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Genetic variability in natural populations, evidence in support of the selectionist view

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Summary. Genetic variability in 6 neotropical anopheline species, analyzed, by zymogram technique, has been described. The results support the 'selectionist' theory of the evolutionary significance of high levels of molecular diversity in natural populations.

Thanks to the monumental work of Lewontin and his group, showing a high level of genetic diversity in *Drosophila pseudoobscura*, by zymogram technique[2,3], the long controversy between the 'classical' school and 'balanced' view finally started to turn around. Until then, according to the 'classical' school championed by H.J. Muller, later led by J.F. Crow and M. Kimura, natural populations possess very low genetic variability. Most mutations are deleterious and are removed by natural selection which thus acts primarily as a cleansing agent of 'wild type' gene pool. A rare favourable mutation may replace the old less-fit gene, leading to evolution of the gene pool[4-6]. On the other extreme, the 'balanced' view proposed by T. Dobzhansky and later led by B. Wallace holds quite an opposite view of the evolutionary process. According to this school, natural populations possess a high level of genetic variation maintained by various forms of balancing selection. An occasional favourable mutation contributes just a simple fraction to the high level of genetic diversity always present. This variation enables populations to adapt to diverse temporal and spatial environmental conditions.

Since then a great amount of genetic diversity has been reported in populations of diverse types of organisms[3,7-9]. These findings thus tended to support the 'balanced' school. With the controversy among the 2 schools on the amount of genetic diversity in populations thus resolved, the next 3 central questions of the population or evolutionary genetics persist; 1. what is the evolutionary significance of this high level of molecular polymorphism or in other words its adaptive significance?; 2. what is the important 'unit of selection'?; 3. what evolutionary mechanisms maintain the high level of genetic variability[3,10,11]. The 1st question again drew diverse answers from 2 schools of thought. Kimura[12], the proponent of the 'classical' school put forward the 'neutral' theory of genic polymorphism suggesting that most of the observed biochemical variation is random and physiologically irrelevant, whereas according to the 'selection' school, this variation is the direct result of balancing forms of natural selection, and is thus not selectively neutral. If so, it would then be necessary to support this with experimental data. Thus the 'balanced vs. classical' controversy changed into 'selectionist vs. neutralist' controversy.

2 types of approaches commonly used can be phrased into the following questions. Does each isoenzyme of a given locus possess a specific physiological or metabolic function and if the enzyme diversity indeed provides the adaptive raw material necessary for a population to explore and adapt to the changing environment, then there should be an overall correlation between the degree and nature of molecular diversity in a population or species and the proportion of the diverse ecological niches it occupies in its geographical distribution range. In other words the more extensive the distribution of a population or species over wide environmental conditions, more genetically heterozygous or polymorphic it should be and conversely if a population or species has its distribution limited or restricted to specialized ecological niches it should be less polymorphic. Far more studies have been addressed to the 1st question[13], for references, than to the second. It is the latter question that our studies have attempted. We initiated studies on genetic structure of 6 neotropical anophelines, of which *A. aquasalis* has a very rigid requirement of salt water during the larval stages and thus the distribution of this species is restricted to the coastal region only. On the other hand, other species (table) are widely distributed, both in coastal and interior regions, and consequently these species have been able to explore regions of high and low

Measures of genetic variability in neotropical anopheline species

Species	No. of enzyme loci analyzed	Proportion of genome heterozygous per individual	Proportion of polymorphic loci crit. 1	crit. 2	Proportion of heterozygous individuals
A. aquasalis	26	0.081	0.23	0.34	0.084
A. darlingi	19	0.21	0.579	0.632	0.125
A. nuneztovari	26	0.171	0.46	0.54	0.111
A. argyritarsis	27	0.188	0.46	0.682	0.113
A. albitarsis	18	0.27	0.42	0.65	0.17
A. evansae	19	0.23	0.56	0.63	0.149

Enzymes used for this table included esterases, ODH, XDH, MDH, ME, HK, GOT, AO of larval stages; LAP of pupal and ACPH and a-GPDH of adults. Crit. 1 = Most common allele with a frequency of 0.95 or less. Crit. 2 = Second most common allele has a frequency not smaller than 0.01.

humidity, with wide ranges of temperature conditions and diverse types of breeding sites.

Analysis of zymograms of various gene-enzyme systems in 6 species (table) shows that only 23% of all the structural gene loci are polymorphic in *A. aquasalis*, compared to 46–58% in other species (crit. 1). Similarly an average individual of this species is likely to have only 8.1% of its genome in heterozygous condition, whereas in other species it is 2–3 times higher. Again *A. aquasalis* possesses fewer of its individuals (8.4%), as heterozygotes, considering all loci studied, than other species (11–17%). From these measures of genetic variability in different anopheline species, we can conclude that our results support the 'selectionist' view of significance of genetic (allozymic) variability.

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Frequency-domain study of the mechanical response of living striated muscle

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Summary. Small-amplitude sinusoidal displacements, in the frequency range 4–100 Hz, were applied to intact whole frog sartorius muscle whilst in a state of tetanus. At low frequencies the muscle was observed to do oscillatory work, while at higher frequencies it tended towards elastic behaviour. Frequency-response plots obtained were compared with those from other muscle preparations. Results were interpreted in terms of mechano-chemical transduction properties of muscle.

Dynamic stiffness and phase values over a range of frequencies have been obtained for a variety of muscle preparations: intact and glycerinated insect fibrillar muscle[2,3]; glycerinated frog sartorius muscle[4]; chemically stimulated frog semitendinosus fibres[5], and intact, whole, frog sartorius muscle[6].

Intact insect fibrillar muscle displayed a minimum in the dynamic stiffness response, while in the phase response both minimum and maximum features were evident[2]. The phase response was negative in the minimum region and positive in the maximum region. Negative phase values imply that the muscle is doing oscillatory work[2]. The same features were observed in glycerinated insect fibrillar muscle. It was concluded that the stiffness and phase responses were not a feature of excitation and contraction coupling but an intrinsic property of the contractile mechanism[3]. Negative values for the minimum-phase region were taken to be a characteristic feature of the oscillatory behaviour of insect fibrillar muscle.

Glycerinated frog fibres displayed the same negative phase region[4], but the frequency range was not extended far enough to define the maximum phase feature. However this emphasized that the capacity for oscillatory work was not peculiar to insect fibrillar muscle. Studies performed on chemically stimulated frog semitendinosus fibres over the frequency range 0.25–133 Hz displayed a minimum in the stiffness response and the minimum-maximum features in the phase response[5].

All the above systems could maintain a state of tetanic contraction for many minutes. Living muscle, stimulated electrically, cannot endure such long periods of tetanic contraction. Halpern and Alpert[6] used a broad-band length pertubation signal which enabled them to obtain dynamic stiffness and phase values over a wide range of frequencies from each 1.3-sec tetanic stimulation. Their results, however, departed from the pattern emerging from the above investigations. They characterized the dynamic stiffness trend by a 2-plateau shape, and only observed positive values for phase. Their study led them to model their response by a first-order transfer function with a finite delay, in contrast to transfer functions of orders 2 and 3 which were necessary for insect fibrillar muscle[7] and chemically stimulated frog fibres[5].

Our investigation was directed at establishing whether dynamic stiffness and phase trends, as observed in insect fibrillar muscle and chemically stimulated frog fibres can be observed in living frog muscle. We used small-amplitude sinusoidal length changes, as were used by all other workers, except Halpern and Alpert[6]. Although requiring more tetanic stimulations than the wide-band signal to define stiffness and phase trends, sinusoidal length changes had the advantage of much greater frequency-domain power for the same time-domain amplitude[8].

Methods. Whole sartorius muscles from the tree climbing frog *(Litoria caerulea)* were dissected and mounted horizontally in a small rectangular glass bath containing oxygenated Ringers solution (NaCl 115 mM, KCl 2.5 mM, $CaCl_2$ 1.8 mM, Na_2HPO_4 2.15 mM, NaH_2PO_4 0.85 mM, pH 6.9). The temperature of the bathing solution was measured using a thermocouple and a Keithley 160 multimeter and controlled by means of a Tauchlora cooling system.

Typical values for muscle weight and length were 32 mg and 2.3 cm. The tendon end of the muscle was firmly secured to the length driver, and the pelvic end to the force gauge. By means of a Spectra Physics 4 mW He-Ne laser, and displaying the diffraction fringes on a translucent screen, the sarcomere length was adjusted to rest length L_o, considered to correspond to an average of 2.2 μm along the muscle.

The muscles were supramaximally stimulated using a single pair of bright platinum wire electrodes. Duration of stimulation was kept to 2 sec and the muscle allowed 2 min rest between tetani.

The frequency of the sinusoidal length displacements was controlled by a digital computer (Hewlett-Packard 2100S) via a voltage from the digital to analog (D/A) interface