Solution to Exercise 3.5 (Version 1, 22/09/14)

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Exercise 3.5

The efficacy of six synthetic insect pheromones is to be tested in the field. Traps are baited with a single pheromone, deployed at dusk, left out overnight, then retrieved the next morning and the insect catches recorded. There is sufficient material to bait six traps with each pheromone.

a) Consider how you might use a RCBD for this experiment if only six traps are available at any one time and all six traps will be placed in the same field, but a different field will be used each night. Are any structural factors confounded? What are the assumptions of this design? Write down the structural component for this design.
b) How would you change your design if the same trap locations were to be used each night and the positions could not be considered homogeneous? Write down the structural component for this design.
c) How might you modify your design if 18 traps are available at any one time? Under which conditions would designs based on CRD, RCBD or Latin square arrangements be preferable?
d) What design might you use if only four pheromones are to be tested, with six traps available at any one time, and enough material for nine replicates of each pheromone?

Solution 3.5

a) Six traps placed in the same field on any one night, but a different field used each night.

With six pheromones and six traps it is possible to test all pheromones simultaneously on any given night. We expect that insect populations might vary between fields (due to differences in environment) and across nights (due to differences in weather) and so wish to use both as blocking factors. In fact, if we change fields on each night, then these two structural factors are confounded, and we can use this combined night-field factor to define blocks. On each night, we randomly allocate the six pheromones to the traps within the field allocated. We repeat the process on the six nights, using a new randomization each night. The resulting design is a RCBD with the six night-field combinations as blocks. It is assumed that the insect catch may vary between nights/fields but that this relative change is the same across all pheromones, i.e. no block × treatment interactions. If we label the night-field combinations (1-6) using factor Night and the traps within night-fields (1-6) using factor Trap, then we can write the structural component for this design as

Night/Trap

One of the many possible randomizations is shown in Table S3.5.1.

Table S3.5.1 A randomization allocating pheromones (A-E) to traps (1-6) within each night-field combination as a RCBD.

Trap	Night					
	1	**2**	**3**	**4**	**5**	**6**
1	E	D	B	E	A	C
2	A	B	D	F	D	F
3	F	C	C	C	F	E
4	B	E	A	B	B	B
5	D	F	E	D	E	A
6	C	A	F	A	C	D

b) Same trap locations used each night and the positions not homogeneous.

If the same trap locations are used each night and cannot be considered homogeneous then trap positions (labelled using factor Trap) are a source of heterogeneity as well as the different nights, and we need to take account of both sources of variation. As the same trap positions are used each night, this is now a crossed structure, written as

Night*Trap

To obtain an efficient design, we constrain the randomization so that by the end of the six nights each pheromone has occurred once at each trap position. This is a Latin square design as introduced in Section 3.3.3. This design assumes no interaction between night and pheromone or between trap position and pheromone.

One of the many possible randomizations is shown in Table S3.5.2.

Table S3.5.2 A randomization allocating pheromones (A-E) to trap positions (1-6) within each night as a Latin square design.

Trap	Night					
position	**1**	**2**	**3**	**4**	**5**	**6**
1	D	C	A	E	F	B
2	A	F	D	B	C	E
3	C	E	F	A	B	D
4	E	A	B	F	D	C
5	B	D	E	C	A	F
6	F	B	C	D	E	A

c) 18 traps available.

There are now 18 traps available but still only 36 samples (six of each pheromone). We might consider the following options:

- Use all 18 traps on each of two nights, and on each night allocate three replicates of each pheromone to traps using a CRD. This design assumes there are no other structural factors to consider each night, i.e. that the environmental conditions at the trap positions each night could

be considered homogeneous. This design is a variation on the standard RCBD as it has two blocks (nights) with three replicates of each treatment in each block. The structural component could again be written Night/Trap but here Night is a factor with two levels and Trap is a factor with eighteen levels numbering traps within nights. This design is completed in two nights, but may sample a narrower range of environmental conditions than the RCBD in part (a).

- Still use only six traps per night and use the RCBD from part (a). Even though more traps are available, it may be preferable to run the experiment for six nights in order to achieve a broader representation of environmental conditions.

- Still use only six traps per night and use the Latin square design from part (a). This has the advantage that it samples six different nights whilst testing the pheromones across the same set of positions.

- Use all 18 traps per night but use three different fields each night to sample a wider range of physical locations. Each field has 6 traps, with the six pheromones randomly allocated to traps within each field. Now traps are nested within fields which are nested within nights. So the structure of this three-level nested design can be written as Night/Field/Trap where now factor Night labels the two nights, factor Field labels the three fields within each night and factor Trap labels the six traps within each field.

- Use all 18 traps per night in the same field but take account of spatial heterogeneity in the field. For example, we might split the field into three zones with different characteristics and use a RCBD in each zone, with each pheromone being tested once in each zone. The structural component would then be written as Night/Zone/Trap. Or we might use a Latin square design to account for an environmental gradient, e.g. distance from a hedge, by defining transects in each of the three zones leading away from the hedge into the field. Trap positions are then allocated at the same six distances into the field in all three transects. Each pheromone should be tested once at each distance from the hedge. The rows of the LS design correspond to the six night × transect combinations and the columns of the design correspond to distance from the hedge. The structural component for this model can be written as Night*Transect*Distance.

The best design depends on the aim of the study and the relative sizes of the different sources of variation. If night-to-night variation is large, it may be sensible to sample as many nights as possible so that representative results are obtained. If trap positions are interchangeable, then use of a RCBD is appropriate. If positions can give large differences in counts (for the same treatment on the same night), then use of the Latin square design can be helpful to eliminate position as a possible cause of bias between treatments (due to an unlucky randomization).

c) Four pheromones to test, six traps and enough pheromone available for nine replicates.

We could take a simple approach here, and just use four traps per night over nine nights, and use one of the approaches outlined in parts (a) or (b).

Alternatively, we consider whether we can find an efficient design to use all six traps on six nights. As we now have only four pheromones, this means that on each night we can have one replicate of two of the pheromones and two replicates of the other two. Following the principle of balance, we would like to add a different pair of extra replicates on each night. In this case we are fortunate, as there are six possible pairs of pheromones (without repeats) that we can add: AB, AC, AD, BC, BD and CD. We allocate each pair to one night (selected at random) and then randomize the allocation of pheromones to traps on each night. Table S3.5.3 shows a randomization generated in this way. Each pheromone occurs nine times in the experiment (with one replicate on three nights and two replicates

on three nights), and there are 13 within-night comparisons for each pair of pheromones. This approach is appropriate if we use different trap locations each night, or if trap positions can be assumed to be interchangeable. If trap positions were known to be heterogeneous then we would try to also balance the occurrence of each treatment at each trap position.

Table S3.5.3 Balanced allocation of four pheromones to six traps across six nights.

	Night					
Trap	**1**	**2**	**3**	**4**	**5**	**6**
1	B	A	B	B	B	C
2	C	B	D	C	D	A
3	D	D	D	A	C	C
4	B	C	A	D	C	D
5	A	A	C	B	A	A
6	D	D	C	A	B	B