A PROPOSED TEST OF THE DIFFERENCE BETWEEN THE MEANS OF MONOZYGOTIC AND DIZYGOTIC TWINS

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The rationale for using an approximate t′ *test of the difference between the means of MZ and DZ twins is presented. This test avoids pooling the among-MZ and among-DZ twin-pair mean squares and has approximate degrees of freedom based upon the relative number of MZ and DZ twin pairs as well as the relative sizes of the among-MZ and among-DZ twin-pair mean squares. Sampling experiments simulating twin studies were used to show that the rate of Type I error for this* t′ *test was appropriate while other tests could give either too many or too few Type I errors depending upon the relative sizes of the mean squares and the relative numbers of MZ and DZ twin pairs.*

INTRODUCTION

Analyses of variance for monozygotic (MZ) and dizygotic (DZ) twins are often combined to estimate genetic variance or heritability. If there is an association between one type of twinning and the trait being studied, then estimates of genetic variance may be biased. Such associations could result in inequality of the means or variances of MZ and DZ twins. In a previous paper, Christian et al. (1974) discussed testing for unequal variance of MZ and DZ twins before choosing an estimate of genetic variance. This paper considers the choice of an appropriate test for the difference between the means of MZ and DZ twins and presents results of a series of simulated twin sampling experiments which compare the Type I error rates of three tests of this difference.

METHODOLOGY

Review of previous twin studies reveals that authors often fail to report whether the means of DZ and MZ twins are compared statistically. If the means are tested, a common practice is to consider the members of the MZ and DZ pairs as samples of independent individuals. The usual independent-samples t-test for the difference between means is then applied. This procedure, which we will designate t_1, ignores the *nested* structure of twin data. Zygosity

4

is a fixed effect (Sokal and Rohlf 1969, pp. 186 ff.), being fixed at or shortly after conception into one of two exhaustive classes (MZ and DZ). The twin pairs nested within zygosities constitute a random effect; finally, individuals nested within twin pairs are another random effect. The test of the fixed effect (zygosity) that is ordinarily appropriate when the sample has this structure is the *mixed model nested* or *hierarchical* analysis of variance (Sokal and Rohlf 1969, pp. 266 ff.).

The usual analysis of variance among and within twin pairs in each zygosity is shown in Table 1 (Christian et al. 1974). The analysis in Table 1 may be used as the first step in the

Table 1. *General analysis of variance model for twin studies*

Source of variation	Monozygotic pairs		Dizygotic pairs	
	df	Mean squares	*df*	Mean squares
Among pairs	$n_{MZ} - 1$	M_{AMZ}	$n_{DZ} - 1$	M_{ADZ}
Within pairs	n_{MZ}	M_{WMZ}	n_{DZ}	M_{WDZ}

df = degrees of freedom.
n_{MZ} = number of monozygotic twin pairs.
n_{DZ} = number of dizygotic twin pairs.

nested analysis referred to above. Allowing for unequal numbers of pairs within zygosities, but recognizing that the number of individuals within a pair is always two, the nested analysis of variance together with the expected values of the mean squares is as shown in Table 2 (Sokal and Rohlf 1969, pp. 268 and 277).

It is evident from the expected values of the mean squares that the appropriate test of the null hypothesis $\mu_{MZ} = \mu_{DZ}$ is $F = M_Z/M_A$. Since this F-test has one degree of freedom

Table 2. *Mixed model nested analysis of variance for twin studies*

Source	*df*	Mean squares	Expected values of mean squares*
Between zygosities	1	M_Z	$\sigma^2 + 2\sigma^2_A + \dfrac{2(\mu_{MZ} - \mu_{DZ})^2}{n_{MZ}^{-1} + n_{DZ}^{-1}}$
Among pairs within zygosities	$n_{MZ} + n_{DZ} - 2$	M_A	$\sigma^2 + 2\sigma^2_A$
Between individuals within pairs	$n_{MZ} + n_{DZ}$	M_W	σ^2

M_A = result of pooling M_{AMZ} and M_{ADZ}.
M_W = result of pooling M_{WMZ} and M_{WDZ}.
σ^2 = parameter variance between individuals within pairs.
σ^2_A = parameter variance among pairs within zygosities, assumed in the analysis in this table to be the *same* in both zygosities.
μ_{MZ} = parameter mean for monozygotic twins.
μ_{DZ} = parameter mean for dizygotic twins.

* Sokal and Rohlf's (1969) quantity $\Sigma \, \alpha^2$ (p. 268) can be shown in this case, because of only two zygosities, to be $1/2 \, (\mu_{MZ} - \mu_{DZ})^2$. Note that the coefficient on $(\mu_{MZ} - \mu_{DZ})^2$ in $E(M_Z)$ is the *harmonic mean* of n_{MZ} and n_{DZ}.

for the numerator, there exists an equivalent t-test such that $F = t^2$. Algebraic manipulation will verify that this equivalent t-test, which we will call t_2, is:

$$t_2 = \left(\overline{Y}_{MZ} - \overline{Y}_{DZ} \right) \bigg/ \sqrt{ M_A \left(\frac{1}{2n_{MZ}} + \frac{1}{2n_{DZ}} \right) } \qquad \text{with } df = n_{MZ} + n_{DZ} - 2 \qquad (1)$$

where $\overline{Y}_{MZ}$ = observed mean for sample of monozygotic twins,

$\quad\quad \overline{Y}_{DZ}$ = observed mean for sample of dizygotic twins.

The independent-samples t-test commonly, though mistakenly, used in this situation is equivalent to pooling M_A and M_W, as follows:

$$t_1 = \left(\overline{Y}_{MZ} - \overline{Y}_{DZ} \right) \bigg/ \sqrt{ \left[\frac{(n_{MZ} + n_{DZ} - 2) M_A + (n_M + n_{DZ}) M_W}{2n_{MZ} + 2n_{DZ} - 2} \right] \left[\frac{1}{2n_{MZ}} + \frac{1}{2n_{DZ}} \right] } \qquad (2)$$

From an examination of the expected values of the mean squares it is evident that pooling of the among and within mean squares can be justified *only* under the assumption $\sigma^2 A = 0$. This assumption is rarely, if ever, justified. The mean square *among* pairs is often several times greater than the corresponding mean square *within* pairs.

The analysis presented above, whether the test used is the F-test or t_2 (1), requires the assumption, among others, that the parameter variance among pairs within zygosities ($\sigma^2 A$) is the *same* in the two zygosities, so that the sums of squares among pairs within the two zygosities may be *pooled* — a pooling which takes place automatically when an analysis of variance of the kind depicted is computed (Sokal and Rohlf 1969, Box 10.4). *However*, Christian et al. (1974) have shown that the expected values of the mean squares among pairs within the two zygosities are *not* the same if there is any genetic variance present, or if environmental variance is different in the two zygosities. It is therefore desirable to construct a test in which these two sources of variance are *not* pooled.

Following Satterthwaite (1946) and Cochran (1951), we can construct an approximate test of the hypothesis $\mu_{MZ} = \mu_{DZ}$ which avoids the pooling of these two potentially unequal sources of variance as follows:

$$t' = \left(\overline{Y}_{MZ} - \overline{Y}_{DZ} \right) \bigg/ \sqrt{ \frac{M_{AMZ}}{2n_{MZ}} + \frac{M_{ADZ}}{2n_{DZ}} } \qquad (3)$$

with

$$\text{approximate degrees of freedom} = \frac{ \left(\frac{M_{AMZ}}{2n_{MZ}} + \frac{M_{ADZ}}{2n_{DZ}} \right)^2 }{ \left[\left(\frac{M_{AMZ}}{2n_{MZ}} \right)^2 \bigg/ n_{MZ} - 1 \right] + \left[\left(\frac{M_{ADZ}}{2n_{DZ}} \right)^2 \bigg/ n_{DZ} - 1 \right] } \qquad (4)$$

[Note: In calculating the approximate degrees of freedom the constant 2 may be cancelled out of both terms in the numerator and both terms in the denominator of (4)].

If $n_{MZ} = n_{DZ}$, then t_2 (1) equals t' (3), although the degrees of freedom will not be the same. The extent to which the approximate df in (4) falls below ($n_{MZ} + n_{DZ} - 2$) is dependent on the ratio of M_{AMZ} to M_{ADZ} and also on the ratio of n_{MZ} to n_{DZ}.

RESULTS AND DISCUSSION

Performance of the three tests (t_1, t_2 and t') of the difference between the means of MZ and DZ twins were compared in a series of simulated twin sampling experiments (Table 3). In the first sampling experiment 1000 replicates each consisting of a simulated sample of 25 pairs of MZ and 25 pairs of DZ twins, were drawn from a normally distributed population with a mean of 0.0, total variance of 1.0, additive genetic variance of 0.5 and environmental covariance for both MZ and DZ twins of 0.5.

Table 3. *The percentage of Type I statistical errors for three methods of testing the difference between the means of MZ and DZ twins. (Three sampling experiments, 1000 twin samples/experiment.)*

Experiment No.	Twin pairs		Average mean squares				Type I errors* (%)		
	n_{MZ}	n_{DZ}	M_{AMZ}	M_{WMZ}	M_{ADZ}	M_{WDZ}	t_1	t_2	t'
1	25	25	1.74	0.28	1.47	0.54	12.2	5.5	5.4
2	10	40	1.69	0.29	1.46	0.54	12.6	6.8	6.1
3	40	10	1.73	0.28	1.48	0.51	11.2	3.9	4.8

t_1 = a t-test treating the twins as unrelated individuals in essence pooling the within and among mean squares.
t_2 = a t-test pooling the among mean squares for MZ and DZ pairs.
t' = an approximate t-test using the unpooled among mean squares.

* A Type I error occurred whenever the t-statistic under study exceeded in absolute value the two-tailed 5% level for Student's t with the degrees of freedom associated with that test. In each experiment 1000 twin samples were generated.

The averages of the four mean squares over the 1000 replicates are very close to their expected values (Christian et al. 1974) and for each twin type the sum of the average mean squares is close to the total of 2.0 expected from the model. The differences between the MZ and DZ means vary from zero only by chance due to sampling variation; therefore an appropriate test statistic should exceed its 5% level in only about 5% of the replicates, i.e., have a Type I error rate of 5%. In the sampling experiment with equal numbers of twin-sets the t' had 5.4% Type I errors while the t_2 test was very close with 5.5%. With equal numbers of twin sets the t' and t_2 tests should be very close because as was pointed out their t-values will be identical but they differ slightly in Type I error rate because in general the t' will have fewer degrees of freedom. The t_1 test had over twice as many Type I errors as it should have (12.2% versus 5%) revealing that it is not an appropriate test when the among mean squares are larger than the within mean squares, as was true in this experiment.

In the second and third sampling experiments the numbers of MZ and DZ twins were varied (10 pairs of MZ and 40 DZ in the second experiment: 40 pairs of MZ and 10 DZ in the third experiment). Again the average mean squares were close to their expected values. The t' test produced 6.1% and 4.8% Type I errors respectively. In both experiments 2 and 3 the t_1 test had an unacceptedly high number of Type I errors (12.6% and 11.2% respectively). In experiment 2 the t_2 test had a Type I error rate of 6.8% while in experiment 3 the t_2 test had a Type I error rate of 3.9%.

In general, if the among pairs mean squares with the larger average have the *larger* degrees of freedom, the test using the pooled among pairs mean squares (t_2) has a Type I error rate *below* its nominal level. The converse is true when the among pairs mean squares with the larger average have the *smaller* degrees of freedom. This is consistent with the results reported by Box (1954).

The t' test therefore appears to have the appropriate Type I error rate with the conditions simulated and in our opinion is the appropriate test of the difference between the means of DZ and MZ twins. The t_1 test will in general have an unacceptably high Type I error rate and for experiments with unequal numbers of MZ and DZ twins the Type I error rate for t_2 may be too high or too low depending upon the relative number of MZ and DZ twins and the relative size of the among-pair mean squares.

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RIASSUNTO

Proposta di un Test per la Differenza fra Medie di Gemelli Monozigotici e Dizigotici

Viene illustrato un test t' approssimato per la differenza fra medie di gemelli MZ e DZ. Il test evita di mettere insieme le medie quadratiche di gemelli MZ e DZ ed ha gradi di libertà approssimati basati sul numero relativo di coppie MZ e DZ, così come sul numero relativo delle medie quadratiche in gemelli MZ e DZ. Esperimenti di simulazione di studi gemellari sono stati usati per dimostrare che il tasso di errori di Tipo I per questo test t' era appropriato, mentre altri test davano troppo pochi o troppi errori di Tipo I, in funzione della relativa entità delle medie quadratiche e del numero di coppie MZ e DZ.

RÉSUMÉ

Proposition d'un Test pour la Différence entre Moyennes de Jumeaux Monozygotiques et Dizygotiques

Un test t' approximé pour la différence entre moyennes de jumeaux MZ et DZ est illustré. Ce test évite de mettre ensemble les moyennes des carrés de jumeaux MZ et DZ et a des degrés de liberté approximés, basés sur le nombre relatif de couples MZ et DZ ainsi que sur le nombre relatif des moyennes des carrés de jumeaux MZ et DZ. Des expériments de simulation d'études gémellaires ont été effectués afin de démontrer que le taux d'erreurs de Type I pour ce test t' était approprié, alors que d'autres tests donnaient un nombre trop petit ou trop élevé d'erreurs de Type I, en fonction de la grandeur relative des moyennes des carrés et du nombre de couples MZ et DZ.

ZUSAMMENFASSUNG

Ein neuer Test für die Errechnung der Unterschiede in den Durchschnittswerten zwischen EZ und ZZ

Beschreibung eines Tests, der die Differenz der Durchschnittswerte bei EZ und bei ZZ hervorbringen soll. Dieser Test t' vermeidet die Verbindung der Quadratdurchschnittsziffern von EZ und ZZ und verfügt über annähernde Freiheitsgrade, die auf der relativen Zahl der EZ- und ZZ-Paare basieren, sowie auf der relativen Zahl der Quadratdurchschnittswerte bei EZ und ZZ. Es wurden simulierte Zwillingsexperimente angestellt, um zu beweisen, daß die Fehlerrate des Typs I für diesen Test t' angemessen war, während andere Tests, je nach der Höhe der Quadratdurchschnittswerte und der Zahl der EZ- und ZZ-Paare, zuviel oder zuwenig Fehler des Typs I ergaben.

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