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Is *Pi* a Selectively Balanced Polymorphism?

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Abstract. A stable polymorphism of the *Pi* alleles M1, M2 and M3 close to their observed frequencies has been predicted, simply by relating fitness to the proportion of each genotype having α_1-antitrypsin (α_1-AT) levels greater than 100 mg/100 ml. By further specifying that the rare S and Z genotypes with low α_1-AT levels have enhanced fertility, equilibria of the M, S and Z alleles have also been predicted close to their observed frequencies; however, none of these latter equilibria is stable, mainly because the predicted fitness of the SS genotype is too great.

Introduction

α_1-Antitrypsin (α_1-AT) is responsible for inhibiting trypsin and some other proteases in human serum. Isoelectric focussing resolves five alleles at polymorphic frequencies at the *Pi* (protease inhibitor) locus and these account for much of the variance in serum α_1-AT levels. These alleles and their approximate frequencies in European populations are: M1~0.75, M2~0.15, M3~0.04, S~0.04 and Z~0.01.

Beckman and Beckman [1980] have found that heterozygotes for the M subtypes (M1, M2 and M3) have higher α_1-AT levels and lower variances than their homozygotes. Since low levels of α_1-AT as expressed in Z and S genotypes have numerous disease associations [see *Clark and Martin,* 1982, for references], it is implied that the higher levels in M1M2, M1M3 and M2M3 heterozygotes confer a selective advantage over M homozygotes through lower disease susceptibility. If true, this would be an important finding because, with the notable exception of the associations of G6PD deficiency and Hb^S with malaria resistance, the adaptive significance of protein polymorphism remains unknown.

A further puzzle is the existence of the Z and S alleles at considerable frequencies despite their associated low levels of α_1-AT and disease susceptibilities. There is some evidence that increased fertility associated with these alleles may provide an explanation [*Gedde-Dahl* et al., 1981; *Clark and Martin,* 1982].

It is our aim in this paper to quantify these considerations and test whether existing data on α_1-AT levels can be related to fitness such that they satisfy the conditions for a selectively balanced polymorphism and predict the observed gene frequencies at the *Pi* locus.

The α_1-AT levels of the different *Pi* genotypes which we use in our calculations are shown in table I. The data of *Beckman and Beckman* [1980] include no levels for S and Z genotypes and *Cook*'s [1974] data were obtained before M-subtyping was available. We shall consider whether the necessary conditions for a stable polymorphism are found first in *Beckman and Beckman's* data for M1, M2 and M3 and then in *Cook*'s data for M, S and Z.

Table I. α_1-AT levels (mg/100 ml), standard deviations and numbers on which they are based

	Beckman and Beckman [1980]				*Cook* [1974]		
	n	mean	SD		n	mean	SD
M1M1	54	256	49	MM	90	257	76
M1M2	88	286	34	MS	59	229	60
M1M3	12	266	22	MZ	59	171	42
M2M2	39	256	56	SS	17	150	35
M2M3	29	281	28	SZ	29	92	28
M3M3	3	277	81	ZZ	45	17	10

Method

Crow and Kimura [1970] discuss the conditions necessary for a stable equilibrium with multiple alleles. For a polymorphism with n alleles, and fitnesses of the genotypes $w_{ij} = w_{ji}$,

$$\Delta = \begin{vmatrix} w_{11} & w_{12} & \dots & w_{1n} \\ w_{21} & w_{22} & \dots & w_{2n} \\ . & . & . & \\ . & . & . & \\ w_{n1} & w_{n2} & \dots & w_{nn} \end{vmatrix}$$

and Δ_i is the determinant obtained by substituting 1's for all the elements in the *ith* column of the determinant. At equilibrium, the frequency of the *ith* allele $p_i = \Delta i / \Sigma \Delta i$ and the average fitness $\hat{\bar{w}} = \Delta / \Sigma \Delta i$. In the three-allele case, the necessary and sufficient conditions for a stable equilibrium are: $\Delta_1 > 0, \Delta_2 > 0, \Delta_3 > 0$, $t_{11} < 0$, where $t_{11} = w_{11} - 2w_{13} + w_{33}$. *Mandel* [1959] has considered this case in more detail and although there is no simple interpretation of these conditions, *Crow and Kimura* [1970] comment that:

(1) The population fitness at equilibrium $\hat{\bar{w}}$ must exceed the fitness of any homozygote.

(2) There can be stable equilibria without each heterozygote being fitter than any homozygote.

(3) Some cases in which all heterozygotes are fitter than all homozygotes are unstable.

Results

M1, M2 and M3

Beckman and Beckman [1980] showed that their three homozygotes were consistent with a combined level of 257 ± 53 and their three heterozygotes' levels with a combined level of 283 ± 33, but that the difference between homozygous and heterozygous levels was highly significant. However, if M1, M2 and M3 are identical in this regard and in their heterozygous levels with S and Z (for which no data are yet published), and if α_1-AT levels bear a direct relationship with genetic fitness, then we should expect that the three alleles would be equally frequent at equilibrium.

This is far from being the case and consequently we have used the values for separate genotypes reported by *Beckman and Beckman* in our calculations. The first hypothesis we tested was that fitnesses, w_{ij},

Table II. Results for equilibrium calculations with M subtypes. For an explanation, see text

	Fitness equivalent to						
	mean	1/SD	mean/SD	>250	>200	>150	>100
Δ_1	-255	4.44	36.6	448	272	17.2	0.39
Δ_2	-180	0.54	13.6	632	221	9.0	0.10
Δ_3	150	3.46	31.6	704	208	4.5	0.02
t_{11}	1	-5.81	-15.5	-36	-30	-7.4	-1.50
$\hat{p}_1$		0.53	0.45	0.25	0.39	0.56	0.76
$\hat{p}_2$		0.06	0.16	0.35	0.31	0.29	0.20
$\hat{p}_3$		0.41	0.39	0.40	0.30	0.15	0.04

were directly proportional to mean α_1-AT levels. Results for this and subsequent hypotheses are shown in table II. Values for Δ_1, Δ_2, Δ_3, t_{11} and $\hat{\bar{w}}$ are given and when conditions for a stable equilibrium are obtained, the expected equilibrium gene frequencies $\hat{p}_1$, $\hat{p}_2$ and $\hat{p}_3$ (corresponding to M1, M2 and M3) are also given. It can be seen that a direct proportionality between fitness and mean activity does not give the conditions for a stable equilibrium in that $\Delta_1, \Delta_2 < 0$ and $t_{11} > 0$.

Beckman and Beckman noted that the homozygous variance is significantly greater than that of heterozygotes and implied that reduced variance is selectively advantageous. We have thus set fitness proportional to the inverse of the standard deviation (1/sd) and also to the coefficient of variation (mean/sd). Both of these predict stable equilibria but neither predicts the correct order of magnitude of the gene frequencies.

More specifically, *Beckman and Beckman* suggest that the important selective variable may be the proportion of a given genotype which falls below a given level of α_1-AT at which deleterious effects become important. From the tabled means and standard deviations we have calculated the proportions of each genotype expected to fall above given thresholds and have equated these to fitnesses. Only the values for thresholds of 250, 200, 150 and 100 mg/100 ml are tabulated but the results are continuous for intermediate values. It can be seen that all these thresholds predict stable equilibria and that below 200 mg/100 ml the predicted gene frequencies are in the right order. At a threshold of 100 mg/100 ml, the expected equilibrium gene frequencies are 0.76, 0.20 and 0.04 which are very close to the values observed in European populations. Note that the predicted frequencies must sum to 1 while, of course, the observed frequencies of these alleles sum to about 0.94. At this point the heterozygote fitnesses are all equivalent at 1.0000 while M1M1 is 0.9993, M2M2 is 0.9973 and M3M3 is 0.9857.

M, S and Z

So far we have invoked only one selective mechanism, that of decreased viability of low α_1-AT levels, to predict a balanced

Table III. Results of one unstable equilibrium calculation for M, S and Z alleles: $T_v = 100$, $T_f = 120$ and $w = (P_v + P_v \cdot P_f)/2$

	Δ_i	$\hat{p}_i$	Fitnesses of genotypes		
			M	S	Z
M	-0.0493	0.92	0.5078	0.5092	0.5309
S	-0.0033	0.06		0.5521	0.3568
Z	-0.0012	0.02			0.0000
	$t_{11} = -0.5539$		$\hat{\bar{w}} = 0.5084$		

polymorphism of the M1, M2 and M3 alleles. This is not adequate to explain the existence of the S and Z alleles; their homozygous and heterozygous levels are less than the M homozygote so they should disappear.

However, an advantage of low α_1-AT levels has been postulated in increasing fertility. Because S and Z are rare, this must be only a moderate advantage in comparison with the accompanying decreased viability. We need to find a combination of two opposing fitness functions which will predict the maintenance of the polymorphism. These are:

(1) P_v, the proportion of individuals of a given genotype who fall above the 'viability threshold', T_v

(2) P_f, the proportion of individuals of a given genotype who fall below the 'fertility threshold', T_f, and so have an increased probability of reproduction.

P_v and P_f are conceptually independent components of fitness, and nett fitness can be simply given by their product. However, it has been postulated that the increased fertility is expressed only in females [*Clark and Martin*, 1982] and consequently we have defined fitness as $(P_v + P_v P_f)/2$.

We varied T_f, between 50 and 250 for three values of $T_v = 75, 100, 125$ (bracketing the value of T_v arrived at in consideration of the M subtypes). No stable equilibrium was found for any pair of T_v and T_f values; there was always at least one negative Δ_i. However, certain combinations of T_v and T_f produced $t_{11} < 0$ and all three Δ_is < 0. *Mandel* [1959] has defined such solutions as non-trivial equilibria which are nevertheless unstable. Such solutions which also predicted the correct order of gene frequencies, were found for: $T_v = 75$, $T_f = 80$; $T_v = 100$, $T_f = 110$–150; $T_v = 125$, $T_f = 140$–240. In all these regions the predicted mean population fitness w was less than that of the SS homozygote and it is presumed that this is the chief cause of the instability. Gene frequencies closest to those observed (M ~ 0.91, S ~ 0.04, Z ~ 0.01) were obtained for the following combinations: $T_v = 75$, $T_f = 80$ (0.96, 0.04, 0.001); $T_v = 100$, $T_f = 120$ (0.92, 0.06, 0.02); $T_v = 125$, $T_f = 140$ (0.95, 0.04, 0.01). These points are also those at which the difference between $\hat{\bar{w}}$ and w_{ss} is minimal. The solution for one of these points is shown in table III.

Discussion

We have successfully predicted a stable equilibrium of the M1, M2 and M3 alleles close to their observed frequencies, simply by specifying that the chief selective mechanism is the avoidance of α_1-AT levels below 100 mg/100 ml. Obviously such a precise threshold is unrealistic since there is certain to be a variance about it. Never-

theless, this level is close to that implied by the disease associations of S and Z genotypes [see *Clark and Martin,* 1982 for references]. The fact that *Beckman and Beckman* [1980] do not report any α_1-AT levels as low as 100 mg/100 ml is not surprising in view of their small numbers, but values this low and lower have been found for MM genotypes by *Cook* [1974] and *Clark* [unpubl.]. The small differences in fitnesses we have predicted between the six MM genotypes (maximum difference $<1.5\%$) are consistent with the small genetic loads implied by the clinical data, yet are quite sufficient for the stable maintenance of polymorphism.

Our attempts to predict a stable equilibrium of the M, S and Z alleles have been less successful. The predicted fitness of SS was always too large to be compatible with stable equilibria. Unstable equilibria were found which predicted gene frequencies close to those observed but the interpretation of these unstable stationary points is difficult. These problems are not surprising given that little is known about the level below which fertility is enhanced, whether this occurs in both sexes or only in females, and the extent of enhancement.

It may also be questioned whether α_1-AT levels in healthy individuals are more pertinent to fitness than the levels attained by different genotypes during infection. The answer awaits more data, as does the search for a stable equilibrium of all five alleles, which will require α_1-AT levels for M subtype heterozygotes with S and Z. Nevertheless, to the best of our knowledge the analysis of the M subtype data is the first case where observed frequencies of three polymorphic alleles have been predicted simply by relating fitness to enzyme levels and their variances.

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