Aust. N. Z. J. Stat. 2019 doi: 10.1111/j.1467-842X.XXX

Multiphase experiments with at least one later laboratory phase. II. Nonorthogonal designs

C. J. Brien[1]

University of South Australia and University of Adelaide

Summary

Principles and laws that apply to nonorthogonal multiphase experiments are developed and illustrated using examples that are nonorthogonal but structure-balanced, not structure, but first-order, balanced or unbalanced, thus exposing the differences between the different design types. The design of such experiments using standard designs, a catalogue of designs and computer searches is exemplified. Factor-allocation diagrams are employed to depict the allocations in the examples, and used in producing the anatomies of designs or, when possible, the related skeleton-analysis-of-variance tables, to assess the properties of designs. The formulation of mixed models based on them is also described. Tools used for structure-balanced experiments are also shown to be applicable to those experiments that are not.

Key words: analysis of variance; laboratory phase; microarray experiments; mixed models; multiple randomizations; nonorthogonal design; optimal design

1. Introduction

Brien (2017) reviewed multiphase experiments, giving an overview of their design and analysis as it is currently practised, via a literature survey. It illustrated the use of the anticipated model, factor-allocation diagrams, anatomies of designs, skeleton analysis-of-variance (ANOVA) tables and a series of allocation models in designing structure-balanced experiments. An existing gap in the literature is a set of principles and laws, similar to those given by Brien et al. (2011), that cover nonorthogonal multiphase experiments. Further, assessing unbalanced designs as described by Brien (2017) has not been demonstrated.

This paper is restricted to the consideration of multiphase experiments whose *marginal mixed model* is comprised of a fixed and a random model of the form:

$$\mathrm{E}(\mathbf{Y}) = \sum_{T\in\mathcal{T}} \mathbf{X}_T\boldsymbol{\tau}_T \quad \text{and} \quad \mathrm{var}(\mathbf{Y}) = \sum_{U\in\mathcal{U}} \phi_U\mathbf{Z}_U\mathbf{Z}_U^\top + \sum_{E\in\mathcal{E}} \phi_E\mathbf{I}, \tag{1}$$

where $\mathcal{T}$ is the set of fixed terms, $\mathcal{E}$ is the set of *identity terms* that uniquely index the observational units, $\mathcal{U}$ is the set of all random terms, excluding the identity terms, $\mathbf{X}_T$ and $\mathbf{Z}_U$ are design matrices for the terms T in $\mathcal{T}$ and U in $\mathcal{U}$, $\boldsymbol{\tau}_T$ is the vector of fixed parameters for T, ϕ_U and ϕ_E are canonical (covariance) components for terms in U and E, and $\mathbf{I}$ is an identity matrix of order equal to the number of observational units. *Canonical components* differ from variance components in that negative ϕ_Us are allowed. The ϕ_Es are constrained to be nonnegative so that the variance matrix is positive semidefinite. The *terms* correspond

[1]School of Information Technology and Mathematical Sciences, University of South Australia, GPO Box 2471, Adelaide, South Australia. 5001.
Email: chris.brien@unisa.edu.au

Acknowledgments. The author is grateful to the two reviewers who asked many searching questions and made myriad suggestions so that this version of the paper is considerably changed and improved from that originally submitted. Rosemary Bailey also provided helpful comments and David Butler assisted in the use of od.

to *generalized factors* formed from combinations of factors, subject to the restriction that a nested factor cannot occur in a term without its nesting factor; the factors are joined by the symbol '$\wedge$' and the levels of a generalized factor are all observed combinations of its constituent factors. For two-phase experiments, it often transpires that the terms in $\mathcal{T}$ are those arising from the factors allocated in the first-phase and those in $\mathcal{U}$ are those corresponding to the first-phase and second-phase units, excluding any identity terms. The *symbolic model* is formed as the sum of the elements of $\mathcal{T}$ followed by a '$|$', then the sum of the elements of $\mathcal{U}$ and $\mathcal{E}$, the latter sum being grouped according to the tiers giving rise to the elements; each of the elements of $\mathcal{E}$ is underlined. Such variance-matrix models are referred to as *canonical-components models* and the set of possible variance matrices for them includes all those for variance-component models (Bailey & Brien 2016).

Principle 3 (I. Minimize variance) ('I.' signifies a principle discussed in Brien et al. 2011) exhorts the designer of comparative, multiphase experiments to minimize the variance of treatment effects estimates, which demands the use of (near-) A-optimal designs. For such designs, with models that conform to (1), the *A-criterion*, the sum of the variances of the treatment estimates, is minimized; for designs under such mixed models that include a fixed intercept, this is equivalent to minimizing the average variance of their pairwise differences (AVPD). It is common practise to consider *fixed-model A-optimality* for block and row-column designs: A-optimality under restricted models in which all effects are fixed, except for residual error (John & Williams 1995). It is is equivalent to maximizing the average efficiency, the harmonic mean of the 'intrablock' or 'intrarow-column' canonical efficiency factors (John & Williams 1995; Brien & Bailey 2009), hereafter efficiency factors. A disadvantage of looking for optimality under the more general class of models can be that the A-criterion changes with the unknown values of the canonical components, although some designs are optimal irrespective of these values. On the other hand, fixed models are not necessarily those that will be employed in the analysis, for example, (i) when blocks, rows or columns are considered random so that combined estimates of treatments can be obtained, or (ii) in multiphase experiments where first-phase units are allocated and terms associated with them contain treatment information, and so must be treated as being random. This begs the question as to whether an optimal design has been obtained for the likely model. The *universally optimal designs* (Kiefer 1975), designs that are A-, D- and E- optimal, form a useful class. Included are orthogonal, balanced (incomplete-) block, (most generalized) Youden square and the lattice square designs, which are also universally optimal under any mixed model in which treatments are fixed (Shah & Sinha 1989; Bailey & Williams 2007).

Section 2 gives an example of a nonorthogonal two-phase example and explains some terminology. Section 3 outlines a scheme for designing multiphase experiments. Section 4 explores the differences between the properties of designs resulting from differences in their degrees of balance and Section 5 considers laws that apply when the numbers of first-phase and second-phase units are equal. Section 6 gives conclusions. Appendices I– III prove some results; Appendices IV and V together list the complete set of principles and laws that have been developed by Brien et al. (2011) and in the present paper. Brien (2017) explains some of the philosophy, notation and terminology used here. A glossary is available from the multitiered experiments web site (Brien et al. 2001–18).

2. A nonorthogonal two-phase example

Example 1. **A small wheat experiment with a Youden square design in the lab phase.** A simpler example of a nonorthogonal multiphase experiment is used to establish concepts and introduce terminology associated with them.

Design identification. A two-phase experiment begins with a field phase in which seven lines of wheat are compared, with each line replicated six times. Discussion with the researcher establishes that a soil fertility trend in one direction is likely, but not in the perpendicular direction. That is, the anticipated model is Lines | Blocks + $\underline{\text{Blocks}\wedge\text{Plots}}$, where the term to the left of the vertical line (|) is assumed fixed and those to the right are assumed random. The underlined term is an identity term. A randomized complete-block design is A-optimal for this model.

In the second phase, a single sample of grain is taken from each of the 42 plots and the produce from the 42 plots is allocated to 42 oven spots for determining the moisture content using the standard oven drying method. The oven can hold seven samples and so six drying runs are needed. It is believed that drying could vary between spots in the oven in a manner that is consistent from one run to the next. Extra variation is also likely between the runs. Main effects would capture these two behaviours and so the anticipated model for the second-phase units is the random model Runs + Spots + $\underline{\text{Runs}\wedge\text{Spots}}$ and a row-column design is required for the allocation of plots to spots. However, each first-phase unit factor cannot be allocated to a second-phase unit factor; in particular, it is impossible to allocate the 42 levels of Blocks∧Plots to the seven levels of Spots. So the allocation to Runs∧Spots needs to consider the allocation of Lines, to avoid an undesirable amount of Lines information being confounded with Spots. When the formulation of the allocation to second-phase units must account for the factors in both first-phase tiers and the allocations are randomizations, the randomizations are termed *randomized-inclusive* (Brien & Bailey 2006, 2009). For the example, both the lines and plots tiers must be accounted for in formulating the randomization to the spots tier. This is in contrast to *composed randomizations*, in which the first-phase allocated factors can be ignored in formulating the second-phase allocation (cf. Example 2).

An A-optimal row-column design for this situation is a 6×7 Youden square in which treatments are orthogonal to rows and 1/36 of the treatments information is confounded with columns. However, a long-standing problem in multiphase experiments is how to keep track of all the sources of variation from the first phase. Here, the risk is that Plots is lost. Principles 7 (I. Allocate all and randomize in laboratory) and 9 (I. Use pseudofactors) are intended to address this problem. So, to preserve the allocation as being of plots factors, yet accounting for Lines, a seven-level pseudofactor P_1 is introduced into the second-phase to become one of the second-phase allocated factors. Each level of P_1 identifies the group of plots receiving a single Lines level; that is, P_1 is the same as Lines in the randomized design and identifies which Lines level that a plot within a block received. The factor-allocation diagram in Figure 1 shows the two randomizations, which are in a chain that proceeds from left to right. The code in Supplementary materials E does the randomizations. It (i) randomizes Lines to Plots within Blocks, (ii) randomizes Blocks and P_1 to Runs and Spots using a Youden square, and (iii) associates P_1 with Blocks∧Plots according to the allocation of Lines in (i) and so ensures the appropriate allocation of Lines to spots.

Applicable terminology. Fundamental to the approach used in this paper is the division of the factors into sets, called *tiers*, according to the allocations in the experiment (see Brien

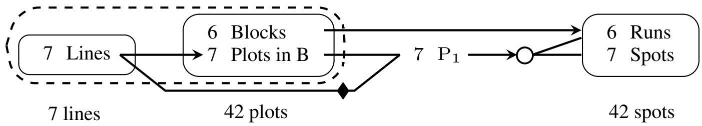


Figure 1. Factor-allocation diagram for Example 1, a small wheat experiment with a Youden square design in the lab phase: lines are allocated to plots, and lines and plots are allocated to spots; the arrows indicate that randomization is employed in the allocation; the line from Lines to P_1 signifies that Lines are used in determining P_1, a pseudofactor for Plots identifying plots assigned the same level of Lines; the solid diamond (⧫) implies that Lines directly determines the pseudofactor; the dashed, rounded rectangle indicates that the factors in the enclosed panels all play a role in randomizing them to spots and so form a pseudotier; the open circle ($\circ$) indicates that a specific design is used in the assignment of P_1 to the combinations of the levels of Runs and Spots, and the lack of a ($\perp$) indicates that it was nonorthogonal; B = Blocks.

et al. (2011) for a comparison with single-set experiment description). As Brien et al. (2011) describe, in two-phase designs that involve two allocations there are three sets of *objects*, each of which has a set of factors, or a tier, that uniquely indexes them: (i) first-phase allocated, (ii) first-phase recipient and (iii) second-phase recipient objects and factors. *Factor-allocation diagrams*, like Figure 1, are used to depict the allocations. The diagrams have a *panel* for each set of objects, each panel listing the factors in the associated tier along with the numbers of levels for the factors and their nesting relationships. Arrows, lines and geometric symbols are used to indicate how the factors in one tier/panel are allocated to those in another. Names are given to sets of objects. The names, written outside the panel, are in lower case to distinguish them from the names of the factors; they are used to collectively refer to their factors and derived constructs like terms and sources. Generic names for the sets of objects in a two-phase design are treatments, first-phase units and second-phase units. In the example the objects are called lines, plots and spots; for example, the 42 plots are the plots in the field and could be labelled as p1, p2, …, p42; they are indexed by the plots factors Blocks and Plots. While spots are also concrete objects, lines are more abstract. The generalized factors derived from a tier group the objects associated with the tier; each group is referred to as an *entity* and the type of grouping as the *entity-type* for that generalized factor. For the example, the set of generalized factors for spots is $\{\text{Runs}, \text{Runs}\wedge\text{Spots}\}$ and the entity-types are a run and a spot; the runs are indexed by Runs and group together seven spots.

Four *species of design*, based on the sets of objects present in two-phase designs, are identified, the last three species involving the second-phase recipient objects:

First-phase design: allocated and recipient objects from the first phase;

Second-phase design: first- and second-phase recipient objects;

Cross-phase design: first-phase allocated objects and second-phase recipient objects;

Two-phase or combined design: all three sets of objects.

The two-phase design combines the second-phase design with the first-phase design and its properties derive from those of the single-phase designs. If the allocation is a composed randomization then just the second-phase design is used and the cross-phase design is not explicitly considered. For randomized-inclusive randomizations, the cross-phase design is usually the primary design; the structure on the first-phase recipient objects is modified for either the first-phase allocation or the cross-phase design, often by adding pseudofactors that account for the first-phase allocated factors (Brien & Bailey 2009). This yields a second-phase design that is used in producing the two-phase design. Sometimes a fourth set of objects is allocated in the second phase only (Brien & Bailey 2006, Example 12); the two second-phase designs form a combined second-phase design.

In the example, the first-phase design is a randomized complete-block design, the cross-phase design a Youden square design and the second-phase design an orthogonal design.

Model formulation. The symbolic form of the *anticipated model* is formed by combining the anticipated models for each phase. The result is the model thought to be relevant *before* the design is obtained and provides the basis for selecting a design. Formulating it makes explicit that the first, fundamental step in choosing a design is to consider what model might be appropriate for an experiment; then a near-optimal design, given the model, is sought. Having chosen a design, the allocation-based mixed models are built. The *initial allocation model* can be derived directly from the factor-allocation diagram: its terms consist of all generalized factors derived from the factors in each panel (tier), with only the treatments factors assumed fixed. When all the allocations are randomizations, it is equivalent to a randomization model. It is modified prior to performing an analysis (see Brien 2017, Section 5) to produce the *prior allocation model*, a starting model for an analysis. The initial allocation model for the example is:

$$\begin{gathered}\text{Lines} \mid \text{Blocks} + \underline{\text{Blocks}\wedge\text{Plots}} + \\ \text{Runs} + \text{Spots} + \underline{\text{Runs}\wedge\text{Spots}}.\end{gathered}$$

Lines is the first-phase allocated factor, the remaining terms in the first line derive from the first-phase recipient factors and the terms in the second line derive from the second-phase, recipient factors. There is a canonical component for each of the random terms, it being denoted by ϕ with a subscript comprised of the initial letters of each factor in the term (e.g. ϕ_{BP}). Here, the initial allocation model is consistent with the anticipated model, confirming that all anticipated sources of variation have been accounted for in the design. A change that might be made to this model in forming the prior allocation model is to assume that Blocks and Runs are fixed; this would avoid the problems that would arise if the estimated sum of their components is negative. Otherwise, the initial and prior allocation models would be the same. A separate term for the pseudofactor P_1 is not included in either model.

Structure balance. To prepare for design evaluation, the key concept of structure balance is now described. Structure balance is a property of pairs of sources. For a tier, there is a (model) *term* for each generalized factor and for each term there is a source. Each *source* is a subspace of the data space; the set of orthogonal sources for a tier is the tier's *structure* and specifies an orthogonal decomposition of the data space. A source is also a subspace of the column space for its term. Consider a tier consisting of two crossed factors A, with a levels, and B, with b levels. The terms are the Mean, A, B and A$\wedge$B: the overall mean parameter and a term with different parameters for the levels of A, for the levels of B and for the combinations of the levels of A and B; they are of dimension 1, a, b and ab. The sources are Mean, A, B and A#B, the overall mean, the A and B main effects and the interaction of A and B; they are of dimension 1, $a-1$, $b-1$ and $(a-1)(b-1)$ and are subspaces of Mean, A, B and A$\wedge$B, respectively. For B nested within A, B [A] represents the source for differences between the levels of B within each level of A and is of dimension $(b-1)a$.

For an allocation of one set of objects to another, designate the allocated and recipient sources as being those derived from the allocated factors and the recipients factors. Considering pairings of allocated with recipient sources, a design is *first-order balanced* if all the efficiency factors for each pair, calculated using their projection matrices (Appendix I), are equal. The design is *structure balanced* if (i) it is first-order balanced, and (ii) for each recipient source, the projection onto it of an allocated source that is (partially) confounded with it is comprised of the full degrees of freedom (DF) of the allocated source and the projection is orthogonal to that of any other allocated source onto it (Brien & Bailey 2009; Brien 2017); a mathematical definition is in Appendix I. It is also said that the allocated tier is structure balanced in relation to the recipient tier. A *structure-orthogonal design* is defined to be a structure-balanced design with all efficiency factors equal to one, while an *orthogonal design* is first-order balanced with all efficiency factors equal to one.

The lack of orthogonality between sources from the same tier in a design that is not structure balanced gives rise to (partial) aliasing. *(Partial) aliasing* occurs between sources from the same tier; it is seen between treatments sources in nonorthogonal two-factor factorial experiments in completely randomized designs. The sources and the amount of aliasing exhibited in the decomposition table depends on the order in which sources are added to the table, since each subspace needs to have removed from it those DF already allocated to sources higher in the table. Aliasing is distinguished from confounding in that *confounding* occurs between a source with allocated factors and a source with only recipient factors that received the allocated factors. Thus, a treatment source might be aliased with another treatment source, whereas treatment sources are confounded with unit sources. This distinction is consistent with their traditional usage in 2^k and 3^k factorial designs, where fractional designs have aliasing between treatment effects and confounded designs have treatments confounded with block effects.

The advantages of structure-balanced designs are discussed by Brien (2017, Section 3). Briefly, sources from the same tier are not (partially) aliased and, as for some first-order balanced designs, all elementary contrasts for treatment sources have equal variance. Also, their decomposition/anatomy, as formed here, is order independent and so unique.

Design evaluation. Heeding Principle 1 (I. Evaluate designs with decomposition/skeleton-ANOVA tables) (see also Appendix IV) the skeleton ANOVA is in Table 1. The R script in Supplementary materials B produces the decomposition table that exhibits the anatomy. Adding the expected mean squares (EMS) to the decomposition table produces the skeleton ANOVA. Bailey & Brien (2016) give expressions for the EMS for structure-balanced designs, which can be realized using rules given by Brien et al. (2011, Web Appendix D) and Brien et al. (2001–18, Multitiered web site). The EMS for a source is obtained from Table 1 as a linear combination of the canonical components plus the source's q-function, if it has one; the components' coefficients are the numbers under the components.

Table 1 shows that Blocks is confounded with Runs, thus conforming to Principle 8 (I. Big with big). However, the decomposition into sources Mean, Blocks and Plots [Blocks] for the plots tier is not structure balanced in relation to the spots tier. While all efficiency factors for the pairs of plots and spots sources are equal to one, the whole of Plots [Blocks] is not combined with Spots, as required for structure balance. Plots [Blocks] has 36 DF, but only six DF are confounded with Spots. Formally, the design can be made structure balanced using a pseudofactor that splits the full Plots [Blocks] into two parts, each of which is confounded with a separate spots source. Such a pseudofactor, labelled $\mathrm{P_S}$, has seven levels and indexes plots with the same Spots level. This results in two orthogonal sources Plots $[\text{Blocks}]_{\mathrm{S}}$ and Plots $[\text{Blocks}]_{\vdash}$ with six and 30 DF, that Table 1 shows are confounded with Spots and Runs#Spots. A mathematical explanation is in Appendix I. Now, because this formulation makes the design structure balanced, there is no partial aliasing, all Lines elementary contrasts have the same variance and the anatomy in Table 1 is unique. Table 1 also shows that Lines

Table 1. Skeleton ANOVA for Example 1, a small wheat experiment with a Youden square design in the lab phase: R = Runs; S = Spots; B = Blocks; P = Plots; L = Lines.

spots tier		plots tier†		lines tier			EMS‡					
Source	DF	Source	DF	Eff†	Source	DF	ϕ_{RS}	ϕ_S	ϕ_R	ϕ_{BP}	ϕ_B	$q(.)$
Mean	1	Mean	1	1	Mean	1						
Runs	5	Blocks	5				1		7	1	7	
Spots	6	Plots $[\mathrm{B}]^{\S}_{\mathrm{S}}$	6	$\frac{1}{36}$	Lines	6	1	6		1		$q(\mathrm{L}_\mathrm{S})$
R#S	30	Plots $[\mathrm{B}]^{\S}_{\vdash}$	30	$\frac{35}{36}$	Lines	6	1			1		$q(\mathrm{L}_\vdash)$
					Residual	24	1			1		

Notes: †The efficiency factors for plots sources confounded with spots sources are one and so are not given. Each Eff is the efficiency factor for the Lines source confounded with a spots source. ‡Each ϕ is a canonical component that, except for ϕ_{RS} and ϕ_{BP}, is allowed to be negative. Their subscripts are comprised of the first letter of each factor in the corresponding term and the numbers in the table are the coefficients of the components in the EMS. Each q-function is the same quadratic function of the expectation as the mean square for that line of the table is of the data; the subscripts of q-functions for the same source indicate the different matrices of their quadratic forms. §Plots $[\mathrm{B}]_\mathrm{S}$ is the part of Plots [B] that corresponds to differences between Blocks-Plots combinations that were assigned to the different levels of Spots and Plots $[\mathrm{B}]_\vdash$ is the part of Plots [B] orthogonal to Plots $[\mathrm{B}]_\mathrm{S}$.

are confounded with Plots [Blocks] and that no Lines information is associated with Blocks, as is expected for a randomized complete-block design. The efficiency factors indicate that nearly all of the information about Lines is confounded with that part of the Plots [Blocks] that is confounded with Runs#Spots. The last two sources are expected to be the smallest sources of variation, as the EMS attest. The design satisfies Principle 3 (I. Minimize variance).

Design optimality. Because the variance matrix for the two-phase design is of the same form as that for a Youden design (see Appendix I), the two-phase design is universally optimal under either the anticipated model or a model in which the only random terms are Blocks$\wedge$Plots and Runs$\wedge$Spots (Shah & Sinha 1989).

3. A scheme for producing comparative, multiphase experiments

A scheme for producing comparative, multiphase designs, that utilizes the concepts introduced via Example 1 and will be used in subsequent examples, is as follows:

1. **Design identification:** Look for a suitable design.
   (i) Obtain a suitable, perhaps fixed-model, near-A-optimal design for the first phase; if feasible, it should be first-order balanced. Formulate its initial allocation model and obtain the corresponding anatomy to check its properties.
   (ii) Determine whether or not a two-phase design that is first-order balanced is possible, conditional on maintaining the properties of the first-phase design.
   If it is possible obtain a near-A-optimal, second-phase or cross-phase design that is first-order balanced, and use it to produce the two-phase design.
   If it is impossible obtain a near-A-optimal, two-phase design, possibly using software that can search for A-optimal designs for mixed models.
2. **Model formulation:** Formulate the initial allocation model for the design.
3. **Design evaluation:** If necessary, check the dispersal of information in the second-phase and two-phase designs by obtaining their anatomies, based on the terms in the initial

allocation model, or, at least for the section(s) of the decomposition that have the most treatment information;

4. **Design optimality:** Use software that can search for A-optimal designs for mixed models to check the A-optimality of the two-phase design.
5. **Extra phases:** Build on the design for the previous phases by repeating the above process for each phase subsequent to the second phase in turn.

This scheme reinforces Principle 1 (I. Evaluate designs with decomposition/skeleton-ANOVA tables) (see also Appendix IV).

4. Designs with different degrees of balance

Brien (2017, Section 3) defines three types of designs, which are extended here to four types: (i) structure orthogonal, (ii) nonorthogonal with structure balance, (iii) not structure, but first-order, balanced, and (iv) unbalanced. The *degree of balance*, being the restrictions placed on the efficiency factor values, decreases as the list is traversed. The first three types of designs derive from the nested hierarchy in which first-order balanced designs include structure-balanced designs, that include structure-orthogonal designs (see Structure balance in Section 2). *Unbalanced designs* are nonorthogonal designs for which there is at least one pair of sources for which there are multiple efficiency factors. In order to explore the differences between designs that differ in the degree of balance, three examples of nonorthogonal two-phase designs are presented; structure-orthogonal two-phase designs are the subject of Brien et al. (2011).

4.1. Nonorthogonal but structure balanced

Theorem 5.1 of Brien & Bailey (2009) justifies the following law that applies to structure-balanced multiphase experiments, such as Example 1.

Multiphase law 2 (Combined structure balance). When the design for a multiphase experiment involves a chain of randomizations and the design for each phase is structure balanced, which includes structure orthogonal, then the complete design is structure balanced. When the combined design is structure balanced, the (canonical) efficiency factors of the component designs multiply so that the efficiency factor for a source is:

For composed randomizations: the product of its efficiency factor with the product of those for sources, from subsequent phases, with which it is confounded.

For randomized-inclusive randomizations: As for composed randomizations, except that the efficiency factors for a component design are those for modified sets of allocated and recipient sources formed from the original sets of sources by including, into the relevant set, sources for appropriate pseudofactors.

(Brien & Bailey 2009, Theorem 5.1(b)). Further, the sum of the efficiency factors for each source that has been allocated is one (a proof is in Appendix II).

Example 1. A small wheat experiment with a Youden square design in the lab phase (continued). Multiphase Laws 1 (I. DF never increase) (see also Appendix V) and 2 (Combined structure balance) are now illustrated for the introductory example.

Applying the laws. For Multiphase law 1 (I. DF never increase), consider just the first phase: the source Plots [Blocks] has 36 DF in total and so its Residual has 30 DF. In the second phase, the splitting of the Plots [Blocks] source between Runs and Runs#Spots sources in the two-phase design results in a reduction to 24 of the Residual DF for testing Lines.

Because the modified first-phase design for the allocation of lines to plots is nonorthogonal, but structure balanced, and the modified second-phase design for the allocation of plots to spots is structure orthogonal, Multiphase Law 2 (Combined structure balance) applies: the two-phase design is structure balanced. Further, the efficiency factors multiply, although indistinctly given that the efficiency factors for the modified second-phase design are one. For example, the efficiency factor for Plots $[\text{Blocks}]_{\text{S}}$ with Spots is one and that for Lines with Plots $[\text{Blocks}]_{\text{S}}$ is 1/36. Thus, the efficiency factor for Lines with Plots $[\text{Blocks}]_{\text{S}}$ combined with Spots is $1 \times (1/36) = 1/36$. Also, the efficiency factors for Lines sum to one.

4.2. First-order balanced

Example 2. A two-phase microarray experiment. Jarrett & Ruggiero (2008, Section 2) gave an example of a two-phase, two-channel microarray experiment.

Design identification. The first-phase design employs a balanced incomplete-block design to assign Treatments to Blocks and Plants, as shown in the two left panels of the factor-allocation diagram in Figure 2. The inter- and intra-block efficiency factors for this design are $2/9$ and $7/9$. It is appropriate when Blocks must consist of three Plants because, for Blocks of this size, variation of Blocks is greater than that of individual Plants. That is, the anticipated model is Treatments | Blocks + $\underline{\text{Blocks}\wedge\text{Plants}}$, where Blocks consist of three Plants. The design is universally optimal for this model.

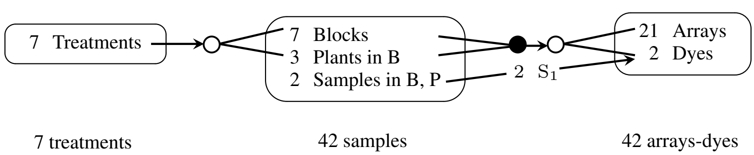


Figure 2. Factor-allocation diagram for Example 2, a two-phase microarray experiment: treatments are randomized to samples and samples to arrays-dyes; the solid circle (•) indicates that the combinations of the levels of Blocks and Plants are to be randomized; the open circle (○) symbols indicate that nonorthogonal designs are used to randomize Treatments to the combinations of Blocks and Plants and the combinations of the levels of Blocks and Plants to the combinations of Arrays and Dyes; S_1 groups the Samples from the Block-Plant combinations that are to be allocated to the same dye; B = Blocks; P = Plants.

In the second-phase, two technical replicates of samples from each plant are assigned to array-dye combinations. These replicates are laboratory replicates, with the plants being the products of the first-phase and the samples being portions derived from the plants. Samples is included in the middle panel of Figure 2. Presumably the replicates are introduced, in accord with Principle 11 (I. Laboratory replication), because they are expected to exhibit appreciable variation. The anticipated model for the second-phase units is Dyes | Arrays + $\underline{\text{Arrays}\wedge\text{Dyes}}$, with Dyes fixed because their difference is systematic rather than random. A row-column design is required for this phase. The second-phase design given by Jarrett & Ruggiero (2008) is recast as seven 2×3 Youden square designs in Table 2 and each Youden square is used to assign the two Samples for the three Plants in a Block to two Dyes $\times$ three

Table 2. Second phase design for Example 2, a two-phase microarray experiment.†

Block	1			2			…	7		
Array	1	2	3	4	5	6	…	19	20	21
Dye										
Green	1	2	3	1	2	3		1	2	3
Red	2	3	1	2	3	1		2	3	1

Notes: † The numbers in the table body are Plant levels within a Block.

Arrays. While Jarrett & Ruggiero (2008, Table 3) include a factor for Sets of three Arrays, it appears that the role of Sets is merely as a device for constructing the design, rather than as a source of variation, because they did not include a term for it in the linear model. Sets should be included if the arrays are processed in batches of three arrays and there is additional Sets variation, in which case, Blocks would be randomized to Sets. The inter- and intra-column efficiency factors for the Youden design are $1/4$ and $3/4$.

The R script in Supplementary materials C produces a randomized layout by randomizing, as shown in Figure 2, (i) Treatments to Blocks$\wedge$Plants, (ii) the two Samples within each Blocks$\wedge$Plants level to the two-levels of the pseudofactor S_1, and (iii) Blocks$\wedge$Plants levels to Arrays-Dyes levels. The second-phase allocation here differs from that in Example 1 in as much as the first-phase allocation of treatments to plants can be ignored in constructing the second-phase design and there is a direct association between the first-phase and second-phase design via Blocks$\wedge$Plants. When the first-phase allocation can be ignored like this, the two randomizations are said to be composed, rather than randomized-inclusive as in Example 1, although both types of randomizations form a chain.

Model formulation. The prior allocation model, with the first-phase factors in the first line and the second-phase factors in the second line, and which differs from the initial allocation model in having Dyes fixed, is:

$$\text{Dyes} + \text{Treatments} \mid \text{Blocks} + \text{Blocks}\wedge\text{Plants} + \underline{\text{Blocks}\wedge\text{Plants}\wedge\text{Samples}} + \\ \text{Arrays} + \underline{\text{Arrays}\wedge\text{Dyes}}.$$

Design evaluation. Because the second-phase design is not orthogonal, it does not have a commutative variance structure (Bailey & Brien 2016, Section 3.3) and so, as Jarrett & Ruggiero (2008) noted, does not have a unique set of uncorrelated strata. However, applying the Brien & Bailey (2009) approach leads to the skeleton-ANOVA table in Table 3, which is related to Table 4(b) in Jarrett & Ruggiero (2008). Table 3 is the single orthogonal decomposition that the Brien & Bailey (2009) approach yields. The table is sufficient for portraying the dispersal of Treatments information amongst the combined samples and arrays-dyes sources, the sources being derived from the terms in the initial allocation model. An R script to produce the anatomy underlying Table 3 is in Supplementary materials C

The two-phase design is not structure balanced because the second-phase design is not structure balanced. The latter design is not because part of the 21 DF for Samples [Blocks$\wedge$Plants] is not orthogonal to other samples sources: 14 DF of it is aliased with Plants [Blocks]. This aliasing arises from the nonorthogonality of Plants [Blocks], its 14 DF being confounded with both Arrays and Arrays#Dyes. Because the samples sources Mean, Blocks and Plants [Blocks] occupy 35 DF of the 42 DF available from array-dyes

Table 3. Skeleton ANOVA for Example 2, a two-phase microarray experiment: A = Arrays; D = Dyes; B = Blocks; P = Plants; S = Samples; T = Treatments.

arrays-dyes tier		samples tier			treatments tiers			EMS‡					
Source	DF	Eff†	Source	DF	Eff†	Source	DF	ϕ_{AD}	ϕ_{A}	ϕ_{BPS}	ϕ_{BP}	ϕ_{B}	$q(\cdot)$
Mean	1	1	Mean	1	1	Mean	1						
Arrays	20	1	Blocks	6	$\frac{2}{9}$	Treatments	6	1	2	1	2	6	$\frac{2}{9}q(\mathrm{T}_1)$
		$\frac{1}{4}$	Plants[B]	14	$\frac{7}{36}$	Treatments	6	1	2	1	$\frac{1}{4}2$		$\frac{7}{36}q(\mathrm{T}_2)$
						Residual	8	1	2	1	$\frac{1}{4}2$		
Dyes	1	1	Samples $[\mathrm{B}\wedge\mathrm{P}]_1^{\S}$	1				1		1			$q(\mathrm{D})$
A$\#$D	20	$\frac{3}{4}$	Plants [B]	14	$\frac{21}{36}$	Treatments	6	1		1	$\frac{3}{4}2$		$\frac{21}{36}q(\mathrm{T}_3)$
						Residual	8	1		1	$\frac{3}{4}2$		
		1	Samples $[\mathrm{B}\wedge\mathrm{P}]_{\vdash}^{\S}$	6				1		1			

Notes: †Each Eff is the efficiency factor for either (i) a samples source confounded with an arrays-dyes source or (ii) a treatments source confounded with a samples source combined with an arrays-dyes source. ‡Each ϕ is a canonical component that, except for ϕ_{AD} and ϕ_{BPS}, is allowed to be negative. Their subscripts are comprised of the first letter of each factor in the corresponding term and the numbers in the table are the coefficients of the components in the EMS. Each q-function is the same quadratic function of the expectation as the mean square for that line of the table is of the data; the subscripts of q-functions for the same source relate to the different matrices of their quadratic forms. §Samples $[\mathrm{B}\wedge\mathrm{P}]_1$ is the subspace of the Samples [B∧P] subspace defined by S_1 and Samples $[\mathrm{B}\wedge\mathrm{P}]_{\vdash}$ is another subspace, orthogonal to the first, but confounded with the Arrays $\#$ Dyes subspace.

sources, there is simply insufficient DF to estimate all 21 DF for Samples [Blocks∧Plants]. Nonetheless, the two-phase design is first-order balanced, with all efficiency factors for any source having the same value; that is, with reference to Principle 14 (Maximize the degree of balance), first-order balance is the highest degree of balance achievable here.

The lack of structure balance means that the EMS expressions in Bailey & Brien (2016) do not apply. Appendix III shows how to use these expressions to develop the EMS in Table 3. The coefficients of ϕ_{BPS}, in the sources where Plants [Blocks] is confounded with Arrays and Arrays$\#$Dyes, have increased from the perhaps expected 1/4 and 3/4 to be one for both.

In this case the lack of structure balance has no real impact in that only Samples [Blocks∧Plants] is partially aliased and the estimation of the Treatments effects is unaffected; the substantial advantage of the same standard errors for all elementary Treatments contrasts being retained. Thus, a productive strategy in designing such experiments is to omit from consideration the factors for the portions, such as Samples, and to combine structure-balanced designs constructed for each phase.

Applying the laws. The second-phase design is not structure balanced, and so Multiphase Law 2 (Combined structure balance) does not apply. However, it is first-order balanced. If Samples is omitted from the allocation and the skeleton ANOVA table, then the designs for each phase are structure balanced so that, as per Multiphase law 2 (Combined structure balance), the two-phase design is structure balanced. In these circumstances, the efficiencies for the designs from each phase multiply: the three Treatments efficiency factors are given by $1 \times (2/9)$, $(1/4) \times (7/9)$ and $(3/4) \times (7/9)$, as shown in Table 3 . Being structure balanced, the efficiencies for Plants [Blocks] sum to one, as do those for Treatments. These efficiencies are unchanged when Samples is included in the allocation.

Table 3 indicates that meeting Principle 3 (I. Minimize variance) has been only moderately successful for this design, because the stringent limitation that each array can accommodate only two colours results in just 58% of the Treatments information confounded with the likely smaller sources of variation, Plants [Blocks] and Arrays # Dyes. The 'intrablock estimates' of the Treatments effects are based on this information and so combined estimates of Treatment effects, and hypothesis testing, are likely to be favoured.

Design optimality. In constructing a two-phase design, the optimality of all four design species might be considered. The first-phase design, being a balanced incomplete-block design, is the universally optimal design for the anticipated first-phase model. Ignoring Treatments, the second-phase design, being a generalized Youden design, is universally optimal under a mixed model, but only for the allocated terms Blocks and Blocks$\wedge$Plants both assumed fixed. To be able to estimate Treatments, Blocks$\wedge$Plants must be random; to combine information from Blocks and Blocks$\wedge$Plants, Blocks must also be random. The cross-phase design is a generalized Youden design, which is universally optimal under a mixed model.

However, the question of the optimality of the combined two-phase design remains. We continue the established practice of (i) constructing a near-A-optimal first-phase design and (ii) obtaining a near-A-optimal two-phase design that retains the first-phase design (Brien & Bailey 2006; Smith et al. 2015). Call this form of optimality *first-phase-conditional optimality* and a design that minimizes it a *first-phase-conditionally A-optimal two-phase design*. For more discussion of A-optimality in two-phase designs see Supplementary materials G.

To investigate the design's A-optimality, od (Butler 2019), a package for the R statistical computing environment (R Core Team 2018), was used to search for A-optimal designs (see Supplementary materials H for using od and Supplementary materials H.2 for a fuller account of the searches). Tabu searches with 10,000 loops were performed for the anticipated model. The searches started with the structure-balanced two-phase design, with Samples omitted because its contribution is encapsulated in ϕ_{AD}. It is necessary to specify values for the variance parameters. For mixed models of the form in (1), it seems natural to express the values of the first-phase components as ratios (γs) to the first-phase residual term and the second-phase components as ratios to second-phase residual term, as well as providing the ratio of the first-phase to second-phase residual terms. Here, the values of γ_{A} and γ_{BP}, the ratios of ϕ_{A} and ϕ_{BP} to ϕ_{AD}, and γ_{B}, the ratio of ϕ_{B} to ϕ_{BP}, were set; ϕ_{AD} was always set to 1. Some results were as follows:

Second-phase units under a mixed model: One search targeted an improvement in the A-optimality of the assignment of plots to arrays-dyes, while keeping the allocation of treatments to plots. With γ_{B}, γ_{BP} and γ_{A} set to 1, 2 and 1, respectively, the AVPD for Blocks$\wedge$Plants decreased from 1.430 to 1.144, but the AVPD for Treatments increased from 1.328 to 2.563. Evidently, searching for an A-optimal second-phase design under a mixed model is ineffective.

First-phase-conditionally A-optimal two-phase designs: Another search targeted an improvement in the A-optimality of the assignment of treatments to arrays-dyes, while retaining the allocation of treatments to plots and with γ_{B}, γ_{BP} and γ_{A} set to 1, 2 and 1, respectively. While there was some rearrangement of the design, the decomposition and AVPD were unchanged by od. Additional searches with the same target, but with the 8 combinations of the two values 0.5 and 10 for each of γ_{B}, γ_{BP} and γ_{A}. Both the AVPD for

Treatments and for Blocks$\wedge$Plants were changed for only one combination of the γs and then the decrease was just 0.23%.

Thus, the proposed two-phase design is a suitable design for this experiment being balanced and apparently first-phase-conditionally near-A-optimal.

4.3. Unbalanced

While Examples 1 and 2 demonstrate the advantages of a balanced design, an unbalanced design may be a necessity because a first-order balanced design does not exist for the recipient factors under consideration, or an unbalanced design may have substantial advantages as far as precision is concerned.

Example 3. **A large wheat experiment with noncorresponding field and lab blocks.** Structure-balanced designs are generally impossible for field experiments with many lines and so the design of unbalanced experiments is explored in this context.

Design identification. The first phase involves a field experiment to investigate 150 Lines each to be replicated three times. The experimental area consists of 30 rows by 15 columns. In addition to the possibility of trends across the rows and columns, it is expected that there will be substantial differences between different areas of the experiment, due to both field variation and arising from management practices. The factors and their relationships that reflect this behaviour are shown in the first two panels in Figure 3. Here the Blocks factor corresponds to three sets of ten rows and is included to capture the anticipated major differences between parts of the field; the Columns factor is shown as crossed with the other factors so as to embody differences between entire columns. The first-phase anticipated model is:

$$\text{Blocks} + \text{Lines} \mid \text{Blocks}\wedge\text{Rows} + \text{Columns} + \text{Blocks}\wedge\text{Columns} + \underline{\text{Blocks}\wedge\text{Rows}\wedge\text{Columns}},$$

the terms in this model being those derived from the factor relationships. Blocks is assumed fixed because it seems more likely that their differences are systematic rather than random.

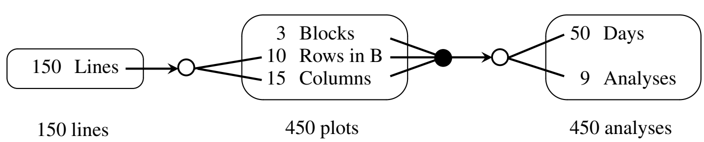


Figure 3. Factor-allocation diagram for Example 3, a large wheat experiment with noncorresponding field and lab blocks: lines are randomly allocated to plots, plots are randomly allocated to analyses; the open circle (○) at the end of the arrow leading from Lines indicates that Lines are allocated to combinations of the levels of Rows and Columns, using a nonorthogonal design; the three lines leading to the solid circle (•) indicate that it is the combinations of Blocks, Rows and Columns that, given the arrow leading from the solid circle, are randomized; the open circle (○) at the end of the arrow indicates that a nonorthogonal design is used in the allocation and the lines from the open circle indicate that they are assigned to the the levels of Days and Analyses; B = Blocks.

The first phase is followed by a second, laboratory phase that involves 50 days; each day, analyses are performed at each of nine consecutive times. The anticipated random terms to be added to the first-phase anticipated model are Days + Analyses + $\underline{\text{Days}\wedge\text{Analyses}}$. Given that there are 10 rows $\times$ 15 columns in the field phase, the second-phase design must of necessity be unbalanced, as shown in the factor-allocation diagram in Figure 3.

Given the anticipated model, a latinized, resolved, row-column design is appropriate for the first phase; the design was latinized in that repetitions of lines within entire columns is avoided and resolved in that each line occurred once and only once in a block. Although an unresolved design confounds slightly more information with Rows#Columns[Blocks], the resolved design isolates Lines from Blocks variation and is more robust to the failure of a replicate. A fixed-model near-A-optimal design was generated using CycDesigN (VSN International 2013) and randomized (see R script in Supplementary materials D). Then a combined design was sought. Three initial designs were constructed: (i) ordered: plots were assigned in order to the analyses, with the plots in standard order for Blocks, Rows and Columns and the analyses in standard order for Days and Analyses; (ii) randomized: plots were completely randomized to the analyses; (iii) row-column: a fixed-model A-optimal row-column design for the cross-phase design that assigns lines to analyses was sought using CycDesigN (Whitaker, Williams & John 2002); then blocks were assigned in order for each Line so that plots from the first block tended to be assigned to the early days and the third block to the later days. All three initial designs were randomized by independently permuting Days and Analyses, as is implicit in Figure 3. In constructing these designs the first-phase allocation of lines to plots was always maintained. This was checked by subjecting the proposed starting design to the function `designAnatomy` from the R package dae (Brien 2018) with two `formulas`: the first specifies the plots sources and the second specifies the lines sources. The decompositions matched those for the original first-phase design.

Model formulation. The initial allocation model for this experiment, which is the same for all three two-phase designs, is the same as the anticipated model, except that Blocks is random in the initial allocation model.

Design evaluation. Table 4 gives the decomposition table for the ordered design. An R script for obtaining the decomposition table is in Supplementary materials D. The orders for Lines (not shown), an order being the number of unique values of the efficiency factors for one source confounded with another, are all greater than one; thus, the design is unbalanced. The order for Lines, when confounded with Rows#Columns[Blocks], is 127. So, in contrast to structure-balanced designs, the variances of elementary contrasts differ for this design. Being unbalanced there is partial aliasing between plots sources and so Table 4 is not unique. Small changes to the decomposition occur if the order of fitting is changed so that Columns is fitted before any other plots sources, but Rows#Columns[Blocks] is unaffected. The unbalanced second-phase design prevents the addition of EMS to the table. There are no Residual sources for any analyses source. However, because all blocks sources other than Rows#Columns[Blocks] are confounded with multiple analyses sources, only the canonical components ϕ_{BRC} and ϕ_{DA} are not separately estimable. In this design, nearly all Days involve only a single block and at most two rows, the best that can be done given 50 Days and three Blocks. The result is that a large amount of Blocks and Rows[Blocks] information confounded with Days, the average efficiencies being 0.97 and 0.77. Two DF for Columns are confounded only with Analyses, but 11 of the 14 DF for Columns are mostly confounded with Days#Analyses. In contrast, the other two designs have more information about these sources confounded with Days#Analyses, their average efficiencies with Days#Analyses being around 0.85. None of the three designs has the full 378 DF for Rows#Columns[Blocks] confounded with Days#Analyses, but the ordered design has 333, as compared with 321 for the other designs. So, when Blocks, Rows to Days are the large sources of variation in the experiment, the ordered design better conforms to Principle 8 (I. Big with big) than the other

Table 4. Decomposition table for Example 3, a large wheat experiment with noncorresponding field and lab blocks: A = Analyses; D = Days; B = Blocks; R = Rows; C = Columns.

analyses tier		plots tier			lines tier		
Source	DF	A eff†	Source	DF	A eff†	Source	DF
Mean	1		Mean	1	1	Mean	1
Days	49	0.97	Blocks	2	0.01	$\text{Lines}^{\dagger}_{1}$	2
		0.77	Rows[B]	27	0.31	$\text{Lines}^{\dagger}_{2}$	27
		0.25	Columns	2	0.35	$\text{Lines}^{\dagger}_{3}$	2
		0.05	B#C	6	0.31	$\text{Lines}^{\dagger}_{4}$	6
		1.00	R#C[B]	12	0.32	$\text{Lines}^{\dagger}_{5}$	12
Analyses	8	0.00	Blocks	2	0.29	$\text{Lines}^{\dagger}_{6}$	2
		1.00	Columns	2	0.31	$\text{Lines}^{\dagger}_{7}$	2
		0.01	B#C	4	0.36	$\text{Lines}^{\dagger}_{8}$	4
D#A	392	0.02	Blocks	2	0.33	$\text{Lines}^{\dagger}_{9}$	2
		0.20	Rows[B]	18	0.32	$\text{Lines}^{\dagger}_{10}$	18
		0.93	Columns	11	0.33	$\text{Lines}^{\dagger}_{11}$	11
		0.77	B#C	28	0.30	$\text{Lines}^{\dagger}_{12}$	28
		1.00	R#C[B]	333	0.65	$\text{Lines}^{\dagger}_{13}$	149
						Residual	184

Notes: †The average efficiency, A eff, is the harmonic mean of the efficiency factors between either (i) a plots source and analyses source or (ii) a lines source and a plots source combined with an analyses source. The 149-dimensional Lines space is disposed across all plots sources; only Lines_{13} is the complete space and there are 115 unique efficiency factors for it.

designs. If Analyses variance is likely greater than Days variance, then allocating Blocks and Rows to Analyses, instead of Days, is expected to be advantageous.

Design optimality. Principle 3 (I. Minimize variance) may not have been fully satisfied, because the optimality of the design is unknown. It is known that first-phase design produced with CycDesigN is fixed-model near-A-optimal. A small study demonstrated that it is near-A-optimal for eight different sets of values of the variance parameters (Supplementary materials H.3). It will be used as the first-phase design in all searches for two-phase designs. Searching for an A-optimal second-phase design for the current situation is futile. To do so, a search for the optimal allocation of the combinations of the term Blocks$\wedge$Rows$\wedge$Columns is needed, but this is an identity term and an interchange of the rows of its design matrix between second-phase units does not change the A-optimality measure. Instead, ordered and randomized second-phase designs were proposed as alternatives. Also, a fixed-model near-A-optimal row-column design has been put forward as a cross-phase design.

To address the question of the optimality of the combined two-phase design, od was used to search for A-optimal designs for the anticipated model for this example. The details and results are presented in Supplementary materials H.4. A summary of the results with respect to the effects of the starting design and the values of the variance parameters is:

1. The first-phase-conditionally near-A-optimal designs derived by od from the ordered starting design are generally superior, over several different settings for the variance parameters, to those derived from the randomized and row-column starting design,

although the differences in the AVPD values are small.

2. The improvements in the AVPD made by od to the ordered starting design were small (up to 3.98%), and no more than 9.20% for any design. A notable change between the ordered starting and od designs is the reduction in Residual DF of 184 for the ordered starting design to 172, making it the same as for the other designs. This results from confounding more Blocks, Rows[Blocks] and Columns information with Days#Analyses.
3. The ordered starting design is robust to different variance parameter values: 3.06% was the maximum decrease, over 32 sets of variance parameters, from the AVPD value for the ordered starting design for one of these sets to that for a design generated with od for the same set, assuming Days and Analyses are random.

On noting that applying the second-phase randomization to an od design will not affect its AVPD values, the best two-phase design, on balance, is a version of the ordered starting design that has been randomized by independently permuting Days and Analyses. It seems to be a near-A-optimal design: (i) it gives close to the lowest value for the AVPD over a range of variance parameters; (ii) compared to the other starting designs, it gives close to the maximum amount of Lines information, about 65%, confounded with Rows#Columns[Blocks] and Days#Analyses; (iii) its 184 Residual DF for Rows#Columns[Blocks] is more than any other design. One cannot be sure because a different initial design that results in a two-phase design with a lower AVPD cannot be ruled out; even so, the differences in the AVPD values for the three initial designs that were tested are small, which gives some grounds for optimism that a near-A-optimal design has been identified.

The current example demonstrates that A-optimality is not the only design attribute to be considered in comparing potential designs and this is encapsulated in the following principle:

Principle 13 (Design selection attributes). In designing a comparative experiment, consider: the A-optimality, degree of balance and available Residual degrees of freedom of potential designs; whether a resolved design has advantages over a more efficient unresolved design.

With respect to balance, the examples above evince the following principle:

Principle 14 (Maximize the degree of balance).

(i) A structure-orthogonal two-phase design is ideal.

(ii) However, if a structure-orthogonal design for a phase is impossible then the next best thing is a nonorthogonal, structure-balanced design. In such a phase, if it includes laboratory replication that requires portions of the products from the previous phase such that the two phases have equal numbers of objects, then omitting the portions in constructing a design for the phase can permit a structure-balanced design. For designs that include portions, first-order balance is acceptable.

(iii) While designs that are at least first-order balanced have desirable properties, unbalanced designs are on occasion appropriate.

5. Consequences of equal numbers of first-phase and second-phase units

All of the examples presented in Section 4 have the same number of first-phase and second-phase units. This prevalent situation arises when there is no laboratory replication so that each measurement in the laboratory phase arises from a different first-phase unit, as in Example 1 which has 42 plots and 42 spots. It also occurs when there is laboratory replication and a different portion of the product from a first-phase unit is required for each replicate (Brien et al. 2011, Section 7.3), as in Example 2. The numbers of units will not be equal when laboratory replication involves non-destructive measurement or reprocessing of the same first-phase unit. An example is twice measuring the protein content of a grain sample from each plot in a wheat experiment using near-infra-red spectroscopy, with only remixing of the grain between measurements.

In this section, the ramifications of the same number of units in two consecutive phases for the properties of a multiphase design are explored. Results in Brien & Bailey (2009), particularly Lemma 4.2, and in Bailey & Brien (2016, Section 8.2.1) have a direct bearing on this situation and their implications are summarized in two laws and a corollary, followed by a discussion of their application to the previously presented Examples 1 and 2 and to Example 4. Then Section 5.1 examines the desirability of and techniques for achieving orthogonal later phases.

Multiphase law 3 (Equal numbers of objects). In an experiment with three or more tiers, for the combined design to be structure balanced when the numbers of objects for two consecutive tiers are equal then, for the allocated tier of the pair, (i) the combined allocation of any tiers to it must be structure balanced in relation to it and, (ii) its structure, possibly refined, for instance by including pseudofactors, must be structure orthogonal in relation to the recipient tier of the pair. Structure orthogonal or not, for the recipient tier of the pair, there are no Residual sources for its sources and its sources are said to be exhausted by those from the allocated tier. (Brien & Bailey 2009, Lemma 4.2 and Section 6)

This law can in some cases, such as Example 2, have the sting in its tail embodied in the following corollary.

Multiphase corollary 3.1 (Structure balance impossible). If the numbers of objects for two consecutive tiers are equal and the structure for the allocated tier, possibly modified, is not structure orthogonal in relation to that of the recipient tier, then the multiphase design using them cannot be structure balanced (Brien & Bailey 2009, Lemma 4.2). It may be first-order balanced.

Multiphase law 4 (Inestimable components). Not all canonical (covariance) components can be estimated when either (i) the numbers of objects for two consecutive tiers are equal, or (ii) the source for the term corresponding to a canonical component is exhausted by sources that are only confounded with it (Bailey & Brien 2016, Section 8.2.1). For canonical-components models, only the sum of the canonical components for the identity terms are estimable when (i) applies.

When this law applies, a mixed model can only be fitted if terms are omitted in forming the prior allocation model such that the components for the remaining terms are estimable; the resulting model is characterised as a 'model of convenience' by Brien & Demétrio (2009).

Example 1. A small wheat experiment with a Youden square design in the lab phase (continued). The laws just introduced are explored for this introductory example from Section 2.

Applying the laws and corollary. Given the equal numbers of plots and spots in the experiment, Multiphase Law 3 (Equal numbers of objects) and its corollary are in play.

Given that the allocation of plots to spots, once the pseudofactor $\mathrm{P_S}$ is included, is structure orthogonal, then Multiphase corollary 3.1 (Structure balance impossible) does not preclude a structure-balanced design. Because the allocation of lines to plots, when based on the lines structure and the modified plots structure is structure balanced, the two conditions in Multiphase Law 3 (Equal numbers of objects) are met so that the combined design is structure balanced. This law forecasts the exhaustion of all the spots sources by the plots sources so that they do not have Residual sources, as revealed in Table 1. Multiphase law 4 (Inestimable components) further predicts that not all canonical components will be estimable. The EMS in Table 1 are linearly dependent such that the only estimable canonical component is ϕ_{S}, although not by ANOVA because all Spots DF are exhausted by Lines; a mixed model analysis produces an estimate by utilizing differences in treatment estimates (Bailey & Brien 2016, Section 6.4). Only sums of the other canonical components are estimable: $\phi_{\mathrm{RS}} + \phi_{\mathrm{BP}}$ and $\phi_{\mathrm{S}} + \phi_{\mathrm{B}}$. Consequently, to successfully fit a mixed-model for this experiment, terms need to be omitted, for example all plots terms: Lines | Runs + Spots + $\underline{\text{Runs}\wedge\text{Spots}}$. The estimates of ϕ_{R} and ϕ_{RS} under this model actually estimate $\phi_{\mathrm{R}} + \phi_{\mathrm{B}}$ and $\phi_{\mathrm{RS}} + \phi_{\mathrm{BP}}$.

Example 2. A two-phase microarray experiment (continued). The new laws also apply to this example from Section 4.2.

Applying the laws and corollary. Multiphase law 3 (Equal numbers of objects) applies and the samples sources exhaust the arrays-dyes sources. The EMS show that Multiphase law 4 (Inestimable components) also applies with Blocks and the two Samples[Blocks$\wedge$Plants] sources each confounded with just one array-dyes source. Consequently, ϕ_{BPS} and ϕ_{B} are not estimable. Because the second-phase design is not structure balanced, Multiphase corollary 3.1 (Structure balance impossible) rules out a structure-balanced two-phase design.

Example 4. A small wheat experiment with incomplete-blocks in the lab phase. This example illustrates Multiphase corollary 3.1: in spite of employing A-optimal designs in the second-phase, because the numbers of first-phase and second-phase units are equal and a nonorthogonal design is unavoidable, the two-phase design must be unbalanced.

Design identification. The situation is the same as in Example 1, except that now there are 10 lines and the laboratory equipment accommodates only four samples per run. Thus, the field phase has 60 plots and the laboratory phase has 15 runs each with four spots. The researcher does not expect consistent differences between the four spots in a run and so the anticipated model is: Lines | Blocks + $\underline{\text{Blocks}\wedge\text{Plots}}$ + Runs + $\underline{\text{Runs}\wedge\text{Spots}}$.

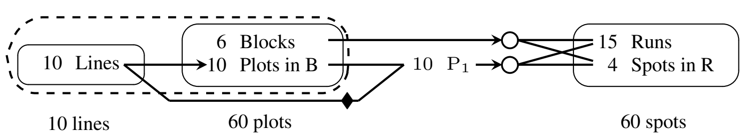


Figure 4. Factor-allocation diagram for Example 4, a small wheat experiment with incomplete-blocks in the lab phase: lines are allocated to plots, and lines and plots are allocated to spots; the arrows indicate that randomization is employed in the allocation; the line from Lines to $\mathrm{P_1}$ with the solid diamond (♦) signifies that Lines directly determine $\mathrm{P_1}$, a pseudofactor for Plots identifying plots assigned the same level of Lines; the dashed, rounded rectangle indicates that the factors in the enclosed panels are combined into a pseudotier for randomizing them to spots; the open circles (∘) indicates that nonorthogonal designs are used in allocating $\mathrm{P_1}$ and Blocks; B = Blocks; R = Runs.

In allocating plots to spots it is again necessary to account for Lines. Like in Example 1, a ten-level pseudofactor P_1 that is the same as Lines is introduced. Given this model and the numbers of levels of the factors, a balanced incomplete-block design is optimal for assigning P_1 to spots and is possible because such a design exists for six replicates of ten treatments (Cochran & Cox 1957, Plan 11.16). It has an intrablock efficiency of 5/6. Further, it is necessary to consider the allocation of Blocks to Runs. Again, an optimal design in the form of a balanced incomplete-block design that allocates four of the six blocks to each run is available (Cochran & Cox 1957, Plan 11.6); it has an intrablock efficiency of 9/10. The two designs used for this phase have to be aligned so that all 60 Blocks-P_1 combinations are present in the design; this is achieved by permuting the order of the Blocks levels within Runs. A permutation is in the R script in Supplementary materials E that constructs and randomizes the two-phase design. The factor-allocation diagram for the design is in Figure 4. It differs from Figure 1 for Example 1 in having Spots nested within Runs and that a nonorthogonal design has to be used in allocating Blocks.

Model formulation. The initial allocation model derived from the factor-allocation diagram is the same as the anticipated model.

Design evaluation. The decomposition table, in Table 5, is based on the anatomy produced using the R script given in Supplementary materials E. It shows the average efficiencies and their orders. Since the orders exceed one, the two-phase design is not structure balanced, and nor is it first-order balanced, as Multiphase corollary 3.1 (Structure balance impossible) asserts. Consequently, the average efficiencies do not sum to one and EMS cannot be added to the table. The efficiency factors for the last, or intrablock, Lines source in Table 5 range from 0.815 to 0.833, averaging 0.827; the design is almost balanced with efficiency factors that are close to the intrablock efficiency factor for the balanced incomplete-block design used to assign P_1. Multiphase law 1 (DF never increase) is activated in that the Residual for Plots [Blocks] has 31 DF as compared to 54 in the first-phase design; this results from the division of Plots [Blocks] between Runs and Runs#Spots, as well as five of its DF being aliased with Blocks.

Table 5. Decomposition table for Example 4, a small wheat experiment with incomplete-blocks in the lab phase: R = Runs; B = Blocks.

spots tier		plots tier				lines tier			
Source	DF	A eff†	Order‡	Source	DF	A eff†	Order‡	Source	DF
Mean	1	1.00	1	Mean	1	1.00	1	Mean	1
Runs	14	0.10	1	Blocks	5	0.04	5	$\text{Lines}_1^{\dagger}$	5
		1.00	1	$\text{Plots [B]}_{\text{R}}^{\S}$	9	0.01	6	$\text{Lines}_2^{\dagger}$	9
Spots [R]	45	0.90	1	Blocks	5	0.01	5	$\text{Lines}_1^{\dagger}$	5
		1.00	1	$\text{Plots [B]}_{\vdash}^{\S}$	40	0.83	6	$\text{Lines}_2^{\dagger}$	9
								Residual	31

Notes: †Each A eff is the harmonic mean of the efficiency factors between either (i) a plots source confounded with a spots source or (ii) a lines source confounded with a plots source combined with a spots source. Lines_1 is the source with five DF that is confounded with Blocks and Lines_2 is source for the full 9 DF that is confounded with Plots [B]. ‡Order is the number of unique values taken by the efficiency factors that are involved in the corresponding A eff. §Plots $[\text{B}]_{\text{R}}$ is the part of Plots [B] that is confounded with Runs and Plots $[\text{B}]_{\vdash}$ is the part of Plots [B] orthogonal to Plots $[\text{B}]_{\text{R}}$.

Design optimality. Again we turn to od to check the first-phase-conditional A-optimality of the two-phase design under the anticipated model. In doing this, (i) to boost the efficiency for the confounding of Lines with Spots [Runs], γ_{R} is set to 10 and ϕ_{RS} to one, (ii) the design obtained using the balanced incomplete-block designs was used as an initial design and both γ_{B} and γ_{BP} set to 0.5, (iii) 15 tabu loops of row interchanges of the Lines design matrix were performed, and (iv) the design produced by od was randomized (see the R script in Supplementary materials E). The changes in the AVPD values and 'intrablock' efficiencies are negligible. While the second-phase is no longer first-order balanced, the intrablock efficiency has only changed from 0.900 to 0.899.

Design robustness was studied by using od to generate 8 first-phase-conditionally near-A-optimal designs for all combinations of the values 0.5 and 10 for γ_{B}, γ_{BP} and γ_{R}. The differences in the AVPD values for the original design and for each od design for the 8 sets of γ values were calculated. Again, the differences were negligible, being less than 0.001.

The originally constructed design is the preferred design, as it would appear to be a first-phase-conditionally near-A-optimal design and retains the balanced second-phase design.

5.1. Orthogonal later phases

The following principle is similar in spirit to Principle 5 (I. Simplicity desirable) and is prompted by Multiphase law 3 (Equal numbers of objects):

Principle 15 (Orthogonal later-phase designs advantageous). If possible, use a structure-orthogonal design for the second, and any subsequent, phase, preferably one that does not require pseudofactors to make it orthogonal.

The motivation arising from Multiphase law 3 (Equal numbers of objects) is that a structure-orthogonal later phase will, if the combined design of earlier phases is structure balanced, combine with the earlier phases to result in a structure-balanced design. Even if the earlier phases of a design are unbalanced, because the structure-orthogonal, later-phase design is A-optimal, constructing an A-optimal combined design is generally straightforward. Also, such a phase will minimize the complexity of the combined design and any further loss in precision If the numbers of units for the first and later phases are equal then, for a design with later structure-orthogonal phases, the initial allocation model for the combined design has commutative variance structure (Bailey & Brien 2016), so that variance parameter estimation is less troublesome than for an alternative design that does not.

The proviso 'if possible' in Principle 15 (Orthogonal later-phase designs advantageous) is important because practical limitations, such as the number of samples that can be processed together, may render its application impossible. Similarly, the need to reduce block size in the later phases to form homogeneous blocks, as advocated in Principle 3 (I. Minimize variance), can be a barrier to structure-orthogonal, later-phase designs.

The preference for not needing pseudofactors to make the design structure orthogonal is because they split sources, which is likely to weaken the attributes, described in Principle 13 (Design selection attributes), of first-phase sources in the two-phase design; in particular, split sources can result in less Residual DF, as in Example 1.

Mechanisms for achieving structure-orthogonal later phases include: (i) have the second-phase units *correspond* exactly to the first-phase units, with the factors in the two tiers being equivalent in the numbers of their levels and the nesting relationships between them;

(ii) *subdivide* first-phase blocks into sub-blocks to form second-phase blocks; (iii) *group* first-phase entities, such as plots, to form blocks such that the source for those entities is orthogonally partitioned and each of the resulting parts of the source is confounded with a single, different, second-phase source; and (iv) *combine* first-phase blocks into superblocks to form second-phase blocks. The grouping of first-phase plots is demonstrated in Example 1; the remaining mechanisms are covered using Example 5.

Example 5. **A beetle damage experiment with corresponding first- and second-phase blocks.** Peacock et al. (2003) described an experiment to investigate a field observation that beetle damage on willows might be inhibiting rust development. While it provides context, the details are changed to provide some novel features.

Design identification. This experiment had a glasshouse and a laboratory phase. Suppose that in the glasshouse phase there are 12 treatments, simulating different extents and timing of beetle damage, and that each is replicated five times so that there are 60 spots in the glasshouse each containing a plant. The willow plants are grown on benches each of which has three spots and it is thought that these spots are relatively homogeneous. Benches are anticipated to vary and benches further apart to differ more than those closer together. The proposed anticipated model for the first phase is:

$$\text{Reps} + \text{Damages} \mid \text{Reps}\wedge\text{Benches} + \underline{\text{Reps}\wedge\text{Benches}\wedge\text{Spots}},$$

where Reps consists of pairs of Benches.

Then a leaf disc is taken from the plant at each spot and these are processed in the laboratory phase. There are 60 discs to be placed onto 20 plates, each with three cells. Conditions are predicted to change during the application of the discs to the cells so that the plates were subdivided into five groups and on each of five occasions all the discs from the same replicates were applied to the four plates in a group. The model that encompasses these laboratory sources of variation is $\text{Occasions} \mid \text{Occasions}\wedge\text{Plates} + \underline{\text{Occasions}\wedge\text{Plates}\wedge\text{Cells}}$; it is added to the first-phase anticipated model.

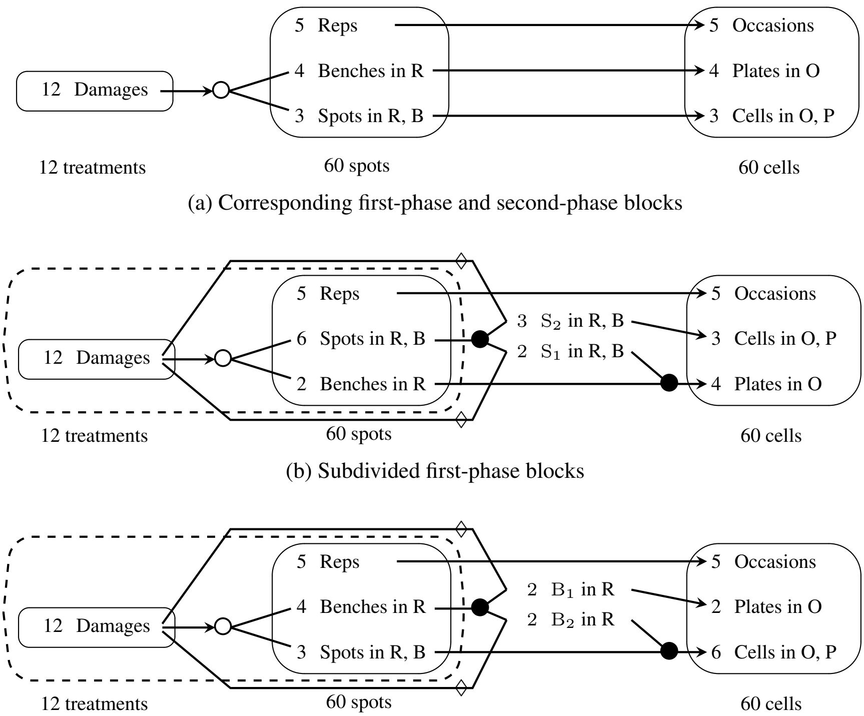
A resolved incomplete-block design for five replicates of 12 treatments in blocks of three plots is chosen for the first-phase design. CycDesigN (Whitaker, Williams & John 2002) was used to obtain a fixed-model, near-A-optimal design (see Table 6). The average efficiency for Damages in relation to Cells [Occasions∧Plates] is 0.698, compared to CycDesigN's upper bound of 0.721. This design fulfils Principle 3 (I. Minimize variance).

Table 6. The unrandomized, resolved, incomplete-block design for assigning Damages in Example 5, a beetle damage experiment with corresponding first- and second-phase blocks; the entries are Damages levels.

Rep	I				II				III				IV				V			
Bench	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Spot																				
1	1	2	3	4	1	3	2	4	1	4	2	3	1	2	3	4	1	3	2	4
2	6	7	8	5	5	7	6	8	8	7	5	6	5	6	7	8	7	5	8	6
3	11	12	9	10	9	11	10	12	10	9	11	12	12	9	10	11	9	11	10	12

The obvious way to assign the spots to the cells is to randomize Reps, Benches and Spots to Occasions, Plates and Cells, respectively, as shown in Figure 5a. This results in

Principles 8 (I. Big with big), 5 (I. Simplicity desirable) and 15 (Orthogonal later-phase designs advantageous) being observed.



(a) Corresponding first-phase and second-phase blocks

(b) Subdivided first-phase blocks

(c) Combined first-phase blocks

Figure 5. Factor allocation diagram for Example 5, a beetle damage experiment; treatments are randomized to spots and treatments and spots to cells; the open circle (○) signifies that a nonorthogonal design is used in allocating Damages; the upper and lower lines running from Damages to the pair of pseudofactors S_1 and S_2 (B_1 and B_2) indicate that Damages determines these pseudofactors on Spots (Benches); the open diamonds (◊) indicate that the pseudofactors are determined from the Damages using a nonorthogonal design; the left-hand solid circle (•) indicates that the levels of Spots are the combinations of the levels of S_1 and S_2 or that Benches are the combinations of the levels of B_1 and B_2; the right-hand solid circle (•) in (b) indicates that the combinations of the levels of S_1 and Benches are combined when randomized to Plates, as implied by the arrow from the solid circle to Plates (similarly, for B_1, Spots and Cells in (c)); the dashed, rounded rectangle indicates that the factors in the enclosed panels are combined into a pseudotier for randomizing them to cells; R= Reps; B= Benches; O = Occasions; P = Plates.

Design evaluation. To assess the proposed design, use the skeleton ANOVA table in Table 7a. An R script for obtaining a randomized layout for the experiment and the decomposition table underlying Table 7a is provided in Supplementary materials F. The orders of the two Damages sources exceed one; the design is unbalanced. The structure on spots is structure-orthogonal in relation to that on cells, so that the combined design has commutative variance structure, even though the whole two-phase design is unbalanced. Table 7a also makes clear that, due to the confounding in the design, the spots and cells canonical components cannot be separated from each other; only pairs of sums are estimable.

Design optimality. Because of the one-to-one correspondence between the spots and cells decomposition, the properties of the two-phase design are precisely the same as those of the first-phase design (McIntyre 1955): both are fixed-model near-A-optimal. However,

Table 7. Skeleton ANOVA table for Example 5, a beetle damage experiment; O = Occasions; P = Plates; C = Cells; R = Reps; B = Benches; S = Spots; D = Damages

(a) Corresponding first-phase and second-phase blocks

cells tier		spots tier†		treatments tier				EMS‡						
Source	DF	Source	DF	A eff†	Order†	Source	DF	ϕ_{OPC}	ϕ_{OP}	ϕ_{O}	ϕ_{RBS}	ϕ_{RB}	ϕ_{R}	$q(\cdot)$
Mean	1	Mean	1			Mean	1							
Occasions	4	Reps	4					1	3	12	1	3	12	
Plates[O]	15	Benches[R]	15	0.31	4	Damages$_1^\dagger$	9	1	3		1	3		$q(\mathrm{D}_1)$
						Residual	6	1	3		1	3		
Cells[O∧P]	40	Spots[R∧B]	40	0.70	5	Damages$_3^\dagger$	11	1			1			$q(\mathrm{D}_3)$
						Residual	29	1			1			

(b) Subdivided first-phase blocks

cells tier		spots tier†		treatments tier				EMS‡						
Source	DF	Source	DF	A eff†	Order†	Source	DF	ϕ_{OPC}	ϕ_{OP}	ϕ_{O}	ϕ_{RBS}	ϕ_{RB}	ϕ_{R}	$q(\cdot)$
Mean	1	Mean	1			Mean	1							
Occasions	4	Reps	4					1	3	12	1	6	12	
Plates[O]	15	Benches[R]	5	0.20	3	Damages$_1^\dagger$	4	1	3		1	6		$q(\mathrm{D}_1)$
						Residual	1	1	3		1	6		
		Spots[R∧B]$_1^\S$	10	0.09	7	Damages$_2^\dagger$	9	1	3		1			$q(\mathrm{D}_2)$
						Residual	1	1	3		1			
Cells[O∧P]	40	Spots[R∧B]$_2^\S$	40	0.70	5	Damages$_3^\dagger$	11	1			1			$q(\mathrm{D}_3)$
						Residual	29	1			1			

Notes: †The efficiency factors for spots sources confounded with cells sources are one and so are not given. Each A eff is the harmonic mean of the efficiency factors between a treatments source and the combined spots-cells source with which it is confounded. Order is the number of unique values taken by the efficiency factors that are involved in the corresponding A eff. The 11-dimensional Damages space is disposed across multiple sources, with only Damages$_3$ being the complete space and for which there are 5 unique efficiency factors. ‡Each ϕ is a canonical component that, except for ϕ_{OPC} and ϕ_{RBS}, is allowed to be negative. Their subscripts are comprised of the first letter of each factor in the corresponding term and the numbers in the table are the coefficients of the components in the EMS. Each q-function is the same quadratic function of the expectation as the mean square for that line of the table is of the data; the subscripts of q-functions for the same source relate to the different matrices of their quadratic forms.. §Spots[R∧B]$_1$ is that 10-dimensional subspace of the Spots[R∧B] subspace that is defined by the pseudofactor S$_1$; Spots[R∧B]$_2$ is the 40-dimensional subspace defined by S$_2$ which is orthogonal to the Spots[R∧B]$_1$ subspace.

searching for a near-A-optimal design under the anticipated model using od with 15 tabu loops decreased the AVPD value by less than 2.5% over the eight combinations of 0.5 and 10 for the values of γ_{RB}, γ_{RBS} and γ_{OP}; γ_{RB} and γ_{RBS} are the ratios of their ϕs to ϕ_{RBS} and ϕ_{OPC}, respectively, while γ_{OP} and γ_{OPC} are ratios to ϕ_{OPC}; Reps and Occasions are fixed. It would appear that the proposed design is near-A-optimal for the anticipated model as well.

Example 5. A beetle damage experiment with second-phase blocks formed by subdividing first-phase blocks (continued). An alternative method for forming blocks is now considered for this example.

Design identification. Suppose that the experiment involved two benches, each with six spots; otherwise all other aspects of the experiment are the same, as shown in Figure 5b. In these circumstances it is proposed to use a near-A-optimal, resolved incomplete-block design for the first-phase design, for 12 treatments with incomplete blocks of size six. These incomplete blocks are to be subdivided into two blocks of size three for the second phase so that the six spots from a bench occupy two plates of three cells. Hence, Principle 5 (I. Simplicity desirable) cannot be observed because the spots source for Benches has to be split across more than one laboratory source, those for Plates and Cells.

Constructing a two-phase design begins with using the resolved incomplete-block design in Table 6 as the cross-phase design, but with Rep, Bench and Spot in Table 6 now replaced with Occasion, Plate and Cell. This construction of the cross-phase design before the first-phase design mandates the use of Principle 6 (I. Preplan all). Then the first-phase design is developed, followed by the assembling of the second-phase design.

To construct the first-phase design for assigning treatments to spots, pairs of Plates are combined, as shown in Figure 5b, to form blocks of size six for assigning to Benches. The arrangement of Plates (Bench) in Occasions (Rep) in Table 6 is planned so that combining Plates 1 with 2 and Plates 3 with 4 results in four of the six pairs of the treatments within one of the three sets of treatments, $\{1 \ldots 4\}$, $\{5 \ldots 8\}$ and $\{9 \ldots 12\}$ occurring together on the same Bench twice and the other two pairs occurring together once. These three sets have the property that, for the design in Table 6, treatments within a set never occur in the same block. The design produced has average efficiency 0.882, compared with the upper bound of 0.898. It is fixed-model near-A-optimal.

To assign spots to cells, the second-phase design is formed, based on the randomized first-phase design. The Spots source must be split and this is driven by the design in Table 6; the six Spots on a Bench must be assigned to two Plates such that each Plate contains the three correct Damages. To split the Spots source and keep track of which of the six Spots allocated to a Bench are to end up on the same Plate, Principle 9 (I. Use pseudofactors) is deployed. Two pseudofactors are constructed to relabel the spots: S_1 with two levels and S_2 with three levels. The first has the same level and the second different levels for the three spots within a bench that are on the same plate. They are used in randomizing the spots to cells, as depicted in Figure 5b, so that Spots are randomized such that Damages is also suitably randomized. Clearly, the Spots pseudofactors require the outcome of the treatments randomization to spots so that, as foreshadowed, the randomizations are randomized-inclusive.

Design evaluation. The skeleton ANOVA table, for examining the properties of the proposed design, is in Table 7b. An R script for obtaining a randomized layout for the experiment and the decomposition table underlying Table 7b is in Supplementary materials F. The orders of all three Damages sources exceed one; only the lowest degree of balance described in Principle 14 (Maximize the degree of balance), unbalanced, is practicable here. Even so, the structure on spots, extended to include the pseudofactors, is structure-orthogonal in relation to that on cells and so the combined design has commutative variance structure. Splitting the source Spots $[\text{Reps}\wedge\text{Benches}]$ diminishes the properties of the first-phase design in the two-phase design. For the first-phase design, the Residual DF for Spots $[\text{Reps}\wedge\text{Benches}]$ are 39; this is effectively reduced to 29 DF in the laboratory phase, although it cannot be separated from variability arising from Occasions∧Plates∧Cells. Also, it can be deduced from Table 7b that, due to the confounding in the design, there are two pairs of canonical components for which only the sum of the pair's components are estimable.

Design optimality. Again, first-phase-conditionally near-A-optimal two-phase designs were produced using od with 15 tabu loops under the anticipated model over the eight combinations of 0.5 and 10 for the values of γ_{RB}, γ_{RBS} and γ_{OP}. A search involved: (1) a search for a near-A-optimal resolved first-phase design; (2) a search for a resolved two-phase design that is first-phase-conditionally near-A-optimal, starting with the first-phase design and assigning the first three spots to one plate and the other three spots to the second plate within an occasion. The decreases in the AVPD value for the od designs were less than 1.75%. The average efficiency is 0.90 for Damages when confounded with Spots[Reps∧Benches]$_2$ in the first-phase design and is 0.71 when these are confounded with Cells[Occasions∧Plates] in the two-phase design. In all two-phase od designs, the structure-orthogonal second-phase design was kept. Both the original and the od designs are first-phase-conditionally near-A-optimal resolved designs.

A third mechanism for achieving a structure-orthogonal later phase is to combine first-phase blocks to form second-phase blocks. Figure 5c illustrates this for Example 5. An R script for obtaining a randomized layout for this experiment and its decomposition table is in Supplementary materials F. The decomposition table is similar to that in Table 7b.

6. Conclusions

Often, when a multiphase experiment includes a laboratory phase, it and the previous phase have the same number of units. It is true for all of the examples presented and so Multiphase laws 3 (Equal numbers of objects) and 4 (Inestimable components) apply to them. They also comply with Principle 7 (I. Allocate all and randomize in laboratory). Example 1 shows that two A-optimal designs can combine to give an A-optimal design. This example, along with Example 5, illustrates the substantial advantages, outlined in Section 5.1, afforded to two-phase designs for which the second-phase design is structure orthogonal. But, then Example 4 forces us to realize that combining A-optimal designs may not produce an A-optimal two-phase design. Table 8 summarizes further features of the examples.

Suitable two-phase designs were produced for all of the examples by combining two (near-)A-optimal designs. For Examples 1, 2 and 4, the component designs were A-optimal under a mixed model, while for Examples 3 and 5 fixed-model near-A-optimal designs were used. While all two-phase designs seem to be at least first-phase-conditionally near-A-optimal, only for Example 1 is there certainty. For the other examples, the optimality depends on the variance parameter values, which was addressed by demonstrating some insensitivity of the proposed designs to these values. Additional doubt surrounds Example 3 because of the difficulty in identifying a good second-phase design for it. This difficulty is a general problem in designing two-phase experiments in which both phases have the same number of units and each requires an unbalanced design. While (fixed-model) near-A-optimal first-phase and cross-phase designs can usually be produced, identifying a good second-phase design can be difficult, because rather than merely finding a near-A-optimal design, a design that maximizes the confounding of blocks effects from different phases is often required.

An important point permeating the examples, and the theory of Brien & Bailey (2009) and Bailey & Brien (2016), is that, while the cross-phase design can have a role, it is the first-phase and second-phase designs that are fundamental to the attributes of a two-phase design.

In my experience, you neglect the second-phase design at your peril.

Also, advocated is that factors for portions associated with laboratory replication be temporarily omitted in designing a second phase.

For constructing multiphase designs, standard designs (like randomized complete-block and Youden square designs), catalogues of designs (for example Cochran & Cox 1957) and computer searches with both CycDesigN (Whitaker, Williams & John 2002; VSN International 2013) and od (Butler 2019) have been used. Advantages of CycDesigN over od include that variance parameter values are not needed and it gives upper bounds for the average efficiency of its designs so that the closeness to A-optimality of a design can be gauged. However, it only produces single-phase designs, which are not always (near-) A-optimal under a mixed model. On the other hand, od is able to produce designs that are (near-)A-optimal for general mixed models, including those for multiphase experiments.

Section 3 outlines a scheme for producing multiphase designs, an essential ingredient of which is to display a design's anatomy. The anatomy depicts the dispersal of information amongst the sources intrinsic to the design and so supports its evaluation. Key to producing an anatomy is the factor allocation, such as is displayed in a factor-allocation diagram. These diagrams also facilitate the formulation of initial and prior allocation-based mixed models.

Table 8. Summary of examples.

		Design		Combined design		
Example	Allocation	First phase†	Second phase†‡	Balance	A-optimal	Points demonstrated
1. Small wheat	Randomized-inclusive	Orthogonal RCBD	(Orthogonal§; Balanced§, YSD)	Balanced§	Yes	Universally optimal design; first-phase Residual DF decreased (Multiphase law 1).
2. Micro-array	Composed	Balanced§ BIBD	(First-order balanced§, YSDs; Balanced§)	First-order balanced§	First-phase-conditional	Combined structure balance, ignoring Samples (Multiphase law 2); structure balance impossible with Samples (Multiphase corollary 3.1); first-order balance can be acceptable (Principle 14).
3. Large wheat	Composed	Unbalanced RRCD	(Unbalanced, Ordered; Unbalanced)	Unbalanced	First-phase-conditional, possibly near	Ordered starting design can be favoured; resolved design preferred (Principle 13); other properties revealed by anatomy can be important; anatomy insensitive to variance parameter values; first-phase Residual DF decreased (Multiphase law 1)
4. Small wheat	Randomized-inclusive	Orthogonal§ RCBD	(First-order balanced; Balanced§, BIBDs)	Unbalanced	First-phase-conditional, near	Structure balance impossible (Multiphase corollary 3.1); even though first-phase and cross phase designs are structure balanced, the second-phase design is not so that Multiphase law 2 is not met; first-phase Residual DF decreased (Multiphase law 1).
5. Beetle damage	Randomized-inclusive	Unbalanced RIBD	(Orthogonal§; Unbalanced, RIBD)	Unbalanced	First-phase-conditional, near	Structure-orthogonal second phase by corresponding, subdividing or combining; resolved design preferred (Principle 13); first-phase Residual DF decreased (Multiphase law 1).

Notes: †RCBD = Randomized Complete-Block Design; BIBD = Balanced Incomplete-Block Design; RIBD = Resolved Incomplete-Block Design; YSD = Youden Square Design; RRCD = Resolved Row-Column Design. ‡The bracketed pair for each example gives the degree of balance for the, possibly refined, second-phase and the cross-phase designs; the named design is the primary design in the construction of the combined design. §orthogonal = structure orthogonal; balanced = nonorthogonal, but structure balanced; first-order balanced = not structure balanced, but first-order balanced.

Appendix I

Variance matrix for Example 1, a small wheat experiment with a Youden square design in the lab phase

Here, the definitions and notation are those of Brien & Bailey (2009). In particular, a structure is a set of $n \times n$ mutually orthogonal idempotents that sum to a suitably defined identity matrix, where n is the number of observations. Let $\mathcal{P}$ be the set of idempotents corresponding to the sources derived from the recipient factors in an allocation and $\mathcal{Q}$ to those derived from the allocated factors.

Definition *For a structure $\mathcal{Q}$ to be structure-balanced in relation to a second structure $\mathcal{P}$ then the following conditions must be met:*

$$\begin{aligned} \mathbf{QPQ} &= \lambda_{\mathbf{PQ}}\mathbf{Q} \\ \mathbf{Q}_1\mathbf{P}\mathbf{Q}_2 &= \mathbf{0} \end{aligned}$$

for all $\mathbf{P}$ in $\mathcal{P}$ and all $\mathbf{Q}_1$, $\mathbf{Q}_2$ in $\mathcal{Q}$ with $\mathbf{Q}_1 \neq \mathbf{Q}_2$; $\lambda_{\mathbf{PQ}}$ is called the canonical efficiency factor and is the value of the nonzero eigenvalues of $\mathbf{QPQ}$.

For the allocation of the first phase units, plots, to second-phase units, spots, $\mathcal{P}$ is comprised of the projection matrices $\{\mathbf{P}_0, \mathbf{P}_{\mathrm{R}}, \mathbf{P}_{\mathrm{S}}, \mathbf{P}_{\mathrm{RS}}\}$ and $\mathcal{Q}$ is initially comprised of the projection matrices $\{\mathbf{Q}_0, \mathbf{Q}_{\mathrm{B}}, \mathbf{Q}_{\mathrm{BP}}\}$. Now $\mathcal{Q}$ is not structure balanced in relation to $\mathcal{P}$ because $\mathbf{Q}_{\mathrm{BP}}\mathbf{P}\mathbf{Q}_{\mathrm{BP}} \neq \lambda_{\mathbf{PQ}}\mathbf{Q}_{\mathrm{BP}}$ for any $\mathbf{P}$ in $\mathcal{P}$. If we replace $\mathcal{Q}$ with $\{\mathbf{Q}_0, \mathbf{Q}_{\mathrm{B}}, \mathbf{Q}_{\mathrm{BP}_{\mathrm{S}}}, \mathbf{Q}_{\mathrm{BP}_{\vdash}}\}$, where $\mathbf{Q}_{\mathrm{BP}_{\mathrm{S}}} + \mathbf{Q}_{\mathrm{BP}_{\vdash}} = \mathbf{Q}_{\mathrm{BP}}$, $\mathbf{Q}_{\mathrm{BP}_{\mathrm{S}}}\mathbf{Q}_{\mathrm{BP}_{\vdash}} = \mathbf{0}$ and $\mathbf{P}_{\mathrm{S}}\mathbf{Q}_{\mathrm{BP}_{\mathrm{S}}} = \mathbf{P}_{\mathrm{S}}$, then the design is structure balanced because, for every $\mathbf{Q}$ in $\mathcal{Q}$, $\mathbf{QPQ} = \mathbf{Q}$ for just one $\mathbf{P}$ in $\mathcal{P}$.

Now, the variance matrix for the initial allocation model is given by

$$\begin{aligned} \mathbf{V} = &\xi_0\mathbf{P}_0 + \xi_{\mathrm{R}}\mathbf{P}_{\mathrm{R}} + \xi_{\mathrm{S}}\mathbf{P}_{\mathrm{S}} + \xi_{\mathrm{RS}}\mathbf{P}_{\mathrm{RS}} + \\ &\eta_0\mathbf{Q}_0 + \eta_{\mathrm{B}}\mathbf{Q}_{\mathrm{B}} + \eta_{\mathrm{BP}}\mathbf{Q}_{\mathrm{BP}_{\mathrm{S}}} + \eta_{\mathrm{BP}}\mathbf{Q}_{\mathrm{BP}_{\vdash}}. \end{aligned}$$

Combining projection matrices that are equal yields the following expression:

$$\mathbf{V} = (\xi_0 + \eta_0)\mathbf{P}_0 + (\xi_{\mathrm{R}} + \eta_{\mathrm{B}})\mathbf{P}_{\mathrm{R}} + (\xi_{\mathrm{S}} + \eta_{\mathrm{BP}})\mathbf{P}_{\mathrm{S}} + (\xi_{\mathrm{RS}} + \eta_{\mathrm{BP}})\mathbf{P}_{\mathrm{RS}}.$$

That is $\mathbf{V}$ is a linear combination of the set of projection matrices for the Youden square used for the cross-phase design.

Appendix II

The sum of the efficiency factors for a source in a structure-balanced design

Using the notation outlined in Appendix I, it is first noted that, for a source $\mathbf{Q}$ in $\mathcal{Q}$, $\mathbf{Q} = \mathbf{Q}\mathbf{I}_{\mathcal{P}}\mathbf{Q}$, where $\mathbf{I}_{\mathcal{P}} = \sum_{\mathbf{P}\in\mathcal{P}} \mathbf{P}$.

Given that $\mathcal{Q}$ structure-balanced in relation to $\mathcal{P}$ and $\mathcal{P}_{\mathbf{Q}}$ is the set of $\mathbf{P}$ for which $\lambda_{\mathbf{PQ}} \neq 0$, it follows that $\mathbf{Q} = \mathbf{Q}\mathbf{I}_{\mathcal{P}}\mathbf{Q} = \sum_{\mathbf{P}\in\mathcal{P}_{\mathbf{Q}}} \mathbf{QPQ} = \sum_{\mathbf{P}\in\mathcal{P}_{\mathbf{Q}}} \lambda_{\mathbf{PQ}}\mathbf{Q}$.

Hence $\sum_{\mathbf{P}\in\mathcal{P}_{\mathbf{Q}}} \lambda_{\mathbf{PQ}} = 1$.

Appendix III

Deriving the EMS for a design that is structure balanced, except for the inclusion of laboratory replicates in the second phase

The derivation uses the techniques of Bailey & Brien (2016). Let $\mathcal{F}$, $\mathcal{G}$ and $\mathcal{H}$ be the sets of generalized factors for three tiers with (i) $\mathcal{H}$ randomized to $\mathcal{G}$ in the first phase, and (ii) $\mathcal{G}$ and $\mathcal{H}$ randomized to $\mathcal{F}$ in the second phase. Assume that the numbers of objects indexed by the factors in $\mathcal{F}$ and $\mathcal{G}$ are both equal to n. Denote the identity generalized factors for these two sets by $E_{\mathcal{F}}$ and $E_{\mathcal{G}}$ and let $\mathcal{F}^* = \mathcal{F} \setminus E_{\mathcal{F}}$ and $\mathcal{G}^* = \mathcal{G} \setminus E_{\mathcal{G}}$. The variance matrix $\mathbf{V}$, equivalent to that in Section 1, is given by

$$\begin{aligned} \mathbf{V} &= \sum_{F \in \mathcal{F}} \phi_F \mathbf{S}_F + \sum_{G \in \mathcal{G}} \psi_G \mathbf{S}_G \\ &= \sum_{F \in \mathcal{F}^*} \phi_F \mathbf{S}_F + (\phi_{E_{\mathcal{F}}} + \psi_{E_{\mathcal{G}}}) \mathbf{S}_E + \sum_{G \in \mathcal{G}^*} \psi_G \mathbf{S}_G \end{aligned} \tag{1}$$

where each $\mathbf{S}$ is an incidence matrix for its generalized factor, obtained from the design matrix, $\mathbf{Z}$, for the generalized factor as $\mathbf{Z}\mathbf{Z}^\top$, and $\mathbf{S}_E = \mathbf{I}_n$.

Let $\mathcal{P}$, $\mathcal{Q}^*$ and $\mathcal{R}$ be the structures, or sets of mutually-orthogonal projection matrices, for $\mathcal{F}$, $\mathcal{G}^*$ and $\mathcal{H}$, respectively. The variance matrix can be rewritten as

$$\mathbf{V} = \sum_{F \in \mathcal{F}} \xi_F \mathbf{P}_F + \sum_{G \in \mathcal{G}^*} \eta_G \mathbf{Q}_G$$

but, in the present case, it is assumed that $\xi_{E_F} = \phi_{E_{\mathcal{F}}} + \psi_{E_{\mathcal{G}}}$.

Assume that $\mathcal{R}$ is structure balanced in relation to $\mathcal{Q}^*$, with the combined structure being $\mathcal{Q}^* \rhd \mathcal{R}$, and that $\mathcal{Q}^* \rhd \mathcal{R}$ is structure balanced in relation to $\mathcal{P}$, with the combined structure being $\mathcal{P} \rhd (\mathcal{Q}^* \rhd \mathcal{R})$. Then the results of Bailey & Brien (2016) can be applied to provide the EMS for the experiment and these include the component $\psi_{E_{\mathcal{G}}}$ for the term $E_{\mathcal{G}}$ via its inclusion in ξ_{E_F}.

Consider Example 2. The tiers are arrays-dyes, samples and treatments. The sets of generalized factors for these tiers are $\mathcal{F} = \{\text{Mean}, \text{Arrays}, \text{Dyes}, \text{Arrays}\wedge\text{Dyes}\}$, $\mathcal{G} = \{\text{Mean}, \text{Blocks}, \text{Blocks}\wedge\text{Plants}, \text{Blocks}\wedge\text{Plants}\wedge\text{Samples}\}$ and $\mathcal{H} = \{\text{Mean}, \text{Treatments}\}$, with $E_{\mathcal{F}} = \text{Arrays}\wedge\text{Dyes}$ and $E_{\mathcal{G}} = \text{Blocks}\wedge\text{Plants}\wedge\text{Samples}$. Let $\mathcal{P}$, $\mathcal{Q}$ and $\mathcal{R}$ be the sets of mutually-orthogonal projection matrices for the arrays-dyes, samples and treatments tiers; $\mathcal{P} = \{\mathbf{P}_{\text{M}}, \mathbf{P}_{\text{A}}, \mathbf{P}_{\text{D}}, \mathbf{P}_{\text{AD}}\}$, $\mathcal{Q}^* = \{\mathbf{Q}_{\text{M}}, \mathbf{Q}_{\text{B}}, \mathbf{Q}_{\text{BP}}\}$, $\mathcal{Q} = \{\mathcal{Q}^*, \mathbf{Q}_{\text{BPS}}\}$ and $\mathcal{R} = \{\mathbf{R}_{\text{M}}, \mathbf{R}_{\text{T}}\}$, where the subscripts are the first letters of the factors in the generalized factor whose projection matrix it is.

The experiment is structure balanced with respect to $\mathcal{P}$, $\mathcal{Q}^*$ and $\mathcal{R}$, so that the results of Bailey & Brien (2016) apply; use these results, with ξ_{AD} set to $\phi_{\text{AD}} + \psi_{\text{BPS}}$, to obtain the EMS . On the other hand, the experiment is not structure balanced with respect to $\mathcal{P}$, $\mathcal{Q}$ and $\mathcal{R}$ because $\mathcal{P}$ and $\mathcal{Q}$ are not structure balanced. The experiment is first-order balanced.

Appendix IV

Principles for designing multiphase experiments

Fifteen principles are listed here (see also `http://chris.brien.name/multitier/MTMphase.html#labprinc`), of which the first twelve Principles 1–12 were developed in Brien et al. (2011).

The first 3 Principles are the basic principles for designing experiments: 1 (Evaluate designs with decomposition/skeleton-ANOVA tables), 2 (Fundamentals) and 3 (Minimize variance). The latter principle implies that a design that is near-A-optimal for the anticipated model is desirable.

Principles 5 (Simplicity desirable), 6 (Preplan all) and 7 (Allocate all and randomize in laboratory) provide general principles for designing multiphase experiments.

Principles 4 (Split-units), 8 (Big with big), 9 (Use pseudofactors), 10 (Compensating across phases), 11 (Laboratory replication) and 12 (Laboratory treatments) describe techniques to employ in achieving Principle 3 (Minimize variance).

Principles 13–15 elaborate on the attributes to look for in a multiphase design: (i) Principles 13 (Design selection attributes) and 14 (Maximize the degree of balance) moderate the objective of Principle 3 (Minimize variance) to suggest that A-optimality is not the sole consideration in designing an experiment, and (ii) Principles 14 (Maximize the degree of balance) and 15 (Orthogonal later-phase designs advantageous) expand Principle 5 (I. Simplicity desirable).

Principle 1 (Evaluate designs with decomposition/skeleton-ANOVA tables). Formulate the decomposition table or, if possible the skeleton-ANOVA table, using the factor-allocation diagram for an experiment, irrespective of whether its data is to be analysed by ANOVA. (Modification of Brien et al. 2011, Section 3.1)

Principle 2 (Fundamentals). A good experimental design employs: *replication* to provide a measure of random error and sufficient to achieve adequate precision; *randomization* to avoid systematic effects and other biases; and, where appropriate, *blocking* (or local control) to reduce variation among experimental units. (Brien et al. 2011, Section 3.3)

Principle 3 (Minimize variance). Block the entities of an entity-type on the units into groups, to form a new entity-type, if it seems that the entities within the new entity-type will be more homogeneous than if they were ungrouped; assign treatments to the least variable entity-type so that the contribution of other entity-types to the variance of the estimates of treatment effects is reduced as far as is possible. (Brien et al. 2011, Section 3.3)

Principle 4 (Split-units). Confound some treatment sources with unit sources for which greater variation is expected if some treatment factors (i) require larger units than others, (ii) are expected to have a larger effect, or (iii) are of less interest than others. (Brien et al. 2011, Section 3.4)

Principle 5 (Simplicity desirable). Whenever possible, in choosing a design to assign first-phase units to laboratory units, randomize first-phase unit factors that have treatments assigned to them so that sources associated with these factors are confounded with a single laboratory-unit source. (Brien et al. 2011, Section 5)

Principle 6 (Preplan all). If possible, plan all phases of an experiment before commencing it. (Brien et al. 2011, Section 6)

Principle 7 (Allocate all and randomize in laboratory). The laboratory-phase design should *always* allocate *all* the first-phase unit factors, as well as any laboratory treatments, to the laboratory units, using randomization wherever possible. (Brien et al. 2011, Section 6)

Principle 8 (Big with big). Confound big first-phase unit sources that have no treatment sources confounded with them, with potentially big second-phase unit sources. (Brien et al. 2011, Section 6)

Principle 9 (Use pseudofactors). Use pseudofactors to split sources, when necessary, to keep track of all factors in the experiment or to produce structure-balanced designs. (Brien et al. 2011, Section 7.1)

Principle 10 (Compensating across phases). If treatments are confounded with a large source of unit variation in the first phase, then consider confounding this source with a smaller source of variation in the laboratory phase. (Brien et al. 2011, Section 7.2)

Principle 11 (Laboratory replication). Replicated measurement of first-phase units is not required, but is highly desirable when uncontrolled variation in the laboratory phase is large relative to the first phase. It is also needed if the relative magnitudes of field and laboratory variation are to be assessed. (Brien et al. 2011, Section 7.3)

Principle 12 (Laboratory treatments). To minimize the variance of the estimates of laboratory treatment effects, confound them with sources to which only small components of laboratory variation contribute. When also confounding with first-phase unit sources, they too should be as small as possible. (Brien et al. 2011, Section 7.4)

Principle 13 (Design selection attributes). In designing a comparative experiment, consider: the A-optimality, degree of balance and available Residual degrees of freedom of potential designs; whether a resolved design has advantages over a more efficient unresolved design.

Principle 14 (Maximize the degree of balance).

(i) A structure-orthogonal two-phase design is ideal.

(ii) However, if a structure-orthogonal design for a phase is impossible then the next best thing is a nonorthogonal, structure-balanced design. In such a phase, if it includes laboratory replication that requires portions of the products from the previous phase such that the two phases have equal numbers of objects, then omitting the portions in constructing a design for the phase can permit a structure-balanced design. For designs that include portions, first-order balance is acceptable.

(iii) While designs that are at least first-order balanced have desirable properties, unbalanced designs are on occasion appropriate.

Principle 15 (Orthogonal later-phase designs advantageous). If possible, use a structure-orthogonal design for the second, and any subsequent, phase, preferably one that does not require pseudofactors to make it orthogonal.

Appendix V

Laws for designing multiphase experiments

Four laws have been identified (see also `http://chris.brien.name/multitier/MTMphase.html#labprinc`), of which the first was first stated in Brien et al. (2011). These laws relate to the kinds of behaviour that one can expect to encounter in design evaluation.

Multiphase law 1 (DF never increase). The degrees of freedom for sources from a previous phase can never be increased as a result of the design for a subsequent phase. It is possible that the design splits a source from a previous phase into two or more sources, each with fewer degrees of freedom than the original source. (Brien et al. 2011, Section 6)

Multiphase law 2 (Combined structure balance). When the design for a multiphase experiment involves a chain of randomizations and the design for each phase is structure balanced, which includes structure orthogonal, then the complete design is structure balanced. When the combined design is structure balanced, the (canonical) efficiency factors of the component designs multiply so that the efficiency factor for a source is:

For composed randomizations: the product of its efficiency factor with the product of those for sources, from subsequent phases, with which it is confounded.

For randomized-inclusive randomizations: As for composed randomizations, except that the efficiency factors for a component design are those for modified sets of allocated and recipient sources formed from the original sets of sources by including, into the relevant set, sources for appropriate pseudofactors.

(Brien & Bailey 2009, Theorem 5.1(b)). Further, the sum of the efficiency factors for each source that has been allocated is one (Appendix II).

Multiphase law 3 (Equal numbers of objects). In an experiment with three or more tiers, for the combined design to be structure balanced when the numbers of objects for two consecutive tiers are equal then, for the allocated tier of the pair, (i) the combined allocation of any tiers to it must be structure balanced in relation to it and, (ii) its structure, possibly refined, for instance by including pseudofactors, must be structure orthogonal in relation to the recipient tier of the pair. Structure orthogonal or not, for the recipient tier of the pair, there are no Residual sources for its sources and its sources are said to be exhausted by those from the allocated tier. (Brien & Bailey 2009, Lemma 4.2 and Section 6)

Multiphase corollary 3.1 (Structure balance impossible). If the numbers of objects for two consecutive tiers are equal and the structure for the allocated tier, possibly modified, is not structure orthogonal in relation to that of the recipient tier, then the multiphase design using them cannot be structure balanced (Brien & Bailey 2009, Lemma 4.2). It may be first-order balanced.

Multiphase law 4 (Inestimable components). Not all canonical (covariance) components can be estimated when either (i) the numbers of objects for two consecutive tiers are equal, or (ii) the source for the term corresponding to a canonical components is exhausted by sources that are only confounded with it (Bailey & Brien 2016, Section 8.2.1). For canonical-components models, only the sum of the canonical components for the identity terms are estimable when (i) applies.

Supplementary information

Additional supporting information nay be found in the online version of this article at `http://wileyonlinelibrary.com/journal/anzs`.

Supplementary materials A. References.

Supplementary materials B. R script for Example 1, a small wheat experiment with a Youden square design in the lab phas.e

Supplementary materials C. R script for Example 2, a two-phase microarray experiment.

Supplementary materials D. R script for Example 3, a large wheat experiment with noncorresponding field and lab blocks.

Supplementary materials E. R script for Example 4, a small wheat experiment with incomplete-blocks in the lab phase.

Supplementary materials F. R script for Example 5, a beetle damage experiment.

Supplementary materials G. A-optimality in two-phase designs.

Supplementary materials H. Using od to search for A-optimal two-phase designs.

References

BAILEY, R.A. & BRIEN, C.J. (2016). Randomization-based models for multitiered experiments. I. A chain of randomizations. *Annals of Statistics* **44**, 1131–1164.

BAILEY, R.A. & WILLIAMS, E.R. (2007). Optimal nested row-column designs with specified components. *Biometrika* **94**, 459–468.

BRIEN, C.J. (2017). Multiphase experiments in practice: A look back. *Australian & New Zealand Journal of Statistics* **59**, 327–352.

BRIEN, C.J. (2018). *dae: functions useful in the design and ANOVA of experiments.* URL `http://CRAN.R-project.org/package=dae`, (R package version 3.0-23, accessed February 25, 2018).

BRIEN, C.J. & BAILEY, R.A. (2006). Multiple randomizations (with discussion). *Journal of the Royal Statistical Society Series B-Statistical Methodology* **68**, 571–599.

BRIEN, C.J. & BAILEY, R.A. (2009). Decomposition tables for multitiered experiments. I. A chain of randomizations. *Annals of Statistics* **37**, 4184–4213.

BRIEN, C.J., BAILEY, R.A., CORRELL, R.L., HARCH, B.D., PAYNE, R.W. & DEMÉTRIO, C.G.B. (2001–18). Multitiered experiments web site. URL `http://chris.brien.name/multitier/`.

BRIEN, C.J. & DEMÉTRIO, C.G.B. (2009). Formulating mixed models for experiments, including longitudinal experiments. *Journal of Agricultural, Biological, and Environmental Statistics* **14**, 253–280.

BRIEN, C.J., HARCH, B.D., CORRELL, R.L. & BAILEY, R.A. (2011). Multiphase experiments with at least one later laboratory phase. I. Orthogonal designs. *Journal of Agricultural, Biological, and Environmental Statistics* **16**, 422–450.

BUTLER, D.G. (2019). *od: generate optimal experimental designs.* URL `https://mmade.org/`, (R package version 2.0.0, to be made available).

COCHRAN, W.G. & COX, G.M. (1957). *Experimental Designs.* New York: John Wiley & Sons, 2nd edn.

JARRETT, R.G. & RUGGIERO, K. (2008). Design and analysis of two-phase experiments for gene expression microarrays – Part I. *Biometrics* **64**, 208–216.

JOHN, J.A. & WILLIAMS, E.R. (1995). *Cyclic and Computer Generated Designs.* London: Chapman and Hall, 2nd edn.

KIEFER, J. (1975). Construction and optimality of generalized Youden designs. In *A Survey of Statistical Design and Linear Models*, ed. J.N. Srivastava. Amsterdam: North Holland, pp. 333–353. Reprinted in Brown, L. D., Olkin, I., Sacks, J., Wynn, H. P. , editors, (1985) *Jack Carl Kiefer Collected Papers III: Design of Experiments.*, pp. 333–363. Springer-Verlag, New York.

MCINTYRE, G.A. (1955). Design and analysis of two phase experiments. *Biometrics* **11**, 324–334.

PEACOCK, L., HUNTER, T., YAP, M.L. & ARNOLD, G. (2003). Indirect interactions between rust (*Melampsora epitea*) and leaf beetle (*Phratora vulgatissima*) damage on *Salix*. *Phytoparasitica* **31**, 226–235.

R CORE TEAM (2018). *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing. URL `http://www.r-project.org/`, (accessed February 25, 2018).

SHAH, K.R. & SINHA, B.K. (1989). *Theory of optimal designs.* New York: Springer-Verlag.

SMITH, A.B., BUTLER, D.G., CAVANAGH, C.R. & CULLIS, B.R. (2015). Multi-phase variety trials using both composite and individual replicate samples: a model-based design approach. *Journal of Agricultural Science* **153**, 1017–1029.

VSN INTERNATIONAL (2013). *CycDesigN for Windows ver. 5.1*. Hemel Hempstead, UK: VSN International. URL `http://www.vsni.co.uk/software/cycdesign/`, (accessed June 19, 2016).

WHITAKER, D., WILLIAMS, E.R. & JOHN, J.A. (2002). CycDesigN: A package for the computer generation of experimental designs (Version 2.0). CSIRO, Canberra, Australia.