A Test of the Equal Environment Assumption (EEA) in Multivariate Twin Studies

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In the classic twin design, estimation of genetic and environmental effects is based on the assumption that environmental influences are shared to the same extent by monozygotic and dizygotic twins (equal environment assumption, EEA). We explore the conditions in which the EEA can be tested based on multivariate phenotypic data. We focus on the test whether the correlation between shared environmental factors in dizygotic twins (r_C) is less than 1. First, model identification was investigated analytically in Maple and Mx. Second, statistical power was examined in Mx. Third, the amount of bias caused by violation of the EEA was evaluated. Finally, applications to empirical data concern spatial ability in adolescents and aggression in children. Bivariate and trivariate models include several instances in which the EEA can be tested. The number of twin pairs that is needed to detect violation of the EEA with a statistical power of .80 ($\alpha = .05$) varied between 508 and 3576 pairs for the situations considered. The bias in parameter estimates, given misspecification, ranged from 5% to 34% for additive genetic effects, and from 4% to 34% for shared environmental effects. Estimates of the nonshared environmental effects were not biased. The EEA was not violated for spatial ability or aggression. Multivariate data provide sufficient information to test the validity of the EEA. The number of twin pairs that is needed is no greater than the number typically available in most twin registries. The analysis of spatial ability and aggression indicated no detectable violation of the EEA.

Genetic and environmental contributions to individual differences in phenotypic traits can be estimated using genetically informative data. In the case of twin data, the estimates are based on the monozygotic (MZ) and dizygotic (DZ) twin variances and covariances. With such data, a number of competing models can be considered. These models may include additive genetic effects (A), dominant genetic effects (D), shared environmental effects (C), and nonshared environmental effects (E). Estimates of these effects in the twin design are based, inter alia, on the following three assumptions: (i) the additive (dominant) genetic effects correlate 1 (1) in MZ twin pairs and .5 (.25) in DZ twin pairs, (ii) the shared environmental effects correlate 1 in both MZ and DZ twin pairs, and (iii) the nonshared environmental effects do not correlate in MZ and DZ twin pairs. In this article, we explore the possibilities of testing the second assumption, usually referred to as the equal environment assumption (EEA), given bivariate or trivariate data. The EEA implies that the shared environmental influences are equally important in MZ and DZ twin pairs. The validity of the EEA has been debated (Faraone & Biederman, 2000; Joseph, 2000).

It has been shown that MZ twins in childhood more often share playmates, share the same room, and dress more alike than same-sex DZ twins (Loehlin & Nichols, 1976). However, this does not necessarily imply that the EEA is violated. First, the greater environmental similarity in MZ than DZ twins does not have to be related to a greater phenotypic similarity. Second, even if a greater environmental similarity *is* related to a greater phenotypic similarity, this association could be mediated by a greater genetic similarity in MZ than DZ twins (Scarr & Carter-Saltzman, 1979). The EEA is only violated when the correlation between environmental similarity and trait similarity is significantly greater than zero *within* zygosity groups. Eaves et al. (2003) concluded on the basis of simulation studies that the absence of any association between environmental similarity and trait similarity justifies the claim that environmental similarity is not a factor in twin resemblance. However, the counterclaim that the presence of an association between environmental similarity and trait similarity falsifies the EEA is unfounded.

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Several methods have been proposed to detect violation of the EEA. One way of assessing the validity of the EEA is to see whether *within* zygosity groups, differences in environmental experiences are associated with differences in phenotypic traits (Loehlin & Nichols, 1976). The influence of several environmental experiences have been studied, including ratings of treatment similarity (Loehlin & Nichols, 1976; Rowe et al., 2002; Wade et al., 2003), physical similarity (Hettema et al., 1995), and frequency of contact as adults (Kendler, Karkowski, et al., 2000). Loehlin and Nichols (1976) reported low correlations between differential treatment scores and mental ability, personality traits, vocational interests, and interpersonal relationships within zygosity groups. The range of correlations was –.15 to +.22, which is about what one would expect on the basis of change fluctuation. This suggests that the EEA is not violated for a wide variety of phenotypic traits. The validity of the EEA was also confirmed by more recent studies, which included measures of the influence of specified environmental measures on psychiatric diseases, such as major depression, generalized anxiety disorder, panic disorder, phobias, posttraumatic stress disorder, nicotine dependence, marijuana dependence, alcohol dependence, and psychoactive substance use and abuse (Hettema et al., 1995; Kendler & Gardner, 1998; Kendler, Karkowski, et al., 2000; Xian et al., 2000). The EEA has also been found to be tenable for sexual orientation (Kendler, Thornton, et al., 2000). In contrast, possible violation of the EEA was reported for smoking initiation (Kendler & Gardner, 1998), and bulimia (Hettema et al., 1995; Kendler & Gardner, 1998; Rowe et al., 2002).

Another test for the validity of the EEA is provided by twins whose genetic similarity is misperceived by themselves and others (Scarr & Carter-Saltzman, 1979). If beliefs about zygosity determine the extent to which the two members of a twin pair are behaviorally similar, then DZ twins who believe they are MZs should be similar to MZs. Likewise, MZs who believe they are DZ will be as different as true DZs. Scarr and Carter-Saltzman (1979) showed that twin resemblance with respect to cognitive measures was in accordance with the true, not the self-perceived, zygosity. For personality measures, DZs who believed they were MZs were more similar than those who correctly believed they were DZ. However, it turned out that beliefs about zygosity were highly related to genetic similarity at 12 loci, so the segregating genes of these DZ pairs might indeed correlate higher than .5. Recent studies suggest that perceived zygosity does not influence similarity on psychiatric and substance dependence disorders (Kendler & Gardner, 1998; Xian et al., 2000).

Finally, correlations of MZ twins reared together can be compared to correlations of MZ twins reared apart (MZA; Bouchard et al., 1990). MZA twins presumably do not share environmental influences so that the MZA correlation can be interpreted as an heritability estimate. Thus, a violation of the EEA has no consequence for the heritability estimate obtained with the MZA study design. Bouchard et al. (1990) report a heritability of about 70% for mental ability, and a heritability of about 50% for personality variables. As these heritability estimates resemble those based on data from MZ and DZ twins reared together, the EEA seems tenable.

The inclusion of specified familial environmental measures or perceived zygosity provides a means to detect violation of the EEA with respect to these environmental measures. However, a violation of the EEA will not be detected if it is related to factors other than those that are measured. For instance, the EEA was violated for some environmental measures related to bulimia (Hettema et al., 1995; Wade et al., 2003), but not for others (Kendler et al., 1991). Another concern with these methods is that they cannot be applied in the absence of an environmental measure or index of perceived zygosity. The purpose of this article is to explore an alternative approach to testing the validity of the EEA in multivariate data. Given multivariate data, and provided certain conditions are met, the shared environmental correlation can be estimated in DZ twins. If, in these situations, the shared environmental correlation in DZ twins does not deviate significantly from 1, this would suggest that the EEA is tenable. Below, we first identify the conditions in which estimation of the shared environmental correlation in DZ twins is possible. Next, we examine the statistical power to detect a violation of the EEA, and estimate the amount of bias (e.g., overestimation of the genetic effects and underestimation of the shared environmental effects) introduced by the given violation of the EEA. Finally, we apply this method to data on two indicators for spatial ability in 12- to 19-year-old twins and three indicators of maternal rated aggression in 7-year-old twins.

Materials and Methods

Model Description

We employ a standard biometric model to test the EEA in bivariate and trivariate data sets. We assume that the phenotypic MZ and DZ covariance matrices are sufficient statistics as this facilitates the assessment of identifiability. Given m phenotypes (m = 2 or 3 in the present article), the number of observed statistics, that is, (co)variances, is nv*(nv + 1), where nv equals m*2 (we do not consider the means, as they provide no information). However, not all variances and covariances have unique expectations in the twin model. Given m = 2 and nv = 4, the total number of observed statistics is 20. However, the number of statistics with unique expectations is nine, consisting of two variances (for variable 1 and variable 2), one within-subjects covariance, and six between-subjects covariances (three in MZ twins and three in DZ twins). Given m = 3 and nv = 6, the total number of

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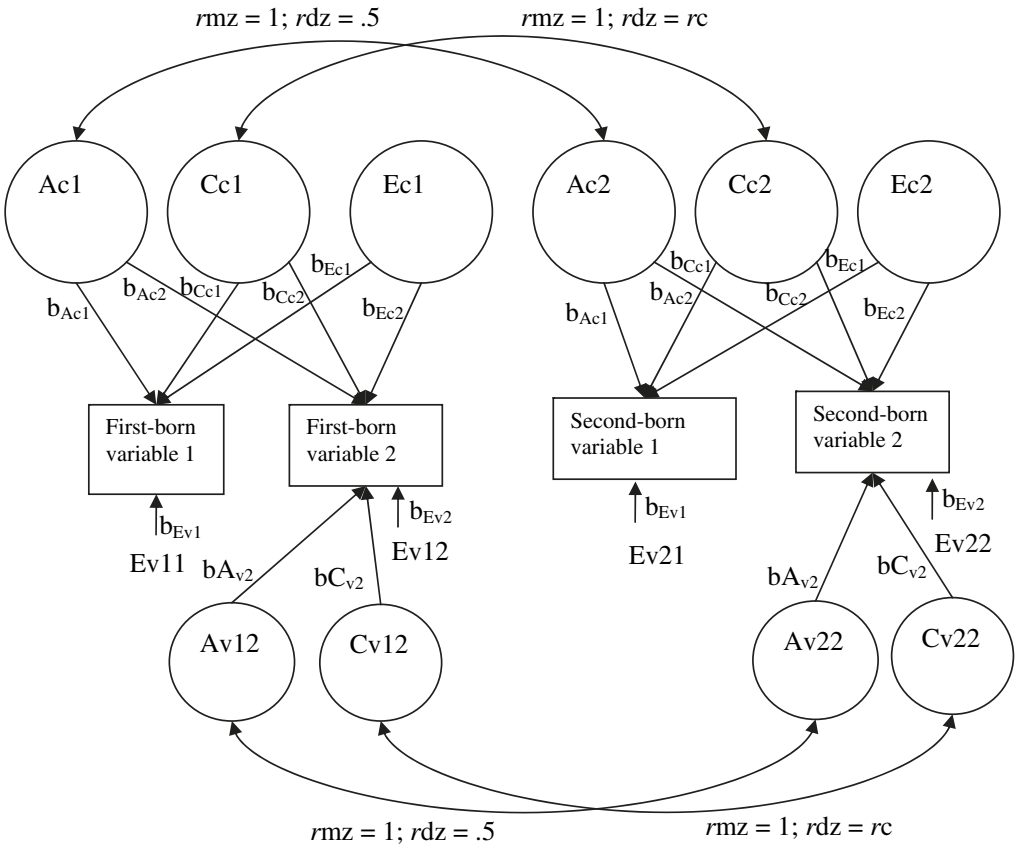

Figure 1

Illustration of the biometric model to test the validity of the equal environment assumption with two observed variables.

Note: Variation in variable 1 and 2 is explained by common (c), and variable specific (v) additive genetic effects (Ac, Av), shared environmental effects (Cc, Cv), and nonshared environmental effects (Ec, Ev). The correlation of A (r_a) is 1 in MZ twins and .5 in DZ twins. The correlation of C (r_c) is 1 in MZ twins, and is freely estimated in DZ twins. In addition, the influence of Av can only be estimated if the influences of Ac are constrained to be equal for variable 1 and variable 2. The r_c of Cv should be equated to the r_c of Cc or should be constrained at 1. Although the model is not identified in its current form, constraints which render the model identified can be made. These constraints are provided in Table 2.

observed statistics in MZ and DZ twins is 42. The number of statistics with unique expectations is 18, consisting of 3 variances, 3 within-subjects covariances, and 12 between-subjects covariances (6 in MZ twins and 6 in DZ twins).

In the standard biometric model, additive genetic influences (A) are assumed to correlate 1 in MZ twins and .5 in DZ twins. The nonshared environmental influences (E) are uncorrelated in MZ and DZ twins. Influences of A, C and E may be common to all observed phenotypes (Ac, Cc, and Ec) or may be variable specific (Av, Cv, and Ev).

In the case of m = 2, nine parameters are estimated in this biometric model, which results in a model that is just identified and no degree of freedom is left to test the validity of the EEA. Below, we explore the constraints that do allow such a test. After establishing that the various constraints do not lead to a decrease in model fit, the validity of the EEA can be tested by constraining the correlation of the Cc variables (r_{Cc}) at 1 in MZ twins, and estimating it freely in DZ twins. The correlation of the Cv variables, r_{Cv}, can be equated to r_{Cc} or fixed to 1. In this article, we chose to equate r_{Cv} and r_{Cc} ($r_{Cv} = r_{Cc} = r_C$). If r_C is significantly lower than 1 in DZ twins, this suggests that the EEA is violated. Graphical representations of the biometric model for two and three observed variables are shown in Figure 1 and Figure 2, respectively.

Simulation Analyses

We established model identification by calculating the null space of the Jacobian of the model. In contrast to the empirical test (necessary, but not sufficient), this test is necessary and sufficient (Bekker et al., 1993). Let σ denote the $q = nv^*(nv + 1)$ dimensional vector of expected (co)variances of both the MZs and the DZs, and let θ denote the p-dimensional vector of free (i.e., unknown, to be estimated) parameters. The $p \times q$ Jacobian matrix simply equals $\partial\sigma/\partial\theta$. As explained by Bekker et al. (1993), the model is identified if the null space is empty. We used Maple (e.g., Heck, 1993) to carry out this test (Maple worksheet is available on e-mail request).

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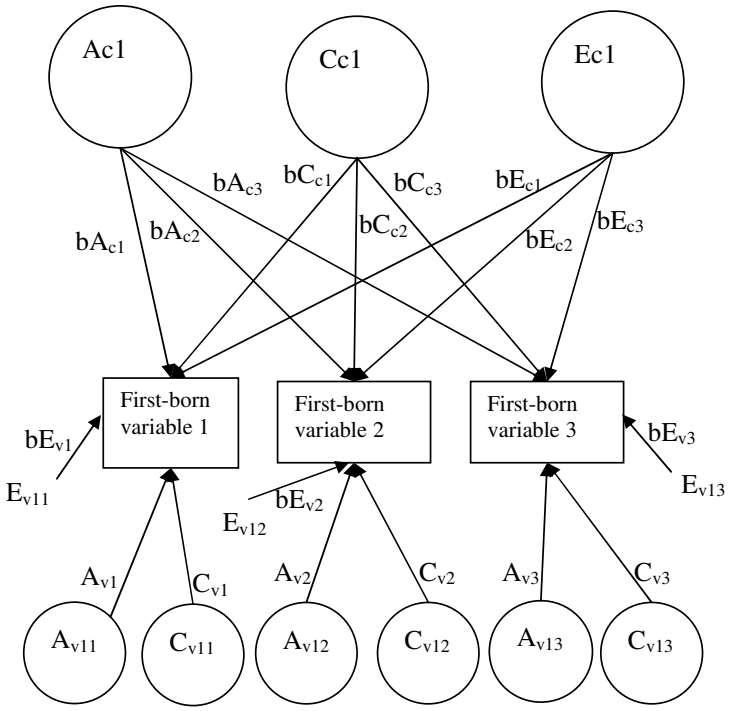

Figure 2

Illustration of the biometric model to test the validity of the equal environment assumption with three observed variables.

Note: Variation in variable 1, 2, and 3 is explained by additive genetic effects (Ac, Av), shared environmental effects (Cc, Cv), and nonshared environmental effects (Ec, Ev). For the sake of clarity, the model is only given for one member of a twin pair. The correlations of Ac and Av are 1 in MZ twins and .5 in DZ twins, and the correlations of Cc and Cv (r_c) are 1 in MZ twins, and freely estimated in DZ twins (but constrained to be equal for Cc and Cv). Although the model is not identified in its current form, eight different constraints which render the model identified can be made. These constraints are provided in Table 3.

To establish if there are certain conditions in which r_C can not be estimated in DZ twins, expected (i.e., population) covariance matrices were calculated in Mx (Neale et al., 2003), given a choice of parameter values. The parameter values of the factor loadings ranged between –5 and 5, and the parameter values chosen for r_C ranged between .3 and .8. Biometric models were fit to these covariance matrices. DZ twins usually outnumber MZ twins, we therefore maintained a 1:2 ratio of MZ to DZ. Model identification was checked empirically by establishing that the true parameter values were recovered, and by computing the confidence intervals of the parameter estimates. If the model is identified, the true parameter values should be recovered exactly regardless of the starting values, the chi-square should equal zero, and the calculation of confidence intervals should pose no computational problems.

Having established model identification, we investigated the statistical power by calculating the number of twin pairs that is required to reject the constrained model with $r_C = 1$ with a probability of .80 and a significance level α of .05. To estimate the bias caused by the violation of the EEA, we compared the simulated standardized genetic and environmental estimates with the estimated standardized genetic and environmental influences when the shared environmental correlation was constrained at 1 in DZ twins (i.e., misspecified).

We performed power calculations for one model with two observed variables, and for one model with three observed variables. In the model with two observed variables, the loadings of Ac on variable 1 and 2 equaled 1 and 2, respectively. The loadings of Cc on variable 1 and 2 equaled 2 and 1, respectively. The loadings of Ec, and Ev equaled 1 on both variables. The loadings of Av and Cv on variable 2 equaled 0. In the model with three observed variables, loadings of Ac equaled 1, 2, and 3; Av equaled 2, 2, and 2; Cc equaled 3, 2, and 1; and Cv equaled 2, 2, and 2, for variable 1, 2, and 3, respectively. Ec and Ev equaled 1 for all three observed variables.

Analysis of Two Indicators for Spatial Ability

Spatial ability data were available for 171 same-sex MZ twin pairs, and 133 same-sex DZ twin pairs aged 12 to 19 years (Osborne, 1980). We did not consider sex differences in view of the relatively small sample size. The two indicators for spatial ability are cube comparison and surface development. In the cube comparison test, each item consists of two cubes of which three sides are visible. The testee has to determine whether the cubes are possibly identical. In the surface development test, the testee has to determine whether a piece of paper with a given form can be folded in to a given three-dimensional form (e.g., a square box).

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Table 1

Overview of the Constraints That Allow Estimation of the Shared Environmental Correlation (r_c) in DZ Twins for the Bivariate Model

	r_c (in DZ twins)	Identifying constraint	*N* parameters	Is the model identified?
Model 1	$r_c = 1$	bEc1 = bEc2 or bEv1 = bEv2	9	Yes
Model 2	r_c = free	bEc1 = bEc2 or bEv1 = bEv2	10	No
Model 3	r_c = free	(bEc1 = bEc2 or bEv1 = bEv2) and bAc1 = bAc2	9	Yes
Model 4	r_c = free	bEc1 = bEc2 and bAv2 = 0	9	Yes

Note: Model description (for explanation of the symbols used, please see Figure 1):

Twin 1, variable 1: $\gamma 11 = b_{Ac1}*Ac1 + b_{Cc1}*Cc1 + b_{Ec1}*Ec1 + b_{Ev1}*Ev11$

Twin 1, variable 2: $\gamma 12 = b_{Ac2}*Ac1 + b_{Cc2}*Cc1 + b_{Ec2}*Ec1 + b_{Av2}*Av12 + b_{Cv2}*Cv12 + b_{Ev2}*Ev12$

Twin 2, variable 1: $\gamma 21 = b_{Ac1}*Ac2 + b_{Cc1}*Cc2 + b_{Ec1}*Ec2 + b_{Ev1}*Ev21$

Twin 2, variable 2: $\gamma 22 = b_{Ac2}*Ac2 + b_{Cc2}*Cc2 + b_{Ec2}*Ec2 + b_{Av2}*Av22 + b_{Cv2}*Cv22 + b_{Ev2}*Ev22$.

Analysis of Three Indicators for Aggression

Mothers of 1534 Dutch twin pairs who are registered with the Netherlands Twin Register (Boomsma et al., 2002) completed the Child Behavior Checklist (CBCL; Achenbach, 1991) and the Conners Parent Rating Scale-Revised: Short version (CPRS-R:S; Conners, 2001) when the children were 7 years old. Subjects were divided into six groups: male MZ (244 pairs), male DZ (269 pairs), female MZ (285 pairs), female DZ (249 pairs), male–female opposite-sex pairs (first-born is male, second-born is female; 243 pairs), and female-male opposite-sex pairs (first-born is female, second-born is male; 244 pairs). The CBCL contains 20 items on aggression, which can be subdivided into two subscales: direct aggression (six items) and relational aggression (14 items; Ligthart et al., 2005). The CPRS-R:S contains six items on oppositional behavior. Analyses were performed on sum scores of these three subscales: direct aggression, relational aggression, and oppositional behavior.

Results

Model Identification

The test based on the Jacobian and the empirical calculations pointed out that a number of bivariate and trivariate models allow estimation of r_C. The constraints that identify the model are summarized in Table 1 and Table 2, for two and three observed variables, respectively. It is possible that additional identifying constraits exist that were not considered in this paper. Before testing the validity of the EEA, it should first be established that the identifying constraint does not lead to a significant decrease in model fit. Otherwise, the possible misfit of the model in which the EEA is assumed to be tenable, can be the result of the misfit of the constraint.

The empirical calculations proved useful to explore specific configurations of parameter values, which rendered the model unidentified. First, the correlation of the shared environment (r_C) in DZ twins should not

Table 2

Overview of the Constraints That Allow Estimation of the Shared Environmental Correlation (r_c) in DZ Twins for the Trivariate Model

	r_c (in DZ twins)	Identifying constraint	*N* parameters	Is the model identified?
Model 1	$r_c = 1$	None	18	Yes
Model 2	r_c = free	None	19	No
Model 3	r_c = free	bAv1 = 0 or bAv2 = 0 or bAv1 = 0	18	Yes
Model 4	r_c = free	bAv1 = bAv2 or bAv1 = bAv3 or bAv2 = bAv3	18	Yes
Model 5	r_c = free	bAc1 = bAc2 or bAc1 = bAc3 or bAc2 = bAc3	18	Yes
Model 6	r_c = free	bAc1 = bCc1 or bAc2 = bCc2 or bAc3 = bCc3	18	Yes
Model 7	r_c = free	bEc1 = bCc1 or bEc2 = bCc2 or bEc3 = bCc3	18	Yes
Model 8	r_c = free	bAv1 = bCv1 or bAv2 = bCv2 or bAv3 = bCv3	18	Yes
Model 9	r_c = free	bAc1 = bAv1 or bAc2 = bAv2 or bAc3 = bAv3	18	Yes
Model 10	r_c = free	bAc1 = bEv1 or bAc2 = bEv2 or bAc3 = bEv3	18	Yes

Note: Model description (for explanation of the symbols used, please see Figure 2):

Twin 1, variable 1: $\gamma 11 = b_{Ac1}*Ac1+b_{Cc1}*Cc1 + b_{Ec1}*Ec1 + b_{Av1}*Av11 + b_{Cv1}*Cv11 + b_{Ev1}*Ev11$

Twin 1, variable 2: $\gamma 12 = b_{Ac2}*Ac1+b_{Cc2}*Cc1 + b_{Ec2}*Ec1 + b_{Av2}*Av12 + b_{Cv2}*Cv12 + b_{Ev2}*Ev12$

Twin 1, variable 3: $\gamma 12 = b_{Ac3}*Ac1+b_{Cc3}*Cc1 + b_{Ec3}*Ec1 + b_{Av3}*Av13 + b_{Cv3}*Cv13 + b_{Ev3}*Ev13$

Twin 2, variable 1: $\gamma 21 = b_{Ac1}*Ac2+b_{Cc1}*Cc2 + b_{Ec1}*Ec2 + b_{Av1}*Av21 + b_{Cv1}*Cv21 + b_{Ev1}*Ev21$

Twin 2, variable 2: $\gamma 22 = b_{Ac2}*Ac2+b_{Cc2}*Cc2 + b_{Ec2}*Ec2 + b_{Av2}*Av22 + b_{Cv2}*Cv22 + b_{Ev2}*Ev22$

Twin 2, variable 3: $\gamma 22 = b_{Ac3}*Ac2+b_{Cc3}*Cc2 + b_{Ec3}*Ec2 + b_{Av3}*Av23 + b_{Cv3}*Cv23 + b_{Ev3}*Ev23$.

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Table 3

Bias in Standardized Parameter Estimates if Violation of the Equal Environment Assumption Is Not Accommodated (True/Biased Estimate)

		Variable 1			Variable 2			Variable 3		
	r_c (DZ)	A	C	E	A	C	E	A	C	E
Model 1 (2 indicators)	.90	14/20	57/50	29/31	57/62	14/10	29/28	—	—	—
	.70	14/38	57/29	29/32	57/70	14/2	29/29	—	—	—
Model 2 (3 indicators)	.90	25/35	65/54	10/10	44/55	44/34	11/11	65/70	25/20	10/10
	.70	25/58	65/31	10/10	44/78	44/11	11/11	65/81	25/9	10/10

Note: A = additive genetic effects, C = shared environmental effects, E = nonshared environmental effects.

r_c is the simulated value of the shared environmental correlation. The estimates of A, C, and E are summed over the common and variable specific factors. The true parameter estimates are obtained with r_c fixed at its true value. The biased parameter estimates are obtained with r_c constrained at 1.

equal .5. Clearly, if r_C is equal to .5, the shared environmental effects cannot be distinguished from the genetic effects, as these also correlate 1 in MZ twins and .5 in DZ twins. Second, the factor loadings of Cc should not be identical for all observed variables. This second condition implies that the observed variables are not allowed to correlate perfectly. Third, the model is not identified if the factor loadings of Ac and Cc are collinear (i.e., the factor loadings of Ac and Cc on an observed variable are the factor loadings of Ac and Cc on another variable, multiplied by a constant). We will illustrate the latter problem for the model with two observed variables V1 and V2 which are observed in twin 1 (T1) and twin 2 (T2):

(I) Covariance (V1T1,V1T2) = $bA_{c1} * r_A * bA_{c1} + bC_{c1} * r_C * bC_{c1}$

(II) Covariance (V1T1,V2T2) = $bA_{c1} * r_A * bA_{c2} + bC_{c1} * r_C * bC_{c2}$

If, for a certain value for a constant c, $bA_{c2} = c * bA_{c1}$, and $bC_{c2} = c * bC_{c1}$, then

(III) Covariance (V1T1,V2T2) = c*Covariance (V1T1,V1T2)

Therefore, it may seem that we observe four covariances (two covariances in MZ twins and two covariances in DZ twins), but two of the covariances are a function of the other two covariances plus the constant c, which results in only three pieces of unique information.

Statistical Power to Detect Violation of the Equal Environment Assumption

The statistical power to detect violation of the EEA depends on the magnitude of the genetic and shared environmental influences. For illustrative purposes, we performed power calculations for one model with two observed variables, and for one model with three observed variables. The parameter values of the models are provided in the methods section. The model with two indicators was identified by constraining the influences of Av and Cv on variable 2 at zero. If r_C equals .70, 618 twin pairs are needed to detect violation of the EEA (for a statistical power of .80 at an α of .05). If r_C equals .90, 3576 twin pairs are needed to detect violation of the EEA. The model with three indicators was identified by constraining Av to be equal for variable 1, 2, and 3. In the case of r_C equals .70, 508 twin pairs are needed to detect violation of the EEA. If r_C equals .90, 2111 twin pairs are needed to detect violation of the EEA.

Bias in Parameter Estimates When Violation of the Equal Environment Assumption is Ignored

How large is the bias in parameter estimates when violation of the EEA is not accommodated in the fitted model? The true standardized influences of A, C, and E are compared to the estimated values when r_C is fixed at 1 in DZ twins for the two models that are described above. The influences of variable specific and common factors in the biometric model are summed. Table 3 summarizes the true and biased standardized estimates for the two models. Depending on the magnitude of the genetic and shared environmental influences, the heritability is overestimated with 5% to 34%, and the shared environmental influences are underestimated with 4% to 34%. The nonshared environmental influences do not show much bias.

Is the Equal Environment Assumption Violated for Spatial Ability?

We tested the validity of the EEA by analyzing data on two indicators of spatial ability: cube comparison and surface development. The results of the model fitting analyses are summarized in Table 4. As was shown before, the number of unique observed statistics in the variance/covariance matrix of these data is nine. First, an ACE model was fit to the data, in which a Cholesky decomposition was specified for A, and C, and a common factor model for E (the full biometric model). This model estimates nine parameters for the covariance structure: three factor loadings for A, C, and E, respectively. Second, before testing the validity of the EEA, we had to find a constraint that identifies the model, but does not lead to a significant decrease in model fit. The loadings of Ac on cube comparison and surface development were significantly different. Therefore, we could not identify the model by constraining these parameters to be equal. The loading of Av on surface development was not

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Table 4

Model-Fitting Results and Parameter Estimates for Two Indicators of Spatial Ability in 304 Twin Pairs

Model	Constraints	–2 log LL	*N* Par	Compared to model	*df*	χ^2	*p*
1. Full biometric model	bEc1 = bEc2, $r_C = 1$	8636.61	17*	—	—	—	—
2. Identifying constraint 1	bEc1 = bEc2, bAc1 = bAc2, $r_C = 1$	8640.54	16	1	1	3.93	.047
3. **Identifying constraint 2**	**bEc1 = bEc2, bAv2 = 0, $r_C = 1$**	**8637.51**	**16**	**1**	**1**	**0.898**	**.343**
4. Violation of the EEA allowed	bEc1 = bEc2, bAv2 = 0, r_C = free	8636.61	17	3	1	0.898	.343

Note: The total number of parameters of the full biometric model = 17, consisting of 8 parameters for the means and 9 parameters for the variance/covariance matrix. The best fitting model is printed in bold.

significantly different from zero, so we identified the model in which the validity of the EEA can be investigated by constraining this loading at zero. Freely estimating r_C did not lead to significant increase in model fit, which suggests that the EEA is not violated. In Table 5, we included the parameter estimates of the best fitting model.

Is the Equal Environment Assumption Violated for Aggression?

We tested the validity of the EEA with respect to aggression by analyzing three indicators for aggression: relational and direct aggression, and oppositional behavior. The model fitting results are shown in Table 6. First, we fitted a standard biometric model. The influences of Av on relational and direct aggression could be equated in both boys and girls, so we used this as the identifying constraint. Next, we fitted the model in which the r_C is estimated in DZ male twins and DZ female twins and opposite-sex twins. We tested if the r_C was significantly lower than 1 in DZ same-sex twins. We did not constrain the r_C in opposite-sex twins at 1, because r_C may be lower than 1 due to different environmental influences in boys and girls. The validity of the EEA was not violated. The parameter estimates of the best fitting model are shown in Table 7.

Discussion

One of the most widely debated assumptions of the twin method is the EEA. The EEA requires that *within* zygosity groups, differences in environmental experiences are not associated with differences in phenotypic traits. In this article, we demonstrated that when two or more variables are observed in both members of a twin pair, the shared environmental correlation can be estimated in DZ twins. By testing whether the shared environmental correlation is significantly lower than 1 in DZ twins, violation of the EEA can be detected in the absence of measured environmental variables.

We showed that estimation of the shared environmental correlation (r_C) is possible if: (i) two or more indicators of a phenotypic trait are measured; (ii) the shared environmental correlation in DZ twins is different from .5; (iii) the factor loadings of Cc are not identical for all observed variables (which implies that the variables are not allowed to correlate perfectly); (iv) the factor loadings of Ac and Cc are not collinear; and (v) an identifying constraint that does not lead to a significant decrease in model fit exists. Condition (ii) does not have to be fulfilled when data from genetically unrelated siblings are available. In genetically unrelated siblings, additive genetic effects do not correlate and the correlation of the shared environmental effects is similar to the DZ shared environmental correlation.

If these five conditions are met, the validity of the EEA can be tested. Although it may seem that an additional requirement is that the influence of the shared environmental effects should be significantly greater than zero in the univariate analyses, this is not the case. In fact, the model is also identified when the shared environmental correlation is lower than .5 in DZ twins. In this case, the correlation of DZ twins is not more than half the MZ correlation and no significant influence of the shared environment would be found in the univariate analyses.

A concern when testing the validity of the EEA is that the correlation in opposite-sex twins may be lower than the MZ and same-sex DZ correlation as a result of different environmental influences in boys and girls. When combining data from opposite-sex and same-sex DZ twins, this may lead to rejection of the validity of the EEA while the lower correlation is actually the result of environmental sex-limitation. Therefore, in case of a significantly lower correlation in opposite-sex twins than in same-sex DZ twins, the data from same-sex DZ twins and opposite-sex twins should not be combined. The validity of the EEA can be tested by constraining the correlation of the shared

Table 5

Overview of the Standardized Genetic and Environmental Influences on the Variance and Covariance of Two Indicators of Spatial Ability

Variable	A	C	E
Cube comparison	17	28	55
Surface development	59	16	24
Covariance cube comparison / surface development	58	39	3

Note: A = additive genetic effects, C = shared environmental effects, E = nonshared environmental effects.

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Table 6

Model-Fitting Results and Parameter Estimates for Three Indicators of Aggression in 1534 Twin Pairs

Model	Constraints	–2 log LL	*N* Par	Compared to model	*df*	χ^2	*p*
1. Full biometric model	r_c =1	40,845.61	73	—	—	—	—
2. **Identifying constraint 1**	**bAv1 = bAv2 (in boys and girls)**	**40,846.99**	**71**	**1**	**2**	**1.38**	**.502**
3. Violation of the EEA allowed	bAv1 = bAv2, r_c = free	40,845.90	73	2	2	1.09	.579

Note: The total number of parameters of the full biometric model = 73, consisting of 36 parameters for the means; and 37 parameters for the variance/covariance matrix (18 in boys, 18 in girls, and the shared environmental correlation in opposite-sex twins). The best fitting model is printed in bold.

environmental influences at 1 in same-sex DZ twins but not in opposite-sex twins.

The statistical power to detect violation of the EEA is acceptable. The number of twin pairs that is needed to detect violation of the EEA is no greater than the number of twin pairs typically available in most twin registries. Additional simulations showed that the power decreases when the factor loadings become more similar for the observed variables (data not shown). This is not surprising, as r_C can not be estimated when the observed variables correlate perfectly (i.e., the multivariate model simplifies to a univariate model).

It was found that ignoring violation of the EEA can sometimes lead to large bias in parameter estimates. The influence of the additive genetic effects is overestimated (5%–34%), and the influence of the shared environmental effects is underestimated (4%–34%).

Analyses of empirical data showed that the EEA is not violated for spatial ability in adolescents or aggression in children. For both phenotypes, the shared environmental correlation was not significantly lower than 1 in DZ twins. This is in accordance with the results of previous studies in which the validity of the EEA was supported (Kendler, Karkowski, et al., 2000; Loehlin & Nichols, 1976; Scarr & Carter-Saltzman, 1979). It is reassuring that the EEA seems to be a valid assumption for most traits, although its validity should be examined whenever possible.

Table 7

Overview of Standardized Genetic and Environmental Influences on the Variance and Covariance of Three Indicators of Aggression

Variable	A girls/boys	C girls/boys	E girls/boys
Relational aggression	63/44	7/37	30/18
Direct aggression	56/37	15/48	30/15
Oppositional	56/43	12/27	32/30
Covariance relational / direct aggression	73/34	5/55	22/11
Covariance relational aggression / oppositional	74/41	3/40	23/19
Covariance direct aggression / oppositional	68/35	2/46	30/19

Note: A = additive genetic effects, C = shared environmental effects, E = nonshared environmental effects.

Although the proposed method is useful for testing the validity of the EEA when no environmental measures are available, it also has a number of limitations. First, if a trait is influenced by dominant genetic influences (e.g., attention-deficit/hyperactivity disorder), shared environmental influences cannot be included in the model and the shared environmental correlation cannot be estimated. Therefore, in the presence of dominance, the proposed model cannot be used to test the validity of the EEA unless phenotypic data from other relatives are available. For example, inclusion of phenotypic data from the parents allows for the estimation of influences of A, C, D, and E on phenotypic variation if these parameters are not age dependent. A second concern is that the interpretation of possible violation of the EEA may be complicated. Imagine that a trait is influenced by a factor that correlates 1 in MZ twins and .7 in DZ twins. This factor can be interpreted as an environmental factor for which the EEA is violated. In contrast, it could also be a genetic factor, which correlates higher than .5 in DZ twins due to assortative mating. Therefore, given that $r_C < 1$ in DZ twins, the EEA is not necessarily violated.

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