Cross-Over Design Experiment (Using SAS)

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ABSTRACT

Cross-over experiments are a special class of repeated measures experiments. Up to now the repeated measures experiments that we have been investigating, are where the experimental unit is measured repeatedly after treatment. Only one treatment is applied to each of the experimental units. Inter observation time do not given other treatments, so it just sort of pure observation from one moment to the next. In this experimental design, the general assumption used is that the experimental units are relatively homogeneous. In experiments involving biological creatures, like animals, human studies, psychology, etc., we will find it difficult to obtain experimental unit as much as we need, which has a homogeneous condition. Because in this study, there are things that physically we can't find a striking difference but basically every individual has the distinction, and this can make the measurements that we do less valid. In addition, the number of treatments with repeats that we demand that we have enough experimental units, but often problems to get it. Cross-Over design experiment, implementing the experiment in which one unit of the experiment received all treatments with a specific sequence and the observations were made at each meal treatment. This design is often also referred to as a Change-Over Design. Using a cross-over experiment allows for an increase in precision when less variability is expected within subjects than between subjects. One feature of cross-over designs with more than two periods, is the ability to measure any possible carryover effects. Carryover effects are when the results in subsequent treatments are influenced by treatments given in previous periods or may also be caused by a "learning effect".

Keywords: *Cross-Over design, SAS, carryover effect*

1. Introduction

In experiments, we known some of the experiment designs are often used. For the experiment with two factors (factorial), we can use split-plot or strip-plot designs. Here, we also have latin-square design, in which its randomization is implemented by creating rows and columns, in which each treatment appears only once in each row and each column, so that automatically amount of replication as many as the treatments. In the design of experiments, we also know that there is design with repeated measurements which usually called repeated measurements design (RMD), in which an experimental unit was given a particular treatment is then observed several times within a certain time interval. Between the observations time not given other treatments, so it just sort of pure observation from one moment to the next.

In previous experimental designs, the general hypothesis that is used is that experimental units are relatively homogeneous. While experiment with the many factors with many levels, will cause the unit to experiment more and more necessary to be so difficult for us to find homogeneous experimental units. Moreover, if the experiments are conducted using biological creatures, such as animal man, psychological studies, etc., then we will find that it is difficult experimental installation as long as we need, which has a homogeneous condition. Because in these studies, there are things that we cannot physically find a difference notable, but, basically, each individual has the distinction, and this can make the measurements we make less valid. In addition, the number of treatments with repeats that we demand that we have enough experimental units, but often problems to get it.

Cross-Over Design (COD), carry out experiments in which one unit of the experiment received all treatments with a specific sequence and the observations were doing at each treatment. This design is often also called change-over or switchover design. It has been used since the 1940s (Cochran, Autrey and Cannon, 1941). COD is often used in medical research which its main purpose is to find a difference in treatments effect of the response is measured. COD can be seen as a mixture of RMD and latin-square design. RMD because of the observations were doing at each treatment. But it's different with RMD that only one treatment on each experimental unit. As latin-square design, because the sequence of treatment using the basic of latin-square design as its randomization.

Following are the reasons for using COD:

1. Research generally involves biological entities, such as animals, humans, in which the two entities have a very large diversity.
2. Cost constraints, because the experimental units are used less.
3. Provide treatment that did not make the experiment a damaged unit.
4. When the effects of the experimental unit for the well-known (the influence of drugs, nutrition, level of knowledge).
5. Sometimes we use the experimental units are scarce, forcing us to use it several times.

COD also has several weaknesses:

1. COD is not suitable if treatment can substantially modify the experimental units (for example, disinfect or destroy).
2. The expected duration of the training time will be longer if you are using COD, albeit with a small number of periods.
3. Congenital effects which called carryover effect may arise in COD.

2. Notation and Model

In COD, each experiment unit is treated with t treatments as t observation. This is a fundamental difference between the COD and RMD generally. In RMD, experimental units received one treatment and then these experiment units will be repeated of observation, or we can say the measurement is done in some time (space). Experimental units did not get another treatment in a time interval of observation.

COD is generally using latin-square design randomizations order to obtain a balanced position in which the treatment was followed by another treatment with the same amount. For example there are three treatments A, B and C, so then there will be three sequence patterns that can be used, so the randomization are as follows:

	Order Position		
Pattern1	2	3	
1	A	B	C
2	B	C	A
3	C	A	B

For example, a researcher has 3n experimental units, each of n experimental units gets one pattern above and n is amount of replication. That is why we can say that COD is a mixture of RMD (in units of the experiment) with latin-square design.

For the previous case, a linear model of COD can be written in the following:

$$y_{ijkm} = \mu + \alpha_i + \beta_j + \tau_k + \gamma_{m(i)} + \varepsilon_{ijkm}$$

i, j, k = 1, 2, …, t ; m = 1, 2, …, n; in this case t=3.
Where

y_{ijkm} = response in i^{th} pattern, j^{th} order position, k^{th} treatment, m^{th} experimental unit
μ = the overall mean effect
α_i = the effect of the i^{th} pattern
β_j = the effect of the j^{th} order position
τ_k = the effect of the k^{th} treatment
$\gamma_{m(i)}$ = the effect m^{th} experimental unit that nested in the i^{th} pattern
ε_{ijkm} = random error

The assumptions used in COD are:

- $\gamma_{m(i)} \sim iid \sim N(o, \sigma_\gamma^2)$
- $\varepsilon_{ijkm} \sim iid \sim N(o, \sigma_\varepsilon^2)$
- $Cov\left(\gamma_{m(i)}, \varepsilon_{ijkm}\right) = 0$

And the table of analysis of variance (ANOVA) is:

Source of Variation	db	SS	MS	F-value
Pattern (P)	t–1	SSP	MSP	MSP/MSE
Order Position (O)	t–1	SSO	MSO	MSO/MSE
Treatment (TR)	t–1	SSTR	MSTR	MSTR/MSE
Experiment unit (S)	t(n-1)	SSS	MSS	MSS/MSE

Error (E)	(t-1)(nt-2)	SSE	MSE
Total (T)	nt-1	SST	

SS=sum square; MS=mean square

$$SST = \sum_{i}^{t}\sum_{j}^{t}\sum_{m}^{n}\left(y_{ijkm} - \bar{y}_{....}\right)^2$$

$$SSP = n\,t\sum_{i}^{t}(\bar{y}_{i...} - \bar{y}_{....})^2$$

$$SSO = n\,t\sum_{j}^{t}\left(\bar{y}_{.j..} - \bar{y}_{....}\right)^2$$

$$SSTR = n\,t\sum_{k}^{t}(\bar{y}_{..k.} - \bar{y}_{....})^2$$

$$SSS = t\sum_{i}^{t}\sum_{m}^{n}(\bar{y}_{i..m} - \bar{y}_{....})^2$$

$$SSE = SST - SSP - SSO - SSTR - SSS$$

Mean square (MS) for each source of variation is formulated with: SS/db

In the COD with more than 2 treatments, there is the possibility of the presence of the effect of learning for the experimental units. This effect is often called a carryover effect, ie the possibility that treatment effects are also caused by the influence of previous treatment applied to the experimental units.
COD model by entering the carryover effect is as follows:

$$y_{ijkm} = \mu + \alpha_i + \beta_j + \tau_k + \gamma_{m(i)} + \lambda_{r(ij)} + \varepsilon_{ijkm}$$

In general, there are effects of pattern, the order position, treatment, experimental unit, and carryover. However, the application code almost all carryover effect λ assuming = 0.

3. An example of COD application and the analysis using SAS

Someone want to know whether there are differences in the number of consumers who buy the apples with 3 different way displays the fruit stores. Say that 3 way displays are A, B and C. There are six stores that can be used as an experimental unit. Observations made for every 100 people who come to the stores, how many of those who buy the apples in the stores they visit. Below is a chart position of treatment:

		Order/period (j)		
Pattern (i)	Store	1	2	3
1	m=1	B	C	A

	m=2	B	C	A
2	m=1	A	B	C
	m=2	A	B	C
3	m=1	C	A	B
	m=2	C	A	B

And following are the result of the observation:

Pattern	store	period	Treatment	response
1	1	1	B	9
1	1	2	C	12
1	1	3	A	15
1	2	1	B	4
1	2	2	C	12
1	2	3	A	9
2	1	1	A	12
2	1	2	B	14
2	1	3	C	3
2	2	1	A	13
2	2	2	B	14
2	2	3	C	3
3	1	1	C	7
3	1	2	A	18
3	1	3	B	6
3	2	1	C	5
3	2	2	A	20
3	2	3	B	4

To analyze the data we can use SAS software. SAS is one of the programming that can be used in the analysis of statistical data with the design of COD.
Here is a SAS program for the case of the example above:

```
data cod;
input pattern store period treatment$ respon;
cards;
1 1 1 B 9
1 1 2 C 12
1 1 3 A 15
1 2 1 B 4
1 2 2 C 12
1 2 3 A 9
2 1 1 A 12
2 1 2 B 14
2 1 3 C 3
```

2 2 1 A 13
2 2 2 B 14
2 2 3 C 3
3 1 1 C 7
3 1 2 A 18
3 1 3 B 6
3 2 1 C 5
3 2 2 A 20
3 2 3 B 4
;
proc print data=cod;
proc anova data=cod;
class pattern store period treatment;
model respon=pattern store(pattern) period treatment;
run;

The important outputs of this program are as follows:

```
     The SAS System

                                   The ANOVA Procedure

Dependent Variable: respon

                                          Sum of
         Source                     DF         Squares     Mean Square    F Value    Pr > F

         Model                       9     443.6666667      49.2962963      19.40    0.0002

         Error                       8      20.3333333       2.5416667

         Corrected Total            17     464.0000000


                       R-Square     Coeff Var      Root MSE    respon Mean

                       0.956178      15.94261      1.594261       10.00000


         Source                     DF        Anova SS     Mean Square    F Value    Pr > F

         pattern                     2       0.3333333       0.1666667       0.07    0.9370
         store(pattern)              3      21.0000000       7.0000000       2.75    0.1120
         period                      2     233.3333333     116.6666667      45.90    <.0001
         treatment                   2     189.0000000      94.5000000      37.18    <.0001


                                   The SAS System

                                   The ANOVA Procedure

Duncan's Multiple Range Test for respon
```

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	2.541667

Number of Means	2	3
Critical Range	2.123	2.212

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	14.5000	6	A
B	8.5000	6	B
B			
B	7.0000	6	C

From the SAS output above we can see that for the model, p-values = 0.0002 which means that the design is quite good with alpha (α) 5%. From the ANOVA also can be concluded that period and treatment significance influence to the response. While further testing using Duncan's test results that treatments A and B, also A and C are different significance, but treatments B and C are not.

Another example:

There are 9 cows that can be used to see the effect of two specific treatments to the dairy cows. For this purpose, the design of experiments made using COD, which means the each cows get all the two treatments.
The following observation:

Period	Treatment					
		Group	1			
		Cow 1	Cow 2	Cow 3	Cow 4	
1	1	29.9	54.0	41.6	28.5	
2	2	27.8	49.7	38.4	26.5	
		Group	2			
		Cow 5	Cow 6	Cow 7	Cow 8	Cow 9
1	2	22.2	55.5	43.5	33.2	18.2

2	1	21.4	49.1	41.3	34.3	17.1

And a SAS program for these data is:

```
data cross;
input per trt seq cow my;
cards;
1 1 1 1 29.9
2 2 1 1 27.8
1 1 1 2 54.0
2 2 1 2 49.7
1 1 1 3 41.6
2 2 1 3 38.4
1 1 1 4 28.5
2 2 1 4 26.5
1 2 2 5 22.2
2 1 2 5 21.4
1 2 2 6 55.5
2 1 2 6 49.1
1 2 2 7 43.5
2 1 2 7 41.3
1 2 2 8 33.2
2 1 2 8 34.3
1 2 2 9 18.2
2 1 2 9 17.1
;
proc mixed;
classes per trt seq cow;
model my = seq trt per/dfm=kr;
random cow(seq);
lsmeans trt;
estimate 'trt 1-2' trt 1 -1;
lsmeans seq;
run;
```

4. Conclusion

COD is effective enough to handle the great diversity of experimental units. There are several options on the COD of them, for example about with or without the carryover effect, which needs further study to determine the most appropriate model for certain COD.

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