18

ANOVA

ONE-WAY ANOVA

The traditional approach to analyzing half-sib data is the **one-way analysis of variance**, based on the linear model

$$z_{ij} = \mu + s_i + e_{ij} \tag{18.1}$$

where z_{ij} is the phenotype of the *j*th offspring of the *i*th father, s_i is the effect of the *i*th father (the **sire effect**), and e_{ij} is the residual error resulting from segregation, dominance, genetic variance among mothers, and environmental variance. Stated another way, e_{ij} is the deviation of the phenotype of the *ij*th individual from the expected value for the *i*th family. As deviations from the linear model, the e_{ij} have expectations equal to zero. We further assume that the e_{ij} are uncorrelated with each other and have common variance σ_e^2, the **within-family variance**. The N sires are assumed to be a random sample of the entire population so that $E(s_i) = 0$. The variance among sire effects (the **among-family variance**) is denoted by σ_s^2.

A basic assumption of linear models underlying ANOVA is that the random factors are uncorrelated with each other. As first recognized by Fisher in his classical 1918 paper, this leads to a key feature — *the analysis of variance partitions the total phenotypic variance into the sum of the variances from each of the contributing factors.* For example, for the half-sib model, the critical assumption is that the residual deviations are uncorrelated with the sire effects, i.e., $\sigma(s_i, e_{ij}) = E(s_i e_{ij}) = 0$. Thus, the total phenotypic variance equals the variance due to sires plus the residual variance,

$$\sigma_z^2 = \sigma_s^2 + \sigma_e^2 \tag{18.2}$$

A second relationship that proves to be very useful is that *the phenotypic covariance between members of the same group equals the variance among groups.* For the model given in Equation 18.1, this can be shown quite simply. Members of the same group (paternal half sibs) share sire effects, but have independent residual deviations, so

$$
\begin{aligned}
\sigma(\text{PHS}) &= \sigma(\,z_{ij}, z_{ik}\,) \\
&= \sigma[\,(\mu + s_i + e_{ij}), (\mu + s_i + e_{ik})\,] \\
&= \sigma(\,s_i, s_i\,) + \sigma(\,s_i, e_{ik}\,) + \sigma(\,e_{ij}, s_i\,) + \sigma(\,e_{ij}, e_{ik}\,) \\
&= \sigma_s^2 \tag{18.3}
\end{aligned}
$$

Thus, the covariance between paternal half sibs equals the variance among sire effects. This is a particularly useful identity since, as we will see below, ANOVA provides a simple means of estimating σ_s^2.

The pure half-sib design employs the simplest possible linear model. However, the general logic just outlined applies to the estimation of variance components in all linear models, including those employed in subsequent chapters. Thus, the steps we have just taken are worth summarizing. First, the linear model is written down. Second, with the assumptions of the model made explicit, an expression for the total phenotypic variance is written in terms of components. Third, the components of variance associated with the model are expressed as covariances between specific classes of relatives. Fourth, using the mechanistic interpretations of phenotypic covariances between relatives outlined in Chapter 7, the observable variance components are used to partition the phenotypic variance into its causal sources. We now demonstrate the practical utility of this approach by showing how ANOVA generates estimates of the within- and among-family components of variance from phenotypic data.

One-way Analysis of Variance

ANOVA uses **sums of squares,** the derivation of which we outline below. Throughout, we use SS to denote an observed sum of squares, and E(SS) to denote its expected value. As we will see shortly, scaled sums of squares, known as **mean squares,** are used to estimate variance components. We use the parallel notation, MS and E(MS), to denote observed and expected mean squares.

Consider the balanced design in which n half sibs are assayed from each of N males, so that there are a total $T = Nn$ individuals in the analysis. The quantity

$$\text{SS}_T = \sum_{i=1}^{N} \sum_{j=1}^{n} (z_{ij} - \overline{z})^2 \tag{18.4}$$

defines the observed **total sum of squares** around the grand mean $\overline{z}$. ANOVA partitions SS_T into components describing variation among the s_i (i.e., among families) and among the e_{ij} within families. This partitioning is readily accomplished by expanding around the observed family means, $\overline{z}_i = \sum_{j=1}^{n} z_{ij}/n$,

$$\text{SS}_T = \sum_{i=1}^{N} \sum_{j=1}^{n} [(z_{ij} - \overline{z}_i) + (\overline{z}_i - \overline{z})]^2$$

$$= \sum_{i=1}^{N} \sum_{j=1}^{n} [(z_{ij} - \overline{z}_i)^2 + 2(z_{ij} - \overline{z}_i)(\overline{z}_i - \overline{z}) + (\overline{z}_i - \overline{z})^2] \tag{18.5}$$

The middle term of this expression is equal to zero, since by the definition of a mean, $\sum_{j=1}^{n} (z_{ij} - \overline{z_i}) = 0$. The third term may be written as $n \sum_{i=1}^{N} (\overline{z}_i - \overline{z})^2$ since

it does not contain j. Thus, the total sum of squares is partitioned into an among- and a within-family component,

$$\text{SS}_T = n \sum_{i=1}^{N} (\overline{z}_i - \overline{z})^2 + \sum_{i=1}^{N} \sum_{j=1}^{n} (z_{ij} - \overline{z}_i)^2$$
$$= \text{SS}_s + \text{SS}_e \tag{18.6}$$

The within-family sum of squares (SS_e) is simply the sum of the squared deviations of individual measures from their observed family means, while the among-family sum of squares (SS_s) is the sum (over all progeny) of the squared deviations of observed family means from the grand mean.

Assuming that the parents are a random sample of the population at large, the sums of squares can be used to obtain unbiased estimates of the within- and among-family components of variance in the following way. We note first that the expected within-family sum of squares is

$$E(\text{SS}_e) = \sum_{i=1}^{N} E \left[\sum_{j=1}^{n} (z_{ij} - \overline{z}_i)^2 \right] = N(n-1)\sigma_e^2 \tag{18.7a}$$

This result follows from the fact that $\sum_{j=1}^{n} (z_{ij} - \overline{z}_i)^2 / (n-1)$ is an unbiased estimate of the variance among sibs in the ith family (Chapter 2) and from our assumption that the variance within each family is equal to σ_e^2.

For the among-family sum of squares, similar reasoning leads to

$$E(\text{SS}_s) = nE \left[\sum_{i=1}^{N} (\overline{z}_i - \overline{z})^2 \right] = n(N-1)\sigma^2(\overline{z}_i) \tag{18.7b}$$

where $\sigma^2(\overline{z}_i)$ is the expected variance of the observed family means, here (with a balanced design) assumed to be the same for all families. Further simplification of this expression is possible. The variance of observed family means is a function of the variance of the true family means, the $(\mu + s_i)$, as well as of their sampling error, the $\overline{e}_i = \overline{z}_i - (\mu + s_i)$. Thus, assuming that the measurement error is independent of the family mean,

$$\sigma^2(\overline{z}_i) = \sigma^2(\mu + s_i) + \sigma^2(\overline{e}_i) \tag{18.8}$$

Since μ is a constant, the first term of this expression is the among-family variance, σ_s^2, while the second is the expected sampling variance of a mean, σ_e^2/n (Chapter 2). Substituting into Equation 18.7b,

$$E(\text{SS}_s) = (N-1)(\sigma_e^2 + n\sigma_s^2) \tag{18.9}$$

Finally, rearranging Equations 18.7a and 18.9, the variance components can be expressed in terms of the expected sums of squares,

$$\sigma_e^2 = \frac{E(\mathrm{SS}_e)}{N(n-1)} \tag{18.10a}$$

$$\sigma_s^2 = \frac{1}{n}\left[\frac{E(\mathrm{SS}_s)}{N-1} - \frac{E(\mathrm{SS}_e)}{N(n-1)}\right] \tag{18.10b}$$

Note that the sums of squares in these expressions are divided by constants. Such weighted sums of squares are the mean squares (MS) referred to above, and the quantities in their denominators are the associated degrees of freedom (df). For the half-sib model,

$$\mathrm{MS}_s = \frac{\mathrm{SS}_s}{N-1} \tag{18.11a}$$

$$\mathrm{MS}_e = \frac{\mathrm{SS}_e}{N(n-1)} \tag{18.11b}$$

are the observed among- and within-family mean squares. Substitution of observed mean squares for their expectations in Equations 18.10a,b yields the following unbiased estimators of σ_s^2, σ_e^2, and σ_z^2,

$$\mathrm{Var}(s) = \frac{\mathrm{MS}_s - \mathrm{MS}_e}{n} \tag{18.12a}$$

$$\mathrm{Var}(e) = \mathrm{MS}_e \tag{18.12b}$$

$$\mathrm{Var}(z) = \mathrm{Var}(s) + \mathrm{Var}(e) \tag{18.12c}$$

A summary of the steps for obtaining the observed mean squares, generalized to allow for unequal family sizes, is given in Table 18.1. This general procedure of estimating variance components from observed mean squares is an example of the **method of moments**, as the unknown variances can be expressed in terms of observable moments (here, the mean squares).

Table 18.1   Summary of a one-way ANOVA involving N independent families, the ith of which contains n_i individuals.

Factor	df	SS	MS	$E(\mathrm{MS})$
Among-families	$N-1$	$\mathrm{SS}_s = \sum_{i=1}^{N} n_i(\bar{z}_i - \bar{z})^2$	$\mathrm{SS}_s/(N-1)$	$\sigma_e^2 + n_0\sigma_s^2$
Within-families	$T-N$	$\mathrm{SS}_e = \sum_{i=1}^{N}\sum_{j=1}^{n_i} (z_{ij} - \bar{z}_i)^2$	$\mathrm{SS}_e/(T-N)$	σ_e^2

Total	$T-1$	$SS_T = \displaystyle\sum_{i=1}^{N}\sum_{j=1}^{n_i}(z_{ij}-\overline{z})^2$	$SS_T/(T-1)$	σ_z^2

Note: The total sample size is $T = \sum_{i=1}^{N} n_i$, and $n_0 = [T - (\sum n_i^2/T)]/(N-1)$, which reduces to n with equal family sizes. Degrees of freedom are denoted by df, observed sums of squares by SS, and expected mean squares by $E(MS)$.

The quantity

$$t_{PHS} = \frac{Var(s)}{Var(z)} \tag{18.13}$$

is the intraclass correlation (Fisher 1918, 1925), discussed previously in Chapter 17.

Ratios of quantities estimated with sampling error are usually biased with respect to their parametric values (Appendix 1), and this is true for the intraclass correlation (Ponzoni and James 1978, Wang et al. 1991). Letting τ be the parametric value, the downward bias is approximately

$$\Delta_t = \tau - E(t_{PHS}) = \frac{2\tau(1-\tau)[(n-1)\tau+1]}{nN} \tag{18.15}$$

In principle, correction for this bias can be made by substituting the observed t_{PHS} for τ in the preceding expression and adding the estimated bias Δ_t to t_{PHS} prior to estimating the heritability with Equation 18.14. The bias in t_{PHS} can be considerable if N is very small (less than 20), but for larger designs it is generally no more than a few percent.

Hypothesis Testing

In obtaining the variance-component estimators, Equations 18.12a,b, we made no assumptions as to how the data or their underlying components (s_i and e_{ij}) were distributed, other than the constraint that they are independent of each other. This distribution-free condition illustrates a useful feature of ANOVA that is not shared by many other estimation procedures — it yields variance-component estimates that are unbiased with respect to the true parametric values (although, as just noted, nonlinear functions, such as ratios, of these estimates will be biased). Unfortunately, this distribution-free property does not extend to the estimation of confidence intervals for the variance components, nor to most traditional methods of hypothesis testing.

Most conventional hypothesis tests involving ANOVA assume normality and homogeneity of error variances. Thus, prior to embarking on an analysis of variance, an attempt should always be made to ensure that the observed data

are on an appropriate scale of measurement (Chapter 11). It should be realized, however, that normality of the observed data does not guarantee normality of the distributions of the underlying factors s_i and e_{ij}.

Assuming that adequate normalization has been accomplished, standard theoretical results can be used to test the hypothesis that the among-family component of variance, and hence the heritability, is significantly greater than zero. We accomplish this by recalling from Appendix 5 that when normally distributed variables with mean zero and variance one (unit normals) are squared, they follow a χ^2 distribution. Dividing an observed sum of squares (SS) by its associated E(MS) transforms the SS into a sum of squared unit normals, which is χ^2-distributed with the associated degrees of freedom. For the one-way ANOVA, from Equations 18.7a, 18.9, and 18.11, the expected mean squares are

$$E(\text{MS}_s) = \sigma_e^2 + n\sigma_s^2 \qquad (18.16\text{a})$$

$$E(\text{MS}_e) = \sigma_e^2 \qquad (18.16\text{b})$$

Thus,

$$\frac{\text{SS}_s}{\sigma_e^2 + n\sigma_s^2} \sim \chi_{N-1}^2 \qquad (18.17\text{a})$$

$$\frac{\text{SS}_e}{\sigma_e^2} \sim \chi_{T-N}^2 \qquad (18.17\text{b})$$

Recall also that the ratio of two χ^2-distributed variables, each divided by its respective degrees of freedom, follows an F distribution (Appendix 5).

Now notice that if $\sigma_s^2 = 0$, the denominators of Equations 18.17a and 18.17b are the same, in which case their ratio is simply SS_s/SS_e. Recalling that $\text{SS}_x/\text{df}_x = \text{MS}_x$,

$$F = \frac{\text{MS}_s}{\text{MS}_e} \qquad (18.18)$$

provides a test of the hypothesis that $E(\text{MS}_s) = E(\text{MS}_e)$, or equivalently that $\sigma_s^2 = 0$. If $\sigma_s^2 > 0$, we expect the ratio of observed mean squares to be greater than one. However, it needs to be significantly larger than one if we are to be confident in our conclusion that $\text{MS}_s > \text{MS}_e$ did not occur just by chance. An explicit test of the null hypothesis of no sire effects is made by referring to standard F-distribution tables and comparing the observed value of F with the critical values associated with $(N - 1)$ and $(T - N)$ degrees of freedom.

Sampling Variance and Standard Errors

In the analysis of heritability, a case can be made that hypothesis testing is of little biological relevance. Because polygenic mutation continually introduces genetic variation into populations, the heritabilities of essentially all characters must be

nonzero, and the only real issue is their absolute magnitude. If an F-test signals nonsignificance, it most likely is a simple consequence of inadequate sample size.

Standard errors provide rough guides to the accuracy of variance-component estimates, and to estimate them, we require the sampling variances of the observed mean squares. Under the assumptions of normality and balanced design, a useful (and general) result is that the observed mean squares extracted from an analysis of variance are distributed independently with expected sampling variance

$$\sigma^2(\text{MS}_x) \simeq \frac{2(\text{MS}_x)^2}{\text{df}_x + 2} \tag{18.19}$$

This fundamental relationship has been used in many contexts in quantitative genetics to derive expressions for variances and covariances of variance components extracted from ANOVA (Tukey 1956, 1957; Smith 1956; Bulmer 1957, 1980; Scheffé 1959). Searle et al. (1992) provide a particularly lucid overview of its utility.

Since the variance-component estimators, Equations 18.12a–c, are linear functions of the observed mean squares, the rules for obtaining variances and covariances of linear functions (Chapter 3 and Appendix 1) can be used in conjunction with Equation 18.19 to obtain the large-sample approximations

$$\text{Var}[\,\text{Var}(e)\,] = \text{Var}(\text{MS}_e) \simeq \frac{2(\text{MS}_e)^2}{T - N + 2} \tag{18.20a}$$

$$\text{Var}[\,\text{Var}(s)\,] = \text{Var}\left[\frac{\text{MS}_s - \text{MS}_e}{n}\right]$$

$$\simeq \frac{2}{n^2}\left(\frac{(\text{MS}_s)^2}{N+1} + \frac{(\text{MS}_e)^2}{T-N+2}\right) \tag{18.20b}$$

$$\text{Cov}[\,\text{Var}(s), \text{Var}(e)\,] = \text{Cov}\left[\left(\frac{\text{MS}_s - \text{MS}_e}{n}\right), \text{MS}_e\right] = -\frac{\text{Var}(\text{MS}_e)}{n}$$

$$\simeq -\frac{2(\text{MS}_e)^2}{n(T-N+2)} \tag{18.20c}$$

$$\text{Var}[\,\text{Var}(z)\,] = \text{Var}[\,\text{Var}(e)\,] + 2\text{Cov}[\,\text{Var}(s), \text{Var}(e)\,] + \text{Var}[\,\text{Var}(s)\,] \tag{18.20d}$$

The standard errors of the estimated within-family, among-family, and total phenotypic variance estimates are obtained by substituting observed mean squares into Equations 18.20a,c,d and taking square roots. Since the accuracy of the resultant standard errors depends on the accuracy of the observed mean squares, the standard errors are not very reliable if the degrees of freedom are small. Hence, the reference to "large-sample" estimators.

Using the techniques in Appendix 1, Osborne and Paterson (1952) showed that the large-sample variance of the intraclass correlation from a balanced one-way ANOVA is

$$\text{Var}(t) \simeq \frac{2(1-t)^2[1+(n-1)t]^2}{Nn(n-1)} \tag{18.21}$$

The standard error of h^2 derived by half-sib analysis is estimated by $4\sqrt{\mathrm{Var}(t)}$. Again, the accuracy of this expression increases with the number of families in an analysis.

Confidence Intervals

Under the assumption of normality, approximate confidence intervals for variance-component estimates can be obtained from the expected distributions of the sums of squares (Harville and Fenech 1985, Searle et al. 1992). For the within-family variance, recalling the distribution of SS_e/σ_e^2 given in Equation 18.17b, the lower and upper values associated with the $100(1-\alpha)\%$ confidence level are simply

$$\frac{\mathrm{SS}_e}{\chi^2_{(T-N),(\alpha/2)}} < \mathrm{Var}(e) < \frac{\mathrm{SS}_e}{\chi^2_{(T-N),(1-\alpha/2)}} \tag{18.22}$$

where $\chi^2_{(T-N),(\alpha/2)}$ and $\chi^2_{(T-N),(1-\alpha/2)}$ are the upper and lower χ^2 values associated with α given $(T-N)$ degrees of freedom. For example, for a 95% confidence interval (2.5% error on each side of the estimate), $\chi^2_{(T-N),0.025}$ is the point at which the probability of obtaining a higher χ^2_{T-N} by chance is 0.025 and $\chi^2_{(T-N),0.975}$ is the point at which the probability of obtaining a higher value is 0.975. These values can be found in tabular form in most elementary statistics texts.

For the among-family variance, the lower and upper confidence limits associated with the $100(1-\alpha)\%$ level are given by

$$\frac{\mathrm{MS}_e}{n}\left[\frac{F}{F_{(N-1),\infty,(\alpha/2)}} - 1 - \left(\frac{F_{(N-1),(T-N),(\alpha/2)}}{F_{(N-1),\infty,(\alpha/2)}} - 1\right)\left(\frac{F_{(N-1),(T-N),(\alpha/2)}}{F}\right)\right]$$

$$\tag{18.23a}$$

and

$$\frac{\mathrm{MS}_e}{n}\left[F \cdot F_{\infty,(N-1),(\alpha/2)} - 1 + \left(1 - \frac{F_{\infty,(N-1),(\alpha/2)}}{F_{(T-N),(N-1),(\alpha/2)}}\right)\left(\frac{1}{F_{(T-N),(N-1),(\alpha/2)}}\right)\right]$$

$$\tag{18.23b}$$

respectively, where the unsubscripted F is the ratio of observed mean squares defined by Equation 18.18, and the F values subscripted by their degrees of freedom are the critical values associated with $\alpha/2$. These values are also obtainable from standard tables.

Assuming normality of the underlying data, the $100(1-\alpha)\%$ confidence interval for the heritability is given by

$$4\left[\frac{(F/F_U)-1}{(F/F_U)+n-1}\right] < h^2 < 4\left[\frac{(F/F_L)-1}{(F/F_L)+n-1}\right] \tag{18.24}$$

(Scheffé 1959, Graybill 1961, Williams 1962). In these expressions, F is again the ratio of observed mean squares defined by Equation 18.18, and F_U and F_L are the upper and lower F values associated with $(\alpha/2)$ at $(N-1), (T-N)$ degrees of freedom. Specifically, $F_U = F_{(N-1),(T-N),(\alpha/2)}$, whereas $F_L = 1/(F_{(T-N),(N-1),(\alpha/2)})$. (See Example 2 for an application of this equation.)

Although somewhat complicated, the preceding expressions are general, provided the data are normally distributed with homogeneous variance (i.e., $s \sim N(0, \sigma_s^2)$ and $e \sim N(0, \sigma_e^2)$ for all families). However, most confidence intervals reported in the literature are approximated by a simpler route. The usual procedure is to assume that the degrees of freedom are large enough that parameter estimates are approximately normally distributed. Then, symmetrical confidence intervals can be computed more simply from the standard errors, e.g., 95% confidence intervals are obtained by multiplying the standard error by 1.96 (Chapter 2). The degree to which this approach can yield biased confidence intervals will be illustrated in Example 2.

Unbalanced Data

Accidental losses or natural mortality almost always cause inequities in family sizes in sib analyses. With unbalanced data, estimates of variance components can still be obtained by the method of moments, but this requires that the definitions of the expected mean squares first be modified appropriately (Table 18.1). All aspects of the unbalanced one-way ANOVA are identical to those outlined for the balanced design, except for the expected among-family mean square, which is no longer $(\sigma_e^2 + n\sigma_s^2)$, but $(\sigma_e^2 + n_0\sigma_s^2)$, where n_0 is a function of the sire-specific family sizes (Table 18.1). Thus, in obtaining estimates of the variance components by the method of moments, we still use Equations 18.12a,b, substituting n_0 for n.

Provided the data are normally distributed, the sums of squares obtained from an unbalanced one-way ANOVA are still independent, and expressions for the sampling variances and covariance of the variance components analogous to Equations 18.20a–d are obtainable,

$$\text{Var}[\,\text{Var}(e)\,] \simeq \frac{2[\,\text{Var}(e)\,]^2}{T - N + 2} \tag{18.27a}$$

$$\text{Var}[\,\text{Var}(s)\,] \simeq \frac{2}{n_0(N+1)} \left\{ \frac{(T-1)[\,\text{Var}(e)\,]^2}{n_0(T-N)} + 2\text{Var}(e)\text{Var}(s) \right.$$
$$\left. + \frac{\sum n_i^2 + (\sum n_i^2/T)^2 - 2\sum n_i^3/T}{n_0(N-1)}[\,\text{Var}(s)\,]^2 \right\} \tag{18.27b}$$

$$\text{Cov}[\,\text{Var}(s), \text{Var}(e)\,] \simeq -\frac{2[\,\text{Var}(e)\,]^2}{n_0(T-N+2)} \tag{18.27c}$$

$$\text{Var}[\,\text{Var}(z)\,] \simeq \text{Var}[\,\text{Var}(e)\,] + 2\text{Cov}[\,\text{Var}(s), \text{Var}(e)\,]$$
$$+ \text{Var}[\,\text{Var}(s)\,] \tag{18.27d}$$

where the summations in Equation 18.27b are over sires. (These expressions contain corrections to results given in Searle et al. 1992).

Comparison of Equation 18.27a with 18.20a shows that the sampling variance of the within-family component of variance is unaffected by lack of balance. In fact, lack of balance does not alter the fact that the within-family sum of squares is χ^2-distributed. Thus, Equation 18.22 can still be used to obtain confidence intervals for the within-family component of variance. Unfortunately, the situation is not so simple with the among-family statistics. If $\sigma_s^2 = 0$, the ratio $F = \mathrm{MS}_s/\mathrm{MS}_e$ still has an F distribution with $(N-1)$ and $(T-N)$ degrees of freedom, so even with an unbalanced design, the ratio of mean squares provides a basis for testing the null hypothesis that $\sigma_s^2 = 0$. Searle et al. (1992, pp. 76–78) outline procedures for estimating confidence intervals for the among-family variance component and for t_{PHS}, but these procedures are quite complicated.

In recent years, maximum likelihood procedures have been developed as an alternative to ANOVA approaches for variance-component estimation. As a consequence of their relative insensitivity to unbalanced designs, these methods have been embraced widely by animal breeders. Unlike ANOVA, maximum likelihood techniques assume normality in *both* the estimation of parameters and hypothesis testing. A broad overview of the use of maximum likelihood methods in quantitative genetics is given in Chapters 26 and 27. For historical completeness, we note that Smith (1956) long ago introduced a weighted ANOVA procedure for unbalanced data that is closely related to maximum likelihood estimation. For the computation of the among-family sum of squares, he proposed that the family means be weighted by the inverse of their sampling variance. As in the case of weighted regression (discussed in the preceding chapter), the weights turn out to be a function of the variance components to be estimated, so an iterative solution is used in the estimation of the variance components. The weights proposed in Smith's (1956) paper are identical to those used in the maximum likelihood solution to the one-factor model (Searle et al. 1992).

Resampling Procedures

To avoid the interpretative pitfalls that can arise with hypothesis tests involving nonnormal and unbalanced data, several computer-based resampling procedures have been developed that make no assumptions about the form of the distribution of the data or the structure of experimental design (Miller 1968, 1974; Efron 1982; Milliken and Johnson 1984; Wu 1986; Little and Rubin 1987; Manly 1991; Crowley 1992). All of these techniques assume that the sample data provide a reasonably good representation of the distribution in the entire population. The data are then used to generate sampling distributions of desired statistics. Three basic approaches are used:

1. The **jackknife** procedure iteratively deletes one unit of the data set, each time using the truncated data to obtain a set of parameter estimates. For

the one-way ANOVA, a different paternal half-sib family is deleted in each analysis, and from the resultant N sets of parameter estimates, one obtains a mean estimate and a standard error for each variance component, heritability, and so on.

2. The **bootstrap** procedure repeatedly draws random samples from the original data set with replacement. With reasonably large sample sizes, the number of ways the data set can be sampled is effectively infinite, and usually a thousand or more analyses are performed to arrive at stable average values for the parameter estimates and their standard errors. Confidence intervals are constructed from the cumulative distribution of the individual estimates. For the one-way ANOVA, bootstrapping would be done over families, as our interest is in the among-family variance.

3. **Permutation tests** randomize the individual data with respect to families, while keeping the overall data structure (number of families and progeny per family) constant. Again, an essentially unlimited number of data sets can be constructed in this way, and from a large number of them, the distribution of the estimated among-family variance can be established under the null hypothesis that $\sigma_s^2 = 0$. This distribution is then used to evaluate the probability of obtaining by chance an estimate of σ_s^2 with a value as extreme as that found with the original data set. Mitchell-Olds (1986) used this approach in a sib analysis of life-history variation in the annual plant *Impatiens capensis* to test for significant heritabilities; later, the delete-one jackknife was applied to the same data (Mitchell-Olds and Bergelson 1990). The two types of analyses led to similar, although not identical, conclusions.

NESTED ANOVA

The linear model for the **nested design** where full-sib families are nested within half-sib families is

$$z_{ijk} = \mu + s_i + d_{ij} + e_{ijk} \tag{18.28a}$$

where z_{ijk} is the phenotype of the *k*th offspring from the family of the *i*th sire and *j*th dam, s_i is the effect of the *i*th sire, d_{ij} is the effect of the *j*th dam mated to the *i*th sire, and e_{ijk} is the residual deviation. As usual, under the assumption that individuals are random members of the same population, the s_i, d_{ij}, and e_{ijk} are defined to be independent random variables with expectations equal to zero. It then follows that the total phenotypic variance is

$$\sigma_z^2 = \sigma_s^2 + \sigma_d^2 + \sigma_e^2 \tag{18.28b}$$

where σ_s^2 is the variance among sires, σ_d^2 is the variance among dams within sires, and σ_e^2 is the variance within full-sib families.

Nested Analysis of Variance

As with one-way ANOVA, estimates of the variance components can be obtained by the method of moments, i.e., by partitioning the total observed sum of squares into components, writing the expected mean squares as linear functions of the variance components, equating the observed mean squares to their expectations, and solving for the variances. Let $\bar{z}_{ij}$ be the mean phenotype of full-sib family ij, $\bar{z}_i$ be the mean phenotype of progeny of sire i, and $\bar{z}$ be the grand mean of the z_{ijk}. The total sum of squared deviations of the z_{ijk} from $\bar{z}$ can be partitioned into components describing deviations of observed sire means from the grand mean, deviations of the full-sib family means from their sire group means, and deviations of individual measures from their full-sib family means (Table 18.3).

The variance-component estimators are given by,

$$\text{Var}(s) = \frac{\text{MS}_s - \text{MS}_e - (k_2/k_1)(\text{MS}_d - \text{MS}_e)}{k_3} \tag{18.32a}$$

$$\text{Var}(d) = \frac{\text{MS}_d - \text{MS}_e}{k_1} \tag{18.32b}$$

$$\text{Var}(e) = \text{MS}_e \tag{18.32c}$$

where k_1, k_2, and k_3 are functions of the experimental design (equal to n, n, and Mn under a completely balanced design, where n is the number of offspring per full-sib family, and M is the number of dams per sire). Table 18.3 gives the general expressions for these quantities.

By analogy with Equation 18.13, the intraclass correlations for paternal half sibs and full sibs are

$$t_{\text{PHS}} = \frac{\text{Cov(PHS)}}{\text{Var}(z)} = \frac{\text{Var}(s)}{\text{Var}(z)} \tag{18.33a}$$

$$t_{\text{FS}} = \frac{\text{Cov(FS)}}{\text{Var}(z)} = \frac{\text{Var}(s) + \text{Var}(d)}{\text{Var}(z)} \tag{18.33b}$$

As in the half-sib design, $4t_{\text{PHS}}$ provides the best estimate of h^2 since it is not inflated by dominance and/or maternal effects. If, however, $\text{Var}(s)$ and $\text{Var}(d)$ are found to be approximately equal, then dominance and maternal effects can be ruled out as significant causal sources of covariance. In that case, the average

Table 18.3   Summary of a nested analysis of variance involving N sires, M_i dams within the ith sire, and n_{ij} offspring within the ijth full-sib family.

Factor	df	Sums of Squares	MS	E(MS)

Sires	$N-1$	$\displaystyle\sum_{i=1}^{N}\sum_{j=1}^{M_i} n_{ij}(\overline{z}_i - \overline{z})^2$	SS_s/df_s	$\sigma_e^2 + k_2\sigma_d^2 + k_3\sigma_s^2$
Dams (sires)	$N(\overline{M}-1)$	$\displaystyle\sum_{i=1}^{N}\sum_{j=1}^{M_i} n_{ij}(\overline{z}_{ij} - \overline{z}_i)^2$	SS_d/df_d	$\sigma_e^2 + k_1\sigma_d^2$
Sibs (dams)	$T - N\overline{M}$	$\displaystyle\sum_{i=1}^{N}\sum_{j=1}^{M_i}\sum_{k=1}^{n_{ij}}(z_{ijk} - \overline{z}_{ij})^2$	SS_e/df_e	σ_e^2
Total	$T-1$	$\displaystyle\sum_{i=1}^{N}\sum_{j=1}^{M_i}\sum_{k=1}^{n_{ij}}(z_{ijk} - \overline{z})^2$		

$$k_1 = \frac{1}{N(\overline{M}-1)}\left(T - \sum_{i=1}^{N}\frac{\sum_j^{M_i} n_{ij}^2}{n_i}\right)$$

$$k_2 = \frac{1}{N-1}\left(\sum_{i=1}^{N}\frac{\sum_j^{M_i} n_{ij}^2}{n_i} - \frac{\sum_i^N \sum_j^{M_i} n_{ij}^2}{T}\right)$$

$$k_3 = \frac{1}{N-1}\left(T - \frac{\sum_i^N n_i^2}{N}\right)$$

Note: T is the total number of individuals in the experiment, $\overline{M}$ is the mean number of dams/sire, and n_i is the total number of offspring of the ith sire. MS denotes an observed mean square, $E(\text{MS})$ denotes its expected value, and df denotes degrees of freedom.

of $\text{Var}(s)$ and $\text{Var}(d)$ provides an estimate of $\sigma_A^2/4$, and when multiplied by $4/\text{Var}(z)$ provides an estimate of h^2. Ideally, such an average should weight the two variance-component estimates by the inverse of their sampling variances (Grossman and Norton 1981).

Hypothesis Testing

Under the assumption of normality and balanced design, standard F ratios can be used to test for significant variation associated with sires and dams. In each case, the numerator of the F ratio is the observed mean square at the level containing the factor of interest, and the denominator is the observed mean square at the next lower level (which incorporates all factors except the one of interest). The test statistic for evaluating whether there is significant variance associated with sires is the F ratio MS_s/MS_d, since under the null hypothesis of $\sigma_s^2 = 0$, the expected value of the numerator is equal to that of the denominator (see Table

18.3). Similarly, the test statistic for significant dam effects is the ratio MS_d/MS_e, as the expected value of the numerator is again equal to that of the denominator under the null hypothesis of $\sigma_d^2 = 0$ (Table 18.3).

With unbalanced designs, hypothesis testing with F ratios becomes more difficult under the nested model. If the data are normally distributed, the logic developed above tells us that

$$F_{N(\overline{M}-1),(T-N\overline{M})} = \frac{MS_d}{MS_e} \tag{18.34a}$$

can still be employed as a test for significant dam effects, since the numerator and denominator have identical expectations under the null hypothesis of $\sigma_d^2 = 0$. However, since the coefficients (k_1 and k_2) associated with σ_d^2 in the mean squares associated with dams and sires are unequal in an unbalanced design (Table 18.3), the numerator and denominator of MS_s/MS_d no longer have equal expectations under the null hypothesis $\sigma_s^2 = 0$. Nevertheless, a linear function of the mean squares can be constructed for the numerator that does fulfill this requirement, leading to the test statistic

$$F_{r,N(\overline{M}-1)} = \frac{k_1 MS_s + (k_2 - k_1)MS_e}{k_2 MS_d} \tag{18.34b}$$

The main problem with this test statistic is the unknown degrees of freedom for the numerator, r. A general solution to this problem was developed by Satterthwaite (1946). Consider a linear function of m observed mean squares

$$Q = c_1 MS_1 + c_2 MS_2 + \cdots + c_m MS_m \tag{18.35a}$$

Satterthwaite showed that $rQ/E(Q)$ is approximately χ^2-distributed with degrees of freedom equal to

$$r = \frac{Q^2}{\displaystyle\sum_{i=1}^{m} \frac{(c_i MS_i)^2}{df_i}} \tag{18.35b}$$

Thus, for example, for the numerator of Equation 18.34b, the degrees of freedom is estimated by

$$r = \frac{Q^2}{\dfrac{(c_s MS_s)^2}{N-1} + \dfrac{(c_e MS_e)^2}{T - N\overline{M}}} \tag{18.35c}$$

where $c_s = k_1/k_2$, $c_e = (k_2 - k_1)/k_2$, and $Q = c_s MS_s + c_e MS_e$. This estimate of r is really only a first-order approximation, as Satterthwaite's derivation assumes that the observed mean squares in the function Q are independently distributed, a condition that is not strictly true with an unbalanced design.

Recalling that the variance associated with sires is an estimate of $\sigma_A^2/4$, the F ratio defined by Equation 18.34b with the numerator degrees of freedom defined by Equation 18.35c provides a test for significant additive genetic variance. Provided that both the sire and dam components of variance are significant, the next question is whether the latter is significantly greater than the former. From Equation 18.31b, it can be seen that a test of the null hypothesis $\sigma_s^2 = \sigma_d^2$ is equivalent to a test for no significant dominance and/or common-environmental effects. This test also requires the construction of a linear function of mean squares whose expectation is equal to the expectation of MS_d under the null hypothesis. The appropriate F ratio is

$$F_{r,N(\overline{M}-1)} = \frac{c_s MS_s + c_e MS_e}{MS_d} \tag{18.36}$$

where $c_s = k_1/(k_2 + k_3)$ and $c_e = (k_2 + k_3 - k_1)/(k_2 + k_3)$. The numerator degrees of freedom is approximated by Equation 18.35c, with $Q = c_s MS_s + c_e MS_e$.

Resampling procedures provide an alternative to F ratios for testing for the significance of variance components under the nested design. The jackknife, with deletion around sire families, has been shown to provide a relatively robust approach for testing for significance of the sire component of variance (Arvesen and Schmitz 1970, Knapp and Bridges 1988, Mitchell-Olds and Bergelson 1990). Presumably, the jackknife or the bootstrap can also be used to test the hypothesis that $\sigma_s^2 = \sigma_d^2$, by referring to the sampling distribution of $\text{Var}(d) - \text{Var}(s)$.

Sampling Error

Under a balanced design (with N sires, M dams per sire, and n progeny per dam), the large-sample variance for t_{PHS} and t_{FS} can be obtained from formulae provided by Osborne and Paterson (1952),

$$\text{Var}(t_{\text{PHS}}) \simeq \frac{2\{(1 - t_{\text{PHS}})(\phi + Mnt_{\text{PHS}})\}^2}{M^2(N-1)n^2}$$
$$+ \frac{2\{[1 + (M-1)t_{\text{PHS}}]\phi\}^2}{M^2 N(M-1)n^2} + \frac{2(n-1)[t_{\text{PHS}}(1 - t_{\text{FS}})]^2}{NMn^2} \tag{18.37a}$$

$$\text{Var}(t_{\text{FS}}) \simeq \frac{2\{t'[\phi + Mnt_{\text{PHS}}]\}^2}{M^2(N-1)n^2}$$
$$+ \frac{2\{[M - (M-1)t']\phi\}^2}{M^2 N(M-1)n^2} + \frac{2\{(1 - t_{\text{FS}})[1 + (n-1)t']\}^2}{MN(n-1)n^2} \tag{18.37b}$$

where $t' = t_{\text{FS}} - t_{\text{PHS}}$, and $\phi = 1 - t_{\text{FS}} + nt'$. The standard error of $h^2 = 4t_{\text{PHS}}$ is $4\sqrt{\text{Var}(t_{\text{PHS}})}$.

A more general procedure for estimating $\text{Var}(t_{\text{PHS}})$, which allows for unbalanced designs, is to use the large-sample estimator for the variance of a ratio given

in Appendix 1. Such a computation requires estimates of the sampling variances and covariances of $\text{Var}(s)$, $\text{Var}(d)$, and $\text{Var}(e)$, expressions for which are given in Hammond and Nicholas (1972) and Searle et al. (1992). If one is willing to assume normality and to ignore the sampling variance of $\text{Var}(z)$ and the sampling covariance between $\text{Var}(z)$, $\text{Var}(s)$, and $\text{Var}(d)$, some fairly simple and conservative (upwardly biased) estimates are possible,

$$\text{Var}(t_{\text{PHS}}) \simeq \frac{\text{Var}(\text{MS}_s) + (k_2/k_1^2)\text{Var}(\text{MS}_d) + [1 - (k_2/k_1)]^2\text{Var}(\text{MS}_e)}{[k_3\text{Var}(z)]^2} \quad (18.38\text{a})$$

$$\text{Var}(t_{\text{FS}}) \simeq \frac{\text{Var}(\text{MS}_s) + k_3^2[\phi^2\text{Var}(\text{MS}_d) + (1 + \phi)^2\text{Var}(\text{MS}_e)]}{[k_3\text{Var}(z)]^2} \quad (18.38\text{b})$$

with

$$\text{Var}(\text{MS}_x) = \frac{2(\text{MS}_x)^2}{\text{df}_x + 2}, \qquad \phi = \frac{(k_2/k_3) - 1}{k_1}$$

and k_1, k_2, and k_3 as defined in Table 18.3 (Dickerson 1969).

Graybill et al. (1956), Broemeling (1969), and Graybill and Wang (1979) provide expressions for the confidence limits of t_{PHS} and t_{FS} for the special case of a balanced design with normally distributed effects. These expressions are not necessarily very robust to violations of the assumptions of balance and normality.

Optimal Design

In the now familiar fashion, the optimal design for a nested analysis of variance is defined to be the combination of N, M, and n, subject to some constraint, that minimizes the sampling variance of the intraclass correlation of interest. Since $4t_{\text{PHS}}$ provides the most reliable estimate of h^2, it will generally be most desirable to minimize $\text{Var}(t_{\text{PHS}})$ as defined in Equation 18.37a, but the solution is quite complicated, as it depends upon both t_{PHS} and t_{FS}. Some feeling for the best design and the sensitivity of $\text{Var}(t_{\text{PHS}})$ to nonoptimal designs can be achieved by substituting different values for the design parameters (N, M, n) and for the possible values of t_{PHS} and t_{FS}.

Robertson (1959a) has shown that when dominance and common environmental effects are absent, the preferred design for estimating t_{PHS} is to use full-sib families of only single individuals, i.e., to rely on the pure half-sib analysis outlined in the previous section. If on the other hand, one desires approximately equal precision in the estimates of t_{PHS} and t_{FS}, it is advisable to allocate at least 3 to 4 females/male and to maintain full-sib families of $\sim 1/(2t_{\text{PHS}})$ (but no less than 2) progeny/female.

Bridges and Knapp (1987) performed simulation studies to evaluate the probability of obtaining negative estimates for σ_A^2 and σ_D^2 under the nested design. Assuming no epistatic or common environmental effects, with designs of moderate size, the probability of obtaining a negative estimate of the additive genetic

variance is usually on the order of only a few percent. However, the probability of obtaining a negative estimate of σ_D^2 is typically about an order of magnitude higher. Thus, although the nested design is often relied on as a means for detecting dominance, it is not particularly powerful in this regard.

Example 3. To evaluate the causal sources of variation in developmental rate in the flour beetle *Tribolium castaneum*, Dawson (1965) estimated the covariances between several types of relatives. Here we focus on the results from a nested design in which 30 males were each mated to three different females, with the goal of assaying 10 progeny per female. Some mortality among the dams and the offspring induced slight inequalities in family sizes, resulting in $k_1 = k_2 = 9.1$ and $k_3 = 25.7$. Following the layout in Table 18.3, the degrees of freedom and observed and expected mean squares for the nested ANOVA are:

Factor	df	Mean squares	E(MS)
Sires	29	5.949	$\sigma_e^2 + 9.1\sigma_d^2 + 25.7\sigma_s^2$
Dams within sires	56	3.925	$\sigma_e^2 + 9.1\sigma_d^2$
Sibs within dams	695	1.314	σ_e^2

From Equations 18.32a–c, the estimated variance components for sires, dams within sires, and sibs within dams are $\text{Var}(s) = 0.079$, $\text{Var}(d) = 0.288$, and $\text{Var}(e) = 1.314$. From Equations 18.33a,b, the intraclass correlations for paternal half sibs and full sibs are $t_{\text{PHS}} = 0.047$ and $t_{\text{FS}} = 0.218$. If all of the resemblance between relatives were due to additive genetic variance, we would expect $t_{\text{FS}} \simeq 2t_{\text{PHS}}$. The fact that t_{FS} is nearly five times t_{PHS} immediately suggests that dominance and/or common maternal effects may be contributing to the covariance between full sibs.

How much confidence can we have in these intraclass correlations? To evaluate the significance of the sire component of variance, we compute $F = 5.949/3.925 = 1.52$. Using an F-distribution table, for 29 and 56 degrees of freedom, we find that there is a 5% chance of observing an F as large as 1.68 by chance. Thus, the hypothesis that $\sigma_s^2 = 0$ cannot be rejected at this level. On the other hand, for the dam component of variance, $F = 3.925/1.314 = 2.99$, which is well above the critical 0.1% value for 56 and 695 degrees of freedom (1.70), implying that a significant fraction of the total variation in developmental rate is attributable to dams.

Approximate standard errors can be obtained for the two intraclass correlations using Equations 18.38a,b. After the appropriate substitutions, we find $\text{Var}(t_{\text{PHS}}) \simeq 0.0098$ and $\text{Var}(t_{\text{FS}}) \simeq 0.0127$. Taking square roots, we arrive at the standard errors $\text{SE}(t_{\text{PHS}}) \simeq 0.099$ and $\text{SE}(t_{\text{FS}}) \simeq 0.113$. As noted in the text, Equations 18.38a,b generally yield conservative (upwardly biased) estimates of the

standard errors. When the more precise Equations 18.37a,b are used, we obtain $\mathrm{SE}(t_{\mathrm{PHS}}) \simeq 0.038$ and $\mathrm{SE}(t_{\mathrm{FS}}) \simeq 0.037$. When compared with the estimates t_{PHS} and t_{FS}, these results are consistent with our conclusion that the dam component of variance is much more significant than the sire component. The heritability of developmental rate is estimated as four times t_{PHS}, and its standard error is four times $\mathrm{SE}(t_{\mathrm{PHS}})$. Thus, Dawson's results yield the estimate $h^2 \simeq 0.19$, but with a standard error of 0.15.