ISOZYMES

Current Topics in Biological and Medical Research Volume 13

Editors
Mario C. Rattazzi

John G. Scandalios

Gregory S. Whitt

ISOZYMES

Current Topics in Biological and Medical Research

Volume 13

Editors

Mario C. Rattazzi

Department of Pediatrics
North Shore University Hospital–Cornell University Medical College
Manhasset, New York

John G. Scandalios

North Carolina State University, Raleigh

Gregory S. Whitt

Department of Ecology, Ethology, and Evolution
University of Illinois, Urbana

Alan R. Liss, Inc., New York

Contributors

Stephen D. Cederbaum, Departments of Psychiatry and Pediatrics, University of California, Los Angeles, Los Angeles, CA 90024 **[181]**

R.N. Chibbar, Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada; present address: National Research Council, Plant Biotechnology Institute, Saskatoon, Saskatchewan S7N 0W9, Canada **[155]**

George J. Dizikes, Department of Psychiatry, University of California, Los Angeles, Los Angeles, CA 90024 **[181]**

D.W. Foltz, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803 **[1]**

Wayne W. Grody, Departments of Pathology and Psychiatry, University of California, Los Angeles, Los Angeles, CA 90024 **[181]**

Alison J. Mack, Department of Botany, Duke University, Durham, NC 27706 **[127]**

E.B. Meyer, Department of Genetics and Development, University of Illinois Urbana-Champaign, Urbana, IL 61801 **[61]**

D.L. Nanney, Department of Genetics and Development, University of Illinois Urbana-Champaign, Urbana, IL 61801 **[61]**

T. Kaye Peterman, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 **[127]**

James N. Siedow, Department of Botany, Duke University, Durham, NC 27706 **[127]**

Athanasios S. Tsaftaris, Department of Genetics and Plant Breeding, University of Thessaloniki, Thessaloniki, Greece 54 006 **[103]**

R.B. van Huystee, Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada **[155]**

E. Zouros, Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada **[1]**

The numbers in brackets are the opening page numbers of the contributors' articles.

Preface

Since the origin of the isozyme concept and the recognition of the biological significance of isozymes in 1959, by Clement L. Markert, these gene products have served as powerful probes of molecular, genetic, developmental, physiological, and evolutionary mechanisms. The present series **Isozymes: Current Topics in Biological and Medical Research**, founded in 1977, has been used to review the progress of research in all the various biological disciplines utilizing isozymes. This series has successfully communicated the fact that the use of isozymes transcends traditional biological disciplines, and has helped lead to the 5th International Congress on Isozymes, held in Kos, Greece, May 26–30, 1986. (The Proceedings of the Congress will be published in this series following the publication of the present volume.)

The general theme of the series continues to be the roles of isozymes in the biology of prokaryotes, plants, man, and other animals. One goal is to provide the reader with the most contemporary concepts and state-of-the-art technology in the area of isozyme research. The other goal is to illustrate the diversity of experimental approaches used to study isozymes and the versatility of isozymes as research tools. The editors believe that it is important to communicate these diverse perspectives which have proven successful in testing hypotheses at different levels of biological organization.

Isozymes are present in the cells of all organisms. Ultimately, all enzymes exist as allelic isozymes, but many also exist as multilocus isozymes, and as isozymes formed by epigenetic events. Because isozymes can have different genetic and molecular bases as well as different functional roles, they are not only intrinsically interesting, from a structural and functional point of view, to protein chemists, molecular and cellular biologists, and physiologists, but are also probes of fundamental mechanisms underlying gene regulation, transmission, and evolution, and can provide an insight into the molecular mechanisms of pathological processes. This series, therefore, is aimed at readers with very diverse backgrounds and interests. By stressing the interdisciplinary nature of isozyme research, the editors hope to stimulate an enhanced appreciation of the diverse roles of isozymes in the molecular ecology of the cell and their evolutionary significance at the organismic and species levels. This volume and successive volumes will continue to provide different general and specific topics, ensuring a wide coverage of established fields as well as burgeoning new ones to which the study of isozymes is significantly contributing.

Mario C. Rattazzi
John G. Scandalios
Gregory S. Whitt

Contents

试读结束，需要全本PDF请购买 www.ertongbook.com

Isozymes: Current Topics in Biological and Medical Research 13:1–59

The Use of Allelic Isozyme Variation for the Study of Heterosis

E. Zouros and D.W. Foltz

Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada (E.Z.), and Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803 (D.W.F.)

© 1987 Alan R. Liss, Inc.

I. INTRODUCTION

This chapter addresses the question of how studies of isozymes have contributed to our understanding of heterosis. From this point of view, it only partially addresses the problem of how enzyme variability is maintained in populations. To the extent that heterosis is caused by single-locus overdominance, the role of heterosis in maintaining genetic variation is quite obvious. But other selective forces can also maintain polymorphism, such as frequency-dependent selection, fluctuation in the sign and magnitude of selection coefficients in time or space, antagonistic pleiotropy, and antagonistic selection among different levels of biological organization. The phenotypic consequences of these types of balancing selection may or may not appear as heterosis [Turelli and Ginzburg, 1983].

Shull's [1914] original definition of heterosis was "a descriptive term for hybrid vigor, irrespective of mechanism." Because "vigor" or "luxuriance" (another word used in definitions of heterosis) are terms that themselves need definition, a more precise definition of heterosis is the phenomenon whereby, for a specified quantifiable character, the average score of progeny of two parental stocks is higher than the score of either stock. The character need not necessarily relate to Darwinian fitness. In this regard, the above definition differs from Dobzhansky's [1952] definition of heterosis, which refers strictly to hybrid superiority in realized fitness. Dobzhansky's definition is appropriate when the primary interest in heterosis is as a mechanism ensuring genetic diversity in populations. It is also operationally useful in the search for "ultimate" explanations of heterosis (i.e., why heterosis evolved), because heterosis, like sex, would not have evolved if it were not associated with a fitness advantage. But having evolved, heterosis may appear under circumstances in which there is no fitness advantage. Sterile interspecific hybrids with heterotic characters (e.g., the mule) are obvious examples. The physiological and molecular basis of heterosis (i.e., "proximal" explanations of heterosis) can be studied in these hybrids as profitably as in fertile intraspecific hybrids.

Because heterosis was originally defined as a property of hybrids, it became closely associated with heterozygosity. With time, heterosis became synonymous with heterozygote superiority, without further reference as to whether the heterozygote is the product of the crossing of two inbred strains or simply the product of a random mating in an outbred population. Thus, heterosis has been used as a synonym of overdominance, a term that strictly denotes that, for a specified quantifiable character, the heterozygote for two allelic genes (e.g., A_1 and A_2) is superior to either corresponding homozygote (A_1A_1 or A_2A_2) but not necessarily to any homozygote that may exist in the

population—e.g., A_3A_3. As pointed out by Lewontin [1974], the relationship between heterosis and overdominance is that between observation and explanation. But overdominance may not be the only explanation of heterosis. To what extent overdominance is the cause of heterosis remains one of the major unresolved problems in population genetics and evolution, and one to which we will return after we have reviewed the contribution of allelic isozyme studies to the problem of heterosis.

Several recent reviews have addressed questions related to the subject matter of this review. Among these, the most relevant is the one by Mitton and Grant [1984]. Other related reviews are by Singh [1984], Zouros and Foltz [1984a], Koehn [1985], Vrijenhoek [1985], and Watt [1985].

II. EXPLANATIONS OF HETEROSIS

A. The "Dominance" and "Overdominance" Hypotheses

The early studies of heterosis have been reviewed in considerable detail by Wright [1977]. As Wright states in the beginning of his review, "It is not possible in experimental studies to make a sharp distinction between those concerned with systems of mating and those concerned with selection since both are always involved." Studies of heterosis always involved some degree of inbreeding, and for many investigators heterosis could be simply viewed as the reversal of inbreeding depression. This view is still prevalent today. Falconer [1981], for example, gives a theoretical treatment of heterosis as "inbreeding depression in reverse." The underlying assumption in this view is that in natural populations several loci segregate for completely or partially recessive deleterious alleles. This explanation of inbreeding depression and of restoration of vigor upon hybridization (heterosis) can be traced to Castle et al. [1906] and came to be known as the "dominance hypothesis." Its early rival was the "stimulation hypothesis" [Shull, 1914], which, according to Wright [1977], is essentially similar to the views of Darwin [1868] on hybridization. According to the stimulation hypothesis, the degree of vigor of an organism is determined by the "dissimilarity" of the gametes from which it is formed. The dissimilarity was thought to act as a stimulus for increased cell division and growth. Despite its vagueness, the fundamental assumption of the stimulation hypothesis is that heterosis is due to heterozygosity per se. In that sense, it is the forerunner of the "overdominance hypothesis"—i.e., the hypothesis that heterozygotes have higher fitness than either homozygote.

The "dominance" and "overdominance" explanations of heterosis make directly opposite predictions about the levels of variation in natural popula-

tions and the mode and tempo of evolution, and they constitute, respectively, the cornerstones of the "classical" and the "balanced" schools of population genetics [Lewontin, 1974]. A numerical illustration of the two hypotheses and how they account for heterosis is given in Table I. The fitnesses (or phenotype scores) of three genotypes determined by two alleles, L_i and L_j segregating at a locus *L*, are specified by the selection and dominance coefficients, *s* and *h*, respectively. The difference in the two models is in the range of values that *h* may assume. (The ranges of *h* given in Table I represent the "rule" under each hypothesis; both hypotheses will occasionally allow *h* to admit values outside this range.) For *h* between 0.5 and zero the favorable allele (L_i) is partially or completely dominant over the unfavorable allele (L_j), so the fitness of L_iL_j is closer to the fitness of L_iL_i than L_jL_j, but it never exceeds L_iL_i. For h_{ij} negative, the fitness of the heterozygote exceeds the fitness of the best homozygote (L_iL_i). In the numerical example i = 1 and j = 2, and, for simplicity, *s* and *h* are assumed to be the same for all four loci. In the multiplicative model the total fitness is the product of one locus fitnesses (e.g., the fitness of pure line #1 is $1 \times 0.9 \times 1 \times 0.9 = 0.81$). In the additive model the total fitness is one plus the differences from one of the single locus fitnesses (e.g., the fitness of the hybrid is $1 + 0.01 - 0.1 + 0.01 + 0.01 = 0.93$). The main conclusion from the table is that regardless of whether fitnesses are multiplicative or additive, both the domi-

TABLE I. A Formulation of the "Dominance" and "Overdominance" Explanations of Heterosis With a Numerical Example

One-locus genotype	Fitness	Dominance hypothesis	Overdominance hypothesis
L_iL_i	1	$1 > s_{ij} > 0$	$1 > s_{ij} > 0$
L_iL_j	$1-h_{ij}s_{ij}$	$0.5 > h_{ij} > 0$	$0 > h_{ij}$
L_jL_j	$1-s_{ij}$		
Example:		s = 0.1, h = 0.1	s = 0.1, h = −0.1
	Genotype:		
	L_1L_1	1	1
	L_1L_2	0.99	1.01
	L_2L_2	0.90	0.90
	Multiplicative model:		
Pure line 1	$A_1A_1,B_2B_2,C_1C_1,D_2D_2$	0.81	0.81
Pure line 2	$A_2A_2,B_2B_2,C_2C_2,D_1D_1$	0.73	0.73
Hybrid	$A_1A_2,B_2B_2,C_1C_2,D_1D_2$	0.87	0.93
	Additive model:		
Pure line 1	$A_1A_1,B_2B_2,C_1C_1,D_2D_2$	0.80	0.80
Pure line 2	$A_2A_2,B_2B_2,C_2C_2,D_1D_1$	0.70	0.70
Hybrid	$A_1A_2,B_2B_2,C_1C_2,D_1D_2$	0.87	0.93

试读结束，需要全本PDF请购买 www.ertongbook.com

nance and the overdominance hypotheses predict that F_1 will perform better than either parental line (i.e., heterosis) and that F_2 will have a lower average score than F_1.

A full discussion of the implications of the two hypotheses in regard to the organization of the gene pools of sexually breeding populations can be found in Lewontin [1974]. We will mention only those points that are relevant to our discussion of the study of heterosis through the use of allelic isozymes. Under the dominance hypothesis, there will be one allele in the population which in the homozygous state will confer the maximum fitness. It follows that selection will keep other alleles in low frequencies and that most individuals will be homozygous for the superior allele. If large random-mating populations are found to contain more than one allele in high frequencies, the most likely explanation will be that these alleles are selectively equivalent (the hypothesis of neutrality). In theory, it is possible to obtain and maintain a superior genotype, and in breeding programs the best strategy will be to select from the population rather than establish inbred lines that upon crossing will produce vigorous hybrids. Under the hypothesis of overdominance, on the other hand, basic selection theory dictates that most loci in a population will be polymorphic and, consequently, a typical individual will be heterozygous for a large fraction of its genome. There can be no best genotype that can breed "true," and the largest possible gains in a breeding experiment will be obtained by cross-fertilizing inbred lines.

Another difference between the two hypotheses, which is not always brought into focus and which is particularly important when considering heterosis, is that the dominance hypothesis will account for heterosis only if heterosis is the manifestation of the effects of more than one locus. Under this hypothesis, one-locus phenotypes will always produce a pattern in which the heterozygote scores somewhere between the two homozygotes. For the overdominance hypothesis, one-locus heterosis is not only possible but is expected to be the common state of affairs (in a strict sense, overdominance and one-locus heterosis are completely equivalent terms). This distinction is important, because if one could decompose a polygenic character into one-locus components, one could, in principle, decide between the two hypotheses. There are several theoretical and practical reasons why such a decomposition is not possible. Yet this reductionist approach may provide useful information about the nature of heterosis. Thus, studies that have examined the in vitro properties of enzyme preparations from individuals of different genotypes have had an important influence on the development of theoretical models bearing on the question of heterosis.

B. Heterosis as a Property of Multilocus Systems

As presented in Table I, the dominance and overdominance hypotheses have one major point in common: the multilocus fitness can be obtained from the one-locus fitnesses. This is not a basic tenet of the dominance or overdominance hypotheses, but it provides a convenience for mathematical analysis. Models that assign fitness values to one-locus genotypes and then proceed to obtain multilocus fitnesses as simple functions of the one-locus fitnesses are vastly easier to analyze than are models in which multilocus fitnesses are the only real quantities and one-locus fitnesses are only statistical deductions. The view that multilocus fitnesses are the only real quantities is particularly favored among enzymologists and biochemists. A strong case for it can be found in Kacser and Burns [1981]. They list several examples in which the in vivo output of an enzyme pathway is a concave function of the dose of a particular enzyme in the pathway. When the flux through a multienzyme pathway is near (or at) the plateau (which must occur most of the time), then changes at individual enzymes will have only a minor effect on the system's output and, by extension, on the phenotype. As a result, at the phenotypic level heterozygotes may not appear different from the superior homozygote (dominance effect), even though at the biochemical level they may appear as intermediate between the two homozygotes. An illustration of this phenomenon, involving the well-studied alcohol dehydrogenase locus of *Drosophila melanogaster*, is given by Briscoe et al. [1975]. The in vitro activity of the enzyme is higher in S/S than F/F homozygotes, but the activity of the F/S heterozygote does not deviate significantly from the midpoint of the two homozygotes. Yet, Briscoe et al. observed that in regard to viability on a medium containing 12.5% ethanol, heterozygotes were as good as the favored S/S homozygotes.

Interestingly, whereas the consideration of a locus as an element embedded within a highly buffered multilocus system seems to reduce the locus's contribution to heterosis, the conditions for the maintenance of polymorphism in such systems are less stringent than when each locus is considered as an independently selected unit. This basically follows from the fact that the genetic load (i.e., the requirement for differences in the reproductive potentials of the various genotypes) is larger when loci segregate independently than when they segregate in groups [Maynard Smith, 1968] or when the effect of single-locus substitutions on fitness follows a concave curve [Gillespie, 1976] or when selection is truncate [Milkman, 1967; Wills, 1981].

III. EVIDENCE OF HETEROSIS FROM ISOZYME STUDIES

As we noted in the Introduction, heterosis has become, in many aspects, synonymous with heterozygote superiority. Heterozygosity can be studied in

several ways. One widely used approach is through hybridization. Pure lines from the same species or stocks from two different hybridizable species are crossed to produce hybrids. The literature on heterosis through hybridization is vast and will not be reviewed here. We will restrict ourselves to studies where heterozygosity is measured in progeny of conspecific individuals. Hybrids between races and species may not always show an increase in fitness, and, in fact, a decrease in fitness may be seen owing to incompatibility between genomes evolving in isolation from each other (for a recent discussion of this point, see Vrijenhoek [1985]). Yet interspecific hybrids behave in many ways like intraspecific ones. An example is the discordance between enzymatic and phenotypic scores. Pasdar et al. [1984] reported faster growth rates in hybrids between green and redear sunfish than in progeny from intraspecific crosses, yet activities for several enzymes in hybrids were intermediate. Enzyme intermediacy in heterozygotes is also the rule in intraspecific crosses (Sect. IV.A).

A. Evidence From Gene Frequencies and Fitness Estimates in Laboratory Populations

Most experimental studies of heterosis have tried to explain the results in terms of either the dominance or overdominance hypotheses. Most notable in this respect is the work on *Drosophila*. The genetic variants used in these studies were mainly fitness modifiers (naturally occurring mutants; genes affecting viability, fertility, developmental time, mating propensity, etc.) and chromosomal inversions. Whereas the evidence from the studies of fitness modifiers is still open to question [see Lewontin, 1974], the studies of inversion polymorphisms have accumulated an impressive body of evidence in support of the overdominance hypothesis [Dobzhansky, 1970]: When fitness differences are observed among homozygotes and heterozygotes for alternative gene arrangements, most often they are in favor of the heterozygote. Unfortunately, overdominance at the gene arrangement level cannot be equated to overdominance at the locus level. There are several reasons for this. Inversions are thought to be unique events, and gene exchange between inverted sections of the genome is severely restricted because of suppression of crossing over. As a result, the effect of heterozygosity at one locus within the inversion cannot be studied independently from the effect of heterozygosity at other such loci, inverted sections tend to accumulate different sets of deleterious genes, and, if the allelic content of enzyme loci within an inversion is found to be different from the content of another inversion, one cannot decide whether this is the result of epistatic selection (coadaptation) or of historic accidents (for a discussion of this problem see Krimbas and Loukas [1980]).

Electrophoretic variation appeared at first to be ideally suited to overcome the problems associated with inversion polymorphisms. One-locus phenotypes can be identified, the primary products of the variants can be studied in vitro, and for many enzymes the physiological functions are known. These properties may allow one to establish a direct connection between genotype and a fitness-related trait (for a discussion of this point see Koehn et al. [1983]).

The first applications of electrophoresis were not specifically designed to answer problems related to heterosis. Merely recording gene or genotype frequencies in an array of populations will not allow one to establish heterozygote superiority. There are several reasons for this limitation. In species with nonoverlapping generations, random mating will establish Hardy-Weinberg proportions in every new generation, thus wiping out any heterozygote excess caused by selection. In populations with overlapping generations, viability gains among older heterozygotes will be swamped by the younger ages, upon which selection has yet to act. One way out of this difficulty is to score the population for several generations in the hope that systematic trends will emerge. In practice, this approach will rarely produce unambiguous results.

If gene frequencies in a natural or laboratory population are recorded over two or more successive generations, it might be possible to implicate selection, but it would be much more difficult to decide among different models of selection. The difficulties with the problem of fitting a selection model and of estimating selection coefficients from a series of observations are formidable (for recent discussions, see Alvarez et al. [1984] and Christiansen [1984]).

Another serious problem with studies of selection in laboratory populations is the possibility of nonrandom associations of allelic isozymes with deleterious genes in the background genotype. It is practically impossible to have an idea of how many chromosomes one must sample from a natural population in order to minimize the probability of linkage disequilibrium in the founding population. Even more unsettling is the possibility that, irrespective of the original sample size, nonrandom associations will be established in the population as a result of selection at loci that are linked to enzyme markers. This possibility has no negative bearing on studies whose interest is in the dynamics of maintenance of enzyme variation, provided the results from the laboratory can be extended to natural populations. But if the interest is in the physiological or molecular basis of heterosis, the question of whether selection acts on the enzyme variant itself or on an adjacent but unseen gene is fundamental, and the possibility of nonrandom associations cannot be ignored.

In spite of these limitations, there have been several serious attempts to estimate fitness components of isozyme genotypes in a variety of organisms in the laboratory. Since these studies have a bearing on the question of heterosis, we will briefly review a few of the most typical of them.

Among the earliest and most comprehensive attempts to estimate fitness components of isozyme genotypes is the work of Yamazaki [1971] on the sex-linked esterase-5 locus of *Drosophila pseudoobscura*. Yamazaki extracted 33 isofemale lines for each of two different electrophoretic alleles from a 15-year-old laboratory population and proceeded to obtain viability, developmental time, and fecundity estimates for each of the three female and two male genotypes. Although significant differences in fitness components were found among some lines, these differences were neither consistent across the lines nor large enough to account for the maintenance of the polymorphism in the original laboratory population. One interesting observation is that fitness estimates for the same genotype varied considerably from line to line, causing the intragenotype variance in fitness estimates to be as large as the intergenotype variance. This observation may mean two things: The slow and fast mobility lines are heterogeneous collections of alleles with the same electrophoretic mobility, or there is a great deal of variation in background genotype among lines carrying the same allele. The first possibility is most likely in view of the work of Coyne et al. [1978], who by means of sequential electrophoresis and heat stability tests demonstrated that at least 30 alleles may segregate at the Est-5 locus in natural populations of *D. pseudoobscura*. If the second explanation is correct, it will mean that nonrandom associations between allelic isozymes and fitness-affecting genes were present in the population from which the lines were extracted.

An experiment similar to that of Yamazaki was undertaken by Marinkovic and Ayala [1975], who studied seven fitness parameters in 30 strains of *Drosophila pseudoobscura* synthesized from crosses of 28 isofemale lines derived from a wild population. The crosses were designed such that the genotypes for two enzyme loci (Pgm-1 and Me-2) were known in the synthetic lines. Contrary to Yamazaki's findings, Marinkovic and Ayala found significant variation in the fitness components of the various isozyme genotypes. They also observed that the fitness value of a given genotype may change or be completely reversed during the life cycle of the insect. Fitness values were also affected by the presence or absence of competition for food.

A case of strong overdominance was reported by Zouros et al. [1982] for the alcohol dehydrogenase locus of the olive fruit fly, *Dacus oleae*. Natural populations of this species contain invariably two Adh alleles, fast (F) and

slow (S), in approximate frequencies of 35% and 65%, respectively. A third allele, intermediate (I), is also present in frequencies less than 1%. When wild flies are brought in the laboratory and forced to establish a colony on an artificial larval substrate (the natural substrate is exclusively the olive fruit), the I allele rapidly increases in frequency, and in less than six generations it reaches 30%, with a compensatory decrease in the frequency of S. The triallelic system quickly arrives at a stable equilibrium. The authors repeated the experiment with samples from different populations and obtained the same result. They used the gene frequency scores for the first 12 generations from colonies originated from two different populations to estimate relative fitnesses for the six genotypes. The mean fitnesses were as follows: II, 0.02; FF, 0.04; SS, 0.08; SF, 0.58; SI, 0.98; IF, 1. These are very high selection differences. Subsequent work by Loukas and Economopoulos (in preparation) showed that the selective agent is the larval substrate. In colonies containing the I allele in high frequencies, these authors replaced the artificial diet with olive fruit and observed that the frequency of I rapidly declined from 30% to 10% (owing to seasonal availability of the olive fruit, the colonies could not be maintained year round on this medium to see if the frequency of I will eventually drop to lower levels). It is clear that the strong overdominance at the Adh locus is associated with a drastic change in the species' environment. Abrupt ecological changes of this kind do not happen in nature very often, and selection pressures of this magnitude are therefore not expected to represent the rule. It must be noticed, however, that the study of Van Delden et al. [1978] of the same locus in *Drosophila melanogaster* has suggested that overdominance, even though of a milder degree, may operate under regular conditions. The fitnesses of homozygotes appear on average to be about 90% those of heterozygotes (estimates given by Mukai and Yamazaki [1980]).

As an example of a study in which estimation of fitness differentials for the various isozyme genotypes has implicated a different type of selection, we cite the work of Nassar [1979] on the polymorphism of Lap in *Drosophila melanogaster*. Nassar calculated fitness estimates under different allele frequencies and found that these estimates were frequency-dependent. The dependence on frequency was more obvious under crowded conditions. Male mating rates also appeared to be frequency-dependent. These results are in accord with previous studies by Kojima and his collaborators [e.g., Huang et al., 1971], which implicated frequency-dependent selection for allelic isozymes in *Drosophila melanogaster*.

There have been several studies that have failed to obtain significant fitness differences between isozyme genotypes. Mukai et al. [1974] measured via-

试读结束，需要全本PDF请购买 www.ertongbook.com