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MITOTIC INSTABILITY IN A III-VIII DUPLICATION STRAIN OF *ASPERGILLUS NIDULANS.*

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ABSTRACT

Strains of *Aspergillus nidulans* with a duplicate chromosome segment are unstable at mitosis. They produce mainly two types of sectors designated as improved and deteriorated sectors. A study of the number of such sectors produced by two unstable strains (III-VIII strains) where a segment of linkage group III is present twice, one copy being in a normal position and the other translocated to linkage group VIII, was carried out. Some of the deteriorated sectors were genetically analysed. The results were compared tho those obtained from previously analysed unstable duplication strains (I-II strains). The overall number of sectors was greater in the III-VIII strains than in the I-II strains; of the two III-VIII strains, the one carrying a determinant of deterioration *(v9)* previously obtained from a I-II strain was more unstable. Although distinction between improved and deteriorated sectors produced was not always clear, conspicuous deteriorated sectors and derivatives from them which were genetically analysed have shown that deterioration can be associated to deletion on the duplicated segments although in two instances determinants of deterioration were found to be located in linkage groups not

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involved in the original duplication. Another characteristic of the deteriorated variants from the III-VIII duplication strains may be the high rate of mitotic crossing-over between the duplicated segment. This is possibly due to the association of mitotic crossing-over and the origin or the presence of deterioration.

INTRODUCTION

Strains of *Aspergillus nidulans* with a duplicate chromosome segment show instability at mitosis. Several strains, each with a different chromosome segment in excess of the standard haploid genome, have so far been examined and all show similar patterns of instability at mitosis (Bainbridge and Roper, 1966; Ball, 1967; Nga and Roper, 1968).

Such instability, never observed in standard haploids, seems to be a feature of all duplication strains of *Aspergillus nidulans*. Duplication strains, which have a characteristic "crinkled" morphology and reduced growth rate, produce sectors showing various degrees of phenotypic improvement. These arise from nuclei which have lost a variable part of one or the other duplicate segment and so, with reduced imbalance, have selective advantage over their duplication parent (Nga and Roper, 1968). Duplication strains produce, infrequently but regularly, sectors with deteriorated morphology. These have been analysed in one duplication strain of *Aspergillus nidulans* which had a terminal segment of linkage groups I present twice, one copy in the normal position and the other translocated to a tip of linkage groups II. Analysis of deteriorated variants from this duplication strain showed that, for most cases, each had a new mutation which segregated as a single gene mutation change. It has been proposed (Azevedo and Roper, 1970) that deterioration resulted from newly-arisen tandem duplication on either duplicate segment and that transposition of the duplicated material to non-duplication regions reduced instability and the transposed element behaved in meiosis as a single gene determinant. The results were all obtained with a single type of duplication, the I-II duplication strains of Nga and Roper (1968). The present work was carried out with duplication strains of a second type, where a segment of linkage group III is present twice, one copy being in a normal position and

the other translocated to linkage group VIII (Bainbridge and Roper, 1966). Sectors arising from these strains were analysed genetically and the results compared to those obtained from the previously analysed strains.

MATERIAL AND METHODS

(i) Media

The minimal medium (MM) was Czapek Dox with 1% (w/v) glucose. Complete medium (CM) contained yeast extract, hydrolyzed casein, hydrolyzed nucleic acids, vitamins, etc. (Pontecorvo, Roper, Hemmons, MacDonald and Bufton 1953). The solid medium contained 1.5% agar.

(ii) Methods of genetic analysis

The general techniques were those of Pontecorvo *et al.*(1953). Diploids were prepared by Roper's technique (1952). Allocation of mutant alleles, duplications and deletions to their linkage groups by mitotic haploidization (Forbes, 1959) was facilitated by the use of *p*-fluorophenylalanine (FPA) (Morpurgo, 1961; Lhoas, 1961). Incubation was at 37°C. Sectors were obtained by inoculation of conidia from the duplication strain in the centre of 9 cm diameter Peter dishes; after 7 days of incubation sectors were scored, isolated and purified.

(iii) Strains

Strains of *Aspergillus nidulans*, all derived from Glasgow stocks, were kept at 5° C on CM slopes. Master strain E (MSE) carrying markers on all eight linkage groups was that of McCully and Forbes (1965) (Fig. 1.a). Following Clutterbuck's (1970) proposal, mutant alleles were designated as follows: (the chromosome on which markers are located is given in brackets)

wA$_3$ (II), *yA*$_2$ (I), white and yellow conidia respectively; *adE*$_{20}$ (I), *biA*$_1$ (I), *nicB*$_8$ (VII), *pyroA*$_4$ (IV), *riboB*$_2$ (VIII), *sB*$_3$ (VI) and *sC*$_{12}$ (III) requirements respectively for adenine, biotin, nicotinic acid, pyridoxine, riboflavin and thiosulphate; *galA*$_1$ (III) and *facA303* (VI), inability to grow on media containing galactose and acetate respectively as the sole carbon source; *suA*$_1$*adE*$_{20}$ (I), suppressor of *adE*$_{20}$; *v*$_9$ (III) determinant of deterioration, obtained from strain with I-II duplication (Azevedo and Roper, 1970). Two duplication strains, both with a segment of linkage group III present twice, one segment in the normal position and the other translocated to linkage group VIII, were used (Fig. 1b). These strains, designated C and D, were obtained as meiotic recombinants from a cross between a III-VIII duplication strain (*biA*$_1$; *wA*$_3$; *pyroB*$_{12}$) with a non duplication strain carrying the *v*$_9$ determinant (*biA*$_1$; *sC*$_{12}$; *v*$_9$). The genetic constitution of strains C and D is shown in Fig. 1b.

(iv) Symbols for variants and diploids

Variants from strain C were labelled C$_1$, C$_2$, etc. Second and later order variants, arising during vegetative growth, were designated so as to show their lineage. This is made clear in Fig. 2. Each subsequent determinant was designated as the variant but with lower case *c*. Haploid components of diploids are separated by the symbol //.

RESULTS

(i) Genetic analysis of strains C and D

Mitotic haploidization of diploids C//MSE and D//MSE (Table I) has shown that both retained the III-VIII duplication, since there was strong selection against ribo$^+$ sectors and it is known (Azevedo and Roper 1970) that sectors with a duplication are selected against on FPA medium. Besides the III-VIII duplication they contained no other translocations since the other linkage groups segregated independently. The presence of gal$^+$ segregants

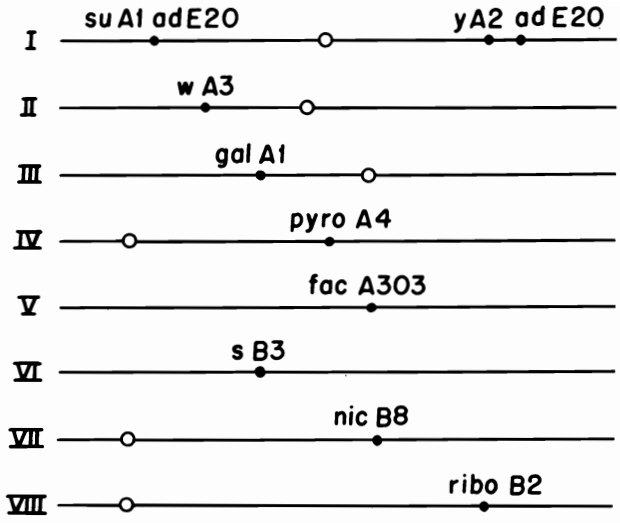

Fig. 1a

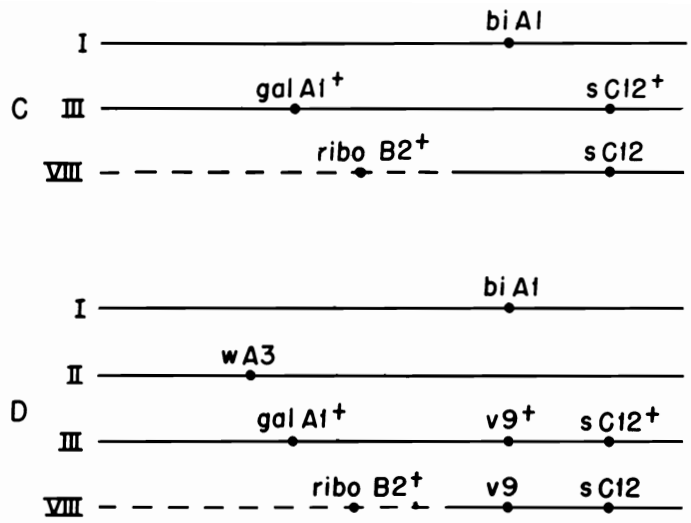

Fig. 1b

Figure 1 - Genotypes of the strains used. *1a)* Master strain. MSE. The centromeres are designated by open circles. Map position are not to scale. *1b)* Duplication strains C and D. Linkage groups I, II and III are shown by unbroken lines; linkage group VIII is shown by broken lines.

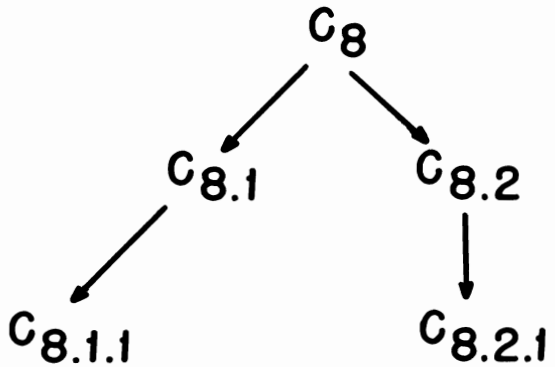

Figure 2 - Lineage of mototic derivatives of C_8.

which have normal morphology and are s^+ indicated that sC_{12} and v_9 markers in strain D were on the translocated III segment and that sC_{12} is also on the translocated segment in strain C. sC_{12} and v_9 were detected, by nutritional and visual means, respectively, in sectors which arise spontaneously from both strains.

(ii) Incidence and phenotype of deteriorated variants.

Spontaneous sectors were detected from strains C and D during growth on plates of CM. However, in contrast to the I-II duplication strains, the distinction between improved and deteriorated sectors was not very clear in strains C and D. Improved sectors were usually detected easily by their improved growth rate, but, in some cases, classification of these was equivocal. In the present work the only sectors classified as deteriorated were those with abnormal, mainly brown, mycelial pigmentation and/or poor con-

Table I – Haploid segregants from diploids MSE//C and MSE//D.

Linkage groups	Marker	Diploid* MSE//C	Diploid* MSE//D
I	bi^+	20***	20
	bi	6	6
II	w	10***	0**
	w	16	26
III	gal^+	6***	7
	gal	20	19
IV	$pyro^+$	7	13
	pyro	19	13
V	ac^+	13	15
	ac	13	11
VI	s^+	10***	8
	s	16	18
VII	nic^+	11	12
	nic	15	14
VIII	$ribo^+$	0	1
	ribo	26	25
	Total	26	26

* The number of independently arisen haploid sectors from the diploids is shown; all sectors were of normal morphology (v_9 not present).

** both MSE and D strains carried the wA_3 marker and were therefore white; so only white sectors were obtained.

*** Although number of segregants for linkage groups I and III (20 and 6) and for linkage groups II and VI (10 and 16) is the same, all parental and recombinant types were found; so, they segregated independently.

idiation. Some sectors from strain D had a deteriorated phenotype but an improved growth rate; this was expected since the loss of part of linkage group III containing the v_9^+ allele, should result in v_9 deteriorated sectors with improved growth rate and these were included in the total count. The number of sectors produced by strains C and D is shown in Table II. D appears to be more unstable than C.

Table II – Number of sectors from strains C and D.

Strain	Colonies analysed	Sectors	Sectors/colony
C	25	44	1.6
D	25	118	4.7

Table III – Mitotic and meiotic segregation of deteriorated phenotype.

Variant	Linkage group involved as determined by haploidization	Meiotic segregation Non deter. : Deter.
C_3	III	534 : 579
C_7	II	202 : 255
C_8	I	60 : 48

(iii) Genetic analysis of variants from strain C

Several sectors with a clear deteriorated phenotype were isolated from strains C and D. These had a distinct morphology,growth rate, conidiation and different degrees of instability, some being more stable than the original strains from which they were derived, and others as unstable as the parental strain or even more unstable. Derivative sectors from some of the deteriorated variants were also isolated. Complete mitotic and meiotic analysis of these variants and their derivatives was not always possible due to the high instability of the variants, or to failure to produce heterokaryons, diploids or hybrid cleistothecia. Also, in a few cases, although diploids were obtained they could not be analysed as they failed to produce haploids. In none of these cases were attempts made to pursue genetic analysis by other crossings. From the deteriorated sectors analysed only key examples will be shown (Table III).

C_3 , like several other similar sectors, was a very unstable deteriorated sector of C; it required thiosulphate and this suggested a deletion which included the locus sC_{12}^+ from the translocated, duplicate segment. Of 18 haploid segregants from diploid C_3//MSE, 4 were deteriorated and 14 were normal. The 4 deteriorated sectors were gal$^+$ (III) ribo$^+$ (VIII). Of the 14 normal (nondeteriorated) sectors, 12 were gal$^-$ribo$^-$and 2 were gal$^-$ribo$^+$; no gal$^+$ ribo$^-$ sectors were obtained (Table IV); this places the determinant of the deterioration c_3 in linkage group III associated with a deletion in the same linkage group. The shortage of types carrying the translocated III segment (the ribo$^+$ haploids) is expected since they are a disadvantage on FPA medium used in haploidization. c_3 behaved in segregation as a single gene and it recombined freely with $galA_1$ in linkage group III.

C_7 was also a deteriorated sector from strain C. Forty-five haploid segregants were obtained from diploid C_7//MSE, 25 being non-deteriorated and white, and 20 deteriorated and non-white. The cross C x MSE gave a 1:1 segregation of deteriorated to non-deteriorated, and the determinant of deterioration c_7 was located in linkage group II, 4.7 units from the wA_3 gene. All other markers segregated as expected. In contrast with other diploids involving the III-VIII duplication, however, a large number of haploid ribo$^+$ sectors were obtained from the diploid C_7//MSE and only few sectors were ribo$^-$. This could suggest a mitotic crossing-over in this strain, $riboB_2$

being now in the chromosome VIII carrying the translocated III segment (Table IV).

Table IV – Mitotic analysis: haploid segregants from diploids MSE//C_3, MSE//C_7 and MSE//C_8.

Diploid	Linkage group *	Marker	Deteriorated	non-deteriorated
MSE//C_3	III - VIII	gal^+ $ribo^+$	4	0
		gal^+ $ribo^-$	0	0
		gal^- $ribo^+$	0	2
		gal^- $ribo^-$	0	12
MSE//C_7	II	w^+	20	0
		w^-	0	25
	VIII	$ribo^+$	9	14
		$ribo^-$	2	1
MSE//C_8	II	bi^+	0	35
		bi	0	0
	IV	$pyro^+$	0	35
		pyro	0	0
	VIII	$ribo^+$	0	0
		ribo	0	35

* only relevant linkage groups are shown. Linkage groups not shown segregated as expected.

C_8, another type of deteriorated sector, was very unstable. All 35 haploid segregants obtained from diploid C_8//MSE were non-deteriorated and bi^+. In the cross C_8 x MSE a 1:1 ratio of deteriorated: non-deteriorated types was found. The determinant of deterioration *(c_8)* was in linkagegroupI, 20.0 units from biA_1. No $pyro^-$ sectors were obtained from diploid C_8//MSE. This suggests that the diploid carried a recessive lethal on chromosome IV of MSE or the diploid C_8//MSE could also have become homozygous for $pyro^+$, due to mitotic crossing-over (Table IV); conclusive evidence for a lethal or mitotic crossing-over was not sought. As expected, there was also a shortage of $ribo^+$ sectors due to duplication.

(iv) Mitotic variants from deteriorated sectors

Deteriorated variants produce second order sectors of different, mainly improved morphology. This also occurs in deteriorated variants from the I-II duplication strain (Azevedo and Roper, 1970). Second order derivatives from C_8 were analysed in detail (Fig. 2). In all cases derivatives of C_8 maintained the determinant of deterioration of C_8 (designated c_8) in linkage group I. $C_{8.1}$ was a thiosulphate-requiring variant of C_8 but mitotic analysis failed to detect any deletion in linkage group III. Only few haploid sectors were obtained, from $C_{8.1}$//MSE; both $ribo^+$ and $ribo^-$ haploid segregants were found, which suggests that linkage group VIII in $C_{8.1}$ no longer contained the duplication. The results are explained by a mitotic crossing-over between the duplicate segments in strain C_8, resulting in homozygosity for the sC_{12} allele in linkage group III. A deletion in the translocated segment may then have given rise to the variant $C_{8.1}$

An analysis was carried out of $C_{8.1.1}$ which was a deteriorated variant from $C_{8.1}$. Haploid segregants from $C_{8.1.1}$//MSE were all gal^- and non-deteriorated. In this case a second determinant of deterioration, probably associated to a deletion ($c_{8.1.1}$) was located in linkage group III. From meiotic analysis it appears that it is linked to the *galA* gene (34 units). Segregation of 128 deteriorated : 48 non-deteriorated colonies was consistent with the presence of two determinants of deterioration in this derivative. The results can be explained by a mitotic crossing-over (as occurred in C_3) and a further deletion in linkage group III which gives the deteriorated phenotype.

In this case a segregation of 2 deteriorated: 1 normal would be expected. Also, no nic$^-$ segregants were found among 16 haploids from diploid $C_{8.1.1}$// MSE, suggesting an apparent recessive lethal in linkage group VII or homozygosity for nic$^+$ due to mitotic crossing-over. $C_{8.2}$ was also a thiosulfate-requiring derivative of C_8. The diploid $C_{8.2}$//MSE gave gal$^-$ and gal$^+$ segregants, and ribo$^-$ and ribo$^+$ segregants, which could be explained in the same way as with $C_{8.1}$ (homozygosity for sC_{12}) with loss of almost all duplication in linkage group VIII since $C_{8.2}$ was more stable than C_8 and gave a 1:1 segregation of deteriorated: non-deteriorated types, as did C_8.

$C_{8.2.1}$ was an improved and stable variant of $C_{8.2}$ and probably lost all of the duplicated translocated segment.

DISCUSSION

The two III-VIII duplication strains used in the present work have features in common with the I-II duplication strain studied by Nga and Roper (1968), although there are also differences. First, as expected and already show (Bainbridge and Roper 1966), both strains C and D produce sectors during vegetative growth, mostly due to the loss of one or another duplication segment. However, the distinction between improved and deteriorated sectors in the III-VIII duplication strains is far from clear. Counts are open to subjective errors but it is evident that deteriorated sectors are very frequent. This is in contrast with the I-II duplication strains which show only rare deteriorated sectors and many improved ones. Also the overall number of sectors is greater in the III-VIII duplication strains than in the I-II duplication strains (Azevedo, 1975). These differences can be explained in the light of the results of Birkett and Roper (1977) who proposed that in the III-VIII duplication strains, deletions in the duplication segment can produce deteriorated phenotypes, a feature also found in the present work. Strain D is more unstable than strain C (Table II). Since both are segregants from the same cross, although they carry different genetic markers, it seems that it is the presence of v_9 in D which is causing this high instability. If v_9 were a transposed segment of the I-II duplication as hypothesised by Azevedo and Roper (1970), strain D would contain a chromosomal element, presumably a small segment

(v_9) in addition to the III-VIII aberration. It is unlikely that v_9 acts on the III-VIII duplication causing high instability. The extra number of sectors, mainly deteriorated ones, in strain D can be attributed to mitotic crossing--over which results in homozygosity for v_9. However, the present analysis does not exclude an effect of v_9 on the III-VIII duplication; the authors intend to carry out a systematic study of the effects of various determinants of deterioration derived from the I-II duplication strain on strains with other duplications.

When deteriorated variants from strains with the I-II duplication are compared to those variants with some phenotypic similarity from strains with the III-VIII duplication, differences become apparent. In the former strains the determinants of deterioration are found mainly to be located in linkage groups not involved in the initial duplication; in only two cases out of more than 50 studied, was deterioration due to deletions in the duplication segment (Azevedo and Roper, 1970; Azevedo, 1971). In the latter, deletion seems to be more frequently associated to deterioration as in two variants (C_3 and $C_{8.1.1}$) out of seven here presented. Birkett and Roper (1977), in a study of deteriorated sectors from a III-VIII duplication strain, suggested that in the principal genotypes isolated from a single deteriorated sector, the initial event was a deletion leaving a "sticky" locus which provoked mitotic crossing-over, and giving several types of deteriorated phenotypes. In two deteriorated variants analysed in the present work, determinants of deterioration were found to be located in linkage groups not involved in the original duplication. Following a hypothesis put forward by Azevedo and Roper (1970) these deteriorated sectors, obtained from the III-VIII duplication strain (C_7 and C_8) can tentatively be explained by transposition of tandem duplications to other regions of the genome. In our work, however, no genetic changes which might have been described as original tandem duplication were detected; this may have been because they are very unstable. In fact very unstable derivatives of strains C and D were obtained but, due to their high instability, they were not amenable to genetic analysis.

Another characteristic of the deteriorated variants from the III-VIII duplication strains may be the high rate of mitotic crossing-over between the duplicated segments as evidenced by the presence of sC_{12} on chromosome III (in C_3 and derivatives of C_8) and by the virtual absence of ribo$^-$sectors in a diploid with the C_7 variant. This is possibly due to the association of mitotic

crossing-over and the origin or the presence of deterioration as suggested by Birkett and Roper (1977). Some of the diploids composed of a normal and a duplication strain, failed to haploidize. This too can be explained by mitotic crossing-over in linkage group VIII of the diploid, leading to homozygosity for the duplication of linkage group VIII. Such a diploid would produce few or no sectors on CM plus FPA because of selection against duplication haploids. Recessive lethals were frequently found in diploids containing deteriorated variants derived from the I-II duplication strains (Azevedo and Roper, 1970). In the present case there were also indications of recessive lethals or of an abnormally high frequency of mitotic crossing-over throughout the genome in the diploid leading to homozygosity of markers. This latter possibility is open to further investigation; however, if it occurred the III-VIII duplication would cause also an increase in the overall rate of mitotic crossing-over in diploids.

Instability is a common genetic feature. Similarities between cases of instability have been pointed out already at the observation level and perhaps also at the mechanism level when such cases are compared in *Aspergillus*, maize, man, Drosophila and bacteria (Nga and Roper, 1968; Roper and Nga, 1969; Azevedo and Roper, 1970; Menezes and Azevedo, 1978). However, differences with regard to instability are found not only between species of fungi like *Aspergillus* and *Neurospora* (Perkins, 1972; Turner, 1977; Newmeyer and Galeazzi, 1977) but also between two duplication strains of *Aspergillus nidulans*. This could reflect the complexity of the phenomenon as suggested by Birkett and Roper (1977). In *A. nidulans* additional duplication strains are available (Birkett and Roper, 1977; Almeida, 1976). A study of these other duplication strains will probably help clarify the process of genetic instability.

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CLIMATIC DETERMINANTS OF THE DISTRIBUTION AND ABUNDANCE OF *DROSOPHILA SUBOBSCURA* AND OTHER SPECIES IN ISRAEL[1]

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ABSTRACT

Species of *Drosophila* were collected from 26 sites distributed throughout the four biogeographic regions of Israel during March to June 1976 and 1977. The relative frequencies of these species were calculated and analyzed in relation to various ecological parameters. Abundance and distribution patterns vary spatially and temporally with general climatic conditions, especially temperature and humidity, and with food requirements. No competition was noted, though a temporal succession pattern does exist. *D. subobscura* was found to prefer cooler, more humid weather thus reaching its highest abundance in early spring and autum. Drosophilids of the *simulans* group *(D. simulans* and *D. melanogaster)* and *D. hydei* prefer warmer weather and replace *D. subobscura* in the late spring and summer. Significant deviations from the expected sex ratios were found in *D. subobscura* and *D. hydei,* and a possible explanation based on sexual differences in food and habitat preferences is discussed. An alternative explanation concerning female diapause in *D. subobscura* is suggested.

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INTRODUCTION

Most species have a complex population structure characterized by series of clinal, peripheral, marginal and isolated populations. (Mayr, 1970). The area inhabited by a species is a spatio-temporal mosaic of favorable habitats. As the frequency in space and time of favorable habitats is reduced, there finally comes a point at which the species is unable to maintain a population, and the species border is reached. This border fluctuates dynamically with the environment (Lewontin, 1974). The species border is one of the most interesting phenomena of evolution and ecology, yet as a scientific problem it has been almost totally ignored (Mayr, 1970). Border populations are not only geographically peripheral but are also ecologically marginal and may be genetically unique. The border region is characterized by a never-ending race between reproductive capacity and mortality due to adverse conditions. Are peripherally isolated populations centers of evolutionary novelties due to the founder effect as Mayr (1970) believes, or, alternatively, is their chance of producing evolutionary novelties nil? This problem is still unresolved due to our ignorance as to the genetic structure and differentiation of marginal border populations. Only detailed quantitative ecological, genetic, behavioral, and developmental analysis of marginal border populations, as compared and contrasted with central populations, may resolve the problem.

Local and seasonal variations are of primary importance in species diversity as shown by Dobzhansky and Pavan (1950), Cooper and Dobzhansky (1965), Birch (1960), and Shorrocks (1975), among others.

The palaearctic *Drosophila subobscura* was chosen for this study for several reasons. First, it ranks among the most polymorphic members of the genus and has been analyzed for inversion polymorphism in both central European, and marginal Israeli populations (Goldschmidt 1956, 1958, Stumm-Zollinger and Goldschmidt 1959, and their references). Second, at present, renewed combined efforts are being made by several European *Drosophila* geneticists to integrate an overall study of this species throughout its range. The renewed studies involve both chromosomal and genic heterozygosities in populations of Portugal, Spain, England, France, Germany, Austria, Switzerland, Italy, Greece and Tunis in North Africa, in an attempt to evaluate the genetic structure and spatial differentiation of the species. A complementary study of marginal populations along the southern border of the

species in the Middle East will be a substantial contribution to the understanding of the overall patterns of the species across its range. Third, we expect not only to study population differentiation but also to investigate potential centers of species formation along the margins of its distribution.

D. subobscura inhabits Europe from Spain to Scandinavia in the north, reaching Lebanon and Israel in the south. It may well extend into Northern Asia (Buzzati-Traverso and Scossiroli, 1955). The activity of this fly in Israel is much more limited than in France or Switzerland, suggesting marginal conditions, (Goldschmidt 1958). An extensive study with the aim at analyzing the actual and potential populations to be found for a variety of variables involving allelic, chromosomal, behavioral and developmental parameters is being pursued in an attempt to derive an evolutionary model of marginal and peripherally isolated populations as potential centers of evolutionary novelties.

The purpose of this paper is to explore the climatic determinants affecting the distribution and abundance of *D. subobscura* in Israel and to compare it with other species found in artificial baits in four different bioclimatic zones in Israel.

This is the first paper in a series dealing with the evolutionary genetics of *D. subobscura* in Israel and it discusses the climatic determinants of population dynamics.

I. *The ecological background*

Drosophila subobscura has a typical Mediterranean pattern of distribution in Israel. European species with their southern distribution limit in Israel penetrate chiefly the more humid northern regions of the country but do not succeed in colonizing the hot and dry southern deserts. This is typically the geographical pattern of distribution of *D. subobscura.*

It is therefore appropriate to briefly describe here the biogeographical and climatic characteristics of Israel and of the regions where the species were collected so as to get an insight into the ecological factors which expand or limit their distribution and mainly the distribution of the "southern marginal" *D. subobscura* species.

Climate – Thornthwaite (1948) classified climates according to moisture and thermal efficiency balance. Based on moisture index there are

four separate climatic regions in Israel, humid, subhumid, semiarid and arid. Thermally, four regions were recognized: a megathermal region and three mesothermal ones. The species described in this study inhabit primarily the humid as well as the first 3 mesothermal regions, avoiding the megathermal, or the high temperature one (see Atlas of Israel, 1970 IV/3). Following are brief descriptions of temperature and rainfall.

Temperature – Several factors determine temperature trends in Israel. First, regionally, temperature increases southwards. This factor does not affect the coastal region because of the sea reverse effect which during most of the year is subject to a reverse temperature pattern: along the coast of Israel the sea is warmer in the north than in the south. Contrary to the lack of temperature differences along the Mediterranean Coast, there is a distinct north-south rise in temperature along the mountainous ridge and the Jordan-Arava depression. Second, in Israel, proximity to the sea causes a decrease in the mean temperature gradient. Third, as elsewhere, increase in altitude brings about a decrease in temperature. Hence, along the ascent from the Mediterranean coast to the mountain ridge, temperature first decreases, and then increases along the descent towards the Jordan-Arava depression (relevant temperature maps in the Atlas of Israel, 1970; IV/1 A-H).

Rainfall – In Israel, the mean annual rainfall (see rainfall in the Atlas of Israel, 1970; IV/2), displays the following patterns: a) Rainfall decreases southwards, from the more humid north to the more arid south; b) Rainfall decreases eastwards from the Mediterranean Sea inland; c) Rainfall increases with elevation above sea level, thus showing a rainfall minimum over the Dead Sea (400m. below sea level) and a maximum on Mount Meron in the Upper Galilee mountains (1200m. above sea level); d) Rainfall depends upon location: slopes exposed to rain bearing winds generally receive larger amounts of rain than those downwind.

Vegetation of Israel – The Mediterranean vegetation territory includes the Galilee Mountains, the northern part of the Rift Valley, the Plain of Yisreel and those of the western slopes of the mountains of Samaria and Judea (see Atlas of Israel, 1970; Zohary 1973). The mean yearly minimum rainfall in this area is around 350mm. and the dominant soil types

are *terra rossa* and *redzina.* Maquis and forests form the climax vegetation over most of the region. Due to deforestation by man, the arboreal climax vegetation has largely been destroyed or partly reduced to dwarf shrub formations classified under *garigue* and *batha.* These involve the following plant formations: *Poteretum spinosae, Cistetum villosae, C. salvifoliae, Calycotemetum villosae* and others. The *Retametea* class inhabits the Mediterranean Coastal belt and comprises a çomplex of plant communities growing on sands, sandy-clay plains and consolidated sand stone formations. In the Mediterranean region of Israel, the *Quercetae* class comprises the zonal communities. Most important are those forming the maquis and the forests. The dominant plant community in the Mediterranean hills and the mountains is that of *Quercus calliprinos* and *Pistacia palestina* which includes evergreen and deciduous trees and shrubs. Along the foothills the predominant group of communities is that of *Ceratonia siliqua* and *Pistacia lentiscus.* The Aleppo pine forests *(Pinus haleppensis)* which once covered vast areas is now found only in scattered remnants. It is in the Mediterranean region that *D. subobscura* chiefly occurs in Israel.

II. *Material and Methods*

Traps were prepared with 250cc. milk bottles filled with 2 to 3 cm of malted barley bait prepared according to Lakovaara et al. (1969). The open bottles were hung on pine or citrus trees at about 60-100cm above the ground, and, when possible, examined twice daily, during the early morning and late afternoon when *Drosophila* activity is at its highest. Care was taken when replacing traps to set them at exactly the same points so that the relative frequencies of the species could not be ascribed to local variation. Samples were collected almost daily from early March to late May during the two consecutive years of 1976 and 1977. Trapping was discontinued during the hot summer months from the third week of June until late September which were the least successful months for *Drosophila* collecting in Israel, although several unsuccessful trials were made during this period in the Carmel Mountains.

Temperature and humidity readings were taken on the night before the traps were set and when the traps were collected. Measures were extracted from the Israel Meterological Service archives, Beit Dagan, Israel.

Twenty-four collection sites were subdivided according to the four biogeographic regions, Coastal Plain, Foothills, Mountains, and Rift Valley. Table I lists the sites, their classification according to region, latitude, longitude, and altitude, and total number of sites per region, and Table II lists the number of sites, samples and flies collected from March to mid June 1976 and 1977 in the four biogeographic regions. For technical reasons, Huqoq, Rishon Le Zion, Petah Tikva, Ein Carmel, and Tamra were not used as sites in 1977, while Kyriat Shemona, Hazor, Rosh Pina, Ginnossar, Ein Feshkha-Jericho, Nueima, Ein Gedi, Yad Mordechai, Ashkelon, Dor, Maayan Zvi, and Beer Sheva were added.

RESULTS

A total of 4006 and 5888 individuals were collected from March until the end of June in 1976 and 1977, respectively. Since, except in the pine forest near the University of Haifa, trapping was sporadic during June, the number of flies during this month is not recorded in Tables III and IV. The species that were attracted to the baits were *D. subobscura* Collin, *D. melanogaster* Meigen, *D. simulans* Sturtevant, *D. hydei* Sturtevant, *D. busckii* Coquillet, and *D. immigrans* Sturtevant. Since both *D. melanogaster* and *D. simulans* appear in Israel and are indistinguishable except for a slight difference in the male external genitalia, they were subsequently scored and analyzed together under the heading *simulans* group.

The general pattern of distribution and abundance may be described as follows:

1. *Coastal Plain: D. subobscura* is the most common species in early Spring. Its abundance is greatly reduced as the hot weather sets in and is almost completely replaced by the *simulans* group and, to a lesser degree, by *D. immigrans* by *summer.*

2. *Foothills: D. subobscura* is found in early spring, but is nearly or completely replaced by the *simulans* group and by *D. hydei* in April and May *D. immigrans* is rare or non-existent in this region.

Table I: Climatic regions of 26 collecting sites in which *Drosophila* was found. Their geographic coordinates and altitude are given in meters.

Climatic Regions	Collecting sites	Latitude	Longitude	Altitude	Sampling (years)	TEMPERATURE Annual	Coldest (Jan)	Hottest (Aug)
	Yad Mordehai	31°35'	34°33'	50	1977	21	15	25
	Ashkelon	31°40'	34°35'	50	1977	"	"	"
	Rishon LeZion	31°58'	34°48'	50	1976	20	13	25
	Petah Tikva	32°05'	34°53'	50	1976	"	"	"
Coastal Plain	Hertzlia	32°10'	34°51'	50	1976, 1977	21	14	26
	Dor	32°36'	34°55'	10	1976, 1977	"	"	"
	Maayan Zvi	32°34'	34°56'	25	1977	"	"	"
	Ein Carmel	32°40'	34°57'	20	1976	"	"	"
	Naharia	33°00'	35°05'	10	1976, 1977	20	13	25
	Maayan Zvi	32°34'	34°56'	100	1976, 1977	21	14	26
Foothills	Tamra	32°51'	35°12'	150	1976	—	—	—
	Tivon (Oranim)	32°43'	35°07'	75	1977	20	14	26
	Beer Sheba	31°14'	34°47'	275	1977	21	13	28
	Kiriat Anavim	31°49'	35°07'	700	1976, 1977	21	13	29
Mountains	Mevàsseret	31°48'	35°10'	600		"	"	"
	Carmel (U.H.)	32°46'	35°01'	480	1976, 1977	21	11	26
	Biria (Zfat)	32°59'	35°30'	850	1976, 1977	17	7	25
	Kiriat Shmonah	33°13'	35°35'	175	1977	21	12	29
	Hazor	32°59'	35°33'	300	1977	19	8	24
	Rosh Pina	32°58'	35°32'	400	1977	"	"	"
	Ginossar	32°51'	35°31'	200	1977	24	15	32
Rift Valley	Hookuk	32°53'	35°30'	0	1976	—	—	—
	Ein Feshcha	31°43'	35°27'	−375	1977	24	16	31
	Jerico	31°51'	35°27'	−260	1977	25	15	33
	Nueima (near Jerico)	31°53'	35°27'	−200	1977	"	"	"
	Ein Gedi	31°27'	35°23'	−350	1977	24	16	31

3. *Mountains: D. subobscura* is by far the most common species throughout the trapping season. The *simulans* group and *D. hydei* were very rare, and *D. busckii* and *D. immigrans* nearly or totally non-existent.

Table II – Number of sites, samples, and individuals collected in 4 biogeographic regions of Israel in which *Drosophila* was trapped from March to mid-June 1976 and from March to mid-June 1977.

Biogeographic regions	Nº of sites		Nº of samples		Nº of flies collected	
	1976	1977	1976	1977	1976	1977
Coastal Plain	6	6	30	18	1095	3184
Foothills	3	2	19	9	1337	322
Mountains	3	4	23	11	1044	1388
Rift Valley	1	7	4	11	530	994
TOTAL	13	19	76	49	4006	5888

4. *Rift Valley:* The *simulans* group are the most common species throughout the season. *D. subobscura* is found in smaller numbers. *D. hydei* is also found to be abundant in this area.

Traps collected from the Coastal Plain, Foothills, and Rift Valley during the month of June revealed almost only flies of the *simulans* group and a few individual specimens of *D. immigrans.* The relative frequencies of each species during March, April, May 1976 and 1977 in the four biogeographic regions is shown in Tables III and IV.

In general, the abundance of the species collected in our traps changed greatly in time, and according to the biogeographical regions. The most pronounced change was exhibited by *D. subobscura,* decreasing in numbers with increasing temperature. In addition, it is worthy of note that several fluxes were observed in the number of *D. subobscura* trapped on particular days at different locations: March 28, 1976 at Dor (Coastal Plain) with maximum-minimum temperatures of 26.4°C and 12.5°C, respectively, and

humidity reaching 99% on the previous day, the total number of individuals was 70 ♀♀ and 24 ♂♂. On March 29 and 30, 1976, at the pine forest collecting site near the University of Haifa (Mountains) with maximum-minimum temperatures of 23.0°C and 11.0°C, respectively, and the humidity reaching 91% on the previous day, the total number of individuals was 39 ♀♀ and 53 ♂♂, and 38 ♀♀and 45 ♂♂, respectively. On April 22, 1976, at the collecting site near the University of Haifa, with maximum-minimum temperatures of 21.0°C and 13.2°C and humidity reaching 91% (64% on the previous day), the total number of individuals was 72 ♀♀ and 100 ♂♂. At the pine forest site near Biriah (Mountains), with a maximum temperature of 17.2°C and a minimum of 9.2°C, and humidity reaching 88% (63% on the previous day), the total number of individuals was 79 ♀♀ and 166 ♂♂.

Significant differences from the 1:1 sex ratio were found in the collected populations of *D. subobscura* and in a lesser degree in *D. hydei.* In contrast to Shorrocks (1975), the preponderance of males was a constant trait of both these species throughout the four biogeographical regions during the collecting season in 1976. This trend continued with *D. subobscura* in 1977, while no trend could be observed in that year with *D. hydei* because of the small sample size. (Refer to Tables III and IV).

Old *D. subobscura* females with shrunken abdomens that were collected in late spring were dissected after being kept for a few days on fresh culture medium. Their spermathecae were examined for sperm and were found to be empty.

DISCUSSION AND CONCLUSIONS

The data clearly indicate that the five *Drosophila* species studied herein have different realized niches varying in space and time apparently in accordance with climatic factors. Although *D. subobscura,* the *simulans* group, and *D. hydei* were all found in three of the four biogeographic regions, their periods of greatest abundance varied and were non-overlapping. It appears that one species replaces the other according to the seasonal variations and

Table III – Changes in the relative frequencies of the different Drosophila species collected in four different geographic regions from March to May, 1976. The figures show the percentages of each species among the total number of *Drosophila* collected during a given period in each region.

Geographic region	Month	*D. subobscura*		*simulans* group		*D. Hydei*		*D. busckii*		*D. immigrans*		Total nº flies
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
Costal Plain	March	26.30	18.42	4.49	2.70	6.74	13.03	6.51	13.70	5.84	2.25	445
	April	3.90	5.74	16.90	16.38	5.91	10.98	7.09	12.33	11.82	8.95	592
	May	0.0	0.0	4.08*	6.12*	0.0	4.08*	16.32	10.20	38.77	20.41	49
Foothills	March	9.15	11.76	5.23	6.53	16.34	37.25	7.20	3.92	0.65*	1.96*	153
	April	12.66	27.07	8.73	11.35	6.98	10.50	11.35	9.17	2.18	0.43	229
	May	0.10	0.63	27.01	28.40	23.83	18.64	0.10*	0.21*	0.63	0.42*	944
Mountains	March	41.23	55.70	0.44*	0.87*	0.44*	0.87	0.0	0.0	0.0	0.44*	228
	April	28.96	61.47	1.05	0.78	3.80	3.80	0.0	0.0	0.13*	0.0	763
	May	*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*	0.0	1
Rift Valley	March	3.97	8.73	11.11	19.84	16.66	19.05	3.97	2.38*	3.17	11.11	126
	April	0.0	0.0	24.26	26.00	10.40	20.05	5.70	4.45	5.20	3.96	404
	May	-	-	-	-	-	-	-	-	-	-	-

*with four or less than four individuals.

Table IV – Changes in the relative frequencies of 5/6 different species of *Drosophila* collected in four climatic zones from March to May 1977. The figures show the percentage of each species among the total number of *Drosophila* collected during a given period in each region.

Zone and Date	*D. subobscura*		*D.simulans* / *D. melanogaster*		*D. hydei*		*D. busckii*		*D. immigrans*		Total nº of flies
Coastal Plain	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
March	6.14	42.25	9.50	10.05	0.56*	0.0	0.56*	4.50	11.17	12.29	179
April	0.40	0.81	29.69	25.23	0.27*	0.94	0.54*	1.48	17.95	29.94	741
May	0.48	0.27	41.41	34.90	2.79	0.37	0.54	2.20	9.66	7.35	1862
Foothills											
March	31.54	55.70	4.70	6.71	1.34*	0.0	0.0	0.0	0.0	0.0	149
April	0.0	0.0	11.76*	11.76*	0.0	0.0	29.41	47.06	0.0	0.0	17
May	0.0	0.0	51.66	48.33	0.0	0.0	0.0	0.0	0.0	0.0	60
Mountains											
March	42.86	57.14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35
April	32.07	43.94	8.84	14.39	0.0	0.50*	0.0	0.0	0.25*	0.0	396
May	14.03	36.50	23.50	9.47	0.0	0.0	0.0	0.0	11.0	5.6	285
Rift Valley											
March	0.17*	0.79*	47.32	46.11	0.52*	1.03	0.35*	3.80*	0.0	0.0	579
April	4.83	11.11	44.92	28.98	0.0	0.97*	1.45*	4.38*	1.45*	2.0*	207
May	-	-	-	-	-	-	-	-	-	-	-

*With four or less than four individuals

according to differences in their feeding and breeding sites. For example, *D. subobscura* may be characterized as a cooler weather species, being abundant throughout most of the year in the mountain region near the University of Haifa. However, as the temperature rises from June to mid-October, the *simulans* group replaces it. In constrast, *D. busckii* as well as *D. immigrans* seem to have narrower realized niches, being rare if not totally absent in the collection sites located in the Foothills or Mountain regions. While no direct evidence of competition was obtained, the patterns reflect that of competitive exclusion. These observations agree with Carson (1971) and Shorrocks (1975) among others, that there is generally a distinct separation between feeding and breeding sites for most species of *Drosophila.*

As a reservation it should be noted that the number of flies collected in each biogeographic region may not be exactly indicative of the population densities at each locality. First, the number of localities and the number of samples is greater in the Coastal Plain than in the other three regions (see Table II). Second, the different species may not be equally attracted to the bait used, thus affecting the relative frequencies calculated. Third, the different species may be affected differently by the insecticides commonly used in the cultivated parts of Israel in the spring.

Notwithstanding this reservation, several specific observations have been noted. In contrast to Shorrocks (1975) and Begon (1975, 1976) who found that it reaches its peak abundance in the autumn and early winter in the British woodlands, *D. subobscura* was noticed to be most abundant in Israel in the spring by Goldschmidt, (1956). Our data confirm the latter observation.

In analyzing the fluxes in number of *D. subobscura* collected, it is suggested that climatic factors may have seriously influenced the local number of flies. The higher temperatures followed by lower temperatures and higher humidity on the next day when the flies were collected, were probably the conditions necessary for the mature pupae to eclose from the puparium. Flies collected on those dates had fully extended abdomens, and were found copulating or in a resting position on the pine trees. Some still had very light chitinous bodies when brought to the laboratory for examination.

In contrast to the suggestion that *D. subobscura* females diapause (Goldschmidt, 1956, and Krimbas 1965, 1967), it is more probable that lower temperatures in the winter diminish their sexual drive as suggested by

Begon (1976). It is also possible that high summer temperatures sterilize the females. This is supported by the fact that no sperm cells were found in the spermathecae of the females collected in late spring.

As mentioned above, significant deviations from the expected 1:1 sex ratio were found in the collected populations of *D. subobscura* and less in *D. hydei.* It is not considered likely that these changes in the sex ratio reflect actual population structures in nature. Pilot laboratory crosses of wild males and wild females revealed no deviation from the expected sex ratios of the offspring. Our data indicate that males of the two species are preferentially attracted to the artificial baits. It is of interest that Cooper and Dobzhansky (1956) found the opposite phenomenon, where females of some species in the *obscura* group were the more frequent visitors to their traps. Dobzhansky and da Cunha (1955) have already noted racial variations in yeast preferences in some Brazilian *Drosophila* species. It is suggested here that adult *D. subobscura* and *D. hydei* have sexual variations in food preferences. While females are attracted by males for copulation, they do look for better habitats in order to oviposite their fertilized eggs. This hypothesis would explain the higher percentage of males trapped near habitats where no other vegetation but pine trees and dry shrubs were to be found.

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CHROMOSOME NUMBERS AND MEIOTIC BEHAVIOR OF SOUTH AMERICAN SPECIES OF THE *BRIZA* COMPLEX (GRAMINEAE)+ *

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ABSTRACT

Chromosome numbers of 20 *taxa* (18 species, including 4 varieties) of *Briza* were determined in pollen mother cells and seedling meristematic tissues. *B. macrostachya, B. brachychaete, B. calotheca, B. itatiaiae, B. jurgensii, B.* aff. *jurgensii* (possibly a new species), *B. rufa* var. *sparsipilosa* and *B. bidentata,* all with 2n = 28, had their chromosome numbers ascertained for the first time. Counts reported in the literature for other species were verified. Meiosis of 7 species and 1 variety studied was shown to be highly regular.

INTRODUCTION

The *Briza* complex (Poeae, Gramineae) presents some interesting taxonomic and evolutionary problems. These species are disjunctively dis-

+ SYSTEMATICS AND EVOLUTION OF THE *BRIZA* COMPLEX (GRAMINEAE). I.

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tributed between Eurasia (8 native species) and temperate and subtropical regions of South America (approximately 20 native species). Some authors consider all species as belonging to only one genus, *Briza*, but others subdivide them into different genera. Matthei (1975), classifies as *Briza* only the Eurasian species and divides the South American ones into three different genera: *Calotheca*, *Chascolytrum* and *Poidium*.

The Eurasian species are all diploids with $2n = 14$ chromosomes, with the exception of *B. minor* ($2n = 10$) and the tetraploid varieties of *B. media* ($2n = 28$). On the other hand, all South American species examined have $2n = 28$ chromosomes. B-chromosomes have been reported for both groups of species (Matthei, 1975; Murray, 1975, 1976b).

In 4 South American species studied by Murray (1976a), meiosis was regular. Nuclear DNA content of 3 South American species was almost equivalent to that of a Eurasian diploid, *B. maxima*, and much less than that of tetraploid plants of *B. media* (Murray, 1975). Analysis of flavonoids showed some common compounds, but others were characteristic of each group of species (Williams and Murray, 1972).

There are inbreeding and outbreeding, annual and perennial Eurasian species. The South American ones appear to be all inbreeding, commonly cleistogamic and perennial (Rosengurtt *et al.*, 1970; Murray, 1974; Matthei, 1975).

The systematics and evolution of the South American species of the *Briza* complex are being studied in a joint project by members of the Genetics and Botany Departments of the Universidade Federal do Rio Grande do Sul. The approaches used are: classical and numerical taxonomy, flavonoid pattern analysis, isozyme pattern analysis, palinology, comparative ontogeny, and chromosome number, karyotype, nuclear DNA content and meiotic behavior. The cytological data were presented in a Master Degree thesis (Sampaio, 1979). The data on chromosome numbers and meiotic behavior will be presented in this paper; those on karyotypes and nuclear DNA content will be published elsewhere.

MATERIAL AND METHODS

The plants were collected at 31 different locations shown in the maps in Fig. 1, and listed below. The number of individual plants (in brack-

kets) of each species (name abbreviated according to Table I) collected at each site is also indicated. MIN = *Briza minor* and MAX = *Briza maxima.*

BRAZIL. 1. Parque Nacional do Itatiaia, Rio de Janeiro State: ITA (3 plants); 2. Serra da Rocinha, Bom Jesus, Rio Grande do Sul State (RS): CAL (1 pl.) SP. (1 pl.); 3. Estrada Bom Jesus-Vacaria, Bom Jesus, RS: MAC (1 pl.) CAL (1 pl.) RU-SPA (1 pl.); 4. Estrada Bom Jesus-Vacaria, Bom Jesus, RS: STR (1 pl.) RU-SPA (1 pl.); 5. Itaimbezinho, Cambará do Sul, RS: LAM (1 pl.) SUB (2 pl.) BRA (4 pl.) CAL (3 pl.) JUR (2 pl.) SP. (4 pl.) POA (1 pl.) UNI (2 pl.); 6. Estrada São Francisco de Paula-Cambará do Sul, Cambará do Sul, RS: LAM (1 pl.) RU-SPA (1 pl.); 7. Estrada São Francisco de Paula--Cambará do Sul, Cambará do Sul, RS: CAL (1 pl.) LAM (1 pl.) SUB (1 pl.); 8. Praia de Itapeva, Torres, RS: ERE (2 pl.) SUB (1 pl.); 9. Cidade de Porto Alegre, Porto Alegre, RS: UNI (1 pl.); 10. Sítio Winge (Restinga), Porto Alegre, RS: MIN (1 pl.) MAC (1 pl.) STR (1 pl.) POA (1 pl.) RU-SPA (1 pl.) UNI (1 pl.); 11. Belém Novo, Porto Alegre, RS: SUB (1 pl.) INT (1 pl.) RU-RU (5 pl.); 12. Itapoã, Viamão, RS: INT (1 pl.) CAL (2 pl.); 13. Estrada Porto Alegre-Pelotas, Camaquã, RS: RU-RU (1 pl.); 14. Estrada São Lourenço do Sul-Canguçu, São Lourenço do Sul, RS: MAX (1 pl.); 15. Estrada Porto Alegre-Pelotas, Pelotas, RS: MIN (1 pl.) MAC (1 pl.) LAM (1 pl.); 16. Estrada Canguçu-Piratini, Canguçú, RS: MAC (1 pl.) RU-RU (1 pl.) UNI (1 pl.); 17. Margens Arroio Piratini Menor, Piratini, RS: INT (2 pl.) BID (3 pl.); 18. Estrada Pinheiro Machado-Bagé, Pinheiro Machado, RS: LAM (1 pl.) INT (1 pl.); POA (1 pl.); 19. Estrada Bagé-Lavras do Sul, Bagé, RS: MAC (1 pl.); 20. Estrada Bagé-Aceguá, Bagé, RS: LAM (1 pl.) POA (1 pl.) RU-RU (1 pl.). 21. Estrada Dom Pedrito-Santana do Livramento, Santana, RS: SUB (1 pl.) INT (1 pl.); 22. Estrada Gramado-Canela, Gramado, RS: CAL (1 pl.); 23. Estrada Vacaria-Porto Alegre, Vacaria, RS: MAC (1 pl.) SCA (1 pl.).

URUGUAY. 24. Aceguá, Cerro Largo Department: INT (1 pl.) POA (1 pl.) UNI (1 pl.); 25. Estrada Chuí-Montevideo, Rocha: MAX (1 pl.) BRI (1 pl.); 26. Estrada Chuí-Montevideo, Maldonado: MIN (1 pl.); 27. Estrada Chuí--Montevideo, Maldonado: BRI (3 pl.) UNI (2 pl.); 28. Balneário Solis, Maldonado: ERE (1 pl.); 29. Estrada Punta del Este-Montevideo, Canelones: BRI (1 pl.); 30. Balneário Carrasco, Montevideo: ERE (2 pl.); 31. Cidade de Montevideo, Montevideo: MAX (1 pl.).

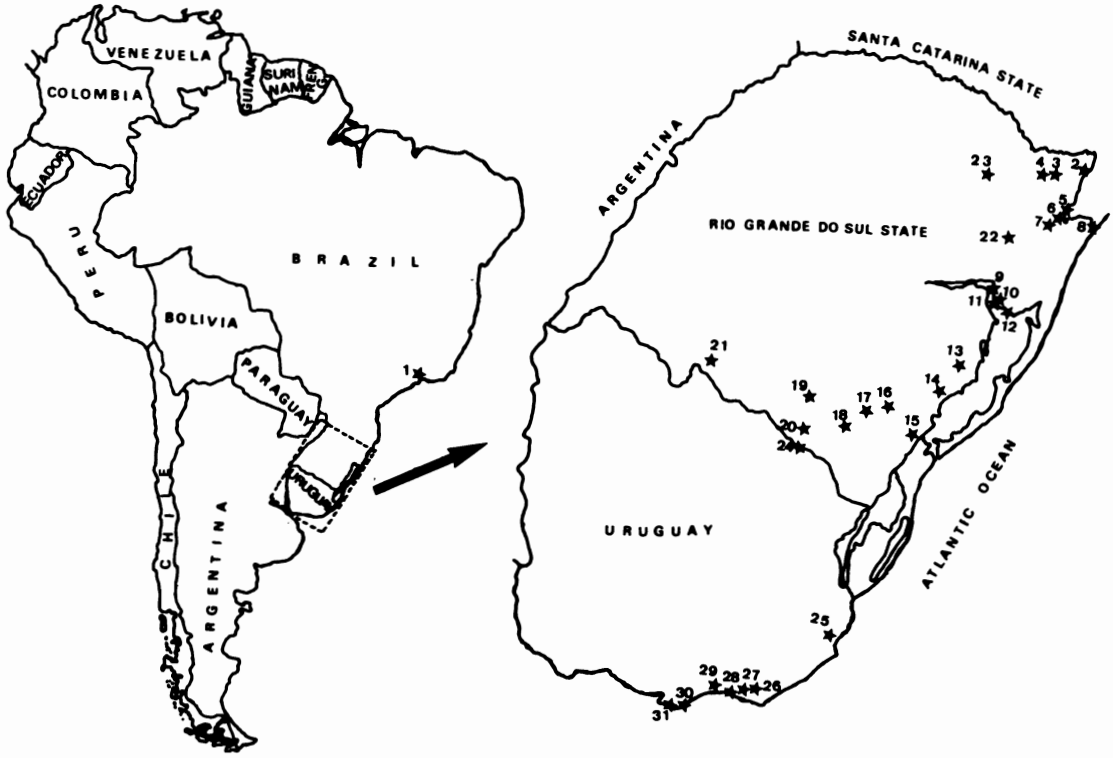

Figure 1 - Geographic origin of the analyzed plants. At right: amplified map of Southern Brazil and Uruguay. The locations are listed in the text.

Each plant was collected individually; the herborized plant, its seeds and/or very young inflorescences received the same collector's number. The herborized plants are being kept at the ICN Herbarium (Department of Botany-UFRGS) as vouchers for this work. These plants can be examined upon request.

For the study of meiosis in pollen mother cells, very young inflorescences were fixed in Newçomer fixative in the field, transferred to 70% ethanol after 24 hours, and kept in a freezer until used. Squash preparations with propionecarmine were made on gelatinized slides.

Mitotic chromosomes were studied in coleoptiles and/or root tips of very young seedlings. Seeds were washed in "Dakin solution" (0,4% sodium hypochlorite solution) for asepsis, sown on moist filter paper in petri dishes, kept at 4°C for a week and then transferred to 17°C. The seedlings were pretreated and fixed according to Murray (1975). Fixed seedlings were treated for 2 to 4 hrs. with a 5% solution of pectinase, hydrolized at 60°C in a 1N HCl solution for 12 to 15 min., and Feulgen-stained for 1 to 2 hrs. Meristematic tissue was cut off and squashed in 45% acetic acid on gelatinized slides. Photographs (Zeiss photomicroscope) and/or camera lucida drawings were used. Permanent slides were prepared using liquid nitrogen or dry ice, and Euparal as mounting media.

RESULTS AND DISCUSSION

Table I contains a review of all available data on chromosome numbers (gametic and diploid), and meiotic behavior of the South American species of *Briza,* including the data reported here. As can be seen, the chromosome numbers of *B. macrostachya, B. scabra, B. brachychaete, B.calotheca , B. itatiaiae, B. jurgensii, B.* aff. *jurgensii* (possibly a new species), *B. bidentata* and *B. rufa* var. *sparsipilosa* are being reported here for the first time.

All the South American species so far studied have 2n = 28 chromosomes. The only exception, a report by Saura (1947) for *B. rufa,* with 2n = 14, was most probably a mistake since it was not verified by any other author. The two introduced Eurasian species, *B. minor* and *B. maxima,* have 2n = 10 and 2n = 14 chromosomes, respectively, as expected.

Meiotic regularity was observed in 14 out of the 15 plants studied of *B. macrostachya, B. subaristata* var. *subaristata, B. subaristata* var. *interrupta, B. lamarckiana, B. scabra, B. rufa* var. *rufa, B. calotheca, B. uniolae* (Table I and Fig. 2). Diakinesis of three of the analyzed species is shown in Fig. 2.

The only exceptional plant, a sample of *B. calotheca,* had certainly more than $2n = 28$ chromosomes, and presented several multiple associations, suggesting a hybrid origin.

The uniformity of the chromosome number of the South American species of *Briza* does not permit any discussion of the generic subdivision suggested by Matthei (1975). Nevertheless, as will be shown in another paper, the analyses of karyotypes and nuclear DNA contents of these species do not support that suggestion (see also Sampaio, 1979).

Since natural and artificial tetraploids of the Eurasian *B. media* show some flavonoids that are characteristic of the South American species, Harborne (1977) suggested that our species evolved from *B. media* through autopolyploidy. But the meiotic regularity shown by the South American species (Table I), the nuclear DNA content (Murray, 1975) and the karyotypes of those species (Sampaio, 1979) do not support that hypothesis. However, one has to take into account that chromosome pairing, at least in polyploids, is a poor criterion on which to base phylogenetic affinities, since many examples are known of genic control of meiotic pairing (Wet and Harlan, 1972). To check Murray's hypothesis (1976a), of an allopolyploid origin of our species, much more data are needed, including those obtained with other approaches.

Table I – Review of all the available data on meiotic behavior and chromosome numbers of South American species of the *Briza* complex, including those obtained in this report.

Species	Meiotic behavior	*chromosome number* n	2n	References
B. brizoides	–	–	28	Bowden and Senn (1962)[1]
BRI	–	–	28,28+1 B	Matthei (1975)
	–	–	28	This report
B. erecta	–	–	28	Bowden and Senn (1962)[1]
ERE	–	–	28	Matthei (1975)
	–	–	28	This report
B. macrostachya	regular	14	28	This report
MAC				
B. lamarckiana	–	–	28	Bowden and Senn (1962)[1]
LAM	–	–	28	Matthei (1975)
	regular	14	28	This report
B. subaristata	–	–	28	Saura (1947)[1]
var. *Subaristata*	–	–	28	Tateoka (1962)
SUB	–	–	28	Davidse and Pohl (1971)
	–	–	28	Reeder (1971)[1]
	–	–	28	Matthei (1975)
	regular	14	28	Murray (1975, 1976a)
	regular	14	28	This report
B. stricta	–	–	28	Saura (1947)[1]
STR	–	–	28	Bowden and Senn (1962)[1]
	regular	14	28	Murray (1975, 1976a)
	–	–	28	This report*
B. subaristata	–	–	28	Bowden and Senn (1962)[1]
var. *interrupta*	regular	14	28	Murray (1975, 1976a)
(=*B. triloba*)	regular	14	28	This report
INT				

Table I (cont.)

Species	meiotic behavior	chromosome number n	2n	References
B. scabra SCA	regular	14	28	This report
B. brachychaete BRA	–	–	28	This report
B. calotheca CAL	regular (except one plant)	14	28	This report
B. itatiaiae ITA	–	–	28	This report
B. jurgensii JUR	–	–	28	This report
B. aff. *jurgensii* (sp.n.?) SP	–	–	28	This report
B. poaemorpha	–	–	28	Matthei (1975)
POA	regular	14	28	Murray (1975, 1976a)
	–	–	28	This report
B. monandra	–	–	28	Matthei (1975)
B. rufa	–	–	14	Saura (1947)(1)
	–	–	28	Matthei (1975)
B.r.var.rufa RU-RU	regular	14	28	This report
*B.r.*var.*sparsipilosa* RU-SPA	–	–	28	This report
B. uniolae	–	–	28	Bowden and Senn (1962)(1)
UNI	–	–	28	Matthei (1975)
	regular	14	28	This report
B. bidentata BID	–	–	28	This report

* *B.* cf.*stricta*

For references see:

(1)Murray, 1975

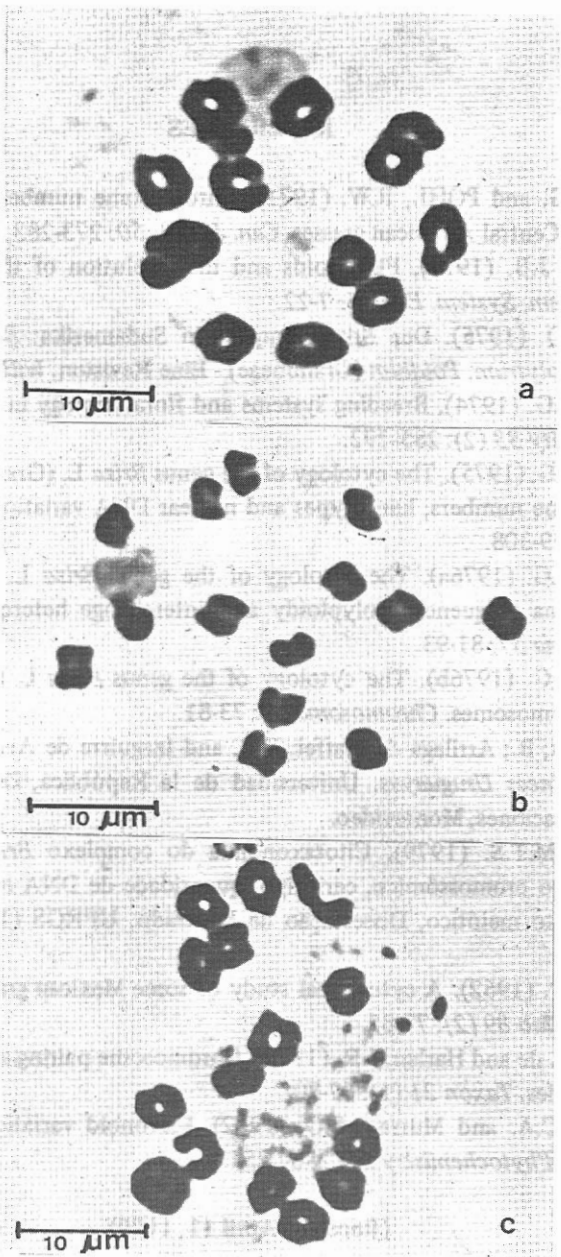

Figure 2 - Diakinesis in pollen mother cells, showing 14 bivalents a)*B. calotheca* b) *B. scabra* c) *B. lamarckiana.*

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ROBERTSONIAN TRANSLOCATION IN IMPORTED BULLS UTILIZED AT ARTIFICIAL INSEMINATION CENTERS IN BRAZIL [1]

L.E.L. Pinheiro [2], I. Ferrari [3], and R.B. Lobo [4]

ABSTRACT

Nineteen imported bulls were submitted to cytogenetic analysis at two artificial insemination centers located in the State of São Paulo, Brazil. Metaphase chromosomes, obtained by temporary cultures of peripheral blood, were treated to visualize G and C chromosome bands. Four out of eleven sires of the Marchigiana breed were carriers of 1/29 Robertsonian translocation. The remaining animals, 8 Fleckview bulls, were shown to have a 60, XY constitution. Analysis of C bands showed that the chromosome with the translocation was a monocentric, although a larger mass of constitutive centromeric chromatin was observed in some metaphases. The possible

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origin of this chromosome aberration is discussed, as well as the consequences of its dispersion throughout the Brazilian herd.

INTRODUCTION

Semen, sires and dams of the major European cattle breeds continue to be imported to Brazil to form new herds or to produce commercial crossings with the Zebu breeds. As a result, synthetic breeds have been created such as Canchim (5/8 Charoles and 3/8 Zebu), Ibage (5/8 Aberdeen Angus and 3/8 Zebu), and Pitangueiras (5/8 Red Poll and 3/8 Zebu).

Applied cytogenetics studies have shown that a large number of European cattle breeds are carriers of centric fusion (Blazak and Eldridge, 1977), with considerably variable constitutions (Eldridge and Balakrishan, 1977). Several reports (Gustavsson, 1969; Refsdal, 1976) indicate decreased fertility in females heterozygous for 1/29 translocation, a fact that can cause severe economic losses (Gustavsson, 1975). As to the breeds studied here (Marchigiana and Fleckview, or Simmental), Harvey (1972) found 2 cases of 1/29 Robertsonian translocation in 42 animals, and 4 cases in 18 animals in a later study (1974).

This report represents one of the first cytogenetic studies of sires used for large scale artificial insemination in this country.

MATERIAL AND METHODS

Nineteen bulls of the *Bos taurus taurus* subspecies were analyzed cytogenetically. Eight of these animals are imported Fleckview bulls, and the remaining 11 are sons born in Brazil of imported Marchigiana parents. The animals are stationed at two artificial insemination centers in the State of São Paulo, Brazil.

Blood (10 cc) was collected from the jugular vein with heparinized disposable syringes. Approximately 0.5 ml of plasma was incubated according to the method of Tambasco (1976), modified for routine use in the Department of Genetics of the Faculty of Medicine of Ribeirão Preto. G–banding was

Table I – Type and effect of Robertsonian translocations found in some cattle breeds.

Chromosomes Involved	Breed	Animals Studied (No.)	Affected Animals (%)	Observed Phenotypic Alteration	Authors
1/29	Norwegian Red (NRF)	430	4.20	None	Amrud, 1969
1/29	Swiss Red (SRB)	2045	14.32	Subfertility in females	Gustavsson, 1969
2/4	Friesian	1 *	–	None	Pollock and Bowman, 1974
1/29	Norwegian Red (NRF)	5 **	–	Subfertility in females	Refsdal, 1976
1/29 ***	Brown Swiss	299	2.40	None	Blazak and Eldridge, 1977

* = One-hundred-and-thirty-nine cows were inseminated with semen from the carrier bull. No lower rate of non-return was noted when compared to females inseminated with semen from a normal bull.

** = The non-return rate of 21,212 female descendants of the 5 carriers was compared to that of 610,714 normal females.

*** = Identification of the smallest chromosome involved remained uncertain.

performed by the technique of Scheres Vac (1972), also modified. C– banding was done by the method described by Popescu (1975). Eleven metaphases were analyzed for each animal in order to exclude the possibility of mosaicism (Hook, 1977), and mitoses were photographed for pairing for C and G bands.

RESULTS

The chromosome constitution of the animals analyzed here was 60, XY, with 58 acrocentric chromosomes in addition to the sex pair, formed by metacentrics. Four animals of the Marchigiana breed (out of 11) were found to have a 59, XY + t (1;29) constitution. The G–banding patterns showed perfect homology of No. 1 with the long arm of the submetacentric resulting from fusion. It was not possible to determine precisely the homology of the short arm with No. 29 since the G–band pattern of the last 5 chromosome pairs did not permit a rigorous characterization (Fig. 1). All the 8 Fleckview bulls had normal karyotypes.

In the karyotype with C bands (Fig. 2) it is possible to see that the centromeric region of the translocated chromosome shows a larger stained area than that of No. 1. The sex chromosomes are negative with respect to pericentromeric chromatin.

DISCUSSION

The pedigrees of the Marchigiana animals showed that 5 bulls were half-sibs on the paternal side. Of these, 3 were heterozygous carriers of 1/29 translocation. The fourth animal also belonged to the same paternal lineage although he was not a brother to the others. The semen of the common ancestor as well as that of his descendants analyzed here is distributed nationally and at least one of the artificial insemination centers in the country has utilized the semen of two carriers in its progeny test program.

As to the constitution of the centric fusion diagnosed in this paper, we may assume that it is 1/29, as already described by Harvey (1974) for the Marchigiana breed. A definite conclusion is not possible because both chromo-

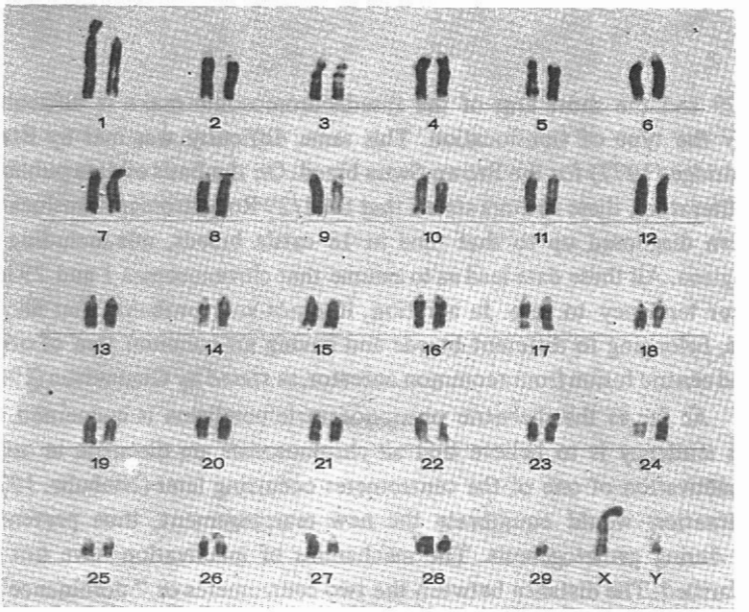

Fig. 1. Heterozygous male for 1/29 translocation. G bands.

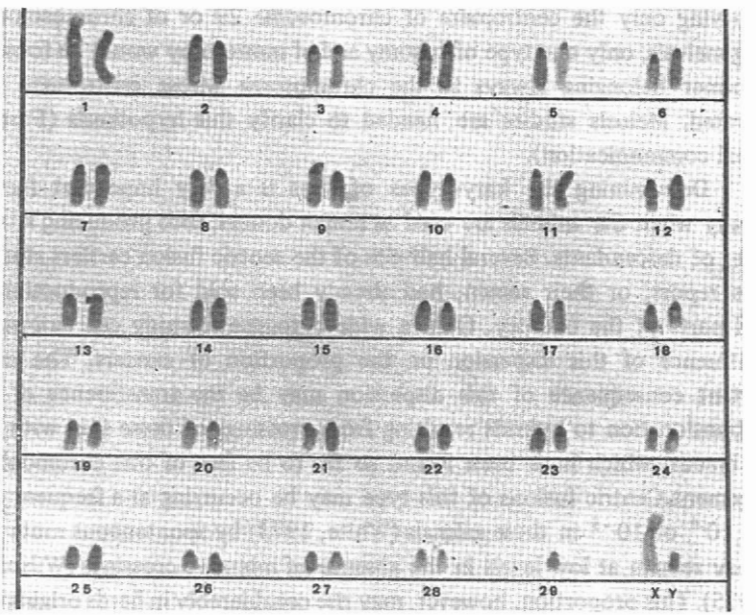

Fig. 2. Karyotype of heterozygous male for 1/29 translocation. C band

some 29 and the short arm of the fused chromosome make it difficult to identify the type of translocation. This same difficulty was met by Blazak and Eldridge (1977) for the Brown Swiss breed. On the basis of data published in the literature, these authors stated that the 1/29 Robertsonian translocation had been diagnosed up to that time in 18 cattle breeds, not including the Marchigiana. All these data lead us to assume that chromosomes 1 and 29 have a greater tendency to fuse. In addition, it is not very probable that all the carriers, belonging to different breeds and having no common tree of origin, received centric fusion from a common ancestor, as stated by Gustavsson (1969).

As far as the dicentric or monocentric condition is concerned, the present tendency is to believe that all chromosomes are dicentric in origin, with inactivation of one of the centromeres occurring later (Niebuhr, 1972). This situation would equilibrate the new rearrangement, thus preventing breaks during gametogenesis. The mechanims of inactivation have not yet been clarified. The distance between the two centromeres or " dominance" of one over the other may have an influence (Niebuhr, 1972). If one of the centromeres predominates over the other, the fused chromosome will behave as if having only the centromere of chromosome 29 or of chromosome 1. During meiosis, only one type of trisomy and of monosomy would be formed, the former belonging always to the chromosome whose centromere was inactivated. Meiosis studies are needed to clarify this hypothesis (Ferrari, personal communication).

Determining the karyotypes of sires is a very important factor, especially when the animals are used as semen donors, thus producing a large number of descendants. Several half-sibs of the centric fusion carriers studied in this report, or their semen, had already been sold for reproduction in several parts of the country. Only a wide cytogenetic study can determine the influence of this dispersion on the proportion of carriers. The most important consequence of this dispersion may be the transference of the 1/29 translocation to hybrids resulting from crossings of these sires with our Zebu breeds, which have been found so far to be free of this chromosome arrangement. Centric fusions of this type may be occurring at a frequency of about 10^{-4} or 10^{-5} in these animals (White, 1973) by spontaneous mutation and may remain at low levels in the absence of intensive crossings (Wilson et al., 1975). This proportion, however, may rise considerably in herds originating from crossings with sires which are carriers of the translocation, such as the

animals in our study. This has already occurred in *Bos taurus taurus* herds because artificial insemination, almost always utilized on a large scale basis, has created clans and harems within a reproductive system which is ideal for increased frequency of chromosome rearrangements (Wilson et al., 1974). This may be the explanation for the high levels of centric fusions detected in this subspecies.

As Wurster and Benirschke (1968) demonstrated in their work, centric fusions may have been the major chromosome rearrangement that took place in the bovoidea superfamily, drastically reducing the ancestral chromosome number (2n = 60). However, no populations with a chromosome number of 2n = 58 or less have been detected as yet, although Eldridge (1975) has found that 60 % of the English White Park breed cattle are carriers of the 1/27 centric fusion. Of these, 40.4 % were homozygotes for the rearrangement (2n = 58). Of the English breeds, the White Park one is considered to have the lowest number of individuals, which means that it has few lineages and large clans with high homozygozis.

In view of the investigations carried out by Gustavsson (1969) and Refsdal (1976) with very wide samples, demonstrating the subfertility of the carriers, and considering the possibility of reaching homozygosis for the 1/29 centric fusion or for any of those that have already been identified, with unpredictable effects, and finally considering the fact the 60, XY and 60,XX constitution still prevails in the populations where natural social living conditions are maintained for these animals, selection against centric fusion may be recommended.

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ASPECTS OF OVARY DEVELOPMENT IN *RHYNCHOSCIARA*

Renato Basile [1]

ABSTRACT

The ovary of *Rhynchosciara* was studied at the light microscope level using whole ovary squashes or sections in order to understand the functional relationship between the different cells of the follicle. The variations in the relationship between the size of the nurse cell and the oöcyte during development are described. The growth of the follicle during the pupa stage is due to oöcyte growth. An adult female with one normal and one underdeveloped ovary was found. The defect must have been the result of a developmental error whereby yolk production was blocked. The other ovary of the same female was apparently normal. The morphology of the underdeveloped adult ovary resembled that of a young pupa. In this ovary, nurse cell chromosomes had a higher degree of polyteny and their number was smaller than normal. rRNA must remain in this organelle, which is overdeveloped.

INTRODUCTION

The last mitotic division of undifferentiated germinative cells in *Rynchosciara* leads to the formation of two cells which differentiate in two distinct ways: one becomes the oöcyte I, which will soon enter meiosis, and

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the other becomes its nurse cell. The nurse cell will pass through several polyploidization cycles, its volume will greatly increase, and its chromosomes will become polytenic during the mid pupa stage. The ensemble of these two main cells plus the small follicle cells surrounding them is called follicle (Basile, 1966, 1969).

At the beginning we simply observe oöcyte I and nurse cell in contact, with the presence of small cells, especially in the proximity of the oöcyte, which probably migrate from the ovary wall. These are somatic cells in whose metaphases the chromosomes of the species can be well analyzed ($2n = 8$), and they are called primordial follicle cells. After a certain number of divisions, these cells will ultimately envelop the nurse cell-oöcyte ensemble and the follicle will be completed (Basile, 1966, 1969).

The nurse as well as the follicle cells are responsible for sending RNA to the oöcyte. Although the oöcyte chromosomes do not transcribe during the whole zygotene cycle their cytoplasm is continually enriched with RNA (Basile, 1966, 1969).

When oöcytes with attached nurse cells are compared to oöcytes without nurse cells a sharp difference in chromosome behavior is observed. In the presence of nurse cells, the oöcyte chromosomes remain practically inactive in terms of transcription, only undergoing reactions related to other aspects of the meiosis mechanism. This is a slow process with a long zygotene, during which the attached cells seem to take on nursing duties, i.e. production of yolk precursors for the future egg. In brief, these are the phenomena that take place in *meroistic ovaries.* In the absence of nurse cells, the activity of the oocyte chromosomes is quite strong, as can be seen by observing their aspect and physiology. These chromosomes usually have a plumulate shape which is evidence of a high transcription rate, as is the case for *panoistic ovaries.* In addition, oöcyte chromosomes, which are clearly redundant, may also exhibit the gene amplification phenomenon, in general of rRNA.

In most of the insects studied the chromosomes are not functionally active in the oöcytes, but are active in nurse and follicle cells, as demonstrated for *Drosophila* (Painter and Reindorp, 1939; Sirlin and Jacob, 1960; see also King, 1970 a), *Calliphora* (Bier, 1957, 1959), *Musca* (Zalokar, 1965), and *Rhynchosciara* (Basile, 1966, 1969).

In general, nurse cells exhibit polyploidy, and their chromosomes maintain a cryptopolytene form with consequent great activity (Basile, 1969).

This polyploidy may be followed by polyteny, which could determine total gene amplification for the cell (see discussion in Ashburner, 1970). The follicle cells exhibit little gene amplification, but they also send RNA to the oöcyte (Basile, 1966, 1969). A very low degree of polyteny can be observed in these cells.

In this paper we present new results obtained from the study of different developmental stages of *Rhynchosciara* follicles, as well as data concerning the development of an abnormal ovary. It is our hope that these data will contribute to a better understanding of the development, morphology and physiology of *Rhynchosciara* ovarian follicles.

MATERIAL AND METHODS

Material

*Rhynchosciara angelae** larvae, pupae and adult flies were utilized. The insects were maintained in the laboratory by the method of Lara *et al.* (1965) modified by Morgante *et al.* (1970).

Methods

Microscope preparations were carried out according to the following techniques:

a) *Squash Technique*

The ovaries, dissected and placed in 0.7 % saline, were squashed after a short period of fixing (5 minutes) in ethanol-acetic acid (3:1), in lactic-acetic orcein (1 %) according to Nicoletti (1959), or in Targa fluid (1969). In the last case, the ovary was dipped in a drop of lactic-acetic orcein (1 %) for 5 minutes, and later squashed in Targa fluid between slides. Strong squashing was used in some cases, light squashing in others, and no squashing at all in still others, when the material was simply observed under the weight of a cover slip. Observations were made with a Zeiss photomicroscope, mostly during phase constrast, when photographs were taken.

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* *Rhynchosciara americana* (Weidmann, 1821) was redescribed by Nonato and Pavan (1951) as *R. angelae*. Their identity was demonstrated by Breuer (1969).

b) *Histological Sections*

To evaluate the size of the main follicle cell (oöcyte and nurse cell), histological sections of the ovary or of the whole insect were made with care taken not to damage the organ. The material was fixed in Bouin's (3 parts picric acid-saturated solution: 1 part Formaldehyde), with or without acetic acid (when acetic acid was used, it was added at the time of use in the following proportions: 95 parts 3:1 picro-formol solution and 5 parts glacial acetic acid) for 2 hours, or in Carnoy solution (3 ethanol: 3 chloroform: 1 glacial acetic acid) for 24 hours. The ovary sections were immersed in the fixing solution. When the whole insect was utilized, the fixing solution was injected into the body cavity with a microneedle, and the animal wasthenplaced in additional fixing solution. After alcohol dehydration and encasement in paraffin, sections of 4 um were prepared.

The stains used were: hematoxilin (1%) and eosin (0.2%) for 5 and 1 minute, respectively; Giemsa (5 to 10 minutes) according to Gude, *et al.* (1955), and methyl green and pyronine according to Brachet (1944). In the last case, fixing was always carried out in Carnoy solution.

RESULTS

1. *Follicle Measurements*

As mentioned in the Introduction, the *Rhynchosciara* follicle consists of two main cells: oöcyte and nurse cell, encased by a layer of follicle cells. A schematic presentation is given in Figure 1. Histological sections of ovaries obtained from insects at different developmental stages (larva, pupa and adult fly) were observed. The two cells were measured in follicles whose sections had been made along their median longitudinal plane, i.e. the A-B plane in Figure 1. To evaluate the size of the two cells we used the diameter of the nurse cell and the a-b distance in the oöcyte (see Figure 1). Measurements were made at one larval stage only since we know that at this stage growth is slow (Basile, 1966, 1969), at 3 pupa stages (young, still without polytene chromosomes in the nurse cells, but in full polyploidy; middle, with typical polytene chromosomes mature, already at the end of the polytene stage), and when the insect had reached the adult stage. The results, together with the respective schematic drawings of the follicles at each of these phases, are shown in Figure

2. Abnormal ovary

When adult *R. angelae* females were dissected so that the ovaries could be isolated, several insects exhibiting only one ovary were found. The frequency of this anomaly is relatively low, about 1: 250. In all cases encountered, the abdomen was carefully dissected in an attempt to find the other ovary. Usually no other ovary was found, and the physiological aspect of the single ovary was perfectly normal. Only one adult fly, of apparently normal aspect, was found to have a normal ovary and an atrophied one. The atrophied ovary was much smaller than normal (about 1/10 the normal size), had few lighter-colored follicles (light yellow, while the normal color is deep yellow), which were also less opaque than normal follicles.

The follicles of this ovary were carefully examined. At first sight they exhibit characteristics of follicles from young pupae (Figure 3), with oocytes that are small in relation to nurse cells. Arrow II in Figure 2 indicates the position which these follicles would occupy, according to their external characteristics, if their development had been normal.

The nurse cells of these ovaries exhibit special characteristics, especially whith respect to the chromosomes and nucleoli. The nucleoli are very large. At times they appear to have originated from a single chromosome (the X one) (Figure 4); at other times, from various chromosomes, in which case they are larger than those organized by a single chromosome (Figure 5). The nurse cell chromosomes are in polyteny (Figures 4 and 5), but are much larger than polytene chromosomes in normal animals (Basile, 1969). They are well differentiated, but their number is small (Figure 6). In addition, zones with good synapsis and zones with different degrees of asynapsis can be observed (Figure 7). In some cases it was possible to observe chromocenters joining chromosome groups (Figure 4).

The chromosomes of the follicle cells in this abnormal ovary also exhibit a reasonable degree of polytene, being much larger than those in follicle cells of normal ovaries. The oöcyte nucleus in these follicles has a normal aspect, in typical zygotene phase (Figure 3), but no accumulation of yolk material is found in its cytoplasm, as observed in normal cases.

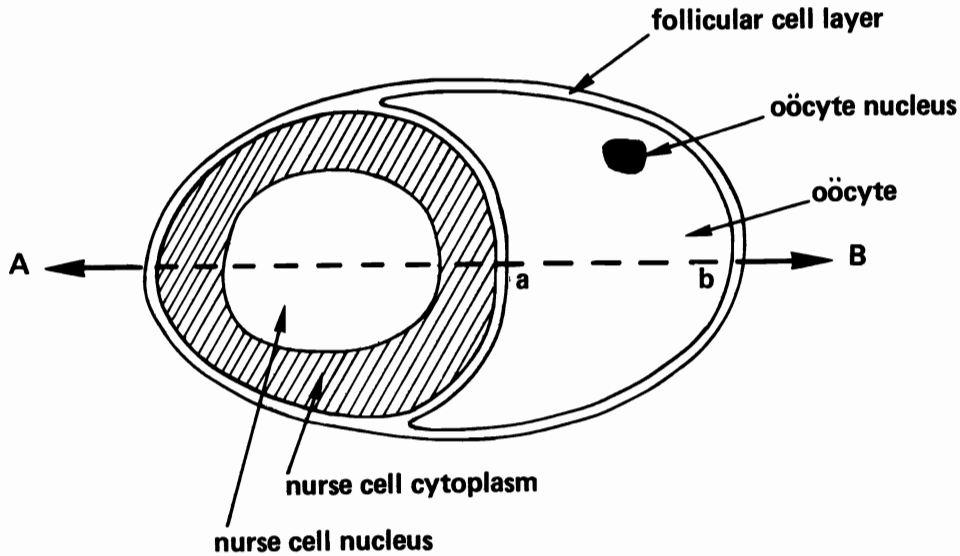

Figure 1. Schematic presentation of a *Rhynchosciara angelae* follicle in median longitudinal section of mid pupa.

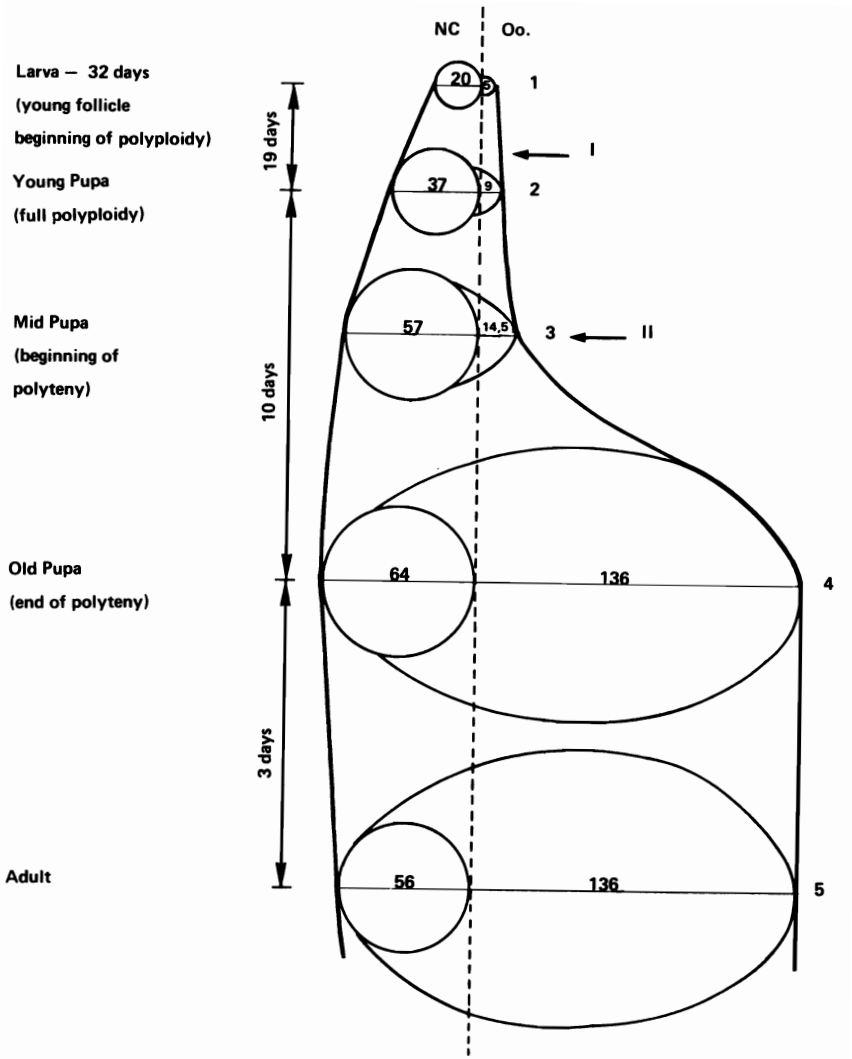

Figure 2. Size relationship between nurse cell (NC) and oöcyte (Oo) during development of an *R. angelae* follicle. The cell sizes given in the drawings (in microns) are the average values of measurements taken in 10 cells during each phase (1 to 5). The days indicated between arrows on the left correspond to the time intervals between the different phases. Note that highest growth occurs during the mid pupa stage (with polytene chromosomes in nurse cells).

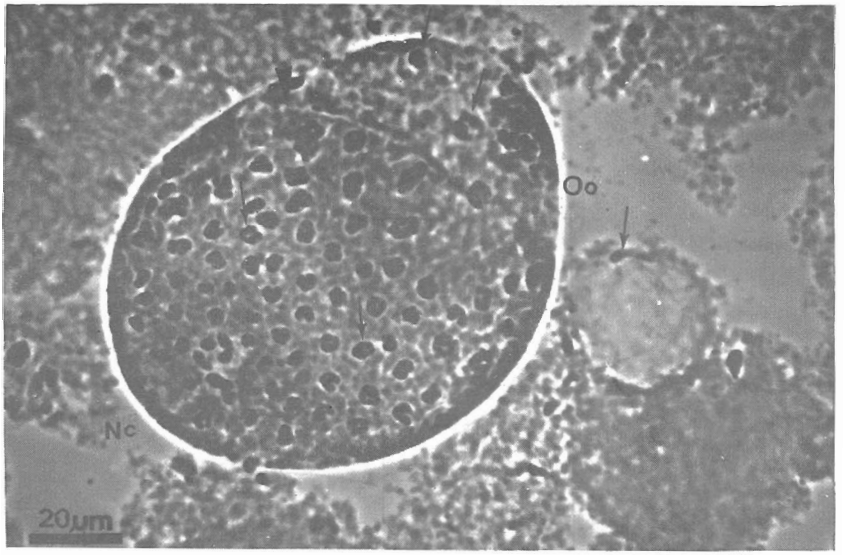

Figure 3 - General aspect of a whole follicle in the abnormal ovary. This preparation was made simply by placing the material, stained with orcein, under the weight of a cover slip. The large arrow indicates the limit between nurse cell (NC) and oöcyte (Oo). Note the nuclei of the follicle cells (small arrows) which have polytene chromosomes. At right, nucleus of a oocyte in zygotene. The arrow on top of the nucleus indicates the chromocenter, of which the limited chromosome is a part.

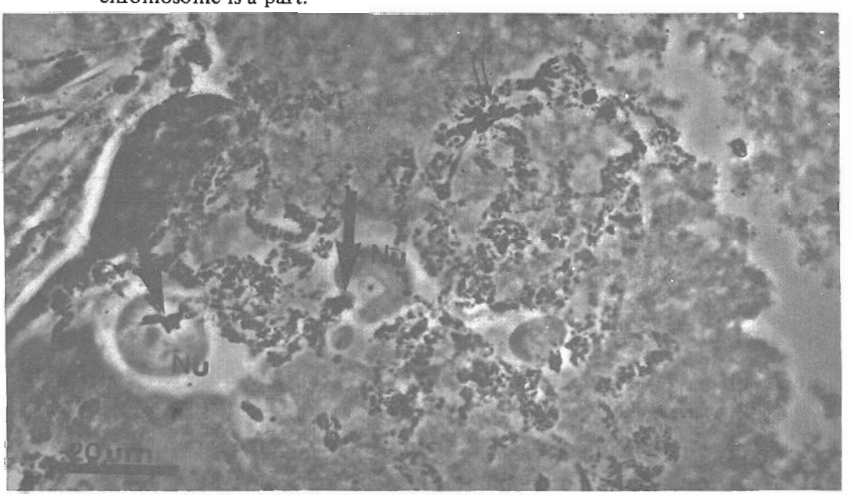

Figure 4 - Overdeveloped nucleoli (Nu) in a nurse cell of the abnormal ovary. The arrows indicate the base of the X chromosome, the nucleolus organizer. Observe the overdeveloped polytene chromosomes in the nurse cells after squashing with orcein. The double arrow indicates chromocenters including chromosome groups.

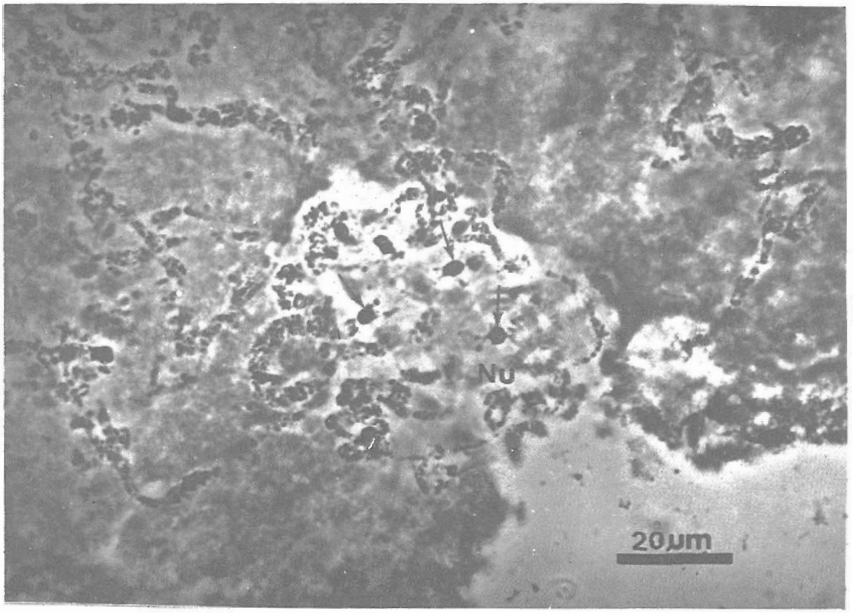

Figure 5- Nucleolus (Nu) resulting from fusion of sigle nucleoli. The arrows indicate the extremities of the X chromosomes, which organize the nucleolus.

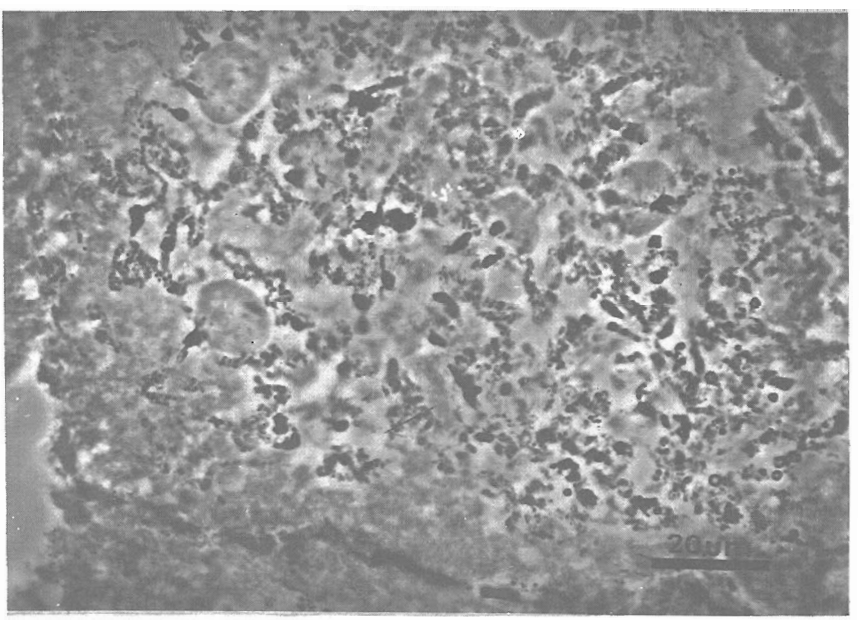

Figure 6- General aspect of nurse cells in the squashed abnormal ovary. Note the large and scarce polytene chromosomes.

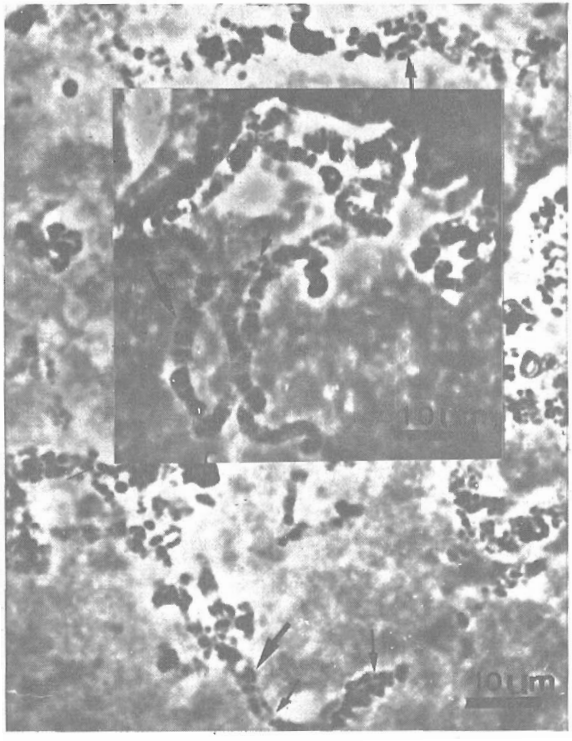

Figure 7. Different degrees of synapsis in chromosomes of nurse cells in the abnormal ovary. The thin arrows indicate less synaptic zones, and the thick arrows, more synaptic zones. At right, a schematic rendering of the different degrees of synapsis in a polytene chromosome.

DISCUSSION

Two types of ovaries are found in insects. The *panoistic* ones are those without nurse cells: there is only a follicle epithelium which covers the oöcytes, as in the case of *Gryllus* (Favard-Séréno and Durand, 1963) The *meroistic* ones are those which, in addition to follicle cells covering the oöcyte, also have one or more nurse cells forming an integral part of the follicle. In *Rhynchosciara,* the ovarian follicle is meroistic and has only one nurse cell (Basile, 1966, 1969).

The size relationship between nurse cell and oöcyte varies during insect development, as shown in Figure 2. It can be observed in this Figure that the nurse cell continues to grow until the advanced pupa stage, with a small decrease in size occurring in the adult fly before fertilization. Its large growth is accompanied by intense polyploidy in the nucleus, which results in a process of endomitosis. Data by King (1970 b) have shown that the "female-sterile" mutant (fs '2' E) of *Drosophila melanogaster* suffers a delay in mitotic replication of the nurse cell chromosomes when in homozygosis, a fact suggesting inhibition of vitellogenesis. Endomitosis in nurse cell nuclei has been observed in other species of insects with meroistic ovaries, such as *Drosophila* (Painter and Reindorp, 1939; King et al., 1956), *Calliphora* (Bier, 1957, 1959), and *Musca* (Zalokar, 1965). The nurse cell greatly increases in size during the polyploidy phase in *Rhynchosciara.* We can observe in Figure 2 that slow growth of the nurse cell takes place during polyploidy: the oöcyte also grows, however ovary growth during this phase is mainly a consequence of nurse cell growth. Basile (1969) showed that the ovary continues to grow during the mid pupa stage when the nurse cell chromosomes are typically polytene. This growth is a consequence of oöcyte growth, a statement which is supported by the data of Benozzati (1975) and Benozzati and Basile (1978), who have shown that, during this developmental stage of the ovary, a gradual increase in total protein content occurs in this organ. The data presented here show that, once polyploidy is over, during the pupa phase when the nurse cell chromosomes are no longer typical polytene chromosomes, the oöcyte practically does not increase in volume. In the adult phase, oöcyte volume actually decreases. On the other hand, during the polytene phase, oöcyte growth is very fast, and an enormous concentration of yolk grains can be observed in the cytoplasm. In other words, the increased ovary size in the pupa is a direct

consequence of the growth and maturation of the oöcyte, which exhibits large amounts of yolk material.

Oöcyte growth caused by yolk material accumulation seems to be a general rule in most species. The more extensive studies were those carried out on amphibians (see Discussion in Wischnitzer, 1966). Panijel (1951) divided cytoplasm synthesis in *Triturus* and *Xenopus* oöcytes into two phases: during the first phase no synthesis of yolk material is presumed to occur, and the oöcyte diameter is supposed to go from 50 μm to 300 μm *(Xenopus)* or 500 μm *(Triturus)*. During this stage, many mitochondria and lipochondria are organized. During the second phase, a greater increase in oöcyte volume is supposed to occur due to yolk production, which starts at the oöcyte periphery and progresses towards the nucleus. The amphibian egg is of the panoistic type and its maturation is quite slow, lasting up to three years (Grant, 1953). The phase of greatest growth occurs during diplotene, i.e. at the beginning of the first meiotic division when the chromosomes are still in plumulate condition (Wischnitzer, 1966).

Other organs may also participate in yolk production, as demonstrated for various organisms: *Aedes* (Roth and Porter, 1962; Hagerdon *et al.*, 1973), *Periplaneta* and *Rhodnius* (Anderson, 1964), *Planorpa* (Bier and Ramamurty, 1964), *Drosophila* (Meyer, apud Bier, 1963), *Platysamia cecropia* (Telfer, 1953), *Hialophora* (Stay, 1965), *Schistocerca* (Hill, 1965). In all these organisms the principal organ responsible for supplying substances related to vitellogenesis is the fat body, which manufactures proteins that are transported to the hemolymph and from there to the developing ovary (Pan *et al.*, 1969; Engelmann, 1969; Brooks, 1969; Hagedorn and Judson, 1972). These proteins exist only in females and have been generically called vitellogenins (Pan et al., 1969).

Basile (1969) showed that, in *Rhynchosciara*, nurse cells supply RNA to the oöcyte during the pupa phase. The oöcytes, in turn, synthetize protein from at least part of this RNA. RNA transport from nurse cells to oöcytes has also been demonstrated for other insects such as *Drosophila* (Sirlin and Jacob, 1960), *Rhodnius* (Vanderberg, 1963), and *Musca* (Bier, 1963; Zalokar, 1965). Benozzati (1975) and Benozzati and Basile (1978) have studied in detail the protein pattern of *R. angelae* ovaries during insect development by the technique of acrylamide gel electrophoresis, showing that female hemolymph exhibits protein patterns very similar to those found in the ovaries. This fact

suggests a possible transport of protein material from other organs to the ovaries through the hemolymph.

The abnormal ovary studied by us was atrophied. Its external development exhibited characteristics of young pupa ovaries (Figure 3) although the ovary was found in an adult fly. Although this was the only ovary of this type we found, the alterations in its cells in relation to normal cells are very interesting, as shown by what follows:

a) The nurse cell polytene chromosomes, are larger and their number is smaller (Figure 6) than is the case for normal cell chromosomes. The degree of polyteny is greater, but there are synaptic and asynaptic zones (Figure 7).

b) The follicle cells exhibited polytene chromosomes with a clearly higher degree of polytene than observed in normal cells. Somehow, in these cells the stimulus towards chromosome polytenization was greater. This fact strongly indicates that the response to the stimulus depends on intracellular conditions since the other ovary in the same insect developed in an apparently normal way.

c) The nurse cells exhibit one or more hypertrophic nucleoli (Figures 4 and 5) which adhere to one or more chromosomes. This fact, in addition to the small development of the oöcyte, which, in turn, has no yolk grains, leads us to postulate the existence of a mechanism which may prevent passage of rRNA from the nurse cell to the oöcyte. The inability to produce yolk may be related to the lower degree of ploidy in the nurse cell, as observed in the case of the "female sterile" mutant of *Drosophila melanogaster* previously mentioned in this paper (King, 1970 b). The larger volume nucleoli may result from fusion of small nucleoli. In a normal situation multiple nucleoli are commonly observed during polytene in the nurse cell. These, however, are always organized by different X chromosomes. In any case, the volumes of these nucleoli, although belonging to larger cells, never reach those discussed here.

We have no data on the possible causes of nonformation or faulty formation of one of the ovaries in *R.angelae* pupae, but studies of the alterations occurring in faultily developed ovaries may lead us to a better understanding of the normal development of this important organ in animals.

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PHENOTYPIC AND GENETIC PARAMETERS OF BUTTERFAT PRODUCTION IN PITANGUEIRAS COWS.

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ABSTRACT

Data from 1,723 records on 483 Pitangueiras cows (5/8 Red Poll x 3/8 Zebu) were analyzed. This single herd was maintained at Três Barras farm, in the State of São Paulo, Brazil. Least-squares analysis of variance showed significant effects for generation, age, season and year for first lactation. Mean and standard error for fat yield were 117.4 ± 0.8 kg, and 4.15 ± 0.02% for fat percent. Repeatability estimated by intraclass correlation was 0.31 ± 0.07. Heritabilities for fat yield estimated from half-sib correlations were 0.11 ± 0.18 for first lactation, 0.14 ± 0.22 for second and 0.69 ± 0.37 for third. Overall heritability estimate was 0.19 ± 0.10.

INTRODUCTION

The importance of the study of butterfat yield in tropical milk breeds has increased considerably with the development of the dairy industry, since this is an important factor for determining the price of milk. Over the last

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few years, however, the tendency has been to express the genetic potential of a breed by the protein content of the milk it produces, so that several investigators in European countries have recommended to select milk herds only on the basis of milk protein content. Nevertheless, the general tendency seems to be to select on the basis of the protein-fat ratio, since this quotient has a medium to high heritability.

Most studies of fat yield carried out in different countries have reported means of 104.7 to 224 kg for first lactation data (Allaire and Henderson, 1966; Bereskin and Touchberry, 1966; Branton *et al.*, 1967; Dutt *et al.*, 1972; Kali *et al.*, 1968; Lobo *et al.*, 1979; White and Nichols, 1965). Several investigators have calculated estimates of repeatability and heritability for fat yield in many countries (Bereskin and Freeman, 1965; Branton *et al.*, 1967; Gacula Jr., *et al.*, 1968; Gill and Allaire, 1976; Lobo *et al.*, 1979; Molinuevo and Lush, 1964, Paul *et al.*, 1971; The objective of this study was to evaluate the influence of environmental effects on fat yield and to estimate repeatability and heritability for a dairy cattle population.

MATERIALS AND METHODS

Data were collected over 13 years (1962 to 1974) from 1,723 records on 483 Pitangueiras cows (5/8 Red Poll x 3/8 Zebu), maintained at Três Barras farm, near Pitangueiras city, State of São Paulo, Brazil. The farm is located at an altitude of 503 m, 21° 00' latitude South, and 48° 41' longitude West of Greenwich. The mean atmospheric temperature in this area ranges from a maximun of 31.9° C to a minimum of 16.2° C, with an annual mean of 24° C. Annual precipitation is 1,346 mm.

Statistical Analysis

The nongenetic effects were estimated by least-squares analysis of variance (Harvey, 1960), according to the following linear model

$$Y_{ijklm} = u + a_i + b_j + c_k + d_l + e_{ijklm}$$

where:

Y_{ijklm} = lactation fat yield; u = least-squares mean; a_i = effect of the i^{th}

generation (i = 1,2); b_j = effect of the j^{th} season (j = 1,2); c_k = effect of the k^{th} year (k = 1,2, ..., 13); d_l = effect of the 1^{th} age (j = 1,2,...,4); and e_{ijklm} is residual within-animal error variance.The effects of generation, season, year and age were considered to be fixed. The usual assumptions about the distribution of the e's were made.

The heritability estimates were calculated only for the second generation cows ($G_2 = G_1 \times G_1$, inter se mating), where the population reached Hardy-Weinberg equilibrium. A least squares analysis of variance was performed using the same model as above, but deleting generation and including sire.

The heritability coefficient (h^2) is computed as the ratio of V_A/V_P, and refers to that proportion of the phenotypic variance (V_P) that is caused by additive genetic variance (V_A). This estimate of heritability is very useful in a predictive role for expressing an estimate of breeding value for an individual and predicting response from selection for improvement of traits of economic importance.

The following formula was used to estimate the heritability coefficient

$$h^2 = \frac{4\,\sigma_S^2}{\sigma_T^2}$$

where σ_S^2 = sire component of variance, and σ_T^2 = total or phenotypic variance. The standard error (S.E.) of this estimate was computed by the formula of Swiger *et al.*, 1964.

Repeatability estimates refer to the proportion of the variance of single measurements that is due to permanent differences between individuals, both genetic and environmental. This index was calculated as a ratio of where σ_C^2 = between cow-component of variance, and σ_W^2 = within cow-component of variance. The genetic interpretation of these components of variance is well documented.

RESULTS AND DISCUSSION

The mean fat percent for 1,723 lactations was 4.15 ± 0.02 % with a coefficient of variation of 6.29 %. These results place the Pitangueiras breed

on the same level as the Zebu and Creole breeds in the tropics (Bodisco *et al.*, 1968; Sharma *et al.*, 1970) and are higher than those obtained by Naufel (1965/66) for the Holstein breed. The butterfat content ranged between 4.13 % (second lactation) and 4.18% (fifth lactation). These results are in agreement with those of Fuenmayor *et al.* (1973), Kohli *et al.* (1961) and Naufel (1965/66). The last author, however, found increased yields only up to the 47-months age groups, with a consistent decrease related to ageing. In general, the increased fat percent according to cow's age is lower than that found for milk or fat yield, probably because its value is calculated as a ratio of the milk and fat yields.

The mean fat yield for first lactation was 105.8 kg with a coefficient of variation of 27.7%. Table I shows consecutive increases in production from the first to the fifth lactation. These increases, expressed as percentages of mean first lactation yields, were +8.1% for the second lactation, +16.6% for the third, +18.6% for the fourth, and 23.5% for the fifth.The variation of fat yield with age found in this work is similar to that described for milk yield in dairy cattle. The European cattle in temperate regions usually attain maximum production at about the seventh lactation; in Zebu cattle in the tropics, maximum production is usually reached by the third or fourth lactation, and the magnitude of the increase in yield from first to maturity is small. Perhaps

Table I - Mean and variability of fat yield (kg) by lactation number.

Lactation number	Number of records	Mean	% of the first lactation mean	C.V.(%)
First	483	105.8 (4.15 %)[a]	100.0	27.7
Second	431	114.4 (4.13 %)	108.1	29.2
Third	357	123.4 (4.14 %)	116.6	26.9
Fourth	257	125.5 (4.15 %)	118.6	28.3
Fifth	195	130.7 (4.18 %)	123.5	31.4
Overall	1723	117.4 (4.15 %)	110.9	29.5

[a] ( ) = mean fat percent.

the causes of the low rate of increase in fat yield from first to maturity in most tropical herds is related to their low milk production levels. It probably has some relation also to the intensity of selection practiced in the evolution of tropical breeds of cattle (Mahadevan, 1966).

Few studies have been carried out in tropical climate regions on the influence of generation, age of cow, season and year of calving on fat yield. Generation effects were significant in all lactations studied (Table II). Thus, the effect studied possibly includes selection and generation, since the genetic material analyzed is in segregation. Cow age had significant effect on fat yield at first, second, fourth and fifth lactation. Similar results have been described by other workers (Gacula Jr. *et al.*, 1968; Dickerson,1940). The least squares constants (Table III) for age effect show that the cow ages most favorable to start fat yield at different lactations were 48 months at first lactation (+6.6 kg), 60 at second (+9.0 kg), 72 at third (+7.2 kg), 84 at fourth (+13.2 kg), and at least 96 at fifth (+15.8 kg).

The season effect was significant only for the first three lactations. Gacula Jr. *et al.*(1968) and Dutt *et al.* (1972) found significant influences for Holstein and Hariana breeds, respectively. Thes least squares constants for season effect ranged from 2.8 to 4.7 kg for dry season. All the constants were negative for the rainy season (Table III).

Table II - Analysis of variance (mean squares) for fat yield.

Source of variation	Lactation number				
	First	Second	Third	Fourth	Fifth
Generation	47 027*	34 056*	48 391*	34 495*	45 603*
Age of cow	4 032*	5 102*	1 886	4 485*	9 052*
Season	3 234*	8 640*	4 793*	1 162	1 965
Year	1 410*	957	470	2 510*	1 138
Error	724	972	945	982	1 321

*P < . 05

In this study the year of calving was found to have significant effect on fat yield only at first and fourth lactation. The least squares constants indicated variations over the years, but did not demonstrate any specific tendency throughout the period studied (1962 to 1974). However, during the years 1962, 1965/68 and 1972/3, the constants for first lactation were negative, thus showing that during those years the Pitangueiras cows produced less fat than during the remaining years.

A knowledge of repeatability estimates for fat yield may be used in selecting for future performance. When the repeatability estimate for a trait is high, culling on the basis of the first lactation should be effective in improving the overall lactation of the herd the next year. In addition, offspring from the superior individuals in the herd should be given preference when selection is made for replacement stock (Lasley, 1978).

The repeatability estimate computed by intraclass correlation was 0.31 ± 0.07. This result shows that fat yield at first lactation must not be utilized to eliminate the worst cows from the herd, since the danger exists of eliminating some potentially good ones. Several investigators have calculated repeatability estimates for fat yield for several breeds. In general, the values obtained vary between 0.41 and 0.71 (Bereskin and Freeman, 1965; Gacula Jr., *et al.*; Madden *et al.*, 1955; Pal *et al.*, 1971, thus indicating greater correlation between the different lactations of one cow. On the other hand, Stonaker's (1953) estimate of 0.5 for the repeatability of fat yield in succeeding lactations, shows that permanent differences between cows, including genetic differences, are present on an adequate scale for improvement in butterfat production to be achieved by the adoption of proper breeding methods.

The heritability estimates for fat yield computed from paternal half-sib correlations were 0.11 ± 0.18 for first lactation, 0.14 ± 0.22 for second, and 0.69 ± 0.39 for third. Numerous studies have been reported on the heritabilities of fat yield in dairy cattle as 0.10 to 0.40 (Bereskin and Freeman, 1965; Branton *et al.*, 1967; Gill and Allaire, 1976; Lasley, 1978; Lobo *et al.*, 1979.

The increase in heritability coefficient observed from first (0.11) to third (0.69) lactation possibly reflects smaller variations in yield among cows during the last lactations because of selection for milk production mainly at first lactation. However, due to the small number of daughters per bull and the high standard errors found, this parameter needs further study in the Pitangueiras breed.

Table III - Least squares constants (kg) for factors affecting fat yield.

Source of variation		Constants (kg)				
		First	Second	Third	Fourth	Fifth
Least squares mean		101.2	110.8	117.4	112.7	109.1
Generation	1	10.8	10.0	13.2	13.5	20.4
	2	-10.8	-10.0	-13.2	-13.5	-20.4
	24	-9.9	–	–	–	–
	36	3.3	-17.8	–	–	–
	48	6.6	-0.4	-11.6	–	–
Age(months)	60	–	9.0	-0.3	-20.8	–
	72	–	9.2	7.2	1.6	-11.9
	84	–	–	4.7	13.2	-3.9
more than	96	–	–	–	6.0	15.8
Season	dry	2.8	4.7	3.9	2.3	3.2
	rainy	-2.8	-4.7	-3.9	-2.3	-3.2
	1962	-20.4	–	–	–	–
	1963	15.8	11.7	–	–	–
	1964	5.4	19.3	-7.2	–	–
	1965	-5.7	-3.2	-3.6	5.4	–
	1966	-3.3	6.5	5.1	-6.7	-0.7
	1967	-0.7	4.6	2.1	10.3	5.4
Year	1968	-0.5	0.6	5.7	12.2	-12.1
	1969	7.4	-2.8	-1.3	12.3	5.3
	1970	6.0	-1.7	3.6	-1.0	10.4
	1971	5.2	-3.3	3.5	9.2	3.7
	1972	-0.9	2.5	-3.9	-12.4	6.8
	1973	-8.3	-4.4	-3.6	-1.1	-2.0
	1974	–	-29.8	-0.4	-28.2	-16.8

CONCLUSIONS

On the basis of our data and analysis, we reached the following conclusions:

a) The general mean fat yield of 117.3 ± 0.8 kg (or 4.15 ± 0.02%) demonstrates the good capacity for butterfat yield of the Pitangueiras breed.

b) Highest fat yield was reached at fifth lactation (130.7 kg).

c) Generation, cow age, season and calving time had significant influence.

d) The 0.31 coefficient of repeatability for fat yield shows the low predictive value of first lactation.

e) The heritability coefficients increased from first (0.11) to third lactation (0.69) without necessarily indicating a greater response to selection during the last lactations.

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