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Designs for animal experiments under two-way blocking structure in the presence of systematic trend

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ABSTRACT

Designs under two-way blocking structure are quite useful in animal experiments. Further, in animal experiments, situations are also quite prevalent where there are evidences of systematic trend component influencing the response. These effects are generally neglected but they may have significant impact on the precision of the experiments, if considered. Suitably choosing a design will nullify the effect of such systematic trend. The present article describes the model under two-way blocking structure incorporating systematic trend component. The condition to nullify the effects of trend component when they are present in the experimental material has been obtained and illustrated under a two-way blocking structure. The analytical procedure has been explained through a hypothetical dataset to show the significance of such effects. The results shows that systematic trend component have significant impact on the precision of the experiments and hence will affect the inference.

Key words: Latin square design, Systematic trend, Trend-free designs, Trend resistance, Two-way blocking

In any scientific investigation, experimentation and drawing valid inferences based on the experimentation are twin essential features. Many factors (e.g. objectives of the experiments, availability of experimental material, cost of the experiment etc) are to be considered while designing an experiment which is to be done carefully for any experiment in order to test hypothesis with acceptable degree of precision. Heterogeneity in the experimental material which is an important problem, can be taken care of while designing of scientific experiments through blocking of experimental material. Heterogeneity in experimental material may present either only due to one source or due to more than one source. In animal experiments, situations are quite prevalent, where we may come across some situations when there is evidence of heterogeneity in the experimental material due to two sources. Consider the following experimental situation:

Experimental situation: In an animal experiment with an object of comparing the effect of different feeds (treatments), let different cows be the experimental units with their milk yield over different time interval of a particular lactation as the variate under study. Suppose breed and age of cows are two factors that correspond to two sources of variation apart from the treatment. Thus, both of them are actually the controlled factors and it is intended to eliminate the variation due to breeds and age of the cows. Here, as there are two sources of variation to be eliminated viz. breed and age, thus, designs based on two-way blocking structure is most preferable for this situations.

In animal experiments, many situations arise in which apart from the known source of variations, the response may also depend on the temporal effect, i.e. the experimental units may get affected by one or more systematic trend present in the experimental material. For the above experimental situation, one can also identify systematic trend component which can affect the milk yield if a fact regarding milk yield can be taken in to consideration as it is well known in advance that the milk yield will decrease as lactation of an animal progress over weeks. Therefore, the experimental output will be more précised within the limited available resources if the above mentioned fact can be included in the model in the form of systematic trend component. One way to account for the presence of trends in the experimental material is to think of suitable designs which are orthogonal to trend effects. Such design may be called as trend-free designs. In case of block design with trend components, it is assumed that the within block trend effects can be represented by orthogonal polynomial of u^{th} degree ($u < k$, where k is the block size). Following is an example of linear trend-free balanced incomplete block design for $v = 5$, $b = 10$, $r = 6$, $k = 3$, $\lambda = 3$ and $p = 1$ (symbols have their usual meaning):

Orthogonal trend component of degree one without normalization is given in the upper row of the array. A lot of work has been done under block design setup incorporating systematic trend component both at national

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–1	0	1
1	2	3
1	2	4
1	2	5
3	4	1
3	5	1
4	5	1
4	2	3
5	2	3
5	2	4
3	4	5

and international level [For example one may refer Jacroux *et al.* (1997), Majumdar and Martin (2002), Lal *et al*. (2007), Bhowmik (2013) etc.]. Different aspects of trend free designs in the presence of interference effects have been studied and series of trend free totally balanced designs have been obtained for different situations by Bhowmik *et al.* (2014, 2015). Sarkar *et al.* (2017) obtained trend resistant neighbour balanced bipartite block designs. Recently Bhowmik *et al.* (2017) also obtained the necessary and sufficient condition for a design under two-way elimination of heterogeneity to be trend free.

In this article, we have defined the model under two-way blocking structure incorporating systematic trend along with the condition for a design under two-way blocking structure to be trend resistance. Further, a comparison among the analytical procedure of experimental data based on designs under two-way blocking with and without trend effects has been made through an illustration using a hypothetical data set.

MATERIAL AND METHODS

Experimental setup and model: Let there are two sources of variability as described in the above experimental situation viz. age and breed of the animal. Thus, model under two-way blocking structure should be used in this situation. Also let, experimenter is having the knowledge about some systematic trend like milk yield of an animal will decrease as lactation of the animal progress over weeks. Therefore, for getting better precision from the experiments, the effect of systematic trend should be included in to the model. Based on the above experimental setup, following fixed effects additive model in matrix notations, can be considered for capturing the effect of systematic trend for designs under two-way blocking structure:

$$Y=\mu 1 + \Delta' \tau + D1\, \rho + D'_2\, \chi + Z\theta + e \qquad ...(1)$$

where, $\mathbf{Y}$ is a n × 1 vector of observations, μ is the general mean, **1** is a n × 1 vector of unity, Δ is a n × v matrix of observations versus treatments i.e. feeds, $\boldsymbol{\tau}$ is a v × 1 vector of treatment effects i.e. feed effects, $\mathbf{D'_1}$ is a n × p incidence matrix of observations versus one source of heterogeneity i.e. breed, $\boldsymbol{\rho}$ is a p × 1 vector of breed effects, $\mathbf{D'_2}$ is a n × q incidence matrix of observations versus another source of heterogeneity i.e. age, $\boldsymbol{\chi}$ is a q × 1 vector of age effects, $\boldsymbol{\theta}$ is a u × 1 vector representing the trend effects. The matrix **Z**, of order n × u, is the matrix of coefficients which is given by $\mathbf{Z=1p} \otimes \mathbf{F}$ where **F** is a q × u matrix with columns representing the (normalized) orthogonal polynomials as it is known that the milk yield of any breed will decrease as lactation progress over weeks. Here, **e** is a n ×1 vector of errors where errors are normally distributed random variable with E(**e**) = **0** and D(**e**) = $\sigma^2\, \mathbf{I}_n$. Based on the model, when the levels of all the factors are equal say v, the information matrix for estimating the contrast pertaining to the effect of treatments viz. feeds under two-way blocking structure can be obtained as:

$$C = v\left(I - \frac{11'}{v}\right) - \frac{1}{v}\Delta ZZ'\Delta' \qquad ...(2)$$

Conditions for designs to be trend free: When one include systematic trend component into the model for proper model specification, an effective way to deal with the situation is the adaption of designs which are resistance to the effect of systematic trend i.e. the design where the trend effect will be nullified. For a completely trend resistant design i.e. for a trend free design, the treatment (feed) effects and the trend effects are orthogonal or nearly orthogonal to each other and the analysis of the design could then be done in the usual manner, as if no trend effect was present. Statistically we can say, a design is said to be trend-free if the adjusted treatment sum of squares arising from the effects of treatments under the model (1) with trend component is same as the adjusted treatment sum of squares under the usual model without trend component. Therefore, for a design to be trend free, a necessary and sufficient condition is Δ Z=0. Thus for a trend free design, when the levels of all the factors are equal say v, the information matrix for estimating the contrast pertaining to the effect of treatments viz. feeds under two-way blocking structure becomes

$$C = v\left(I - \frac{11'}{v}\right) \qquad ...(3)$$

This will be same if one obtains the expression of the information matrix under the usual model for two-way blocking structure without trend component. It can be noted that for a nearly trend-free design ΔZ will be very small quantity near to zero. Nearly trend-free designs are useful when it is not possible to obtain a completely trend-free design for a given situation.

Trend-free designs: Following is an example of a trend-free design based on two way-blocking structure (say breed and age are two source of blocking with five number of levels for both the factor) for five number of treatments say feeds.

Age→ Breed ↓	1 (-2) [-0.632]	2 (-1) [-0.316]	3 (0)] [0	4 (1) [0.316]	5 (2) [0.632]
1	A	B	C	D	E
2	B	C	D	E	A
3	C	D	E	A	B
4	D	E	A	B	C
5	E	A	B	C	D

Here, different letter indicates different feeds, number in first column indicates different breeds, number in first row without bracket indicates different ages. Since, due to the known fact that, as the lactation of an animal progresses over weeks, the milk yield decreases, and hence trend effect has also been incorporated into the model. Here numbers in () indicates orthogonal trend component of degree one of size five without normalization. These coefficients, has been used to measure trend effects. The numbers in [ ] are normalized orthogonal trend component of degree one of size five. Therefore based on the model one can choose **F** as

$$F=\left[\frac{-2}{\sqrt{10}} \quad \frac{-1}{\sqrt{10}} \quad 0 \quad \frac{1}{\sqrt{10}} \quad \frac{2}{\sqrt{10}}\right]' = \left[-0.632\ -0.316\ 0\ 0.316\ 0.632\right]'$$

Since, the above design is a trend free design, therefore, the adjusted treatment sum of squares arising from the effects of treatments under the model (1) with trend component is same as the adjusted treatment sum of squares under the usual model without trend component.

Analytical procedure with and without systematic trend: When, there is evidence of systematic trend components in the experimental material they should be included in the model for proper model specification as these remote effects may affect the response and hence will play a direct role in affecting the precision of experiments and interpretation of the results. Therefore, if these effects are not included, results may be erroneous. One can split up the sources of variation and degrees of freedom in the ANOVA table with respect to design under two-way blocking structure involving systematic trend component of degree u and n number of experimental units [In case of linear trend as the case described in experimental situation i.e. when only one trend is considered, u will be equal to 1] as follows:

Table 1. Split up of sources of variation and degrees of freedom in the ANOVA table with respect to design under two-way blocking structure involving systematic trend component of degree u

Source of variation	Degrees of freedom (DF)
Treatments	v-1
First factor of heterogeneity (e.g. breeds)	p - 1
Second factor of heterogeneity (e.g. age of animal)	q - 1
Trend	u
Error	By subtraction
Total	n - 1

where the symbols have their usual meaning as defined earlier. Following is an illustration based on hypothetical data of milk yield (in quintals) of cow.

*Illustration:*Consider an animal experiment with the major purpose of comparing five different feeds. Let, the observations are milk yield (in quintals) in a particular time period (say, week) within a lactation of different breeds of cows along with different age groups. Since, milk yield of any breed will decrease as the period of lactation progresses, therefore, in this experiment, there is also an evidence of systematic trend component which may affect the precision of the experiment. Following is the layout of the experiment along with hypothetical data set:

Trend→ (Normalized) Breed ↓	-0.632	-0.316	0	0.316	0.632
1	2.665(1) [A]	2.095 (2) [B]	1.500(3) [C]	0.743 (4) D]	0.385 (5) [E]
2	3.702 (3) [B	3.107 (4) [C]	2.250 (5) [D]	1.018 (1) [E]	1.098(2)] [A
3	3.740(5) [C]	2.007(1) [D]	1.702(2) [E]	1.774(3) [A]	1.193(4) [B]
4	3.093(2) [D]	2.867(3) [E]	3.128(4) [A]	2.536(5) [B]	0.685(1) [C]
5	4.479(4) [E]	5.095(5) [A]	2.763(1) [B]	2.236(2) [C]	1.391(3) [D]

Here, numbers in bold letter indicates milk yield in quintals, number in first column indicates different breeds of cow, number in () indicates different age group of a particular breed, letters in [ ] indicates different feeds. Values in the first row are normalized orthogonal polynomial coefficient of degree 1 which are used to measure the trend effect. Here, the trend component is linear as only one trend has been captured.

RESULTS AND DISCUSSION

Initially, the above data set was analyzed by using the usual three-way Analysis of Variance technique (ANOVA) through SAS 9.3 with three known sources of variation as feeds, breeds and age group without considering the trend information. It has been observed that none of the effects came out to be significant. Thereafter, by considering trend effect, the same data set was analyzed once again by incorporating a linear trend component in to the model based on equation (1). Interestingly, it has been observed that the main effect of interest i.e. the effects of feed came out to be significant at 1% level of significance i.e. by considering trend component, it has been seen that effects of feeds became significantly different from each other at 1% level of significance. Further, we have also found that the effect of breed, age and trend all came out to be significant at 1% level of significance. Thus, one can say that, trend effects from the experimental material can play a significant role in the precision of the experiment. So, when there are evidences of trend effects, one has to consider these effects in to the model and analyze the data accordingly for drawing valid conclusions based on the experiment. The significance of the effects due to the inclusion of systematic trend component therefore throws light on the importance of these type of remote but important effects on experimental precision and interpretation of results. ANOVA of the above data also reveals that, the sum of squares of all the three sources of variations are unaffected by the presence or absence of trend component although the interpretation has been changes significantly. This is so because, here the layout of the experiments is based on a trend free designs under two-way blocking structure. Since, experimenter used trend free design, therefore the sum of squares of all the three different source of variability remains same in both the presence or absence of systematic trend component.

Thus, in animal experiments, where there may be evidences of two sources of variability apart from the treatment applied to the experimental material, response may also get affected by systematic trend. The effect of trend although remote, still may have high influence on response and hence should be incorporated in to the model for proper model specification. The trend-free design based on two way-blocking structure would nullify the effects of common trend effects and thus will increase the precision of the experiments. Therefore, trend-free designs based on two way-blocking structure would be useful for researchers involved in animal experiments.

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