Rev. Brasil. Genet. I, 3, 181-191 (1978)
(Brazil. J. Genetics)

EFFECTIVE SIZE OF MONOECIOUS POPULATIONS SUBMITTED TO ARTIFICIAL SELECTION

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ABSTRACT

A procedure is outlined, which can be used to calculate the variance of effective size for monoecious populations submitted to specific artificial selection schemes. The expression obtained is similar to that of Crow and Kimura, except for the fact that the variance of the number of effective gametes contributed by the parents also contains a covariance between the number of male and female gametes. Also presented is a measure of a net effective size, encompassing a complete selection cycle, when more then one generation or step of gametic sampling is required to complete the cycle. In this case, effective size turns out be the harmonic mean of the effective sizes of all steps, within a cycle, divided by number of steps. Examples of application are given for three selection schemes.

INTRODUCTION

The role of effective population size in artificial selection programs has been discussed by many authors (Robertson, 1960, 1961; Enfield, 1969;

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Baker and Curnow, 1969; Rawlings, 1970; Comstock, 1974; Vencovsky and Godoy, 1976).

Aspects such as selection limits, the half-life of a selection process, a measure to evaluate depletion of genetic variability due to drift, and comparisons of the long range potential of different selection schemes all require or are dependent upon an exact knowledge of the effective size involved. It is well known, on the other hand, that recurrent selection encompasses a great number of different schemes and variations. As a consequence, straightforward expressions to compute effective size values are not available to the breeder, for specific selection schemes. It is the purpose of this paper to present means for calculating the effective size for a given selection procedure in monoecious species. Included are also those procedures which contain more than one generation or sampling step of gametes per cycle.

METHODS

Subsequent derivations refer to the concept of effective population size ($N_{e(v)}$) based on the variance of allelic frequency due to genetic drift. The simplified symbol N_e will be used to represent $N_{e(v)}$.

Let us consider a finite set of N monoecious individuals, in generation t -1. To represent a selection process, F individuals are taken from N to contribute female and male gametes to the next generation. A number R will be taken from the remaining N-F individuals to contribute only male gametes. This reasoning could be reversed by supposing that the R individuals will contribute female gametes only. This model evidently does not apply only to individual selection but may also include other procedures of recurrent selection. On these terms, selection proportions are:

$$u = \frac{F}{N}, \text{ for female gametes;}$$

$$v = \frac{F + R}{N} = \frac{M}{N}, \text{ for male gametes.}$$

To generalize, we may suppose that $0 < F \leqslant N$ and that $0 \leqslant R \leqslant (N-F)$. In fact, if $F = N$, we have the conditions reported by Crow and Kimura (1970), where all individuals in generations t-1 potentially contribute gametes to generate zygotes, with no selection. If $F < N$ and $R = 0$, for example, we have a situation where selection is practiced on both sexes with equal intensity. Also, $F < N$ and $R = N - F$ represent selection on one sex only, in this case the female one.

By considering locus B, b (neutral with respect to the selection process and to natural fitness) as a marker to measure the variance of allelic frequency and to obtain the N_e value, it is possible to represent the number of individuals involved as shown in Table I.

Table I. Number of individuals per genotype before and after the process of selection.

Genotypes	Before selection	in F	in R
BB	n_2	f_2	r_2
Bb	n_1	f_1	r_1
bb	n_0	f_0	r_0
Totals	N	F	R

Since the withdrawal of F individuals is without replacement and the locus is neutral, an f_i value ($i = 0, 1, 2$) is random variable with double hypergeometric distribution (Feller, 1965). The distribution of r_i is the same, but conditioned to that of f_i.

Since the derivation of N_e is based on the variance of $\delta q = q_t - q_{t-1}$, where q_{t-1} is the frequency of B in the parental set, and q_t the frequency of B in the offspring, it is necessary to introduce measures of the number of B alleles transferred to the next generation. Thus, let us consider:

d_{ij} : as number of female gametes contributed by the j^{th} parent with genotype i; $j = 1, 2, \ldots, f_i$;
$i = 0, 1, 2$; $d_{ij} = 0$ for $j > f_i$;

e_j : as the number of B female gametes contributed by the j^{th} parent; $j = 1, 2, ..., f_i$; $e_j = 0$ for $j > f_i$;

g_{ij} : as the number of male gametes contributed by the j^{th} parent with genotype i; $j = 1, 2, ..., ... m_i$, where $m_i = f_i + r_i$; $g_{ij} = 0$ for $j > m_i$;

h_j : as the number of B male gametes contributed by the j^{th} heterozygote; $j = 1, 2, ..., m_i$; $h_j = 0$ for $j > m_i$;

$\bar{d}_s$ and $\bar{g}_s$: as the average number of female and male gametes, respectively, within sets of selected F and M individuals, respectively;

V_{ds} and V_{gs} : as the variance of d_{ig} and g_{ij}, within sets of selected F and M individuals, respectively;

$\bar{d}$ and $\bar{g}$: as the overall mean of d_{ij} and g_{ij}, respectively, in relation to the total group of N parents;

$k_{ij} = d_{ij} + g_{ij}$: the total number of gametes contributed by the j^{th} parent with genotype i;

V_k : the variance of k_{ij} among individuals in the initial group of size N.

In artificial selection, a cycle often contains more than one step or generation where genetic drift can act. This may happen, for example, with family selection, where remnant seeds of selected families are used for intercrossing to reconstitute the population. New progenies are then obtained for the next cycle. As a consequence, the N_e value must apply to the complete selection cycle. Thus, changes in frequency q may occur in each step within a cycle. Hence, from cycles t-1 to t:

$$q_t = q_{t-1} + \delta q_{1t} + \delta q_{2t} + ... + \delta q_{mt}$$

for m phases. Thus:

$$q_t - q_{t-1} = \delta q = \delta q_{1t} + \delta q_{2t} + \ldots + \delta q_{mt}, \text{ and}$$

$$V(\delta q) = \sum_{\ell} V(\delta q_{\ell t}) \qquad \ell = 1, 2, \ldots, m.$$

It follows that N_e must be measured by:

$$N_e = \frac{q_{t-1}(1 - q_{t-1})}{2 \sum_{\ell} V(q_{\ell t})}, \text{ for the whole cycle,}$$

which includes the accumulated effect of drift within a selection cycle of m phases.

RESULTS

Following derivations described by Crow and Kimura (1970) to obtain N_e, we have:

$$\delta q = \frac{1}{N\bar{K}} [\sum_{j=1}^{n_2} d_{2j} + \sum_{j=1}^{n_1} e_j + \sum_{j=1}^{n_2} g_{2j} + \sum_{j=1}^{n_1} h_j] - q_{t-1},$$

where $\bar{k} = \bar{d} + \bar{g}$ is the average number of female plus male gametes contributed by individuals in set of size N. Operating with the expression for δq, and given the hypergeometric distributions already mentioned, it follows, in terms of mathematical expectation that:

$$N^2 \bar{k}^2 V(\delta q) = \frac{V_k}{N-1} [N(n_2 + \frac{1}{4} n_1) - (n_2 + \frac{1}{4} n_1)] + \frac{1}{4} n_1 \bar{k}.$$

From this expression, as shown by Crow and Kimura (1970):

$$N_e = \frac{N\bar{K}}{\frac{s^2{}_k}{\bar{k}}(1 + \alpha) + (1 - \alpha)} \qquad (1)$$

where $s^2_k = \frac{N}{N-1} V_k$ and α is a measure of departure from Hardy equilibrium in the parental generation (t - 1).

However, we now have:

$$V_k = u\,V_{ds} + u\,(1-u)\,\bar{d}^2_s + v\,V_{gs} + v(1-v)\,\bar{g}^2_s + 2\,u(1-v)\,\bar{d}_s\,\bar{g}_s; \quad \ldots\ldots\ldots\ldots \text{(2) for } u \leqslant v$$

The last term on the right side of expression (2) measures the covariance between the number of female and male gametes contributed by the parents, which was considered to be zero by Crow and Kimura (1970), since they did not assume the possibility of groups of individuals being discarded before reproduction. Now, if $u > v$ in V_k, i.e. if selection is more intense on male than on female gametes, then $2u\,(1-v)$ must be replaced by $2v\,(1-u)$.

For a selection cycle with two steps of genetic sampling, or two generations, we have:

$$N_e = \frac{N_{e1} \cdot N_{e2}}{N_{e1} + N_{e2} - 0.5} \simeq \frac{N_{e1} \cdot N_{e2}}{N_{e1} + N_{e2}}$$

where N_{e1} refers to the first stage, in which a δq_{1t} random deviation occurs around q_{t-1}, N_{e2} to the second stage, and relative to deviation δq_{2t} around $q_{t-1} + \delta q_{1t}$. N_{e1} and N_{e2} are calculated considering expressions (1) and (2). Also, for this case:

$$N_e \simeq \frac{N_{e1} \cdot N_{e2}}{N_{e1} + N_{e2}} = \frac{\bar{N}_{eh}}{2}$$

With m stages, where drift may occur during a selection cycle:

$$N_e \simeq \frac{\bar{N}_{eh}}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3)}$$

where $\bar{N}_{eh}$ is the harmonic mean of $N_{e1}, N_{e2} \ldots N_{em}$.

DISCUSSION

Some examples are given for illustration:

a. Panmictic population; individual selection, based on any criterion, only on female gametes; F selected plants are crossed at random with the whole set of N parents; seeds are taken at random from these F plants to reconstitute the population of size N. In this case:

$$u = F/N;\ v = 1;\ \overline{d} = 1;\ \overline{g} = 1;\ \overline{k} = 2;$$

gametes taken at random follow a binomial distribution, thus:

$$V_{ds} = N(1/F)(1-1/F),\ V_{gs} = V_g = N(1/N)(1-1/N)$$

It follows that:

$$u\,V_{ds} = 1 - 1/F$$

$$u(1-u)\,\overline{d}_s^2 = N/F - 1$$

$$v\,V_{gs} = 1 - 1/N$$

$$v(1-v)\,\overline{g}_s^2 = 0$$

$$2u(1-v)\,\overline{d}_s\,\overline{g}_s = 0.\ \text{Therefore:}$$

$$V_k = 1 - 1/F + N/F - 1 + 1 - 1/N = (N-1)(1/F + 1/N)$$

$$s_k^2 = N/F + 1 = 1/u + 1$$

Assuming Hardy-Weinberg equilibrium in the parental population:

$\alpha \simeq 0$, and

$$N_e = \frac{2N}{0.5\,(1/u+1)+1} = N\,\frac{4u}{3u+1}$$

With no selection, $u = 1$ and $N_e = N$. With 10% selection $u = 0.10$ and $N_e = 0.31\ N$.

b. Same selection process as in example a., except that an equal number of seeds (N/F) is taken from each selected plant. Now $V_{ds} = 0$, the other quantities remaining unchanged. Thus:

$$V_k = u(1 - u)\,\overline{d}_s^2 + v\,V_{gs}$$

$$V_k = N/F - 1/N = 1/u - 1/N, \text{ giving}$$

$$N_e = N \frac{4u}{2u.+ 1}$$

When $u = 0.10$, we obtain $N_e = 0.33$ N, which, when compared with $N_e = 0.31$ N, indicates that controlling the number of contributed female gametes does not produce a considerable increase in effective size when selection is relatively intense. With a weaker selection, for example $u = 0.5$, the gametic control on the female side produces a more compensating effect, since $N_e = 0.8$ N and $N_e = N$ for the two cases shown (a and b).

c. Individual selection in self-fertilizing homozygous species; selection of F plants from an initial set of N; equal number of seeds (N/F) taken from each selected plant to reconstitute the initial population of N individuals.

Now:

$$\alpha = 1;$$

$$u = v = F/N;\ \overline{d}_s = \overline{g}_s = N/F;\ \overline{k} = 2$$

$$V_{ds} = V_{gs} = 0$$

$$V_k = u(1 - u)\,\overline{d}_s^2 + v(1 - v)\,\overline{g}_s + 2u(1 - v)\,\overline{d}_s\,\overline{g}_s$$

$$s_k^2 = \frac{N}{N - 1} \cdot \frac{4(1 - u)}{u}, \text{ giving}$$

$$N_e \simeq N \frac{u}{2(1-u)} \text{ if } \frac{N}{N-1} \simeq 1.$$

Note that when $u = 1$, i.e. in the absence of selection, $N_e = \infty$ as expected. For $0 \leqslant u \leqslant 0.5$ we will have N_e between 0.5 uN and 0.5 N.

d. To visualize the case of a selection process containing more than one step or generation in a cycle, consider the following: an initial panmictic population with N individuals, selection of F individuals based on any selection unit; selfing of each selected individual and retention of P plants per progeny; random mating between FP plants and sampling of N plants among the offspring to reconstitute the population. In this case there are two generations per cycle; one to obtain S_1 progenies, and the other to generate the new N plants. By applying the procedures described in the previous examples, we obtain:

i. for the first generation:

$$N_{e1} = \frac{F}{1 - u + 1/(2P)}$$

ii. for the second generation

$$N_{e2} = N = \frac{F}{u}$$

where $u = F/N$ is the selection proportion. On this basis, for the whole cycle:

$$N_e \simeq \frac{\overline{N}_{eh}}{2} = \frac{F}{1 + 1/(2P)} = N \frac{u}{1 + 1/(2P)}$$

As may be seen, in this case, the number F of selected plants is predominant, in determing the N_e value, regardless of the number (N) of plants kept at the end of each cycle. On the other hand the number of offspring (P) retained per S_1 progeny also participates in the effective size, especially when P is small. However, under experimental conditions, it is usually possible to take a sufficiently large P so as to obtain $1/(2P) = 0$.

e. Same as in example d. except that an equal number (P) of offspring is taken per S_1 progeny for intercrossing.

Now

$$N_e = \frac{F}{1 + 1/P}$$

Again, due to the additional generation of intercrossing among S_1 remnant offspring, N_e will always be less than F. Also, controlling the number (P) of offspring per progeny has little effect on N_e when P is sufficienty large.

In general, since $N_e \simeq (1/m)\, \overline{N}_{eh}$, it is obvious that processes requiring a larger number of generations per cycle (m) are more vulnerable to the effect of drift than those with one generation cycles. The well known problem of bottlenecks is also evident in this connection, and reinforces the necessity of controlling effective size in all steps of a cycle.

Examples a an b served to indicate that the relative increase in effective size, attainable through a control on the number of contributed gametes by parental plants to the offspring, depends upon the selection proportion. With high selection pressure, a gametic control will not counterbalance the effect of genetic drift caused by selection. The breeder however, should decide wether or not to make plant-to-plant hand pollinations, to control male gametes, or to count equal number of seeds per parental plant, to control female gametes. The critical point is that a given proportional increase in an N_e value is more important for small effective size values than for large ones, insofar as the selection limited is concerned.

ACKNOWLEDGMENT

The author is much indebted to Prof. R.D. Comstock, of the University of Minnesota, for his advice and patience.

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Rev. Brasil. Genet. I, 3, 193-202 (1978)
(Brazil. J. Genetics)

GENETIC VARIABILITY AND DISEQUILIBRIUM IN THE MAJOR ESTERASE LOCUS IN FOUR SPECIES OF THE *CARDINI* GROUP OF *DROSOPHILA*

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ABSTRACT

Samples of natural populations of *Drosophila cardini, cardinoides, neocardini itambacuriensis* and *polymorpha* showed significant disequilibrium in the esterase *est* 4 locus with respect to the Hardy-Weinberg rule. However, analysis of F_1 sàmples from wild inseminated females collected from two sites showed equilibrium frequencies. This fact suggests that different selection pressures could be operating from egg to adult and during the adult stage. The allele *est* $4^{1.0}$ is the most frequent in all collection sites of *D. polymorpha* and in two sites for *D. cardini*. The most frequent allele in the *cardinoides* population is one with the highest electrophoretic mobility (*est* $4^{1.1}$). Populations of *neocardini* show a different major allele in each locality so far studied, *est* $4^{0.80}$ and the null allele (*est* 4^{0}).

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INTRODUCTION

The *cardini* group of *Drosophila* contains 16 species, eight restricted to the Greater and Lesser Antilles, and eight to continental tropical and subtropical America. W.B. Heed has made the largest contribution to our knowledge of the genetics, reproductive isolation, color polymorphism, cytology, and phylogeny of this group (Heed, 1962; Heed and Krishnamurty, 1959; Heed and Blake, 1963; Heed and Russel, 1971). This species group has the greatest degree of color polymorphism among neo-tropical *Drosophila*.

The studies of Da Cunha (1949, 1955) on *polymorpha* and *neocardini*, Stalker (1953) and Heed (1962) on *acutilabella*, Heed and Blake (1963), and Martinez and Cordeiro (1970) on *polymorpha* have provided some information on the genetic control of this polymorphism although our understanding of it is quite incomplete.

We have carried out isoenzyme analysis of the esterases of this group to learn more about their population genetics, and to eventually correlate color and enzyme polymorphism.

MATERIAL AND METHODS

The flies were collected in southern Brazil in native woods by sweeping a net over banana-orange (1:1) bait fermented with baker's yeast. In Ponta do Caximbo, Porto Alegre, collections were made over naturally decaying fruits of *Aleurites molucana* (Euphorbiaceae). *Drosophila polymorpha* samples were obtained from Eldorado (Guaiba County), Restinga (Porto Alegre County), Iguaçu National Park, State of Paraná, Morro do Ferro, and Control wood in the region of high radiation background in Poços de Caldas, State of Minas Gerais. *Drosophila cardini* were collected in Eldorado, Restinga, and Ponta do Caximbo, and *Drosophila neocardini itambacuriensis* (Da Cunha, 1955) in Restinga and Ponta do Caximbo.

The captured wild flies and the F_1 generation from inseminated wild females were aged 8 days and stored frozen at -25° C. Gels of hydrolyzed starch (12%) or polyacrylamide (5 or 7%) were used in horizontal plates, with Poulik's (1957) discontinuous buffer system. A 10 V/cm electric field was applied across the gels for 3 to 4 hours until the migrating front was 10 cm from the sample slot line. The gels were incubated at 38^{0} C for 20 minutes in 0.1 M phosphate buffer and then in a mixture containing: 1% of alpha - and 1% beta-naphthylacetate and 1% fast blue BB salt until the major esterase bands appeared.

RESULTS

Most of the isozymes of the *est* 4 locus in *D. polymorpha* are shown in Figure 1. We observed the same pattern in the four species studied. The relative mobility was used when comparing different gels. The *est* $4^{1.0}$ was used as the reference marker for the alleles. Nine alleles were detected in *Drosophila polymorpha* (Table I). It was possible to distinguish 12 electrophoretic alleles in *Drosophila cardini* with relative mobilities ranging from 0.25 to 1.1 (Table II). Four alleles slower than those present in *polymorpha* were detected but no null allele was observed in the 255 individuals analyzed. *Drosophila n. itambacuriensis* showed 11 alleles (Table II). This locus is less variable in *D. cardinoides*. Heterozygosity was calculated according to Lewontin and Hubby (1966), and also by the formula $h = 1 - x_i^2$ (Nei, 1975), where x_i is the frequency of the i^{th} allele. The same result was obtained with both calculations and these data are included in Tables I and II. The most common allele in the six samples of *polymorpha* is the *est* $4^{1.0}$. Samples of *cardini* collected in Eldorado and Restinga in February and March '71 also have *est* $4^{1.0}$ as their major allele. However, in the Caximbo sample of April '74 *est* $4^{0.95}$ is the most frequent allele. In *D. n. itambacuriensis* the most frequent alleles are *est* $4^{0.80}$ in the Restinga sample of March '73 and *est* 4^{0} null allele in the Caximbo sample of April '76. The only sample of *D. cardinoides* (Caximbo, April '74) shows predominance of *est* $4^{1.1}$.

To estimate the equilibrium frequency of zygotic proportions we reduced the data to three classes: 1) the homozygotes for the most frequent allele, 2) the pooled other homozygotes, and 3) the pooled heterozygotes. This was done because several allele frequencies were so low that some zygotic combinations did not appear. Except for F_1 samples from Eldorado and Tainhas and the Iguaçu sample of *D. polymorpha*, all other samples

Table I - Frequencies of the alleles of *est* 4 and heterozygosity in several populations of *Drosophila polymorpha* from South Brazil.

Localities	Collecting Dates	Genomes Analyzed	est $4^{1.1}$	est $4^{1.05}$	est $4^{1.0}$	est $4^{.95}$	est $4^{.88}$	est $4^{.85}$	est $4^{.75}$	est $4^{.70}$	est 4^{0}	Heterozygosity
Eldorado	2/1971	382	.005	.031	.757	.146	.037	.018	.003	.003	–	.403
Eldorado *+	6/1973	256	.008	.063	.656	.191	.039	.031	.012	–	–	.526
Eldorado	6/1973	234	.026	.085	.645	.180	.026	.038	–	–	–	.541
Restinga	3/1971	310	.003	.045	.765	.085	.061	.019	.006	.003	.013	.401
Tainhas	3/1972	98	.020	.031	.776	.122	–	.031	–	–	.020	.380
Tainhas *+	4/1973	164	.006	.067	.659	.183	.067	.012	.006	–	–	.523
Iguaçu *	3/1972	72	.014	.042	.805	.111	–	.028	–	–	–	.337
Poços de Caldas	1/1972	360	.005	.016	.882	.077	.005	.011	.002	–	–	.216
TOTAL		1876										

+ Data from F_1 samples

* Samples in equilibrium according to Hardy-Weinberg rule.

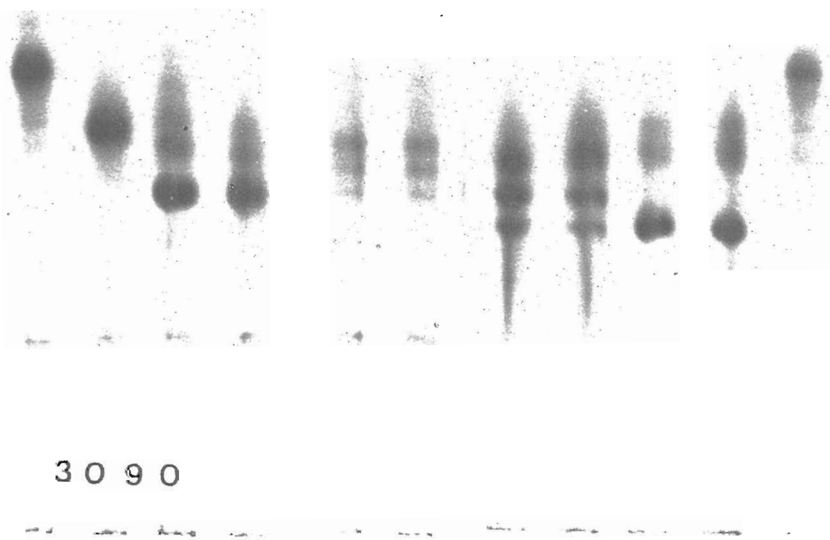

Figure 1 - Phenotypes of the est 4 loci in D. polymorpha. From left to right: est $4^{1.1}$ / est $4^{1.1}$; est $4^{1.0}$ / est $4^{1.0}$; est $4^{.85}$ / est $4^{.85}$; est $4^{.85}$ / est $4^{.85}$; est $4^{.95}$ / est $4^{.85}$; est $4^{.95}$ / est $4^{.85}$; est $4^{.88}$ / est $4^{.75}$; est $4^{.88}$ / est $4^{.75}$; est $4^{.75}$ / est $4^{.75}$; est $4^{.75}$ / est $4^{.75}$; est $4^{1.1}$ / est $4^{1.1}$.

Electrophoresis was carried out in 7% polyacrylamide gels, and esterase activity was detected as described in the Methods section.

Table II - Frequencies of the alleles of *est* 4 and heterozygosity in populations of *D. cardini*, *D. itambacuriensis* and *D. cardinoides*.

Species	Localities	Collecting Dates	Genomes Analyzed	est $4^{1.1}$	est $4^{1.0}$	est $4^{.95}$	est $4^{.88}$	est $4^{.85}$	est $4^{.80}$	est $4^{.75}$	est $4^{.70}$	est $4^{.65}$	est $4^{.60}$	est $4^{.55}$	est $4^{.25}$	est 4^{0}	Heterozygosity
	Eldorado	2/1971	130	.146	.338	.231	.031	.015	.023	.139	–	.031	.046	–	–	–	.787
	Restinga	3/1971	264	.004	.299	.152	.144	.030	.049	–	.087	.178	.057	–	–	–	.821
D. cardini	Ponta do Caximbo	4/1974	116	–	.009	.207	.155	.112	–	.086	.069	.146	.069	.095	.052	–	.870
	TOTAL		510														
	Restinga	3/1971	78	–	.051	.090	.180	.051	.269	.064	.141	.051	.103	–	–	–	.845
D. neocardini itambacuriensis	Ponta do Caximbo	4/1974	128	–	.016	–	–	.055	.109	.086	.148	–	.110	.023	–	.453	.738
	TOTAL		206														
D. cardinoides	Ponta do Caximbo	4/1974	284	.644	.187	.074	.039	.007	.018	–	–	.003	–	–	–	.023	.542

of this species and the other three are not in agreement with the equilibrium frequencies predicted by the Hardy-Weinberg rule. There is an excess of homozygotes in the other samples of *D. polymorpha, D. cardinoides* and *D. n. itambacuriensis* from Caximbo. However, the sample of *D. n. itambacuriensis* from Restinga and the three samples of *cardini* show only an excess of one of the homozygote classes but also an excess of heterozygotes.

DISCUSSION

Drosophila polymorpha populations consistently showed a distinctly higher frequency for *est* $4^{1.0}$ than for any other allele. The other two species studied in more than one sample showed a different allele at the highest frequency for each collecting site. These interspecific variations of the most common allele are not the rule in *Drosophila* species, and different species usually do not share the same allele as the major one. We do not know the causes of these variations.

It is interesting to note that the naturally decaying fruits of *Aleurites molucana* at Caximbo attracted exclusively *D. cardini, D. cardinoides, D. neocardini,* some *polymorpha* and a few *simulans* individuals. A trap of banana and orange fermented with baker's yeast, placed at 20 meters from the *Aleurites* fermenting fruits, exhibited a varied fauna with 12 to 15 commonly occurring *Drosophila* species and only a few *Cardini* group individuals. The Caximbo results appear to differ from the others in the three first species. Unfortunately we have not analyzed other samples from this location which seems to be quite specialized for the *cardini* group of *Drosophila.*

If natural sites were more completely analyzed, the microgeographic (microecologic) structure of gene distribution might demonstrate that the prevalence of one allele is a niche character. The excess of homozygotes in populations of *D. polymorpha* may be explained by the Wahlund's principle, if a pooling of subdivided populations is occurring. The pooling of low frequency alleles and the grouping together of their heterozygotes, as we did to estimate the equilibrium frequency of zygotic proportions, might also be inappropriate.

Another possibility is that heterozygotes have an advantage (heterotic selection) from the egg stage to the pupa stage, while the adult homozygotes are better adjusted to several specialized niches due to diversifying selection. Da Cunha (1949), studying color polymorphism, also found an excess of homozygotes in samples of adults of *D. polymorpha* from nature, and excellent agreement with the Hardy-Weinberg rule in laboratory cultures, when the adults were not submitted to selection pressure. This is suggested in our data by the contrasting results of the wild fly samples (adult samples) and the F_1 sample of Eldorado and Tainhas (Table I). However, the F_1 sample of *D. polymorpha* from Poços de Caldas showed an excess of homozygotes also, which might be explained by very low larval competition. Another problem is the predominance of the null allele (*est* 4^0) since a great portion of heterozygotes would bear it and would be scored as homozygotes. Only an F_1 analysis could clarify this point.

ACKNOWLEDGMENTS

The technical assistance of Mrs. Gladis S. Correa, Miss Nena B. Morales and Mr. Danubio Ramila is gratefully recognized. We thank Dr. M. Ashburner and Dr. Helga Winge for their participation in the collection of *Drosophila* in Iguaçu and Restinga. This work was supported in part by grants from CNPq and Universidade Federal do Rio Grande so Sul.

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(Received July 25, 1978)

Rev. Brasil. Genet. I, 3, 203-214 (1978)
(Brazil. J. Genetics).

ON THE PHENOTYPIC IDENTIFICATION OF CREOLE CATTLE

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ABSTRACT

Creole cattle are a potentially rich source of protein and milk in marginally productive regions. Before they can be studied, subjected to genetic improvement and put to practical use, they must be identifiable reliably. Experts and non-experts were compared on their ability to identify Creole cattle phenotypes from photographs. Although the experts showed a high level of agreement in certain cases, their overall success was disappointing. Nevertheless, the degree of agreement among experts was higher than that of non-experts on a variety of measures. The results point to the necessity of devising better definitions of the Creole race.

INTRODUCTION

Creole cattle (*Bos taurus*) have almost disappeared, but are currently receiving increased, albeit slight and belated, attention as a potentially rich source of protein and milk in marginally productive regions of Latin America. Introduced to the Western Hemisphere by Iberian colonists in the 16th

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Century, the race is known by a variety of names: *Criollo* in most of Spanish--speaking America, *Ganado Corriente* or *Chinampo* in parts of Mexico, *Crioulo* or *Caracu* in Brazil, and *Longhorn, Spanish Cattle* or *Scrub Cattle* in the U.S.A. In some localities, particularly in Colombia, Creole stock is subdivided into breeds such as Blanco Orejinegro, Valleeaucano, Chino--Santandereano, San Marinero, Costeño con Cuernos and Romo Sinuano. The Creole race has adapted itself to an extraordinary range of environmental diversity in temperature, humidity, altitude, parasitic infestation, predatory pressure, nutritional resources, and human management practices (e.g. Calles Salguero, 1971; Rouse, 1977). As Inchausti and Tagle (1967) pointed out, Creole cattle are especially adept in poorer areas where climatic, topographic and nutritive conditions are difficult for the more specialized breeds.

Little is known about the heterogeneity of phenotypes which served as the progenitors of the Creole cattle seen today. Nevertheless, after more than 400 years of selective breeding for economic and aesthetic reasons, idiosyncratic inbreeding in isolated populations, hybridization with other cattle, and natural selection in radically different environments, phenotypic variation (with the possible exception of certain recurring coloration patterns) in Creoles is remarkable (e.g. Rouse, 1977). Creoles are so variable, in fact, that one author (Winsberg, 1968) defined them simply as animals "of unknown ancestry". Although several attempts have been made to provide identification criteria (e.g. Inchausti and Tagle, 1967; Payne, 1970; Rouse, 1970, 1977), the sum of the available descriptions and published photographs present a kaleidoscope of variation rather than a tabloid of uniformity. Moreover, when traveling in areas where Creoles are still raised, one encounters considerable disagreement amongst the growers and other cattle experts as to which animals are "true Creoles". Identification of Creoles by blood groups, while promising, is not yet a reality. For example, Miller (1966) reported blood type data on a sample of Longhorns, but as Owen, Stormont and Irwin (1947) explained several years ago in a treatise which is still true, serological analyses provide only probabilistic statements concerning racial identity - not an absolute determination: "... breed differences are not of the 'all-or-none' variety, but depend on contrasting frequencies..." Even though continuing investigations and technological advances are enhancing the use of blood typing for possible Creole identification, ultimately the serological findings must be evaluated in the light of morphological characteristics, such as conformation and coloration.

Obviously, before Creole cattle can be studied, subjected to genetic manipulations and put to practical use in a rigorous, scientific manner, they must be identifiable reliably. Instead of digressing to a discussion of the merits of the Platonic notion of "ideal types" existing in a reality independent of human perceptions, let us assume for present purposes that the creation of categories of biological phenomena is a human accomplishment. That is, if humans invented the names of categories (for example the concept of "Creole cattle") and want to employ the categories in some way, then it follows that a reasonable starting point for examining the identification of Creoles would be to study the ability of humans to use perceptual information in discriminating between Creoles and other varieties of cattle. Ascertaining human abilities to make reliable descriminations involves comparing the judgments of individuals with regard to the consistency of their assigning particular objects to particular categories.

To recapitulate, the research reported here concerns the phenotypic identification of Creole cattle. If the process of identification is perceptual, then a study of the identification of Creole cattle calls for a perceptual approach to the problem, at least initially. Unorthodox though it may be, the theme of the present investigation is more psychological than taxonomic in the classical sense.

METHODS

The 44 subjects who participated in the investigation consisted of two groups of 22 persons each. One group was made up of specialists working with bovine cattle in various scientific and educational institutions in the Argentine Republic. They are called "experts" in the remainder of this report. The second group ("non-experts") worked in the same or similar institutions in Argentina, but their specialties were in some biological field other than bovine cattle. The two groups of subjects were similar in age distribution, level of formal education and professional status.

All 44 subjects were shown a set of 22 photographs ("stimuli") of bovine cattle found in several regions of northern Argentina. The animals selected for the research were chosen more or less at random, but with some effort to present a wide range of morphological variation, excluding descendents of obviously common European breeds. Simultaneously, with seeing the protographs, the subjects were given a questionnaire designed to elicit information related to discriminating between Creole and non-Creole cattle. There were four tasks involved in responding to the questionnaire:

(1) For each of the 22 stimuli, the subjects made a binary ("sí" or "no") judgment as to whether or not the animal in the photograph was a Creole.

(2) Also for each stimulus, the subjects indicated the subjective degree of confidence they had in their judgments, where degree of confidence was defined on a scale ranging from 0 (pure conjecture) to 100 (absolute certainty).

(3) Space was provided for the subjects to record the criteria they used in making their judgments.

(4) The subjects were asked to indicate the number of years of direct experience they had with Creole cattle.

The photographs of the cattle, in addition to being variable because of intrinsic differences among the animals, differed in technical clarity as well. In order to ascertain the extent to which the results might have been influenced by photographic quality rather than variation in the cattle and the subjects, it was necessary to contrive a measure of the clarity of the photographs. A third group of 22 subjects (students, professors, secretaries and passers-by in the Department of Psychology, University of California, Davis) rated the photographic clarity of the stimuli on a scale of 0 (indiscernible) to 10 (excellent).

RESULTS AND INTERPRETATIONS

Differences Between Experts and Non-Experts

The responses of the experts and non-experts were compared on the tasks mentioned in the Methods section, except for the third one (criteria for judgments), which provided very little useful information for present purposes. The mean degree of confidence, degree of agreement, and clarity of each stimulus are shown in Table I. If the number of positive responses (Σ sí) is 16 or more in the table, there was statistically significant agreement ($p < .05$) that the animal in question is a Creole; 6 or less positive responses indicates that the animal is not a Creole. Significance levels were based on z-score transformations, using the assumption that the response distribution was symmetrical. Clearly, the experts differed from the non-experts in many ways. Among the differences were the following:

(1) Stimuli number 1 and number 22 were photographs of the same animal with its body oriented differently. Twenty of the 22 experts (90.9%) responded in the same way to the two stimuli, whereas only 12 of the 22 non-experts (45.4%) showed inter-stimulus consistency. The difference between the two groups on the measure is statistically significant (χ^2 = 7.33; df = 1, $p < .01$).

(2) The correlation coefficient (Table II) between degree of agreement (i.e., the difference between the number of "sís" and "nos") of expert and non-experts was negative and statistically significant ($r = -0.557$, df = 20, $p < .01$).

(3) The experts agreed statistically more often than did the non--experts (see Table I), where the ratio was 16:7 significant agreements for experts and non-experts, respectively (χ^2 = 7.38, df = 1, $p < .01$). Moreover, the total number of times the experts answered "sí" was not different from the total number of times the non-experts said "sí" ($\chi^2 < 0.001$, df = 1, $p > .05$). That is to say, the superior agreement on the part of the experts is attributable to the similarity in their judgments rather than a differential tendency for experts and non-experts to answer "sí".

(4) Upon examining all 484 responses made by each group (22 responses X 22 stimuli), the experts agreed a total of 242 times (exactly 50%, where chance performance was 25%), whereas the overall agreement for non-experts was 156 (32.23%). The difference between the two groups was statistically significant (χ^2 = 31.56, df = 1, $p < .001$). Even though the experts once again showed a superior ability to agree with one another, the finding that their overall agreement was only 50% is disappointing.

(5) The mean degree of confidence expressed by the two groups distinguished them statistically ($t = 2.292$, df = 20, $p < .05$).

(6) Although the apparent discrepancy in reported experience with Creole cattle (where the means were 13.54 and 4.95 years for experts

and non-experts, respectively) is not statistically significant ($t < 1.0$, $df = 20$, $p > .05$), it is probably significant in reality. The lack of a statistically significant t-test is explainable in part by large within-group variation, particularly among the experts, where the inclusive range was from 1 to 62 years.

Correlational Analyses

Correlation coefficients for degree of agreement, degree of confidence, and clarity of photographs are shown in Table II. The only statistically significant relationships that were found were between experts and non--experts, where degree of agreement was negatively related, as has already been mentioned, and degree of confidence was positively related.

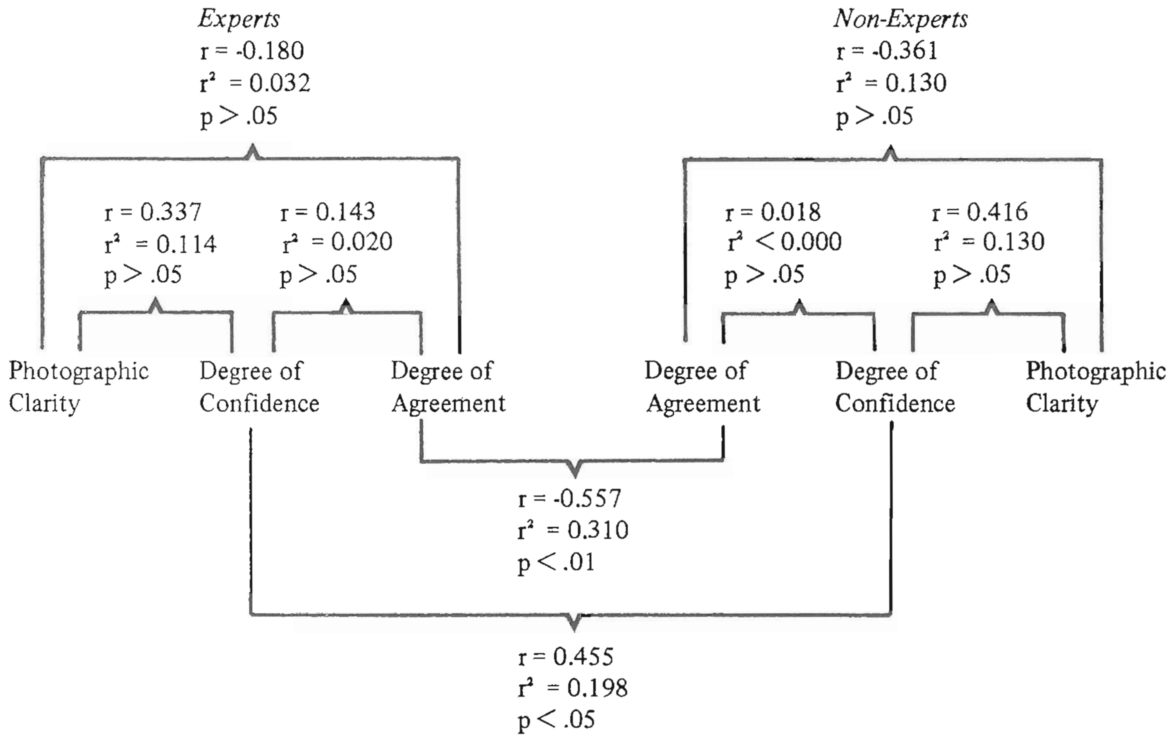
Within the two groups of subjects, the forms of the correlations were similar but not statistically significant. In both cases, the relationship between confidence and photographic clarity was higher than the relationship between confidence and agreement, as might be expected. Also in both groups the relationship between agreement and photographic clarity was negative, which was not expected and which would have been very difficult to explain had the correlations been statistically significant. Even so, the findings suggest that poor photography was not responsible for poor agreement between subjects.

Correlations were also computed between the number of times each individual subject agreed with the majority of experts on the 16 stimuli which produced significant agreement and reported years of experience. The coefficients were $r = 0.054$, $r^2 = 0.003$, $p > .05$ for experts and $r = -0.033$, $r^2 = 0.001$, $p > .05$ for non-experts. Note that the two variables were not strictly independent in the case of the experts because in at least some cases the values for agreed-upon stimuli included individual agreement values. Nevertheless, if the study reported here was a valid test, then experience is unrelated to success in agreeing with experts in identifying Creole cattle. However, there are several reasons for rejecting such an interpretation. Some of them are mathematical. For example, there may not have been enough variation in years of experience (although the range was large) and/or expert opinions to produce a large correlation coefficient. Furthermore,

Table I - Stimulus Number (S) with Mean Photographic Clarity Rating ($\bar{X}_{cl}$): Degree of Agreement ($A = \Sigma sí - \Sigma no/$), Sum of Positive Responses ($\Sigma sí$), Standard Scores of Positive Responses ($z_{sí}$), Bilateral Probability of $z_{sí}$ (p) and Mean Degree of Confidence ($\bar{X}_{co}$) for Experts and Non-Experts.

Stimuli		Experts					Non-Experts				
S	$\bar{X}_{cl}$	A	$\Sigma sí$	$z_{sí}$	p	$\bar{X}_{co}$	A	$\Sigma sí$	$z_{sí}$	p	$\bar{X}_{co}$
1	5.3	12	4	-2.98	< .01	84.5	10	16	2.13	< .05	53.2
2	4.2	4	13	0.85		68.2	12	5	-2.13	< .05	67.7
3	8.0	10	16	2.13	< .05	76.4	4	13	0.85		61.4
4	5.6	4	13	0.85		77.3	8	7	-1.70		55.4
5	4.0	8	15	1.70		73.2	6	8	-1.28		58.6
6	7.5	10	16	2.13	< .05	68.2	2	10	-0.43		67.7
7	8.2	14	18	2.98	< .01	77.7	4	9	-0.85		55.9
8	6.3	14	4	-2.98	< .01	83.6	6	14	1.28		63.2
9	5.1	4	9	-0.85		71.8	6	14	1.28		56.8
10	4.2	18	2	-3.84	< .01	72.3	8	7	-1.70		64.1
11	3.5	12	5	-2.56	< .05	66.8	10	6	-2.13	< .05	50.0
12	5.4	10	16	2.13	< .05	65.4	10	16	2.13	< .05	55.9
13	6.2	12	5	-2.56	< .05	72.7	4	9	-0.85		57.3
14	5.7	16	3	-3.41	< .01	79.1	12	17	2.56	< .05	57.3
15	4.6	16	19	3.41	< .01	65.0	12	17	2.56	< .05	79.1
16	3.9	16	19	3.41	< .01	68.6	10	16	2.13	< .05	59.5
17	8.8	6	14	1.28		79.1	8	7	-1.70		56.4
18	7.3	0	11	0.00		68.2	4	13	0.85		51.8
19	4.0	12	18	2.98	< .01	70.9	0	11	0.00		57.7
20	6.6	12	17	2.56	< .05	70.4	6	14	1.28		62.7
21	5.3	22	22	4.69	< .01	74.1	8	15	1.70		62.3
22	5.3	10	6	-2.13	< .05	79.1	6	8	-1.28		62.7

Table II - Correlations between Degree of Agreement, Degree of Confidence and Photographic Clarity for Experts and Non-Experts.



upon examining the difference between the two correlations ($z_{r_1} - z_{r_2} = 0.087$, $\delta_{z_{r_1} - z_{r_2}} = 33$; $t = 2.636$, $df = 20$, $p < .02$), it appears that experience may help experts somewhat, but if anything, it has a negative effect on non--experts. The finding suggests the possibility that a third, unmeasured variable such as rigorous training is involved, and that this third, hypothetical variable could be used in improving the subjects' performance.

DISCUSSION

There were three principal findings in this research. First, the notion of "expert" is a valid concept. Experts do exist, and their judgments concerning Creole cattle were different from those of non-experts. Second, the experts showed a high degree of agreement on some stimuli, but not all of them. The observation that their overall agreement was only 50% indicates a need for considerable improvement if research on Creole cattle is to continue. Third, the lack of agreement between experts was independent of the photographic quality of the stimuli and the amount of confidence they had in their judgments. This, in combination with the finding that experts can agree very well on particular animals, suggests that: (1) the procedures used in this study were valid and (2) an important aspect of the problem concerns developing better criteria for defining the Creole race.

If the points made in the preceding paragraph are correct, then the next task in improving the identification of Creoles should be taxonomic. One of the most important decisions that must be made initially is whether Creole cattle are to be thought of as a particular phenotype or as a distribution of phenotypes. Clearly, the population of animals that can reasonably be called "Creoles" does not represent a single phenotype. On the contrary, possibly the most salient characteristic that distinguishes Creoles from other cattle is not their size, shape or coloration, but variation in those and other traits. That is, the characteristic most typical of the Creole race may be variation itself. Apparently, a useful definition of Creole cattle will require specifying the limits of the population with respect to many traits, rather than trying to create a type which may not exist in reality, or if it does exist is so rare that it is not representative of the population as a whole.

Some scientific consequences of creating inappropriate typologies have been discussed at length by Ghiselin (1966), Hull (1967), Mayr (1957, 1959), Rollins (1965) and Sokal and Camin (1965). There is little need to repeat the arguments here. The alternative to a typological classification implies the use of some form of phenetic, numerical taxonomy (e.g. Sokal, 1966; Sokal and Sneath, 1963), although some of the traits of interest will be qualitative rather than quantitative.

Apart from the considerations just mentioned, there are no explicit rules for deciding how to devise a useful classification. As Cowan (1955) remarked, "All classifications are subjective, and, like religious and political opinions, have a large element of aesthetic unreason about them. A classification consists of two elements: the objects to be classified, and the subject who feels the urge to classify them. There cannot be a classification without a classifier or taxonomist, and no two taxonomists will approach objects in the same way". Success in defining the Creole race will require a clever and systematic combination of accumulated experience, creativity and fortuitous suppositions.

ACKNOWLEDGMENTS

Data for this research were collected while the author was, under the auspices of the Organization of American States, a visiting professor in the Instituto Nacional de Tecnología Agropecuaria (INTA) in Castelar, Angentina, where he received indispensable cooperation and help, particularly on the part of Ing. Alejo von der Pahlen. So many people gave valuable assistance to the project that it is impossible to thank them all individually. Therefore, only their affiliated institutions will be mentioned with grateful appreciation: El INTA in Balcarce, Buenos Aires, Castelar, Corrientes, Formosa, Leales, Nueva Orán and Salta; El Instituto de Biología & Medicina Experimental in Buenos Aires; La Universidad de Tucumán; SELSA in Zapala, Neuquen; and most especially El Instituto de Investigaciones Médicas in Rosario. Also, particular thanks are owed to the many ranchers, gauchos and other kind Argentines in various parts of the republic who openly shared the hospitality of their homes, their time and their knowledge.

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(Received September 24, 1978)

Rev. Brasil. Genet. I, 3, 215-226 (1978)
(Brazil. J. Genetics)

MORPHOLOGICAL AND FUNCTIONAL STUDIES OF THE SMALL INTESTINE IN INHERITED ICHTHYOSIS

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ABSTRACT

The possible association between inherited ichthyosis and morphological alterations of the jejunal mucosa and/or intestinal malabsorption was investigated. Six cases of ichthyosis vulgaris, four cases of non-bullous congenital ichthyosiform erythrodermia and one case of X-linked ichthyosis were studied. The patients were submitted to the following intestinal absorption tests: measurement of total fecal fat content, determination of feces radioactivity after ingestion of I^{131} oleic acid, and D-xylose test. Jejunal biopsies were taken from the patients and the following morphological measurements were taken: total mucosa height (TMH), villi height (VH), VH/TMH index, enterocyte height (EH), and intraepithelial lymphocytes/epithelial cells ratio. The results of the intestinal absorption tests were normal in all patients, indicating that there is no association between inherited ichthyosis and intestinal

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malabsorption. A probable light partial atrophy of the villi was detected in one case of ichthyosis vulgaris and in two cases of non-bullous C.I.E., but no pathological significance could be attributed to them.

INTRODUCTION

Among the hypotheses advanced to explain the physiopathology of ichthyosiform dermatoses, the one related to possible alterations in the metabolism and/or intestinal absorption of lipids is particularly outstanding (Esterly, 1968, and Fry et al., 1966). Several observations are at the basis of this hypothesis: 1) patients with Sjögren-Larsson syndrome and intestinal malabsorption showed complete regression of intestinal malabsorption and of ichthyosiform skin alterations when submitted to a diet in which normal lipids had been replaced with medium-chain triglycerides (Guilleminault et al., 1973; Hooft et al., 1967 a, b); 2) prolonged use of triparanol (MER-29), a drug causing post-squalene blocking in the synthesis of cholesterol (Horlick and Avigan, 1963), leads to the onset of acquired ichthyosis (Wilkelmann et al., 1963), with intestinal malabsorption and morphological alterations of the jejunal mucosa appearing before skin lesions (Roe and Rice, 1948). Since the synthesis of cholesterol in the skin of ichthyosis vulgaris patients is normal (Summerly and Yardley, 1967), and since triparanol is responsible for the appearance of intestinal malabsorption followed by acquired ichthyosis, it can be presumed that the skin alteration is a consequence of the drug-induced intestinal malabsorption; 3) morphological and histoenzymological alterations of the intestinal mucosa in two forms of inherited ichthyosis were described by Fry et al. (1966); 4) acquired ichthyosis is frequently found in patients with intestinal malabsorption (Wells, 1962).

The objective of this research was to study the possible association between inherited ichthyosis and intestinal malabsorption and/or morphological alterations of the jejunal mucosa.

SUBJECTS AND METHODS

A general physical and dermatological examination, a genealogic survey and histopathological skin examinations were carried out to diagnose and classify the types of ichthyosis affecting the patients. The criterion used for classification was that of Wells and Kerr (1965) modified by Esterly (1968).

Eleven ichthyosis patients were selected: six with ichthyosis vulgaris (autosomal dominant), four with congenital ichthyosiform erythroderma (C.I.E.) of the non-bullous type (autosomal recessive), and one with X-linked ichthyosis (recessive).

None of the patients was affected by any other diseases which might interfere with the process of intestinal absorption. The patients were selected for this study independently of possible digestive symptoms. During the period of investigation of intestinal absorption, no medication for hyperkeratosis treatment or of any kind that might interfere with intestinal absorption was administered to the patients. All patients volunteered for hospital admission in order to be submitted to the tests.

The following techniques were used:

1. Study of Intestinal Absorption

Measurement of total fecal fat content. The patients were submitted to a diet containing 100 g fat/day. Feces were collected over a 72 hour period. Chemical determination of the total amount of fat in the feces was performed according to the method of van de Kamer (1949).

Measurement of fecal radioactivity after ingestion of I^{131} oleic acid. This determination was carried out and interpreted by the method adopted by Meneghelli et al. (1972).

D-xylose test. Each patient ingested 25 g of D-xylose, and urine was collected five hours after ingestion; chemical measurement of D-xylose was carried out by the technique of Roe and Rice (1948).

2. Morphological Studies of the Jejunal Mucosa

Intestinal biopsies were taken from the patients at the level of the duodenojejunal flexure by means of an intestinal tube with a Crosby type capsule; the position of the capsule was controlled radioscopically. The biopsy material was placed on filter paper with the luminal surface of the mucosa looking up, with proper care taken to avoid shrinkage. The material was then placed in a 10% formaldehyde solution and later processed histologically. Special care was taken to align the material during inclusion in order to avoid artifacts due to tangential sectioning of the specimen. Quantitative methods were used on the histological sections due to their discriminatory power in the diagnosis of minor morphological alterations of the intestinal mucosa (Rubin and Dobbins, 1967).

Measurement of total mucosa height (TMH), villi height (VH) and of the VH/TMH index. Micrometric measurements of TMH and VH were carried out with a microscope fitted with a viewfinder (Visopan-Reichert) which had been calibrated with a millimiter ruler. TMH was taken as the distance between the fibers of the muscularis mucosae and the apex of the villus. The value for VH was measured from the cripta-villus transition point up to the apex of the villus (Salazar de Souza and Cunha, 1969). These measurements were made in ten different regions of the mucosal biopsy, and the final result represents the average value. The VH/TMH index was obtained by calculating the ratio of the two values.

Measurement of enterocyte height (EH). EH was taken as the distance between the basal membrane and the edge of the enterocyte (Salem and Truelove, 1965).

Measurement of intraepithelial lymphocytes/epithelial cells ratio. A sample of 200-300 epithelial nuclei was counted starting from the base of the villi. The intraepithelial lymphocytes found in the same area were also counted, and the ratio of the two values was obtained (Ferguson and Murray, 1971).

3. Radiology study and intestinal transit time study.

This study was carried out by standard radiological methods (Adlersberg et al., 1954).

RESULTS

1. Intestinal Absorption

Total fecal fat content (Table I). The results for the seven cases in which it was possible to use the method were within normal limits. Only one patient, case 2, showed digestive symptoms. This patient had intestinal constipation lasting up to 5 days, possibly of a functional nature.

Fecal radioactivity after ingestion of I^{131} oleic acid (Table I). Enteral excretion of I^{131} oleic acid could be measured in six patients. The results were all within normal limits.

D-xylose test (Table I). D-xylose levels in 5 hour urine from the 9 cases who were submitted to this test were similar to those found in normal individuals.

2. Morphology of the Jejunal Mucosa

Total mucosa height (TMH), villi height (VH), and VH/TMH index (Table II). VH/TMH indexes of less than 0.66, considered to be the lower normal limit for North Americans and Europeans (Salazar de Souza and Cunha, 1969), were obtained for cases 4, 9 and 10. According to the classification by these authors, these three cases would be considered as having light partial atrophy of the villi (VH/TMH index between 0.50 and 0.66). As to the absolute values for VH and TMH, the average values of the measurements made in all patients were much lower than those found by other authors (Madanagopalan et al., 1965; Shiner and Doniach, 1960). However, only the VH values for cases 4 and 9 were below the lower limits established by these authors.

Enterocyte height (EH) (Table II). The results for all ichthyosis patients studied and controls were within the normal limits established by Shiner and Doniach (1960).

Intraepithelial lymphocytes/epithelial cells ratio (Table II). The results for cases 3, 4 and 5 (all of them ichthyosis vulgaris) showed a slight increase in relation to the upper limit established by Ferguson and Murray (1971). The results for the other patients and the controls were within the limits established by these authors.

3. Radiology study and intestinal transit time study.

The results were normal for all 11 patients.

DISCUSSION

1. Intestinal Absorption

Our data showed the absence of intestinal malabsorption in the 11

Table I - Intestinal tests in cases of Inherited Ichthyosis.

		Total fat content of the feces	Radioactivity in the feces after ingestion of I^{131} oleic acid		D-xylose in urine
Case	Type of ichthyosis	G. of fat in the feces/24hr[a]	% of excreted radioactivity/72hr[b]	Total weight of feces/72hr	G. D-xylose 5h urine[c]
1	Ichthyosis vulgaris	2.68	3.20	508.9 g	7.89
2	Ichthyosis vulgaris	*	*	—	6.98
3	Ichthyosis vulgaris	1.56	*	571.2 g	15.24
4	Ichthyosis vulgaris	1.93	7.05	408.7 g	10.42
5	Ichthyosis vulgaris	3.80	1.07	615.8 g	11.63
6	Ichthyosis vulgaris	*	*	—	*
7	Non-Bullous C.I.E.	*	*	—	7.13
8	Non-Bullous C.I.E.	1.71	2.24	354.4 g	6.91
9	Non-Bullous C.I.E.	1.30	0.24	260.3 g	4.80
10	Non-Bullous C.I.E.	1.82	3.29	922.3 g	5.09
11	X-Linked Ichthyosis	*	*	—	*

* Test could not be carried out

[a] Normal up to 6 g/24 hr

[b] Normal up to 7% enteral excretion/72 hr (Texter, Jr. et al., 1964)

[c] Normal when higher than 4 g/5 hr (Texter, Jr. et al., 1964)

Table II - Villi height (VH), total mucosa height (TMH) and enterocyte height (EH) expressed in microns[a], VH/TMH index and intraepithelial lymphocytes/epithelial ratio, in cases of ichthyosis and in controls.

Case	Type of ichthyosis	VH	TMH	EH	VH/TMH Index[b]	Lymphocytes/Epithelial cells ratio[c]
1	Ichthyosis vulgaris	*	*	*	*	*
2	Ichthyosis vulgaris	*	*	*	*	*
3	Ichthyosis vulgaris	*	*	42 (36-48)	*	47%
4	Ichthyosis vulgaris	295 (247-329)	457 (392-495)	44 (38-48)	0.64	44%
5	Ichthyosis vulgaris	346 (227-495)	449 (330-618)	32 (28-38)	0.77	41%
6	Ichthyosis vulgaris	392 (227-515)	532 (350-660)	35 (28-40)	0.73	28%
7	Non-Bullous C.I.E.	*	*	*	*	*
8	Non-Bullous C.I.E.	*	*	28 (24-34)	*	*
9	Non-Bullous C.I.E.	265 (206-329)	439 (289-495)	32 (28-40)	0.60	32%
10	Non-Bullous C.I.E.	317 (144-432)	532 (247-660)	35 (26-44)	0.59	*
11	X-Linked ichthyosis	398 (289-453)	534 (412-618)	29 (22-32)	0.74	37%
Controls						
1		474 (350-660)	618 (474-825)	35 (30-40)	0.76	21%
2		387 (268-515)	493 (371-639)	31 (26-36)	0.78	27%
3		331 (247-453)	457 (329-618)	35 (30-40)	0.72	35%
4		*	*	38 (30-42)	*	36%
5		*	*	32 (24-38)	*	14%

* Biopsy was unsuccessful

[a] The values represent the average ($\overline{x}$) of 10 measurements and their respective variation limits.
Normal values - VH = 430 (320-570μ); TMH = 600 (400-835μ); EH = 34 (29-41μ) (Shiner and Doniach, 1960)

[b] A VH/TMH index of $\geqslant$ 0.66 is considered normal (Salazar de Souza and Cunha, 1969)

[c] The normal limit of the intraepithelial lymphocytes/epithelial cells ratio is 6 to 40% (Ferguson and Murray, 1971)

patients studied. This conclusion is based on the following facts: a) with the exception of case 2, the patients had no history of chronic diarrhea nor any digestive symptoms; b) the amount of total fat in the feces and the radioactivity level in the feces after ingestion of I^{131} oleic acid were normal in all cases (Table I); c) the D-xylose test (Table I) gave normal results in all cases.

Since total fecal fat content, determined by the technique of van de Kamer et al. (1949) is the standard method for the detection of steatorrhea, and since the determination of fecal radioactivity levels after ingestion of I^{131} oleic acid is considered by Braunstein and Song (1975) to be a useful screening method for steatorrhea, we can conclude that no steatorrhea was present in our group of ichthyosis patients. The D-xylose test has been variously criticized as to its value in the detection of alterations in the intestinal absorption process (Krawitt and Beeken, 1975), but is still widely chosen for this purpose (Kendall, 1973).

These findings are in agreement with those of Fry et al. (1966) who, even though they described morphological alterations of the jejunal mucosa in 7 out of 10 ichthyosis cases studied, did not find any alterations of the intestinal absorption process in any of them. Our data also support the opinion of Marks and Shuster (1970) who believe that malabsorption is not the general mechanism producing acquired ichthyosis, since they only found one case of steatorrhea in a group of 10 cases of acquired ichthyosis.

2. Morphology of the Intestinal Mucosa

The VH and TMH measurements of our controls are clearly lower than the corresponding values in the literature (Chelli et al., 1964; Jos, 1962; Madanagopalan, 1965; Shiner and Doniach, 1960), but the values for the VH/TMH index and EH are similar to those found in the literature. Although the number of controls available for our study was small, the discrepancy found for the VH and TMH values can still be considered as probably representing a difference between our population and those of Europe and North America, due to different environmental conditions (nutrition, intestinal parasites, etc.). These different conditions may have a decisive influence on the reduction of VH and TMH values both in the patients' group and in the controls. However, the VH value, especially in cases 4 and 9, was quite different from that of the controls. The VH/TMH index was below the 0.66 limit in these two cases as well as in case 10. These reductions may represent a light partial atrophy of the villi or simply a normal spectrum of variation within our population. It is difficult, however, to attribute a pathological significance to the first hypothesis (light partial atrophy of the villi) since none of the alterations considered to be important factors for the definition of pathology of the intestinal mucosa (Chelli et al., 1964) was observed in the villi epithelium or in any other elements of the mucosa of these patients.

Fry et al. (1966) used stereoscopy for the morphological study of the intestinal mucosa of 10 ichthyosis cases. Of the 10 patients, 7 were considered to have abnormal mucosa. Five of the 7 abnormal cases had flat leaf-like villi, which, however, according to Shiner and Doniach (1960), cannot be considered to be abnormal elements since their histological aspect was normal.

Alterations in the morphology of the intestinal mucosa have been described in a large number of dermatoses, especially psoriasis, eczema, rosacea, lichen planum, pityriasis rubra pilaris and dermatitis herpetiformis (Marks and Shuster, 1970). These authors reported an 8% incidence of convoluted mucosa in a group of 200 patients with different types of dermatoses; an 8% incidence was also found in the "control" group, and 30% incidence was found in 28 cases of dermatitis herpetiformis. Thus they concluded that no correlation exists between generalized dermatoses and abnormalities of the intestinal mucosa; if anything, this correlation would be limited to dermatitis herpetiformis. They justify the large number of patients with different dermatoses described as having abnormal intestinal mucosa on the basis of the high incidence of such alterations found in the general population (8%), considering that the bulk of the research was carried out in the same region (Newcastle upon Tyne). The data obtained by Salem and Truelove (1965) in another region of the United Kingdom (Oxford) confirm those of Marks and Shuster (1970). However, a study by Barry et al. (1971) in Bristol disclosed a higher incidence of convoluted mucosa in psoriasis and other chronic dermatoses than in the general population.

On the basis of our results, we can conclude that no morphological alterations with a pathological significance were found in our group, but the possibility of a light partial atrophy of the villi for case 4 (ichthyosis vulgaris) and cases 9 and 10 (non-bullous C.I.E.), without any serious clinical implications, could not be excluded.

3. Lymphocyte Infiltration in the Mucosa.

Fry el al. (1966) described an important lymphocyte infiltration only in one of the patients with bullous C.I.E. studied. Our results demonstrate an increase in lymphocyte infiltration of the intestinal mucosa in three patients with ichthyosis vulgaris (cases 3, 4 and 5, Table II). These abnormal results may have little or no pathological significance because of the small difference they represent in relation to the normality index established by Ferguson and Murray (1971). They are probably due to the different conditions in which individuals from tropical zones live (intestinal parasitoses, nutrition etc.).

ACKNOWLEDGMENTS

We wish to thank Dr. Luis Marino Bechelli, Dr. Paulo Frederico Becker, Dr. Fritz Köberle, Dr. Ricardo B. de Oliveira, Dr. Roberto Dantas, and Dr. José Alberto Mello de Oliveira for their support of this work and helpful suggestions. We are grateful to Mrs. Zuleica Custódio, Mrs. Nadja Naira N. Zambell, Mr. Armando V. Verceze for their technical help, to Mrs. Leticia B. Caetano for typing the manuscript, and to Mrs. Elettra Greene for translating the text. We are grateful for the cooperation of the Radiology and Nursing Services of the Hospital das Clínicas of the Faculty of Medicine of Ribeirão Preto, University of São Paulo.

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