

General Two-way Block Design: GTWBD

ST-302: Design, Planning and Analysis of Experiments

Outline of the Topic

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Linear Model

The model for GTWBD with v treatments and b blocks is

$$Y_{ijk} = \mu + \tau_i + \beta_j + \epsilon_{ijk};$$

$$i = 1, 2, \dots, v,$$

$$j = 1, 2, \dots, b,$$

$$k = 1, 2, \dots, n_{ij}$$

- v =number of treatments
- b =number of blocks
- n_{ij} is non-negative integer representing number of times i^{th} treatment appears in j^{th} block

Assumptions about the Linear Model

- Y_{ijk} is observed response from k^{th} experimental unit receiving i^{th} treatment in j^{th} block
- μ is the common effect (Effect without any treatment)
- τ_i is the effect of i^{th} treatment
- β_j is the effect of j^{th} block
- n_{ij} Number of times i^{th} treatment is replicated in j^{th} block and $n_{ij} \geq 0$
- ϵ_{ijk} is the random error associated with Y_{ijk}
- $\epsilon \sim N_n(\mathbf{0}, \sigma^2 \mathbf{I}_n)$, where $\sigma^2 > 0$ is an unknown constant.

Some notations

- $\sum_{i=1}^v \sum_{j=1}^b n_{ij} = n_{..}$ = Total Number of observations
- $\sum_{j=1}^b n_{ij} = n_{i.} = r_i$ = Replication of i^{th} treatment, $i = 1, 2, \dots, v$
- $\sum_{i=1}^v n_{ij} = n_{.j} = k_j$ = Size of j^{th} Block, $j = 1, 2, \dots, b$
- $\sum_{i=1}^v r_i = \sum_{j=1}^b k_j = n$
- $N = v \times b$ Incidence matrix of integers

$$N = \begin{pmatrix} n_{11} & n_{12} & \cdots & n_{1b} \\ n_{21} & n_{22} & \cdots & n_{2b} \\ \vdots & \vdots & \ddots & \vdots \\ n_{v1} & n_{v2} & \cdots & n_{vb} \end{pmatrix}$$

Some notations...

- $\sum_{i=1}^v \sum_{j=1}^b \sum_{k=1}^{n_{ij}} Y_{ijk} = G$ = Grand total
- $\sum_{j=1}^b \sum_{k=1}^{n_{ij}} Y_{ijk} = T_i = i^{th}$ Treatment total, $i = 1, 2, \dots, v$
- $\sum_{i=1}^v \sum_{k=1}^{n_{ij}} Y_{ijk} = B_j = j^{th}$ Block total, $j = 1, 2, \dots, b$
- $\sum_{i=1}^v T_i = \sum_{j=1}^b B_j = G$

Some notations...

- Vector of replications

$$\underline{r} = (r_1 \quad r_2 \quad \cdots \quad r_v)'$$

- Vector of block sizes

$$\underline{k} = (k_1 \quad k_2 \quad \cdots \quad k_b)'$$

- $R = diag(r_1, r_2, \dots, r_v) : v \times v$ diagonal matrix
- $K = diag(k_1, k_2, \dots, k_b) : b \times b$ diagonal matrix

Some notations...

- Vector of Treatment Totals

$$\underline{T} = (T_1 \quad T_2 \quad \cdots \quad T_v)'$$

- Vector of block totals

$$\underline{B} = (B_1 \quad B_2 \quad \cdots \quad B_b)'$$

- $E_{vb} = v \times b$ matrix with all elements one

Some Interrelations

- $\underline{T}'E_{v1} = G$: Sum of treatment totals is grand total
- $\underline{B}'E_{b1} = G$: Sum of block totals is grand total
- $\underline{Y}'E_{n1} = G$: Sum of all observations is grand total
- $NE_{b1} = \underline{r}$: Row sums of N-matrix are replications of treatments
- $N'E_{v1} = \underline{k}$: Column sums of N-matrix are Block sizes
- $E_{1v}NE_{b1} = \sum_{i=1}^v \sum_{j=1}^b n_{ij} = n$
- $\underline{r}'E_{v1} = n$
- $\underline{k}'E_{b1} = n$

Example

Consider a GTWBD with $v = 4$ treatments and $b = 5$ blocks as follows.

B_1	B_2	B_3	B_4	B_5
A	A	C	C	C
A	B	D	D	D
B	B			

Here the incidence matrix is as follows.

$$N = \begin{pmatrix} 2 & 1 & 0 & 0 & 0 \\ 1 & 2 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 1 \end{pmatrix}$$

Further $(r_1, r_2, r_3, r_4) = (3, 3, 3, 3)$ and
 $(k_1, k_2, k_3, k_4, k_5) = (3, 3, 2, 2, 2)$

Example...

Then the model for this GTWBD is

$$Y_{ijk} = \mu + \tau_i + \beta_j + \epsilon_{ijk}; i = 1 : 4; j = 1 : 5, k = 1, : n_{ij}, n_{ij} \in N$$

This GTWBD with $n = 12$, we can express in GLM setup with following quantities:

- \underline{Y} : 12×1 vector of known observations
- $\underline{\beta}$: $(1 + v + b) \times 1$ vector of unknown parameters
- Δ : $n \times v$ design matrix of treatments
- D : $n \times b$ design matrix of blocks

Example...

This GTWBD can be expressed in the GLM setup as follows.

$$\begin{pmatrix} Y_{111} \\ Y_{112} \\ Y_{121} \\ \hline Y_{211} \\ Y_{221} \\ Y_{222} \\ \hline Y_{331} \\ Y_{341} \\ Y_{345} \\ \hline Y_{431} \\ Y_{441} \\ Y_{451} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 & | & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & | & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & | & 0 & 1 & 0 & 0 & 0 \\ \hline 1 & 0 & 1 & 0 & 0 & | & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & | & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & | & 0 & 1 & 0 & 0 & 0 \\ \hline 1 & 0 & 0 & 1 & 0 & | & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & | & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 & | & 0 & 0 & 0 & 0 & 1 \\ \hline 1 & 0 & 0 & 0 & 1 & | & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 1 & | & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 & | & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} \mu \\ \tau_1 \\ \tau_2 \\ \tau_3 \\ \tau_4 \\ \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_4 \\ \beta_5 \end{pmatrix} + \begin{pmatrix} \varepsilon_{111} \\ \varepsilon_{112} \\ \varepsilon_{121} \\ \varepsilon_{211} \\ \varepsilon_{221} \\ \varepsilon_{222} \\ \varepsilon_{331} \\ \varepsilon_{341} \\ \varepsilon_{345} \\ \varepsilon_{431} \\ \varepsilon_{441} \\ \varepsilon_{451} \end{pmatrix}$$

Example...

Observations for design matrices:

- Design matrix (DM) has three parts: E_{n1}, Δ and D
- All entries of DM are values of indicator variables
- Column sums of Δ -matrix are replications of treatments
- Column sums of D -matrix are block sizes
- Inner product of Y and first column of DM =G
- Inner product of Y and columns of Δ =respective treatment totals T_1, T_2, T_3, T_4
- Inner product of Y and columns of D =respective block totals B_1, B_2, B_3, B_4, B_5
- Inner product of i^{th} column of Δ and j^{th} column of $D=n_{ij}$

Some relations:

Observations for design matrices:

- Column sums of Δ : $\Delta'E_{n1} = \underline{r}$
- Column sums of D : $D'E_{n1} = \underline{k}$
- $\Delta'D = N$
- $\Delta'\Delta = R$
- $D'D = K$
- $\underline{Y}'E_{n1} = G$
- $\Delta'\underline{Y} = \underline{T}$
- $D'\underline{Y} = \underline{B}$

GTWBD in GLM setup:

A GTWBD with v treatments and b blocks has model:

$$\underline{Y} = \mu E_{n1} + \Delta \underline{\tau} + D \underline{\beta} + \underline{\varepsilon}$$

where

- \underline{Y} : $n \times 1$ vector of known observations
- $\underline{\tau}$: $v \times 1$ vector of unknown treatment parameters
- $\underline{\beta}$: $b \times 1$ vector of unknown block parameters
- Δ : $n \times v$ design matrix corresponding to treatments
- D : $n \times b$ design matrix corresponding to blocks
- $\underline{\varepsilon}$: $n \times 1$ vector of random errors

Normal equations of GTWBD in GLM setup:

The GLM for GTWBD with v treatments and b blocks can be written as:

$$\underline{Y} = \begin{pmatrix} E_{n1} & \Delta & D \end{pmatrix} \begin{pmatrix} \mu \\ \tau \\ \beta \end{pmatrix} + \underline{\varepsilon}$$

Normal equations can be obtained as:

$$(\begin{pmatrix} E_{n1} & \Delta & D \end{pmatrix}') \underline{Y} = (\begin{pmatrix} E_{n1} & \Delta & D \end{pmatrix})' (\begin{pmatrix} E_{n1} & \Delta & D \end{pmatrix}) \begin{pmatrix} \hat{\mu} \\ \hat{\tau} \\ \hat{\beta} \end{pmatrix}$$

Normal equations of GTWBD ...

Normal equations can be obtained as,

$$\begin{pmatrix} E_{n1} \\ \Delta' \\ D' \end{pmatrix} \underline{Y} = \begin{pmatrix} E_{n1} \\ \Delta' \\ D' \end{pmatrix} \begin{pmatrix} E_{n1} & \Delta & D \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\tau} \\ \hat{\beta} \end{pmatrix}$$

Simplifying we get,

$$\begin{pmatrix} E_{n1}\underline{Y} \\ \Delta'\underline{Y} \\ D'\underline{Y} \end{pmatrix} = \begin{pmatrix} E_{n1}E_{1n} & E_{n1}\Delta & E_{n1}D \\ \Delta'E_{n1} & \Delta'\Delta & \Delta'D \\ D'E_{n1} & D'\Delta & D'D \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\tau} \\ \hat{\beta} \end{pmatrix}$$

Using the interrelations,

$$\begin{pmatrix} G \\ T \\ B \end{pmatrix} = \begin{pmatrix} n & \underline{r}' & \underline{k}' \\ \underline{r} & R & N \\ \underline{k} & N' & K \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\tau} \\ \hat{\beta} \end{pmatrix}$$

Characterizations of Normal equations:

The following are $(1 + v + b)$ equations.

$$\begin{pmatrix} G \\ \underline{T} \\ \underline{B} \end{pmatrix} = \begin{pmatrix} n & \underline{r}' & \underline{k}' \\ \underline{r} & R & N \\ \underline{k} & N' & K \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\tau} \\ \hat{\beta} \end{pmatrix}$$

But all are not linearly independent.

$$G = n\hat{\mu} + \underline{r}'\hat{\tau} + \underline{k}'\hat{\beta}$$

$$\underline{T} = \underline{r}\hat{\mu} + R\hat{\tau} + N\hat{\beta}$$

$$\underline{B} = \underline{k}\hat{\mu} + N'\hat{\tau} + K\hat{\beta}$$

Characterizations of Normal equations...

To see the linear dependence of equations we express them in the following form.

$$G = n\hat{\mu} + \sum_{i=1}^v r_i \hat{\tau}_i + \sum_{j=1}^b k_j \hat{\beta}_j$$

$$T_i = r_i \hat{\mu} + r_i \hat{\tau}_i + \sum_{j=1}^b n_{ij} \hat{\beta}_j; i = 1, 2, \dots, v$$

$$B_j = k_j \hat{\mu} + \sum_{i=1}^v n_{ij} \hat{\tau}_i + k_j \hat{\beta}_j; j = 1, 2, \dots, b$$

Observe that:

- Adding equations in A we get first equation.
- Adding equations in B we get first equation.
- Number of linearly independent equations are less than $(1 + b + v) - 2$.

Reduced normal equations...

We eliminate $\hat{\beta}$ to obtain normal equations in terms of $\hat{\tau}$

$$\hat{\underline{\beta}} = K^{-1} (\underline{B} - \underline{k}\hat{\mu} - N'\hat{\underline{\tau}})$$

Substituting in the following equation,

$$\begin{aligned}\underline{T} &= \underline{r}\hat{\mu} + R\hat{\underline{\tau}} + N\hat{\underline{\beta}} \\ &= \underline{r}\hat{\mu} + R\hat{\underline{\tau}} + NK^{-1} (\underline{B} - \underline{k}\hat{\mu} - N'\hat{\underline{\tau}}) \\ &= \underline{r}\hat{\mu} + R\hat{\underline{\tau}} + NK^{-1}\underline{B} - NK^{-1}\underline{k}\hat{\mu} - NK^{-1}N'\hat{\underline{\tau}} \\ &= \underline{r}\hat{\mu} + R\hat{\underline{\tau}} + NK^{-1}\underline{B} - \underline{r}\hat{\mu} - NK^{-1}N'\hat{\underline{\tau}}\end{aligned}$$

$$\underline{T} - NK^{-1}\underline{B} = R\hat{\underline{\tau}} - NK^{-1}N'\hat{\underline{\tau}}$$

$$\underline{T} - NK^{-1}\underline{B} = (R - NK^{-1}N')\hat{\underline{\tau}}$$

$$\underline{Q} = C\hat{\underline{\tau}} \quad \text{where } \underline{Q} = \underline{T} - NK^{-1}\underline{B}, C = (R - NK^{-1}N')$$

Reduced Normal Equations...

T

The reduced normal equations for treatments are:

$$\underline{Q} = \underline{C}\hat{\tau}$$

where $\underline{Q} = \underline{T} - NK^{-1}\underline{B}$ and $\underline{C} = (R - NK^{-1}N')$

- \underline{Q} is $v \times 1$ vector and is called vector of treatment totals adjusted for blocks.
- \underline{C} is $v \times v$ symmetric matrix
- \underline{Q} is also called LHS of Reduced Normal Equations
- The difference between the two treatment totals:
 \underline{T} is vector of treatment totals (unadjusted)
 \underline{Q} is vector of adjusted (for blocks) treatment totals

Characterizations of Reduced Normal Equations...

- ① C is a symmetric matrix.

$$\begin{aligned}C' &= (R - NK^{-1}N')' \\&= (R - NK^{-1}N') \\&= C\end{aligned}$$

Characterizations of Reduced Normal Equations...

- ① C is a singular matrix.

$$\begin{aligned}
 CE_{v1} &= (R - NK^{-1}N') E_{v1} \\
 &= RE_{v1} - NK^{-1}N'E_{v1} \\
 &= \underline{r} - NK^{-1}\underline{k} as N'E_{v1} = \underline{k} \\
 &= \underline{r} - NE_{b1} as K^{-1}\underline{k} = E_{b1} \\
 &= \underline{r} - \underline{r} as NE_{b1} = \underline{r} \\
 &= \underline{0}
 \end{aligned}$$

Thus $CE_{v1} = \underline{0}$ interprets that all row sums of C-matrix are zero.

Or sum of all columns of C-matrix is $\underline{0}$ and all columns are not linearly independent.

Hence $\text{rank}(C) < v$

That is C is symmetric singular matrix.

Characterizations of Reduced Normal Equations...

$$\textcircled{1} \quad E(\underline{Q}) = C\underline{\tau}$$

$$\begin{aligned}
 E(\underline{Q}) &= E(\underline{T} - NK^{-1}\underline{B}) \\
 &= E(\Delta'\underline{Y} - NK^{-1}D'\underline{Y}) \\
 &= (\Delta' - NK^{-1}D') E(\underline{Y}) \\
 &= (\Delta' - NK^{-1}D') E(\mu E_{n1} + \Delta\underline{\tau} + D\underline{\beta} + \underline{\varepsilon}) \\
 &= (\Delta' - NK^{-1}D') (\mu E_{n1} + \Delta\underline{\tau} + D\underline{\beta}) \\
 &= \Delta' (\mu E_{n1} + \Delta\underline{\tau} + D\underline{\beta}) - NK^{-1}D' (\mu E_{n1} + \Delta\underline{\tau} + D\underline{\beta}) \\
 &= \mu \Delta' E_{n1} + \Delta' \Delta\underline{\tau} + \Delta' D\underline{\beta} \\
 &\quad - NK^{-1}D' \mu E_{n1} + -NK^{-1}D' \Delta\underline{\tau} - NK^{-1}D' D\underline{\beta} \\
 &= \mu \underline{r} + R\underline{\tau} + N\underline{\beta} - \underline{r}\mu - NK^{-1}N'\underline{\tau} - N\underline{\beta} \\
 &= (R - NK^{-1}N') \underline{\tau} \\
 &= C\underline{\tau}
 \end{aligned}$$

Characterizations of Reduced Normal Equations...

- $C_{\underline{\tau}}$ contains v linear parametric functions which are estimable.
 - If $C'_{(i)}$ is the i^{th} row of C then $C'_{(i)}\underline{\tau}, i = 1, 2, \dots, v$ are estimable parametric functions.
 - $C'_{(i)}\underline{\tau}, i = 1, 2, \dots, v$ are not linearly independent estimable parametric functions as $\text{rank}(C) \leq v - 1$
 - $C'_{(i)}\underline{\tau}, i = 1, 2, \dots, v$ are contrast (linear parametric function whose coefficients add to zero) as each row sum of C-matrix is zero.
 - $C_{\underline{\tau}}$ contains v linear estimable parametric functions which are contrast in τ'_i s.
 - If Q_i is the i^{th} element of \underline{Q} then Q_i is BLUE of $C'_{(i)}\underline{\tau}$ or $C'_{(i)}\hat{\underline{\tau}} = Q_i$, for $i = 1, 2, \dots, v$
- ① $\text{rank}(C) \leq v - 1$
As C is $v \times v$ singular matrix, $\text{rank}(C) < v \leq v - 1$

Characterizations of Reduced Normal Equations...

① $E_{1v} \underline{Q} = 0$

$$\begin{aligned} E_{1v} \underline{Q} &= E_{1v} (\underline{T} - NK^{-1} \underline{B}) \\ &= E_{1v} \underline{T} - E_{1v} NK^{-1} \underline{B} \\ &= G - \underline{k}' K^{-1} \underline{B} \\ &= G - E_{1b} \underline{B} \\ &= G - G \\ &= 0 \end{aligned}$$

It is same as $\underline{Q}E_{v1} = 0$

It means sum of elements of vector of adjusted treatment total is 0.

Characterizations of Reduced Normal Equations...

① $Cov(\underline{Q}) = C\sigma^2$

$$Cov(\underline{Q})$$

$$= Cov(\underline{T} - NK^{-1}\underline{B})$$

$$= Cov(\Delta'\underline{Y} - NK^{-1}D'\underline{Y})$$

$$= (\Delta' - NK^{-1}D') Cov(\underline{Y}) (\Delta' - NK^{-1}D')'$$

$$= (\Delta' - NK^{-1}D') \sigma^2 I_n (\Delta - DK^{-1}N')$$

$$= (\Delta' (\Delta - DK^{-1}N') - NK^{-1}D' (\Delta - DK^{-1}N')) \sigma^2$$

$$= (R - NK^{-1}N' - NK^{-1}N' - NK^{-1}KK^{-1}N') \sigma^2$$

$$= (R - NK^{-1}N') \sigma^2$$

$$= C\sigma^2$$

Characterizations of Reduced Normal Equations...

① $\underline{Q} \sim N_v (\underline{C}\underline{\tau}, C\sigma^2)$

$$\begin{aligned}\underline{Q} &= (\underline{T} - NK^{-1}\underline{B}) \\ &= (\Delta'\underline{Y} - NK^{-1}D'\underline{Y}) \\ &= (\Delta' - NK^{-1}D')\underline{Y}\end{aligned}$$

Observe the characterizations of \underline{Q}

- Each element of \underline{Q} is linear combination of \underline{Y} i.e. \underline{Q} is collection of v linear combinations of \underline{Y}
- $\underline{Y} \sim N_n (\underline{0}, \sigma^2 I_n)$ distribution
- $E(\underline{Q}) = \underline{C}\underline{\tau}$ and $Cov(\underline{Q}) = C\sigma^2$
- Hence $\underline{Q} \sim N_v (\underline{C}\underline{\tau}, C\sigma^2)$ distribution.

Characterizations of Reduced Normal Equations...

- ① $\underline{Q} = C\underline{\tau}$ is a consistent system of equations

As the reduced normal equations are obtained by eliminating one set of parameters and original system of equations is consistent hence the set of reduced normal equations is consistent.

Some results:

- ① $Cov(\underline{T}) = R\sigma^2$
- ② $Cov(\underline{B}) = K\sigma^2$
- ③ $Cov(\underline{T}, \underline{B}) = N\sigma^2$
- ④ $Cov(\underline{Q}, \underline{B}) = O$
- ⑤ $Cov(\underline{T}, \underline{P}) = O$
- ⑥ $Cov(\underline{Q}, \underline{P}) = -CR^{-1}N\sigma^2 \text{ or } -NK^{-1}D\sigma^2$

VCM

- $Cov(\underline{T}) = R\sigma^2$

$$Cov(\underline{T})$$

$$= Cov(\Delta' \underline{Y})$$

$$= \Delta' Cov(\underline{Y}) \Delta$$

$$= \Delta' (\sigma^2 I_n) \Delta$$

$$= \Delta' \Delta \sigma^2$$

$$= R\sigma^2$$

$$\therefore Cov(\underline{Y}) = \sigma^2 I_n$$

$$\therefore \Delta' \Delta = R$$

VCM

- $Cov(\underline{B}) = K\sigma^2$

$$\begin{aligned} & Cov(\underline{B}) \\ &= Cov(D'\underline{Y}) \\ &= D'Cov(\underline{Y})D \\ &= D'(\sigma^2 I_n)D \quad \therefore Cov(\underline{Y}) = \sigma^2 I_n \\ &= D'D\sigma^2 \\ &= K\sigma^2 \quad \therefore D'D = K \end{aligned}$$

VCM

- $Cov(\underline{T}, \underline{B}) = N\sigma^2$

$$\begin{aligned} & Cov(\underline{T}, \underline{B}) \\ &= Cov(\Delta' \underline{Y}, D' \underline{Y}) \\ &= \Delta' Cov(\underline{Y}) D \\ &= \Delta' (\sigma^2 I_n) D && \because Cov(\underline{Y}) = \sigma^2 I_n \\ &= \Delta' D \sigma^2 \\ &= N\sigma^2 && \because \Delta' D = N \end{aligned}$$

Independence of adjusted treatment total and unadjusted block totals

- $\text{Cov}(\underline{Q}, \underline{B}) = O$
$$\begin{aligned}\text{Cov}(\underline{Q}, \underline{B}) &= \text{Cov}(\underline{T} - NK^{-1}\underline{B}, \underline{B}) \\ &= \text{Cov}(\Delta'\underline{Y} - NK^{-1}D'\underline{Y}, D'\underline{Y}) \\ &= (\Delta' - NK^{-1}D') \text{Cov}(\underline{Y})(D')' \\ &= (\Delta' - NK^{-1}D') \sigma^2 I_n D \\ &= (\Delta' - NK^{-1}D') D \sigma^2 \\ &= (\Delta' D - NK^{-1}D'D) \sigma^2 \\ &= (N - NK^{-1}K) \sigma^2 \\ &= (N - N) \sigma^2 \\ &= O\end{aligned}$$

Independence between unadjusted treatment total and adjusted block totals

- $Cov(\underline{T}, \underline{P}) = O$

$$\begin{aligned} Cov(\underline{T}, \underline{P}) &= Cov(\underline{T}, \underline{B} - N'R^{-1}\underline{T}) \\ &= Cov(\Delta'\underline{Y}, D'\underline{Y} - N'R^{-1}\Delta'\underline{Y}) \\ &= Cov(\Delta'\underline{Y}, (D' - N'R^{-1}\Delta')\underline{Y}) \\ &= (\Delta')Cov(\underline{Y})(D' - N'R^{-1}\Delta')' \\ &= (\Delta')(D - \Delta R^{-1}N)\sigma^2 \\ &= (\Delta'D - \Delta'\Delta R^{-1}N)\sigma^2 \\ &= (N - RR^{-1}N)\sigma^2 \\ &= (N - N)\sigma^2 \\ &= O \end{aligned}$$