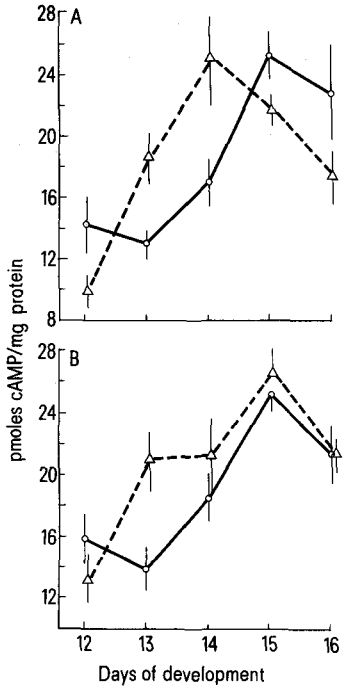
less distinguishable from those of normal fetuses except on day 13 were *bm/bm* fore limbs had higher cAMP levels than did the congenic C57BL/6J fetuses.

The prenatal differences in limb bud cAMP levels observed here between *bm/bm* and C57BL/6J may be related to the dwarfed phenotype observed in adult *bm/bm* mice. Using the model of Miller and coworkers[13], the results found here for back limb buds can be interpreted to explain the dwarfed limbs and undersulfated cartilage proteoglycans observed in postnatal *bm/bm*. The higher levels of cAMP on days 13 and 14 (back limbs) could be related to suppressed cell division of chondrocytes and the consequent dwarfed skeleton in adult *bm/bm* mice. The lower levels of cAMP on days 15 and 16 may relate to undersulfation of matrix proteoglycans observed later in *bm/bm*. Such a hypothesis assumes the presence of 'critical periods' and is less readily applied to the front limb bud data. However, these results are important in combining observations on postnatal *bm/bm* limbs (dwarfed with undersulfated cartilage proteoglycans) with models about cAMP's role in chondrogenesis derived from other systems. Deficient cartilage concentrations of cAMP have been reported in chicks homozygous for the nanomelia mutation[14] – a recessive lethal trait characterized by a severe reduction in size of all cartilaginous structures. The correlational nature of the results here and elsewhere on adenine nucleotides must always be remembered. Until extensive cause and effect results are obtained, differences in cAMP or APS levels must be viewed as merely suggestive.



Means and SE for concentrations of cAMP in paired limb buds of fetuses homozygous for brachymorphic (*bm/bm;* about 22 fetuses per day) or normal, syngeneic controls (+/+; about 17 fetuses per day) by day of gestation. *A* Hind limb buds; *B* fore limb buds; –△–, *bm/bm;* –○–, +/+.

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Genetic analysis of modifier variability in *Drosophila subobscura*[1]

G. Alvarez, P. Martínez, C. Zapata, M. Santos and A. Fontdevila[2]

Departamento de Genética, Facultad de Biología, Universidad de Santiago, Santiago de Compostela (Spain), 11 February 1981

Summary. The previously detected modifier variability acting on the expression of the Bare (*Ba*) locus in *Drosophila subobscura* is demonstrated to be due to polygenes situated along the chromosome O. From crosses between isogenic lines of high and low modifier effect we ascertained the presence of approximately 5 modifier loci.

Modifier variability is one type of genetic variability frequently used to explain many evolutionary changes which cannot be understood by considering only structural loci[3–5]. In spite of the great modifier variability acting on the expression of structural genes that has been found[6,7], little research has been devoted to the quantification of this type of variability in natural populations. With this in mind, we have studied the modifier variability in one of the chromosomes of *Drosophila subobscura* (the so-called O chromosome) in a natural population, inasmuch as it affects the expression of the dominant morphological mutant Bare (*Ba*)[8]. This mutant is precisely located on chromosome O of this species, and its phenotypic effect is to reduce variably the number of bristles[9].

The problem that arises is to define whether the variability we have studied is mainly due to the structural locus (isoallelic variability in wild O chromosomes) or to typical modifiers, namely allelic substitutions in loci on the chro-

mosome O other than the *Ba* locus (polygenic loci). Although, in general, quantitative genetic variation is described in terms of typical polygenes, the possibility is not excluded that isoallelic variation constitutes an important component of this variation. Thus, Green[10] has found several wild-type isoalleles acting on the eye color of *Drosophila melanogaster*, and Milkman[11] and Scharloo[12] have detected some isoalleles involved in several selection experiments. On the other hand, Thompson[13] found that ci^+ (cubitus interruptus) isoalleles did not contribute to quantitative variability of *veinlet* expression.

The genetic basis for the variability we are studying could be established by considering that the effects of recombination on isoalleles at the *Ba* locus and on polygenic loci distributed along the chromosome O must be very different. If modifier variability of the wild O chromosomes is mainly due to polygenic loci, crossing over between chromosomes of high and low modifier effect will produce several recombinant chromosomes which will present intermediate modifier effects. On the contrary, if isoalleles at the *Ba* locus are principally responsible for the variability, crossing over between the 2 chromosomes will not be able to produce chromosomes of intermediate modifier effect and a bimodal distribution corresponding to the 2 parental chromosomes will arise.

To test these arguments, we performed an experiment with isogenic lines for the chromosome O of high and low modifier effect (lines 220 and 57, respectively[8]). First of all, we obtained $+_{220}/+_{57}$ heterozygotes, which were mated with individuals of the balanced lethal *Va/Ba* strain (*Va*=Varicose, a dominant wing venation mutant) in 2 different ways, separately: via male and via female crosses (fig. 1). In both via male and via female crosses, a single *Va/+* male of the offspring was mated to *Va/Ba* females, and the bristles of 40 *Ba/+* and 40 *Va/Ba* progeny individuals were counted (fig. 1). 12 bristles per individual (4 dorso-centrals, 4 scutellars, 2 supra-alars and 2 post-alars) were considered in our experiment. The difference (D) in mean number of bristles between *Ba/+* and *Va/Ba* (control) individuals was used as a measure of the modifier effect of the chromosome O. A total of 83 *Va/+* males were studied: 40 from via male and 43 from via female crosses.

The results obtained (fig. 2) show a clear bimodal distribution of D-values with only 2 classes in the via male crosses (absence of crossing over in the male), while several intermediate classes corresponding to the recombinant chromosomes appear in the via female crosses. These results clearly demonstrate that the modifier variability acting on the *Ba* locus is mainly polygenic, i.e. due to several loci situated on the chromosome O. However, this conclusion does not fully exclude the possibility that isoalleles at the *Ba* gene can effectuate a slight contribution to the modifier variability of the natural population studied.

Another interesting question is to define the number of polygenes or effective factors determining the modifier variability we are considering. Recently, a controversy on the number of polygenic loci involved in quantitative genetic traits has arisen and the problem remains an open question today. Thus, while Thoday and Thompson[14], and Thompson[15-16] have argued that many continuous distributions could be explained by segregation at a few loci, Vetta[17] has defended the common hypothesis that quantitative variation is produced by segregation in a large number of loci. The distribution of our D-values of chromosomes from via female crosses can be interpreted in terms of 6 discrete classes, as can easily be observed in figure 2. This is confirmed by χ^2 tests of heterogeneity[18], which were performed by using the variance of D-values from via male crosses as an estimate of the parametric variance. With a minimum of 6 classes these tests do not detect statistical signification inside each class. On this basis, a model of 5 polygenic loci could explain the distribution we observe. This assumption is consistent with an experiment on the location of modifiers that we have performed by using Thoday's method[19] (unpublished results), which detects 5 effective factors distributed along the chromosome O. If these conclusions become definitive, we could explain the modifier variability of wild O chromosomes affecting the expression of the *Ba* gene in terms of a relatively small number of modifier loci.

Finally, we would like to point out that modifier variability and the main locus on which this variability is acting, could be considered as a particular case of a multilocus system. In the last years, multilocus systems have attracted the attention of many workers[20,21] as an alternative to the single gene as the unit of selection. Unfortunately, it is not easy to find other alternative units on which evolutionary forces could be operating. However, modifier systems could probably constitute evolutionary units themselves, and they could be

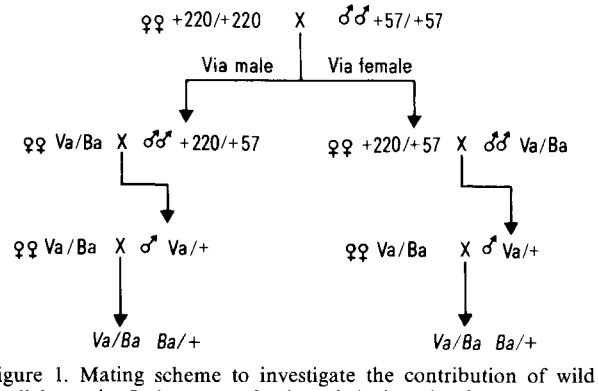

Figure 1. Mating scheme to investigate the contribution of wild isoalleles at the *Ba* locus, or of polygenic loci on the chromosome O affecting the expression of the *Ba* mutant.

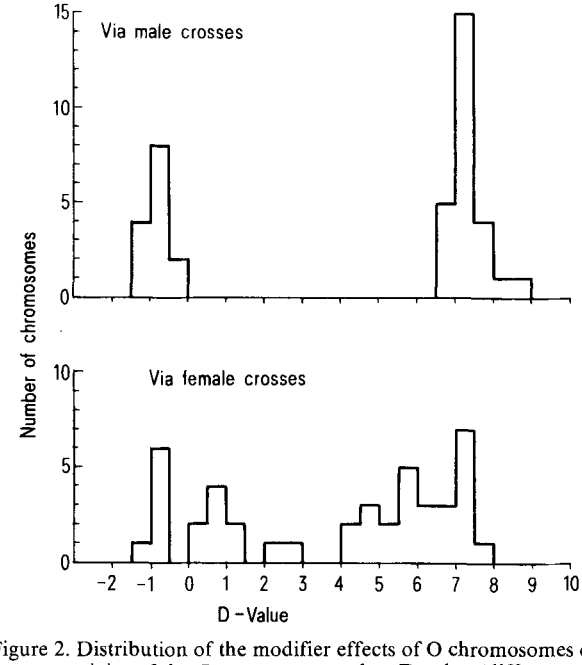

Figure 2. Distribution of the modifier effects of O chromosomes on the expressivity of the *Ba* gene measured as D-value (difference in mean number of bristles between *Ba/+* and *Va/Ba* individuals).

analyzed with relatively little effort. The results reported in this article constitute an attempt to characterize such a system in preparation for further studies. On this basis, we are presently investigating the question of where selective forces are operating within a modifier system, in the hope of finding out whether selection is principally acting on modifiers of major genes, or whether selective changes are occurring primarily on the major genes themselves.

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2 Present address: Departamento de Genética, Facultad de Ciencias, Universidad Autónoma de Barcelona, Bellaterra (Spain).
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Mercury selection of allozyme genotypes in shrimps

E. Nevo, T. Perl, A. Beiles and D. Wool[1]

Institute of Evolution, Haifa University, Haifa 31999 (Israel), and Department of Zoology, Tel Aviv University, Tel Aviv (Israel), 29 December 1980

Summary. The effects of mercury pollution on the allozymic variation of 15 phosphoglucomutase (PGM) genotypes was tested in the Mediterranean shrimp *Palaemon elegans* in 79 laboratory tests involving 2765 shrimps, with 1560 survivors (767 test and 793 control). Our results indicate differential tolerance of genotypes in variable mercury concentrations, suggesting that they are adaptive. The genetic structure can possibly be explored and potentially be used as a monitoring system for the quality and quantity of marine pollutants.

The proportion of adaptive[2] or neutral[3] allozyme polymorphisms, commonly found in natural populations, is still a major unresolved problem of evolutionary genetics. Usually, tests utilize differences in gene frequencies in natural populations observed after the operation of unknown natural forces. In contrast with this routine, we have tested in controlled experiments the cause-effect influence of mercury on allozymic variation in the shrimp *Palaemon elegans*. Our results indicate differential tolerance of some phosphoglucomutase (PGM) genotypes in variable mercury concentrations, suggesting that they are adaptive.

The shrimp *Palaemon elegans* is a widespread species in the Eastern Atlantic and Mediterranean coasts in rocky pools and lagoons[4,5]. This species was chosen because of its abundance and small size, which permitted the testing of relatively large samples. We have decided to concentrate on the PGM system owing to its high variability in comparison with 24 other tested systems. Animals were collected from rocky pools near Haifa and introduced into 10 aquaria in the laboratory. Fresh water was pumped for the experiments from 30 m depth at the Shikmona National Institute of Oceanography. Conditions in all aquaria were identical (22 °C; pH = 8.3, and constant aeration). No food was provided during any of the experiments which involved 0.02–0.40 ppm $HgCl_2$ and lasted 1–11 days according to concentration (table). All tests conducted simultaneously were matched with only 1 control. The test and the control differed solely in the addition of the pollutant to the former. Each experiment included at least 25 shrimps (for experimental details, see Nevo et al.[6]). We conducted 79 experiments involving 2765 shrimps. The survivors were deep-frozen (−80 °C) and 1560 shrimps (767 test and 793 control) were homogenized and studied by horizontal starch gel electrophoresis[7]. To avoid temporal variation in gene frequencies, we employed the method of paired comparison design, where control and tests were randomly taken from the same batch of shrimps and run simultaneously. Differential survivorship of genotypes was analyzed by the Wilcoxon matched-pairs signed-ranks test[8].

Results and discussion. A total of 5 alleles were found and designated: S^-, *S*, *M*, *F*, F^+ for *slow*, *medium*, and *fast*

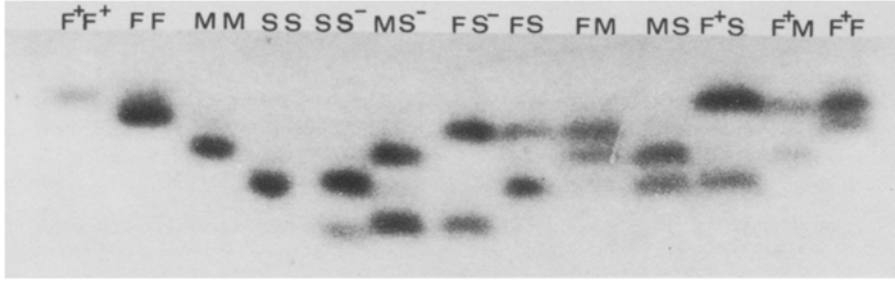

Figure 1. Zymogram of phosphoglucomutase (PGM) of *Palaemon elegans*, involving all possible genotypes except S^-S^- and F^+S^-.