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EFFECTS OF PARITY ON REPRODUCTIVE PERFORMANCE IN LINES OF MICE SELECTED FOR LITTER SIZE OR BODY WEIGHT[1]

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ABSTRACT

Reproductive performance of five lines (L) of mice, where females to be evaluated were reared in three postnatal litter sizes (N), was compared from first to second or third parity (P). Lines were selected for 17 generations as follows: large litter size (L^+), large 6-week body weight (W^+), small litter size and large 6-week body weight (L^-W^+), large litter size and small 6-week body weight (L^+W^-) and randomly (K). Levels of N were 8, 12 and 16. The objective was to determine the magnitude of parity effects and interactions involving parity for litter size, litter weight, gain and feed efficiency and female weight and feed intake measured from parturition to weaning at 21 days. The

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L x N x P interaction was not significant for any trait. The N x P interactions were significant for female body weight at parturition and female weight change from 12-21 days postpartum, but neither interaction was biologically important. Thus, the effect of N on litter size and other reproductive traits persisted over parities. The L x P interactions were significant for litter size at birth, 12 and 21 days, litter birth weight and female weight at parturition, which was interpreted to mean that the criterion of selection had modified the influence of parity on these traits. Litter weights, gains and efficiency and female body weights increased with parity, while exhibiting no important interactions. Pooled repeatabilities for litter size were 33 ± .04, 16 ± .07 and 18 ± .06 for parities 1 and 2, 1 and 3, and 2 and 3, respectively. Repeatability between parities 1 and 2 for litter weights, gains and feed efficiency ranged from .22 to .45, except for litter feed efficiency from 12 to 21 days which was .01 ± .06.

INTRODUCTION

Litter size in a polytocous species is a complex character influenced by both genetic and environmental factors. In mice, litter size at birth readily responds to selection (Falconer, 1960a; Bateman, 1966; Bradford, 1968; Joakimsen and Baker, 1977; Eisen, 1978; Bakker *et al.*, 1978). There is a positive genetic correlation between litter size at birth and adult body weight in mice (Joakimsen and Baker, 1977; Eisen, 1978). Selection for adult body weight has generally yielded positive correlated responses in litter size (see review by Roberts, 1965; Eisen, 1974), although exceptions have been reported (Bradford, 1971). Litter size also has responded to selection when this trait was included in a selection index (Doolittle *et al.*, 1972; Eisen, 1978). First parity litter size at birth (total number born or total number born alive) has been the selection criterion most frequently adopted to modify litter size genetically, with no attention given to litter size at subsequent parities. In unselected lines of mice, litter size tends to increase in early parities, then plateaus and eventually declines (Biggers *et al.*, 1962; Rugh and Wohlfromm, 1967; Wallinga and Bakker, 1978). Much less is known about the influence of selection for litter size or body weight on reproductive performance in later parities.

A major environmental component influencing the litter size pheno-

type is the litter size in which a female is reared (postnatal litter size). Females reared in large litters have smaller body weights at sexual maturity and also give birth to smaller litters. This negative postnatal maternal effect is mediated partially through body weight of the mother (Falconer, 1965; Eisen, 1970; Machin and Page, 1973; Nelson and Robison, 1976). Eisen and Durrant (1980a, b) reported that the postnatal maternal effect on litter size did not differ among lines selected for litter size and/or 6-week body weight. No data are available to determine whether the maternal effect persists in subsequent parities, however.

The present experiment was designed to determine if parity effects on reproductive performance and litter growth are uniform over 1) a set of five lines which differ widely in litter size at birth and 6-week body weight and 2) three levels of postnatal litter sizes (8, 12 or 16). Repeatability for litter size at birth and for the other traits was estimated also.

MATERIALS AND METHODS

The lines used in this experiment had undergone 17 generations of selection as follows: L^+ for large litter size at birth, W^+ for large 6-week body weight, L^-W^+ for a selection index designed to decrease litter size and increase 6-week body weight, and L^+W^- for a selection index designed to increase litter size and decrease 6-week body weight (Eisen, 1978). An unselected control line (K) was included also. Litter size at birth was defined as the total number of live plus dead pups born, and excluded litters of zero due to infertile matings. Only first parity litters were used during the selection experiment, and they were standardized to eight pups at one day of age.

The present experimental design was described previously (Eisen and Durrant, 1980a, b). Briefly, litters from each line were randomly standardized to eight (N8), 12 (N12) or 16 (N16) pups on the day of parturition. Females and males of the same line and reared in the same postnatal litter size were mated in cages of one male and two females with the avoidance of sib matings. Mean mating age for first parity females was 84 days. Females were permitted to rear all their pups up to a total of 16. Litters having more than 16 pups born alive were restricted to 16 on the day of birth. Litters were weaned at 3 weeks

of age. When all females had weaned their litters, they were remated to a random sample of males from the same line. The experimental design and the number of females per subclass for litter size at birth are presented in Table I. The traits which were recorded in the first two or three parities are given in Table II.

The data were analyzed as a split-plot experiment with unequal subclass numbers (Harvey, 1975). The statistical model was $Y_{ijk\ell m} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{ijk} + \delta_\ell + (\alpha\delta)_{i\ell} + (\beta\delta)_{j\ell} + (\alpha\beta\delta)_{ij\ell} + \Sigma_{ijk\ell m}$ where μ = overall mean, $\alpha_i = i^{th}$ line effect (i= 1,...5,), $\beta_j = j^{th}$ postnatal litter size effect (j = 1,2,3), δ_ℓ = parity effect (ℓ = 1,2,3), $(\alpha\beta)_{ij}$, $(\alpha\delta)_{i\ell}$, $(\beta\delta)_{j\ell}$ and $(\alpha\beta\delta)_{ij\ell}$ = interaction effects, γ_{ijk} = whole-plot error, and $\Sigma_{ijk\ell m}$ = split-plot error. The present paper is concerned primarily with ascertaining the importance of parity effects and interactions with parity, which were tested statistically by

Table I - Number of females per subclass for litter size at birth[a,b,c].

Line	Postnatal litter size								
	N8			N12			N16		
	Parity			Parity			Parity		
	1	2	3	1	2	3	1	2	3
L^+	48	16	15	47	19	12	48	19	14
W^+	49	14	11	49	17	12	47	19	16
L^-W^+	47	18	16	47	18	17	48	19	18
K	49	18	16	49	19	18	50	20	19
L^+W^-	50	20	17	44	20	20	45	19	17

[a] Number of observations for other traits within a parity were similar.

[b] Traits recorded in the third parity were litter size at birth, number born alive, litter birth weight, female weight at parturition and litter weight/female weight at parturition.

[c] A random sample of females within each line by postnatal litter size subclass was chosen to produce the 2nd and 3rd litters.

Table II - Tests of significance for effects of parity (P), line x parity (LxP), postnatal litter size x parity (NxP) and LxNxP.

Trait	F-ratio P	LxP	NxP	LxNxP	Split-plot error mean square[a]
			Parities 1, 2 and 3		
Litter size at birth	6.57**	5.52**	.65	1.32	9.07
Number born alive	7.54**	6.02**	.58	1.11	9.97
Litter birth wt (g)	11.20**	6.08**	.99	1.13	24.07
Female wt partur.(g)	1240.73**	10.01**	2.37*	1.05	3.50
Litter wt/female wt partur.	59.68**	6.32**	1.04	1.21	.011
			Parities 1 and 2		
Litter size birth	9.28**	3.90**	.01	.67	7.92
12 days	4.85*	5.03**	.29	.30	6.05
21 days	4.35*	4.80**	.29	.25	6.23
Number born alive	8.37**	5.46**	.03	.67	8.29
Litter wt (g) birth	9.21**	6.14**	.36	.63	20.54
12 days	15.19**	.66	.49	.82	183.52
21 days	6.08*	1.75	.50	.79	767.42
Litter wt gain (g) (0-12 days)	17.47**	.45	.54	1.25	113.28
(12-21 days)	10.67**	1.08	.35	.89	260.87
Female wt (g) partur.	1115.77**	6.16**	3.86*	.77	3.24
12 days pp	313.91**	1.85	1.80	.86	7.04
21 days pp	507.48**	.37	.80	1.60	4.47
Female wt change (g) (0-12 days)	20.55**	.87	.15	.82	7.49
(12-21 days)	.05	1.19	3.69*	.97	7.83
Feed intake (g/day) (0-12 days)	3.08	1.88	.59	.75	3.16
(12-21 days)	5.80*	1.14	.66	.46	9.83
Female feed eff. (0-12 days)[b]	22.91**	1.14	.08	.73	1.88
Litter feed eff. (0-12 days)[b]	49.55**	1.62	.69	1.62	12.18
(12-21 days)[b]	5.13*	1.75	.35	.92	36.16
Litter wt/female wt partur.	11.42**	5.97**	.35	.57	.010
12 days	3.29	.75	.37	1.46	.060
21 days	3.23	1.93	1.07	1.44	.41

*P < .05, **P < .01.

[a] Error degrees of freedom vary from 481 to 497 for three parities and from 249 to 260 for two parities.

[b] Female feed eff. = 100 x female gain/feed intake; litter feed eff. = 100 x litter gain/feed intake.

the split-plot error variance in the analysis of variance. If interaction effects involving parity were absent for a particular trait, then only the main effects of parity are presented. Where interactions of parity by line or parity by postnatal litter size were statistically significant, the appropriate subclass means were compared. Detailed analyses of line and postnatal litter size differences for first parity traits were reported by Eisen and Durrant (1980a,b).

Repeatability of traits between the i^{th} and j^{th} parity was estimated as $r_{ij} = \sigma^2_\gamma / (\sigma^2_\gamma + \sigma^2)$ where σ^2_γ represents the variance among females and σ^2 is the variance within females. Repeatability estimates were pooled within line by postnatal litter size subgroups after tests of heterogeneity among repeatability estimates were conducted. Repeatabilities were calculated separately for pairs of parities. In addition, repeatability estimates for litter size at birth between first and second parities were also available for dams of the females. The standard error of repeatability was based on the formula given by Falconer (1960b).

RESULTS

Parity and Interactions with Parity. The split-plot portion of the analyses of variance is presented in Table II. For those traits measured in the first three parities, the levels of significance are in complete agreement with the results of parities 1 and 2.

The three-way interaction of line by postnatal litter size by parity was not significant for any trait.

Postnatal litter size by parity interactions were significant ($P < .05$) for female body weight at parturition and weight change from 12 to 21 days postpartum. The subclass means for female body weight at parturition (Table III) indicate that the interaction was not a result of change in the ranking of main effects. Within each parity, body weight at parturition declined ($P < .05$) with increasing postnatal litter size and, within each level of postnatal litter size, body weight increased ($P < .05$) with each successive parity. The interaction observed for female weight change from 12 to 21 days of lactation resulted from a greater weight loss in N12 and N16 mice than in N8 for parity 1, while the reverse was true in parity 2. The differences, however, were not large enough

to suggest that the interaction was of any biological consequence. Although the postnatal litter size by parity interaction for litter size at birth was not statistically significant, a plot of these subclass means reveals an interesting trend (Figure 1). The negative influence of postnatal litter size on litter size at birth observed for parity 1 persisted in parity 2, which was accompanied by the negative influence of postnatal litter size on female body weight at parturition. However, in parity 3 the postnatal litter size effect on litter size at birth is absent. The conclusion is that the magnitude of postnatal maternal environmental effects influencing the traits of first parity females (Eisen and Durrant, 1980a, b) persists into at least the second parity, but the results of the third parity are not consistent regarding litter size.

Line by parity interactions were significant for litter size at birth, 12 and 21 days, litter birth weight, female body weight at parturition and litter weight/female body weight at parturition (Table II). The line by parity subclass means (Table IV) reveal the basis for the interactions.

The line by parity interaction for litter size at birth is emphasized since the significant line by parity interactions in number born alive and litter size at 12 and 21 days follow a similar pattern. Litter size increased ($P < .05$) from parity 1 to 2 in the L^+W^- and K lines and tended to increase in L^-W^+. The L^+ and W^+ lines did not show an increase in litter size in parity 2. These results agree with data on the dams of the females utilized in the present study, except that W^+ mice had a larger litter size in parity 2 (Eisen and Durrant, 1980a). In the third parity, the lines selected directly (L^+) or indirectly (L^+W^-) for high litter size declined markedly ($P < .05$) in litter size at birth, while K, L^-W^+ and W^+ did not change much from parity 2. Parity 3 females of L^+ and L^+W^- did not differ ($P > .05$) in litter size at birth, but both lines had higher means than the W^+ and K lines; the latter two lines did not differ ($P > .05$). The L^-W^+ line still had the lowest litter size of the five lines examined. Results in parity 3 were similar to the second parity in some respects, but the differences clearly were reduced because of the lower litter size in L^+ and L^+W^-. Thus, line differences in fecundity in parity 1, reported by Eisen and Durrant (1980a), clearly changed in magnitude in parities 2 and 3.

The line by parity interaction for litter birth weight followed a pattern similar to that for number born alive with few exceptions. The high correlation between litter birth weight and number born alive accounts for this relationship (Eisen and Durrant, 1980a).

The line by parity interaction for female body weight at parturition was due to a change in the magnitude of line differences across parities, as the ranking of lines did not change. The L^+W^- females were significantly ($P < .05$) larger than controls in parity 3. All lines demonstrated an increase in body weight at parturition from parity 1 to 3. The line by parity interaction for litter weight/female weight ratio at parturition is more complex. The ratio declined linearly with parity in the L^+ and W^+ lines, but evidenced a non-linear decline in L^-W^+, L^+W^- and K. The line differences changed noticeably at each parity, and were a function of how litter birth weight and female weight at parturition were altered.

Parity means for traits not demonstrating interactions of biological importance are listed in Table V. Litter weights and gains increased ($P < .01$ except 12-21 day gain, $P < .05$) in parity 2 by about 6%. Body weight of the

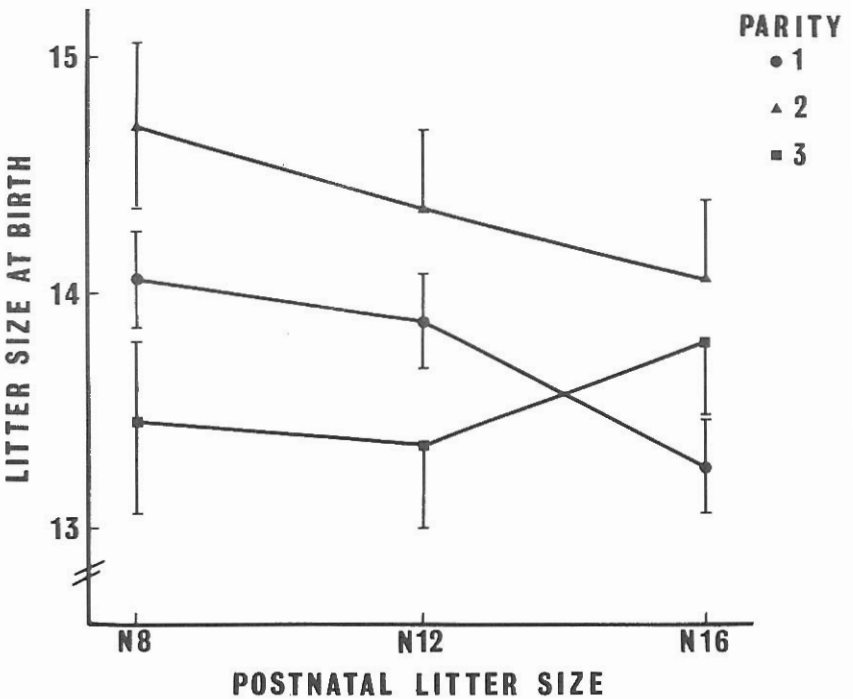

Figure 1 - Postnatal litter size by parity subclass means for litter size at birth. Standard errors of each mean are represented by a vertical line.

dam at parturition and during lactation also increased ($P < .01$) with parity. Females in their first parity gained more ($P < .01$) weight during the first 12 days of lactation but incurred no difference in weight loss in the last 9 days of lactation. Feed intake of the dam in the first 12 days of lactation did not differ between parities, but feed intake from 12 to 21 days was higher ($P < .05$) in the second parity. Litter feed efficiency was greater ($P < .01$) for parity 2 but litter weight/female weight ratios at 12 and 21 days were not different.

Table III - Postnatal litter size x parity least squares means for traits having a significant NxP interaction and tests of significance for parity effects within postnatal litter size and postnatal litter size effects within parity.

Postnatal litter size	Female wt partur.(g) Parity 1	2	3	Female wt change (12 to 21 days) Parity 1	2
N8	$43.46^{a,A}$	$48.95^{b,A}$	$51.28^{c,A}$	$-7.95^{a,A}$	$-9.08^{b,A}$
N12	$42.58^{a,B}$	$47.49^{b,B}$	$50.69^{c,B}$	$-8.76^{a,B}$	$-8.57^{a,A}$
N16	$41.17^{a,C}$	$45.48^{b,C}$	$48.44^{c,C}$	$-8.77^{a,B}$	$-8.50^{a,A}$
SE[d]	.12-.13	.19-.20	.20-.21	.18-.19	.29-.31

abc Means within a row which do not share common lower case superscripts are significantly different at $P < .05$.

ABC Means within a column which do not share common upper case superscripts are significantly different at $P < .05$.

d Range of standard errors.

Table IV - Line x parity least squares means for traits having a significant interaction and tests of significance for parity effects within lines and line effects within parities.

Line	Litter size at birth			Number born alive			Litter size at 12 days		Litter size at 21 days	
	Parity			Parity			Parity		Parity	
	1	2	3	1	2	3	1	2	1	2
L^+	$17.42^{a,A}$	$17.01^{a,A}$	$14.59^{b,A}$	$16.94^{a,A}$	$16.35^{a,A}$	$14.00^{b,A}$	$14.65^{a,A}$	$14.20^{a,A}$	$14.45^{a,A}$	$14.15^{a,A}$
W^+	$14.09^{a,B}$	$13.71^{a,B}$	$13.39^{a,B}$	$13.95^{a,B}$	$13.27^{a,B}$	$12.92^{a,B}$	$13.15^{a,B}$	$12.40^{a,B}$	$13.11^{a,B}$	$12.40^{a,B}$
L^-W^+	$10.78^{a,C}$	$11.56^{ab,C}$	$11.89^{b,C}$	$10.33^{a,C}$	$11.36^{b,C}$	$11.48^{b,C}$	$10.08^{a,C}$	$11.27^{b,C}$	$10.06^{a,C}$	$11.22^{b,C}$
K^-	$11.99^{a,D}$	$13.27^{b,B}$	$13.44^{b,B}$	$11.87^{a,D}$	$13.19^{b,B}$	$13.27^{b,B}$	$11.80^{a,D}$	$12.83^{b,B}$	$11.73^{a,D}$	$12.68^{b,B}$
L^+W^-	$14.29^{a,B}$	$16.45^{b,A}$	$14.28^{a,AB}$	$13.95^{a,B}$	$16.25^{b,A}$	$13.57^{a,AB}$	$12.96^{a,B}$	$14.39^{b,A}$	$12.87^{a,B}$	$14.37^{b,A}$
SE[d]	.25-.26	.39-.43	.41-.49	.25-.26	.38-.41	.40-.48	.20-.21	.32-.36	.20-.21	.33-.36

Line	Litter birth wt(g)			Female body wt partur. (g)			Litter wt/female wt (partur.)		
	Parity			Parity			Parity		
	1	2	3	1	2	3	1	2	3
L^+	$27.46^{a,A}$	$26.15^{a,A}$	$22.71^{b,A}$	$44.60^{a,A}$	$48.98^{b,A}$	$50.76^{c,A}$	$.61^{a,A}$	$.54^{b,A}$	$.45^{c,AC}$
W^+	$26.01^{a,B}$	$25.46^{ab,A}$	$23.62^{b,A}$	$50.40^{a,B}$	$56.62^{b,B}$	$60.34^{c,B}$	$.52^{a,B}$	$.45^{b,B}$	$.39^{c,B}$
L^-W^+	$18.52^{a,C}$	$20.35^{b,B}$	$20.19^{b,B}$	$40.99^{a,C}$	$46.28^{b,C}$	$49.33^{c,C}$	$.45^{a,C}$	$.44^{ab,B}$	$.41^{b,AB}$
K^-	$20.17^{a,D}$	$22.49^{b,C}$	$21.87^{b,AB}$	$37.76^{a,D}$	$42.04^{b,D}$	$44.40^{c,D}$	$.53^{a,B}$	$.54^{a,A}$	$.49^{b,C}$
L^+W^-	$22.56^{a,E}$	$26.39^{b,A}$	$22.15^{a,AB}$	$38.29^{a,D}$	$42.61^{b,D}$	$45.81^{c,E}$	$.59^{a,D}$	$.62^{b,C}$	$.48^{c,AC}$
SE[d]	.40-.42	.64-.70	.67-.80	.16-.17	.24-.27	.26-.29	.0086-.0088	.0135-.0147	.0141-.0167

[abc] Means within a row which do not share common lower case superscripts are significantly different at $P < .05$.

[ABCDE] Means within a column which do not share common upper case superscripts are significantly different at $P < .05$.

[d] Range of standard errors.

Table V - Least squares parity means for traits showing no biologically important interactions.

Trait	Parity 1	2	3
Litter wt (g) 12 days	92.37[a]	97.74[b]	
21 days	160.16[a]	170.13[b]	
Litter wt gain (g) (0-12 days)	70.16[a]	74.38[b]	
(12-21 days)	67.63[a]	72.29[b]	
Female wt (g) partur.	42.41[a]	47.31[b]	50.13[c]
12 days pp	50.48[a]	54.27[b]	
21 days pp	41.96[a]	45.55[b]	
Female wt change (g) (0-12 days)	8.07[a]	6.89[b]	
(12-21 days)	-8.49[a]	-8.72[a]	
Feed intake (g/day) (0-12 days)	17.57[a]	17.33[a]	
(12-21 days)	24.50[a]	25.49[b]	
Female feed eff. (0-12 days)	3.84[a]	3.24[b]	
Litter feed eff. (0-12 days)	33.00[a]	35.38[b]	
(12-21 days)	29.75[a]	30.80[b]	
Litter wt/female wt 12 days	1.83[a]	1.80[a]	
21 days	3.85[a]	3.74[a]	

[abc] Row means not having a common superscript are significantly different at $P < .01$ except 21-day litter wt, 12-21 day feed intake and 12-21 day litter feed efficiency which are significant at $P < .05$.

Repeatability of Litter Size at Birth. Repeatability estimates of litter size at birth are presented in Table VI. Repeatabilities for daughters were first pooled within postnatal litter size classes after χ^2-tests of heterogeneity were found to be non-significant. Repeatabilities among lines within pairs of parities were also found to be homogeneous. Pooled repeatabilities between first and second parities were .39 ± .05 for dams and .27 ± .06 for daughters. Since these estimates did not differ significantly, they were pooled to provide an

Table VI - Repeatability of litter size at birth[a]

	Parities 1 and 2								
	Dams			Daughters[b]			Pooled		
Line	DF	r_{12}	SE	DF	r_{12}	SE	DF	r_{12}	SE
L^+	49	.29	.13	51	.44	.11	100	.37	.09
W^+	49	.37	.12	47	.27	.14	96	.32	.09
L^-W^+	47	.61	.09	51	.28	.13	98	.46	.07
K	55	.27	.12	54	.09	.13	109	.18	.09
L^+W^-	48	.38	.38	56	.22	.13	114	.31	.09
Pooled	252	.39	.05	259	.27	.06	511	.33	.04
χ^{2c}		5.69^{NS}			3.89^{NS}			5.30^{NS}	

	Parities 1 and 3			Parities 2 and 3		
	Daughters[b]			Daughters[b]		
Line	DF	r_{13}	SE	DF	r_{23}	SE
L^+	37	.02	.16	37	.36	.14
W^+	34	.32	.15	33	.07	.17
L^-W^+	47	.26	.14	45	.26	.14
K	49	.12	.14	49	.05	.14
L^+W^-	50	.01	.16	50	.18	.14
Pooled	221	.16	.07	218	.18	.06
χ^{2c}		3.69^{NS}			3.12^{NS}	

NS = not significant.

[a] r_{ij} = repeatability between parities i and j, ij = 1, 2, 3; SE = standard error; DF = degrees of freedom.

[b] Data on daughters are pooled within postnatal litter size levels.

[c] Chi-squares test of heterogeneity of repeatabilities among lines, 4 degrees of freedom.

estimate of repeatability of .33 ± .04 for litter size at birth between first and second parities. The pooled repeatability estimates for parities 1 and 3 and parities 2 and 3 were somewhat lower (.16 ± .07, .18 ± .06).

Repeatability of Other Traits. Preliminary analysis indicated that the repeatabilities of the traits listed in Table VII were homogeneous across line

Table VII - Repeatabilities and standard errors of traits of the female[a]

Trait	r_{12}	SE	r_{13}	SE	r_{23}	SE
Litter size 12 days	.22	.06				
21 days	.20	.06				
Number born alive	.27	.06	.15	.07	.21	.06
Litter wt birth	.30	.06	.14	.07	.22	.06
12 days	.43	.05				
21 days	.35	.06				
Litter wt gain (0-12 days)	.45	.05				
(12-21 days)	.22	.06				
Female wt partur.	.77	.03	.71	.03	.77	.03
12 days pp	.57	.04				
21 days pp	.65	.04				
Female wt change (0-12 days)	.18	.06				
(12-21 days)	.23	.06				
Feed intake (0-12 days)	.35	.06				
(12-21 days)	.37	.05				
Female feed eff. (0-12 days)	.16	.06				
Litter feed eff. (0-12 days)	.39	.05				
(12-21 days)	.01	.06				
Litter wt/female wt partur.	.28	.06	.08	.07	.19	.06
12 days	.36	.06				
21 days	.36	.06				

[a] Pooled within line by postnatal litter size subclasses; r_{ij} = repeatability between parities i and j, i, j = 1,2,3; SE = standard error.

by postnatal litter size subclasses; therefore, only pooled estimates are presented. Repeatabilities of number born alive and litter size at 12 and 21 days were similar to the repeatability of litter size at birth. Litter weight repeatability varied from .30 ± .06 at birth to .43 ± .05 at 12 days. Repeatability of litter weight gain from birth to 12 days (.45 ± .05) was higher than from 12 to 21 days (.22 ± .06). Litter feed efficiency and litter weight/female weight had repeatabilities within this range, except for litter feed efficiency between 12 and 21 days which was not different from zero. Feed intake had repeatabilities of .35 ± .06 and .37 ± .05. Female body weight at parturition, midlactation and weaning had the highest repeatabilities.

DISCUSSION

The effects of parity on litter size at birth in unselected mice that were interval bred (young were weaned prior to mating) have been well documented (Murray, 1934; Roberts, 1961; Biggers *et al.*, 1962; Rugh and Wahlfrohmm, 1967; Wallinga and Bakker, 1978). Litter size increases initially, followed by a plateau and then a decline. The results of the first three parities in the K control line are in agreement with these findings. In a randombred strain where females were left with males on a continuous basis, there was no increase in litter size with birth order (Wallinga and Bakker, 1978). This outcome was possibly caused by the greater stress on the dam when pregnancy occurred during lactation.

The effect of parity on fecundity is typically confounded with age and body weight of the female at mating. Kennedy and Kennedy (1972) designed an experiment to determine the importance of age, parity and body weight on corpora lutea counts. They reported that corpora lutea counts were greater in 14-week-old parous females than in 14-week-old virgins, and were greater in 14-week-old virgins than in sexually mature 6-week-old virgins. After adjustment for female body weight at the time they were assigned to a male, there was no difference in numbers of corpora lutea among the three groups. Kennedy and Kennedy (1972) concluded that age and parity effects on litter size were due to differences in body weight at mating. In support of this conclusion, Machin and Page (1973) found that litter size in primiparous mice increased with age at mating (6, 10 and 16 weeks) which could be

attributed to the increased body weight at mating. It is apparent, however, from the present study that results obtained in unselected lines cannot be extrapolated to lines selected for litter size and/or body weight. For example, female body weight increased with parity in all lines, whereas litter size did not.

The L^+ line failed to show an increase in litter size in parity 2 and declined in parity 3. Wallinga and Bakker (1978) observed the same trend in a line selected for large litter size, except that the decline did not occur until parity 4. Selection for large litter size increased ovulation rate to a high level in sexually mature nulliparous females (Joakimsen and Baker, 1977; Bakker *et al.*, 1978; Durrant *et al.*, 1980). Thus, increases in body weight associated with second or third parities are unlikely to result in a further increase in ovulation rate. Related to these results was the increase in litter size in second parity L^+W^- females. Considering the relatively small body size of L^+W^- females at their first parity mating and the relatively greater increase in size of these females during first parity gestation compared to controls, an increase in ovulation rate in parity 2 seems likely. The explanation for the decline in litter size in parity 3 of L^+W^- mice must await evaluation of implantation rates and fetal survival.

Selection for increased body weight in the W^+ line has resulted in a positive correlated response in litter size (Eisen, 1978) and ovulation rate (Durrant *et al.*, 1980). The absence of an increase in the second and third parities of the W^+ line conflict with findings in a previous generation (Eisen and Durrant, 1980a). Roberts (1961) found that a cross of two lines selected for large body weight and then selected further for large body weight for ten generations had a greater litter size than unselected mice, but there was no trend in litter size over the first four parities. A line that Falconer (1953) had selected for increased body weight showed a positive correlated response in litter size at first parity and increased over four parities followed by a rapid decline (Roberts, 1961). The large body size strains had a shorter reproductive life than small strains.

The index line selected for small litter size and large body weight (L^-W^+) exhibited an increase in litter size with parity. Data on successive parities in lines selected for reduced litter size do not appear to be published. Selection for small body weight exhibited a negative correlated response in

litter size (Falconer, 1953), but litter size increased with parity and eventually declined (Roberts, 1961).

Selection for small litter size resulted in a decreased ovulation rate, lower implantation rate and lower embryo survival to day 16 (Joakimsen and Baker, 1977). In contrast, Falconer (1960a) reported that selection for low litter size led to a decline in embryo survival and no decrease in ovulation rate. Selection for small body weight resulted in a decline in litter size primarily due to a lowered ovulation rate since embryo survival was not altered (Elliot *et al.*, 1968). It is apparent that a decrease in litter size due to direct or indirect selection for small litter size may be the result of different physiological factors. Consequently, similar parity effects on litter size may occur for different reasons, and these need to be investigated further.

Considering the fact that females are larger during their second parity and litter growth is increased, the parity effect on lactational feed consumption is trivial. The second parity females are more mature, and consequently litter growth and litter feed efficiency is enhanced for all lines and postnatal litter sizes. This result is expected as mammary gland development in the second parity is expected to increase lactational yield. However, Wallinga and Bakker (1978) reported no influence of the first four parities on litter weight at 5, 12 or 21 days in a line selected for large litter size and in unselected controls.

Repeatability measures the proportion of phenotypic variance accounted for by genetic, maternal and permanent environmental effects. Repeatability of litter size between first and second parity of .33 ± .04 was lower than .51 ± .07 obtained from the base population (Hanrahan and Eisen, 1974). Repeatabilities of litter weight, gain and feed efficiency between parities 1 and 2 were generally between .30 and .45, being slightly higher between birth and 12 days postpartum than between 12 and 21 days postpartum. Wallinga and Bakker (1978) reported litter size repeatabilities of .14 to .23 in a high litter size selected line and .47 in a control. Falconer (1960b) found a repeatability of .45 for number of young born alive from first and second litters.

The present findings demonstrate that selection for litter size and/or body weight can modify the influence of parity on litter size. In contrast, the postnatal maternal environmental effect persisted at least into parity 2. The second order interaction of line by postnatal litter size by parity was of no biological importance for litter size or any of the other traits examined.

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RESUMO

O desempenho reprodutivo de cinco linhagens (L) de camundongos, em que as fêmeas a serem avaliadas foram criadas em três tamanhos de ninhada pós-natais (N), foi comparado da segunda à terceira paridade (P). As linhagens foram selecionadas durante 17 gerações, como segue: tamanho de ninhada grande (L^+), peso corporal grande à 6ª semana de idade (W^+); tamanho de ninhada pequeno e peso corporal grande à 6ª semana (L^-W^+); tamanho de ninhada grande e peso corporal pequeno à 6ª semana (L^+W^-), e ao acaso (K). Os níveis de N foram 8, 12 e 16. O objetivo foi o de determinar a magnitude dos efeitos da paridade e as interações envolvendo a paridade para tamanho de ninhada, ganho de peso e eficiência de alimentação, peso das fêmeas e ingestão de alimento pelas mesmas, medidos desde o parto até o desmame aos 21 dias. A interação L x N x P não foi significativa para nenhum caráter. As interações N x P foram significativas para o peso corporal das fêmeas na data do parto e para mudança do mesmo do 12º ao 21º dia após o parto; nenhuma das interações foi biologicamente importante. Assim, o efeito de N no tamanho da ninhada e em outros caráteres reprodutivos persistiu ao longo das paridades. As interações L x P foram significativas para tamanho da ninhada ao nascer, no 1º e 21º dia, para peso ao nascer da ninhada e para peso da fêmea na data do parto, o que foi interpretado como sinal de que o critério de seleção tinha modificado a influência da paridade nestes caráteres. Os pesos ao nascer, ganhos de peso das ninhadas e eficiência, e os pesos corporais das fêmeas aumentaram com a paridade, mas não apresentaram interações importantes. As repetibilidades combinadas para o tamanho da ninhada foram 33 ± 0,04, 16 ± 0,07 e 18 ± 0,06 respectivamente para as paridades 1 e 2, 1 e 3 e 2 e 3. A repetibilidade entre as paridades 1 e 2 para os pesos ao nascer, ganhos de peso das ninhadas e eficiência de alimentação variaram de 0,22 a 0,45, com a exceção da eficiência de alimentação do 12º ao 21º dia, que foi de 0,1 ± 0,06.

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ALLOZYME AND INVERSION POLYMORPHISMS IN CHILEAN NATURAL POPULATIONS OF *DROSOPHILA FLAVOPILOSA**

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ABSTRACT

Drosophila flavopilosa in Chile is found exclusively associated with the flowers of *Cestrum parqui* (Solanaceae). Natural populations of the species are polymorphic for the gene sequences in the fifth chromosome due to the presence of four independent inversions, and for allozyme variation at the *Lap*, *Est-1* and *Est-2* loci. A study of five natural populations along an altitudinal gradient in the Maipo Valley near Santiago (Chile), from sea level to an elevation of 1250 m, reveals that the frequency of the two most abundant gene arrangements follows a clinal distribution in relation to the altitude. In contrast, geographic differences in allozyme polymorphism are found only with respect to some alleles of the *Est-2* locus. These variations do not follow an altitudinal gradient. The distribution of the observed variations at the *Lap* and *Est-1* loci is rather uniform.

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INTRODUCTION

Drosophila flavopilosa is a widely distributed species in the neotropical region, that has been recorded in Brazil. Uruguay, Bolivia, Peru and Chile (Brncic, 1976). The species is an exclusive flower-breeding organism that in Chile is always associated with the solanaceous shrub *Cestrum parqui* (Brncic, 1962, 1966). Chilean populations of the species are polymorphic for the banding sequences in the fifth chromosome due to the existence of four paracentric inversions. Quantitative analysis of the polymorphism demonstrated that there are seasonal fluctuations and two kinds of geographic variations, a North-South gradient and an altitudinal one. The altitudinal gradient of the frequencies of the two most conspicuous inversions, *inv. A* and *inv. B*, is specially pronounced along the Maipo Valley, near Santiago, Chile (Lat. 33^0 40' S) (Brncic, 1972).

The present paper intends to discuss whether a connection exists between the altitudinal clines of the inversion polymorphism in *D. flavopilosa* and allozyme polymorphisms. The allozyme variations were studied electrophoretically for two enzyme systems: *esterase* (two sites), and *leucine aminopeptidase* (one locus).

MATERIAL AND METHODS

For the present study samples of flowers of *C. parqui* were brought into the laboratory in Santiago from five sites in the Maipo Valley: *El Tabo* at sea level, *Algarrobo*, approximately 20 km further North from the first location, also at sea level, *Vizcachas* (818 m above sea level), *Melocotón* (1015 m) and *San Gabriel* (1250 m). All samples were collected in April and May of 1978.

The following procedure was employed with each collection. Upon arrival at the laboratory, more than 200 *C. parqui* flowers were opened, to determine the percentage containing preadult forms of the fly. A rough estimation of the abundance of the species was obtained with this procedure. The percentage of flowers containing preadults was, 37 at *Algarrobo*, 54 at *El Tabo*, 33 at *Vizcachas*, 41 at *Melocotón* and 29 at *San Gabriel*. The larvae of *D. flavopilosa* continued to develop until the adult stage inside the flowers

which were brought to the laboratory. To analyze the gene arrangements, the larger larvae were dissected and their salivary glands were stained by means of the aceto-lacto-orcein rapid squash methods.

To determine allozyme polymorphisms, live emerged adults were sent alive to Porto Alegre, Brasil, where they were frozen at 20^0, C until electrophoretic analysis. Individual flies were homogenized in 5 μl deionized water and absorbed onto wicks of Whatman Nº 3 filter paper (0.4 x 0.2 cm). Each wick was applied to horizontal polyacrylamide (6 %) gels combined with a discontinuous tris-citrate buffer (Poulik, 1957) for Esterase (*Est*) and Leucine aminopeptidase (*Lap*). An electric field of 10 v/cm was applied across the gels for 3 to 4 hours or until the migrating front was 9 cm from the sample slot line. After the bands appeared, the reaction was stopped by washing the gel with water and adding 100 ml of the fixing solution of methanol: water: acetic acid (5:5:1). The staining methods used are similar to those described in a previous paper (Napp and Brncic, 1978).

The allozyme variants were identified by their relative electrophoretic mobilities, the reference being the most common allele in *D. cestri*, a species of the *flavopilosa* group already analyzed (Napp and Brncic, 1978).

RESULTS

Table I shows the frequencies of the different gene arrangements found in heterozygous condition in the five natural populations along the Maipo Valley. The observed frequencies were very similar to those already reported for the same sites in previous years (Brncic, 1972, 1976). Three of the gene arrangements, *inversion A* and *B*, and the *standard* gene order were well represented. The other two, *inversions C* and *D* were in low frequencies. As previously reported (Brncic, *op. cit.*), the frequencies of the *A* heterokaryotypes are lower at the localities near the coast (*Algarrobo* and *El Tabo*), but gradually increase with altitude, reaching over 50 percent at San Gabriel (1250 m). In contrast, inversion *B* is more abundant near sea level and tends to decrease with altitude.

The observed allozyme polymorphisms for the *Lap*, *Est-1* and *Est-2* loci are shown in Tables II, III and IV. Five alleles in the *Lap* locus were

detected (Table I), $Lap^{1.00}$ being the most frequent one in all populations with the exception of *El Tabo*, where $Lap^{.95}$ was predominant. $Lap^{1.00}$ was also reported to be the predominant one in *D. cestri*, a member of the same group of species of *Drosophila* (Napp and Brncic, 1978). No significant interpopulational differences were observed regarding the allele frequencies. Six alleles were electrophoretically detected in the *Est-1* locus (Table III). There is interlocality variation regarding the frequency of the most abundant variant, $Est\text{-}1^{.97}$. Chi-square analysis gives values of 9.97 with 4 df ($p = 0.05 - 0.02$). Nevertheless, the observed variation does not follow an altitudinal cline.

The *Est-2* locus shows six electrophoretic detectable variants (Table IV). Contrary to what is seen with *Lap* and *Est-1* polymorphisms, there are clear geographic differences regarding the allele frequencies. In the *Algarrobo* population the predominant allele was $Est\text{-}2^{.96}$ and in the other four the most frequent was $Est\text{-}2^{1.04}$. Chi-square analysis for the observed interlocality differences gives significant values for all the alleles with the exception of $Est\text{-}2^{1.00}$, which shows little variability. However, there is no evidence of a clinal frequency variation corresponding to the altitudinal gradient of the samples studied.

Table I - Frequencies of inversions found in heterozygous condition in the V-R chromosome of *D. flavopilosa* in populations at different altitudes in central Chile.

SITES	ALTITUDE (in m)	COLLECTING DATES	LARVAE TESTED	NONE	HETEROZYGOUS INVERSIONS				
					A	B	C	D	OTHERS*
ALGARROBO	10	MAY, 1978	116	.578	.103	.293	.026	–	–
EL TABO	10	APRIL, 1978	100	.560	.130	.270	.020	.010	.010
VIZCACHAS	818	APRIL, 1978	140	.500	.357	.129	.007	.007	–
MELOCOTÓN	1015	MAY, 1978	92	.489	.402	.076	.022	–	.011
SAN GABRIEL	1250	MAY, 1978	102	.539	.402.	.029	.010	.020	–
TOTAL			550		**	**			

* Represents the double Heterozygotes, i.e A + B, A + C.

** $P < 0.001$ using the chi-square test (D.F. 4).

Table II - Frequencies of the alleles of *Lap* and heterozygosity in populations of *D. flavopilosa* at different altitudes in central Chile.

SITES	ALTITUDE (in m)	COLLECTING DATES	GENOMES ANALYZED	.91	.93	.95	1.00	1.03	HETERO-ZYGOSITY
ALGARROBO	10	MAY, 1978	86	–	.058	.430	.477	.035	.480
EL TABO	10	APRIL, 1978	212	.005	.024	.505	.424	.024	.552
VIZCACHAS	818	APRIL, 1978	174	–	.017	.471	.489	.023	.460
MELOCOTÓN	1015	MAY, 1978	156	–	.026	.423	.525	.026	.538
SAN GABRIEL	1250	APRIL, 1978	154	–	.020	.448	.513	.019	.519
TOTAL			782						

Table III - Frequencies of the alleles of *Est-1* and Heterozygosity in populations of *D. flavopilosa* at different altitudes in central Chile.

SITES	ALTITUDE (in m)	COLLECTING DATES	GENOMES ANALYZED	.91	.95	.97	1.00	1.03	1.06	HETERO-ZYGOSITY
ALGARROBO	10	MAY, 1978	78	.013	.179	.372	.218	.115	.103	.641
EL TABO	10	APRIL, 1978	174	.023	.172	.454	.213	.086	.052	.697
VIZCACHAS	818	APRIL, 1978	198	.025	.157	.303	.247	.172	.096	.641
MELOCOTÓN	1015	MAY, 1978	146	.041	.185	.370	.206	.130	.068	.699
SAN GABRIEL	1250	MAY, 1978	142	.070	.232	.331	.148	.120	.099	.648
TOTAL			738			*				

*P = 0.02 - 0.05 using the chi-square test (D.F. 4).

Table IV - Frequencies of the alleles of *Est-2* and Heterozygosity in populations of *D. flavopilosa* at different altitudes in central Chile.

SITES	ALTITUDE (in m)	COLLECTING DATES	GENOMES ANALYZED	.89	.94	.96	1.00	1.04	1.07	HETERO-ZYGOSITY
ALGARROBO	10	MAY, 1978	76	.158	.210	.382	.224	.026	–	.368
EL TABO	10	APRIL, 1978	218	.005	.028	.179	.206	.330	.252	.404
VIZCACHAS	818	APRIL, 1978	204	.029	.034	.103	.304	.348	.181	.294
MELOCOTÓN	1015	MAY, 1978	164	.024	.201	.146	.275	.342	.012	.390
SAN GABRIEL	1250	MAY, 1978	142	.035	.148	.183	.303	.317	.014	.142
TOTAL			804	*	*	*		*	*	

*P < 0.01 using the chi-square test (D.F. 4)

DISCUSSION

The altitudinal gradients of the inversion polymorphism observed in *D. flavopilosa* have been discussed fully in previous publications (Brncic, 1966, 1972). Some experiments carried out under controlled laboratory conditions lead to the conclusion that the temperature at which the larvae develop represent a critical factor for the viability of the *A* and *B* heterokaryotypes (Brncic, 1968). *A* heterokaryotypes seem to be advantageous at 25° C, while *B* heterokaryotypes seem to be advantageous at 16° C. *Algarrobo* and *El Tabo*, at sea level, have a lower mean temperature than *Vizcachas, Melocotón* and *San Gabriel* (the coldest site) in the pre-andean zone. Therefore, the clinal frequency variation of the inversions is assumed to be dependent of the temperature gradient at the different elevations in the Maipo Valley. This interpretation is also in agreement with the already reported seasonal fluctuations in the chromosomal polymorphism, where the *A* arrangement is more abundant during the summer months while the *B* arrangement tends to increase during the coldest season. As a result of the increase in *A* inversions concomitant with the decrease of *B* in some seasons of the year or at certain elevations and the reverse tendency in other seasons or elevations, the total inversion heterozygosity is maintained at a more or less constant level (Brncic, 1972, 1976).

Contrary to what is seen with the inversion polymorphism, the allozyme polymorphisms for *Lap* and *Est-1* seem to be rather uniform throughout the five natural populations of *D. flavopilosa* located at different altitudes. Geographic differences in allele frequencies seem to exist only in the *Est-2* locus, and the observed variation at this locus does not show an altitudinal cline. The most pronounced differences were observed just between the *Algarrobo* and *El Tabo* populations, both at sea level and separated from each other by a distance of only 20 km. This is not surprising, given the data from other *Drosophila* species showing that samples of flies collected, within a 40 x 75 m area, can be differentiated genetically by their allozyme gene frequencies (Richmond, 1978). For example, this author reports that in *D. affinis*, differentiated populations with respect to six allozyme loci could be found 25 meters apart. Moreover, Taylor and Powell (1977) found that populations of *D. persimilis* separated by a distance of only 200 meters, exhibit significant differences in the allele frequency of seven allozyme loci.

It is difficult to decide whether the observed interlocality frequency differences of *Est-2* alleles, are due to random drift or to selection response. However, the effective size of the studied populations of *D. flavopilosa,* estimated by the abundance of the plant-host and the number of flowers of *C. parqui,* containing preadult forms, are large enough to prevent random changes. Yet, the efficacy of genetic drift depends upon another demographic parameter, the migration rate. Most *Drosophila* species have a high dispersive ability which permits genetic differentiation within a rather reduced area. Moreover, contrary to the effects of selection, drift acts uniformly over all loci. Nevertheless, no noticeable variations in allelic frequencies were detected in *Est-1* and *Lap* loci, or in the *Est-2*$^{1.00}$ allele. All these considerations suggest that genetic drift is not the key factor in the maintenance of the observed microspatial differences. Therefore, changes at the *Est-2* locus should be ascribed to selection rather than to random drift. In spite of the narrow substrate utilized by *D. flavopilosa,* the flowers of *C. parqui,* it is possible that some environmental heterogeneity exist. This heterogeneity could be related to the microgeographic genetic differentiation at some electrophoretically detectable alleles. There is evidence in *Drosophila* that small changes in the environment can induce clear modifications in the frequencies of structural genes (Powell and Wistrand, 1978; Richmond, 1978).

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RESUMO

A *Drosophila flavopilosa* no Chile é encontrada exclusivamente associada às flores do *Cestrum parqui* (Solanaceae). As populações naturais da

espécie são polimórficas para as sequências gênicas do quinto cromossomo, devido à presença de quatro inversões independentes, e para variação alozímica nos locos *Lap*, *Est-1* e *Est-2*. Um estudo de cinco populações naturais ao longo de um gradiente de altitude no Vale de Maipo, perto de Santiago (Chile), desde o nível do mar até uma elevação de 1250 m, revela que a frequência dos dois arranjos gênicos mais abundantes segue uma distribuição clinal em relação à altitude. Em contraste, diferenças geográficas de polimorfismo alozímico são encontradas apenas em relação a alguns alelos do loco *Est-2*. Estas variações não seguem um gradiente de altitude. A distribuição das variações observadas nos locos *Lap* e *Est-1* é bastante uniforme.

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ENVIRONMENTAL AND GENETIC EFFECTS ON LENGTH OF LACTATION IN A BRAZILIAN TROPICAL DAIRY BREED

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ABSTRACT

Data from 1,723 records on 483 Pitangueiras cows were analyzed. The Pitangueiras breed was synthesized by crossing pure Red Poll (RP) males with 1/4 RP 3/4 Zebu females in the humid tropic area of Pitangueiras city, near São Paulo, Brazil. Mean length of lactation was 281 days (range 100 to 390) with mean milk yield of 2835 kg. Least-squares analysis of variance showed that within lactation number older animals had longer lactations, (17.2 to 50.7 days), as did animals freshening in the dry season (.6 to 9.8 days). Repeatability estimated by intraclass correlation was .15 ± .06. Heritabilities for lactation length estimated from paternal half-sib correlations were .33 ± .22 for first lactation, .34 ± .25 for second and .16 ± .28 for third. Overall estimate was .29.

INTRODUCTION

An interesting aspect of milk production in tropical dairy cattle is

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length of lactation. Variability appears much greater in tropical than in temperate dairy cattle with the former having a relatively high proportion of short lactations. Most European dairy cattle in temperate zones produce milk at profitable levels for 305 days. This length is the most common for evaluating performance of dairy cows and it corresponds to a 12-month calving interval. With Zebu cattle, however, lactations usually are shorter than 305 days; this often has resulted in the recording of total lactation yields without limiting the period of production. The mean length of lactation in Zebu cattle varies considerably between breeds (Benintendi *et al.*, 1965/6; Carmo and Prata, 1961; Correa, 1956; Dutt and Singh, 1961; Mahadevan *et al.*, 1962). The objective of the present study was to evaluate several environmental and genetic effects on lactation length in a new tropical Brazilian breed, the Pitangueiras.

MATERIAL AND METHODS

Animals and Management

Data used were collected during 13 years (1962 - 1974 inclusive) from records on 483 Pitangueiras cows (5/8 Red Poll x 3/8 Zebu) with 1,723 lactations, maintained at Três Barras farm, in the state of São Paulo, Brazil. Details of the climate and management program have been presented earlier (Lobo *et al.*, 1979).

In this herd, young heifers are placed with bulls for mating when they reach 320 kg body weight. Milking cows are mated during the first heat period after the 60th day postpartum. They are treated for reproductive disorders such as trychomonosis or endometriosis as required. Cows with serious reproductive problems and those which fail to reproduce for more than two consecutive years are culled from the herd. The major selection pressure applied was for milk yield with some attention to animal development and typical traits of the breed.

Statistical Analysis

Limitations in computer capability prevented estimation of all desired parameters in a single least squares analysis. Data were partitioned into subsets, therefore, and mathematical models revised appropriately. To

obtain estimates of non-genetic effects with maximum utilization of data, the data were sorted by lactation number and each lactation analyzed separately (Harvey, 1960).

The underlying model used was

$$Y_{ijkmn} = u + c_i + a_j + s_k + p_m + e_{ijkmn}$$

where:

Y_{ijkmn} = length of lactation; u = least squares mean; c_i = effect of the i^{th} cross (i = 1, 2); a_j = effect of the j^{th} age (j = 1, 2, 3, 4); s_k = effect of the k^{th} season (k = 1, 2); p_m = effect of the m^{th} year (m = 1, 2, ..., 13); and e_{ijkmn} is random error common to a single record. Cross, age, season and year were considered to be fixed effects. The errors were assumed to be independently and normally distributed with mean zero and variance σ_e^2.

Repeatability was estimated from a subset of data from 68 cows which had four lactations each. The model consisted of lactation number, cow and residual. Heritability was determined on P_2 (i.e. $P_1 \times P_1$) animals only with lactation numbers 1, 2 and 3 analyzed separately. Model included age, year, sire and residual.

Estimate of repeatability was obtained by intra-class correlation,

$$t = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2},$$

where σ_c^2 represented among-cow variance and σ_e^2 within-cow variance. Heritability was estimated by paternal half-sib correlation,

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2},$$

where σ_s^2 was the sire variance and σ_e^2 the error variance. A pooled within-lactation estimate of heritability also was obtained.

RESULTS AND DISCUSSION

Table I shows the means, standard errors and coefficients of variation (C.V.) for lactation length in the first five lactations. The average lactation length for 1,723 records was 281.3 ± 1.4 days (C.V. = 20.5%). Mean milk yield was 2835.4 ± 20.1 kg (C.V. = 29.4%). The shortest length was 100 days, and longest 390 days. Only 66.6% of first lactation cows reached a minimum length of 305 days of milking. However, this value is higher than that described by Mahadevan (1955) for the Red Sindhi breed, where approximately 50% of lactations recorded terminated before 300 days. Consequently it is not surprising to note that lactation yields of cows in the tropics are highly correlated with the length of lactation.

Table I - Means and variability in lactation lengths (days)

Lactation number	Number of records	Mean ± S.E.[a]	C.V.[b] (%)
First	483	284.2 ± 2.6	19.7
Second	431	277.6 ± 2.8	20.7
Third	357	282.0 ± 3.1	20.7
Fourth	257	281.2 ± 3.7	21.1
Fifth	195	280.7 ± 4.2	21.0
Total	1,723	281.3 ± 1.4	20.5

[a]S.E. = Standard error; [b]C.V. – Coefficient of variation

In tropical Brazil, mean lengths of lactation for milk-producing Zebu breeds vary between 247 and 263 days (Correa, 1956; Carmo and Prata, 1961; Benintendi *et al.*, 1965/6), and between 240 and 258 days for *Bos taurus* x *Bos indicus* crosses (Jordão, 1968; Lobo, 1974). This work suggests that animals of Zebu breeds have short lactation periods, which doubtless are due to a combination of genetic and environmental factors.

Perhaps more attention should be paid to genetic and management implications of lactation length and its genetic as well as environmental

correlation with milk yield. Some researchers indeed consider it important to take length of lactation into account in selecting milk cows for production in hot climates.

Compared to other data, the 281-day value obtained for the Pitangueiras was higher than those for Hariana cattle in India (Dutt and Singh, 1961), African Zebu cattle in Uganda (Mahadevan *et al.*, 1962), Sahiwal cattle in Hissar, India (Malik and Sindhu, 1968), and Native cattle in Iraq (Kassir *et al.*, 1969). With respect to *Bos taurus* x *Bos indicus* crosses, the Pitangueiras cattle have shorter periods of lactation than those found for Sahiwal x African Zebu in Uganda (Mahadevan *et al.*, 1962), Schwyz x Criollo in Venezuela (Cevallos *et al.*, 1968) or Jamaica Hope in Jamaica (Wellington *et al.*, 1970).

Although length of lactation in this study was quite variable, its coefficient of variation (20.5%) was lower than reported for most studies carried out in the tropics (Correa, 1956; Kassir *et al.*, 1969; Ohri and Singh, 1971; Venkateshwarlu *et al.*, (1973). Values cited by these authors varied between 28 and 35%, suggesting ample possibilities for improving the trait, if such variation is mostly additive genetic.

Means for first lactation were 284.2 days, for second 277.6 days, for third 282.0 days, for fourth 281.2 days and for fifth 280.7 days. The decrease from first to second lactation followed by an increase in the third is a phenomenon which has been noted several times for cattle raised in tropical countries. Wilson (1959) observed variations up to the sixth lactation. According to these authors, such results may be attributed to feeding deficiencies since the cows were kept on poor grazing grounds and received no concentrate supplements. Oscillation between odd and even-numbered lactations, considered by some authors to be characteristic of tropical cattle, could not really be confirmed in this study, since the differences were not statistically significant.

According to Mahadevan (1953, 1955), short lactations are not a trait peculiar to Zebu cattle, but rather a characteristic shared by all cattle submitted to environmental conditions prevailing in tropical areas, whether Zebu, European or crossbreeds. Bodisco *et al.*, (1968), however, studying the Schwyz (Brown Swiss) breed, concluded that the tropical environment does not interfere with length of lactation. A later report by Bodisco *et al.*, (1971) confirmed this. These authors declared that European breeds specialized for

Table II - Least-squares constants for factors affecting length of lactation.

Effects		Constants (days)				
		First	Second	Third	Fourth	Fifth
Least-squares means		276.5	277.8	271.7	264.3	264.1
Group[a]	P_1	9.6	4.0	9.9	7.4	15.5
	P_2	-9.6	-4.0	-9.9	-7.4	-15.5
Age (mo)	24	-8.7	–	–	–	–
	36	8.5	-23.1	–	–	–
	48	.2	-.2	-30.2	–	–
	60	–	7.9	1.5	-25.8	–
	72	–	15.2	8.2	6.2	-5.0
	84	–	–	20.5	10.9	-8.8
	96	–	–	–	8.7	13.8
Season dry		3.1	4.5	4.9	.3	2.0
rainy		-3.1	-4.5	-4.9	-.3	-2.0
Year	62	-47.6	–	–	–	–
	63	31.2	40.2	–	–	–
	64	2.9	3.9	-26.7	–	–
	65	-13.5	-1.0	-12.9	5.8	–
	66	-9.0	-6.8	-3.8	-34.1	-25.5
	67	18.7	-5.1	5.1	15.4	16.5
	68	-3.3	21.8	19.1	55.1	-8.9
	69	16.7	15.2	22.6	26.7	13.2
	70	.4	-16.6	-4.6	-10.1	1.2
	71	5.2	-21.7	.1	8.9	-12.0
	72	-5.2	-4.5	-11.3	-13.4	15.3
	73	3.5	-1.6	3.1	10.1	17.7
	74	–	-23.8	9.3	-64.4	-17.5

[a] P_1 = 5/8 Red Poll x 3/8 Zebu

$P_2 = P_1 \times P_1$

milk production can produce the same amounts of milk in the tropics as they do in their temperate environments.

No doubt, more genetic studies of tropical breeds are needed, but, on the basis of the data obtained in this study, there is no evidence that a tropical environment reduces the length of lactation.

The least-squares constants for cross varied between 4.0 and 15.5 days favoring P_1 cows from first through fifth lactation (Table II). Differences between P_1 and P_2 (i.e. $P_1 \times P_1$) crosses were significant overall.

Parturition in the rainy season resulted in reduced lactation lengths ranging from .58 (fourth lactation) to 9.80 days (third lactation). Weighted mean effect was 6.57 days. Dutt and Singh (1961), working with Hariana cows, also observed a significant season of calving effect. According to these authors, cows which calved in Winter had longest lactation periods (281 days), whereas those which calved in the Fall had shortened lengths (240 days). In the present study, cows freshening in the dry season were offered supplementary feed, which could have resulted in the longer lactations.

Table III - Least squares analysis of variance (mean squares) for lactation length.

Source of variation	Lactation order				
	First	Second	Third	Fourth	Fifth
Group	37174*	5393	27397*	10543	26433*
Age of cow	7409	6747	6151	3661	10493*
Season	4159	7899	7619	1800	770
Year	6042*	6922*	4311	14657*	4230
Error	2981	3187	3259	3028	3248

*$P < .05$

Estimate of repeatability was .15 ± .06, suggesting a real though slight tendency for the cows to repeat their lactation lengths from year to year. This estimate was based on a small (68 cows) and select (only cows with four lactations) data set, so it must be viewed with caution. Repeatability also is useful, of course, in suggesting the upper limit of heritability. The estimate

was lower than those reported by Singh and Desai (1961) for Hariana (.28), Mahadevan *et al.* (1962) for African Zebu cattle (.33), Wellington *et al.* (1970) for Jamaica Hope (.23), and Dutt *et al.* (1974) for the Tharparkar breed (.41).

Heritabilities for lactation length were .33 ± .22 for first lactation, .34 ± .25 for second and .16 ± .28 for third. These values suggest there is a reasonable amount of additive genetic variance in this trait. According to Singh and Desai (1961) and Venkateshwarlu *et al.* (1973), this trait is quite highly heritable and could be improved in tropical countries by selection. Overall (pooled) estimate was .29. These estimates obtained for the Pitangueiras herd were the first to be determined for this breed and may provide a guideline for choosing future effective breeding methods. However, they were higher than the estimate of repeatability.

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RESUMO

Foram analisados dados de 1.723 registros de 483 vacas da raça Pitangueiras. A raça Pitangueiras foi sintetisada pelo cruzamento de machos puros Red Poll (RP) com fêmeas 1/4 Red Poll x 3/4 Zebu, na área tropical úmida da cidade de Pitangueiras, perto de São Paulo, Brasil. A duração média da lactação foi de 281 dias (variando de 100 a 390), com uma produção leiteira média de 2.835 kg. A análise de variância pelo método dos quadrados mínimos mostrou que os animais mais velhos tiveram lactações mais prolongadas (de 17,2 a 50,7 dias), acima da média, o mesmo ocorrendo com os animais paridos na época seca (de 0,6 a 9,8 dias). A repetibilidade estimada pela correlação intraclasse foi de 0,15 ± 0,06. As herdabilidades para duração da

lactação estimadas a partir das correlações entre meio-irmãos paternos foram de 0,33 ± 0,22 para a primeira lactação, 0,34 ± 0,25 para a segunda e 0,16 ± 0,28 para a terceira. A estimativa global foi de 0,29.

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DE NOVO BALANCED RECIPROCAL TRANSLOCATION 46,XX, t (16;22) (p13; q11) IN A DYSMORPHIC FEMALE*

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ABSTRACT

Multiple congenital anomalies were found in a female child with a *de novo* translocation involving a chromosome number 16 and a number 22, with no apparent loss of chromosome material. The karyotype was 46,XX,t (16;22) (q13;q11). The clinical and dermatoglyphic features of the patient are described and the karyotype-phenotype association discussed.

INTRODUCTION

Apparently balanced autosomal translocations have been described in individuals with a wide variety of congenital malformations. Nearly all chromosomes have been involved in these cases (Biederman and Bowen, 1976;

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Francke, 1972; Fried *et al.*, 1977; Jacobs *et al.*, 1978; Miller *et al.*, 1977; Serville *et al.*, 1974; Skovby and Niebuhr, 1974; Tharapel *et al.*, 1977; Tinning *et al.*, 1975; Verma *et al.*, 1976; Wajntal *et al.*, 1978). A detailed analysis of these presumptive balanced translocations is very important for understanding their possible clinical implications. This knowledge is fundamental for evaluating balanced rearrangements when found in prenatal diagnosis. Information about translocations can also contribute to our understanding of the structure and function of genetic material.

This report describes what we believe to be the first case of a *de novo* translocation between chromosomes number 16 and number 22 associated with multiple congenital anomalies.

CASE REPORT

APF was born at term in August 1976, when the mother was 26 and the father 29 years old. Birth weight was 3300 g and length, 50 cm; pregnancy and delivery were normal. The patient had a healthy sister born in 1975 and the mother had had a previous miscarriage. The parents were healthy and unrelated. There were no cases of congenital malformations in the family.

Physical examination at the age of 12 months showed a healthy female child with a peculiar facial appearance (Figure 1). The head was microcephalic with a flat occipital bone and widely open fontanelles. The hair was thin and frontally low-implanted. The face was dominated by prominent and tearing eyes with a moderate degree of hypertelorism, presence of epicanthal folds and lowered palpebral fissures. A bilateral congenital glaucoma was diagnosed with the horizontal corneal diameter measuring 13 mm at the right and 14 mm at the left. The irises filled out the palpebral fissures. Iridodonesis, blue sclerae, coloboma of iris, bilateral subluxation of the crystalline lens and nystagmus were present. The mouth was small with down-slanted fissures and high-arched palate. There was great difficulty in swallowing. The ears were low-set and the nasal bridge was flat. The neck was short, the chest showed thoracic kyphoscoliosis and pectus excavatum, without detectable cardiorespiratory anomalies. The lower limbs had small patellae and prominent heels. Generalized hypotonia and generalized articular hyperextensibility, with

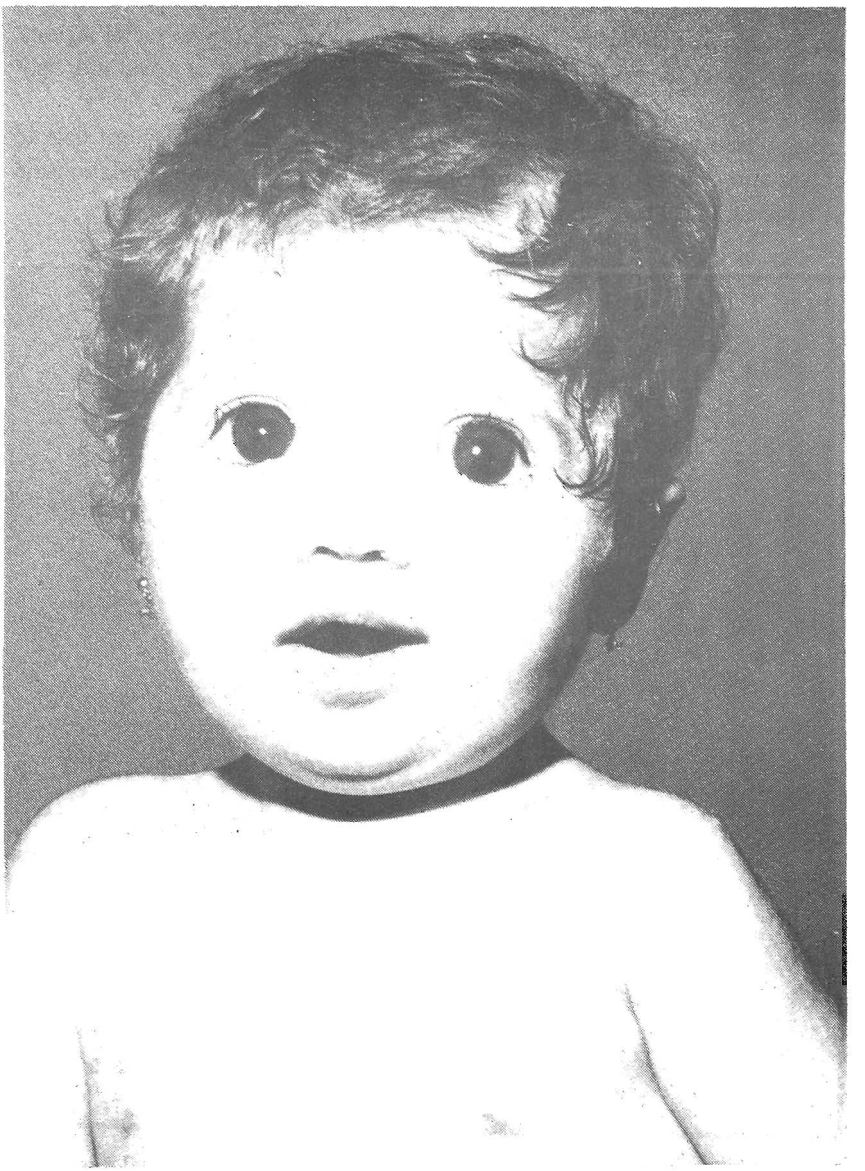

Figure 1. Face of the patient; aged 12 months.

tendency to maintain the lower limbs abducted were observed. Cutaneous haemangiomas were present in the frontal, occipital and lumbar-sacral regions. Lanuginous areas were found on the left thigh, scapular regions and right forearm.

Neuropsychomotor development was retarded. She was not able to sit without support, she was quiet and had a normal emotional relationship with her family. The haematological findings were normal.

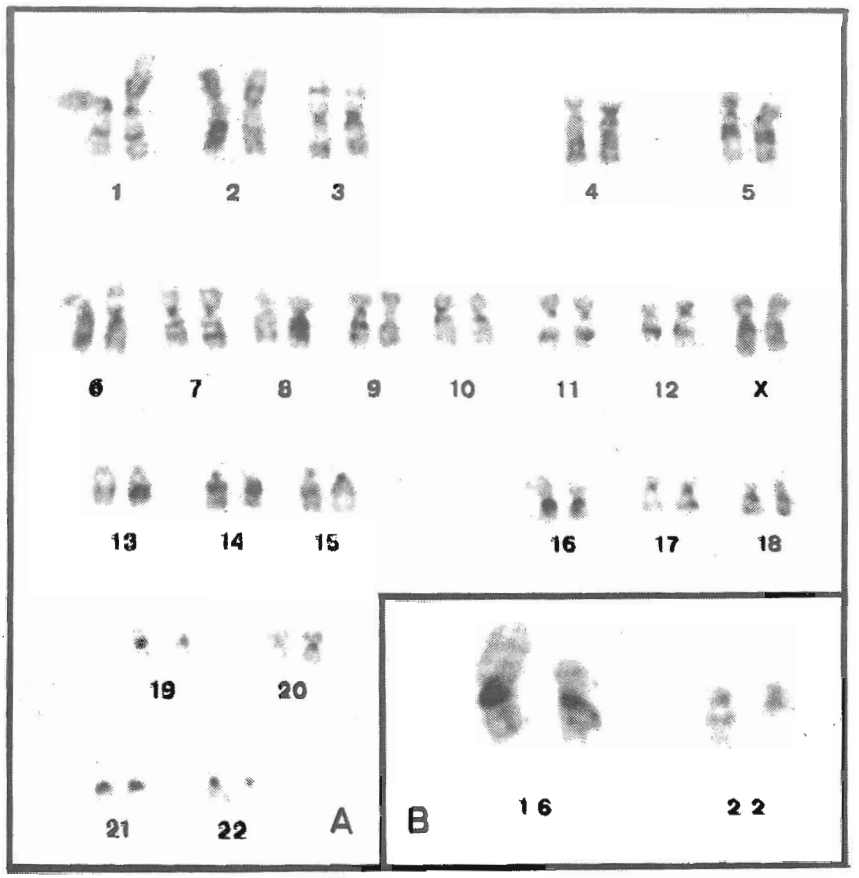

Figure 2. G- banded karyotype of the patient (A) and the partial karyotype illustrating the translocation (B).

Cytogenetics studies

Chromosome investigation was performed on peripheral blood lymphocytes. After G- and C- banding the karyotype of the patient (Figure 2) was found to be 46,XX,t (16;22) (p12;q11). Her parents had normal chromosomes. No increase in breakage rate was noted.

Dermatoglyphics

The patients had 3 whorls, 6 ulnar and one radial loop on her fingers. The mainline formulae were 11. 9. 7. 5"-t-A^{u}.W/L. O.L.O. at right palm and 11. 9. 7. 5"-t-A^{u}.W/V. O.L.O. at left. There was a simian line and an atd angle

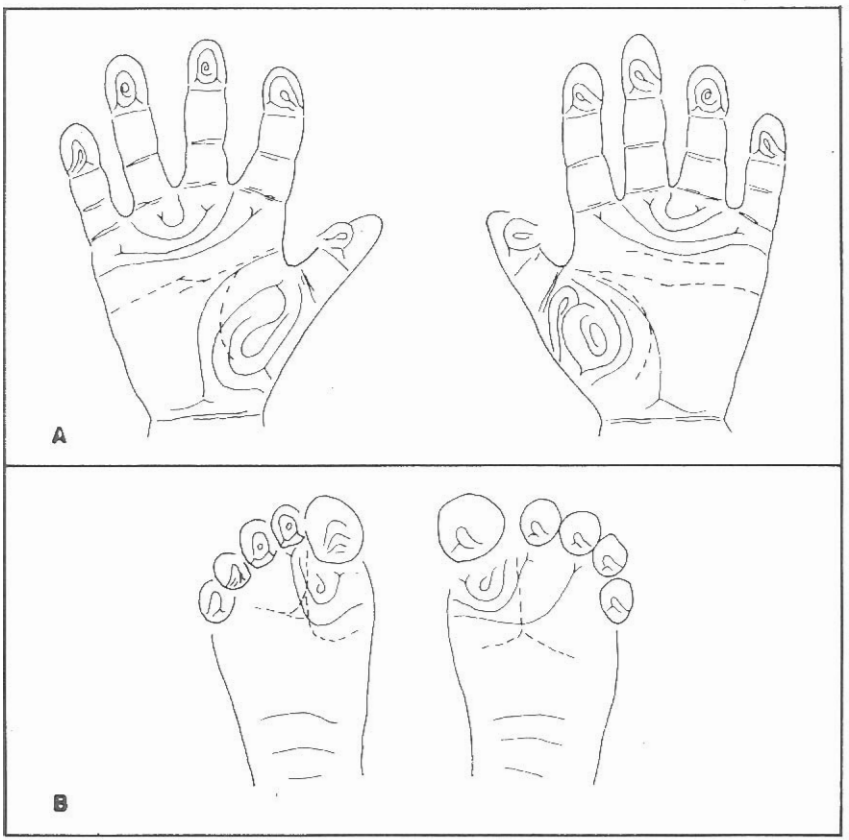

Figure 3. Diagram of palmar (A) and plantar (B) dermatoglyphic patterns of the patient.

of 43° on the right and a transitional flexion crease and an atd angle of 46° on the left. A distal loop in the hallucal area was present bilaterally on the soles of her feet (Figure 3).

DISCUSSION

Balanced structural rearrangements are infrequently found in newborn series (Table I), and most often have no effect on the phenotype. Most of the known cases of balanced reciprocal translocations have been detected because of offspring with partial monosomy or partial trysomy or because of recurrent abortions (Grouchy, 1976).

Table I - Frequency of balanced reciprocal translocations in newborn series.

References	Number of newborns	rcp (%)	rcp with CM (%)
Friedrich and Nielsen, 73	5049	0.14	0.02
Bochkov *et al.*, 74	2500	0.08	–
Jacobs *et al.*, 74	11680	0.09	0.01
Hamerton *et al.*, 75	14069	0.08	0.02
Lin *et al.*, 76	930	0.11	–
Maeda, 77	2201	–	–
Kuleshov *et al.*, 78	6000	0.12	0.05
Total	42429	0.09	0.02

CM = congenital malformation

The observation of balanced reciprocal translocations both in clinically healthy and in mentally retarded carriers in the same family (Aymé *et al.*, 1979); Knuutila *et al.*, 1977; Neu *et al.* (1974) may suggest a casual association. However, Aymé *et al.*, (1979), considering the slightly increased incidence of familial chromosome rearrangements among mentally retarded children demonstrated by Funderburk *et al.* (1977), assumed that the presence of such rearrangements in phenotypically abnormal children is not

merely coincidental, even though the chromosome aberration has been transmitted by a healthy parent. The abnormal phenotypes, in these situations, may be induced by a submicroscopic loss of chromosome material or by the gene complement supplied by the intact chromosome. Moreover, the frequency of association between translocations and multiple congenital anomalies is greater than the randomly expected frequency based on the incidence of such anomalies is newborns (Aurias *et al.*, 1978).

Recent cytogenetic studies in mentally retarded individuals have shown an increase in the number of balanced chromosome rearrangements (Breg, 1977; Funderburk *et al.*, 1977; Jacobs, 1974; Jacobs *et al.*, 1978). The congenital anomalies in these translocation carriers may be due to a mutation occurring at the chromosome breakpoint, a position effect (Biederman and Bowen, 1976) or to an undetectable aneuploidy. Funderburk *et al.* (1977) also suggested that the rearrangement may have a deleterious effect on the ovum or spermatozoan prior to fertilization, thus leading to abnormal development.

A chromosome 22 with deletion of the long arm (Philadelphia chromosome) has been observed in several haematological diseases, and occurs in about 90% of the cases of chronic myelocytic leukemia (CML). The deleted segment in most of these patients is translocated to 9q, but there are references of translocations involving chromosomes 1-4,6,7,9-11,13-17,19,21 and 22 (Fleischman *et al.*, 1977; Jotterand-Bellomo, 1978; Mammon *et al.*, 1976; Verma and Dosik, 1979).

The genesis of the typical or atypical Ph^1 chromosome has been considered recently and the precise location of the breakpoint has already been the subject of much debate. There are no concordant abnormal phenotypes in all cases of Ph^1 carriers, even when they have the same breakpoint location; and there are several breakpoints (22q11 → 22q13) which can result in the CML phenotypes (Fitzgerald, 1977; Panani, *et al.*, 1979; Watt and Page, 1978, for references). The patient described in our study has to date a normal haematological profile which will be followed up.

Megalocornea, easily noted in this patient, is a rare condition generally considered an isolated genetic defect, transmitted as an X-linked or autosomal recessive trait (McKusick, 1978). However, Frank *et al.* (1973) described a patient with normal karyotype and megalocornea associated with multiple skeletal anomalies, several of which are also present in our patient:

flat occipital bone, widely open fontanelles, pectus excavatum and kyphoscoliosis in the thoracic region. There was no agreement with respect to some findings which are absent in our patient: normal intraocular pressure, congenital heart defects, clubbing feet and flexion deformity of the digits of the hands. Although lacking familial information, and taking into consideration the presence of parental consanguinity, Frank *et al.* (1973) suggested an autosomal recessive inheritance for the newfound genetic entity.

In the present case we cannot exclude the possibility of a casual karyotype-phenotype association. Nevertheless, the occurrence of signs indicative of chromosomal aberrations suggests that the clinical picture must be the effect of the rearrangement. Also, if the present case and the patient studied by Frank *et al.* (1973) are examples of the same genetic entity, it is possible that the chromosomal rearrangement has permitted the manifestation of a rare recessive gene.

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RESUMO

Foram encontradas anomalias congênitas múltiplas em uma criança de sexo feminino, com uma translocação *de novo* envolvendo um cromossomo nº 16 em um cromossomo nº 22, sem perda aparente de material cromossômico. O cariótipo foi 46,XX,t (16;22) (q13;q11). Descrevemos os aspectos clínicos e dermatoglíficos da paciente, discutindo a associação cariótipo-fenótipo.

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A SÍNDROME DE DOWN: ASPECTOS ETIOLÓGICOS*

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ABSTRACT

A review of factors related to the etiology of mongolism. Special emphasis is given to parental non-disjunction as caused by delayed fertilization as well as to seasonal distribution of Down syndrome.

INTRODUÇÃO

Em 1866, a síndrome de Down foi descrita pela primeira vez por John Langdon Down. Na primeira metade do século XX foi extensivamente documentada a associação da síndrome com o aumento da idade dos pais, especialmente por Penrose. Em 1959, Lejeune, Turpin e Gautier mostraram que os indivíduos afetados apresentavam trissomia de um cromossomo do grupo G. Na maioria dos casos observa-se trissomia simples. Em cerca de 2% do total dos casos ocorre translocação D/G ou G/G; se considerarmos, no entanto, mães com menos de 26 anos, a incidência da translocação é maior, atingindo 6% (Beçak, 1977). Finalmente, em cerca de 3%, ocorre mosaicismo, em uma estimativa feita para 4835 casos estudados (Kasahara e col., 1977).

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INCIDÊNCIA

A incidência varia entre 1 e 2 por mil, tendo sido exaustivamente pesquisada por diversos autores. No Brasil, Delascio e col. (1966) em um estudo prospectivo na Maternidade Leonor Mendes de Barro encontraram entre recem-nascidos, 0,86‰ de mongolóides; em estudos retrospectivos no mesmo hospital, Altimari e Beçak (1968) encontraram, entre recém-nascidos, a freqüência de 1,21‰ e, numa amostra maior, 1,13‰ (1969). Ainda no mesmo hospital, Milstein-Moscati e col. (1979), em estudo retrospectivo, encontraram 278 mongolóides em 174.745 nascimentos consecutivos, no período de 1962 a 1976, portanto 1,59‰, o que é compatível com a estimativa de Martello e Frota-Pessoa (1969) para o Brasil (1,7‰). Saldanha e Cavalcanti (1963) relataram 0,79‰ em um estudo retrospectivo no Hospital das Clínicas; Mello da Silva (1972), em estudo prospectivo, menciona 1,7‰; Xavier e col. (1978) descreveram uma incidência de 1,66‰ na cidade de Ribeirão Preto, em 1972.

As variações podem refletir flutuação da amostragem, diferenças reais de freqüência ou ainda diferenças de padronização no diagnóstico da síndrome, nos diferentes centros.

As freqüências mais baixas observadas em maternidades quando comparadas com as estimativas de Martello e Frota-Pessoa (1969) poderiam ser devidas ao fato de que provavelmente as mães idosas estariam menos representadas nas maternidades do que na população geral (Martello e Frota-Pessoa, 1976).

A etiologia da síndrome tem sido discutida e investigada desde longa data. Inicialmente foi observado que os mongolóides eram geralmente os últimos membros de uma irmandade. Entretanto, vários investigadores demonstraram que a associação com a paridade é uma decorrência do efeito da idade materna (Penrose, 1934a e b; Bleyer, 1938; Stark e Mantel, 1966; Matsunaga, 1967).

ASPECTOS CITOGENÉTICOS

Na espécie humana, devido à alta correlação entre incidência de mongolismo e idade materna, acredita-se que o cromossomo 21 extra é quase sempre originário de uma não-disjunção na mãe. O avanço da metodologia,

principalmente através das técnicas de bandeamento cromossômico, possibilitou um estudo mais objetivo sobre a origem do autossomo adicional. Através das variantes estruturais e associação de satélites, pode-se determinar com exatidão a origem do cromossomo extra; entretanto a maioria dos casos estudados não é informativa (Licznerski e Lindsten, 1972; Robinson, 1973; Mutton, 1973; Sasaki e Hara, 1973; Uchida, 1973; Mikkelsen e col., 1976; Wagenbichler e col., 1976; Hansson e Mikkelsen, 1976; Langenbeck e col., 1976; Jacobs e Morton, 1977; Nikawa e col., 1977; Magenis e col., 1977; Rett e col., 1977; Hansson e Mikkelsen, 1978; Hassold e Matsuyama, 1979; Mattei e col., 1979; Arena, 1979).

Mattei e col. (1979), sumarizando os casos informativos citados na literatura, a respeito da não-disjunção do cromossomo 21 na oogênese e espermatogênese, mostraram que, em um total de 170 casos informativos, 62% dos casos são devidos a erro na primeira divisão meiótica materna. Cerca de 15% provêm de erro na 2ª divisão meiótica materna. Cerca de 12% são originários da meiose paterna I e 11% são de origem paterna na meiose II.

Até recentemente toda a informação a respeito da constituição cromossômica dos gametas humanos era obtida indiretamente. Rudak e col. (1978) desenvolveram uma técnica para analisar os cromossomos de espermatozóides humanos diretamente, através da sua ativação em óvulos de hamster. A freqüência de aneuploidia encontrada em uma amostra pequena foi de 5%.

Um aumento significante de associação de satélites do cromossomo 21 foi encontrado nas mães, mas não nos pais de mongolóides. Entretanto, em todos os casos de não-disjunção paterna, havia maior tendência para associação de satélites do cromossomo 21 que na amostra controle. Esses resultados indicam uma correlação entre tendência de associação de satélites e aumento do risco da não-disjunção (Nilsson e col., 1974).

Numerosos fatores têm sido investigados com o propósito de elucidar as causas da não-disjunção. Tanto fatores hereditários como ambientais podem resultar em anomalias cromossômicas.

EFEITO DA IDADE DOS PAIS

Vários trabalhos a partir de Jenkins, em 1933, e Penrose, em 1933, demonstraram haver uma associação entre mongolismo e idade materna, sendo

que a curva de distribuição dos nascimentos de mongolóides, segundo a idade materna, é bimodal, indicando que há dois tipos de mongolismo: um independente da idade materna (40%; classe A) e outro dependente da idade materna (60%; classe B) (Penrose e Smith, 1966). Na classe A estão incluídos trissômicos e translocados e na classe B a grande maioria é constituida por trissômicos (Hamerton, 1971).

A freqüência da não-disjunção aumenta com a idade materna, especialmente após os 35 anos. A estimativa de afetados por mongolismo é de 0,1% para mães de menos de 35 anos, alcançando 3,5% para mães de 45 a 49 anos (Frota-Pessoa, 1978). Considerou-se durante muito tempo que a idade paterna não tinha efeito etiológico. No entanto, estudos posteriores, em que foi analisada a distribuição da idade paterna, mantendo a idade materna controlada, mostrou um aumento brusco da incidência no caso de pais com 55 anos ou acima de 55 anos (Stene e Stene, 1977; Stene e col., 1977; Matsunaga e col., 1978), embora, o efeito da idade paterna seja moderado comparando-se com o efeito da idade materna (Matsunaga e col., 1978).

Segundo alguns autores, as diferenças entre a intensidade do efeito da idade materna e paterna na incidência relativa da síndrome de Down estão ligadas às diferenças entre a gametogênese feminina e a masculina. Segundo Hamerton (1971), a probabilidade de um zigoto se formar a partir de um óvulo aneuplóide é maior do que a partir de um espermatozóide aneuplóide. No homem, a não-disjunção na meiose I resulta em 50% de gametas nulissômicos e 50% de gametas com um cromossomo adicional. Quando ocorre na meiose II, 50% serão aneuplóides. Na mulher, se a não-disjunção ocorrer na meiose I, 100% dos ovócitos serão anormais e na meiose II, se não considerarmos a divisão dos corpúsculos polares, também 100% dos ovócitos serão aneuplóides. Por outro lado, enquanto na espermatogênese existe uma produção contínua de espermatozóides, durante a vida do individuo, e a sua formação a partir de uma gônia leva apenas dois meses, a meiose nos ovários é interrompida na fase de dictióteno, antes ou um pouco depois do nascimento. Apenas os ovócitos dos folículos maduros saem do estágio de dictióteno prosseguindo a meiose reducional, e produzindo ovócito de 2ª ordem e corpúsculo polar, que são lançados na trompa. É na trompa que o ovócito de 2ª ordem inicia a segunda divisão meiótica, que não se completa até que ocorra a fertilização.

Durante a sua maturação no ovário, o ovócito encontra-se bem protegido. Quando é lançado na trompa, passa a um meio muito diferente daque-

le em que permaneceu durante vários anos,o que o torna mais vulnerável e aumenta a probabilidade de não-disjunção. É, pois, plausível perguntar se alterações do ovócito que poderiam levar à não-disjunção não se processam de uma forma mais acelerada fora do ovário, ficando a célula mais exposta aos fatores ambientais.

Também durante a clivagem do embrião poderão ocorrer alterações que resultem em anomalias cromossômicas. Pelo menos 35% dos abortos espontâneos no primeiro trimestre de gravidez têm aberrações cromossômicas (Milunsky e Atkins, 1977). Trabalhos recentes utilizando amniocentese mostraram uma discrepância entre a incidência de mongolismo ao nascer e a encontrada na amniocentese (Kardon e col., 1978; Spielman e col., 1978; Hook e Chambers, 1977; Meredith e col., 1978).

Hook (1978), analisando dados obtidos em vários centros, na Grã-Bretanha, Estados Unidos e Suécia, ajustados para idade, sugere que existe um diferencial de 30% entre as freqüências observadas na amniocentese e aquelas observadas ao nascimento.

Spielman e col. (1978) detectaram um número de fetos trissômicos próximos ao esperado em mulheres entre 35-39 anos. Entretanto, para mulheres entre 40-44 anos, o número observado é o dobro do esperado. Esses resultados podem ser parcialmente explicados pelos achados de Creasy e Polani (1978), que propõem que 20% dos fetos com síndrome de Down que conseguem alcançar 16 semanas de gestação se perdem antes do nascimento.

Devido à discrepância existente entre a incidência encontrada ao nascer e nos estudos pré-natais, alguns pesquisadores como Kardon e col. (1978) e Spielman e col. (1978), discutem se o risco citado no aconselhamento pré-natal dever ser o obtido através dos estudos de amniocenteses ou o de incidência ao nascer. Concordando com Wyatt (1978), achamos que todos devem ser informados da existência das discrepâncias entre a freqüência ao nascer e a freqüência encontrada na amniocentese mas o risco utilizado no aconselhamento deve ser o risco ao nascer.

As mudanças associadas com o envelhecimento dos indivíduos, bem como das células, podem estar relacionadas com as anomalias cromossômicas. Segundo Henderson e Edwards (1968) há uma freqüência mais baixa de quiasmas em ovócitos que ovulam tardiamente na vida reprodutiva; esta mudança pode impedir a disjunção meiótica normal.

Outros sinais de envelhecimento do ovócito em animais e que podem

resultar em triploidia são a deterioração da função do fuso impedindo a formação do corpúsculo polar e a degradação dos mecanismos ligados à defesa contra a polispermia (Austin, 1976). Para Penrose (1965), o envelhecimento celular acarretaria um acúmulo de alterações físicas e químicas que levam à degeneração do fuso celular.

De uma maneira genérica poderíamos dizer que as causas estudadas da não-disjunção podem ser agrupadas em dois tipos: as devidas a factores intrínsecos ao material genético e as associadas a fatores extrínsecos ao material genético, isto é, ambientais. No primeiro caso, estariam o controle gênico da não-disjunção e as anomalias estruturais que poderiam interferir com a disjunção normal. No segundo caso, os agentes biológicos, físicos e químicos. Existe sempre uma interação em maior ou menor grau entre os fatores intrínsecos e extrínsecos.

CONTROLE GÊNICO

Segundo alguns pesquisadores, existem genes controladores da não-disjunção. Para Hamerton (1971), a ocorrência de dois ou mais afetados por aneuploidias simples na mesma irmandade sugere a existência de genes controladores da não-disjunção mitótica ou meiótica em famílias com vários indivíduos aneuplóides quanto a cromossomos sexuais e/ou autossomos dos grupos D, E ou G. O primeiro indivíduo XYY descrito na literatura foi, por exemplo, descoberto porque tinha uma filha mongolóide (Hauschka e col., 1962). Entretanto, essas hipóteses não foram confirmadas (Inberg e Davis, 1970).

EMPARELHAMENTO DISTRIBUTIVO

Um outro tipo de hipótese para justificar a ocorrência de aneuploidia na mesma família é a hipótese do "emparelhamento distributivo" (Grell e Valencia, 1964; Grell, 1971). Na meiose de *Drosophila melanogaster*, primeiro ocorre o pareamento homólogo que é seguido por um segundo tipo de pareamento, o pareamento distributivo, que determina a segregação. Os cromossomos em que ocorreu permuta e estão presumivelmente ligados por quiasma separam-se regularmente e não têm oportunidade de envolvimento com um heterólogo. Cromossomos que não tiveram permuta formam um conjunto, cujos

membros podem parear homologamente ou não, ou mesmo não parear. Dependendo da composição do conjunto, existe a possibilidade de associação não homóloga seguida de não-disjunção de homólogos e produção de aneuplóides. As preferências de pareamento mostradas nesse estágio dependem da similaridade no comprimento dos cromossomos.

A hipótese do emparelhamento distributivo foi testada em *Drosophila*. Sua aplicação na gametogênese humana é especulativa. Alguns pesquisadores (Dekaban e col., 1964) descreveram uma alta freqüência de anomalias cromossômicas nas famílias de um grupo não selecionado de mongolóides, o que os levou a sugerir que certas variantes cromossômicas podem aumentar a freqüência de não-disjunção de outros cromossomos, durante a meiose. Foram observados cromossomos marcadores ou satélites gigantes nos acrocêntricos (Wahrman e Fried, 1970) e parece haver uma correlação positiva entre associação de satélites e aumento do risco da não-disjunção.

AÇÃO DA ANTITRIPSINA ALFA-1

Fineman e col. (1976) sugerem que uma diminuição da atividade de antitripsina alfa-1 é também um fator etiológico na trissomia 21. A antitripsina alfa-1 (α_1AT) é um inibidor de proteases celulares. A herança desse fator polimórfico é por codominância. Fineman e col. (1976) verificaram um aumento da freqüência de heterozigotos para variantes de α_1AT em indivíduos com mosaicismo dos cromossomos sexuais ou trissomia 21. Esses autores encontraram uma associação significante de trissomia 21 com variantes alélicas α_1AT, aparentemente confinadas a indivíduos nascidos de mães com mais de 35 anos. Entretanto, os autores não explicam como o aumento da idade materna e a diminuição da atividade da α_1AT (associada com variantes heterozigotas de α_1AT) interagem para produzir filhos cromossomicamente anormais.

AÇÃO DE HORMÔNIOS

Hormônios da tireóide podem, por exemplo, aumentar a tendência de associação de satélites dos cromossomos 21 e 14 (Nilson e col., 1974,1975). O mesmo fenômeno também foi encontrado quando células de indivíduos

sadios são cultivadas em um meio com soro de pacientes hipertireoideanos. Esse fenômeno poderia decorrer de um prolongamento do período de síntese de RNA ribossômico, pelas regiões organizadoras do nucléolo desses cromossomos, por influência de hormônios. A mesma explicação é válida para a observação de Merz e col. (1966), sobre a variação de número e tamanho dos nucléolos, em mulheres hipertireoideanas. Há muito tempo que se estabeleceu uma relação entre disfunção da tireóide e incidência de mongolismo (Dollinger, 1921). Fialkow e col. (1965) verificaram que as mães, mas não os pais, de crianças mongolóides têm uma freqüência significativamente superior de auto-anticorpos tireoideanos, em relação a indivíduos do grupo controle, na mesma faixa etária.

Resultados aparentemente contraditórios foram encontrados em relação ao uso de contraceptivos orais. Vários estudos referentes ao uso de contraceptivos orais, em mulheres que deram à luz, não mostraram nenhum excesso de anomalias congênitas em seus filhos (Peterson, 1969; Robinson, 1971; Kay, 1976). Entretanto, estudos epidemiológicos e clínicos de abortos espontâneos mostraram que hormônios tomados pela mãe, antes da gravidez, podem causar anomalias cromossômicas nas células germinativas ou zigoto (Carr, 1970; Alberman e col., 1976). As incidências de várias anomalias foram aumentadas, mas não foi possível relacioná-las com a dose de estrógenos ou progesterona, nos anticoncepcionais usados. Se existe um efeito nos cromossomos dos embriões concebidos após a interrupção do uso de contraceptivos orais, ele seria devido ao fato de os hormônios usados não terem sido completamente eliminados do organismo, ou de o funcionamento do organismo não ter retornado ao normal na época da concepção. Ambas as alternativas são possíveis pois ocorre um ligeiro atraso na concepção, depois da interrupção do uso dos contraceptivos.

Ciari e col. (1972) sugerem que o anticoncepcional interfere no processo reprodutivo. Verificaram que o tempo de concepção das mulheres que tomaram o anticoncepcional durante um ano é igual ao do grupo controle. Entretanto, para as que tomaram o mesmo produto durante 2 a 3 anos, o intervalo de tempo até a concepção é muito maior do que no grupo controle.

Janerich e col. (1976), comparando a história de uso de contraceptivo oral em um grupo de 103 mães de crianças com síndrome de Down e um número igual de controles que tiveram filhos normais, não encontraram maior freqüência de usuárias de pílulas anticoncepcionais, quer na época da gravidez

quer no ano precedente entre as mães de crianças anormais. Seus dados mostram que há menos usuárias da pílula entre as mães de crianças com síndrome de Down.

Boué e Boué (1973) verificaram que a freqüência de abortos espontâneos era significativamente maior quando as mulheres eram tratadas com hormônios, não só durante o ciclo em que ocorreu a concepção, como também durante os ciclos precedentes.

Harlap (1976) não encontrou diferenças significativas quanto ao risco de malformações, em crianças nascidas após tratamento de indução de ovulação. De acordo com Janerich e Jacobson (1977 b), podem-se explicar os resultados contraditórios da seguinte forma: embora não haja um aumento na incidência de síndrome de Down associado ao uso de contraceptivos orais, não se pode afastar a possibilidade de que a pílula causa um aumento nas trissomias entre os abortos espontâneos. Aparentemente não há um aumento de incidência de abortos espontâneos nas usuárias da pílula. Segundo os autores (Janerich e col., 1976; Janerich e Jacobson, 1977 b) isso poderia ser explicado pela possibilidade de a pílula agir sobre os mecanismos de divisão celular na oogênese; entretanto não agiria sobre o mecanismo de rejeição materna a fetos defeituosos. Como muitas dessas rejeições provalmente resultam em abortos precoces, é possível que não sejam reconhecidas e talvez seja por isso que não há um aumento aparente na incidência de abortos nas usuárias da pílula.

Segundo Janerich e Jacobson (1977 a) a ovogênese seria também afetada por alterações hormonais endógenas. Assim, o aspecto sazonal (acúmulo de afetados em certos períodos do ano) da síndrome de Down estaria associado ao aspecto sazonal da concentração hormonal no sistema endócrino feminino. Flutuações nos níveis de receptores de estradiol em mulheres parecem coincidir com a flutuação sazonal bimodal nos meses de concepção da síndrome de Down. Esses receptores são proteínas intracelulares que são mediadores essenciais da ação hormonal. Quando aumentasse a concentração dos receptores, haveria um risco maior de síndrome de Down. Os hormônios da ad-renal poderiam ter um papel protetor contra a síndrome de Down, através da modificação da concentração dos receptores de estradiol no folículo em desenvolvimento. As secreções da ad-renal diminuem com a idade; assim, essa proteção diminuiria com a aproximação da menopausa (Janerich e Jacobson, 1977 b).

A síndrome de Down apresenta aspecto sazonal em lugares onde sua

incidência é relativamente alta, tendo sido verificado na Holanda (Jongbloet, 1975 a) e Israel (Harlap, 1974), mas não nos Estados Unidos (Mac Mahon e col., 1953).

Em resultados de amniocentese praticada exclusivamente devido à idade materna, Kardon e col. (1978) encontraram, em uma pequena série, freqüências semelhantes de trissomia 18 e trissomia 21, havendo uma agregação temporal, com 6 das 11 anormalidades ocorrendo entre dezembro de 1977 e fevereiro de 1978.

Tentando explicar para o caso da trissomia do 21, poderíamos dizer que existem dois grupos de trissômicos. O grupo A, de freqüência mais ou menos constante, e o grupo B, que apresentaria uma variação no espaço e no tempo. Essa variação seria causada por fatores endógenos e exógenos, havendo grande interação entre eles. Tanto os fatores endógenos como os exógenos seriam ambientais em relação aos cromossomos. Assim, por exemplo, as diferenças de incidência entre duas localidades seriam atribuidas a uma discrepância no grupo B. Para que uma mudança temporal fosse percebida, seria necessário um aumento de B em relação a A. Em locais onde a freqüência é baixa, B seria relativamente pequeno e, mesmo que houvesse uma variação de B, ela passaria desapercebida.

O grupo B incluiria os fatores hormonais e os agentes químicos, físicos e biológicos.

AÇÃO DE RADIAÇÃO

Tanto as radiações, como os agentes químicos e infecciosos, podem induzir aberrações numéricas e estruturais em células germinativas humanas (Hausmann, 1974).

Nos estudos sobre os efeitos genéticos das bombas atômicas em Hiroshima e Nagasaki, não foi encontrada associação significante entre incidência de mongolismo e exposição materna (mulheres presentes na cidade na época dos bombardeios). Pelo contrário, segundo os dados de Schull e Neel (1962), a incidência no caso de mães não expostas (1,27‰) foi mais alta do que no caso das expostas (0,54‰). Por outro lado, verificou-se que mulheres submetidas a raios X tinham uma freqüência de prole aneuplóide significantemen-

te maior do que mulheres que não receberam radiação (Uchida e Curtis, 1961; Uchida, 1971).

Associando esses resultados a um número muito grande de trabalhos que mostram o efeito deletério das radiações, Rotblat (1978) sugere que os sobreviventes de Hiroshima e Nagasaki podem constituir uma população selecionada; somente os indivíduos geneticamente mais resistentes sobreviveram aos ferimentos e ao trauma. Essa hipótese pode ser válida ou não, mas parece que esses sobreviventes estão em uma categoria especial, não constituindo uma amostra adequada como base para calcular o risco de radiação para outras populações.

Em Kerala, uma população exposta a uma dose de radiação natural acima dos 3 rad/30 anos, geralmente encontrada na grande maioria das regiões, mostrou uma prevalência maior de síndrome de Down, quando comparada com uma população vizinha exposta a doses mais baixas (Kochupillai e col., 1976).

No Brasil, em estudos feitos em áreas de alta radiação natural não foi encontrado efeito claro nas análises de abortos, mortalidade pré-natal, anomalias e anormalidades em geral (Freire-Maia, 1975). Os efeitos biológicos das ondas eletro-magnéticas têm sido estudados, com resultados controvertidos. Há uma sugestão de que uma proporção significativamente maior de pais de propósitos, do que de pais de controles, foram expostos ao radar e/ou algum outro tipo de micro-ondas no serviço militar ou na indústria (Cohen e col., 1977). Dadas as dificuldades metodológicas envolvidas, a questão permanece em aberto. Entretanto, pesquisas nesse campo são de grande importância, uma vez que o uso do radar e micro-ondas tem aumentado muito ultimamente.

AÇÃO DE AGENTES INFECCIOSOS

Pleidell, em 1957, notou em muitos casos agrupamentos de 2 ou 3 crianças com síndrome de Down, nascidas próximas no tempo e no espaço. Stoller e Collman (1965 a,b), fizeram um estudo retrospectivo da ocorrência da síndrome de Down entre 1942-64 em Vitória, Austrália. Com base nos seus resultados, emitiram a hipótese da existência de um vírus de incubação longa, afetando em geral, mas não exclusivamente, o óvulo da mãe idosa, de uma forma direta, ou indiretamente através de algum padrão imunológico. De

todas as moléstias infecciosas estudadas, só a hepatite infecciosa apresentou picos de incidência 9 meses antes dos picos de incidência de mongolismo.

Estes resultados não foram, porém, confirmados (Stark e Fraumeni, 1966; Leck, 1966). Os dados de Leck (1966) sugerem que pequenos agrupamentos tendem a ocorrer em certa localidade em um mesmo intervalo de aproximadamente 100 dias, mas que estes agrupamentos não ocorrem simultaneamente em lugares que distam mais de 4 km, concluindo que a incidência da síndrome de Down pode ser afetada por um agente epidêmico, mas sem grande importância etiológica.

Nielsen e col. (1973) verificaram que existe um pico de rúbeola e sarampo dois meses antes do pico de concepção de crianças com aneuploidia dos cromossomos sexuais. Por outro lado, o pico das aneuploidias dos cromossomos sexuais está nos meses de mais alta temperatura média na Dinamarca. A temperatura dificilmente seria a causa direta, podendo agir através de diferenças em alimentação, drogas, atividade física, hábitos sexuais, infecções e outros fatores que mudam com a temperatura.

Os resultados obtidos até agora sobre a variação sazonal da freqüência de nascimentos de crianças com anomalias cromossômicas são inconclusivos, porque, na sua maioria, são contraditórios ou se baseiam em pequenas amostras e discrepâncias não significantes. Alguns pesquisadores encontraram variações sazonais para anomalias dos cromossomos sexuais (Frøland, 1967; Nielsen e Friedrich, 1969; Robinson e col., 1969; Jongbloet, 1971). Outros não encontraram essa variação (Court Brown e col., 1964; Tünte e Nierman, 1968). Em relação a aneuploidias nos autossomos, os resultados são também contraditórios (Stoller e Collmann, 1965a, 1965b; Stark e Mantel, 1966; Ceccarelli e Torbidoni, 1967; Richards, 1967; Kogon e col., 1967; Baird e Miller, 1968; Shapiro, 1970; Anderson e col., 1970; Kardon e col., 1970; Cohen, 1971; Nielsen e col., 1973; Harlap, 1974).

A incidência de aberrações não é a mesma em todas as localidades, bem como não é a mesma a variação temporal em diferentes localidades. Portanto, baseando-se nos resultados de vários autores, conclui-se que parece existir uma variação temporal e espacial nas incidências das aneuploidias.

Nielsen e col. (1973), na Dinamarca, não encontraram efeito sazonal na variação da freqüência em 791 casos de trissomia do 21, mas verificaram efeito sazonal na variação de freqüência de não-disjunção dos cromossomos

sexuais. Para esses pesquisadores, haveria diferença na etiologia da não-disjunção dos cromossomos sexuais e dos autossomos.

Harlap (1974), analisando um série de recém-nascidos de Jerusalém, verificou que, ao lado de uma incidência muito alta da síndrome de Down, existia uma distribuição bimodal ao longo do ano. Esse padrão se repetiu durante 7 anos, com exceção de 9 meses após a guerra dos 6 dias, em que não ocorreu o pico esperado. Segundo a hipótese de Janerich e Jacobson, esse fenômeno decorre de uma situação de "stress" onde há maior atividade da adrenal que funcionaria como proteção, prevenindo a síndrome de Down (Janerich e Jacobson, 1977b).

Segundo Ford (1973), uma diminuição na concentração de estrógeno leva a uma elevação do pH no trato reprodutivo feminino. Quando o pH é elevado por 15 a 30 minutos em culturas de fibroblastos fetais humanos, há alteração cromossômica em 60% das células por poliploidia e aneuploidia. As células são sensíveis a elevação do pH em todas as fases do ciclo, mas principalmente durante a replicação do DNA. Por outro lado, o trato reprodutivo feminino não tem um sistema de manutenção do pH constante tão eficiente como outras partes do organismo, e pode variar o seu pH mais de 0,2 unidades, quando exposto a pequenas mudanças de estrógeno. Esse mecanismo poderia operar logo depois da ovulação, (erro na 2ª divisão meiótica) produzindo um gameta anormal, ou mais tarde (erro em uma divisão mitótica inicial) dando origem a mosaicismo cromossômico no zigoto.

FERTILIZAÇÃO ATRASADA

Na maioria dos mamíferos a fertilização ocorre logo depois da ovulação, assegurando assim a integridade do ovo. Isso acontece pelo fato de a fêmea aceitar o macho somente durante o estro, que está intimamente associado à ovulação e ao acasalamento. Na espécie humana, as relações sexuais ocorrem em qualquer fase do ciclo. É difícil estimar o efeito do processo de envelhecimento isoladamente, pois numerosos fatores agem conjuntamente.

A exposição aos fluidos do oviduto durante as mudanças hormonais do período pós-ovulatório, bem como uma deposição gradual de mucina durante a passagem através do oviduto, podem alterar o ovo.

Os efeitos da fertilização atrasada têm sido estudados desde 1934

(Hammond, 1934). A fertilização atrasada reduz o tamanho da cria, induz segmentação anormal e causa desenvolvimento anormal dos fetos em coelhos, cobaias e ratos. Também foi demonstrado um aumento da incidência de polispermia e desenvolvimento anormal dos pronúcleos em ratos (Hammond, 1934; Chang, 1952; Blandau e Young, 1939; Blandau e Jordan, 1941; Austin, 1956; Odor e Blandau, 1956; Blandau, 1952). Em hamsters submetidos a fertilização atrasada (Chang e Fernandez-Cano, 1958), 50% dos óvulos eram incapazes de ser fertilizados cerca de 4 a 5 horas depois da hora estimada da ovulação. Dos óvulos fertilizados, cerca de 50% apresentaram um procedimento anômalo no estágio pronuclear e durante a clivagem.

As fêmeas de hamster apresentam um declínio acentuado no número de recém-nascidos por cria, depois que atingem 14 meses de idade (Soderwall e col., 1960). Sumarizando os resultados obtidos por vários autores, pode-se dizer que esta redução resulta de maior reabsorção de embriões nas fêmeas velhas assim como de perdas antes da implantação e de atraso na habilidade do útero de formar células deciduais, durante a gravidez normal. Por outro lado, a presença de um grande número de ovos não viáveis e o atraso no desenvolvimento dos embriões de fêmeas velhas podem resultar de um atraso de fertilização. A maioria dos óvulos obtidos de fêmeas velhas, cruzadas com machos jovens, mostraram um atraso na fertilização. Essa diferença não foi atribuída a uma ovulação atrasada, mas a uma penetração prolongada na zona pelúcida e vitelo, pelo espermatozóide, aumentando o tempo da fertilização (Parkening e Soderwall, 1975). A maioria dos óvulos de fêmeas velhas de hamsters se mantêm não fertilizados 2 a 5 horas a mais do que os de fêmeas jovens. O atraso na fertilização por algumas horas, no hamster, afeta a viabilidade do óvulo. Experiências com inseminação artificial algumas horas após o tempo propício mostraram que 17% dos embriões formados apresentavam aberrações cromossômicas (Yamamoto e Ingalls, 1972).

Trabalhos recentes mostraram que, em hamster, hipóxia, fertilização atrasada, idade materna e alteração do equilíbrio hidrogênio-iônico (Ingalls e Yamamoto, 1972; Adachi e Ingalls, 1976) podem, conjunta ou separadamente, ter uma influência decisiva na ocorrência de poliploidia e aneuploidia (Yamamoto e Ingalls, 1972).

Adachi e Ingalls (1976), submetendo fêmeas de hamster, acasaladas no estro, a um atraso de fertilização, combinado com hipóxia, obtiveram embriões triplóides onde somente cariótipos com XXX ou XXY ocorriam, suge-

rindo que a anomalia provavelmente provinha dos óvulos. Esses pesquisadores trabalharam somente com animais adultos jovens, excluindo, portanto, o fator da idade materna. O envelhecimento do óvulo foi considerado só a partir da ovulação. O grau do desequilíbrio hidrogênio-iônico foi determinado pela intensidade da hipoxia. Aumentando o "stress" sobre os animais, o número de embriões mortos e de anomalias cromossômicas na prenhez apresentou um aumento significante. Esse trabalho demonstrou que o acasalamento e a concepção fisiologicamente normais no controle estão associados a cariótipos normais na prole e que, por outro lado, a perda fetal e embrionária pode estar associada ao envelhecimento dos óvulos, devido a circunstâncias da concepção e não ao envelhecimento materno, uma vez que todos os animais usados eram adultos jovens.

German (1968) propôs uma hipótese segundo a qual o mongolismo seria o resultado de fertilização atrasada devido a relações sexuais esporádicas ou de freqüência diminuída. Baseou-se em experimentos com animais, em considerações tiradas dos hábitos sexuais humanos e na correlação entre mongolismo e idade materna. Fez uma associação entre duração de casamentos e incidência de mongolismo. Usou os dados de Kinsey e col. (1953) sobre a freqüência de relações sexuais da mulher acima de 35 anos considerando que, com o aumento da duração do casamento, a freqüência de relações diminui. Conseqüentemente, a proporção do tempo em que os espermatozóides aptos estão presentes no oviduto também é diminuída. Assim, ovócitos lançados no oviduto no caso de mulheres mais velhas poderiam ser fertilizados após um período mais longo, Durante esse período, enquanto o ovócito permanece fora do ovário, sem completar a meiose, podem ocorrer alterações, sendo a trissomia G um dos efeitos possíveis.

A comprovação dessa hipótese poderia ter implicações importantes no aconselhamento genético de pais de um filho com trissomia do 21 e também no aconselhamento genético em geral, como educação preventiva.

O trabalho de German não foi confirmado por vários pesquisadores, como Cannings e Cannings (1968), Matsunaga e Maruyama (1969), James (1968) e Penrose e Berg (1968).

Os estudos de German foram retrospectivos, baseados em dados do trabalho de Kinsey, duração de casamento, idade materna e incidência de mongolismo. Foi, portanto, um estudo com abordagem indireta. Os trabalhos

que se seguiram também foram retrospectivos e basearam-se em pressupostos teóricos e modelos matemáticos.

Na pesquisa que estamos desenvolvendo, pretendemos verificar se existe alguma associação entre hábitos sexuais e incidência de mongolismo. Não partimos de nenhum pressuposto e usamos uma abordagem direta, baseando-nos em entrevista psicológica.

De um modo geral, podemos dizer que a técnica de entrevista usada está de acordo com Jourard (1972), Bleger (1972), Garret (1974), Trait (1973), Crano (1973), Myrdal (1968) e Kinsey e col. (1948, 1953), tendo sido realizado previamente um treinamento específico do entrevistador. Nossos resultados preliminares (Milstein-Moscati e Beçak, 1978), mostraram que relações sexuais infreqüêntes, com longos intervalos de abstinência durante o período de concepção, eram mais comuns em mães de crianças com síndrome de Down que em mães de uma amostra controle e esta associação parece ser independente da idade materna. Não há necessariamente uma relação de causa e efeito podendo refletir outros fatores.

A fecundação do óvulo no tempo ótimo pode não depender só da disponibilidade de espermatózoides no trato sexual feminino. Assim, por exemplo, a ovulação precoce ou tardia em relação ao ciclo hormonal normal da mulher pode resultar em liberação de um óvulo que não esteja em condições ótimas e que poderia predispor a uma não-disjunção cromossômica.

Compatíveis com os nossos resultados estão os resultados obtidos por James (1974), que demonstraram que a idade do marido está associada à freqüência de relações sexuais quando a idade da mulher é controlada. Por outro lado, trabalhos recentes sobre o efeito da idade paterna (Matsunaga e col., 1978; Stene e Stene, 1977; Stene e col., 1977). Stene e col. (1977) chegaram à conclusão de que, com o aumento da idade, os homens têm um risco crescente de ter um filho mongolóide. Acima da idade 55 anos este risco é significante. Mantendo a idade materna controlada, a incidência relativa de síndrome de Down apresenta uma subida brusca de 55 anos para cima.

Os diferentes aspectos que apresentamos mostram várias facetas que afetam a etiologia da síndrome de Down. Esses aspectos são passíveis de inúmeras interpretações sob o ponto de vista biológico, social e psicológico. É evidente que muitos dos fatos são controvertidos, sendo necessárias investigações mais amplas para esclarece-los. No entanto já se pode afirmar que os fa-

tores etiológicos são diversos e que todos eles devem ser levados em consideração ao se proceder ao Aconselhamento Genético.

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SUMÁRIO

Trabalho de revisão que aborda aspectos referentes à etiologia do mongolismo e analisa, principalmente, os fatores que levam a não-disjunção cromossômica nos pais, enfatizando a defasagem entre ovulação e fecundação, bem como a distribuição sazonal da Síndrome de Down.

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