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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Genetica. — *Studies on the phosphoglucomutase* (PGM) *polymorphism in two successive years in natural populations of* Drosophila melanogaster (*). Nota di GIOVANNI TRIPPA, ADA LOVERRE, CLAUDIA BARBERIO, LAURA ULIZZI e ROSARIA SCOZZARI, presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — Allo scopo di valutare l'importanza della deriva genetica e della selezione naturale nei confronti del polimorfismo della fosfoglucomutasi (PGM) in *Drosophila melanogaster* sono stati raccolti campioni di differenti popolazioni naturali dell'Italia Meridionale in due anni successivi (ottobre 1971 e ottobre 1972). È stata quindi studiata la distribuzione delle frequenze geniche per gli alleli *Pgm* e ne è stato verificato il grado di costanza da un anno all'altro, dopo che queste popolazioni avevano subito una drastica riduzione numerica invernale (effetto « collo di bottiglia »). I risultati ottenuti mostrano che tutte le popolazioni esaminate sono polimorfiche per gli alleli Pgm^A e Pgm^B. Essi costituiscono un buon punto di partenza per stimare le dimensioni formali delle popolazioni (effective breeding sizes) in esame. Questi a loro volta rappresentano la base di qualsiasi tentativo di stimare il peso della selezione dalla differenza tra variazioni di frequenze osservate e variazioni attese dalla sola deriva genetica.

INTRODUCTION

One of the most challenging problems of evolutionary genetics is the estimate of the proportion of structural genes with common alleles which the selection is acting upon. Clearly this estimate is the product of the proportion of the polymorphic genes by the proportion, among these genes, of those with at least two not selectively neutral common alleles.

In order to make a reliable estimate of any frequency one needs:

1) an unselected sample of known size where to measure this frequency,
2) a method for classifying the single observations according to the relevant criteria. This method must be reasonably efficient and its degree of efficiency must be known.

The first of the required estimates has been obtained for many species [1, 8]. In fact, the polymorphic genes—out of a reasonably unselected sample of known size of structural genes—have been counted by a method (electrophoresis) with a high and known efficiency (~ 0.3). It is now believed that most, if not all, the structural genes are polymorphic. Until relatively

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(**) Nella seduta del 12 gennaio 1974.

recent times none of the required criteria for obtaining this estimate was fulfilled: the genes were identified only through their variation and the efficiency of the available methods for discovering the polymorphic genes was unknown and surely very low. When genetic heterogeneity was searched for at a phenotypic level rather far from the primary product of the gene, the structural alleles which failed to express themselves at such phenotypic level could not be detected by definition. Thus the alleles which previously could be examined made up a quite biased sample. On the contrary, we have now available for several species a sample of structural alleles of known and reasonably large size, which may be considered unbiased if one accepts that electrophoretic differences are representative of all the structural differences.

In conclusion, the " only " thing still needed now is a method with a known and high efficiency for classifying a given number of common alleles, one by one, as a selectively neutral or not neutral allele.

In principle, a *direct* approach measuring the variations between the fitnesses of the relevant common genotypes for a reasonably large number of genes would be quite adequate for this purpose. However, the sensitivity of such methods is so low that only major selective effects would be discovered. On the other hand, we know—from empirical results as well as on the basis of theoretical considerations—that the selective forces involved among electrophoretic alleles discovered by chance are, as a rule, small if present at all. One is then obliged to turn to *indirect* approaches, namely to look for *magnified consequences* of the small selective forces under study rather than to these forces themselves, much in the same way as for structural genes heterogeneity, which can be chemically recognized only at the extremely magnified level of protein heterogeneity. In favourable cases in fact selection is additive over several generations.

A number of expected consequences of a prolonged action of selection have been proposed as suitable for the present purpose. These tests are usually based on the idea that any spatial (*i.e.*, between populations) or temporal (*i.e.*, between different generations within the same population) pattern of gene frequencies incompatible with chance alone suggests selection.

It is worth emphasizing a serious difficulty at the level of *interpretation* of the results, if they point to the occurrence of a selective effect. It consists in the fact that it is impossible in practice, with statistical data only, to exclude that coselection due to a linkage-disequilibrium effect is the factor responsible for the findings suggesting that selection is working on the gene under study. A conclusive evidence for that may be obtained only by identifying the mechanisms through which the suggested selective effect operates.

The test adopted in this paper consists in measuring the changes of gene frequencies of a structural gene in natural populations submitted to strong genetic drift (bottle neck effect) every year in order to ascertain whether or not selection buffered the expected variations of these frequencies.

Phosphoglucomutase (PGM) and *Drosophila melanogaster* have been chosen for these experiments.

METHODS

The samples studied were obtained from seven (in October 1971) and six (in October 1972) natural populations of *Drosophila melanogaster*, collected in Puglia (Castellaneta, Otranto and Corato) and in Sicily (Ranna, Pedalino, Vittoria and Archi). The PGM electrophoretic phenotypes of single adult flies were determined according to the technique of Spencer *et al.*, 1964 [9], adapted by Trippa *et al.*, 1970 [10] to single fly homogenates.

Before determining the PGM phenotype, the six different samples were examined for excluding the presence of individuals belonging to the *Drosophila simulans* species. Male flies were identified stereoscopically and *melanogaster* males were subsequently crossed with virgin females of *Gl Sb/Ubx* stock. A progeny test was adopted to classify females as belonging either to *melanogaster* or to *simulans* species. In this way it was also possible to recover new alleles and synthesize new laboratory stocks, confirming at the same time the genetic basis for all the observed variations.

RESULTS

The examined populations showed the different phenotypes determined by the presence of a series of codominant alleles at the same autosomal locus previously described (11–12), that is Pgm^A, Pgm^B, Pgm^C, Pgm^D, Pgm^E and Pgm^F.

Table I shows the pattern of genetic electrophoretic variations and the comparisons between the *Pgm* gene frequencies found in two consecutive years in the populations under study.

All of them turned out to be polymorphic for at least two alleles, Pgm^A and Pgm^B. All the testable populations were in Hardy–Weinberg equilibrium for these alleles.

DISCUSSION

The data of Table I show that Pgm^A is the most common allele in all the populations studied.

In a previous work [11] one of the points unresolved was whether the alleles Pgm^C, Pgm^D, Pgm^E and Pgm^F could be considered as common or as variant alleles, given the limited sizes of the samples examined. To answer this question we have increased the sample size and reexamined, after a period of time, the "*same*" (from a geographical point of view only) populations for the distribution of these gene frequencies. On the basis of the data presented in Table I, it is now possible to answer the question with greater precision and to consider the Pgm^A and Pgm^B alleles as common and the remaining alleles as variant ones.

TABLE I

The distribution of Pgm *gene frequencies in seven samples of* Drosophila melanogaster *collected in two consecutive years* (1971* *and* 1972**) *from each of seven different localities.*

POPULATIONS	Number of tested individuals	Pgm^A	$Pgm^B \pm$ S.E.	Pgm^C	Pgm^D	Pgm^E	Pgm^F
Castellaneta	213*	90.6	8.3±1.3	0.7	0.2	—	0.2
	544**	93.6	5.5±0.7	0.3	0.5	0.1	—
Otranto	296*	93.2	5.4±0.9	0.5	0.8	—	—
	531**	92.6	6.9±0.8	0.3	—	0.1	0.2
Corato	233*	94.4	5.2±1.0	0 2	—	0.2	—
	157**	93.9	5.1±1.2	0.3	—	0.6	—
Ranna	206*	94.7	4 1±1.0	1.0	0.2	—	—
	434**	93.2	5.5±0 8	0.3	0.7	0.1	0.1
Pedalino	162*	94.1	5 9±1.3	—	—	—	—
Vittoria	46*	79.3	20.7±4 2	—	—	—	—
	382**	95.3	3.9±0.7	0.3	0.1	0.4	—
Archi	200*	99 2	0.8±0.4	—	—	—	—
	498**	94 8	4.4±0.6	0.2	0.5	—	0.1

The present data on the Pgm^A gene frequencies are still insufficient for estimating the effective breeding sizes, N_e's, of the populations examined. They therefore represent the necessary premise for estimating this parameter from further temporal variations to be observed in the same populations (Trippa *et al.*, in preparation). This parameter is a fundamental one, not only from an ecological point of view, but even more because it is the basis for evaluating the relative weight of genetic drift and selection in determining the Pgm^A gene frequencies.

Another problem discussed in the above mentioned paper [11] was whether to consider the variant alleles with the same electrophoretic mobility found in different populations as the same structural alleles or as different alleles with the same electrophoretic behaviour. Since the less common alleles turned out to be variant alleles and were found in two consecutive years, in spite of the " bottle neck " effect, these data suggest that they represent different isoelectrophoretic alleles in different populations.

A question suggested by this type of considerations is: how these variant alleles, in spite of the fact that they probably disappear by mere chance during winter, can attain every year the frequencies observed in the natural populations.

A possible mechanism, other than mutation, for the appearance of new alleles could be intragenic recombination between alleles which are different

from each other in, at least, two different sites. That is to say a mechanism " which can be stated as ' polymorphism generates more polymorphism ' in that mutation-like events are more likely to occur in germ cells of heterozygotes than in those of the homozygote " [13].

Available data [13–14] point out to a rate of intragenic recombination lying between 10^{-2} and 10^{-3}. Therefore, not only spontaneous mutations but probably also intragenic recombinations generate new alleles.

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