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CROSSBREEDING PARAMETERS OF RABBIT MOTION UNDER OPEN FIELD TEST CONDITIONS

RAFAY J., FL'AK P.

Research Institute of Animal Production, Hlohovská 2, 949 92 NITRA, Slovakia

ABSTRACT : The biometric - genetic analyses of movement ability of New Zealand White (NZ) rabbits, Wild (W) rabbits and their reciprocal crosses (NZ x W or W x NZ) in the open field test showed that the mean values of grid crossing of NZ rabbits are in the first five means greater than in W rabbits. Means of grid crossing of reciprocal hybrids have intermediate values. Correlations between subsequent minutes of observations of grid crossing are in the most cases low and non significant. The highly statistical significance between genotypes, time of observations and interaction term genotype x time was caused mainly by the direct genetic effect (4.91) grid crossings, direct maternal effect (3.48) grid crossings and by heterosis (2.63) grid crossings. The hypothesis was confirmed that movement activity in domestic and wild rabbits and their reciprocal crosses has a genetic basis.

RÉSUME : Détermination par croisements réciproques de l'effet du génotype sur le nombre de déplacements par minute de lapins testés en "open field"..
Le nombre de déplacements par minute lors des tests en "open field" d'une durée de 5 mn (grille de 9 carrés de 0,81 m²) a été mesuré chez des lapins Néo-Zélandais Blancs (NZ), de lapins de garenne (W) et de leurs croisements réciproques (NZ x W et W x NZ). Vingt lapins de 107 jours de chaque sexe ont été testés pour chaque génotype. Le nombre des déplacements par minute d'observation est plus important pour les lapins NZ (7,04/mn) que pour les lapins W (1,69/mn). Pour les lapins croisés, les valeurs sont proches de celles des lapins W. Les corrélations entre les valeurs observées au cours des minutes consécutives sont dans la plupart des cas faibles ou non significatives. La signification statistique élevée observée pour le modèle incluant les effets du génotype, le moment de l'observation et l'interaction génotype x moment, résulte principalement de l'importance de l'effet génétique direct (4,91 mouvements), de l'effet maternel direct (- 3,48 mouvements) et l'hétérosis (- 2,63 mouvements par minute). Ce travail confirme l'hypothèse de l'existence d'une base génétique aux différences de mobilité observées entre les lapins domestiques, sauvages et leurs croisements.

INTRODUCTION

The contribution of experimental methods in the research of genetic determination of animal behaviour resides in the fact that it possible to study the cause as the interaction between the environment conditions and state of organism. The need to quantify objectively the adaptive abilities in intensively bred farm rabbits led to the utilization of behavioural tests derived from the basic ethologic-psychological methodical procedures. These tests give certain information about the reactivity and emotional state in the tested animals. Open field test is one of these criteria (FAURE, 1981) which is used in the study of behavioural activities in laboratory and farm animals. It is based on the measurement of behaviour of animals in a limited area in dependence on variable values of the environment such as the movement from known to the unknown environment, manipulation and transfer to the experimental facilities, and the exposition in the testing environment. Data obtained in the test show the possibilities of utilization of breeding methods in selection for behavioural characteristics connected with aggressiveness, temperament and other reaction to outer stimulation (e.g., noise, light, etc.)

The reactions of animals in the open field test were described and examined by DENENBERG (1969) and MARTINEK (1976). FUJITA (1980) used an open field test to characterize genetical determination motility activities in two lines of rats. BEILHARZ and COX (1976) have presented a behavioural response of two different breeds of pig, KOVALCÍKOVÁ and KOVALCÍK (1982) demonstrated motion differences in open field on Slovak Spotted breed of cattle with comparison to Lowland Black and White breeds.

Observation of behaviour of wild and domestic rabbits was presented by MODRZEJEWSKI *et al.* (1976) and TOKARSKI *et al.* (1984). Motor activity of wild and domesticated New Zealand White rabbits as well as their reciprocal crossbreds was investigated in open field test by ZELNÍK *et al.* (1990). The reaction of wild rabbits was lowest from the four observed groups, a high initial activity in the first minute of the five minute period of observation. In subsequent 4 minutes, the frequency of grid crossing evidently diminished. New Zealand White rabbits, particularly the females, reacted in a different manner, with an increasing tendency of motor activity with higher values in the last five minutes. The mobility reaction of hybrids was the highest. Authors have estimated habituation coefficients for all animals from a logarithmic transformation of function $y = b \times a$ and by the analysis of variance which showed genetic differences between groups.

Table 1 : Basic statistical characteristics of rabbit grid crossings by genotype, sex and minutes observation, n = 20

	Males				Females				
Minute	$\bar{X}$	**S**	S_X	**V %**	$\bar{X}$	**S**	S_x	**V %**	**t-test**
1 New Zealand White (NZ)									
1	4.50	1.47	0.33	32.64	3.85	1.78	0.39	46.36	1.26
2	5.60	1.73	0.39	30.87	5.70	1.52	0.34	26.76	0.19
3	7.60	1.50	0.34	19.75	7.20	1.99	0.44	27.63	0.72
4	8.30	1.81	0.40	21.80	8.80	1.54	0.34	17.53	0.94
5	9.15	1.63	0.36	17.83	9.75	1.33	0.29	13.67	1.27
2 Wild (W)									
1	0.55	0.60	0.13	109.6	0.95	1.15	0.26	120.62	1.38
2	1.30	1.21	0.27	93.71	1.25	0.86	1.19	68.05	0.15
3	1.95	1.10	0.25	56.36	1.40	1.50	0.34	107.20	1.32
4	2.90	1.71	0.28	59.09	2.25	1.21	1.27	53.71	1.39
5	2.35	1.18	0.26	50.30	2.05	1.00	0.22	48.72	0.87
3 Wild (males) x New Zealand White (females) (W x NZ)									
1	1.05	0.89	0.20	84.48	1.50	1.00	0.22	66.67	1.51
2	1.65	0.81	0.18	49.26	1.70	0.97	0.22	57.57	0.18
3	2.35	0.99	0.22	42.05	2.45	1.00	0.22	40.76	0.32
4	2.30	0.92	0.21	40.15	2.35	0.81	0.18	34.58	0.18
5	2.10	0.91	0.20	43.42	2.15	0.93	0.21	43.41	0.17
4 New Zealand White (males) x Wild (females) (NZ x W)									
1	0.80	0.77	0.17	95.97	0.90	0.91	0.20	101.32	0.37
2	1.25	0.72	0.16	57.30	1.45	0.76	0.17	52.36	0.86
3	1.45	0.69	0.15	47.33	1.55	0.69	0.15	44.28	0.46
4	1.50	1.00	0.22	66.66	2.00	0.92	0.20	45.88	1.65
5	2.05	0.94	0.21	46.07	2.25	0.79	0.17	34.95	0.73

Table 2 : Correlations of rabbit grid crossings between subsequent minutes of observations by genotype and sex, n = 20

	Males					Females				
Minute	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
1 New Zealand White (NZ)										
1	1.000	0.041	0.024	-0.059	-0.165	1.000	-0.075	-0.110	0.199	0.514^{+}
2		1.000	-0.085	-0.010	-0.060		1.000	0.125	-0.184	-0.326
3			1.000	0.066	0.112			1.000	-0.364	-0.060
4				1.000	0.269				1.000	0.435
5					1.000					1.000
2 Wild (W)										
1	1.000	-0.164	-0.115	0.259	-0.357	1.000	0.337	-0.141	0.048	-0.320
2		1.000	0.326	-0.287	0.289		1.000	-0.124	0.141	0.047
3			1.000	-0.226	0.176			1.000	-0.261	-0.014
4				1.000	0.174				1.000	0.621^{++}
5					1.000					1.000
3 Wild x New Zealand White (W x NZ)										
1	1.000	-0.413	-0.081	-0.019	-0.137	1.000	0.108	0.026	-0.162	-0.141
2		1.000	-0.36	0.217	-0.163		1.000	0.146	-0.258	0.282
3			1.000	0.283	0.018			1.000	-0.334	-0.133
4				1.000	-0.163				1.000	-0.073
5					1.000					1.000
New Zealand White x Wild (NZ x W)										
1	1.000	-0.287	-0.120	-0.137	-0.131	1.000	-0.160	0.177	0.189	-0.037
2		1.000	0.509^{+}	0.257	-0.097		1.000	0.308	0.151	-0.375
3			1.000	0.038	-0.118			1.000	-0.334	-0.268
4				1.000	0.307				1.000	-0.073
5					1.000					1.000

MATERIALS AND METHODS

The experimental animals consisted of four genetic groups of rabbits. The first two groups were purebred populations:
1. New Zealand White breed (NZ) and
2. Wild (W) rabbits.
Second two groups were reciprocal crosses:
3. Wild (male) x New Zealand (female) (W x NZ) and
4. New Zealand (male) x Wild (female) (NZ x W) .
In each genetic group we observed 40 animals (20 males and 20 females). The studied animals of the mentioned genotypes came from balanced litters ($6 \leq n \leq 8$). The reproductive and fattening phases of rearing were performed in one breeding hall in cages of flat deck type. The animals were fed pelleted feed (crude protein 18,5 %, fiber 14,0 %, and ME 10,5 MJ). The average conditions of microclimate during the rearing of studied rabbits were as follows : air temperature $17 \pm 3°C$, relative humidity 73 ± 5 %, photoperiodic regime 16:8 h (light:dark). The animals were weaned at the age 35 of days and transferred into cages of 3 animals. Animals were marked individually by tattooing at the age of 56 days. They were used in the test to the age of 107 days.

The open field equipment was modified according to the description by ZELNIK *et al.* (1990) and it was placed in a room acoustically and light isolated from surrounding. The square area with black surface of the total size 7.29 m^2 was divided by white lines into 9 equal squares (with the area 0.81 m^2 each) marked by identification numbers. The source of light lighted equally the whole testing area, and it was placed over the centre of the equipment in the height 1 of 500 mm. The open field equipment was surrounded by 600 mm in high glass panels.

The whole experiment was performed within 10 days. Two animals of each genotype and sex were tested each day (one animal in the morning and one in the afternoon). Sixteen animals were tested daily. The area of open field was cleaned with moist cloth between the individual tests (to eliminate the smell traces of the previous animal). The observer was behind a mirror glass 2 m distant from open field equipment, out of the animal's field of vision. The animal was carried over from the rearing cage into the room with the installed open field during 1 min. Animal was placed into the central square at the beginning, and the number of line crossings on the open field were recorded in one minute intervals during 5 minutes. Experimental conditions of

Table 3 : Basic statistical characteristics and average correlation coefficients of rabbit grid crossing between consecutive minutes of observation (males and females pooled together) after analysed genotypes, n = 20

	Average statistics				Correlation				
Minute	$\bar{X}$	**S**	S_x	**V %**	**1**	**2**	**3**	**4**	**5**
1 New Zealand White (NZ)									
1	4.18	1.65	0.26	39.5	1.000	-0.017	-0.043	0.070	0.175[h]
2	5.65	1.61	0.25	28.5		1.000	0.020	-0.097	-0.133
3	7.40	1.75	0.28	23.7			1.000	-0.149	0.026
4	8.55	1.68	0.27	19.6				1.000	0.352[+]
5	9.45	1.50	0.24	15.9					1.000
2 Wild (W)									
1	0.75	0.93	0.15	123.6	1.000	0.087	-0.128	0.153	-0.333
2	1.28	1.04	0.16	81.4		1.000	0.101	-0.073	0.168
3	1.68	1.33	0.21	79.3			1.000	-0.244	0.081
4	2.58	1.50	0.24	58.3				1.000	-0.224[h]
5	2.20	1.09	0.17	49.6					1.000
3 Wild x New Zealand White (W x NZ)									
1	1.28	0.96	0.15	75.3	1.000	-0.153	-0.027	-0.091	-0.139
2	1.68	0.89	0.14	53.3		1.000	0.055	-0.020	0.060
3	2.40	0.98	0.16	40.9			1.000	-0.026	-0.058
4	2.33	0.86	0.14	36.9				1.000	-0.118
5	2.13	0.91	0.14	42.9					1.000
4 New Zealand White x Wild (NZ x W)									
1	0.85	0.83	0.13	98.1	1.000	-0.223	0.028	0.026	-0.084
2	1.35	0.74	0.12	54.5		1.000	0.403[+,+']	0.204	-0.236
3	1.50	0.68	0.11	45.3			1.000	-0.148	-0.193
4	1.75	0.98	0.16	56.0				1.000	0.117
5	2.15	0.86	0.14	40.2					1.000

h : heterogeneity of sample correlation coefficients

Table 4 : Two-factor analysis of variance of grid crossing with repeated measurements in the same animals

Source of variation		df	MS	F
Genotypes G		3	1421.6633	1045.193^{++}
Animals in genotype A:G		156	1.3602	
Time (minutes)	T	4	137.6481	96.638^{++}
Interaction	GT	12	28.4165	19.950^{++}
Error	TA:G	624	1.4244	

Table 5 : Basic statistical characteristics of grid crossing for genotype and means for minutes - total.

Genotype	1 NZ	2 W	3 W x NZ	4 NZ x W	
$\bar{X}$	7.04	1.69	1.96	1.52	
S	2.51	1.35	1.01	0.92	
S_x	0.48	0.10	0.07	0.07	
V %	35.7	79.6	51.4	60.8	
Minutes	1st	2nd	3rd	4th	5th
	1.76	2.49	3.24	3.80	3.98

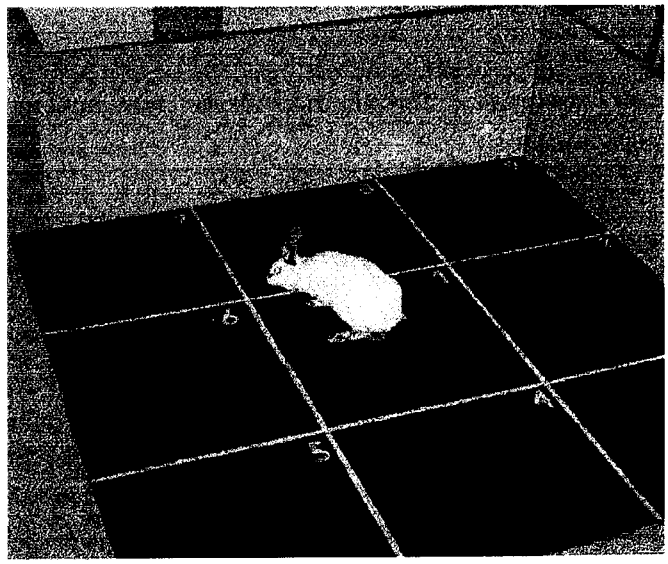
microclimate in the testing room were as follows : air temperature 22 ± 0.5°C, relative humidity 70 %, illumination level in the open field area 120 lux.

Purpose of the work was an observation of grid crossings of animals (their motility) during the first five minutes.

These observations were statistically analyzed by the following methods:

1. For all subgroups (i.e. genotype x sex x minute) were calculated by arithmetic means, standard deviations, standard error of means and coefficient of variation, and correlations between all minutes in the smallest subgroups.
2. The significance of differences between sexes in subgroup genotype x minutes were tested by the Student t-test. Differences between correlation coefficients were tested by the FISHER, DONNER and ROSNER criteria with calculations of mean correlation coefficients.
3. For non significant differences in grid crossings between sexes in subgroups (genotype x minutes), were observations for males and females were pooled, and differences between genotypes, times of observations (minutes) were estimated by the two-factor analysis of variance with repeated measurements on the same individuals, on the basis of the linear model:

$$y_{ijk} = (\mu + g_i + a_{k:i} + t_j + (gt)_{ij} + e_{ijk} .$$



Rabbit in open field during testing

In the model the effects are:

y_{ijk} = observation of k-animal in i-genotype and j-treatment general mean
μ = general mean
g_i = fixed effect of i-genotype
$ak_{:i}$ = random effect k-animal nested in i-genotype
t_j = fixed effect of j-time
$(gt)_{ij}$ = fixed interaction term of genotype x time
e_{ijk} = experimental error distributed N $(0,\sigma^2_e)$ which is actually the random interaction term animal x time in i-group $(ta)_{jk:i}$.

4. Elementary contrasts between genotypes means involving linear contrasts of genotype means were tested for statistical significance for genetic and non-genetic effects.

The statistical methods were according to GROFÍK and FL'AK (1990) and by using of statistical programs for the calculator (HP 9820A) developed by FL'AK (1975-1996).

RESULTS AND DISCUSSION

The basic statistical characteristics of grid crossings by genotype, sex and minutes are in Table 1. Presented in Table 2 are correlations of rabbit grid crossings by genetic group between subsequent minutes for males and females. Statistical significance between means of sexes in minutes was not observed, therefore we will in the next paper discuss only the results of genotypes together.

Basic statistical characteristics and average correlations by genetic group are presented in Table 3.

Table 6 : Coefficients c_i for multiple linear contrasts and estimated linear contrasts of rabbit grid crossing.

L_i		Coefficients c_i for genotype A = NZ, B = W				Estimated linear contrasts		
Linear contrasts		A	B	BA	AB	L	S_L	t
L_1	DI	-1	1	1	-1	-4.910	0.165	29.77
L_2	DRC	0	0	-1	-1	-3.480	0.117	29.84
L_3	h	-½	-½	½	½	-2.630	0.082	31.89
L_4	ABh^I	-½	-½	1	0	-2.410	0.101	23.86
L_5	BAh^I	-½	-½	0	1	-2.850	0.101	28.22
L_6	A-B	1	-1	0	0	5.350	0.117	45.87
L_7	A-AB	1	0	-1	0	5.085	0.117	43.60
L_8	A-BA	1	0	0	-1	5.525	0.117	47.37
L_9	B-AB	0	1	-1	0	-0.265	0.117	2.27
L_{10}	B-BA	0	1	0	-1	0.175	0.117	1.50
L_{11}	AB-BA	0	0	1	-1	0.440	0.117	3.77

DI : direct genetic effect ; DRC : difference in reciprocal crosses or direct maternal effect ; h^I: individual heterosis ; ABh^I and BAh^I : are individual heterosis effects from crossing of AB or BA
$t_{0.05}$ (156) = 1.98 ; $t_{0.01}$ (156) = 2.61

In NZ rabbits, we observed in the 1st minute a mean of 4.18 and in the 5th minute a mean of 9.45 grid crossings. In W rabbits, 0.75 or 2.20 mean grid crossings were observed. Standard deviations were higher in NZ rabbits than in wild rabbits with a range from 0.93 in the 1st minute for W rabbits to 1.75 grid crossings in the 3rd minute for NZ rabbits. In the reciprocally crosses, we observed in the 1st , 2nd, and 3rd minutes in W.NZ, intermediate values of grid crossing and in the 4th and 5th minutes (lower mean values than in parental purebreds). This table obviously shows higher standard deviations in purebreeds, particularly NZ.

In the most cases, we observed homogeneity of correlation coefficients of grid crossings between minutes (Table 3.) Heterogeneity was observed only between the 1st and 5th minutes in NZ rabbits and between the 4th and 5th minutes in W rabbits. Significant or highly significant mean correlation coefficients were observed between the 4th and 5th minutes in NZ rabbits ($r = 0.3523^{+}$) and between 2nd and 3rd minute in reciprocal crosses NZ.W ($r = 0.4083^{++}$).

Results from two-factor analysis of variance with repeated measurements on the same individuals are presented in Table 4. The F criteria in the table were statistically highly significant for genotypes, times of observations of grid crossings, and for the interaction term of genotype x time of observations of grid crossing. The last F test refers to the non-parallelism of regressions for grid crossings in minutes between genotypes. In Table 5, we present statistical characteristics of grid crossings by genotype and cumulative means of minutes for a total description of our population. Maximal grid crossings were observed in NZ rabbits (7.04) and minimal mobility was noticed in the group of crossbred animals (NZ.W = 1.52). Without regard to genotype, the average grid crossings in the 1st minute was 1.7, in 2nd minute was 2.49, 3rd minute was 3.24, and in the final two minutes was approximately equal to 3.80 or/and 3.98. For consideration of genetic or non-genetic determination of grid crossings, we present multiple linear contrasts, which were calculated on the basis of coefficients c_i for multiple linear contrasts (Table 6).

All linear contrasts, besides the contrast W - W.NZ, are statistically significant or highly significant. Direct genetic effects yielded -4.91^{++} grid crossings and direct maternal effects (e.g. difference in reciprocal crosses) yielded -3.48^{++} grid crossings. We observed a highly significant individual heterosis effect of -2.63^{++}, calculated from both types of crossings (-2.41 for NZ.W and -2.85 for W.NZ). Elementary contrasts showed significant and/or highly significant differences in square crossings (L6-L9) among the genotypes evaluated.

Results obtained from the biometric - genetic analyses clearly show that the grid crossing of crossbred rabbits is genetically determined by the direct and maternal effects of parental breeds and by individual heterosis. Our results are similar to the results of ZELNÍK *et al.* (1990) who studied behavioural activity of rabbits in open field for the classification of rabbit movement.

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