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RESPONSE SURFACE METHODS OF FITTING

STOCHASTIC BIOLOGICAL MODELS

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CATHERINE ANN MACKEN

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ABSTRACT

Response surface methods are discussed, with emphasis on the particular experimentation problems encountered in their use. A brief outline of simulation and modelling is given. This includes an indication of the role of randomness.

Two specific uses of computer simulation of biological phenomena are considered. The first is fitting growth curves to some cell growth data. This was done largely to develop techniques. The second and more significant use is in fitting stochastic selection values to some genotypic frequency data. To date, only deterministic estimates have been found from this data.

Attention is given to the careful design of simulation experiments, in order to reduce the number of simulation runs needed. Response surface methods were used and proved to be efficient experimentation techniques.

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## INTRODUCTION

A lack of exact analytical solutions to a mathematical system implies that numerical methods are needed to be able to study the system.

Simulation is the technique of imitating as best as possible the behaviour of a system. Using a mathematical model of the system, the experimenter can observe the effect that a different set of parameter values has on the outcome of the model by running a simulation trial using those parameter values. This technique has become practical since the advent of high speed computers. Stochastic models which previously defied solution by the mathematical analysts can now be studied by simulation.

The experimenter generally aims to estimate those values of the parameters which make the model as close to the real life situation as possible. Some criterion is needed for stating just how close the model is. If the simulated data is compared with observed data from the real life system, then the distance between them would be a measure of the goodness of fit of the model. The experimenter thus wishes to estimate those values of the parameters which make the distance as short as possible. An average distance must be taken to account for the variation in a stochastic model.

Thus experimentation, particularly on a stochastic model, involves many simulation runs. It is desirable to keep the number of runs down as much as possible. This means that it is important to plan experimentation so that the least distance is found with maximum efficiency. There are several alternative plans of experimentation available. Response surface methods were chosen for the following study since they involve experimental designs which are economical of simulation runs.

## Literature Survey

### Hoel and Mitchell

Hoel and Mitchell's (1971) paper first brought to notice the problem of fitting stochastic models using response surface methods. They proposed three competing stochastic models for the growth of a cell population and studied the goodness-of-fit of each model to the experimental data by measuring the sum of the squared differences between the simulated trials and the experimental data. They viewed the expectation of this distance as a response surface over the parameter space of the model, then using response surface methods optimized the fit of the model. The competing models were fitted to some data of Kubitschek (1962) on the growth of colonies of E.coli cells.

### Response Surface Methods

A variable classified as a response can be explained or predicted by means of a functional relationship with a prespecified number of independent variables called factors. The functional relationship defines a response surface and measures of the response taken at different factor levels are points on this surface. Response surface methods provide a means of studying the functional relationship.

Initial interest in the use of response surface methodology was generated by Box and Wilson (1951). They first set forth the fundamentals and underlying philosophy of the use of this package of techniques and Box (1952) later extended this work for linear models. Davies (1956) edited an important textbook with a chapter dealing with the exploration of response surfaces.

There has been extensive development of second order designs. Box and Hunter (1957) studied rotatable second-order designs in general and central composite designs in particular. Hunter (1954)



discussed the problem of finding a stationary point on a fitted second-order response surface and pointed out that a general second-order response surface could be transformed into a canonical form. Box and Hunter (1954) developed a method of setting a confidence region on this stationary point.

Box and Draper (1959) considered the problem of choosing a design such that a polynomial of degree  $d_1$  might be most closely fitted to a response surface whose true representation is a polynomial of degree  $d_2 > d_1$ . Subject to this condition they chose their designs such that inadequate fit of the closest possible polynomial representation had a high chance of detection.

Since Box and Draper's, many other papers have been published on this subject. Hill and Hunter (1966) gave a review of the literature with particular emphasis on applications of the methodology. More recent publications were by V.J. Thomas (1971) who, in his M.Sc. thesis, concentrated on second-order designs including conditions for orthogonality of estimates; and by Myers (1971) whose textbook gave a comprehensive study of response surface methodology.

#### Response Surface Methods and Simulation

Modern use of the word 'simulation' traces its origin to the work of von Neumann and Ulam in the late 1940's when they coined the term "Monte Carlo analysis" to apply to a mathematical technique they used to solve certain involved nuclear-shielding problems. An interesting history of the technique is given in Hammersley and Handscomb (1964). In the early 1950's, the advent of high speed computers made simulation much more feasible. It is now a standard technique dealt with in many texts, including that of Naylor, Balintfy, Burdick and Chu (1966).

Computer simulation techniques have made it possible to perform a type of pseudo-experiment in areas where real-world experiments were otherwise

impossible or impractical.

Simulation has also enabled study of models for which the nature of the model as much as the nature of the equations prohibits analytical solution of the equations. Such a situation may arise, for example, upon introduction of stochastic variation to parameters of a model, thus making closed forms for maximum likelihood parameter estimates not only difficult but no longer possible to obtain.

Hence an increasing concern with experimental design, response surface methods in particular.

Hufschmidt (1962) analysed, using response surface methods, the response surface obtained from simulation of a simplified river-basin system. He gave in detail an account of the complete experimental plan undertaken. Burdick and Naylor (1969) gave a general discussion of response surface methods applied to problems in Economics. They used simulation to study a model in a situation where real-world experiments would not have been feasible. Hoel and Mitchell (1971) used simulation and response surface methods to fit a model to some experimental data. Hunter and Naylor (1970) referred to a specific example in order to discuss in detail the experimental design problems encountered when using simulation to explore response surfaces.

### Selection

Allard, Kahler and Weir (in press) used genotypic frequency data from barley populations to obtain maximum likelihood estimates of selective values. They made selective value estimates from a pair of consecutive generations, then averaged these estimates over several pairs of generations.

The next step might be to study the effect of allowing stochastic variation of selective values. Jain and Marshall (1968) reviewed

the literature and found support for the idea of varying selection values. They examined by means of computer simulation the effect on genotypic equilibria of random fluctuations from generation to generation in selective values. They concentrated on values distributed according to a normal distribution. Barker and Butcher (1966) also studied the effect of generation - to - generation fluctuations in selective values. They chose selective values from a uniform distribution and, using simulation, observed quasi-fixation of genes in a population.

## RESPONSE SURFACE METHODS

### Basic Concepts

It is assumed that the experimenter is concerned with a system involving some response  $\eta$  which depends on input variables  $\xi_1, \xi_2, \dots, \xi_k$ . These, the natural variables, should be distinguished from the coded or design variables, the latter ( $x_i$ 's) normally being simple linear functions of the former.

For example, if the experimenter wishes  $\xi_i$  to take a maximum value of  $\xi_{imax}$  and a minimum value of  $\xi_{imin}$  with  $n$  equally spaced values  $\xi_{iu}$  ( $u = 1, 2, \dots, n$ ) between, then a common linear function is

$$x_{iu} = \frac{\xi_{iu} - \frac{1}{2}(\xi_{imax} + \xi_{imin})}{(\xi_{imax} - \xi_{imin})/(n-1)}$$

where  $x_{iu}$  is then called the  $u^{\text{th}}$  level of factor  $i$

and  $\xi_{iu}$  is the value of the natural variable to which this factor level corresponds.

It is further assumed that the  $\xi_i$ 's can be controlled by the experimenter with negligible error.

In general, the response function can be written

$$\eta = f(\xi_1, \xi_2, \dots, \xi_k)$$

where the form of  $f$  is unknown and perhaps extremely complicated. The response surface is defined by  $f$ . The success of response surface methods depends on the approximation of  $f$  by a low order polynomial in some region of the independent variables.

The experimenter is, then, generally interested in finding in the smallest number of experiments (1) what value of the factors are optimum as far as the response is concerned, and (2) a suitable approximating

function to  $f$  for the purpose of predicting future response.

Now, a suitable approximating function can be obtained by applying the Taylor series expansion to  $f$  around the origin (in factor-level notation the origin is  $x_1 = x_2 = \dots = x_k = 0$ ).

Then

$$f = f_{\underline{\underline{x}}=\underline{\underline{0}}} + \sum_{i=1}^k \left( \frac{\partial f}{\partial x_i} \right)_{\underline{\underline{x}}=\underline{\underline{0}}} x_i + \sum_{i,j=1}^k \left( \frac{\partial^2 f}{\partial x_i \partial x_j} \right)_{\underline{\underline{x}}=\underline{\underline{0}}} x_i x_j + \dots$$

This series may be truncated at any point to give any desired closeness of fit (i.e. approximation) to the surface. The truncated polynomial is then called the fitted surface.

A first order model of the response function would be

$$\eta = f_{\underline{\underline{x}}=\underline{\underline{0}}} + \left( \frac{\partial f}{\partial x_1} \right)_{\underline{\underline{x}}=\underline{\underline{0}}} x_1 + \left( \frac{\partial f}{\partial x_2} \right)_{\underline{\underline{x}}=\underline{\underline{0}}} x_2 + \dots + \left( \frac{\partial f}{\partial x_k} \right)_{\underline{\underline{x}}=\underline{\underline{0}}} x_k$$

or, in the usual notation

$$\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k$$

where

$$\beta_0 = f_{\underline{\underline{x}}=\underline{\underline{0}}}; \quad \beta_i = \left( \frac{\partial f}{\partial x_i} \right)_{\underline{\underline{x}}=\underline{\underline{0}}}$$

The first order model is often useful when the experimenter is interested in studying  $f$  in narrow regions of  $x_1, x_2, \dots, x_k$ ; that is, where little curvature in  $f$  is present. In a wider area it provides a rough approximation to the surface. The experimenter might use a second order approximating function to study the shape of the surface more closely.

$$\text{e.g.} \quad \eta = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i,j=1}^k \beta_{ij} x_i x_j$$

where  $\beta_0$  and  $\beta_i$  are as before, and

$$\beta_{ij} = \frac{1}{2!} \left( \frac{\partial^2 f}{\partial x_i \partial x_j} \right)_{\underline{\underline{x}}=\underline{\underline{0}}}$$

The response surface study can now be thought of as being one in which the topography of an area is being explored. The top of a "hill" or "mound" represents a point of maximum response. The bottom of a "valley" represents a point of minimum response.

At times, models of order greater than two are used.

### Experimentation

While the investigation would be planned so that experimental runs were made in the supposed region of optimum response, the experimenter often starts his work with complete ignorance of the proper region. In this case, the experimental plan would develop into a sequential determination. Some starting point is chosen. This point will most probably be remote from the optimum. From here, the experimenter can systematically work his way towards the desirable conditions. The experimental region is then in the general vicinity of the optimum and analysis of the fitted surface - probably using a second order model - can proceed.

The experimenter is immediately confronted with the problem of choosing a starting point. To aid the selection, a grid search could be performed. Each factor is allowed to vary over a specified range of levels. All possible combinations of factors at their various levels are then tested and the point of lowest response can be used as a starting point for further experimentation.

But how are the range of factor levels to be chosen? From his experience with the system being studied, it would be hoped that the experimenter could narrow down the entire parameter space to some region of operability i.e. the parameter values would have to lie within the bounds of this region for experiments to take any real meaning. The experimenter would then again be looked to, for guidance in choosing a sub-region of this region of operability. The sub-region, called the

region of interest, would specify the range of parameter values within which interest is confined and the optimum was likely to occur. Experimentation would then be concentrated on this smaller sub-region.

A grid search should cover the region of interest. Thus the problem of range of factor levels is solved. But how many points should be included in the grid? The first problem to note is that the response function is generally stochastic in nature. The observed response,  $y$ , would then be subject to unavoidable uncontrolled factors and would vary in repeated observations, having mean  $\eta$  and variance  $\sigma^2$ . If the grid search covered the region minutely, stochastic variation would conceal true differences in response. Groups of experiments would give similar responses, wasting information from many of the grid points. Should the response be deterministic, a close grid would accurately determine a point of low response. The number of experiments needed would be enormous, but the experimenter would have the advantage of a clear idea of the relationship between the response and the factors. For more than one or two factors though, it would be quicker to perform fewer grid experiments and concentrate on experiments to lead toward the optimum. Unfortunately, an open grid search over a deterministic or stochastic response does introduce the possibility of reaching a local optimum only.

The initial point of lowest response chosen from the grid can be used as a centre about which to concentrate further experimentation. Unless the experimenter knows otherwise (from prior information about the system), he must assume that he is remote from the true optimum and must aim to estimate the line leading to a better response.

The steepest descent procedure<sup>1</sup> is a method whereby the experimenter proceeds sequentially along the path of steepest descent, that is, along

1. The discussion following refers to seeking a minimum response. The process is similar, but with signs reversed, for seeking a maximum response.

the path of maximum decrease in response, according to the following steps:

- (1) fit a first order model about the centre point,
- (2) use the information from step 1 to locate a path of steepest descent,
- (3) conduct experiments along the path until no further decrease in response is evident,
- (4) steps 1, 2 and 3 are then repeated using the point of minimum response on the line as the centre of the design for fitting the next first order model,
- (5) if lack of fit of the first order is significant, then fit a second or higher model and analyse the fitted surface.

Discussing each step in turn:

- (1) It is extremely important that a decision be made at the outset regarding what experimental design points are to be used. These design points give the factor levels to be used in experimentation. The coefficients  $\beta_0, \beta_1, \dots$  in the models given above are estimated from data taken by the experimenter. Good experimental design will accomplish the estimation with maximum effectiveness.

A simple  $2^k$  factorial design will estimate the  $\beta_i$ 's. A fractional factorial may be more economical with design points yet still give estimates of the  $\beta_i$ 's - especially as the number of factors under study increases. If  $b_0, b_1, \dots$  are estimates of  $\beta_0, \beta_1, \dots$ , then the first order response function is

$$\hat{y} = b_0 + \sum_{i=1}^k b_i x_i$$

The decision regarding which experimental design is used is often a very critical one. Variances of the estimates of  $\beta_0, \beta_1, \dots$  are dependent on the design, and are minimized when the "spread" of the points in the design is greatest. However, in many cases, a model is assumed which is not an adequate approximation to the true system mechanism. As a result, the model coefficients are biased by terms that are of



order higher than the order of the assumed model. The extent of these biases can be altered by the choice of design. As spread of the design points is increased to minimise variance, so bias increases, since the fitted surface is less capable of giving an adequate representation of the true response surface. Bearing these two considerations in mind, the experimenter must choose the design which best suits his particular situation.

The choice of step sizes i.e. "spread" of design points, is thus far from straightforward. Minimum variance or minimum bias imply two opposing design objectives. Minimum variance of the  $b_i$ 's requires a large, "spread-out", design, but minimum bias requires a small, close, design. The lack of fit with a larger design is more significant.

Step sizes should also be chosen to ensure that the  $b_i$ 's are of approximately the same order of magnitude. Then the effects of all factors are nearly equal. (If little change in effect is produced by an increased step size, then the factor may have negligible effect. If increased step size produces a large change in effect, then the factor is near its optimum value. In this case the step size should be left small.)

(2) To understand the need for approximately equal  $b_i$ 's, consider the first order model again.

$$\hat{y} = b_0 + \sum_{i=1}^k b_i x_i$$

This is a planar approximation to the response surface. Movement along the steepest slope of this fitted plane would produce the greatest change in response. It can be shown (see Myers (1971), for example) that the steepest slope of the plane is traced out by the points with co-ordinates  $(x_1, x_2, \dots, x_k)$  in the ratio  $(b_1, b_2, \dots, b_k)$ . If any of the  $b_i$  are very small relative to the others, successive planar approximations in the steepest descent procedure will lead the experimenter in a "zig-zag"

fashion towards the optimum. Progress to the optimum will be slower, with the factor corresponding to the small  $b_i$  moving steadily toward the optimum, but other factors overshooting the optimum. Step sizes corresponding to those factors with large  $b_i$ 's will be large relative to step sizes corresponding to those factors with small  $b_i$ 's.

(3) In general, it is found that experiments should be conducted along the path of descent until two successive experiments give an increased response. At this stage, it can safely be assumed that further movement in the same direction would not produce any decrease in response. The step size should then be reduced and experimentation concentrated about the point of lowest response on the steepest descent line. A good plan for one factor,  $x_1$ , would be that shown in figure 3.1.

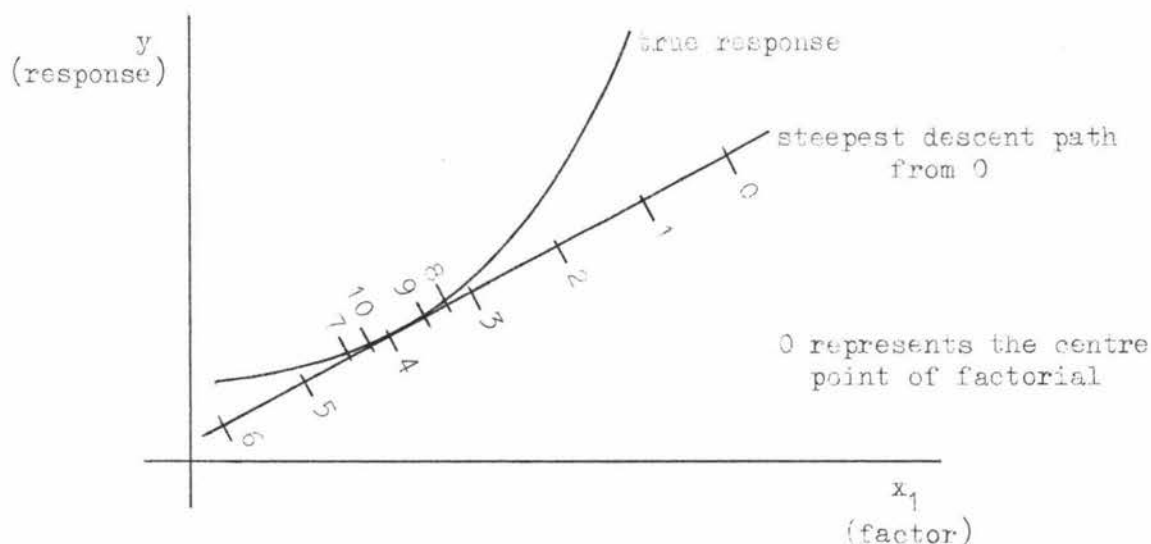


Figure 3.1 Path of Steepest Descent

By continually halving the step size, then conducting two more experiments - one on each side of the current minimum response on the line of steepest descent - the experimenter will "spiral in" on the optimum value on this line. To decide when to stop "spiralling", the experimenter should consider the desired accuracy of the final estimate of the effects. If all effects are wanted to two decimal place accuracy, then an error of  $\pm 0.01$  in every effect will cause, at most, an error of  $\pm (0.01 \sum_{i=1}^k |b_i|)$  in the response. Hence, experimentation should

continue until a change in response of only  $\pm (0.01 \sum_{i=1}^k |b_i|)$  occurs.

When first setting out on the path of steepest descent, step sizes are fairly arbitrary but from experience it seems wise to ensure that the factor changing least has a step size smaller than the step size of the factorial design.

(4) A new first order design can then be fitted with its centre at the point of minimum response found in step (3). The experimenter then returns to step (1) and repeats the process.

(5) Eventually the lack of fit of the first order model will become significant. This lack of fit can be tested in an analysis of variance. First order effects become negligible or interactions become significant. (The factorial design should allow at least some of the interactions to be estimated for just this check.)

In any case, experiments along the line of "steepest descent" will produce no evident decrease in response. The analysis of variance method is to be preferred however, since then no experiments will be wasted in testing for lack of fit.

If the experimenter follows the theory below, information from the design points used to set up this last first order design can be absorbed into the next experimental outlay. Once again, no experiments will be wasted.

The experimenter must now fit a higher order model in order to better approximate the surface. The next step up from a first order model is a second order model. A second order approximation to the response function would be

$$\hat{y} = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i,j=1}^k b_{ij} x_i x_j$$

where  $b_i$  estimates  $\beta_i$

and  $b_{ij}$  estimates  $\beta_{ij}$ .

Experimental designs for fitting a second order response surface

must involve at least three levels of each variable so that the  $2k + \binom{k}{2} + 1$  coefficients in the model can be estimated. The obvious choice of design would be a  $3^k$  factorial. However, for  $k > 3$  the number of observations required are far in excess of the number of parameters to be estimated.

Box and Wilson (1951) introduced a workable alternative to the  $3^k$  factorial system through the development of central composite designs. They are first order designs augmented by additional axial points to allow estimation of the coefficients of second order designs. The experiments of the first order design are still used - there is no wastage.

The axial points have co-ordinates  $(x_1, x_2, \dots, x_k)$  where  $(x_1, x_2, \dots, x_k)$  has the form

$$\begin{aligned} & (\pm\delta, 0, \dots, 0) \\ \text{or } & (0, \pm\delta, \dots, 0) \\ & \dots\dots\dots \\ \text{or } & (0, 0, \dots, \pm\delta) \end{aligned}$$

Thomas (1971) showed that, by careful specification of  $\delta$ , any orthogonal first order design can be augmented in this manner to form an orthogonal second order design. Orthogonal estimates of the  $\beta$ 's are then possible. These axial points are essential for non-singularity of the second order design matrix.

In particular, if  $\delta$  is chosen such that

$$\sum_u x_{iu}^2 \sum_u x_{ju}^2 = n \sum_u x_{iu}^2 x_{ju}^2 \quad (i \neq j)$$

where  $u$  is the level of the factor

and  $n$  is the total number of points in the design;

and if the quadratic terms are transformed to the new variables  $\zeta_i$  according to

$$\zeta_{iu} = x_{iu}^2 - \frac{1}{n} \sum_u x_{iu}^2$$

then  $\xi_i$  will be orthogonal to the mean, the first order terms and the interaction terms. The "mean"  $b_0^*$ , as estimated from this central composite design, corresponds to

$$b_0 + \frac{1}{n} (\sum_i (\sum_u x_{iu}^2))$$

where  $b_0$  is the true mean of the design.

Centre points may be added, if the response is stochastic, to obtain an estimate of experimental error. This will not affect orthogonality provided the value of  $\delta$  is adjusted accordingly.

An added advantage of using central composite designs is that they are also rotatable. The experimenter does not know before his experiment is run, what will be the orientation of the system. A rotatable design estimates the response with the same precision at all points equally distant from the centre of the design. If this were not the case, there would be a certain "imbalance" in the reliability of experimental results from equidistant points in different directions from the centre.

To analyse the shape of the surface, the second order model can be expressed in its canonical form.

$$\text{e.g. } \hat{y} = \lambda_1 Y_1^2 + \lambda_2 Y_2^2 + \dots + \lambda_k Y_k^2$$

Standard texts, for example Myers (1971), describe the necessary techniques of translation and rotation of axes to transform from the original model to this form.

The experienced experimenter can learn much from canonical analysis of the fitted surface. Davies (1956) and others give useful assistance. For example, suppose a three factor model had the canonical form

$$\hat{y} = 16.3Y_1^2 + 3.9Y_2^2 + 0.1Y_3^2$$

then (i) all  $\lambda_i > 0$  implies the contours of constant response are

ellipsoidal. Any movement away from their centre ( $y_1 = y_2 = y_3 = 0$ ) would result in an increase in  $\hat{y}$ .

(ii)  $\lambda_3 = 0.1$  is very small compared to  $\lambda_1 = 16.3$  and  $\lambda_2 = 3.9$ .

The contours are attenuated along the  $Y_3$  axis, and movement along this axis would result in very little increase in  $\hat{y}$ .

(iii) the effect of factor  $Y_3$  is almost negligible.

Further experiments could be performed along the  $Y_3$  axis, and information from these included to estimate more accurately the  $\lambda_i$ 's (see Box and Wilson (1951)).

If the stationary point of the fitted surface is estimated in conjunction with the canonical analysis, then more still can be learnt. For example, the experimenter may find that the stationary point is remote from the design. But the fitted model only has meaning in the region of the design and will not provide a meaningful estimate of the co-ordinates of the stationary point or the corresponding response if they lie outside the region. The experimenter must move closer to the optimum. A useful plan is to conduct experiments along the canonical axis giving the greatest decrease in response (if the minimum is sought).

But how is this stationary point found?

From the second order model

$$\hat{y} = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i,j=1}^k b_{ij} x_i x_j$$

differentiating with respect to  $x_1, x_2, \dots, x_k$  in turn will give a stationary value. Whether it be a maximum or minimum depends on whether the matrix of second derivatives of  $\hat{y}$  with respect to  $x_1, x_2, \dots, x_k$  is negative definite or positive definite respectively.

Solving

$$\begin{bmatrix} 2b_{11} & b_{12} & b_{13} & \dots & b_{1k} \\ b_{12} & 2b_{22} & b_{23} & \dots & b_{2k} \\ b_{13} & b_{23} & 2b_{33} & \dots & b_{3k} \\ \dots & \dots & \dots & \dots & \dots \\ b_{1k} & b_{2k} & b_{3k} & \dots & 2b_{kk} \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ \dots \\ x_k \end{bmatrix} = - \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ \dots \\ b_k \end{bmatrix}$$

will thus give the stationary point.

This stationary point analysis is a very important part of the final analysis of the shape of the surface.

As a final word of caution to the experimenter, care must be exercised in choosing a step size for the design to estimate the parameters of this second order model. If the step size is too small, the stationary point will frequently lie outside the region of the design, particularly if the response function is stochastic. In spite of this, the experimenter may be very close to the optimum, so close that any attempt to conduct experiments toward the optimum would fail due to stochastic variation concealing actual changes in response.

If the step size is too large, an estimate of the expected response at the stationary point will be very inaccurate.

It is very difficult to state a criterion for step size. Behaviour of the response function along the immediately previous steepest descent could guide the experimenter, but experience seems to be the best judge. Since it is not known at what stage the first order factorial design will have to be augmented, the experimenter must bear the problem in mind at all times.

## SIMULATION AND MODELLING

### Basic Concepts

Simulation is essentially a working analogy. Analogy means similarity of properties or relations, without identity. When analogous systems can be constructed, measurements or observations made on one of these systems may be used to predict the reaction of the others. Simulation involves the construction of a working mathematical model presenting this similarity of properties or relationships with the natural system under study. New models generally need to be made to fit a specific situation with the required precision.

A model is a set of abstractions from the characteristics of a real system. It must incorporate most of the useful aspects of a system but without becoming so complex that it is difficult to understand and manipulate.

Once the model has been defined in mathematical terms it can be investigated by simulation techniques. Because of the complexity of the system studied, analog and digital computers are almost always necessary for simulation studies. Computer simulation is restricted to logical and mathematical models, whose greatest advantage lies in their ability to provide precise quantitative predictions while still encompassing the intricacies of the real world.

Rationale for computer simulation would be:

- (1) running experiments on models involving stochastic parameters.
- (2) solving deterministic mathematical problems which cannot be solved easily (if at all) by strictly deterministic methods. It may be possible to obtain approximate solutions to these problems by simulating a stochastic process with statistical properties satisfying the functional relationships or solution requirements of the problem.



When the system under study is variable, one method of observing variability in the model is by random sampling. A simulation is essentially a random sample of outcomes of the model. Since a variable model is the result of stochastic parameters, a random sample of outcomes can be drawn by randomly choosing values for these parameters from the probability distribution functions which define their variation. This is Monte Carlo sampling. Monte Carlo methods have been developed for simulating most of the well-known probability distributions as well as any empirical distribution.

The Monte Carlo method is therefore a simulation technique for problems having a probabilistic or stochastic basis - solution of probability problems by practical methods involving sampling experiments.

Main features of the Monte Carlo method are:

- (1) since the process involves random sampling, a ready supply of random elements must be available to a user of the technique.
- (2) the random samples are taken from the probability distribution function of the parameters concerned.
- (3) to get a good estimate of the expected outcome of the model, many samples must be taken. Repetition of the sampling process implies that a large number of random variates is required per simulation.

The essence of good simulation by Monte Carlo methods thus lies in a good source of random numbers.

The problem of sampling from any distribution is that of transforming a random number representing the uniform  $[0, 1]$  variate, (which most random number generators will generate), by means of the inverse cumulative distribution function, since  $F(x)$  (the cumulative distribution function of  $X$ , a random variable) has a uniform distribution on the interval  $[0, 1]$ .

To prove this let  $Y = F(X)$

$$\begin{aligned}
 \text{then prob } (Y \leq y) &= \text{prob } (F(X) \leq y) \\
 &= \text{prob } (X \leq F^{-1}(y)) \\
 &= F(F^{-1}(y)) \\
 &= y
 \end{aligned}$$

$$\begin{aligned}
 \text{hence prob } (Y \leq y) &= 0 \quad \text{if } y < 0 \\
 &= y \quad \text{if } 0 \leq y \leq 1 \\
 &= 1 \quad \text{if } 1 \leq y
 \end{aligned}$$

So provided a value can be drawn that is randomly distributed on  $[0, 1]$ , then it can be transformed to find any (continuously) distributed random variable.

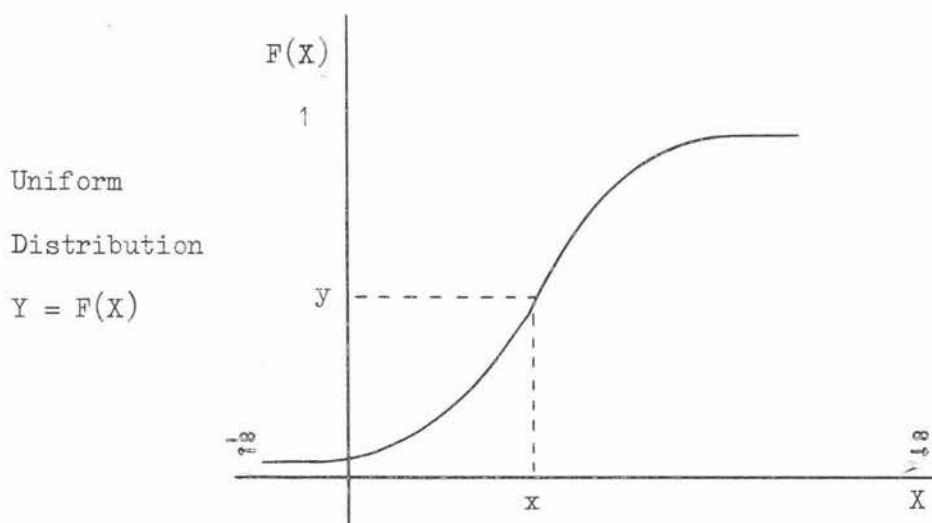


Figure 4.1 Transformation of a uniform random  $y$  to a random  $x$  with distribution function  $f$ .

There must obviously be some means of obtaining large numbers of uniformly distributed random variables. Since this discussion refers to computer simulation, the following will be expressed to some extent in computer terminology.

The two most important methods of obtaining the required random variables are:

(1) lookup of tables of random numbers stored in the computer. However,

this lookup would be slow and to store a large number of random digits would take a lot of room.

- (2) generation of pseudo-random numbers. These numbers, although not random in the strictest sense, have the advantage of being reproducible and fast to obtain.

### Random Number Generation

Most successful "random number" generators are special cases of the following scheme:

if  $X_0 : (X_0 \geq 0)$  is the starting value,  
 $a : (a \geq 0)$  is the multiplier,  
 $c : (c \geq 0)$  is the increment,  
 and  $m : (m > X_0, a, c)$  is the modulus of the sequence

then the desired sequence of numbers  $X_n$  is attained by setting

$$X_{n+1} \equiv aX_n + c \pmod{m} \text{ for } n \geq 0$$

This is a "linear congruential sequence". The sequence will eventually cycle back to give  $X_N = X_0$  for some  $N$ . Careful choice of  $X_0$ ,  $a$  and  $c$  will maximise  $N$ . For computational convenience  $m$  is generally chosen to be the word size of the machine e.g.

$$m = 2^{16} \text{ on the IBM 1130}$$

Sometimes the transformation mentioned earlier from a uniform to some other distribution is awkward to perform. The "composition technique" can then be used in these situations. Two or more variates independently distributed but with the same density function are chosen. They are then combined in such a way that together they compose an approximation to the distribution.

For example, to generate a number from the normal distribution (which has an awkward cumulative distribution function) choose  $n$  values  $x_i$  from a uniform  $[0, 1]$  distribution (mean  $\frac{1}{2}$ , variance  $\frac{1}{12}$ ).

$$\text{If } \bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$$

then from the Central Limit Theorem

$$y = \frac{\bar{x} - \frac{1}{2}}{\sqrt{1/12n}} \sim N(0, 1) \text{ as } n \text{ becomes large.}$$

For most practical purposes  $n = 12$  is large enough, and convenient, since then

$$y = \sum_{i=1}^{12} x_i - 6$$

### Test of "Randomness"

It would be hoped that a sequence generated by any of the above methods behaved as though it were random. There are many statistical tests which will check for randomness. To check them properly, an exhausting variety of tests should be performed. However, the few listed below would quickly give an indication of bad statistical properties. A more detailed account of some possible statistical tests may be found in a standard text, for example Knuth (1968).

- (1) a check on the sequence mean and standard deviation - these can be compared with the population mean and standard deviation.
- (2) a comparison of the number of each of the digits 0, 1, 2, ..., 9 in a sequence of length  $N$ , with the number of digits of each type expected in a completely random sequence. Each digit is expected  $\frac{N}{10}$  times if completely random. A  $\chi^2$  test

$$\sum_{\text{type of digit}} \frac{(\text{expected occurrence} - \text{observed occurrence})^2}{\frac{N}{10}}$$

tests the hypothesis that the observed sequence of digits is random.

- (3) any serial correlation between numbers in a sequence should be checked for. Suppose the sequence  $X_i$  is of length  $N$  with mean  $\bar{x}$ .

$$\text{Then } r_k = \frac{\sum_{i=1}^{N-k} (x_i - \bar{x})(x_{i+k} - \bar{x})}{(N-k) s^2} \quad \text{for all } k = 1, 2, \dots, N$$

$$\text{where } s^2 = \frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N - 1}$$

gives the serial correlation between numbers a distance  $k$  apart. For a completely random sequence,  $r_k$  would have a value of approximately zero.

- (4) runs within the sequence should be tested by a gap test, poker test and so on.

### Testing the Model

Once the tentative mathematical model is set up, it must be tested with actual data to see whether the simulated data are reasonable enough.

Hope (1968) discusses a Monte Carlo procedure for testing the fit of a model.

First, a reference set is constructed. If lack of fit of the model is to be judged significant at the 5% level, then the reference set consists of 19 random simulations. (If considered significant at the 1% level, the reference set consists of 99 random simulations).

A test criterion is considered, for ranking the observed data relative to the members of the reference set. If the test criterion of the observed data is ranked more extreme than the corresponding values of all members of the reference set, then lack of fit is significant. For example, the test criterion might be the distance of one set of data from all the others. If the distance corresponding to the observed data is greater than the distance corresponding to any of the members of the reference set, then there is only a 5% (or 1%, depending on the size of the reference set) chance that the reference set might represent the real world situation. That is, the model fails to give an accurate account of what really happens in nature.

While the Monte Carlo procedure tests the fit of the original hypothesized model, an analysis of variance will test for lack of fit of a fitted linear (i.e. additive) polynomial model.

Once the model has been tested, it can be modified accordingly. This modified model needs to be tested and modified again until a simulation close enough to reality results. However, simulation is only a best representation of reality, a guide to thinking - not reality itself.

## CELL GROWTH DATA

To develop the response surface methodology, considerable study was given to the work of Hoel and Mitchell (1971). Thus this first example of model-fitting follows closely the procedures described in their paper.

### General Procedure

A stochastic model for the growth of a cell population was proposed. To fit this model to experimental data, repeated computer simulations were performed and the distance between the experimental data and simulated trials was measured. This distance depended on the values of the parameters on the model and hence to make the model fit as closely as possible to the data, those values of the parameters which minimized the distance had to be estimated. Each time a new set of values was tested more simulations had to be performed. This could have become expensive in time and money so good experimental design played an important part in keeping the number of simulation runs down.

Once the model was fitted a Monte Carlo test and an analysis of variance were carried out to check on the goodness-of-fit of the model and the accuracy of estimation.

Using these methods the experimenter can consider his data in the light of stochastic models for which mathematical results are not available.

### The Data

Kubitschek (1962) studied the growth rate of several colonies of *Escherichia coli* by recording the generation time of the cells. Each colony had an initial size of two, and recordings were taken until they reached a size of 63. The particular experimental data used were the results of observations on colony 1.1 of his study.

### The Cellular Proliferation Model

Experimentation was concentrated on one only of the competing models studied by Hoel and Mitchell. This model, originally proposed by Kretchmar, was claimed by Hoel and Mitchell to give the best fit to the population growth function  $N(t)$  associated with Kubitschek's data.

Kretchmar's model:

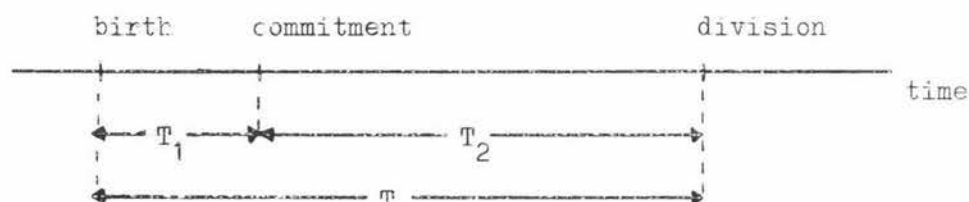


Figure 5.1

*E. coli* cells reproduce by division. The model considers the generation time  $T$  of a cell to be composed of two independent parts (see fig. 5.1).

- (i)  $T_1$ , a random interval representing the time from the birth of a cell until it is committed to divide and
- (ii)  $T_2$ , a random interval representing the time from commitment to divide until division is completed.

Thus the generation time is  $T = T_1 + T_2$

Kretchmar (1969) postulated that whatever inhibits cell division as the population size increases, acts to lengthen  $T_1$  rather than  $T_2$ . That is, once the cell is committed to divide it does so without regard to the size of the population. Formally:-

- (i) probability that a cell commits itself to division in the interval  $(t, t+\Delta t)$  is  $g(N(t))\Delta t$ , and
- (ii) once a cell is committed to divide, the time remaining until division is completed has a probability density  $f(t_2)$ .

The function  $g$  and density  $f$  can be specified arbitrarily, in whatever way the model builder thinks is appropriate.

To fit Kretchmar's model to Kubitschek's data, Hoel and Mitchell



assumed that

(i)  $g(N(t)) = \lambda[N(t)]^{-\alpha}$ , so that if  $\alpha > 0$  this decreases with increasing  $N$ , and

(ii)  $T_2 = T_0$ , where  $T_0$  is a constant

$$\begin{aligned} \text{i.e. } f(t_2) &= 1 \quad \text{if } t_2 = T_0 \\ &= 0 \quad \text{otherwise} \end{aligned}$$

### Fitting the Model

Given Kubitschek's data, the parameters of the postulated model had to be chosen in such a way that the simulated growth function would correspond to the observed growth function as closely as the model would allow.

As mentioned earlier, the fit of the model is gauged by using some measure of the distance between the observed growth function and the simulated growth function. The distance measure may be arbitrarily chosen, and in this instance was defined by  $S$  where

$$S^2 = \sum_{i=1}^k (t_i - s_i)^2$$

for  $t_i$ , the time of the  $i^{\text{th}}$  birth in the observed growth function

$s_i$ , the time of the  $i^{\text{th}}$  birth in the simulated growth function

$k$ , the total number of births in the recorded history of the population.

A different  $S$  value was produced by each repetition of the simulation since  $S$  was a random variable with a distribution dependent on the parameter vector. An average of  $S$  taken over a number of runs gave an estimate  $\bar{S}$  of  $E(S)$ , the expectation of  $S$ . It was this  $\bar{S}$  that was viewed as the response variable over the parameter space. Fitting the model as closely as possible to the data was then equivalent to finding that value of the parameter vector which optimized (i.e. minimized) the response.

An important design problem immediately arose. How many simulation

runs at a point would have to be performed in order that  $\bar{S}$  provided an accurate estimate of  $E(S)$ ? A natural desire for many runs at a point giving more accuracy had to be balanced against considerations of time and cost.

The results in table V.1 show an example of thirty runs at a point giving thirty  $S$  values. Average values,  $\bar{S}$ , taken over five, ten, twenty and thirty runs are also shown. For each different sample size,  $\bar{S}$  was evaluated two more times, each time using a different set of random numbers. Results are shown in table V.2. The time taken to make a single estimation of  $E(S)$  is also shown in this table.

Table V.1 Sample of 30 simulations at the point  $(\lambda, \alpha, \tau_0) = (10, 1.29, 22.7)$ .

1	22.11		11	29.40		21	29.61	
2	24.56		12	35.03		22	21.98	
3	22.33		13	19.94		23	17.31	
4	19.60	$\bar{S}$ over 5 runs	14	20.99		24	30.68	
5	25.53	$22.8 \pm 1.0$	15	21.69		25	25.48	
6	24.60		16	19.78 <sup>a</sup>		26	25.22	
7	21.00		17	21.72		27	24.83	
8	22.75		18	20.76		28	18.71	
9	26.05		19	23.72		29	23.68	
10	25.28	$\bar{S}$ over 10 runs	20	20.12	$\bar{S}$ over 20 runs	30	17.31	$\bar{S}$ over 30 runs
		$23.4 \pm 0.7$			$23.4 \pm 0.8$			$23.3 \pm 0.7$

a. In calculating  $\bar{S}$ , the point  $i = 16$  was not included.

This point corresponded to a cell whose generation time was so long, and whose daughters' generation times were so short, that it was omitted from the calculations.

Table V.2

Comparison of  $\bar{S}$  calculated at the point  $(\lambda, \alpha, \tau_0) = (10, 1.29, 22.7)$  from varying sample sizes.

Number of runs	Time	$\bar{S}$		
5	57 secs	$22.8 \pm 1.0$	$23.9 \pm 0.9$	$25.4 \pm 2.9$
10	1 min 56 secs	$23.4 \pm 0.7$	$23.3 \pm 1.6$	$23.2 \pm 1.4$
20	3 min 55 secs	$23.4 \pm 0.8$	$22.2 \pm 0.9$	$21.0 \pm 0.8$
30	5 min 53 secs	$23.3 \pm 0.7$	$21.1 \pm 0.7$	$22.6 \pm 0.8$

Noting the results in table V.1 for five runs at a point showed a large variance, a t-test was carried out to set a confidence interval on the mean response  $\bar{S}$  as an estimate of  $E(S)$ . With parameter vector  $(\lambda, \alpha, \tau) = (10, 1.29, 22.7)$  a 95% confidence in the response estimate was given by an interval 5.6 standard deviations (i.e. 5.8 units) wide. This fact, together with the observed wide variation in  $S$  values, indicated that while five runs only were quick to perform, the chance of missing some high or low responses was too large to be ignored.

The three remaining sample sizes (10, 20 or 30 runs at a point) show that variation in the estimate did not significantly decrease with increasing sample size after 10 runs. Other relevant considerations were that repeated estimates within each sample size still continued to differ from each other even with as many as 30 runs at a point. A doubling in sample size only decreased the confidence interval on  $\bar{S}$  as an estimate of  $E(S)$  by a factor of  $\sqrt{2}$  but increased by a factor of 2 the time taken to make the estimation. Since good design aims to keep the number of simulations down as far as accuracy will permit, it was decided to calculate estimates from a sample of size 10. Estimation from samples of this size had reasonable accuracy while enjoying the benefits of less

time spent on simulating and calculations. There seemed no point in going to larger sample sizes.

### Seeking the Optimum

The region of operability was defined by:-

$$\lambda > 0; \alpha > 0; \tau_0 > 0$$

from consideration of the biological system.

The region of interest was defined by:-

$$5 \leq \lambda \leq 15; \quad 1.00 \leq \alpha \leq 2.00; \quad 15.0 \leq \tau_0 \leq 25.0$$

from consideration of Hoel and Mitchell's results.

An open grid search was performed first to aid the choice of a likely region of low response on which to concentrate experimentation. All combinations of the proposed levels of the three factors were tested. Factor levels corresponded to:

$$\lambda : 5, 10, 15$$

$$\alpha : 1.00, 1.50, 2.00$$

$$\tau_0 : 15.0, 20.0, 25.0$$

Thus the grid search entailed experimentation at 27 points. From this, an initial parameter vector (10, 1.25, 22.5) was chosen as the centre of a region of low response. It was also used for the centre of the initial first order design - a  $2^3$  factorial with step sizes (1.0, 0.1, 1.0). An analysis of variance of the parameter estimates for this linear approximation indicated that the sums of squares attributable to lack of fit and first order interaction terms were each highly significant. The original  $2^3$  factorial was then augmented by six axial points and six centre points to form a second-order (central composite) design. The co-ordinates of the axial points were

$$(\pm \delta, 0, 0), (0, \pm \delta, 0), (0, 0, \pm \delta) \text{ (in factor level notation)}$$

where  $\delta = 1.542649$  was the value of  $\delta$  necessary to allow orthogonal estimates of the parameters from a design using six centre points.

The parameter vector at the stationary point of the fitted quadratic surface was (10, 1.23, 22.8) to the same accuracy as that quoted by Hoel and Mitchell.

An analysis of variance carried out on the quadratic approximation to the surface still showed lack of fit to be significant. This could have resulted from

- (1) Kretchmar's model not being an accurate description of the population growth function. (Pielou (1968) points out that more than one different model may be fitted equally well to some observed data, but there is no way of telling which model, if any, is the correct one.) or
- (2) a third or higher order polynomial was needed to give a better approximation to the response surface. However, any move towards fitting a third order polynomial approximation to the surface was viewed with the greatest reluctance. The work involved would be considerable and lack of fit might still be significant if the model was in fact not good enough.

A Monte Carlo test was carried out on the model with parameter vectors (10, 1.23, 22.8) and (10, 1.29, 22.7) using a distance test criterion as described in section 4. Results were:

- (1) Parameter vector: (10, 1.23, 22.8)

Ranked distances: 14.8 15.4 15.6 15.6 16.6 16.9 17.1 17.4  
 17.6 17.7 17.8 17.9 18.2 18.8 18.9 19.2  
 21.6 21.6 22.4 24.2

Experimental data: 22.4

- (2) Parameter vector: (10, 1.29, 22.7)

Ranked distances: 15.9 16.6 16.6 17.7 17.7 17.8 18.5 18.8  
 18.9 19.3 19.4 19.8 20.3 20.7 22.5 23.5  
 23.9 25.9 26.7 26.9

Experimental data: 23.5

(The random number generator giving uniformly distributed random variables was set at the same initial value in both cases.)

Although the Monte Carlo test did not come as close to rejecting the model for Hoel and Mitchell's optimal parameter values, distances were consistently larger than distances for the Monte Carlo test on (10, 1.23, 22.8). There was no positive reason for rejecting the model.

Responses obtained at the stationary point (10, 1.23, 22.8) were  $(23.5 \pm 1.5)$ ;  $(20.4 \pm 0.6)$ ;  $(21.3 \pm 0.8)$ ;  $(21.5 \pm 1.5)$ ;  $(21.4 \pm 0.7)$  with an average value of  $(21.5 \pm 0.5)$ .

Expected value of the response at the stationary point was  $(23.3 \pm 1.3)$ .

Responses obtained at the centre point of the design were  $(23.1 \pm 1.6)$ ;  $(22.7 \pm 0.7)$ ;  $(23.7 \pm 1.1)$ ;  $(23.7 \pm 1.4)$ ;  $(24.6 \pm 1.1)$  with an average value of  $(23.6 \pm 0.5)$ .

Responses at the stationary point of the fitted surface were significantly lower than responses at the centre point of the design, confirming the choice of the stationary point to give the optimal parameter values. So best fit of the model was attained at  $\hat{\lambda} = 10$ ;  $\hat{\alpha} = 1.23$ ;  $\hat{\tau}_0 = 22.8$

There was a discrepancy between Hoel and Mitchell's results and those quoted above for the optimal parameter values. Hoel and Mitchell claimed optimal parameter values of (10, 1.29, 22,7).

Consider the canonical form of the quadratic representation of the fitted response surface:

$$\bar{S} - 23.3 = 16.7X_1^2 + 3.1X_2^2 + 1.0X_3^2$$

$$\text{where } X_1 = -0.08 (\lambda - 10) + 0.50 (\alpha - 1.23) + 0.86 (\tau_0 - 22.8)$$

$$X_2 = 0.14 (\lambda - 10) + 0.86 (\alpha - 1.23) + 0.49 (\tau_0 - 22.8)$$

$$X_3 = 0.99 (\lambda - 10) - 0.08 (\alpha - 1.23) + 0.13 (\tau_0 - 22.8)$$

Coefficients of  $X_2$  and  $X_3$  are small compared with the coefficient of  $X_1$ . The term in  $X_3$  could be neglected without introducing much error.

Now if the "true" value of  $\alpha$  were 1.29 say, then an error 0.06 in the estimate  $\hat{\alpha}$  would cause a difference of approximately 0.47 in the response  $\bar{S}$ .

$$\text{i.e. } (\bar{S} - 0.47) - 23.3 = 16.7Y_1^2 + 3.1Y_2^2 + 1.0X_3^2$$

where  $Y_1 = -0.08 (\lambda - 10) + 0.50 (\alpha - 1.29) + 0.86 (\tau_0 - 22.8)$

$$Y_2 = 0.14 (\lambda - 10) + 0.86 (\alpha - 1.29) - 0.49 (\tau_0 - 22.8)$$

and  $X_3$  is unchanged.

But  $(\bar{S} - 0.47)$  is well within one standard deviation of the mean  $\bar{S}$  at the point (10, 1.23, 22.8) and hence  $(\bar{S} - 0.47)$  is not significantly different from  $\bar{S}$ .  $\hat{\alpha} = 1.23$  is then an acceptable estimate of  $\alpha$ .

Apparently therefore, discrepancies between Hoel and Mitchell's results and those reported above could be attributed to stochastic variation. Any difference in results need not necessarily imply that the results were incorrect.

#### The Simulation Program

Suppose the colony initially has  $N = 2$  cells.

The number of cells not committed to divide (set  $S_1$ ) is  $N_1 = 2$ .

The number of cells committed to divide but having not yet completed the division process (set  $S_2$ ) is  $N_2 = 0$ .

A vector  $\{Q(i)\}$  ( $i = 1, 2, \dots, N_2$ ), stores in descending order of magnitude the times to division of the cells in set  $S_2$  at any time  $t$ .

Thus the time at which any cell in  $S_2$  is destined to divide is known at time  $t$ .

If an "event" is a commitment to divide:

The probability of an event occurring in time  $(t, t+\Delta t)$  is

$$r \quad \lambda N_1 (N(t))^{-\alpha} \Delta t$$



which is a Poisson process, so that time between successive events is distributed as a negative exponential. Hence the probability of no events occurring before time  $t$  is

$$e^{-\lambda N_1(N(t))^{-\alpha} t}$$

leaving the probability of an event occurring before time  $t$  to be

$$1 - e^{-\lambda N_1(N(t))^{-\alpha} t}$$

- (1) A random value,  $W$  say, representing the time to the next commitment of a cell in  $S_1$  must now be generated.

By definition

$$1 - e^{-\lambda N_1(N(t))^{-\alpha} u} = F(u)$$

where

$$\lambda N_1(N(t))^{-\alpha} e^{-\lambda N_1(N(t))^{-\alpha} u} = f(u)$$

and  $f(u)$  gives the probability of the next event occurring at time  $t$ . Hence by the theory of section 4, to generate a random value,  $w$ , for the time to the next event, a random value,  $r$  say, distributed uniformly on the interval  $[0, 1]$  must be chosen and

$$r = F(w) = 1 - e^{-\lambda N_1(N(t))^{-\alpha} w}$$

giving

$$w = \frac{-(N(t))^{-\alpha}}{\lambda N_1(t)} \log(1-r) = F^{-1}(r)$$

- (2) If this value  $w$  is less than the shortest time which must elapse until the next cell divides (i.e.  $w < Q(N_2)$ ) then:

- (i) a commitment takes place

$$N_1 \leftarrow N_1 - 1$$

$$N_2 \leftarrow N_2 + 1$$

- (ii) time  $t$  is advanced by  $w$

$$t \leftarrow t + w$$

(iii)  $w$  is subtracted from all elements of the  $Q$ -vector

$$Q(i+1) \leftarrow Q(i) \quad i = 1, 2, \dots, N2 - 1$$

$$Q(i) \leftarrow Q(i) - w \quad i = 2, 3, \dots, N2$$

$$Q(1) \leftarrow \tau_0$$

and the process is started over at step 1.

(3) If this value  $w$  is greater than the shortest time which must elapse until the next cell divides (i.e.  $w > Q(N2)$ ) then:

(i) a division takes place

$$N1 \leftarrow N1 + 2$$

$$N2 \leftarrow N2 - 1$$

$$N \leftarrow N + 1$$

(ii) time  $t$  is advanced by  $w$

$$t \leftarrow t + w$$

(iii)  $w$  is subtracted from all elements of the  $Q$ -vector

$$Q(i) \leftarrow Q(i) - w \quad i = 1, 2, \dots, N2$$

and the process is started over at step 1.

The simulations were continued until the cell colony reached a population size of 63.

Thus a set of values  $s_i$  were generated which represented the time of the  $i^{\text{th}}$  cell division. Table V.3 gives a portion of a simulation trial carried out at the optimal parameter values. Table V.4 gives the final results of this trial.

All work was done on an IBM 1130 computer. The IBM subroutine RANDU was used for generating uniformly distributed random numbers. This package uses the linear congruential method mentioned in section 4.

#### Evaluation of the Methods

Although the grid search was open, a good starting value for the parameter vector was chosen. In fact it was so close to the optimal

value that a first order design could not estimate a line of steepest descent which would in fact descend to points of lower response. Consequently the first order design was immediately augmented to form a central composite design. The entire experimentation plan involved only 49 points; 27 from the grid search, 8 from the first order design, 2 from testing the "steepest descent" and a further 12 to form the second order design. These 49 points would take about  $1\frac{1}{2}$  hours of CPU time on the IBM 1130. Compare this with Hoel and Mitchell's 1 hour on a GE430 - a bigger machine. Hoel and Mitchell do not give the number of simulations that they ran at a point.

Although the stationary point of the fitted surface was taken as the point of optimal response, stochastic variation was such that one response of  $23.5 \pm 1.6$  at the stationary point was higher than a response of  $23.1 \pm 1.6$  at the centre point of the second order design. Although lower responses could be obtained at other points, the stationary point was expected to give the optimal parameter values.

Table V.3 Example of the simulation procedure at optimal parameter values.

Random number w	Q - vector					Population size			Time		Comment
	Q(1)	Q(2)	Q(3)	Q(4)	Q(5)	N	N1	N2	Observed	Simulated	
	0.728					3	2	1	22.6	22.8	<u>Starting values</u>
0.1987	22.800	0.529				3	1	2		23.0	
0.5513	22.271					4	3	1	28.5	23.5	<u>Division</u>
0.0592	22.800	22.211				4	2	2		23.6	
0.1048	22.800	22.695	22.106			4	1	3		23.7	
1.3605	22.800	21.439	21.335	20.746		4	0	4		25.1	
a	2.054	0.694	0.589			5	2	3	44.9	45.8	<u>Division</u>
0.0551	22.800	1.999	0.638	0.533		5	1	4		45.9	
0.0825	22.800	22.717	1.916	0.556	0.451	5	0	5		45.9	

a. Where no random value is given, all cells are committed to divide and the next event must be a division - no random number need be chosen.

Table V.4 Simulated data giving the lowest of ten responses at optimal parameter values.

Generation 1		Generation 2		Generation 3		Generation 4	
Observed	Simulated	Observed	Simulated	Observed	Simulated	Observed	Simulated
22.6	22.8	44.9	45.8	66.6	69.6	89.3	93.0
28.5	23.5	45.7	46.4	67.0	69.7	91.5	93.8
		46.2	46.5	67.1	71.1	93.1	94.5
		46.2	48.8	67.6	71.2	93.3	95.1
				68.6	71.4	93.5	95.4
				68.9	71.7	94.5	97.0
				69.0	72.2	96.3	97.3
				89.0*	74.3*	97.5	97.4
						98.5	97.5
						98.6	97.6
						98.9	97.8
						98.9	98.0
						99.7	98.1
						100.0	99.0
						102.9	101.0
						103.0	103.4

\* Omitted from the calculations.

Table V.4 (cont'd)

Generation 5		Generation 5 cont'd	
Observed	Simulated	Observed	Simulated
115.3	114.4	123.0	122.3
115.7	115.0	123.0	124.1
116.7	116.8	123.1	124.3
118.3	117.9	123.6	125.2
118.8	118.8	125.2	125.7
119.2	119.1	125.9	125.8
119.3	119.2	126.3	129.3
119.3	119.3	126.4	129.6
119.7	119.6	126.6	131.1
120.5	119.7	130.8	132.5
120.7	119.8	132.4	134.8
120.9	120.0	132.6	139.6
121.1	120.4	141.0	141.2
121.8	120.5	141.3	141.4
122.0	120.7	144.1	142.9
122.6	121.7		

### SELECTION ESTIMATION

A very large study has been made on Composite Cross V, an experimental population of barley grown at Davis, California every year since 1937. Seed was saved from each generation and a record exists of the population structure over that period.

Changes in gene and genotypic frequencies at four esterase loci were monitored over 25 generations to obtain experimental evidence concerning the balance of forces responsible for:

- (1) the marked differences in allelic frequencies observed among barleys from different ecogeographical regions of the world, and
- (2) the extensive allelic variation found within local populations of barley.

One hypothesis to account for the observed patterns is that the various alleles confer different properties on the individual, to the extent of altering its contribution (through seed set) to the next generation. Allard, Kahler and Weir (in press) showed the frequencies observed and the patterns amongst them were certainly not consistent with those expected for neutral genes in an infinitely large population with the observed amounts of self-pollination and outcrossing. They also showed mutations and migration could be excluded as the sources of the observed higher than expected heterozygosity. Some form of selection was then considered as a possible explanation.

Allard, Kahler and Weir used the genotypic frequency data to obtain maximum likelihood estimates of genotypic fitness values. They estimated one set of values from each pair of consecutive generations. Their final fitness estimates were average values taken over the sets of estimates from several generation pairs.

It was decided in this work to allow stochastic variation of

fitness values. The system should then be more realistic, for deterministic estimates imply that selection is constant over the span of generations - rather an artificial situation.

### The Data

The four esterase loci of CCV were called A, B, C and D. A and C each had three alleles while B and D each had two alleles.

### The Model

For a mixed selfing and random mating population, as was CCV, the genotypic transition equations are:

$$f'_{ii} = \frac{s}{w} (w_{ii}f_{ii} + \frac{1}{2} \sum_{m \neq i} w_{im}f_{im}) + \frac{t}{w} \frac{(\sum_m w_{im}f_{im})^2}{m} \dots\dots(1)$$

$$f'_{ij} = \frac{sw_{ij}}{2w} f_{ij} + \frac{t}{w} \frac{(\sum_n w_{in}f_{in})(\sum_n w_{nj}f_{nj})}{n} \quad i \neq j$$

where in any generation  $s$  is the (constant) amount of selfing and  $t = 1 - s$  is the amount of outcrossing,  $w_{ij}$  is the relative fitness for the genotype with alleles  $a_i$  and  $a_j$ , and sums are over the integers  $1, 2, \dots, k$  for a  $k$ -allele locus. For deterministic selection, substituting observed frequencies  $f_{ij}$  and solving these equations for  $w_{ij}$  gave the maximum likelihood estimates of the  $w_{ij}$ .

Introduction of stochastic variation to the fitness values made it impossible to solve for the  $w_{ij}$ 's analytically. Thus simulation techniques became important.

Stochastic variation was introduced in two different ways; the population fitness values were hypothesised to fluctuate from generation to generation according to:

- (1) a normal distribution
- and (2) a uniform distribution.



These hypotheses came from works by Jain and Marshall (1968) who studied the effects of normally distributed fitness values on genotypic equilibria, and Barker and Butcher (1966) who studied the effects of uniformly distributed fitness values on quasi-fixation of genes. Estimation procedures employed by Allard, Kahler and Weir could not now be used to estimate stochastic fitness values. Instead response surface methods and simulation were used.

In addition to the introduction of stochastic variation, estimation procedures used took account of the amount of information in each generation. Essentially, actual numbers rather than frequencies of each genotype were used for estimation at the different loci. An appropriate weight, or confidence, was thus placed on the information from the different generations. Homozygotes then had more bearing on the final estimates than did heterozygotes - logically so, since heterozygotes were so infrequent that any deductions made from their numbers would have to be treated with considerable caution. Compare this data usage with that of Allard, Kahler and Weir's. Their maximum likelihood estimation used genotypic frequencies, thus giving equal importance to homozygote and heterozygote data over the generations.

A further modification in estimation procedures was introduced by estimating fitness values this time not from several pairs of generations, as did Allard, Kahler and Weir, but from the span of generations taken all at once. This should take a closer account of the overall changes in the genotypic frequencies observed.

#### Measuring the Response

Consider the B locus, for which genotypic data was known in generations 4, 5, 6, 14, 15, 16, 17, 24, 25 and 26.

A set of genotypic fitness values was chosen from the distribution under consideration. Then using the genotypic data from experimental

generation  $n - 1$ , an expected number of each genotype in generation  $n$  was calculated using the transition equations (1). The expected (simulated) data for generation  $n$  were then compared with the observed data for generation  $n$  and the distance measured between the two.

If  $N_{ij}^{(n)}$  is the observed number of genotype  $B_i B_j$  in generation  $n$  and  $EN_{ij}^{(n)}$  is the expected number of genotype  $B_i B_j$  in generation  $n$  then the response had a value

$$\sum_i \sum_j (N_{ij}^{(n)} - EN_{ij}^{(n)})^2$$

A new set of fitness values was then chosen from the distribution under consideration and the process was repeated for the next consecutive pair of generations.

The final distance between the observed and the simulated genotypic data for the  $i^{\text{th}}$  simulation run was then

$$S_i^2 = \sum_n \left( \sum_i \sum_j (N_{ij}^{(n)} - EN_{ij}^{(n)})^2 \right)$$

for  $n = 5, 6, 15, 16, 17, 25, 26$

An average value of these  $S_i^2$  taken over  $m$  simulation runs was then viewed as the response variable in the parameter space. "Best" fitness estimates minimized the distance between observed and expected data; that is, minimized the response. Response surface methods were used to seek this minimum.

All simulation was performed on an IBM 1130. Random numbers from the normal distribution were generated using GAUSS, an IBM supplied subroutine operating according to the central limit method mentioned earlier. Tests on a sample of these numbers showed they were significantly more skew than the population of normal random numbers. Other sample characteristics up to kurtosis did not differ significantly from the expected values, and so GAUSS generates numbers which, apart

from skewness, are very nearly normal.

### Estimation Procedure

Fitness value estimates were first calculated deterministically. Then, by allowing stochastic variation, it should be possible to achieve a lower response than that achieved using deterministic fitness values; that is, it should be possible to get a shorter distance between observed and simulated data.

Before the response could be measured a decision had to be made on how many simulation trials to make at a point. From considerations of time and accuracy (as in cell growth work), an optimal number of 20 runs at a point was decided upon.

But a problem even more fundamental to the measurement of the response was that of how to specify fitness values. Since these values are relative they cannot be determined uniquely, but only to within a constant multiple of each other. This allows one of the values to be specified arbitrarily. Other values are measured with the specified value as unit size.

It was decided to fix  $w_{11}$  at 1.00 in all estimation. All estimates were then specified relative to  $w_{11}$ . Allard, Kahler and Weir used the same relationship between their fitness values so comparison of results from the different estimation procedures was easy.

The A and C loci each had three alleles, while the B and D loci each had two. Since  $w_{11}$  was fixed, only fitnesses  $w_{12}$  and  $w_{22}$  need to be estimated at the B and D loci. When compared with the problem of estimating fitnesses  $w_{22}$ ,  $w_{33}$ ,  $w_{12}$ ,  $w_{13}$  and  $w_{23}$  at the A and C loci, it was decided to develop techniques on the B and D loci. Two different methods of estimation were used;

(1) deterministic estimates of fitnesses were made. Then stochastic

variation was introduced. The fitnesses were each drawn from the distribution under study, using the earlier deterministic estimates as the appropriate (constant) means and a variance  $\sigma^2$ . The same variance was used for the distribution of both fitness values. That value of  $\sigma$  giving a minimum response was used, together with the means of the distributions, to specify the starting value for further experimentation.

- (2) Experimentation started with a grid search over the three-factor  $(w_{22}, w_{12}, \sigma)$  space. The two fitness values were chosen from their appropriate distributions as in (1), but no prespecified values were used for the distribution means. The grid point giving the lowest response was then used as the starting point for further experimentation.

Table VI.1 shows the results of estimation of fitness values at the B locus by each of the two methods with fitnesses distributed both uniformly and normally. The first method of estimation gave consistently better results (i.e. lower response). If variance in the fitness values was introduced at the very beginning of experimentation, the response surface varied so widely that fitness value estimates were rather inaccurate.

In view of this fact, it was decided to follow the first method of estimation given above at the A and C loci. Experience showed that in moving from deterministic to stochastic estimation, a first order approximation could be omitted: for the variance  $\sigma^2$  had only a small effect which meant that the starting value for stochastic estimation procedures was very close to the optimum response -- so close that no descent to a lower response was possible.

Table VI.1 Comparison of estimation methods at the B locus.

Responses at Given Points				Fitness Estimates				Method <sup>b</sup>
(Actual response) <sup>a</sup> Centre pt. $\pm$ s.d.		(Expected response) Stationary pt. $\pm$ s.d.	(Actual response) <sup>a</sup> Stationary pt. $\pm$ s.d.	w <sub>11</sub>	w <sub>22</sub>	w <sub>12</sub>	$\sigma$	
Normally distributed fitnesses {	346.72 $\pm$ 2.94	346.97 $\pm$ 0.79	345.33 $\pm$ 1.50	1.00	1.10	0.33	0.01	(1)
	361.78 $\pm$ 3.36	351.32 $\pm$ 0.66	353.94 $\pm$ 2.65	1.00	1.09	1.00	0.02	(2)
Uniformly distributed fitnesses {	345.90 $\pm$ 3.34	345.42 $\pm$ 0.58	347.74 $\pm$ 2.42	1.00	1.10	0.31	0.02	(1)
	362.01 $\pm$ 3.79	353.25 $\pm$ 1.15	353.47 $\pm$ 2.53	1.00	1.07	1.05	0.02	(2)

a. Actual responses are averages over six measurements.

b. For explanation of methods see Estimation Procedure Pg. 45.

The experimental designs used to fit the models to the response surface at the A and C loci are of particular interest. The following are degrees-of-freedom analyses for the different designs.

(1) Deterministic estimation

Source	First order model	Second order model
First order effects	5	5
Second order effects	10	15
Error	1	6
<hr/>		
Total	16	26

Design for the first order model:

$1/2$  rep of  $2^5$  factorial with the five-factor interaction  $w_{22}w_{33}w_{12}w_{13}w_{23}$  confounded with the mean; one centre point was also added.

Design for the second order model:

the complete first order design with 10 axial points added.

(2) Stochastic estimation

<u>Degrees of Freedom</u>		
Source	First order model	Second order model
First order effects	6	6
Second order effects	7	21
Error	3	22
<hr/>		
Total	16	49

Design of the first order model:

$1/4$  rep of  $2^6$  factorial with three three-factor interactions -  $w_{22}w_{33}w_{23}\sigma, w_{12}w_{13}w_{23}\sigma$  and their generalized interaction

$w_{22}w_{33}w_{12}w_{13}$  - confounded with the mean.

Design of the second order model:

$1/2$  rep of  $2^6$  factorial with the six-factor interaction

$w_{22}w_{33}w_{12}w_{13}w_{23}$  confounded with the mean; 6 centre points and 12 axial points were also added.

Some of the reasons for using these particular designs to estimate the stochastic fitnesses were

- (1) since the first order model may have to be fitted more than once (at different stages of the steepest descent procedure), it is most important to keep the number of first order design points down. A full  $2^6$  factorial would have too many points, so it was decided to use a fractional replicate of the  $2^6$  factorial. The confounding scheme used to obtain a quarter replicate caused the two-factor interactions to be confounded into seven groups with one degree of freedom for each group. An estimate of only a representative interaction from each group was possible. This did not matter at the then stage, since the interactions were merely used as a guide to lack of fit of the first order model.
- (2) to estimate all second order effects for a second order model, a half replicate was needed. Axial points are necessary to obtain a non-singular precision matrix. Centre points are needed to separate experimental error from lack of fit error before a test on lack of fit can be made.
- (3) half and quarter replicates used in their indicated situations gave orthogonal estimates of the model parameters.

#### Check on Techniques

A check on the simulation programs and estimation procedures was performed first. Checking was done at the B locus only using artificial

data. The artificial genotypic data was generated as follows:

(a) a set of artificial fitness values was arbitrarily chosen

e.g. (1.00, 0.88, 0.61)

(b) Taking the observed genotypic frequencies from generation 4 as starting values, the transition equations (1) were used to generate expected frequencies for generations 5, 6, 14, 15, 16, 17, 24, 25 and 26.

(c) By multiplying the expected frequencies from each generation by the total number of plants in the experimental sample for the same generation, a set of artificial genotypic data was generated.

Only deterministic fitness estimates were made for checking on procedures.

Region of operability was defined by:-

$$w_{11}, w_{22}, w_{12} > 0$$

from considerations of the biological system.

Region of interest was defined by:-

$$w_{11} = 1; \quad 0 \leq w_{22}, w_{12} \leq 2$$

from considerations of Allard, Kahler and Weir's results.

A grid search was first performed. Since  $w_{11}$  was fixed, experiments at all combinations of three levels of each of the other factors involved only nine points. The first order design ( $2^2$  factorial) gave a line of steepest descent leading to a much lower response. By repetition of the first order design - steepest descent sequence several times experimentation quickly came very close to the eventual optimum without wasting experimental points on insignificant changes in response values. The eventual optimal parameter values arrived at from fitting a second-order model were (1.00, 0.88, 0.52) with response estimate 3.78. First order designs and steepest descent only gave corresponding estimates (1.00, 0.89, 0.52) and 3.88. Compare these results with the true fitnesses (1.00, 0.88, 0.61).



Deterministic fitness values generate a response surface with no stochastic variation. It is possible to get very good estimates of parameter values from first order designs only. Hence second order designs can make little improvement on estimates. However, small numbers of heterozygotes caused inaccuracy in estimation of the heterozygote fitness. A large change in  $w_{12}$  will cause only a small change in the response. This explains the discrepancy between the artificial fitness values and their estimates.

#### Results from Experimental Data

Tables VI.2, VI.3, VI.4, VI.5 give estimates of genotypic fitness values, made at loci A, B, C, D respectively, using response surface methods. For comparison the maximum likelihood estimates of Allard, Kahler and Weir are also shown.

Whereas variances of estimates given by Allard, Kahler and Weir reflect sampling error in genotypic frequencies and error in the estimation procedures, other variances quoted in the table ( $\sigma$ ) reflect environmental effects.  $\sigma$  is a parameter of the model.

Table VI.2 Selection estimates at the A locus.

Response Estimates at Given Points			Selection Estimates at Stat. Point							Method <sup>b</sup> of Estimation
(Actual) <sup>a</sup> Centre $\pm$ sd	(Expected) Stationary $\pm$ sd	(Actual) <sup>a</sup> Stationary $\pm$ sd	w <sub>11</sub>	w <sub>22</sub>	w <sub>33</sub>	w <sub>12</sub>	w <sub>13</sub>	w <sub>23</sub>	$\sigma$	
522.56	518.54	520.83	1.00	1.02	0.47	2.95	0.01*	0.50	-	I
526.54 $\pm$ 3.13	505.61 $\pm$ 0.45	531.72 $\pm$ 1.29	1.00	1.03	0.57	3.04	0.01*	0.49	0.017	II
519.47 $\pm$ 3.12	511.28 $\pm$ 0.82	535.42 $\pm$ 2.58	1.00	1.03	0.57	3.11	0.01*	0.49	0.028	III
		756.45	1.00	0.99	0.90	1.07	1.44	2.69	-	IV
				(0.02)	(0.05)	(0.13)	(0.27)	(1.78)	-	

\* Significantly negative, but actual response at the stationary point calculated using this arbitrary value.

a. Actual responses are averages over six measurements.

b. Methods of estimation: I - deterministic estimates

II - normally distributed fitnesses

III - uniformly distributed fitnesses

IV - maximum likelihood estimates (variances in brackets)

- Allard, Kahler, and Weir

Table VI.3 Selection estimates at the B locus.

Responses at Given Points			Selection Estimates at Stationary Point				Method <sup>b</sup> of Estimation
(Actual) <sup>a</sup> Centre $\pm$ sd	(Expected) Stationary $\pm$ sd	(Actual) <sup>a</sup> Stationary $\pm$ sd	w <sub>11</sub>	w <sub>22</sub>	w <sub>12</sub>	$\sigma$	
345.49	345.49	345.49	1.00	1.09	0.35	—	I
346.72 $\pm$ 2.94	346.97 $\pm$ 0.79	345.33 $\pm$ 1.50	1.00	1.10	0.33	0.01	II
345.90 $\pm$ 3.34	345.42 $\pm$ 0.58	345.74 $\pm$ 2.42	1.00	1.10	0.31	0.017	III
		356.25	1.00	1.08	0.13	—	IV
				(0.05)	(0.16)	—	

a. Actual responses are averages over six measurements.

b. Methods of estimation: I — deterministic estimates

II — normally distributed fitnesses

III — uniformly distributed fitnesses

IV — maximum likelihood estimates (variances in brackets)

— Allard, Kahler, and Weir

Table VI.4 Selection estimates at the C locus.

Responses at Given Points			Selection Estimates at Stat. Point							Method <sup>b</sup> of Estimation
(Actual) <sup>a</sup> Centre $\pm$ sd	(Expected) Stationary $\pm$ sd	(Actual) <sup>a</sup> Stationary $\pm$ sd	w <sub>11</sub>	w <sub>22</sub>	w <sub>33</sub>	w <sub>12</sub>	w <sub>13</sub>	w <sub>23</sub>	$\sigma$	
1061.54	1058.36	1061.11	1.00	1.20	1.08	2.11	0.01*	2.47	-	I
1064.85 $\pm$ 4.24	1052.42 $\pm$ 0.57	1066.03 $\pm$ 3.00	1.00	1.21	1.07	2.42	0.01*	2.47	0.040	II
1066.74 $\pm$ 3.62	1069.96 $\pm$ 0.81	1070.12 $\pm$ 2.88	1.00	1.19	1.02	2.51	0.29	2.46	0.040	III
		1278.85	1.00	1.17	1.90	1.15	0.83	1.74	-	IV
				(0.03)	(0.14)	(0.13)	(0.31)	(0.80)	-	

\* Significantly negative but actual response at the stationary point  
calculated using this arbitrary value.

a. Actual responses are averages over six measurements.

b. Methods of estimation: I - deterministic estimates

II - normally distributed fitnesses

III - uniformly distributed fitnesses

IV - maximum likelihood estimates (variances in brackets)

Table VI.5 Selection estimates at the D locus.

Responses at the Given Points			Selection Estimates at Stationary Point				Method <sup>b</sup> of Estimation
(Actual) <sup>a</sup> Centre $\pm$ sd	(Expected) Stationary $\pm$ sd	(Actual) <sup>a</sup> Stationary $\pm$ sd	w <sub>11</sub>	w <sub>22</sub>	w <sub>12</sub>	$\sigma$	
205.44	205.31	205.50	1.00	0.73	0.23	-	I
207.30 $\pm$ 19.89	204.74 $\pm$ 4.07	205.67 $\pm$ 10.11	1.00	0.74	0.19	0.022	II
214.73 $\pm$ 14.29	215.52 $\pm$ 2.90	211.03 $\pm$ 17.30	1.00	0.73	0.23	0.033	III
		215.29	1.00	0.73	2.38	-	IV
				(0.03)	(2.38)		

a. Actual responses are averages over six measurements.

b. Methods of estimation: I - deterministic estimates

II - normally distributed fitnesses

III - uniformly distributed fitnesses

IV - maximum likelihood estimates (variances in brackets)

- Allard, Kahler, and Weir

While it seems desirable to allow stochastic variation in selection values, further work than that reported here is necessary. Such natural phenomena as changing weather in different years will introduce "noise" into the system described by the genotypic transition equations (1) and it would be desirable to take account of this noise. This preliminary analysis merely imposes noise onto selection values, and there is no guarantee that the imposed noise should mimic the natural noise. It will generally reduce the chance of fitting the data in fact. One possible direction for further work to take is in the comparison in variation in genotypic frequencies, from those predicted by the equations, caused by sampling errors with the actual variation. Tests could be established to decide whether or not any excess variation was due to variation in selection intensities.

Estimation of the fitness values by maximum likelihood and by response surface procedures gave similar results for homozygote fitnesses, but heterozygote fitnesses exhibited marked differences. However the response surface estimates were calculated from weighted data. Confidence in the results was increased since undue importance was not attached to the heterozygote data.

One failing of the methods was observed. Because no non-negativity constraints were built into the model,  $w_{13}$  estimates at the A and C loci were negative for deterministic estimation. Allard, Kahler and Weir also obtained some negative estimates at these loci from their maximum likelihood procedures. They regarded these negative estimates as approximations to zero. When a variation in the fitness values was allowed, all but one  $w_{13}$  estimates were significantly different from (i.e. not within two standard deviations of) the small positive values quoted in the tables of results. However, the canonical analysis showed that, even in the deterministic case, a change to a positive fitness would make little difference in the response.

It should be noted that the experimental data was extremely variable for the 1,3 genotype making it hard for the fitted model to give an adequate representation of the system in this respect. Apparently, with the given outcrossing among the barley plants, only a negative fitness could account for the dramatic decreases in heterozygote frequency. What was earlier classed as a failing of the model would seem to be therefore a failing of the data.

It can be concluded that an unreal result (the negative estimates) follows from an unreal situation (forcing fitnesses to remain fixed over all generations). Introducing stochastic variation in the fitnesses helps restore realism to the model with a corresponding increase in the realism of the estimates. (There was one positive  $w_{13}$  value).

The estimated variance of the fitness values was very small for both uniformly and normally distributed fitnesses. There was no evidence for supposing that either distribution better represented the character of the fluctuations in the genotypic fitness values.

## DISCUSSION

Response surface methods for seeking optimum conditions were effective means of fitting the stochastic models under consideration. One of their great advantages lies in their being able to use information from earlier experiments to continually improve the experimentation plan. When each experiment is a number of computer simulation runs at a point, it is desirable not to waste valuable computer time - or money. Experimental design becomes important. The sequential nature of response surface methods causes no wastage and thus makes it possible to economize on the total number of runs needed to reach the optimum conditions sought.

To put the response surface methodology into practice, a lot of careful thought is essential. Even a question as seemingly simple as the positioning of points in a first order design has no clear cut answer. It seems inevitable that some time must be spent in learning by trial and error the design details best suited to a particular situation.

Simulation was used in order to study models involving random elements. The genotypic fitness estimation (Chapter 6) highlights the particular attributes of the technique. Given the genotypic transition equations, it can be seen that maximum likelihood estimates of the fitnesses, even if the fitnesses are regarded as deterministic, would be very difficult (if at all possible) to calculate when the genotypic data were used in the manner described in Chapter 6. With the introduction of stochastic variation in the fitnesses, these estimates would have been impossible to obtain by the maximum likelihood method.

Simulation introduces new possibilities of modelling. Models may be increased in complexity often bringing a corresponding increase in realism. (Recall again the genotypic fitnesses estimation.) Efficient experimental design makes it a reasonable proposition to study these improved models.



APPENDIX 1Cell Growth Simulation Program

The following program was written for an IBM 1130, to simulate the growth of the cell population described in Section V.

```

C--MAIN PROGRAM
C-----CALLS SUBPROGRAM CAMO1 WHICH CALCULATES
C-----E(S) , MEASURE OF DISTANCE OF
C-----SIMULATION DATA FROM EXPTL DATA
      DIMENSION X(63),Y(63)
      COMMON Y,IX,X,MX
      READ (2,311) (X(I),I=3,63)
      READ(2,311)
C--TYPE IN IX, THE STARTING VAL FOR RANDU
      READ (6,401) IX
      WRITE (1,402)
      READ (6,404) MX
C--SET DATSW1      ON**GO TO END
C      OFF*PERFORM CALCNS FOR NEXT OF PARAM VALS
      1 WRITE (1,400)
C--PAUSE ALLOWS FOR THE SETTING OF DATSW1
      PAUSE
      CALL DATSW(1,IRR)
      GO TO (100,101),IRR
      101 CALL CAMO1
      GO TO 1
      100 CALL EXIT
      311 FORMAT (F7.2)
      400 FORMAT ( SET DATSW1      ON GO TO END**OFF CONTINUE )
      401 FORMAT (I5)
      402 FORMAT (' ENTER NO OF SIMN RUNS, MX, TO BE EXECUTED')
      404 FORMAT (I2)
      END

```

```

C  SUBROUTINE SIMULATES GROWTH CURVE DATA
C  THEN CALCULATES THE RESPONSE S
C
C  EXPLANATION OF VARIABLE NAMES USED
C  XLAMB,ALPHA,TO  PARAMS. OF MODEL
C  NCELL           NO. OF CELLS FINALLY IN COLONY
C  N1              NO. OF CELLS INITIALLY IN COLONY
C  N2              NO. OF CELLS WAITING TO DIVIDE
C  IX              INITIALIZATION VAL FOR SUBR. RANDU
C  W               RANDOM NO. FROM DISTRIBN.
C  X               MINIMUM OF (QMIN,W)
C  TIME,T          TIME TO NEXT DIVISION
C  Q               VECTOR OF TIMES TO DIVISION
C  NPTR            MARKER FOR END OF Q VECTOR
C  KK              INDEX OF NO. OF EXPTL. PTS.
C  M               INDEX OF NO. OF SIMN. RUNS
C

```

```

      SUBROUTINE CAM01
      DIMENSION Q(200),X(63),Y(63),SSQR(30),SQRT(30)
      COMMON Y,IX,X,MX
      MX = MX
C---TYPE IN LAMBA, ALPHA, AND TO
      10 WRITE (1,308)
         READ (6,301) XLAMB,ALPHA,TO
         IF (XLAMB-999.) 11,12,11
      11 WRITE (3,302) XLAMB,ALPHA,TO
C

```

```

C---RESPONSE CALCULATION
C-----

```

```

      DO 20 M = 1,MX
      DO 1 I = 1,200
      Q(I) = 0.0
      1 CONTINUE
      NPTR = 0
      N1 = 2
      N2 = 0
      NN = 1
      NCELL = N1 + N2
      T = 0.0
      59 IF (NCELL-63) 60,50,50
      60 XN1 = N1
      XCELL = NCELL
      IF (N1)40,5,40
      40 CALL RANDU(IX,IY,R)
      IX = IY
      W = -(XCELL**ALPHA)/(XN1*XLAMB)*ALOG(1.0-R)
      IF (NPTR) 17,16,17
C

```

```

C
C---EVENT MUST BE A DECISION TO DIVIDE
C-----

```

```

      16 XX = W
C---ADVANCE TIME
      T = T + XX
      NPTR = NPTR + 1
      Q(NPTR) = TO
C---NEW POPULATION SIZES
      N1 = N1 - 1
      N2 = N2 + 1

```

```

      NN = NN + 1
      GO TO 59
17 IF (W - Q(NPTR)) 3,4,5
C
C--EVENT IS A DECISION TO DIVIDE
C-----
      3 XX = W
C--NEW POPULATION SIZES
      N1 = N1 - 1
      N2 = N2 + 1
C--ADVANCE TIME
18 DO 6 K = 1,NPTR
      Q(K) = Q(K) - XX
      6 CONTINUE
      T = T + XX
C--CHANGE Q-VECTOR
      NPTR = NPTR + 1
      MM = NPTR - K
      DO 13 L = 1,MM
      LL = NPTR - L
      Q(LL + 1) = Q(LL)
13 CONTINUE
      Q(1) = T0
      NN = NN + 1
      NCELL = N1 + N2
      GO TO 59
C
C--EVENT IS A DIVISION
C-----
      5 XX = Q(NPTR)
C--NEW POPULATION SIZES
      N1 = N1 + 2
      N2 = N2 - 1
C--ADVANCE TIME
      DO 15 K = 1,NPTR
      Q(K) = Q(K) - XX
15 CONTINUE
      T = T + XX
      NPTR = NPTR - 1
      NCELL = N1 + N2
      IF (NCELL-63)30,30,31
31 WRITE (1,303)
30 Y(NCELL) = T
      GO TO 59
C
C--EVENT IS A DECISION AND DIVISION
C-----
      4 XX = W
C--NEW POPULATION SIZES
      N1 = N1 + 1
      NCELL = N1 + N2
      IF (NCELL-63)32,32,33
33 WRITE (1,303)
C--ADVANCE TIME
32 TIME = T + XX
      Y(NCELL) = TIME
      GO TO 18

```

C--NOW CALCULATE S FOR EACH SIMN. RUN

```

C
50 SUM = 0.0
   DO 21 I = 3,63
   IF (I-16) 8,21,8
   8 SQR = (X(I)-Y(I))**2.0
   SUM = SUM + SQR
21 CONTINUE
   SSQR(M) = SUM
   SQRT(M) = SSQR(M)**0.5
   WRITE (3,312) M,SSQR(M),SQRT(M)
20 CONTINUE
C--NOW CALCULATE E(S) FOR EACH SET OF RUNS

```

```

   XSQRT = 0.0
   DO 22 MK = 1,MX
   XSQRT = XSQRT + SQRT(MK)
22 CONTINUE
   SQRTM = XSQRT/XMX
   WRITE (2,320) SQRTM
C--SIGNIFICANCE TESTS

```

```

   CHI = 0.
   DO 23 MK = 1,MX
   CHI = CHI + (SQRT(MK)-SQRTM)**2.0
23 CONTINUE
   CHISQ = CHI/(SQRTM**2.)
   VAR = CHI/(XMX-1.)
   SD = VAR**0.5
   SDM = SD/(XMX**0.5)
C--OUTPUT RESULTS

```

```

   WRITE (3,315) SQRTM,CHISQ
   WRITE (3,318) SD,SDM
301 FORMAT (3F5.0)
302 FORMAT (' EXPTL PTS ARE ',F5.2,' ',F5.2,' ',F5.2)
303 FORMAT (' BOOBOO* NCELL TOO LARGE')
308 FORMAT (' ENTER LAMBDA, ALPHA, TO IN 3F5.0')
312 FORMAT (' RUN NO. = ',I2,' ',SSQR = ',F11.4,' SQRT = ',F7.2)
315 FORMAT (' 1H ',31X,' MEAN SQRT. = ',F9.5,' 5X ',CHISQ = ',F7.4)
318 FORMAT (' ',45X,' SD = ',F7.4,' SDM = ',F7.4,'//')
320 FORMAT (F12.8)
   GO TO 10
12 RETURN
END

```

## APPENDIX 2

### Selection Estimation Simulation Program

The following program was written for an IBM 1130, to simulate the fluctuations in genotypic fitnesses described in Section VI.

```

** RESPONSE ESTIMATES
C**RESPONSE SURFACE ESTIMATES OF FITNESS VALUES*****
C**PROGRAM ESTIMATES DISTANCE BETWEEN OBSERVED DATA
C**AND EXPECTED VALUES FOR STOCHASTIC
C**FITNESS VALUES. DATA FROM ALLARD, KAHLER, WEIR (1972)
C
C-----REGION OF OPERABILITY      U(I,J) POSITIVE
C-----REGION OF INTEREST         U(I,J)S TWEEN 0 AND 3
C
C**PROGRAM EXITS BY CHOOSING MANUAL INPUT OPTION AND THEN
C**AN INPUT OF 999. WITH SWITCH 12 ON WILL CALL EXIT
C
      DIMENSION F(10,6),EF(10,6),XN(10,6),EN(10,6),XNSUM(10)
      DIMENSION X2(50),X3(50),X4(50),X5(50),X6(50),SIG(50)
      DIMENSION RESP(20)
C**SET UP DESIGN MATRIX FOR AUTOMATIC FACTORIAL
C
      DATA X2/16*1.0,16*-1.0,2.0,-2.0,16*0.0/
      DATA X3/8*1.0,8*-1.0,8*1.0,8*-1.0,2*0.0,2.0,-2.0,14*0.0/
      DATA X4/4*1.0,4*-1.0,4*1.0,4*-1.0,4*1.0,4*-1.0,4*1.0,
14*-1.0,4*0.0,2.0,-2.0,12*0.0/
      DATA X5/2*1.0,2*-1.0,2*1.0,2*-1.0,2*1.0,2*-1.0,2*1.0,
12*-1.0,2*1.0,2*-1.0,2*1.0,2*-1.0,2*1.0,2*-1.0,2*1.0,
22*-1.0,6*0.0,2.0,-2.0,10*0.0/
      DATA X6/1.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,
11.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,
2,1.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,1.0,-1.0,8*0.0,
32.0,-2.0,8*0.0/
      DATA SIG/-1.0,2*1.0,-1.0,1.0,2*-1.0,2*1.0,2*-1.0,1.0,
1-1.0,2*1.0,-1.0,1.0,2*-1.0,1.0,-1.0,2*1.0,2*-1.0,2*1.0,
2,-1.0,1.0,2*-1.0,1.0,10*0.0,2.0,-2.0,6*0.0/
C
      COMMON MX,MY
      MX = 3
      MY = 2
      T = 0.005729
      U1 = 1.00000
      NGEN = 10
      IX = 3
      WRITE (1,533)
      WRITE (1,536)
      WRITE (1,520)
      WRITE (1,521)
      WRITE (1,513)
      PAUSE
      WRITE (3,100)
      WRITE (3,120)
C
C**EXPLANATION OF TERMS USED

```

```

C**U1,U2,U3,U4,U5 AND U6 ARE FITNESSES OF GENOTYPES
C      11,22,33,12,13 AND 23 RESPECTIVELY
C**XN(I,J) ARE NUMBERS OF EACH GENOTYPE
C**F(I,J) ARE FREQUENCIES OF GENOTYPES
C**EF(I,J) ARE EXPECTED FREQUENCIES OF GENOTYPES
C**XNSUM(I) ARE TOTAL NUMBERS IN EACH GENERATION
C
C**READ IN OBSERVED DATA
      DO 20 I = 1,NGEN
20  READ (2,108) XN(I,1),XN(I,2),XN(I,3),XN(I,4),XN(I,5),
      1XN(I,6)
      CALL MATIN(ICODE,XNSUM,10,IROW,ICOL,IS,IER)
      READ (2,351)
C
C**CONVERT INPUT QUANTITIES TO FREQUENCIES
C
      DO 1 I = 1,NGEN
      F(I,1) = XN(I,1)/XNSUM(I)
      F(I,2) = XN(I,2)/XNSUM(I)
      F(I,3) = XN(I,3)/XNSUM(I)
      F(I,4) = XN(I,4)/(XNSUM(I)*2.)
      F(I,5) = XN(I,5)/(XNSUM(I)*2.)
      F(I,6) = XN(I,6)/(XNSUM(I)*2.)
1  CONTINUE
      CALL MXOUT(1,F,10,6,0,60,120,1)
      WRITE (3,105)
      WRITE (3,103)
14  CALL DATSW(0,ISW0)
      GO TO (902,13),ISW0
C
C**OPTION 1 --- AUTOMATIC DESCENT
C*****
C---CALCULATE STEP SIZE RATIOS
902  WRITE (1,1003)
      READ (6,1002) A,B,C,D,E,G,H
      RAT2 = A/H
      RAT3 = B/H
      RAT4 = C/H
      RAT5 = D/H
      RAT6 = E/H
      RATSG = G/H
      WRITE (1,1001)
C---INPUT STARTING VALUE AND STEP SIZES
      READ (6,1000) XMU2,XMU3,XMU4,XMU5,XMU6,SIGM,DU2,DU3,DU4,
      1DU5,DU6,DSIG
15  CONTINUE
      CALL DATSW(0,ISW0)
      GO TO (906,13),ISW0
C---CALCULATE CO-ORD OF POINT ON STEEPEST DESCENT
906  XMU2 = XMU2 + DU2*RAT2
      XMU3 = XMU3 + DU3*RAT3
      XMU4 = XMU4 + DU4*RAT4
      XMU5 = XMU5 + DU5*RAT5
      XMU6 = XMU6 + DU6*RAT6
      SIGMA = SIGM + DSIG*RATSG
      GO TO 10
13  CALL DATSW(13,ISW13)
      GO TO (515,516), ISW13

```

```

C
C**OPTION 2 --- INSERT PARAM. VALS MANUALLY
C          FROM TYPEWRITER
C*****
  515 READ (6,107) XMU2,XMU3,XMU4,XMU5,XMU6,SIGMA
      IF (XMU2 - 999.) 517,11,517
  517 GO TO 10
C
C**OPTION 3 --- PERFORM AUTOMATIC FACTORIAL
C*****
C--READ CENTRE PT OF DESIGN AND THE DIST. FROM 0 TO 1
C-- AND NUMBER OF POINTS IN FACTORIAL
  516 WRITE (1,106)
      READ (6,107) XM2,XM3,XM4,XM5,XM6,XSIG
      READ (6,109) XR2,XR3,XR4,XR5,XR6,XRSIG,NPTS
      IJ = 1
  501 II = IJ
C--CALCULATE CO-ORDS OF POINT IN FACTORIAL DESIGN
      XMU2 = XM2 + XR2*X2(II)
      XMU3 = XM3 + XR3*X3(II)
      XMU4 = XM4 + XR4*X4(II)
      XMU5 = XM5 + XR5*X5(II)
      XMU6 = XM6 + XR6*X6(II)
      SIGMA = XSIG + XRSIG*SIG(II)
      IJ = IJ + 1
C
C
C**RESPONSE CALCULATION
C*****
  10 DO 6 KK = 1,20
      SK = 0.0
      DO 5 L = 1,9
          IF (L-3) 30,5,30
      30 IF (L-7) 31,5,31
      31 CALL DATSW(15,ISW15)
          GO TO (510,511) , ISW15
C--DRAW RANDOM VALUES FROM DESIRED DISTRIBUTION
C--NORMAL DISTRIBUTION
  510 CALL GAUSS(IX,SIGMA,XMU2,U2)
      CALL GAUSS(IX,SIGMA,XMU3,U3)
      CALL GAUSS(IX,SIGMA,XMU4,U4)
      CALL GAUSS(IX,SIGMA,XMU5,U5)
      CALL GAUSS(IX,SIGMA,XMU6,U6)
      GO TO 512
C--UNIFORM DISTRIBUTION
  511 SPRED = (3.*SIGMA)**0.5
      CALL RANDU(IX,IY,YFL)
      IX = IY
      U2 = XMU2 + (2.*YFL-1.)*SPRED
      CALL RANDU(IX,IY,YFL)
      IX = IY
      U3 = XMU3 + (2.*YFL-1.)*SPRED
      IX = IY
      CALL RANDU(IX,IY,YFL)
      U4 = XMU4 + (2.*YFL-1.)*SPRED
      IX = IY
      CALL RANDU(IX,IY,YFL)
      U5 = XMU5 + (2.*YFL-1.)*SPRED

```

```

IX = IY
CALL RANDU(IX,IY,YFL)
U6 = XMU6 + (2.*YFL-1.)*SPRED
IX = IY
512 CONTINUE
C--CALCULATE MEAN FITNESS
U = F(L,1)+U2*F(L,2)+U3*F(L,3)+2.*U4*F(L,4)+2.*U5*F(L,5)
1+2.*U6*F(L,6)
C--FORM INTERMEDIATE RESULTS
XP1 = U1*F(L,1)/U
XP2 = U2*F(L,2)/U
XP3 = U3*F(L,3)/U
XP4 = U4*F(L,4)/U
XP5 = U5*F(L,5)/U
XP6 = U6*F(L,6)/U
TOTP1 = XP1 + XP4 + XP5
TOTP2 = XP2 + XP4 + XP6
TOTP3 = XP3 + XP5 + XP6
C--HENCE EXPECTED FREQUENCIES
EF(L+1,1) = (1.-T)*(TOTP1+XP1)/2. + T*(TOTP1**2.)
EF(L+1,2) = (1.-T)*(TOTP2+XP2)/2. + T*(TOTP2**2.)
EF(L+1,3) = (1.-T)*(TOTP3+XP3)/2. + T*(TOTP3**2.)
EF(L+1,4) = (1.-T)*XP4 + 2.*T*TOTP1*TOTP2
EF(L+1,5) = (1.-T)*XP5 + 2.*T*TOTP1*TOTP3
EF(L+1,6) = (1.-T)*XP6 + 2.*T*TOTP2*TOTP3
C--HENCE EXPECTED QUANTITIES
DO 3 J = 1,6
EN(L+1,J) = EF(L+1,J)*XNSUM(L+1)
C--SUM OF SQUARES (EXP - OBSV)
SK = SK + (EN(L+1,J)-XN(L+1,J))**2.
3 CONTINUE
5 CONTINUE
C
C**FINAL RESULTS
RESP(KK) = SK**0.5
6 CONTINUE
VAR = 0.0
RESPM = 0.0
DO 7 I = 1,20
7 RESPM = RESPM + RESP(I)/20.
DO 9 I = 1,20
9 VAR = VAR + (RESP(I)-RESPM)**2.0/19.
SD = VAR**0.5
C
C**OUTPUT RESULTS
C*****
WRITE (1,352) RESPM
CALL DATSW (14,ISW14)
GO TO (516,514), ISW14
514 WRITE (3,104) U1,XMU2,XMU3,XMU4,XMU5,XMU6,SIGMA,RESPM,SD
GO TO (15,905), ISW0
905 GO TO (13,519), ISW13
C--IF FACTORIAL BEING EXECUTED THE RESPONSES PUNCHED ON CARDS
519 WRITE (2,350) RESPM
IF (II-NPTS) 501,11,11
C
C**RESET SWITCHES
C*****

```



```

11 WRITE (1,535)
   PAUSE
   CALL DATSW(12,ISW12)
   GO TO (522,14),ISW12
522 CALL EXIT
100 FORMAT (' RESP SURFACE ESTIMATES OF SELECTION VALUES')
103 FORMAT (' ',2X,'U1',5X,'U2',5X,'U3',5X,'U4',5X,'U5',5X,
1'U6',5X,'SIGMA',5X,'RESPONSE',8X,'SD')
104 FORMAT (' ',6(F5.3,2X),F6.4,5X,F10.4,4X,F8.4)
105 FORMAT (' GENOTYPIC FREQUENCIES F11,F22,F33,F12,
1F13 AND F23',//)
106 FORMAT (' TYPE IN CENTRE PT, STEP SIZES, NO. FACT PTS IN
1 6F7.0,(6F7.0,I2))
107 FORMAT (6F7.0)
108 FORMAT (6F5.0)
109 FORMAT (6F7.0,I2)
120 FORMAT (' U1 FIXED AT 1.00')
350 FORMAT (F12.7)
351 FORMAT (I2)
352 FORMAT (F12.7)
513 FORMAT (' SWITCH 15 ON NORMAL**OFF UNIFORM DISTRIBN. OF
1RAND. NOS.')
520 FORMAT (' SWITCH 13 ONTYPE IN VALS ** OFF DO FACTORIAL')
521 FORMAT (' SWITCH 14 ON TO ENTER NEW STEP SIZES')
533 FORMAT (' SWITCH 0 ON AUTOMATIC DESCENT**OFF TRY OTHER
1OPTIONS')
535 FORMAT (' RESET SWITCHES')
536 FORMAT (' SWITCH 12 ON CALL EXIT ** OFF CONTINUE')
1000 FORMAT (12F7.0)
1001 FORMAT (' ENTER XMU2,XMU3,XMU4,XMU5,XMU6,SIGM,DU2,DU3,
1DU4,DU5,DU6,DSIG, IN 12F7.0')
1002 FORMAT (7F8.0)
1003 FORMAT (' WRITE IN VALS NEEDED TO CALC STEP SIZE RATIOS
1IN 7F8.0')
   END

```

## Bibliography

- Allard, R.W., Kahler, A.L., and Weir, B.S. The effects of selection on esterase allozymes in a barley population. *Genetics* (in press)
- Barker, J.S.F., and Butcher, J.C. 1966. A simulation study of quasi-fixation of genes due to random fluctuation of selection intensities. *Genetics* 53 (2): 261-268
- Box, G.E.P. 1952. Multifactor designs of first order. *Biometrika* 39: 49-57
- Box, G.E.P., and Draper, N.R. 1959. A basis for the selection of a response surface design. *J. Amer. Statist. Assn.* 54: 622-654
- Box, G.E.P., and Hunter, J.S. 1954. A confidence region for the solution of a set of simultaneous equations with an application to experimental design. *Biometrika* 41: 190-199
- Box, G.E.P., and Hunter, J.S. 1957. Multifactor designs for exploring response surfaces. *Ann. Math. Statist.* 28: 195-241
- Box, G.E.P., and Wilson, K.B. 1951. On the experimental attainment of optimum conditions. *J. R. Statist. Soc. (B)* 13: 18-34
- Davies, O.L. 1956 Design and analysis of industrial experiments. 2nd ed. New York: Hafner Publ. Co.
- Hammersley, J.M., and Handscomb, D.C. 1964. Monte Carlo methods. London: Methuen and Co. Ltd.
- Hill, W.J., and Hunter, W.G. 1966. A review of response surface methodology: a literature survey. *Technometrics* 8: 571-590
- Hoel, D.G., and Mitchell, T.J. 1971. The simulation, fitting, and testing of a cellular proliferation model. *Biometrics* 27: 191-199
- Hope, A.C.A. 1968. A simplified Monte Carlo procedure. *J. R. Statist. Soc. (B)* 30: 582-598
- Hufschmidt, M.M. 1962. Analysis by simulation: examination of response surface. Maass, et. al. Design of water resource systems. New York: Macmillan and Co. Ltd.

- Hunter, J.S. 1954. Searching for optimum conditions. Trans. N. Y. Acad. Sci. 2: 124-132
- Hunter, J.S., and Naylor, J.H. 1970. Experimental designs for computer simulation experiments. Management Sci. 16: 422-434
- Jain, S.K., and Marshall, D.R. 1968. Simulation of models involving mixed selfing and random mating. Heredity 23: 411-432
- Knuth, D.E. 1968. The art of computer programming. V2. Seminumerical algorithms. Reading: Addison-Wesley Pub. Co.
- Kubitschek, H.E. 1962. Normal distribution of cell generation rate. Exptl. Cell Res. 26: 439-450
- Myers, R.H. 1971. Response surface methodology. Boston: Allyn and Bacon
- Naylor, T.H., Balintfy, J.L., Burdick, D.S., and Chu, K. 1966. Computer simulation techniques. New York: Wiley
- Pielou, E.C. 1968. An introduction to mathematical ecology. New York: Wiley-Interscience
- Thomas, V.J. 1971. Some aspects of second order response methodology. Master's thesis, Massey University, Palmerston North, New Zealand.