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

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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.

to arrangements (st and *I*) of chromosome 5. On the other hand, cross B (strain 4s) yields only two genotypes, strongly suggesting an association of the PGM alleles (95 and 100) with arrangements (st and *s*) of chromosome 4. Since both crosses are mutually exclusive we can safely assign the PGM locus to the linkage group of chromosome 4.

Backcross C was performed to estimate the relative distance of the PGM locus from the breakage point of inversion *s* of chromosome 4. Recombination data indicate that the PGM locus is outside the inversion 4*s* at 15.62 morgans from one of its breakage points. Considering that the map distance from the proximal breakpoint of the inversion *s* (4F1c) to the centromere is smaller than the recorded percentage of recombination, it is most probable that the locus of PGM is situated within the cytological interval A–B of chromosome 4 (the region A corresponding to the telomere).

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7 PGM^{95} is one allelomorph which migrates 5 mm towards the cathode from allelomorph PGM^{100} in *D. buzzatii* taken as standard.

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Tandem gene duplication and fixed heterozygosity in the parasitic wasp, *Trichogramma marylandense*[1]

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Summary. All males and females of the parasitic wasp *Trichogramma marylandense* exhibit the 5-band ME phenotype normally found in heterozygous individuals. Since no diploid males were found and all males are hemizygous at both PGI and PGM loci, it is concluded that the permanent heterozygosity at this ME locus in *T. marylandense* is the result of tandem gene duplication.
Key words. Trichogramma; malic enzyme; gene duplication; fixed heterozygosity.

The existence of duplications of genetic loci was inferred in *Drosophila* by Sturtevant[2] early in this century. Roberts and Baker[3] postulated that the four esterase loci in *Drosophila montana* are evolutionarily related through a process of duplication of the original locus. Costa et al.[4] reported a duplication of the esterase 6 locus in a wild population of *D. melanogaster* and there is evidence of duplicate genes for alcohol dehydrogenase in *D. mojavensis*[5,6]. Duplication of the hexokinase locus in dipterans has also been suggested[7]. However, studies in enzyme gene duplication are mostly limited to the vertebrates, especially in fishes[8]. Here I report, to the best of my knowledge, the first published case of enzyme gene duplication in Hymenoptera.

Trichogramma are minute wasps that parasitize eggs of many Lepidoptera and a few species of Diptera and Coleoptera. They have been widely used in biological control projects in various parts of the world. The genetic basis of electrophoretic variations of malic enzyme (ME; E.C. 1.1.1.40), phosphoglucose isomerase (PGI; E.C. 5.3.1.1.) and phosphoglucomutase (PGM; E.C. 2.7.5.1.) in *Trichogramma* was established by progeny analyses[9]. It was found that ME allozymes in this group of insects function as a tetramer and are controlled by fast and slow alleles at a single locus, with heterozygotes exhibiting a 5-band phenotype. Both PGI and PGM have a single locus and each has four codominant alleles. However, PGI is a dimer and PGM is a monomer in *Trichogramma*.

As in other hymenopterous insects, *Trichogramma* males are haploid and females diploid. Therefore, only females can be heterozygous for any locus. However, regardless of the sexes, all wasps from two cultures of *Trichogramma marylandense* collected in Beltsville, Maryland, showed the 5-band ME phenotype. A chromosome number of n = 2SM + 2T + 1A has been reported in *Trichogramma*[10]. Although diploid males were found in a strain of *Trichogramma* from Japan[11], no diploid males have been found in these two cultures. This rules out the possibility that these male wasps with the 5-band ME phenotypes are diploid and heterozygous at this ME locus.

Gene duplication by polyploidization has been promoted as a major evolutionary phenomenon in vertebrates[12,13]. However, since no diploid males were found, the possibility of polyploidization as the cause of this ME gene duplication can be ruled out. This is further supported by allozymic studies of both PGI and PGM[9]. Although variations in PGI and PGM were found in these two cultures of *T. marylandense* with heterozygous females showing 3-band phenotype in PGI and 2-band phenotype in PGM, all males are hemizygous (with only one-band phenotype) at both loci. Therefore, the permanent heterozygosity at this ME locus in both diploid females and haploid males in this *Trichogramma* species is the result of tandem gene duplication. These two cultures have been established for more than 20 generations and still no segregation of the 5-band genotype was observed, indicating that these two loci are tightly linked.

Gene duplication and fixed heterozygosity have been reported in the diploid plant *Clarkia franciscana*[14] and the cultivated soybean *Glycine max*[15]. The present finding of ME gene duplication could facilitate the study of the evolution of Hymenoptera in general and the genus *Trichogramma* in particular. As to the origin of ME gene duplication in this species, we can only speculate at this stage. It could result from unequal crossing-over between two homologous chromosomes in a heterozygote during meiosis, or from duplication during replication followed by a mutation in one of the genes. In either case, if this duplication means a selective advantage, every member of the population would eventually become homozygous for this duplication which would result in the fixed heterozygosity observed in this species.

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