Theor Appl Genet (1986) 73:27–30

A comparison between the full diallel cross and the simplified triple-test cross

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Received August 21, 1985; Accepted July 7, 1986
Communicated by A. Robertson

Summary. The simplified triple-test cross (sTTC) is a mating design that, because of its economic use of the experimental material as compared with other designs, seems very attractive. In theory, its power is almost equal to that of more elaborate designs such as the diallel cross. To evaluate the merits of both designs in a genetic analysis of mouse behavior, the results of a previous replicated 4×4 diallel cross (Crusio and van Abeelen 1986) were reanalyzed as a sTTC. We found that, at least with the fairly low number of strains employed, the sTTC analysis is clearly inferior to the diallel cross. This finding, in combination with some theoretical considerations, leads to the conclusion that the sTTC design is not a very useful one for such studies.

Key words: Quantitative genetics – Simplified triple-test cross – Diallel cross – Mouse behavior

Introduction

One important aim of theoretical quantitative genetics is the development of crossing designs that, as economically as possible, provide reliable information about the genetic variability existing in a population. The diallel cross is probably the design most frequently used for this purpose. In the diallel, a set of n inbred parents is intercrossed in all n^2 possible ways. A more economic design is the half-diallel cross, where reciprocal crosses are omitted, leaving $n(n+1)/2$ crosses. The merits of several alternative analyses of this design have been compared by Singh and Paroda (1984) and Singh and Singh (1984).

Kearsey and Jinks (1968) introduced the triple-test cross, which in its simplified form (sTTC), developed by Jinks et al. (1969), consists of the n inbred parents crossed to two phenotypically extreme tester lines. When the parents are included in the design, $3n$ crosses have to be bred. Thus, the sTTC is more economic than the diallel cross for $n > 3$ and more economic than the half-diallel for $n > 5$. Jinks et al. (1969) compared the merits of the sTTC with those of the diallel cross by analyzing data on two different variables extracted from a 20×20 full diallel cross and they found close agreement between the results rendered by both analyses. Fulker (1972) constructed sTTC's from six different diallel crosses ($6 \leqq n \leqq 8$) and, generally, also found a close resemblance between the results of the two analyses.

Previously, we examined the genetic underpinnings of mouse exploratory behavior in a novel environment by analyzing a 4×4 diallel cross, replicated five times (Crusio and van Abeelen 1986). To compare the merits of the sTTC with those of the diallel cross, employing this relatively small n, part of the data collected in our diallel were rearranged to form a sTTC design and analyzed accordingly.

Materials and methods

Mice and observation

The inbred mouse strains used were C57BL/6J // Nmg (B), DBA/2J // Nmg (D), C3H/St // Nmg (H), and CPB-K // Nmg (K). All relevant information concerning breeding, maintenance, behavioral observation procedure, open-field, ethogram, etc. can be found in Crusio and van Abeelen (1986). Briefly, 3-month-old single males were placed in the center of an illuminated open-field and observed directly and continuously for 20 min. The open-field, an observation cage measuring 109×49×49 cm, contained a prismatic metal object which was attached to the back wall, 5 cm above the floor,

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providing the mice with an opportunity for exploratory object-leaning and object-sniffing. Locomotor activity and the frequencies of rearing, leaning against the wall, object-leaning, sniffing, object-sniffing, jumping, gnawing, grooming, defecation, and urination were registered manually on counters. Durations of grooming and freezing were recorded with stopwatches.

Analysis

To satisfy the usual assumptions of homogeneity of variances, homoscedasticity, and normality of distributions, we applied a scaling procedure following the requirements and methods described by Crusio et al. (1984). Those transformations that proved to be the most adequate ones in the diallel-cross analysis (Crusio and van Abeelen 1986) were also used here.

The data for the sTTC analysis were extracted from our full diallel cross (Crusio and van Abeelen 1986). In this way, appropriate, i.e. phenotypically extreme, testers could be selected for each variable; the strain scoring highest is designated L_1, the lowest L_2. Dams were used as testers and the inbred strains provided the sires. The diallel had been replicated five times, which means that for each F_1 hybrid 5 litters were available. Because of an additional replication of the leading diagonal of the diallel, 10 litters were available for each inbred group. To avoid biases due to unequal cell sizes, 5 litters were randomly taken from each inbred strain to provide for the parental generations. The extra litters were not used, except those of the two testers; these were used for the within-tester crosses so that values independent of those from the parentals were obtained. Each litter consisted of three animals; litter means were used as the experimental units.

The analytical procedures of the sTTC have been described in full by Jinks et al. (1969) and Fulker (1972), and the reader is referred to these authors for the relevant details. Briefly, for each inbred strain we calculate the sums ($L_{1i}+L_{2i}$) of, and the differences ($L_{1i}-L_{2i}$) between, the scores of the tester×strain F_1's. We also calculate $L_{1i}+L_{2i}-P_i$ for each strain. The sums of squares of these values provide the values for the Sums-, the Differences-, and the Epistasis-items, respectively, and their significance is tested against the within-cell variance. If the Epistasis-item is nonsignificant, the Sums-item tests for additive genetic variation and the Differences-item for dominance. Their expected mean squares equal $E+\frac{1}{2}\beta D$ and $E+\frac{1}{2}\beta H$, respectively (β denotes the number of replications). The error mean square estimates E, of course. From these estimates, the narrow and the broad heritability and the degree of dominance can be calculated. Sometimes negative estimates for D or H are obtained due to sampling error. If so, this parameter is taken as zero in subsequent calculations.

A significant Pearson product-moment correlation, corrected for small n (Sachs 1974), between the sums and differences is an indication of directional dominance. The sign of this correlation coefficient will be opposite to that of the direction of the dominance (Hewitt 1980).

Table 1. Results of the simplified triple-test cross analysis of the inbred mouse strains C57BL/6 (B), DBA/2 (D), C3H/St (H), and CPB-K (K)

Variable		Locomotion	Rearing	Leaning	Object-leaning	Sniffing	Object-sniffing	Jumping
Transformation		raw	$\sqrt{x}+\sqrt{(x+1)}$	$\sqrt{x}+\sqrt{(x+1)}$	raw	raw	raw	$x^{1/3}$
L_1		B	B	H	H	B	H	H
L_2		K	K	K	K	D	K	K
	df	*F*-values						
Sums	3,48	4.83**	1.81	3.27*	4.82**	1.27	3.78*	2.85*
Differences	3,48	1.51	4.42**	4.61**	0.63	1.16	2.20	0.37
Epistasis	3,48	1.85	4.14*	1.41	0.48	0.30	1.25	1.24
		Correlation						
r_{S-D}	2	−0.23	−0.67	0.06	−0.90	−0.66	−0.98*	−0.96*

Variable		Gnawing	Defecation	Urination	Groom frequency	Groom duration
Transformation		$\sqrt{x}+\sqrt{(x+1)}$	$\sqrt{x}+\sqrt{(x+1)}$	$1/(x+1)$	$x^{1/3}$	$x^{1/3}$
L_1		D	D	K	D	D
L_2		K	B	D	B	B
	df	*F*-values				
Sums	3,48	5.89**	0.40	3.90*	2.66	10.70***
Differences	3,48	2.40	1.45	2.65	1.92	8.17***
Epistasis	3,48	1.04	0.79	1.81	0.42	0.44
		Correlation				
r_{S-D}	2	0.83	−0.53	0.14	0.05	0.69

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 2. Comparison between the results of the diallel-cross analysis and the simplified triple-test-cross analysis

Variable transformation	Locomotion raw		Rearing $\sqrt{x}+\sqrt{(x+1)}$		Leaning $\sqrt{x}+\sqrt{(x+1)}$		Object-leaning raw	
	sTTC	Diallel[a]	sTTC	Diallel	sTTC	Diallel	sTTC	Diallel
E	2967.7	1979.3	6.87	6.12	3.42	3.37	42.95	57.63
D	4546.2	12792.2	2.24 (ns)	6.47	3.11	6.04	65.67	34.43
H	602.7 (ns)	5572.8	9.40	19.89	4.94	3.77	0.00 (ns)	45.26
$\sqrt{H/D}$	0.36	0.66	2.05	1.75	1.26	0.79	0.00	1.15
$h^2_{(n)}$	0.42	0.49	0.11	0.03	0.25	0.46	0.43	0.12
$h^2_{(b)}$	0.45	0.64	0.34	0.40	0.45	0.58	0.43	0.22
Directional dominance	none	none	none	for high	none	none	none	none
Epistasis	none	none	present	present	none	none	none	none

Variable transformation	Sniffing raw		Object-sniffing raw		Jumping $x^{1/3}$		Gnawing $\sqrt{x}+\sqrt{(x+1)}$	
	sTTC	Diallel	sTTC	Diallel	sTTC	Diallel	sTTC	Diallel
E	6776.4	3289.6	88.86	116.54	0.41	0.27	0.50	0.46
D	725.1 (ns)	6711.9	98.66	142.13	0.38	0.19	0.98	0.71
H	440.2 (ns)	2027.0	42.81 (ns)	51.33	0.00 (ns)	0.43	0.28 (ns)	0.24
$\sqrt{H/D}$	0.78	0.55	0.66	0.60	0.00	1.52	0.54	0.59
$h^2_{(n)}$	0.05	0.24	0.33	0.24	0.32	0.07	0.46	0.31
$h_{(b)}$	0.07	0.31	0.40	0.30	0.32	0.26	0.53	0.38
Directional dominance	none	none	for high	for high	for high	for high	none	for low
Epistasis	none	none	none	none	none	present	none	none

Variable transformation	Defecation $\sqrt{x}+\sqrt{(x+1)}$		Urination $1/(x+1)$		Groom frequency $x^{1/3}$		Groom duration $x^{1/3}$	
	sTTC	Diallel	sTTC	Diallel	sTTC	Diallel	sTTC	Diallel
E	1.64	1.21	0.03	0.03	0.07	0.06	0.41	0.34
D	0.00 (ns)	0.34	0.03	0.00 (ns)	0.05 (ns)	0.06	1.61	1.83
H	0.29 (ns)	0.93	0.02 (ns)	0.02	0.03 (ns)	0.03	1.19	1.64
$\sqrt{H/D}$	–	1.65	0.76	4.80	0.75	0.76	0.86	0.95
$h^2_{(n)}$	0.00	0.07	0.33	0.00	0.23	0.16	0.53	0.35
$h^2_{(b)}$	0.04	0.22	0.43	0.12	0.30	0.25	0.73	0.64
Directional dominance	none	none	none	none	none	none	none	none
Epistasis	none	present	none	none	none	none	none	none

[a] See Crusio et al. (1984) and Crusio and van Abeelen (1986)

Results

The results of the sTTC analyses are entered in Table 1. Additive genetic variation was found for leaning, object-leaning, object-sniffing, jumping, gnawing, urination, and grooming duration and ambidirectional dominance was found for rearing, leaning, and grooming duration. In two cases, object-sniffing and jumping, no dominance was detected although the correlation between sums and differences was significant. According to Jinks et al. (1969) this situation is not diagnostic for the presence of dominance but trivial, probably arising as the result of sampling error. Epistasis was detected for rearing only.

The genetic parameters estimated in the sTTC and the conclusions on the genetic underpinnings of the

phenotypes studied are presented in Table 2, together with those from our previous diallel cross (Crusio and van Abeelen 1986).

Discussion

Evidently, many discrepancies exist between the results of the diallel and the sTTC (Table 2). The former detected additive genetic variation with regard to all phenotypes, except urination. The sTTC failed to find such effects in five cases, but did find them for urination. Dominance became apparent for all phenotypes in the diallel cross but the sTTC clearly indicated dominance for only three out of twelve variables and in one of these cases (rearing) disagreement existed about the nature of this dominance. Epistasis or other violations of the assumptions were found to exist for three phenotypes in the diallel cross. The sTTC agreed with this for rearing only.

Several theoretical and practical problems are connected with the analysis of a simplified triple-test cross. Firstly, it ignores any reciprocal effects. With consistent maternal effects present or, if measurements are taken from males only, with sex-linkage present, two possible complications arise. These are illustrated in Table 3 in the case of a single-gene difference; the extension to the polygenic case follows readily. The first complication is encountered when sires are used as testers: the Sums- and Epistasis-items will be upwardly biased, the Differences-item is free from any bias. The other complication occurs in the more usual case in which dams are used as testers, as was done here. Both the Differences- and the Sums-items are then free from any bias but the Epistasis-item will be inflated. However, even with this bias, the test for epistasis in the sTTC proved to be very weak, as compared with the joint-regression analysis of array variances and covariances in the diallel cross. It is noteworthy that the only case where the Epistasis-item showed significance (rearing) was also the only case where Hayman's *c*-item, indicating the presence of reciprocal effects (Hayman 1954), was significant (Crusio and van Abeelen 1986). The detection of dominance apparently is also a fairly futile undertaking here. A second difficulty is bound to appear if genotype-treatment interactions have to be analyzed. Often the testers are not extreme scorers in respect of these interactions and there is a large chance that testers adequate to analyze the main genetic effects become inadequate in the analysis of genotype-treatment interactions. We refer to Virk and Jinks (1977), who investigated the disadvantageous consequences for the analysis if inadequate testers are used.

Table 3. The contributions of maternal effects and/or sex-linkage to the test statistics of a simplified triple-test cross in the case of a single-gene difference

Inbred-line genotype Frequency	Male testers		Female testers	
	AA u_a	aa v_a	AA u_a	aa v_a
L_1 (AA)	$+d_{ra}$[a]	$-d_{ra}$	$+d_{ra}$	$+d_{ra}$
L_2 (aa)	$+d_{ra}$	$-d_{ra}$	$-d_{ra}$	$-d_{ra}$
$L_{1i}+L_{2i}$	$+2d_{ra}$	$-2d_{ra}$	0	0
$L_{1i}-L_{2i}$	0	0	$+2d_{ra}$	$+2d_{ra}$
$L_{1i}+L_{2i}-P_i$	$+d_{ra}$	$-d_{ra}$	$-d_{ra}$	$+d_{ra}$

[a] d_{ra} equals the maternal effect of locus A ($=d_{ma}$), or the effect of additive genes located on the X-chromosome ($=d_{xa}$), or a combination of both. See Mather and Jinks (1982) for a more elaborate discussion of the properties of d_x and d_m. To simplify the table, only the contributions of reciprocal effects are entered, omitting the normal additive effects and dominance deviations

These considerations lead to the following conclusions: 1. When employing a low number of strains, the sTTC analysis is clearly inferior to the diallel cross. 2. If reciprocal differences are present, some items in the sTTC analysis are biased. This problem might be overcome by randomization of parentals or by breeding both reciprocals of each F_1 cross, but the latter would eliminate the main advantage of this breeding design over the diallel cross, namely its economy. 3. Often the sTTC will be an inadequate approach to analyze genotype-treatment interactions. 4. Another serious drawback is that, in general, only one or a few phenotypes can be investigated because of the requirement of extreme testers.

Acknowledgements. We thank Anton Buis and Ron Engels for their skillful biotechnical assistance and Professor A.P. van Overbeeke for reading a first version of the manuscript.

References

Crusio WE, Kerbusch JML, Abeelen JHF van (1984) The replicated diallel cross: A generalized method of analysis. Behav Genet 14:81–104

Crusio WE, Abeelen JHF van (1986) The genetic architecture of behavioural responses to novelty in mice. Heredity 56: 55–63

Fulker DW (1972) Applications of a simplified triple-test cross. Behav Genet 2:185–198

Hayman BI (1954) The analysis of variance of diallel tables. Biometrics 10:235–244

Hewitt JK (1980) A note on the test for the direction of dominance in the triple test cross in the presence of genotype × environment interaction. Heredity 45:293–295

Jinks JL, Perkins JM, Breese EL (1969) A general method of detecting additive, dominance and epistatic variation for metrical traits. 2. Application to inbred lines. Heredity 24: 45–57

Kearsey MJ, Jinks JL (1968) A general method of detecting additive, dominance and epistatic variation for metrical traits. 1. Theory. Heredity 23:403–409

Mather K, Jinks JL (1982) Biometrical genetics, 3rd edn. Chapman and Hall, London

Sachs L (1974) Angewandte Statistik, 4. Aufl. Springer, Berlin

Sing M, Singh RK (1984) A comparison of different methods of half-diallel analysis. Theor Appl Genet 67:323–326

Singh O, Paroda RS (1984) A comparison of different diallel analyses. Theor Appl Genet 67:541–545

Virk DS, Jinks JL (1977) The consequences of using inadequate testers in the simplified triple test-cross. Heredity 38: 237–251