## 3. <u>Testing the hypotheses</u>.

The hypotheses from which the formulae of the previous section were deduced are

- (i) Homozygous parents.
- (ii) No multiple allelism.

(iii) Genes independently distributed in the parents.

(iv) No genic interaction on the scale in use.

When these hold good the difference Wr - Vr has the value  $W_r-V_r = \Sigma u_i v_i (d_i^2 - h_i^2) = W-V$  independently of r. Heterogeneity of this difference indicates that one or more of the hypotheses fail and two methods are available for testing this.

In the first, the variance of Wr - Vr is compared with a theoretical variance obtained from the diallel table to give an approximate  $\chi^2$  test of significance. As the form of the theoretical variance is complicated, only the numerical results of its use will be quoted.

The second method uses the graph of Wr against Vr which should be a straight line of unit slope. The statistical inequality  $Wr^2 \leq VrVp$  means that all the points (Vr, Wr) of the graph line on that part of the line Wr - Vr = W - V inside the parabola  $Wr^2 = VrVp$ . Though (Vr, Wr) are not a set of independent observations, but are correlated second degree statistics, an approximate test of the validity of the above hypotheses is to fit a regression line to the points (Vr, Wr) in the usual way. Failure of the hypotheses is indicated either by a nonsignificant regression in a sufficiently extensive experiment or, when the regression is significant, by a significant deviation of the slope from unity. Non-significance of regression may also arise if all  $h_i = 0$ , but this may be tested separately.

All the formulae of this and the previous section apply to the means of  $F_2s$  from selfed  $F_1s$  if  $1/2h_i$  is substituted for  $h_i$  so that  $F_2$  data may be tested and analyzed in the same way. Further, the graph of  $F_2$  array variances (or covariances) against  $F_1$  variances (or covariances) should be a straight line of slope 1/2. A linear test which is also available is that  $P + F_1 - 2F_2$  should be zero. The empirical value can be compared with the standard deviation derived from the environmental variation of parental,  $F_1$  and  $F_2$  means to give a test of significance.

When failure of the hypotheses has been demonstrated it is not easy to decide from  $F_1s$  alone just which ones have failed. However, the possible use of an unsuitable scale can be detected by plotting Wr - Vr against the array means to see if there is a definite trend from which a new scale may be deduced by the usual variance stabilization method. When it is not possible to remove the variation in Wr - Vr by rescaling, the (Vr,Wr) graph can be examined. It may show some points deviating markedly from the line of unit slope through (V,W) though even these must of course be inside the limiting parabola. The four hypotheses are discussed in turn and we suppose for convenience that in the graph the OW axis is vertical and the OV axis horizontal.

When there is no dominance  $(h_i = 0)$  the line is a tangent to the limiting parabola, but if there is dominance the line is a chord cutting off an area of the parabola which increases as the amount of dominance increases.

- (i) Heterozygosity in a parent moves the corresponding point above the line and reduces the apparent amount of dominance.
- (ii) As long as there is no segregation, multiple allelism can be regarded as polygenic biallelism exhibiting genic interaction or distributional association.
- (iii) Correlated association of alleles in the parents causes points to deviate either side of the line.

(iv) Genic interaction moves the corresponding points either above or below the line depending on whether it moves the double heterozygote nearer to or further away from the mid-homozygote.

When there is little dominance, neither (i) nor (iii) causes any trouble while in (iv) the limiting parabola forces any deviation to be below the line which accords with the fact that interaction must move the double heterozygote away from the mid homozygote in this case.

Usually in practice only one or two points deviate strongly and then the results from the progeny of the corresponding parents can be removed from the diallel table and the theory of section 2 applied to the smaller subtable.

<u>Maize-yields</u> (Kinman and Sprague, 1945). This is a set of  $F_1$  and  $F_2$  means from a diallel cross of 10 lines labelled Hy, R46, B2, WF9, 38-11, K159, Oh07, Oh04, WV7 and A14 in the original paper and now renumbered 1 to 10 respectively.

The graph of Wr - Vr against array means revealed no trend so that resealing is not suggested.

 $F_1$ . The first test of heterogeneity of Wr - Vr gave  $\chi^2_9 = 32.756$  which is highly significant (P< .001). The greatest improvement was obtained by removing the progeny of line (1) (P = .05 - .02) and then either line (3) (P = .20 - .10) or line (7) (P = .30 - .20).

The regressions in the second method were all highly significant (P< .001). For the whole table  $b = 0.676 \pm 0.108$  which on removing the progeny of line (1) improved to be =  $0.754 \pm 0.098$ . Removing (1) and (3) gave  $b = 0.788 \pm 0.075$  while (1) and (7) gave  $b = 0.808 \pm 0.086$  which is not significantly different from b = 1.000.

 $F_{\rm 2}.$  The first method gave  $\chi^2_{\rm 9}$  = 7.263 which is not significant (P = .70 - .50).

The slope of the regression line was  $b = .707 \pm .114$  which barely differs significantly from 1.000 (P = .05 - .02). Removing (3) which lies farthest from the regression line only improved the slope slightly.

As expected the reduced heterozygosity in  $\mathsf{F}_2$  makes it more difficult to detect anomalies.

<u>Linear test</u>.  $P + F_1 - 2F_2 = 5.66$  which, using what we estimate to be the errors of the means is highly significantly different from zero. This is reduced to 3.41 by removing line (3) and the further removal of line (2) reduces it to 1.74.

These tests show conclusively that interaction of some sort was present, probably in many of the crosses, but that it had altered considerably over the two years in which the  $F_1$  and  $F_2$  were grown. However line (3), i.e. (B2), was picked out by all the tests.

In our Nicotiana experiments we have found little evidence of interaction in flowering time, but in height a few lines exhibit marked interaction and do so consistently in  $F_1$  repeated over two years and also in an  $F_2$  and a backcross.