sediment, accounting for most of the protein present, was centrifuged and washed three times with cold buffer A. For chemical and physical analyses, the crystals were dissolved in 0.05*M* sodium phosphate buffer, *p*H 7.0, containing 1 percent NaCl.

The crystals of asialoceruloplasmin are anisotropic, blue, hexagonal prisms terminating at both ends in shallow pyramids. The prisms are longer in the crystals formed from 7 percent protein solutions than in those from 3 percent (Fig. 1). Their shape resembles that of crystals of cytochrome b_2 freed of DNA (*4*). The crystals may be reversibly decolorized by the addition of sodium ascorbate, and suspensions of the crystals in an 0.10*M* sodium acetate buffer, *p*H 5.0, catalyze the oxidation of *p*-phenylenediamine.

Crystals can also be obtained from partially desialized ceruloplasmin. After a shorter period of incubation, three volumes of an ethanol-chloroform mixture (9:1 by volume) are added to inactivate neuraminidase and to precipitate the protein which crystallizes after re-solution in buffer A (Table 1).

When neuraminidase from *Vibrio cholerae* (*5*) (250 unit/ml) and ceruloplasmin (3 percent) are incubated for 22 hours at 25°C in buffer A containing $2 \times 10^{-3}M$ $CaCl_2$, crystals are formed which are identical to those obtained after treatment with the *Cl. perfringens* enzyme. To our knowledge this is the first time a glycoprotein, freed of a portion of its carbohydrate, has been crystallized.

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Polymorphism in an Inbreeding Population under Models Involving Underdominance

Abstract. *Models of selection favoring homozygotes over the heterozygotes and involving frequency-dependency in their competitive abilities were simulated in order to determine the conditions for maintaining stable polymorphism at a diallelic locus in large inbreeding populations. With heavier inbreeding, frequency-dependency could increasingly override the effects of underdominance in both pure stand and the competing ability components of fitness in terms of yielding stable nontrivial equilibriums. The significance of such selection models is discussed for the retention of variability in inbreeding populations with a minimum of segregational load and higher overall stability in contrast to the overdominance models.*

Many plant species undergo mixed selfing and random mating; of these the predominant selfers such as barley, lima bean, and wild oat have been investigated recently for the various factors maintaining genetic polymorphism in their populations (*1*). These studies have essentially explored the models involving overdominance (selection favoring heterozygotes) or the so-called marginal overdominance arising from various epistatic situations, genetic homeostasis, or disassortative mating. With overdominance, in particular for the case of predominant selfing, the polymorphic populations at equilibrium involve high segregational load and appear to have restrictive stability conditions in that the fate of polymorphisms is precariously tied with the fitness of a few heterozygous individuals in the population. Harding *et al.* (*2*) reported a case of frequency-dependent selection ensuring a constant hybridity level, although in this case overdominance itself was sufficient for maintaining the locus (*S, s*) polymorphic without frequency-dependency.

Lewontin (*3*) analyzed frequency-dependent selection with a general

Table 1. Notation and recurrence equations.

Genotypes	A_1A_1	A_1A_2	A_2A_2
Relative proportions of genotypes after selection in nth generation	$f_1^{(n)}$	$f_2^{(n)}$	$f_3^{(n)}$
Allelic frequencies	$p^{(n)} = f_1^{(n)} + \frac{1}{2}f_2^{(n)}$, $q^{(n)} = 1-p^{(n)}$		
Zygotic proportions prior to selection in $(n+1)$th generation	$z_1^{(n+1)} = s[f_1^{(n)} + \frac{1}{4}f_2^{(n)}] + tp^{(n)2}$ $z_2^{(n+1)} = \frac{1}{2}s[f_2^{(n)}] + 2tp^{(n)}q^{(n)}$ $z_3^{(n+1)} = s[f_3^{(n)} + \frac{1}{4}f_2^{(n)}] + tq^{(n)2}$		(1)
Relative proportions of genotypes after selection in $(n+1)$th generation	$f_1^{(n+1)} = [x + b_{12}z_2^{(n+1)} + b_{13}z_3^{(n+1)}]z_1^{(n+1)}/\overline{W}$, $f_2^{(n+1)} = [b_{21}z_1^{(n+1)} + 1 + b_{23}z_3^{(n+1)}]z_2^{(n+1)}/\overline{W}$, $f_3^{(n+1)} = [b_{31}z_1^{(n+1)} + b_{32}z_2^{(n+1)} + y]z_3^{(n+1)}/\overline{W}$		(2)

Table 2. Examples of equilibriums, and the parameters giving the genotypic composition and marginal fitnesses (W_i); (initially $p = 0.2$, $F = 0$).

s	x, y	N	Genotypic proportions			p	F	$\overline{W}_1$	$\overline{W}_2$	$\overline{W}_3$
			f_1	f_2	f_3					
0.0	0.4, 0.5	53	0.252	0.521	0.227	0.513	−0.042	0.704	0.760	0.697
	.5, .5	98	.479	.442	.079	.700	− .052	.670	.721	.601
	.6, .5	67	1.0	0	0	1.0	1.0			
	.4, .6	180	0.128	.421	.451	0.339	0.060	.843	.711	.779
	.5, .6	102	.345	.482	.174	.586	.008	.750	.741	.755
	.6, .6	46	1.0	0	0	1.0	1.0			
0.95	0.7, 1.1	66	0.692	0.016	0.292	0.700	0.962	0.923	0.508	0.935
	.8, 1.1	177	.935	.005	.060	.937	.959	.846	.502	.869
	.9, 1.1	83	1.0	0	0	1.0	1.0			
	.8, 1.3	89	0.468	.014	.518	0.475	0.972	1.192	.507	1.190
	.9, 1.3	85	.712	.012	.277	.718	.971	1.110	.506	1.128
	1.0, 1.3	288	.963	.002	.035	.964	.968	1.027	.501	1.060
	1.1, 1.3	60	1.0	0	0	1.0	1.0			

method of finding equilibrium properties and clearly established that, at a stable equilibrium under random mating, the marginal selective values could show underdominance. Sakai (*4*) and, more recently, Schutz *et al.* (*5*) investigated models involving the so-called pure reproductive values of genotypes and their competing ability in mixtures as two identifiable components of relative fitnesses in the heterogeneous populations. Frequency-dependency was invoked by way of weighting the competitive abilities with the relative proportions of various "neighbor pairs." There is now available ample evidence from competition studies that such ecological-genetic models are realistic, widespread, and indeed permit various selective strategies associated with the different competing genotypes. In this report we describe a simulation study of models involving frequency-dependency and underdominance at a marker locus segregating in large inbreeding populations. The model is described by the recursion equations for genotypic frequency changes at a locus (A_1, A_2), developed by combining Eqs. 1 and 2 given in Table 1, where (x, 1, and y) are pure stand fitness values; b_{ij} are competing abilities of ith genotype in competition wth jth genotype, as defined by Schutz *et al.* (*5*); $\bar{W}$ is the usual normalizing factor to give $\Sigma f_i = 1$; and s and t are the relative proportions of selfing and random mating, respectively. Thus, selection is assumed to occur only during the zygotic stages and, therefore, male and female gametic arrays are identical. As noted by Jain and Workman (*6*), we can reduce these recurrence relations to two simultaneous equations in allelic frequency, p, and fixation index, $F = 1 - f_2/2pq$, to be solved for $\Delta p = \Delta F = 0$ at genotypic equilibriums. Iterative solutions by Monte Carlo procedure were obtained for various combinations of selective values (x, y, b_{ij}), defining $\Delta f_i < 10^{-5}$ as equilibrium, or for regions of fixation $p > 0.99995$ or < 0.00005 taken as $p = 1$ or 0, respectively (to allow for round-off errors).

Consider the underdominance model of competing abilities given by the following set of b_{ij}'s:

$$b_{12} = \tfrac{1}{4},\ b_{21} = -\tfrac{1}{2},$$
$$b_{13} = \tfrac{3}{4},\ b_{31} = -\tfrac{1}{4},$$
$$b_{23} = -\tfrac{1}{2},\ b_{32} = \tfrac{1}{2},$$

and (x, y) in the range of 0 to 2 ($x, y > 1$, underdominance; $x, y < 1$, overdominance). In Hayman's model (*7*), $x, y < 1$ and for all $i \neq j$, $b_{ij} = 0$. Computer runs for various combinations allowed us to determine the range of selection parameters allowing nontrivial equilibriums ($0 < p < 1$, $F < 0$ in region C, $F > 0$ in region D, and $p = 1$ and 0 for regions A and B, respectively) as shown in the phase diagrams (Fig. 1, $s = 0$, $s = 0.2$; Fig. 2, $s = 0.8$, $s = 0.95$) for the underdominance model as given by the above b_{ij} set. Note the following three significant points: (i) region C of stable nontrivial equilibriums becomes larger as s increases; (ii) underdominance in both pure stand (x, y) and competing ability (b_{ij}) allows large regions for such equilibriums under high values of s with frequency dependency overriding the dispersive effect of underdominance; and (iii) asymmetry in b_{ij} is balanced by the asymmetrical (x, y).

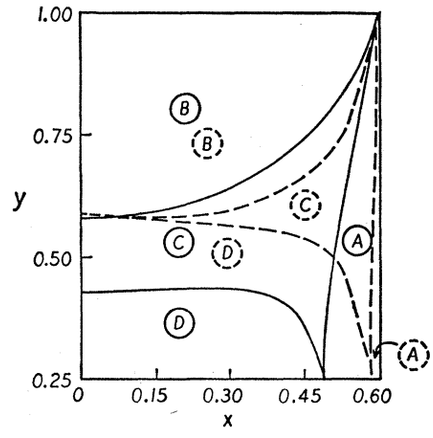

Fig. 1. Phase diagrams for underdominance model showing regions A ($p = 1$), B ($p = 0$), C ($0 < p < 1$, $F < 0$), and D ($0 < p < 1$, $F > 0$) in terms of bounds on (x, y) giving various equilibriums. Two levels of selfing are given by $s = 0$ (------) and $s = 0.2$ (——).

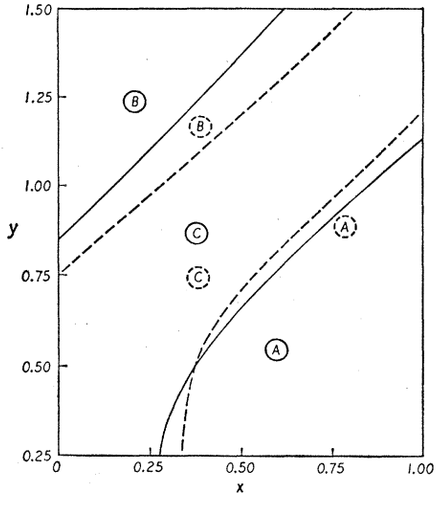

Fig. 2. Phase diagrams for underdominance model showing equilibrium conditions for $s = 0.80$ (------) and $s = 0.95$ (——).

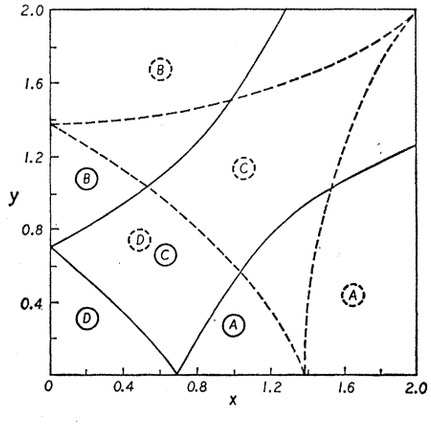

Fig. 3. Phase diagrams for Clarke-O'Donald model showing equilibrium conditions for $s = 0.20$ (------) and $s = 0.95$ (——).

Table 2 gives some examples of parameters describing equilibriums for a few points chosen in the C region. Note that the negative or positive values of F under $s = 0$ indicate an excess or deficiency of heterozygotes, respectively, which reflect marginal over- or underdominance as also indicated by the values of marginal fitness, $\bar{W}_i$, defined so that $f_i^{(n+1)} = f_i^{(n)} \bar{W}_i^{(n)} / \bar{W}^{(n)}$. For $s = 0.95$, without selection, F [$F = s/(2 - s)$] is expected to be 0.905 but note that F is larger for all sets given here; clearly, underdominance is not playing any role in terms of the position of boundaries of C region. Under heavy inbreeding, this model is robust in the sense that genetic variation is maintained in the form of homozygotes rather than heterozygotes, which results in minimum segregational load and highly stable equilibriums in the face of stochastic variations. In fact, the estimates of selective values (x, y) from certain sets of equilibrium frequencies, ignoring frequency-dependency, would give $x, y > 1$ [for example, see (*10*)]. In such cases one might rule out prima facie the maintenance of polymorphisms. Note that in barley, wild oat, and several other species perhaps such cases were present but were otherwise presumed to approach fixation. Also, in the study of Harding *et al.* (*2*) a higher outcrossing rate could be assumed, and yet the model discussed above can account for the observed results on the frequency-dependent selective values of heterozygotes. Frequency-dependent t could also be invoked here (*8*).

Under a frequency-dependency model (*9*) in which the selective values are given by $w_1 = x(1 - k_1 f_1)$, $w_2 =$

$(1 - k_2 f_2)$, and $w_3 = y\,(1 - k_3 f_3)$, we showed earlier that the regions allowing stable polymorphism are increasingly larger for overdominance models and higher values of s (for example, see Fig. 3) (k_i, constants to make w_i, selective values, functions of the frequencies f_i). Computer simulation of models involving overdominance in b_{ij} values also gave significantly wider regions for heavy inbreeding. However, in nature the underdominance models discussed above cannot be assumed a priori to be any less frequent than the very widely hypothesized overdominance situations. The role of frequency-dependency merits further detailed analyses in terms of both genetic and ecological aspects of variation in natural populations. Some recent developments in *Drosophila* research (*10*) indeed clearly attest this viewpoint.

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Azotobacter Cysts: Reactivation by White Light after Inactivation by Ultraviolet Radiation

Abstract. *Cysts of* Azotobacter vinelandii *12837 inactivated by ultraviolet radiation can be reactivated by white light. This photoreactivation mechanism is not seen in the vegetative cells of the same organism.*

Harm and Rupert (*1*) propose that photoreactivation in bacteria exposed to ultraviolet radiation is brought about by a photochemical reaction involving a photoreactivating enzyme. They state that the reaction is characterized by the Michaelis–Menton equation for enzyme mediated reactions:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where E is the photoreactivating enzyme; S is repairable ultraviolet-damaged DNA; and P is repaired DNA. Photoreactivation in many different organisms results in a dose-reduction phenomenon. Harm *et al.* (*2*) state that this dose-reduction parameter is determined by k_3, which is a light-dependent photolysis of the photoreactivating enzyme–DNA complex resulting in repaired DNA. This implies that in the absence of the photoreactivating enzyme (*3*) or light, repairable ultraviolet-damaged DNA will not be repaired, and the cell will be damaged. There are three assumptions in this theory: (i) the concentration of photoreactivating enzyme is not limiting within the doses of ultraviolet used; (ii) the activity of photoreactivating enzyme does not vary; and (iii) the reaction rate constants do not change. If these three assumptions are correct, the effect of photoreactivation is that of dose reduction as commonly seen.

Azotobacter lends itself well to certain types of experiments (*4*). These organisms form cysts which are not totally inactive metabolically; that is, they will oxidize substrates but they probably do not synthesize proteins (*5*). We assumed that these cysts would undergo photoreactivation as proposed (*1*) only if they were metabolically active. Photoreactvation of the *Azotobacter* cysts has not been reported. Washed vegetative cells of *A. vinelandii* 12837 grown in Burk's medium with 1 percent glucose for 18 hours in cultures at 30°C were placed in petri plates to a layer depth of less than 1 mm, exposed to a flux of light of approximately 2537-Å wavelength and approximately 100 erg mm^{-2} sec^{-1}, and kept constantly in motion to insure uniform exposure. Photoreactivation was obtained by subjecting the irradiated bacteria, in test tubes, to the light emitted by a 500-watt Sylvania Photoflood lamp located approximately 10 cm from the tubes in a running-water bath at 12° to 15°C. Controls were subjected to identical manipulations, except that the tubes were wrapped in aluminum foil. Survival rates were obtained by plate counts on Burk's medium after the cultures were incubated for 48 to 72 hours at 30°C.

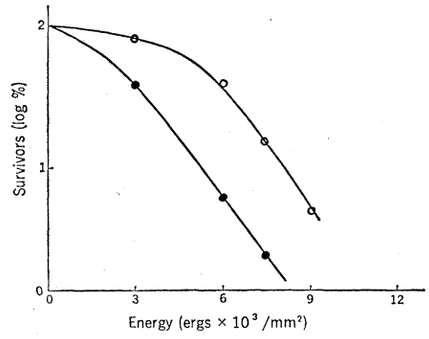

Fig. 1. Ultraviolet inactivation (solid circles) and photoreactivation (open circles) of vegetative cells of *Azotobacter vinelandii* 12837.

Photoreactivation of the vegetative cells follows a simple dose-reduction mechanism and our assumptions are tenable (Fig. 1). The coefficients of regression of the rectilinear portions of these lines are -0.03 (-0.0302) for the death curve and -0.03 (-0.0300) for the photoreactivation curve.

The organism was induced to encyst by culturing it on plates of Burk's medium containing 0.2 percent *n*-butanol in lieu of glucose. The cysts were treated in the same manner as were the vegetative cells. Cysts of *A. vinelandii* 12837 could be photoreactivated (Fig. 2) in the same manner as the vegetative cells of the same organism could. Further analysis of photoreactivation of the cysts showed a higher rate of photoreactivation at higher ultraviolet doses. The inactivation curve has a slope of -0.05 (-0.0481), and the photoreactivation curve has a slope of -0.02 (-0.0198). Photoreactivation of cysts of this organism does not represent the classical dose-reduction phenomenon but rather an ultraviolet-enhanced photoreactivation. The kinetics of cyst photoreacti-

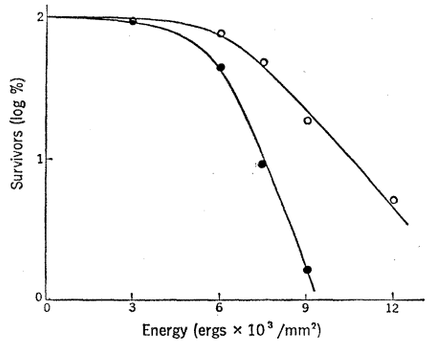

Fig. 2. Ultraviolet inactivation (solid circles) and photoreactivation (open circles) of cysts of *Azotobacter vinelandii* 12837.