Trend free Designs for Animal Experiments under Two Sources of Heterogeneity

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1. Introduction

Designing an experiment which is an essential component of any scientific investigation and need to be done carefully, forms the basis for testing any hypothesis with acceptable degree of precision. While, designing an experiment, experimenter need to investigate many factors like objective of the study, availability of resources, cost of experiment, heterogeneity or variability in the experimental material etc.
Through proper designing of an experiment, the heterogeneity in the experimental material, which is an important problem to be dealt with, can be taken care by grouping of experimental material in such a way that units within the group are more homogenous as compare to between the group. In agricultural and allied experiment, there may be one source of heterogeneity or may be more than one source of heterogeneity. Heterogeneity in more than one direction within the experimental material is very common particularly in animal experiments. Following is an experimental situation:

Experimental situation: In an animal experiment, in order to compare the effect of different feeds (treatments), let the experimental units are different cows with and variate under study is their milk yield over different time interval of a particular lactation. In this situations, the experimenter can consider breed and age of cows as two other sources of heterogeneity. Thus, both breed and age of cows are actually the controlled factors and the experimenter is intended to eliminate the variation due to breeds and age of the cows. Here, **designs under two source of heterogeneity** is most preferable.

In animal experiments, there are many situations where the response may also depend on the temporal effect apart from known sources of variations i.e. some systematic trend component may effect the experimental units within the experimental material. For the above experimental situation, a systematic trend component can be identified if a fact regarding milk yield can be taken in to consideration. The fact is as lactation of an animal progress over weeks, milk yield will decrease. Therefore, if the above mentioned fact can be included in the model in the form of systematic trend component, the experimental output will be more précised within the limited available resources. To account for the presence of trends in the experimental material, use of **trend-free designs** is an effective solution as in such designs treatments are orthogonal to trend effects (Bradley and Yeh, 1980). A lot of work is available in literature which deals with different aspect of trend free designs [For example Bradley and Yeh (1980), Yeh and Bradley (1983), Jacroux *et al.* (1997), Bhowmik *et al.* (2012), Bhowmik (2013), Bhowmik *et al.* (2014), Bhowmik *et al.* (2015), Bhowmik *et al.* (2017), Bhowmik *et al.* (2018),etc.]. In this article, the model incorporating systematic trend under two source of heterogeneity has been defined and the condition for a design under two-source of heterogeneity to be trend free has been highlighted. Further, a list of designs with number of treatments v (prime) less than equal to 15 has also been presented.

2. Experimental Setup and Model

As described in the above experimental situation, let there are two sources of heterogeneity viz. age and breed of the animal apart from feed (treatment). 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 and hence following fixed effects additive model in matrix notations, can be considered:

$$\mathbf{Y} = \mu\mathbf{1} + \boldsymbol{\Delta}'\boldsymbol{\tau} + \mathbf{D}'_1\boldsymbol{\rho} + \mathbf{D}'_2\boldsymbol{\chi} + \mathbf{Z}\boldsymbol{\theta} + \mathbf{e}$$

where, **Y** is a n × 1 vector of observations, μ is the general mean, **1** is a n × 1 vector of unity, $\boldsymbol{\Delta}'$ is a n × v matrix of observations versus treatments i.e. feeds, **τ** 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, **ρ** 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, **χ** is a q × 1 vector of age effects, **θ** 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 $\mathbf{Z} = \mathbf{1}_p \otimes \mathbf{F}$ 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 viz age, breed and feed are equal say v, the information matrix for estimating the contrast pertaining to the effect of treatments viz. feeds for designs under two sources of heterogeneity can be obtained as:

$$\mathbf{C} = v\left(\mathbf{I} - \frac{\mathbf{11}'}{v}\right) - \frac{1}{v}\boldsymbol{\Delta}\mathbf{Z}\mathbf{Z}'\boldsymbol{\Delta}'$$

3.Condition for Designs to be Trend Free

An effective way to deal with the above mentioned situation is the adaption of designs which are resistance to the effect of systematic trend i.e. use of trend free designs. In such designs the trend effect will be nullified as in such scenario, the treatment (feed) effects and the trend effects are orthogonal to each other. As a result, one can carry out the analysis in usual manner, as if no trend effect was present.

Statistically, for a trend-free designs, the adjusted treatment sum of squares arising from the effects of treatments under the above model with trend component is same as the adjusted treatment sum of squares under the usual model without trend component. Hence, under the above experimental setup, for a design to be trend free, the necessary and sufficient condition is $\boldsymbol{\Delta}\mathbf{Z} = \mathbf{0}$. Therefore, the information matrix for estimating the contrast pertaining to the effect of treatments viz. feeds under two source of heterogeneity with all the factors at equal level becomes

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

This will be same if one obtains the expression of the information matrix under the usual model for designs under two source of heterogeneity -without trend component.

4. Trend Free Designs

Following are trend free designs under two source of heterogeneity with v≤ 15 [here v is a prime number]

Design_1 (for v = 3)			
	-1	**0**	**1**
1	A 1	B 2	C 3
2	C 2	A 3	B 1
3	B 3	C 1	A 2

Design_2 (for v=5)					
	-2	**-1**	**0**	**1**	**2**
1	A 1	B 2	C 3	D 4	E 5
2	C 2	D 3	E 4	A 5	B 1
3	E 3	A 4	B 5	C 1	D 2
4	B 4	C 5	D 1	E 2	A 3
5	D 5	E 1	A 2	B 3	C 4

Design_3 (for v=7)							
	-3	**-2**	**-1**	**0**	**1**	**2**	**3**
1	A 1	B 2	C 3	D 4	E 5	F 6	G 7
2	C 2	D 3	E 4	F 5	G 6	A 7	B 1
3	E 3	F 4	G 5	A 6	B 7	C 1	D 2
4	G 4	A 5	B 6	C 7	D 1	E 2	F 3
5	B 5	C 6	D 7	E 1	F 2	G 3	A 4
6	D 6	E 7	F 1	G 2	A 3	B 4	C 5
7	F 7	G 1	A 2	B 3	C 4	D 5	E 6

Design_4 (for v=11)											
	-5	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**
1	A 1	B 2	C 3	D 4	E 5	F 6	G 7	H 8	I 9	J 10	K 11
2	C 2	D 3	E 4	F 5	G 6	H 7	I 8	J 9	K 10	A 11	B 1
3	E 3	F 4	G 5	H 6	I 7	J 8	K 9	A 10	B 11	C 1	D 2
4	G 4	H 5	I 6	J 7	K 8	A 9	B 10	C 11	D 1	E 2	F 3
5	I 5	J 6	K 7	A 8	B 9	C 10	D 11	E 1	F 2	G 3	H 4
6	K 6	A 7	B 8	C 9	D 10	E 11	F 1	G 2	H 3	I 4	J 5

7	B 7	C 8	D 9	E 10	F 11	G 1	H 2	I 3	J 4	K 5	A 6
8	D 8	E 9	F 10	G 11	H 1	I 2	J 3	K 4	A 5	B 6	C 7
9	F 9	G 10	H 11	I 1	J 2	K 3	A 4	B 5	C 6	D 7	E 8
10	H 10	I 11	J 1	K 2	A 3	B 4	C 5	D 6	E 7	F 8	G 9
11	J 11	K 1	A 2	B 3	C 4	D 5	E 6	F 7	G 8	H 9	I 10

Design_5 (for v=13)													
	-6	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
1	A 1	B 2	C 3	D 4	E 5	F 6	G 7	H 8	I 9	J 10	K 11	L 12	M 13
2	C 2	D 3	E 4	F 5	G 6	H 7	I 8	J 9	K 10	L 11	M 12	A 13	B 1
3	E 3	F 4	G 5	H 6	I 7	J 8	K 9	L 10	M 11	A 12	B 13	C 1	D 2
4	G 4	H 5	I 6	J 7	K 8	L 9	M 10	A 11	B 12	C 13	D 1	E 2	F 3
5	I 5	J 6	K 7	L 8	M 9	A 10	B 11	C 12	D 13	E 1	F 2	G 3	H 4
6	K 6	L 7	M 8	A 9	B 10	C 11	D 12	E 13	F 1	G 2	H 3	I 4	J 5
7	M 7	A 8	B 9	C 10	D 11	E 12	F 13	G 1	H 2	I 3	J 4	K 5	L 6
8	B 8	C 9	D 10	E 11	F 12	G 13	H 1	I 2	J 3	K 4	L 5	M 6	A 7
9	D 9	E 10	F 11	G 12	H 13	I 1	J 2	K 3	L 4	M 5	A 6	B 7	C 8
10	F 10	G 11	H 12	I 13	J 1	K 2	L 3	M 4	A 5	B 6	C 7	D 8	E 9
11	H 11	I 12	J 13	K 1	L 2	M 3	A 4	B 5	C 6	D 7	E 8	F 9	G 10
12	J 12	K 13	L 1	M 2	A 3	B 4	C 5	D 6	E 7	F 8	G 9	H 10	I 11
13	L 13	M 1	A 2	B 3	C 4	D 5	E 6	F 7	G 8	H 9	I 10	J 11	K 12

Here, column represents non-normalized liner trend component, row represent one source of heterogeneity, alphabets within row-column intersection represents another source of heterogeneity and different number represents different treatments.

5. Conclusion

Since, all the above designs are trend free designs, 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.

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