The rates at which duplicate genes are silenced may be changing over the course of evolution. In the early evolutionary stages, soon after a disomic pattern of transmission has been established, the 2 loci should be sufficiently similar in structure and regulation, that the silencing of one of these loci would not have serious consequences. In the later evolutionary stages, the 2 loci may have diverged sufficiently in their structure and regulation that a loss of expression at either locus would tend to be selected against. This tendency for duplicate gene expression to be retained would result from both the structural and regulatory divergence of the duplicate genes. One consequence of the divergence in gene structure is that the multiple locus isozymes may eventually acquire different kinetic and physical properties and then come to occupy different metabolic niches of adaptive significance to the organism[24]. Another consequence of this gene divergence is the differential regulation of these duplicate genes during embryogenesis and in the differentiated adult tissues. Such structural and regulatory divergence has been reported for some duplicate loci in all tetraploid fishes examined to date[9, 15, 25, 26]. The extent of this divergence of duplicate locus expression should be able to serve as a very useful gauge of the relative rates of divergence of gene regulation at different periods after a gene duplication event. Furthermore, an estimate of the extent of such regulatory divergence would be helpful in determining how tightly coupled are the rates of evolution of structural and regulatory genes.

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Allozyme constitution of two standard strains of *Drosophila subobscura*[1]

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Summary. 2 reference strains of *Drosophila subobscura* ('Küsnacht' and 'ch-cu-Athens'), widely used for the study of inversion polymorphism in this species, were investigated with respect to their allozyme composition by starch gel electrophoresis in 18 different enzyme systems coded by 18 different gene loci. Both strains are monomorphic for all 18 loci with only 1 exception. A standardized method of designation is proposed, to allow a direct comparison of all enzyme data in the *Drosophila obscura* species group.

Strains. Natural populations of Drosophila subobscura, a European species of the Drosophila obscura group, have been intensively studied for the last 2 decades by several authors with respect to chromosomal inversion polymorphism. For these investigations, mainly 2 different strains have been used as standard strains. Both are homozygous for known chromosomal gene arrangements. The strain 'Küsnacht' originates from a small number of flies caught wild at Küsnacht (Switzerland) and is homokaryotypic for the basic 'Standard' arrangements in all 6 chromosomes[3]. It was used by Mainx[4] and Kunze-Mühl[5] for drawing the chromosomal map of the giant chromosomes of Drosophila subobscura. The other strain is called 'ch-cu-Athens'. It is chromosomally identical with 'Küsnacht' with the exception of being homozygous for the gene arrangement O_{3+4} of chromosome O. (For the designation of gene arrangements see Kunze-Mühl and Sperlich[6].) It is also homozygous for the recessive visible mutant alleles 'cherry' and 'curled', thus any possible contamination with other strains can easily be recognized. The strain is a descendant of the 'β-ch-cu-stock' described by Koske and Maynard-Smith[7]. It was mainly used as a standard strain by Krimbas and coworkers for their studies on inversion polymorphism in Greece.

Symbols for allozymes. Whereas, for inversions of Drosophila subobscura, a common system of designation proposed by Kunze-Mühl and Sperlich[6] has been accepted by practically all investigators, this is not the case for allozymes and the alleles coding for them. Since different authors used different symbols and different strains as controls, some confusion exists. It is the intention of this paper to help to make the situation for D. subobscura more clear.

The terminology we are going to propose here is the one which was used by Lakovaara and Saura[8, 9], Saura et al.[10], Saura[11] and Lakovaara et al.[12–14]. These authors studied the geographical variation of allozyme polymorphism in natural populations of D. subobscura and D. obscura and

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Alleles at different loci of Drosophila subobscura

Locus	Alleles Küsnacht strain	ch-cu-Athens strain	Most common allele in natural populations
Adh	80	80	80
Aph-3	100	100	107, 100
Aph-5	97	97	97
Est-4	100	100	100
Est-8	112	115	112, 113, 115
Est-10	101, 99	99	101, 99
α-Gpdh	100	100	100
Hk-1	101	101	99, 101
Hk-3	96	96	96
Idh	105	105	105
Lap-4	106	104	104, 106
Mdh-2	96	96	96
Me	106	106	106
Odh	100	100	100
Pgm	101	101	101
Sod (To)	100	100	100

the interspecific variation within the D. obscura group. In all the studies mentioned, the symbols for the enzyme loci and their alleles have been arranged in a consistent fashion according to the designation used by several American authors, e.g. Hubby, Lewontin, Prakash, Ayala, Powell and others. The enzyme loci are designated by the abbreviation of the trivial name of the enzyme (e.g. Est, Adh, Mdh. etc.). When several forms of a specific enzyme exist, each coded by a different gene locus, hyphenated numbers are added to the enzyme symbols (e.g. Est-4, Est-8, Est-10), increasing with anodal migration of the corresponding enzymes. When only a single form of a specific enzyme has been found, no index number is given. Electrophoretically separable allozymes and the corresponding allele symbols are identified by an index number according to the mobility of the allozyme in electrophoresis (e.g. Mdh-2^{100}, Mdh-2^{96}, Mdh-2^{105}). In order to make comparisons possible between species of the D. obscura group, D. obscura was chosen for reference. Consequently, the basic value 100 is given to the most common allele of this species. All other corresponding or homologous allozyme bands of any species of the D. obscura group should be designated by an index number according to their difference in migration measured in the gel to the D. obscura standard allozyme band in mm. As a standard procedure for electrophoresis, the starch gel technique described by Lakovaara and Saura[8,9] is used. In D. subobscura the most common allozyme of the Idh enzyme is e.g. Idh^{105}. Under standard conditions of electrophoresis, it moves 5 mm farther towards the anode than Idh^{100} of D. obscura.

Genic constitution of the marker strains. Although strains of D. obscura are available and single individuals could be and often are used as standards in every electrophoretic analysis of D. subobscura, in many cases it is still desirable to have a control strain of D. subobscura. Such a reference strain is especially important in the case where crossings are made in order to check the Mendelian inheritance of an observed banding pattern. Hence, we studied the 2 strains 'Küsnacht' and 'ch-cu-Athens' and the allozyme constitution at 18 loci were determined. The results are given in the table. Using the same enzyme assay and methods as in the studies of Lakovaara and Saura[8], not only the 'Küsnacht' and the 'ch-cu-Athens' strain of D. subobscura were run on the same gel, but also usually controls of other strains of D. subobscura from different geographical origin. In addition, some individuals from other species were electrophoresed on the same gel, including D. obscura, D. bifasciata and D. ambigua in order to make comparisons possible. The number of individuals used for the analysis of a given enzyme system lies between 30 and 50 in each strain. As can be seen from the table, 'ch-cu-Athens' appears to be homozygous for all loci studied; 'Küsnacht' is polymorphic only for Est-10. The 2 strains differ from each other at Lap-4 and Est-8. Both of these loci are highly polymorphic in natural populations of D. subobscura[10].

All earlier investigations on enzyme polymorphism have been made by electrophoresing adult flies only. Aph-3, however, can be analyzed more reliably in larvae. All other enzyme systems listed in the table can be studied both in larvae and in adult flies with the exception of Aph-5, α-Gpdh and Hk-1. The latter 3 enzymes are not active in larvae of D. subobscura. Additional bands in the Lap-4 system can be observed when larval leucine amino peptidases are stained. Some difficulties exist with respect to esterases since different authors have described several esterase systems in D. subobscura. Our Est-4 is a cholinesterase. Loukas and Krimbas[15] found out that an Est locus, which they call Est-9, is 'a complex locus consisting of several very closely linked loci with active and silent alleles'. Our Est-8 is probably the most rapidly staining part of the products of that complex locus. Our Est-10 corresponds to the most anodal group of esterase bands. It is 2-banded in heterozygotes. There are very large differences in activity levels of Est-10 varying from silent to very strongly staining bands. These variations are seen among individuals within the same strain as well as among individuals from different strains. The reasons for this variability are unknown. In some 'ch-cu-Athens' gels, Est-10^{99} has 2 bands and resembles the situation in Est-$10^{99/101}$ heterozygotes. We believe, however, that 'ch-cu-Athens' is homozygous for Est-$10^{99/99}$ and that the 2-bandedness is due to an artefact. According to our experience, the variation of esterases in D. subobscura is very complicated and different electrophoretic techniques, or even uncontrolled variations of the same technique, may give different results for these enzymes.

Additional information not listed in the table is available for fumarase (Fu) and mitochondrial malate dehydrogenase (Mdh-1). Both enzymes show no variation in the 'Küsnacht' and 'ch-cu-Athens' strains. With the usual starch gel electrophoresis, Mdh-1 migrates cathodally. Since there are no published data for these enzymes in D. obscura, no reference can be made to the most common allele of this species. Fu and Mdh-1 allozymes present in 'Küsnacht' and 'ch-cu-Athens' are, however, most probably the most common ones of D. subobscura.

The table contains not only the data for the 2 standard strains but also a list of the most common alleles of the 18 enzyme loci observed in our laboratory strains as well as in natural populations of D. subobscura investigated by Saura et al.[10]. When there is a clearly established most common allele at a given locus, this allele is listed in the table. When there are several common alleles, none of which predominates, the most common ones are given. It is clear from the table that neither of the 2 standard strains is homozygous for a rare allele.

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