Wisconsin fishes. Wisconsin Conservation Commission, Madison 1935.
4 New, J.G., Copeia *1962*, 213.
5 Bailey, R.M., and Allum, M.O., Misc. Publ. Univ. Mich. Mus. Zool. *119* (1962).
6 Brown, C.D., Fishes of Montana. Big Sky Books, Missoula 1971.
7 Legendre, P., Can. J. Zool. *48* (1970) 1167.
8 Joswiak, G.R., Stasiak, R.H., and Moore, W.S., Can. J. Zool. *60* (1982) 968.
9 Moore, W.S., in: Evolutionary Genetics of Unisexual Fishes. Ed. B.L. Turner. Plenum Press, New York and London 1984.
10 Joswiak, G.R., Starnes, W.C., and Moore, W.S., Copeia *1980*, 913.
11 Howell, W.M., and Black, D.A., Experientia *36* (1980) 1014.
12 Ohno, S., Muramota, J., and Christian, L., Chromosoma *23* (1967) 1.
13 Kobaysi, H., Kawashima, Y., and Takeuchi, N., Jap. J. Ichthyol. *17* (1970) 153.
14 Kobaysi, H., and Hashida, M., Jap. Women Univ. J. *24* (1977).
15 Swanson, C.P., Metz, T., and Yong, W.J., Cytogenetics. Prentice Hall, 1981.
16 Dingerkus, G.P., Occ. Pap. Calif. Acad. Sci. *134* (1979) 111.
17 Neff, N.A., and Smith, G.R., Syst. Zool. *28* (1979) 176.
18 Joswiak, G.R., and Moore, W.S., Am. Midl. Nat. *108* (1982) 398.

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Chromosomal localization of the locus PGM (phosphoglucomutase) in *Drosophila buzzatii*

C. Pla*, J.B. Toral, H. Naveira and A. Fontdevila

Departamento de Genética, Facultad de Ciencias, Universidad Autónoma, Bellaterra, Barcelona (Spain), 26 March 1984

Summary. The locus PGM of *D. buzzatii* is localized in the linkage group of chromosome 4, outside the region blocked by the inversion 4s and with a recombination percentage of about 16% from the inversion breakpoint.
Key words. Drosophila buzzatii; phosphoclucomutase locus; chromosome 4; inversion breakpoint; recombination percentage.

Investigations with several *Drosophila* species of the *repleta* group *(D. mojavensis, D. arizonensis* and *D. mulleri)* to establish the role of different chromosomes in the determination of hybrid sterility[1], hybrid inviability[2], sexual isolation[3] and speciation[4] are handicapped by the impossibility of distinguishing the effect of chromosome 4 from that of chromosome 5. The reason is that electrophoretic markers are impossible to assign to one of these specific chromosomes, because both chromosomes do not show inversion polymorphism and are homosequential in the three above species. On the contrary, *D. buzzatii* and its sibling species *D. serido* (*repleta* group, *mulleri* subgroup) show enough inversion differences in those chromosomes to allow the allocation of some allozyme loci. The allozyme locus PGM has been assigned by exclusion to either chromosome 4 or 5 in those species[5] and by homology it is reasonable to locate it at the same chromosomal elements in *D. buzzatii* and *D. serido*. The present work deals with the precise chromosomal localization of the PGM locus using several methods. The feasibility of chromosome marking means that *D. buzzatii* and *D. serido* are excellent species to study correlations between chromosomal factors and reproductive isolation.

Two strains of *D. buzzatii*, 4s and 5I, homokaryotypic for one inversion on chromosomes 4 and 5, respectively, have been used in the present study. Strain 4s carries inversion *s* on chromosome 4[6]. Strain 5I carries inversion *In(5)F2b; F2e*, which appeared in a genetically unstable stock produced by introgression of a chromosomal section from *D. serido* in the *D. buzzatii* genome. This inversion includes nearly the same segment as is contained in inversion 5*g*, which is fixed in *D. buzzatii* and therefore practically represents a reversion of 5*g*. Both strains 4s and 5I carry the allele 100 of PGM in homozygous condition[7].

Females of each strain were crossed with males of stock M16 (kindly supplied by J.S.F. Barker), homocaryotypic for standard arrangements (4st and 5st), and homozygous for allele 95 of PGM[7]. F_1 males were backcrossed to females M16 in order to test for segregation of the PGM locus in relation to inversions *s* and *I*, and, consequently, its linkage group. At the same time, F_1 females were similarly backcrossed to M16 males, in order to find out frequencies of recombination between the PGM locus and the breakage point of the inversion in the same chromosome. The analysis was performed in third instar larvae of the backcross progeny; the same larvae were used for checking chromosomes and enzymes.

Table 1 shows the results from each of the backcrosses. In the progeny of cross A (strain 5I) we find all four possible genotypes, showing an independent segregation of PGM alleles with respect

Results of backcross progenies with *D. buzzatii* strains 4s and 5I

Backcross:	A	B	C
Genotype [a]	$♀♀^{st}/_{st}\ ^{95}/_{95} \times ♂♂^{st}/_{I}\ ^{95}/_{100}$	$♀♀^{st}/_{st}\ ^{95}/_{95} \times ♂♂^{st}/_{s}\ ^{95}/_{100}$	$♀♀^{st}/_{s}\ ^{95}/_{100} \times ♂♂^{st}/_{st}\ ^{95}/_{95}$
$^{st}/_{st}\ ^{95}/_{95}$	9	3	26
$^{st}/_{st}\ ^{95}/_{100}$	5	0	6[b]
$^{st}/_{s}\ ^{95}/_{95}$	–	0	4[b]
$^{st}/_{s}\ ^{95}/_{100}$	–	14	28
$^{st}/_{I}\ ^{95}/_{95}$	3	–	–
$^{st}/_{I}\ ^{95}/_{100}$	7	–	–

Recombination fraction $= \frac{10}{64} = 15.62\%$

[a] 95 and 100 stand for allelomorphs PGM^{95} and PGM^{100}, respectively; [b] recombinant frequencies.