LINKING OF BIO-ASSAY CONTRASTS AND FACTORIAL CONTRASTS

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Introduction

Bliss (1940) while re-examining an experiment by Coward (1938) on the assay of vitamin D from the ash content of the femur of the rat, noticed that the estimation of relative-potency and its error can be facilitated by adopting a factorial type of analysis. Further, Bliss (1952) considered two and three dose factorial assays. However, it appears that a general link between bio-assays and factorial contrasts has not been discussed in literature. We have thus presented, here, a general link between the bio-assay and factorial contrasts which will enable us to use the traditional confounded designs for factorial experiments in bio-assays.

2. The Link

Consider a $2K$-point symmetrical parallel line (SPL) assay. The $(2K-1)$ $d.f$ between doses can be split-up into $(2K-1)$ orthogonal contrasts, each with single $d.f.$ Following notations of Finney (1952), these contrasts are $L_1, L_2, \ldots, L_{K-1}, L_1', L_2', \ldots, L'_{K-1}$ and L_p (L_m and L'_m denote the sums and differences of mth power contrasts of dose effects of the two preparations, L_p denotes the difference between the totals of the standard and test preparation effects, *i.e.*, the 'preparation contrast'). On the other hand, consider an asymmetrical factorial experiment with two factors, viz. X at two levels 0 and 1, and A at K, $(K \geqslant 2)$ levels $0,1,2,\ldots,(K-1)$. The $(2K-1)$ $d.f.$ of this $2 \times K$ factorial experiment can be split up into three components namely, (1) the main effect X with one $d.f.$, (*ii*) the main effect A with $(K-1)$ $d.f.$, and (*iii*) the interaction XA with $(K-1)$ $d.f.$ Further, using the orthogonal polynomials (Fisher and Yates, 1963, Table *XXIII*) of a set of K equally spaced levels the components A and XA can be split up into orthogonal components each with 1 $d.f.$ Thus if we denote A_i (and similarly XA_i), $i = 1,2,\ldots,(K-1)$, as the ith power contrast then the totality of $(2K-1)$ $d.f.$ of the above factorial experiment can be split up into following $(2K-1)$ orthogonal contrasts : $X, A_1, A_2, \ldots, A_{K-1}, XA_1, \ldots, XA_{K-1}$.

The correspondence between the $2K$ treatment combinations (basic treatments) of the factorial experiment and the $2K$ doses of the SPL assay can be defined as below.

$$(X_o\, a_j) \equiv s_{j+1}\ ;\ (x_1\, a_j) \equiv t_{j+1}, j=0,1,2,\ldots,(K-1), \qquad \ldots(1)$$

where $(x_i\, a_j)$ is the treatment combination, at the ith level of the factor X and jth level of factor A, in the $2 \times K$ factorial experiment, $s_1, s_2, \ldots, s_k$ are effects of the K doses (on the logarithmic scale) of the 'standard' preparation and $t_1, t_2, \ldots, t_K$ are the effects of the K doses (on the logarithmic scale) of the 'test' preparation both arranged in the same order of magnitude and the doses of both the preparations are equally spaced on the logarithmic scale.

From the above definitions, it can be easily verified that the link-relations connecting the bio-assay contrasts with factorial contrasts are,
$L_p = x,\ L_i = A_i$ and $L'_i = XA_i,\ i = 1, 2, \ldots, (K-1)$

For example, consider a 6-point *SPL* assay. The coefficients of the contrasts required in this assay are shown in Table I (we assume that $s_1 < s_2 < s_3$ and $t_1 < t_2 < t_3$).

Further, a set of five orthogonal contrasts in the 2×3 factorial experiment can be written as

$$X = x_0a_0 - x_0a_1 - x_0a_2 + x_1a_0 + x_1a_1 + x_1a_2$$
$$A_1 = -x_0a_0 + x_0a_2 - x_1a_0 + x_1a_2$$
$$A_2 = x_0a_0 - 2x_0a_1 + x_0a_2 + x_1a_0 - 2x_1a_1 + x_1a_2$$
$$XA_1 = x_0a_0 - x_0a_2 - x_1a_0 + x_1a_2$$
$$XA_2 = -x_0a_0 + 2x_0a_1 - x_0a_2 + x_1a_0 - 2x_1a_1 + x_1a_2$$

It is now evident that if we adopt the following linking set up,

$x_0a_0 \equiv s_1,\ x_0a_1 \equiv s_2,\ x_0a_2 \equiv s_3,$

$x_1a_0 \equiv t_1,\ x_1a_1 \equiv t_2,\ x_1a_2 \equiv t_3,$

then the link-relations between the contrasts of 6-point SPL assay and the contrasts of 2×3 factorial experiment are as given below,

$$L_p = X,\ L_1 = A_1,\ L_2 = A_2,\ L'_1 = XA_1,\ L'_2 = XA_2$$

The above approach, however, does not offer a general link between bio-assay contrasts and factorial effects. For, the questions like, what are the link-relations between the contrasts of a 8-point SPL assay and the effects of 2^3 factorial experiment ?, what are the link-relations between the contrasts of a 12-point SPL assay and a 3×2^2 asymmetrical factorial experiment ? etc., are still to be considered. All such questions can be answered and in fact a general link can be given by adopting a suitable linking set-up using the following result of Das and Jain (1970) presented in form of a theorem.

Theorem. Let there be n independent observations $y_1, y_2, \ldots, y_n$ drawn from a Population with variance σ^2 and let $A_1, A_2, \ldots A_{n-1}$ be $(n-1)$ orthogonal contrasts among the observations. Now, if L_j be any other contrast among the observations, then L_j can be expressed as a linear function of A_i ($i=1, 2 \ldots, n-1$) through the following relation, $L_j = \sum_i C_{ji} A_i / d_i$ where $C_{ji}\ \sigma^2$ is the covariance between L_j and A_i and d_i is the sum of squares of coefficients of observations in A_i. The variance of $L_j = \sigma^2 \sum_i C_{ji}^2 / d_i$.

3. A General Method of Linking

Let $s_1, s_2, \ldots s_k$ and $t_1, t_2 \ldots t_k$ denote the log-doses of a $2K$-point SPL assay and let $p_1^{m1}\ p_2^{mr} \ldots p_r^{mn}$, where p_i is a prime and m_i is a positive integer, denote $2K$ factorial-combinations. Clearly if $r=1$, then $2K=p_1^{m1}$ and so the corresponding factorial experiment is a symmetrial one with m_1 factors each at p_1 levels. And if $r>1$, the corresponding factorial experiment is an asymmetrical one with $\sum_{i=1}^{r} m_i$ factors.

The method of linking consists in first determining the corresponding factorial experiment (depending on $r=1$, or >1), then adopting a suitable linking set up *i.e.*, the isomorphic correspondence between the $2K$ treatment combinations of the factorial experiment and the $2K$ doses of the SPL assay, and finally obtaining the *link-relations* between bio-assay contrasts and factorial contrasts using the Theorem mentioned above.

It may be noted that there should be a factor at two levels in the factorial experiment corresponding to a SPL assay, to represent the two preparations. Thus the main effect of this factor at 2 levels is a contrast of $2K$ treatment combinations with ± 1 as the coefficients. A convenient way of choosing a linking set up is to associate the K doses of test preparation with the K treatment combinations of the above main effect whose coefficients are with plus sign and the K doses of standard pre-paration with K treatment combinations whose coefficients are with minus sign. Thus the above main effect will correspond to the contrast L_p in the SPL assay.

As an example, consider a 8-point SPL assay. As $8=2^3$, so the corresponding symmetrical experiment is with 3 factors each at two levels, say 0 and 1. Let us denote the three factors by A, B and C and a treatment combination at ith level of A, jth level of B, and lth level of C in the usual way by $(i\,j\,l)$, where $i, j, l=0, 1$. For it, the coecffiients of bio-assay contrasts are shown in Table II and the factorial contrasts in Table III (we are assuming $s_1<s_2<s_3<s_4$ and $t_1<t_2<t_3<t_4$).

A suitable linking set up may now be chosen by associating the contrast A with L_p, *i.e.*, by adopting the followiug linking set up of basic treatments.

$$(000)\equiv s_1,\ (010)\equiv s_2,\ (001)\equiv s_3,\ (011)\equiv s_4$$

$$(100)\equiv t_1,\ (110)\equiv t_2,\ (101)\equiv t_3,\ (111)\equiv t_4$$

Finally, to obtain the link-relations, we need the covariance multipliers C_{ji}'s which can be obtained as the sum of the products of the corresponding coefficients in the two contrasts as shown in the tables II and III. These C_{ji}'s and the link-relations expressing the bio-assay contrasts in terms of factorial effects are shown in Table IV. Since for all the factorial contrasts in Table III, $d_i=8$ so $L_j=\sum_i C_{ji}\, A_i/8$.

4. A Remark

Link-relations for asymmetrical parallel line assays (different number of doses in the two preparations) and for multiple SPL assays (with more than one test preparations) can be easily obtained by using the present method. Some additional illustrations and the use of confounded factorial design in bio-assays is reserved for a further communication.

5. Summary

Since in bio-assays all the contrasts are not of equal importance, we may think of using a confounded design. Contrasts, which are not of major importance, may be partially or completely confounded. Use can be made of usual confounded factorial designs in bio-assays, if it is possible to establish a link between the bio-assay and factorial contrasts. This problem has been attempted in this paper and a general link is established for Parallel Line Assays.

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TABLE I

Contrasts for 6-point parallel line assay

Contrast	*log-dose : tr. comb.* :	s_1 $(x_0\ a_0)$	s_2 $(x_0\ a_1)$	s_3 $(x_0\ a_2)$	t_1 $(x_1\ a_0)$	t_2 $(x_1\ a_1)$	t_3 $(x_1 a_2)$
L_p		−1	−1	−1	1	1	1
L_1		−1	0	1	−1	0	1
L'_1		1	0	−1	−1	0	1
L_2		1	−2	1	1	−2	1
L'_2		−1	2	−1	1	−2	1

TABLE II

Contrasts for 8 Point SPL Assay

Contrast	log dose							
	s_1	s_2	s_3	s_4	t_1	t_2	t_3	t_4
L_p	−1	−1	−1	−1	1	1	1	1
L_1	−3	−1	1	3	−3	−1	1	3
L_1'	3	1	−1	−3	−3	−1	1	3
L_2	1	−1	−1	1	1	−1	−1	1
L_2'	−1	1	1	−1	1	−1	−1	1
L_3	−1	3	−3	1	−1	3	−3	1
L_3'	1	−3	3	−1	−1	3	−3	1

TABLE III

Effects (Contrasts) for 2^3 Factorial Experiment

Contrast	Treatment Combinations							
	(000)	(100)	(010)	(110)	(001)	(101)	(011)	(111)
A	−1	1	−1	1	−1	1	−1	1
B	−1	−1	1	1	−1	−1	1	1
AB	1	−1	−1	1	1	−1	−1	1
C	−1	−1	−1	−1	1	1	1	1
AC	1	−1	1	−1	−1	1	−1	1
BC	1	1	−1	−1	−1	−1	1	1
ABC	−1	1	1	−1	1	−1	−1	1

TABLE IV

Covariances of the two sets of contrasts and the consequent link relations

Bio-assay Contrast	Factorial Effects							*Link Relations* L_j
	A	*B*	*C*	*AB*	*AC*	*BC*	*ABC*	
L_p	8	0	0	0	0	0	0	$L_p=A$
L_1	0	8	16	0	0	0	0	$L_1=B+2C$
L'_1	0	0	0	8	16	0	0	$L'_1=AB+2AC$
L_2	0	0	0	0	0	8	0	$L_2=BC$
L'_2	0	0	0	0	0	0	8	$L'_2=ABC$
L_3	0	16	−8	0	0	0	0	$L_3=2B-C$
L'_3	0	0	0	16	−8	0	0	$L'_3=2AB-C$

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