Statistical considerations in the construction of designs for two-colour microarray experiments

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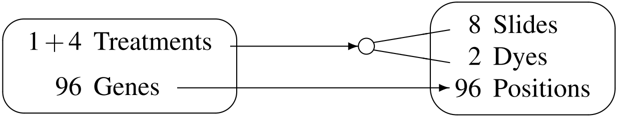
A small microarray experiment



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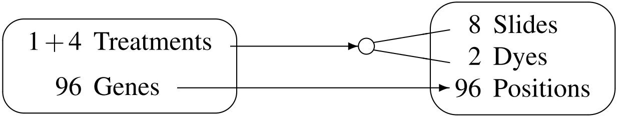

- There is 1 ‘control’ treatment (labelled 0) and 4 other treatments.

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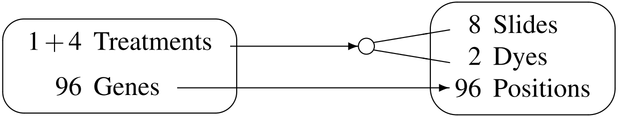

- There is 1 ‘control’ treatment (labelled 0) and 4 other treatments.
- ○ shows that we need to know a specific (non-orthogonal) design for the allocation of the treatments to the dye-slide combinations, such as

	slides							
	1	2	3	4	5	6	7	8
red	0	1	0	2	0	3	0	4
green	1	0	2	0	3	0	4	0

Representation of the design as an oriented graph

Treatments are vertices; slides are edges, oriented from green to red.

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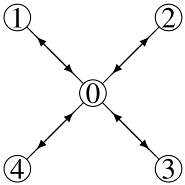

double
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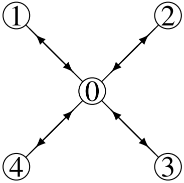

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	1	2	3	4	5	6	7	8
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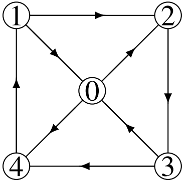
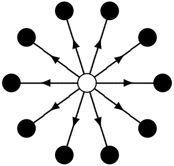


better

Two applications

Large Brazilian forestry experiment (v. Julio Bueno)

10 varieties of tree are being grown in several states for 5 years. It is proposed to compare each with control using a single-reference design with 10 slides. Is this a good use of resources?



Large number of mutations in yeast (Hughes et al., *Cell*, **102**)

300 mutant varieties of yeast were compared with wild-type yeast using a double-reference design with 600 slides. Was that the best use of resources?

Biologists versus mathematicians: Designs for 6 treatments

12 blocks (edges)

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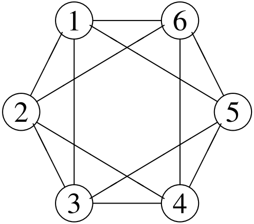

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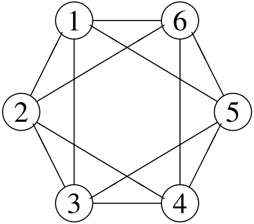

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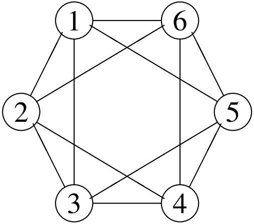

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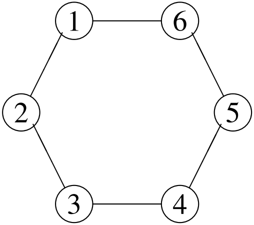

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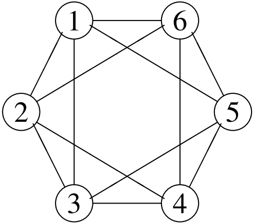

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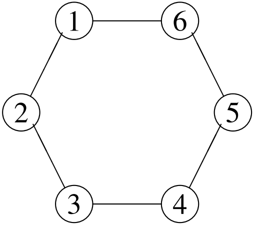

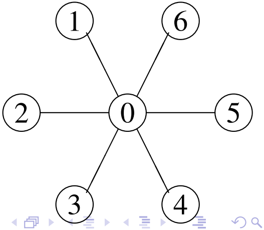

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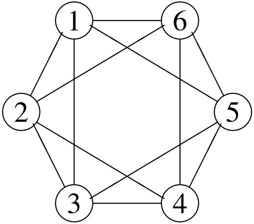

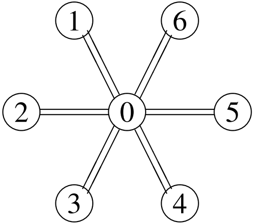

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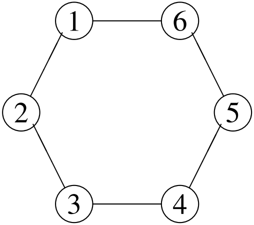

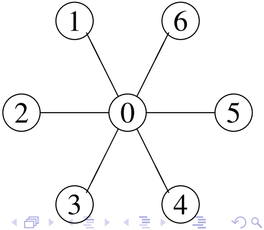

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To estimate all the $\tau_i - \tau_j$, we need $b \geq t - 1$.

Optimality criteria

If there are just 2 treatments, we want V_{12}, the variance of the estimator of $\tau_1 - \tau_2$, to be small and we want the confidence interval I_{12} for $\tau_1 - \tau_2$ to be small.

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In general,	a design is A-optimal if it minimizes the sum of the variances of the estimators of the pairwise differences;	a design is D-optimal if it minimizes the volume of the confidence ellipsoid for the vector $(\tau_1, \ldots, \tau_t)$ subject to $\sum \tau_i = 0$.

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If $t = 2$ then A-optimal = D-optimal.

Temporarily ignore the dyes

We will come back to them later.

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- Designs which are good on the A-criterion are also good on the D-criterion …

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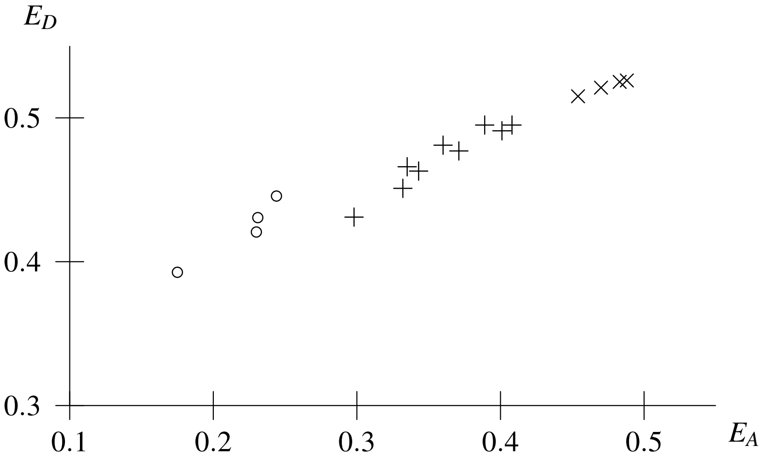
- Designs which are good on the A-criterion are also good on the D-criterion …
- … and vice versa.
- The best designs have equal replication.
- The best designs are symmetric.
- V_{ij}, the variance of the estimator of $\tau_i - \tau_j$, is usually smaller if the distance between vertices i and j in the graph is smaller.

Typical behaviour of the optimality criteria



Optimality criteria for all connected equireplicate designs with 8 treatments in 12 blocks of size 2: graphs with edge-connectivity 3, 2, 1 are shown as $\times$, $+$, $\circ$ respectively

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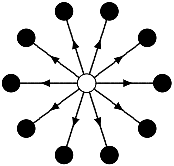

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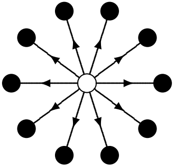

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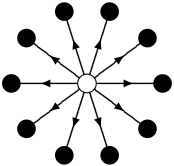

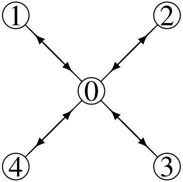

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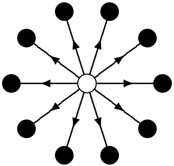

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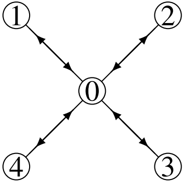

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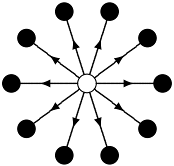

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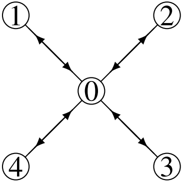

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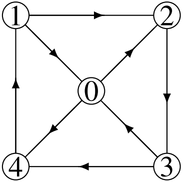

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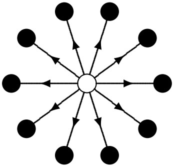

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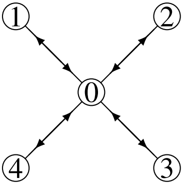

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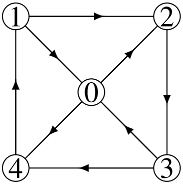


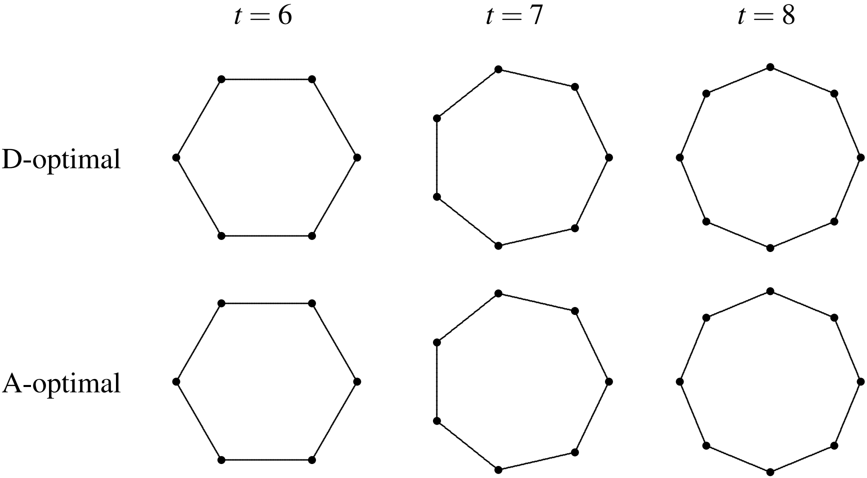
3

What happens when $b = t$?

Computer investigation by

- Jones and Eccleston (1980)
- Kerr and Churchill (2001)
- Wit, Nobile and Khanin (2005)
- Ceraudo (2005).

Optimal designs when $b = t$



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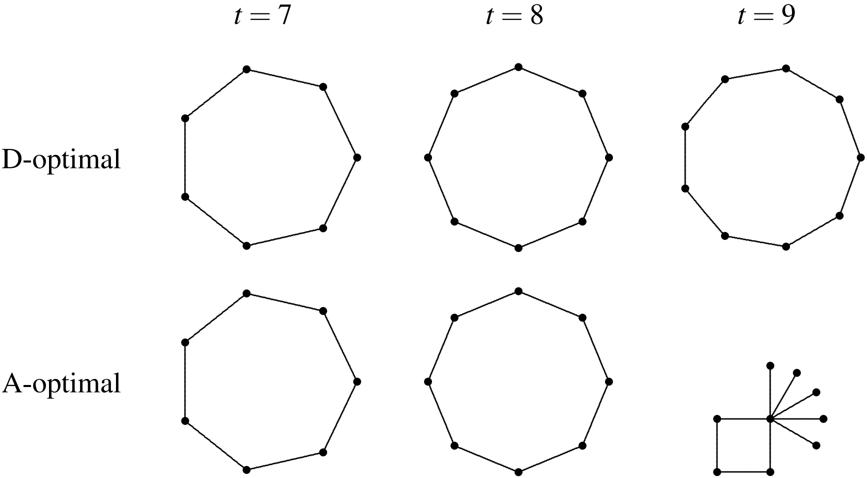

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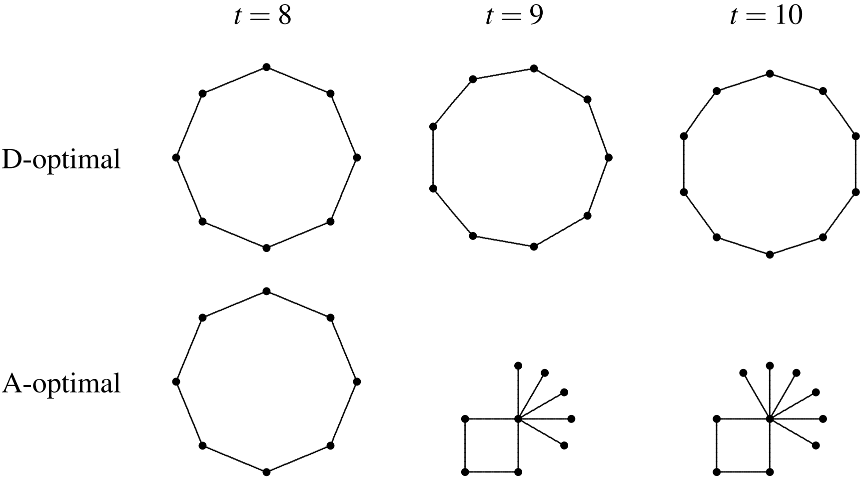

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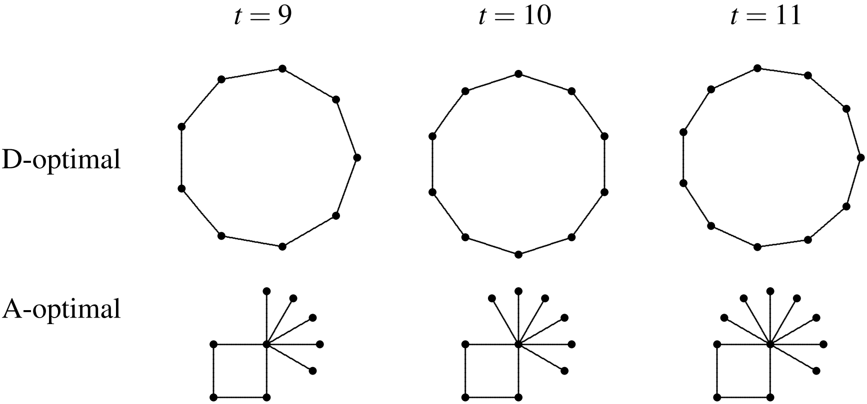

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$$E_D = \frac{(t \times \text{number of spanning trees})^{1/(t-1)}}{2\bar{r}}$$

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number of ways of removing $b - t + 1$ edges without disconnecting the graph, (which is easy to calculate by hand when $b - t$ is small)

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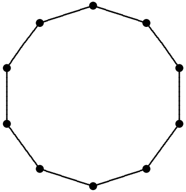
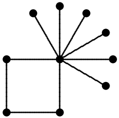


10 spanning trees



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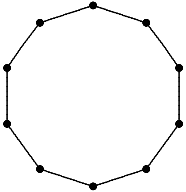

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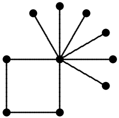

4 spanning trees

The loop design is uniquely D-optimal when $b = t$.

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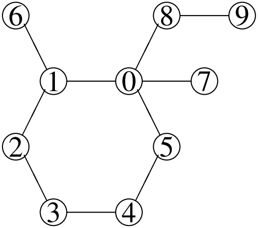
If $b = t$, the graph contains a single circuit.



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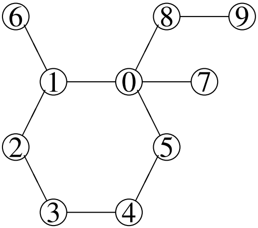

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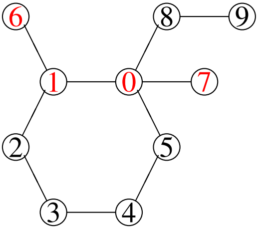

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$$V_{67} = V_{61} + V_{10} + V_{07} = V_{10} + 4\sigma^2$$

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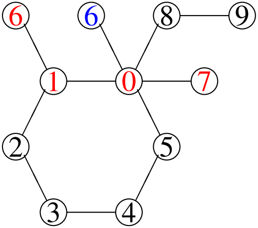

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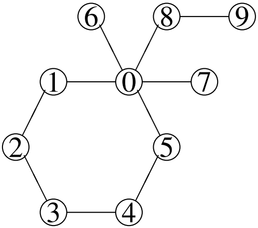

$$V_{67} = V_{60} + V_{07} = 4\sigma^2$$

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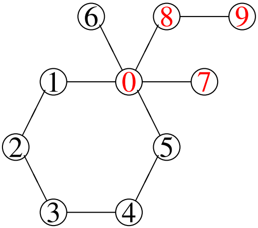

$$V_{97} = V_{98} + V_{80} + V_{07} = V_{80} + 4\sigma^2$$

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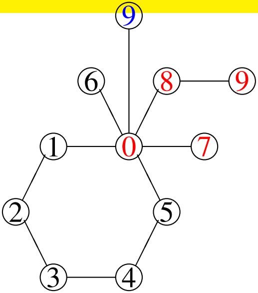

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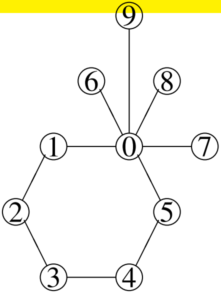


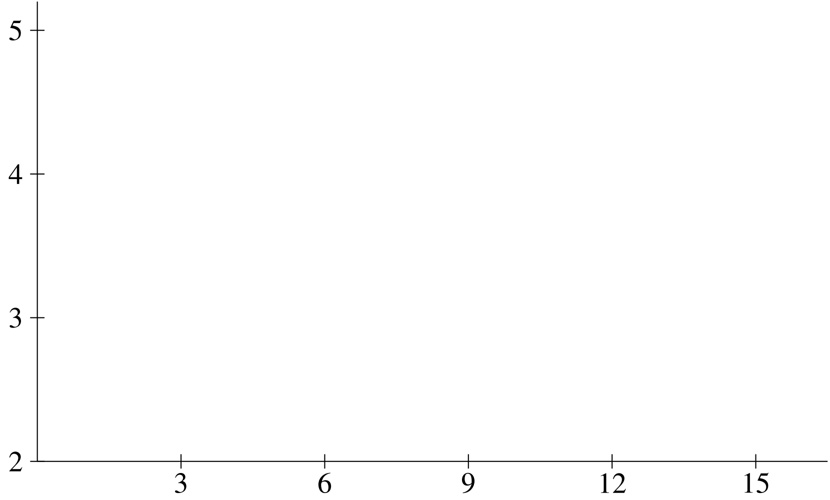
If $b = t$, the graph contains a single circuit.

Let $V_{ij} =$ variance of estimator of $\tau_i - \tau_j$.

For a given size of circuit, the total variance is minimized when everything outside the circuit is attached to the same vertex of the circuit.

A-optimality: continued

Average pairwise variance is a cubic function of the size of the circuit.



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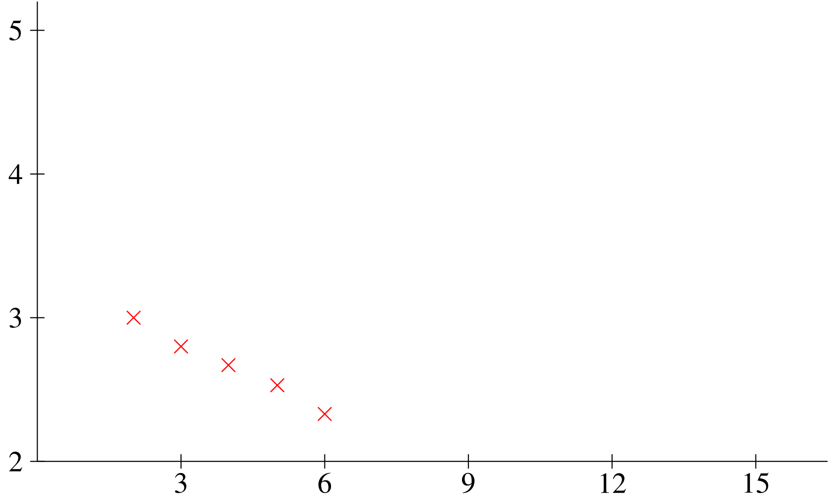

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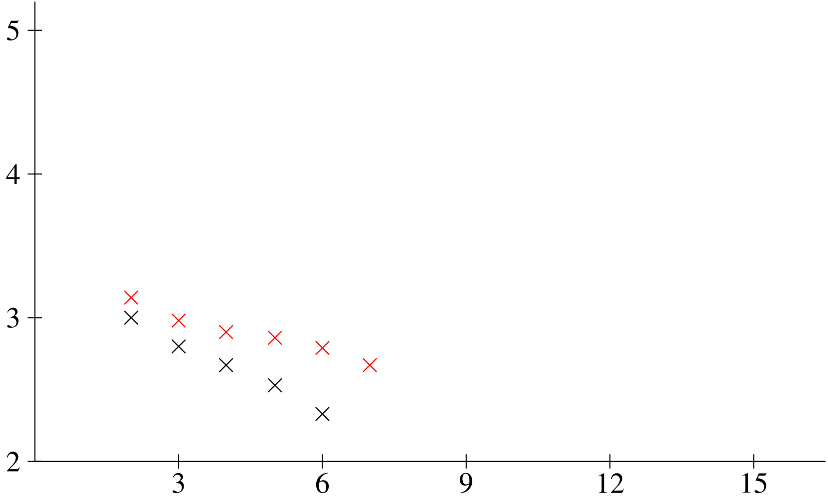

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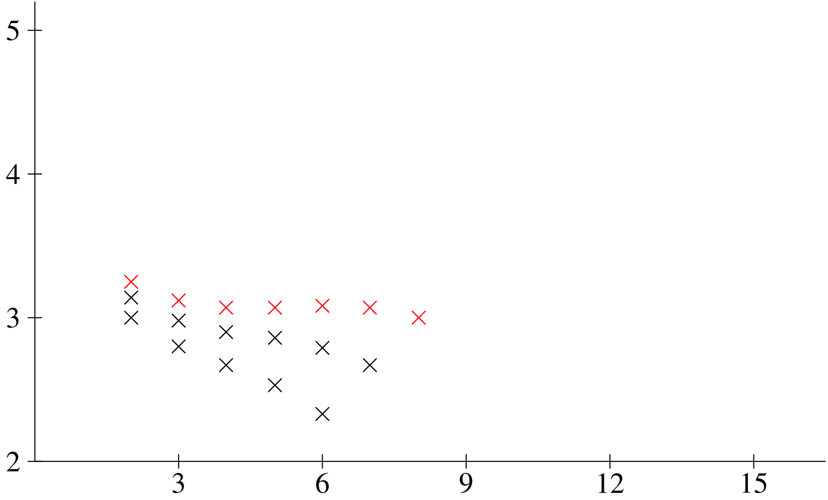

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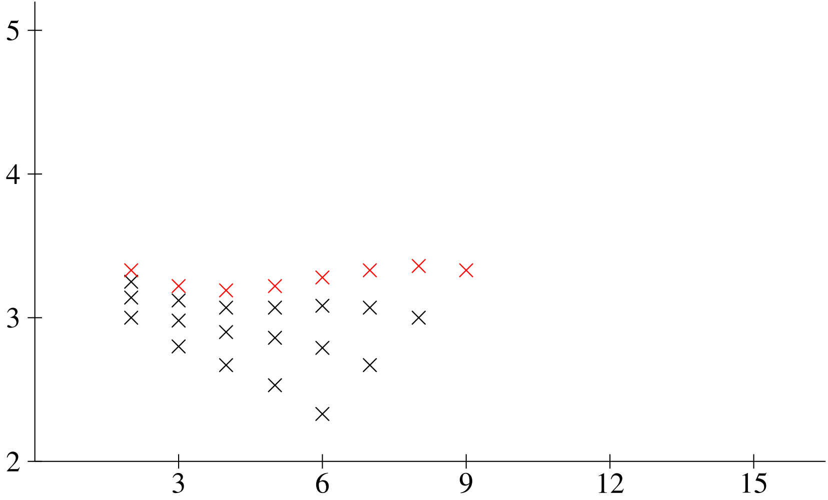

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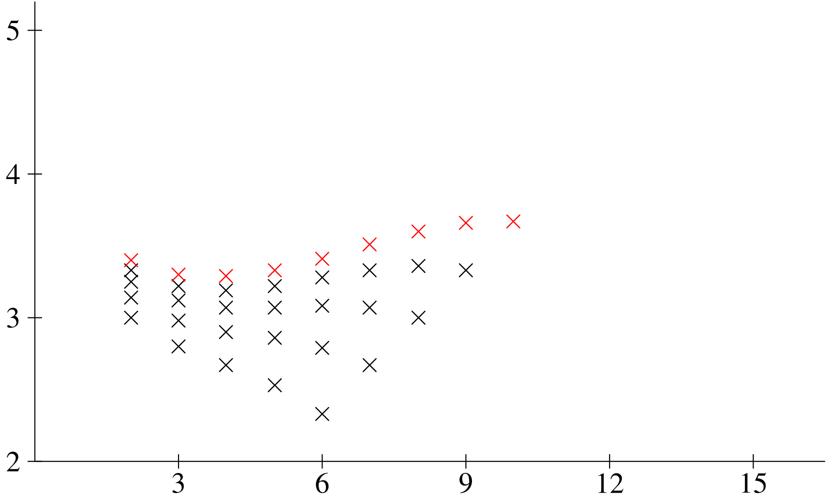

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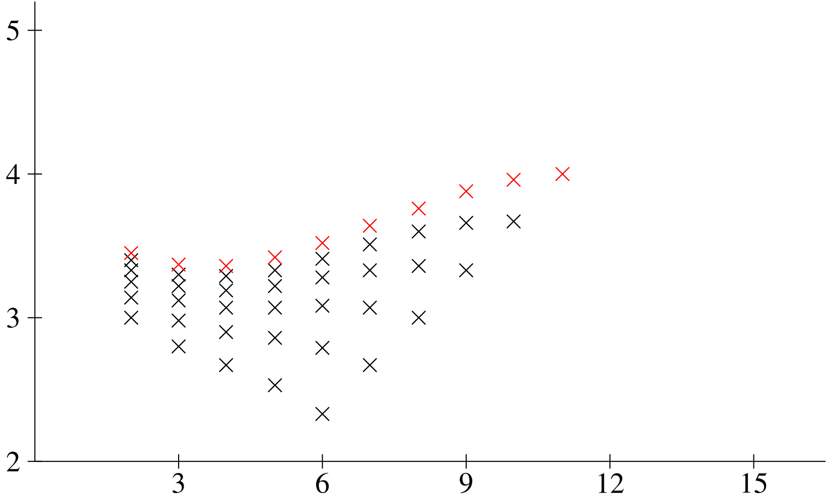

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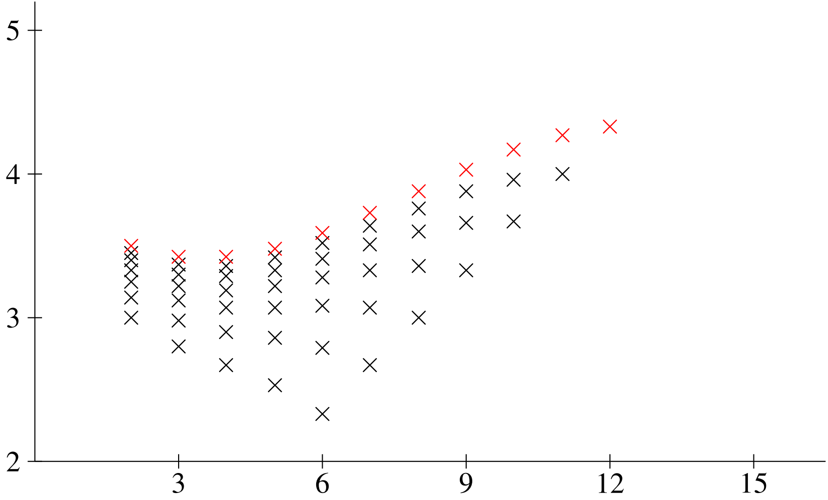

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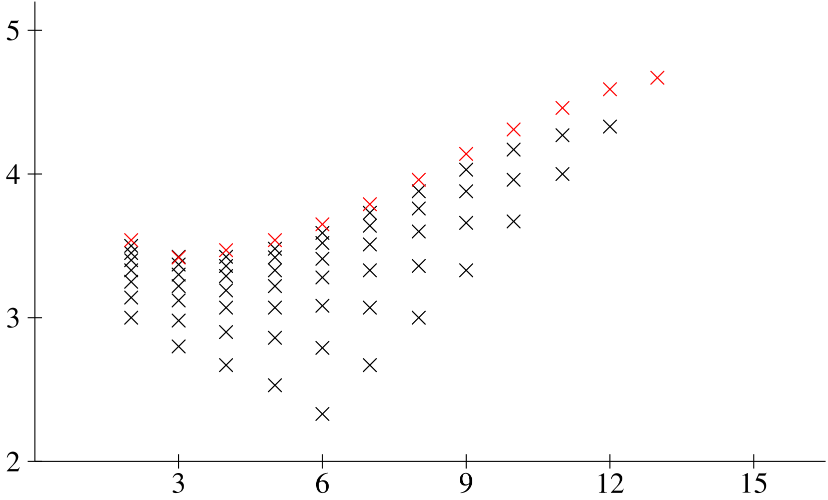

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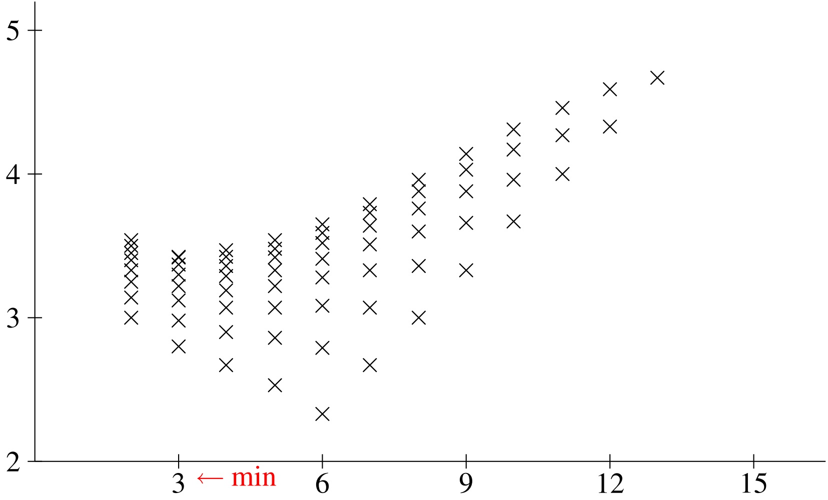
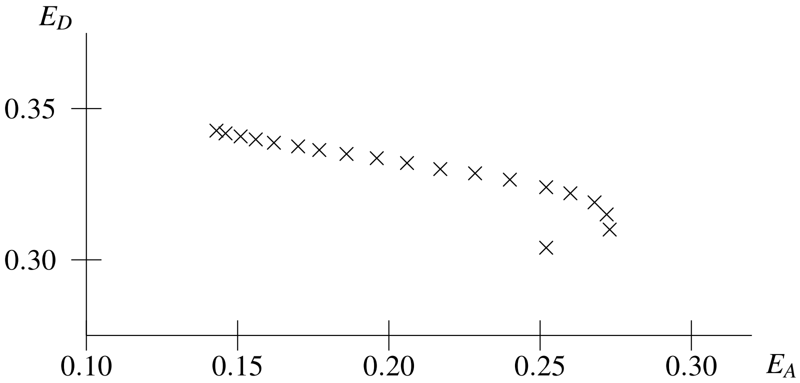


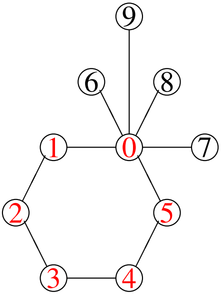
Optimality criteria for designs for 20 treatments in 20 blocks



The two criteria give essentially reverse rankings.

Assigning colours to a circuit with leaves

The difference between the colours can be estimated only from the circuit.



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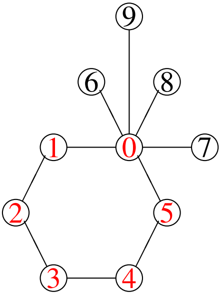

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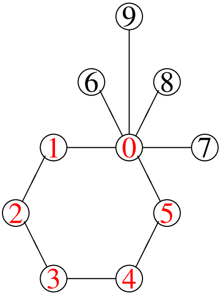

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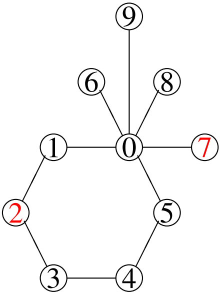

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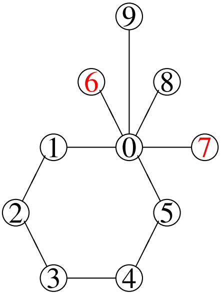

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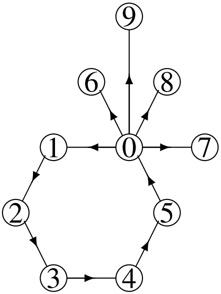
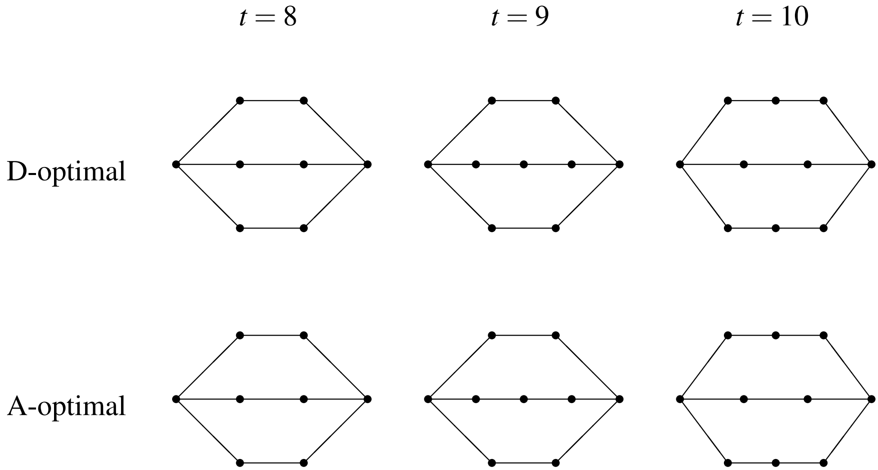


What happens when $b = t + 1$?

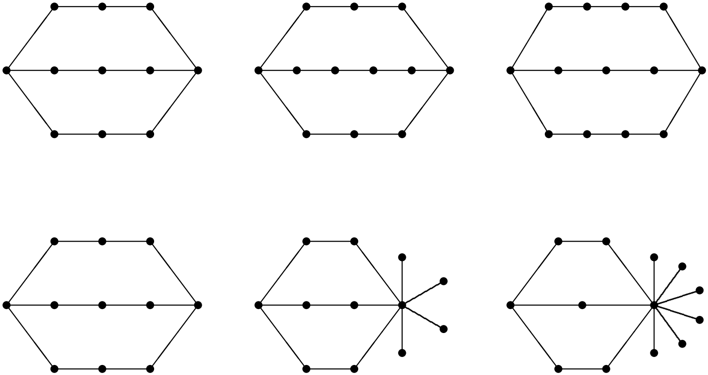
A similar analysis shows that the A-optimality and D-optimality criteria conflict when $t \geq 12$.

Optimal designs when $b = t + 1$



Optimal designs when $b = t + 1$

	$t = 11$	$t = 12$	$t = 13$
D-optimal			
A-optimal			



What happens for larger values of $b - t$?

Bad news theorem

Given any fixed value of $b - t$, there is a threshold T such that when $t \geq T$ the A- and D-optimality criteria conflict.

In fact, when $t \geq T$, the A-better designs have many vertices of valency 1 (leaves) attached to single vertex of some small graph, whereas the D-better designs have no leaves.

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Good news theorem

Inserting 1 or 2 (or sometimes 3) vertices into the edges of a graph with no leaves gives a lower average pairwise variance than attaching the extra vertices to a single vertex of that graph.

Strategy for choosing a design when $b \geq 9t/8$

1. Choose the best equireplicate design with replication 3 (or with replication 4), including dye allocation.
2. Insert up to 2 treatments in each edge.

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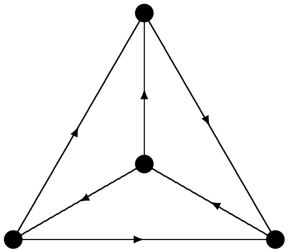

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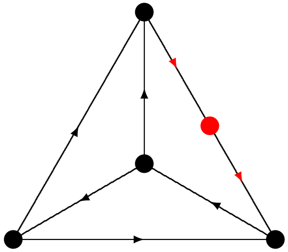

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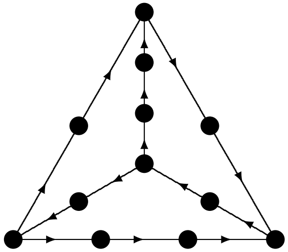

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1. Ignore the colours.
2. Find the best graph with all vertices having valency 4 (smaller problem, can use symmetry to speed up the search).
3. Euler's Theorem (for bridges of Königsberg) says that the arrows can be put on the edges in such a way that every vertex has two edges coming in and two edges going out.

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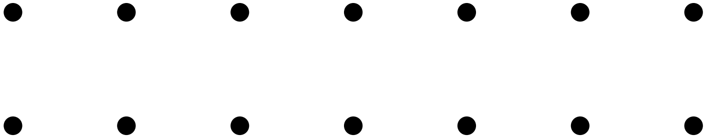
4. Using the algorithm from Hall's Marriage Theorem,
   (also König's Theorem)
   orient the edges so that
   each lower vertex has 2 out-edges and 1 in-edge and
   each upper vertex has 1 out-edge and 2 in-edges.

Example for 14 treatments with replication 3



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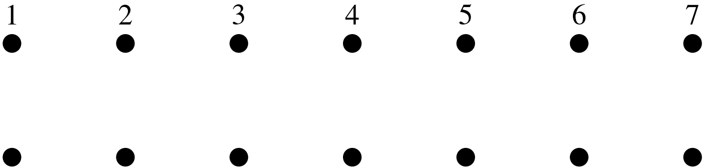

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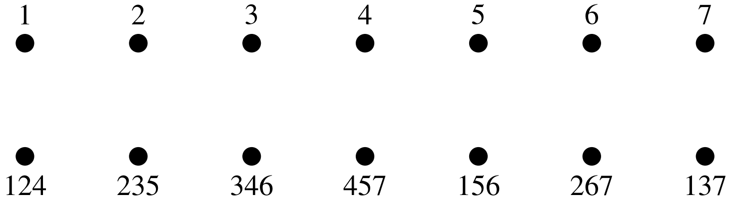

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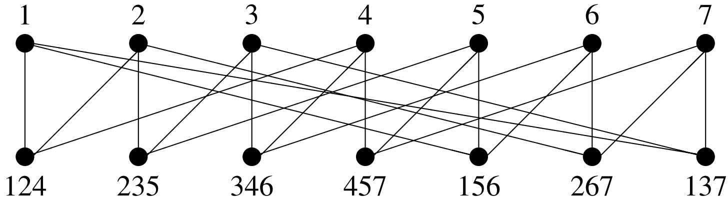

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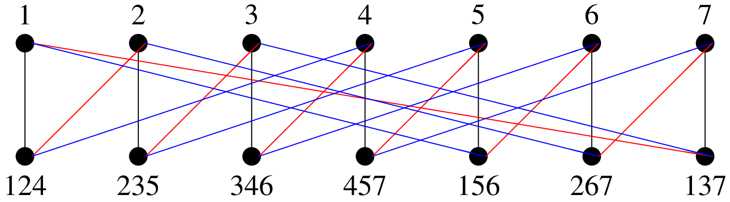

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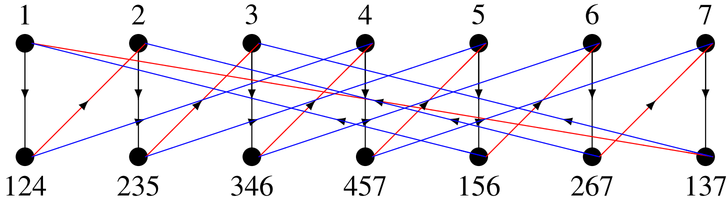

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4. Glue many triangles to a single vertex of some small graph.
   - Needs $b \approx 3t/2$.
   - Few spanning trees, but no pairwise variance is bigger than $2.67\sigma^2$.