
Anthony Hilton


Richard Armstrong

Stat Note 9

In the ninth of a series of articles about statistics for biologists, **Anthony Hilton** and **Richard Armstrong** discuss:

The one-way analysis of variance (random effects model): the 'nested' or 'hierarchical' design

In a previous article in *Microbiologist* (Armstrong & Hilton, 2004), we described a one-way analysis of variance (1-way ANOVA) in a randomised design. In a 1-way ANOVA, an individual observation is classified according to which group or treatment it belongs and observations within each group are a random sample of the relevant population. The scenario to illustrate this analysis compared the degree of bacterial contamination on 2p coins collected from three types of premises, *viz.*, a butcher's shop, a sandwich shop, and a newsagent. A sample of four coins was collected at random from each location and the number of bacterial colonies present on each coin was estimated. This ANOVA can be considered to be a 'fixed effects model' because the objective is to estimate the differences between the three premises, which are regarded as 'fixed' or discrete effects. There is, however, an alternative model called the 'random effects' model in which the objective is not to measure fixed effects but to estimate the degree of variation of a particular measurement and to compare different sources of variation in space and time. These designs are often called 'nested' or 'hierarchical' designs (Snedecor & Cochran, 1980).

The Scenario

The contribution of hands contaminated with pathogenic microorganisms to the spread of infectious disease has been recognised for many years. Of particular importance are communal areas where shared facilities of a tactile nature may present an increased opportunity for cross-contamination of the fingers. A study was therefore undertaken to determine the role of computer keyboards in a University communal computer laboratory as a source of microbial contamination of the hands. The data presented in this Statnote relate to a component of the study to determine the aerobic colony count (ACC) of ten selected keyboards with samples taken from two keys per keyboard determined at 9am and 5pm. Ten keyboards were selected randomly from those available in the computer laboratory and samples taken from two keys per keyboard (the 'a' and 'z' keys) using a cotton swab moistened in sterile distilled water (SDW). The swab was returned to 1ml of SDW and the swab agitated to release the microorganisms recovered from the surface into the dilutent. A 0.1ml sample of the SDW was plated onto nutrient agar and incubated at 30°C for 24 hours following which the colony forming units (cfu's) per millilitre was calculated. The data obtained are detailed in Table 1.

Linear models

There is a commonly used notation to describe the basic model of an ANOVA. The subscript 'i' is used to denote the group or class (i.e. the treatment group), 'i' taking the values '1 to a', whereas the subscript 'j' designates the members of the class, 'j' taking the values '1 to n' (hence, 'a' groups and 'n' replicates or observations per group). Within class 'i', the observations x_{ij} are assumed to be normally distributed about a mean μ with variance σ^2. This linear model can be written thus:

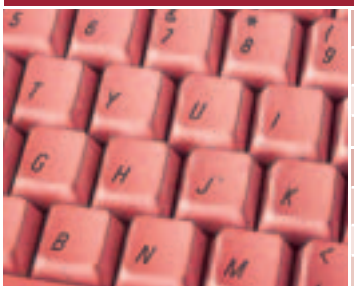
$$x_{ij} = \mu + a_i + e_{ij} \quad \text{.....1}$$

Hence, any observed value x_{ij} is the sum of three parts: 1) the overall mean of all the observations (μ), 2) a treatment or class deviation 'a_i', and 3) a random element 'e_{ij}' taken from a normally distributed population. The random element reflects the combined effects of natural variation that exists between replications and errors of measurement. The more complex types of ANOVA can be derived from this simple linear model by the addition of one or more further terms to equation 1.

Random effects model

Equation 1 describes a 'fixed effects' model in which the a_i

Table 1. Aerobic Colony Count recovered from the 'a' and 'z' keys of computer keyboards in communal use sampled at 9am and 5pm



Keybd:	Keyboard 1				Keyboard 2				Keyboard 3				Keyboard 4				Keyboard 5			
Key:	A		Z		A		Z		A		Z		A		Z		A		Z	
Time:	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
cfu ml^{-1}	170	210	55	90	437	450	200	179	210	350	5	140	560	470	10	93	47	166	12	63
Keybd:	**Keyboard 6**				**Keyboard 7**				**Keyboard 8**				**Keyboard 9**				**Keyboard 10**			
Key:	**A**		**Z**		**A**		**Z**		**A**		**Z**		**A**		**Z**		**A**		**Z**	
Time:	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**
cfu ml^{-1}	921	1043	237	178	34	21	0	8	585	658	34	67	647	457	34	56	78	67	24	3

are fixed quantities to be estimated. The corresponding 'random effects' model is similar, but the symbols KB, (representing keyboards) and K (representing keys) are included. The difference between this model and equation 1 is that KB_i and K_{ij} are considered to be random variables and the term e_{ijk} refers to errors of measurement and to the fact that microbial content is determined on two occasions (am and pm). This model can be written thus:

$$x_{ij} = \mu + KB_i + K_{ij} + e_{ijk} \quad \text{.....2}$$

Analysis of variance

The ANOVA of the data is shown in Table 2. In a random effects model, it is possible to calculate the 'components of variance' (sample estimates s^2, population values σ^2) and these are often more informative than F-tests (Table 1). The components of variance are estimates of the variance of the measurements made between keyboards (σ^2KB), between keys within a keyboard (σ^2K), and between determinations (am/pm) within an individual key (σ^2D) and can be calculated from the ANOVA. In the example quoted, the analysis suggested that the variance between keyboards was essentially zero compared with that due to keys (74707.6) which in turn, was more than 20 times that due to variation between determinations (3379.33).

This experiment provides two important pieces of information. First, there is little significant variation between keyboards or between measurements made in the morning and the afternoon compared with that between keys. This result suggests that in a future study to estimate the degree of microbial contamination on keyboards, a simpler sampling strategy could be employed involving fewer keyboards and a single sample time. Second, the difference in microbial contamination of the two keys is substantial and therefore, to improve the accuracy of estimates of contamination of a keyboard as a whole, more keys should be sampled from each keyboard. Although the experiment was not designed to test the difference between specific keys, the results suggest the hypothesis that a more frequently used key such as 'a' may have a considerably greater degree of contamination than the more rarely used 'z' key and this hypothesis may be tested by a more rigorous experiment. These results emphasise the usefulness of the random effects model in preliminary experiments designed to estimate different sources of variation and to plan appropriate sample strategies.

How to distinguish random and fixed effect factors

It is often necessary to identify whether a 'fixed' or 'random' effect model is the most appropriate in each experimental context. This is essential in more complex factorial-type designs in which there may be a mixture of both 'fixed' and 'random' effect factors ('mixed' models') (Snedecor & Cochran 1980). One way of deciding whether a factor is 'fixed' or 'random' is to imagine the effect of changing one of the levels of the factor (Ridgman 1975). If this substantially alters the experiment, for example, by substituting a confectioners shop in our previous scenario (Armstrong & Hilton 2004), then it is a fixed effect factor. By contrast, if we considered it the same experiment, for example, substituting a different keyboard or key would have little effect on the overall objectives of the experiment and it would be a 'random' effect factor. Hence, a random effect factor is only a sample of the possible levels of the factor and the intent is to generalise to all levels whereas a 'fixed' factor contains all levels of the factor that are of interest in the experimental design (Norman & Streiner, 1994). Whether a particular factor is considered to be random or fixed sometimes depends on the context. For example, the two keys measured were originally regarded as a sample from the population of keys on the keyboard. However, having selected the 'a' and 'z' key and found a significant component of variance associated with them, one could envisage an experiment to investigate the specific difference between such keys. In this new experiment, we would deliberately want to study the 'a' and 'z' keys and the factor 'key' would now become a fixed effect factor.

Table 2. A one-way analysis of variance (ANOVA), random effects model with three levels

Variation	SS	DF	MS (s^2)	σ^2 estimated
1. Keyboards	1110632.23	9	123403.581 =	$\sigma^2_D+2\sigma^2_K+4\sigma^2_{KB}$
2. Keys within keybs.	1527945.25	10	152794.525 =	$\sigma^2_D+2\sigma^2_K$
1. Keyboards	67586.5	20	3379.33 =	σ^2_D

Components of variance:	Estimated variance
Between keyboards (σ^2_{KB})	0
Between keys within a keybd. (σ^2_K)	74707.6
Between am/pm within a key. (σ^2_D)	3379.33

SS = sums of squares, DF = degrees of freedom, MS = mean square

Conclusion

There is an alternative model of the 1-way ANOVA called the 'random effects' model or 'nested' design in which the objective is not to test specific effects but to estimate the degree of variation of a particular measurement and to compare different sources of variation that influence the measurement in space and/or time. The most important statistics from a random effects model are the components of variance which estimate the variance associated with each of the sources of variation influencing a measurement. The nested design is particularly useful in preliminary experiments designed to estimate different sources of variation and in the planning of appropriate sampling strategies.

references

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Dr Anthony* Hilton and Dr Richard Armstrong**
*Pharmaceutical Sciences and **Vision Sciences, Aston University, Birmingham, UK