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REPRODUCTIVE ISOLATION IN THE SALTANS GROUP OF *DROSOPHILA*. IV. THE STURTEVANTI SUBGROUP

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ABSTRACT

The reproductive isolation in the sturtevanti subgroup was studied by pair mating and mass mating crosses involving 3 species in the subgroup: *D. sturtevanti, D. milleri* and *D. magalhaesi*. The results showed high and variable degrees of reproductive isolation among combinations. The isolation indices showed stronger isolation at the insemination level (Ii) than at the fertilization level (Fi) in every case. Two different interpretations of the species derivation in the subgroup were presented and discussed on the basis of the available data.

INTRODUCTION

This paper is part of a series concerned with isolation studies in the saltans group of *Drosophila*. In the preceding papers hybridization tests of species and strains in the saltans, parasaltans and elliptica subgroups were reported (Bicudo, 1973, 1978; Bicudo and Prioli, 1978a, b). Data on intraspecific and interspecific mating tests of species belonging to the sturtevanti subgroup are now presented.

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The sturtevanti subgroup was established on a morphological basis by Magalhães (1962) to include *D. rectangularis, D. sturtevanti* and *D. milleri.* Two other species — *D. magalhãesi* and *D. dacunhai* — were added by Mourão and Bicudo (1967), bringing to 5 the number of species accepted today as belonging to the subgroup.

The geographical distribution of *D. sturtevanti* is the largest among the species in the subgroup and even in the saltans group as a whole. It includes almost the entire distribution area of the group, from Mexico to the south of Brazil and the Caribbean Islands. In contrast, according to the available data, the other members of the subgroup exhibit a very restrict geographical distribution. Magalhães (1962) reported *D. milleri* exclusively in El Yunque, Puerto Rico (where *D. sturtevanti* was also recorded by the same author), and *D. rectangularis* in Orizaba and Tixtla, Mexico. Mourão and Bicudo (1967) reported *D. magalhãesi* in Eldorado, Brazil, and *D. dacunhai* in Kingston and Ocho Rios (Jamaica).

The 5 members in the sturtevanti subgroup are morphologically very similar, being distinguishable only by some minor details (Magalhães and Bjornberg, 1957; Magalhães, 1962; Mourão and Bicudo, 1967). They constitute close genetic systems which are, in general, considered important tools in speciation studies.

The data presented in this paper provide information on the degrees of isolation and on the isolating mechanisms operating among 3 species in the subgroup. An interpretation of the phylogenetic relationships of these species based on their isolation patterns is also presented and discussed.

MATERIALS AND METHODS

Drosophila milleri, D. magalhãesi and *D. sturtevanti* were available for the present study. The geographical origins and the symbols of the strains used are given in Table I. Except for St (from Mirassol), all of these strains have been maintained for a long time in the laboratory.

The methods followed were basically the same as described in detail in the first paper of this series (Bicudo, 1973). In summary, interspecific and intraspecific reciprocal pair mating crosses replicated twice were analyzed 30

days after preparation with respect to fertility (frequency of fertile crosses) and fecundity (average number of progeny). Females of sterile crosses were also examined for the presence of spermatozoa in their reproductive tracts.

The fertility of the F_1 progeny was studied by means of mass mating endocrosses. In cases of no F_2 production, F_1 males and females were separately backcrossed to both parental species.

Fertility and fecundity were also studied in mass mating tests involving *D. sturtevanti* and *D. milleri*. Two mass crosses were prepared in every case using 10 pairs per vial with two changes of food. The crosses were classified with respect to their fecundity as: low productivity (+), medium productivity (++), and high productivity (+++). These classes included crosses which yielded less than 10, less than 50 and more than 50 descendants respectively.

The experiments and the stocks were maintained on banana culture medium at a temperature of 25^0 C$\pm$ 1^0C. Virgin flies aged 7 days were used in the experiments.

Table I - Species and strains used in this study

Species	Symbols	Origin
D. sturtevanti	St	Mirassol (SP), Brazil
	St_1	El Yunque, Puerto Rico
D. milleri	Mi	El Yunque, Puerto Rico
D. magalhãesi	Mag	Eldorado (RS), Brazil

RESULTS

The Results of interspecific pair mating crosses are shown in Table II. Both directions of the combination St x Mi produced fertile crosses. The combinations St x Mag and Mag x Mi produced fertile crosses in a single direction; in both cases the sterile direction involved Mag females.

The combination St x Mi showed 21.28 % fertile crosses when St females and Mi males were used, and 3.28 % in the reciprocal crosses. The difference between both directions of crosses was statistically significant

($P < 0.001$). The combinations St x Mag and Mag x Mi produced low frequencies of fertile crosses in the single fertile direction, as follows: Mi x Mag – 1.44 % and St x Mag – 0.96 %.

The highest percentages of inseminated females found in the sterile crosses were obtained in the crosses Mi females x Mag males (21.62 %) and St females x Mi males (14.28 %). A significant difference with respect to the number of inseminated females was found between the reciprocal crosses in the combinations St x Mi ($\chi_1^2 = 4.61, P < 0.05$) and Mi x Mag ($\chi_1^2 = 6.89, P < 0.01$).

The highest average number of progeny was shown by St females x Mi males (15.60), followed in decreasing order by Mi females x Mag males (12.0), and Mi females x St males (5.0). The single fertile cross of St x Mag yielded only larvae.

With respect to the fertility of F_1, St x Mi showed fertile females and males in the direction of crosses involving St females and Mi males, and fertile females and sterile males in the reciprocal crosses. The single fertile direction of crosses in the combination Mag x Mi yielded fertile females and sterile males.

The results of intraspecific crosses are presented in Table III. Fertility was highest in St (67.06 %) followed in decreasing order by Mi (62.10 %) and Mag (46.67 %). The differences were significant for Mag versus St ($\chi_1^2 = 7.77, P < 0.01$) and for Mag versus Mi ($\chi_1^2 = 3.92, P < 0.05$). Fecundity was highest in St, closely followed in decreasing order by Mag and Mi. The Student's "t" test for comparison of the average numbers of progeny indicated that the differences are not significant. Inseminated females among those dissected from sterile crosses were detected in a single intraspecific cross: St x St (18.18 %).

A comparison of the data in Tables II and III shows that, as expected, the percentages of fertile crosses and their fecundity are clearly lower in the interspecific crosses than in the intraspecific ones.

Tables IV and V present isolation indices calculated from the data in Tables II and III, respectively, as proposed by Bicudo (1973). These indices are: Ti (total isolation), represented by the percentage of crosses not yielding offspring; Ii (isolation at the insemination level) represented by the percentage of uninseminated females; Fi (isolation at the fertilization level) represented by the percentage of inseminated females which did not yield hybrids; and Ai (average isolation), i.e. the average of the Ti values in both directions of

crosses in the interspecific combinations. The highest values for ti in the interspecific combinations (Table IV) were found in the crosses involving Mag females and equals 100 %, while the lowest values (which are still high) were obtained in both directions of crosses between St and Mi (78.72% and 96.72 %). The Ai value of the latter combination equals 87.72., while in the combinations St x Mag and Mi x Mag the same index equals 99.52 and 99.28, respectively. The Ii values obtained were in general considerably greater than the Fi values.

In the intrastrain crosses (Table V) the highest Ti value was obtained in Mag x Mag (53.33 %), followed in decreasing order by Mi x Mi (37.90) and St x St (32.93).

The isolation observed in Mi and Mag endocrosses occurred exclusively at the insemination level. Isolation at the fertilization level was only detected in St endocrosses. However, in this case the Ii value was also greather than the F_1 value.

Table VI shows data on mass crosses prepared using *D. sturtevanti* from El Yunque (St_1) or Mirassol (St) and *D. milleri* from El Yunque (Mi). It also shows data on the intraspecific crosses between *D. sturtevanti* from Mirassol and El Yunque, prepared for control. The results showed stronger isolation between sympatric strains than between allopatric ones. However, hybrids were produced in both cases.

Table II - Results of interpecific pair mating crosses.

Types of crosses F	M	Number of crosses	Fertile crosses Nº (%)	Dissected females	Inseminated females Nº (%)	Average number of progeny within 30 days	Fertility of F_1
St	Mi	94	20 (21.28)	63	9 (14.28)	15.60	Fert. FM
Mi	St	61	2 (3.28)	44	3 (6.82)	5.0	Fert. F; Ster. M
St	Mag	104	1 (0.96)	98	2 (2.04)	–	–
Mag	St	156	0	120	0	–	–
Mag	Mi	92	0	66	4 (6.06)	–	–
Mi	Mag	139	2 (1.44)	74	16 (21.62)	12.0	Fert. F; Ster. M

St = *D. sturtevanti;* Mi = *D. milleri;* Mag = *D. magalhãesi;* F = females; M = males; Fert. = fertile; Ster. = sterile.

Table III - Results of intraspecific pair mating crosses.

Type of crosses F	M	Number of crosses	Fertile crosses Nº (%)	Dissected females	Spermed females Nº (%)	Average number of progeny
Mi	Mi	124	77 (62.10)	23	0	52.34
St	St	167	112 (67.06)	33	6 (18.18)	60.22
Mag	Mag	60	28 (46.67)	18	0	58.71

For symbols, cf. Table II.

Table IV - Measures of isolation (in percentage) calculated from Table II.

Type of crosses F	M	Ti	Ii	Fi	Ai
St	Mi	78.72	67.48	11.24	87.72
Mi	St	96.72	90.13	6.59	
St	Mag	99.04	97.02	2.02	99.52
Mag	St	100.00	100.00	0.00	
Mi	Mag	98.56	77.25	21.31	99.28
Mag	Mi	100.00	93.93	6.07	

Ti = total isolation; Ii = isolation at the insemination level; Fi = isolation at the fertilization level; Ai = average isolation. See text for explanation.

Table V - Measures of isolation (in percentage) calculated from Table III.

Type of crosses F	M	Ti	Ii	Fi
Mi	Mi	37.90	37.90	0.00
St	St	32.93	26.94	5.99
Mag	Mag	53.33	53.33	0.00

For symbols, cf. Table IV.

Table VI - Results of interspecific crosses using sympatric and allopatric strains of *D. milleri* and *D. sturtevanti.*

Type of crosses F	M	Number of fertile crosses	Fecundity
St_1	Mi	1	+
Mi	St_1	0	–
St	Mi	2	++
Mi	St	1	+
St_1	St	2	+++
St	St_1	2	+++

For control, intraspecific crosses of *D. sturtevanti* strains are also included. Two mass crosses were prepared in every case. F = females; M = males. For symbols of strains, cf. Table I.

DISCUSSION

Reproductive isolation data on the sturtevanti subgroup were presented by Mourão and Bicudo (1967) in the same paper in which the species *D. magalhãesi* and *D. dacunhai* were described. The authors used reciprocal pair mating tests in two series of crosses involving D. *dacunhai, D. magalhãesi* and *D. sturtevanti* (*milleri* was not available at that time for study). In one of these series, *D. dacunhai* (from Kingston, Jamaica) and *D. magalhãesi* (from the State of Rio Grande do Sul, Brazil) were crossed to *D. sturtevanti* (from Brazil, Peru and Trinidad). In the other series, *D. dacunhai* (from Kingston, Ocho Rios and an unnamed place, all of them in Jamaica) was crossed to *D. sturtevanti* (from Costa Rica and Trinidad). In both series, the number of fertile crosses in a total of 100 performed in every type of cross was computed. The results showed a high degree of reproductive isolation between *D. dacunhai* or *D. magalhãesi* and *D. sturtevanti.* In the crosses between *D. sturtevanti* and *D. dacunhai* the isolation was stronger when *D. sturtevanti* females and *D. dacunhai* males were involved. In the crosses between *D. sturtevanti* and *D. magalhãesi,* the isolation was stronger when *D. sturtevanti* males and *D. magalhãesi* females were used. Mourão and Bicudo (1967) also intercrossed *D. magalhãesi* and *D. dacunhai.* The crosses involving *D. magalhãesi* females

and *D. dacunhai* males showed complete isolation while the reciprocal ones produced some fertile crosses.

In this paper, a more extensive study of reproductive isolation using *D. magalhãesi, D. sturtevanti* and *D. milleri* is presented. The availability of *D. milleri* was very useful; it made possible to check, by crossability, the evolutionary status of that species, previously established on a morphological basis in relation to the other members in the same subgroup.

The reproductive isolation was strong in every combination analyzed in this study. However it was stronger in *D. magalhãesi* x *D. sturtevanti* and *D. magalhãesi* x *D. milleri* than in *D. sturtevanti* x *D. milleri.* In the combination *D. sturtevanti* x *D. milleri* crosses in both directions were fertile, although with different degrees of fertility. In turn, the combinations *D. magalhãesi* x *D. sturtevanti* and *D. magalhãesi* x *D. milleri* exhibited a low fertility in a single direction of the crosses, in both cases involving *D. magalhãesi* males; the reciprocal crosses were completely sterile. The isolation indices showed Ii values greater than F_1 values in every case, indicating that the isolating mechanisms which prevent insemination are stronger than those which prevent the production of hybrids by inseminated females. The nature of the premating barrier was not analyzed but very probably sexual isolation is the mechanism acting to prevent insemination in these combinations. The action of mechanical isolation is less probable because the male genitalia are very similar in the 3 species studied.

Partial sterility of F_1 hybrids was another isolating barrier found between the 3 species analyzed. In the combination D. sturtevanti x D. milleri, male and female hybrids were fertile in the direction *D. sturtevanti* females x *D. milleri* males while males were sterile and females were fertile in the reciprocal crosses. The same direction of parental crosses which yielded less progeny (*D. milleri* females x *D. sturtevanti* males) also yielded sterile males, showing that the incompatibility of both species is greater when *D. milleri* females are used. Hybrid sterility was also observed in the intercrosses involving *D. milleri* females x *D. magalhãesi* males: male F_1 progeny was sterile while female progeny had low productivity.

The decreased productivity in the interspecific crosses when compared to intraspecific ones and also the detection of some inseminated females which did not produce descendants are indicative of other postmating mechanisms operating among the species studied, involving gametic isolation or hybrid inviability in very early stages of development.

The hybridization of allopatric and sympatric strains of *D. sturtevanti* and *D. milleri* in the laboratory was also focused upon in this study. Extensive data on reproductive isolation of *Drosophila* have shown complete isolation between sympatric strains of different species while allopatric strains present a variable degree of crossability. This was observed, for example, in the saltans subgroup, in crosses between strains of *D. prosaltans* and *D. saltans* (Bicudo, 1973). Complete isolation of sympatric strains is a mechanism which prevents interspecific hybridization in nature.

In the interspecific crosses *D. milleri* x *D. sturtevanti,* crossability was lower between sympatric strains than between allopatric ones. However, progeny was yielded both when sympatric and allopatric strains were used.

Hybrids have also been mentioned to occur, in the laboratory, in interspecific crosses involving sympatric strains of other *Drosophila* species, such as *D. milleri* and *D. arizonensis* (Bicudo and Richardson, 1978). Since natural hybrids of these strains have never been found, additional isolating mechanisms are presumed to operate in nature perhaps involving habitat selection which has been considered an important factor in reproductive isolation (for an example, see Parsons, 1977). *Drosophila arizonensis* and *D. milleri* explore different genera of cacti as oviposition sites and larval substrates. But, in the case of species in the saltans group, ecological data are very limited. *Drosophila sturtevanti* was reported to be bred from citrus (Heed, 1957), and several kinds of fallen fruits such as jaca, genipapo, mango and others (Dobzhansky and Pavan, 1950; Knapp, 1953; Pavan, 1959). Pipkin (1965, in Throckmorton, 1974) mentioned this species as being bred from fallen fruits and blossoms. On the other hand, artificial baits prepared with tomato, papaya, orange, watermelon and banana showed that *D. sturtevanti* prefers banana, followed by citrus (Mourão, 1966). However, as far as we know, no information of this kind on the *D. milleri* species is available. In spite of this, the possibility of habitat selection cannot be discarded.

An attempt was made to interpret the phylogenetic relationships among the 4 species in the *D. sturtevanti* subgroup already submitted to isolation studies. It followed Kaneshiro's (1976) assumption that females of ancestral species show strong sexual discrimination against males of the more derived species since these males exhibit only a part of the total courtship pattern of conspecific males.

In every combination with *D. magalhãesi* the isolation was stronger when *D. magalhãesi* females were used than in the reciprocal crosses. These results were observed in *D. magalhãesi* females x *D. sturtevanti* males and *D. magalhãesi* females x *D. milleri* males. Data presented by Mourão and Bicudo (1967) also showed similar results in the combinations *D. magalhãesi* females x *D. sturtevanti* males and *D. magalhãesi* females x *D. dacunhai* males.

Thus, based on Kaneshiro's hypothesis, *D. magalhãesi* should be considered to be the ancestral among the 4 species. On the other hand, in the crosses between *D. sturtevanti* and *D. milleri* the isolation was stronger in the direction involving *D. milleri* females while data reported by Mourão and Bicudo (1967) on crosses between *D. dacunhai* and *D. sturtevanti* showed stronger isolation when *D. sturtevanti* females were used.

The phylogenetic sequence of derivation based on the isolation patterns would be:

D. magalhãesi → *D. milleri* → *D. sturtevanti* → *D. dacunhai*

However, the wide geographical distribution of *D. sturtevanti* (Magalhães, 1962) and its degree of inversion polymorphism (18 heterozygous inversions were detected by Knapp, 1953) could indicate that this species is the ancestral in the subgroup. *Drosophila magalhãesi* located at the extreme south in the geographical area of the subgroup could be the result of a differentiation process in the limits of *D. sturtevanti* distribution. Besides, *D. milleri* and *D. dacunhai,* reported respectively in Puerto Rico and Jamaica could result from local changes in genetic composition in response to environmental conditions, probably under the influence of a founder event. Another interpretation, following the indication based on Kaneshiro's hypothesis, would be that *D. sturtevanti,* although more recent than *D. milleri* and *D. magalhãesi,* attained such a wide range (and perhaps displaced the other species within its distribution area) because it proved to be a very successful evolutionary experience in the subgroup.

The information available on the subgroup is not sufficient to allow a decision to be made between these two possibilities. A comparison of chromosomal arrangements between the species might be useful to clarify this point and will be done in a next step.

Another aspect worth analyzing in the sturtevanti subgroup and probably helpful to get new information on its speciation process concerns *D. sturtevanti.* The large distribution area of this species, which includes many of the Caribbean Islands and extends from Mexico to southern Brazil

on the mainland, apparently provides many different environments.Their demands could lead to changes in genetic composition under control of natural selection.Thus the detection of populations in the evolutionary status of semispecies, or even new species, would not be unexpected in a study using strains of different origins. Some incipient sexual isolation between *D. sturtevanti* strains from different regions has already been shown by Dobzhansky (1944). The higher isolation indices computed on preference mating were obtained in tests involving strains from Tamazunchale (Mexico) and Bertioga (Brazil), and strains from Quiriguá (Guatemala) and Belém or Bertioga (Brazil). The large distribution area plus the great number of flies in the collections apparently make this species unique in the saltans group for studies of genetic changes involved in the speciation process.

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THE FREEMAN – SHELDON SYNDROME

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ABSTRACT

A Brazilian child born to nonconsanguineous parents and presenting the typical features of Freeman-Sheldon syndrome (cranio-carpo-tarsal dysplasia) is described. Fragmentation of the sacral bone was also observed. Clinical and genetical aspects of the syndrome are discussed.

INTRODUCTION

Thirty four persons with a syndrome consisting of peculiar facial and skeletal anomalies have been reported since Freeman and Sheldon (1938) described two unrelated children affected by it. This rare genetic entity has received the following designations: cranio-carpo-tarsal dysplasia (Gorlin,

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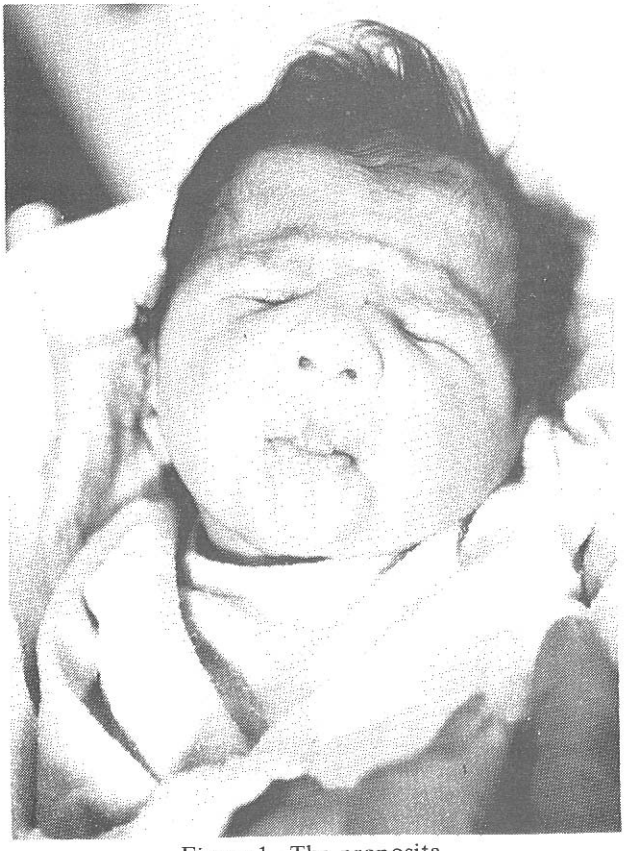
1969) chiro-cheilo-podalic syndrome (Pitanguy and Bisaggio, 1969), Freeman-Sheldon syndrome (Otto, 1953), whistling face deformity (Sharma and Tandon, 1970), whistling face–windmill vane hand syndrome (Walker, 1969). The nature of the basic defect is unknown, but the distribution of affected patients within the kindreds provides presumptive evidence that the majority of the cases are due to an autosomal dominant gene while others have autosomal recessive inheritance. The purpose of this paper is to discuss the clinical and genetical features of this syndrome, and to present a new patient, bearing its typical signs as well as fragmentation of the sacral bone.

CASE REPORT

The proposita, white, female, six days of age (Figure 1), born to nonconsanguineous healthy parents has a brother and a sister, both normal. Normal pregnancy, without exposure to X–rays or drugs. Delivery by pelvic presentation. She was acyanotic but did not cry immediately. Birth weight 2,570g, cranial, chest and abdominal circumferences, 34cm, 31cm and 30cm, respectively. She had been examined when 3 days old while showing difficulty in swallowing and fits of vomiting. When she was one month old, a new examination showed respiratory infection, which recurred until her death at 2 months, from aspirative pneumonia.

Physical examination – Cutaneous ridge in supra-orbitary regions, eyes deeply set, blepharophymosis, convergent strabismus, antimongoloid slants, epicanthus, small nose with broad nasal bridge, hypoplastic alae nasi, antiverted nose, large philtrum, microstomy, high arched palate, microglossy, micrognathism, prominent ears, nasolabial sulci visible only at the sides of the nose, chin with marked H-shaped sulci. Lips protruding as if in whistling. Face stiff and immobile. Long, broad fronto-dorsally compressed skull. Short and broad neck with cutaneous excess. Ulnar deviation of fingers, bilateral camptodactily and clinodactily of fifth fingers. Pterygium in the second phalanx of fifth fingers. Contracture of all fingers bilaterally. Severe bilateral club-foot. Normal thorax. Diastesis of abdominal recti muscles with umbilical hernia. Generalized hypotrophy, deep tendon reflexes decreased.

Blood tests – Ionogram, urine, glycosis, GOT, GPT, LDH, CPK, aldolase, alkaline phosphatase, thyroid hormones, normal.



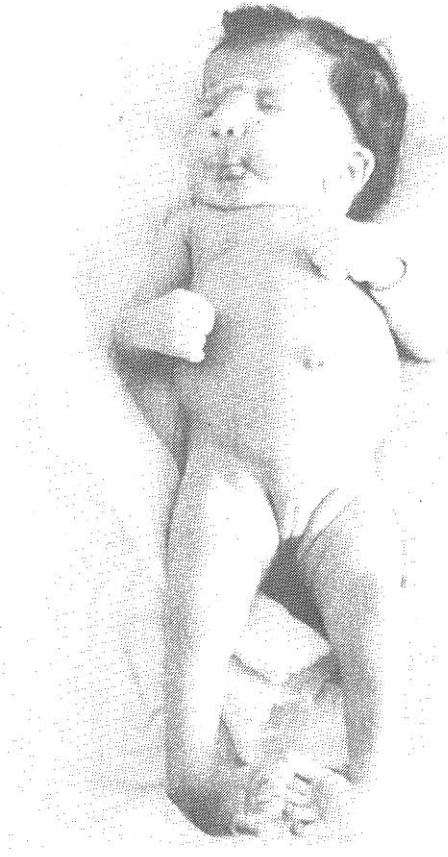


Figure 1 - The proposita.

X-rays – Kyphoscoliosis, bilateral club-foot, fragmentation of sacral bone, skull with a steep and flattened anterior cerebral fossa, cranio-facial disproportion, ulnar deviation of the fingers.

DISCUSSION

The main signs in the patients described by Freeman and Sheldon (1938) were microstomy, small nose, broad nasal bridge, hypertelorism, supra-orbitary ridge, enophthalmia, large philtrum, ulnar deviation of the fingers, contractures of the fingers and bilateral club-foot.

Table I presents the sign frequencies for the Freeman-Sheldon syndrome taken from 33 published cases plus the one described here. The percentages refer to the frequency of patients for which the sign was recorded among the 34 affected. Since, in a number of instances, no reference is made in the original reports to the presence or absence of a given sign, the frequencies in Table I must be considered to be slight underestimates. For instance, if one or more of the 7 patients for whom nothing is said about the nasal bridge actually had a broad nasal bridge the frequency of the sign would exceed 80 %. Moreover, if the cases in which no statement is made about the sign are disregarded, five of the signs included in the 60-80 % group in Table I would then have a frequency of more than 80 %. These are: broad nasal bridge, micrognathia, high arched palate, blepharophymosis and hypoplastic alae nasi. On the same basis, four other signs would all pass from the third to the second section in Table I. These are: antimongoloid slant, clinodactily, enophthalmia and short stature.

In spite of the variety of clusters in which the signs present themselves in different patients, there is no evidence of clinical heterogeneity among the 34 cases. Indeed, the variation of signs among the affected in different families does not seem greater than among affected members in a same family. For instance, Pfeiffer *et al.* (1972) reported an affected mother and daughter. All the important signs were present in the child but the mother showed no impairment in the feet, although she exhibited most of the facial and hand traits typical of the syndrome. In the Negro family described by Walker (1969) the main signs of the syndrome were present in the proposita, in her father and

Table I - Sign frequencies in Freeman–Sheldon syndrome.

Signs	*Present*	*Absent*	*Not stated*
Signs present in 80 % or more of the patients:			
Microstomia	31	2	1
Large philtrum	32	2	0
Small nose	30	3	1
Contracture of fingers	31	3	0
Ulnar deviation of fingers	30	4	0
Club foot	29	2	3
Signs present in 60 % or more but less than 80 % of the patients:			
Broad nasal bridge	27	0	7
Micrognathia	26	2	6
High arched palate	24	4	6
Blepharophymosis	23	4	7
Epicanthus	22	11	1
Puffy cheeks	22	9	3
H-shaped sulcus on the chin	22	7	5
Hypertelorism	21	9	4
Supra-orbital ridge	20	9	5
Hypoplastic alae nasi	20	4	10
Signs present in less than 60 % of patients			
Antimongoloid slant	19	7	8
Clinodactily	19	6	9
Enophthalmia	16	9	9
Kyphoscoliosis	14	10	10
Short and broad neck	10	14	10
Flattened anterior cerebral fossa	14	10	10
Convergent strabismus	12	11	11
Short stature	17	10	7
Difficulty in swallowing	9	8	17
Umbilical hernia	3	25	6
Spina bifida occulta	3	19	12
Low-set ears	2	23	9
Inguinal hernia	1	27	6
Fragmented sacrum	1	33	0

in her paternal grandmother, although only the proposita had kyphoscoliosis; but one of her sisters showed only camptodactily and other mild skeletal deformities as possible manifestations of the same dominant gene. Also in a family studied by Gross-Kieselstein *et al.* (1971) with mother and daughter affected, only the mother had kyphoscoliosis. In the family described by Pitanguy and Bisaggio (1969), one of the 5 persons affected (in three generations) lacked the following signs which were present in the other 4 affected: microstomia, high arched palate, blepharophymosis, large philtrum, ulnar deviation of the fingers and club-foot. One of the two affected brothers reported by Rosti (1971) lacked epicanthus, enophthalmia and short neck, although such signs were present in the other. In the family described by Alves and Azevêdo (1977) with two sibs affected, the girl lacked the following signs which were present in the brother: kyphoscoliosis, spina bifida occulta, blepharophymosis, small nose, contracture of fingers and small stature. On the other hand, the boy lacked broad neck and low-set ears, which were present in the girl.

Genetical heterogeneity seems clearly indicated by the pedigrees, but no clinical distinction can be made between the probable autosomal dominant and autosomal recessive cases.

In relation to recurrence patterns, the 34 patients can be classified as follows:

A. Fifteen affected (6 males and 9 females) distributed among 5 families according to the autosomal dominant pattern in two or three sucessive generations (Walker, 1969; Pitanguy and Bisaggio, 1969; Fraser *et al.*, 1970; Gross-Kieselstein *et al.*, 1971; Pfeiffer *et al.*, 1972).
B. Four affected (3 males and 1 female) in 2 families with 2 sibs each from normal couples, one of which consisted of first cousins (Alves and Azevêdo, 1977) while the other was non-consanguineous (Rosti, 1971).
C. Fifteen isolated cases (7 males and 8 females: Freeman and Sheldon, 1938; Otto, 1953; Kültz, 1961; Burian, 1963; Cervenka *et al.*, 1970; Rintala, 1968; Weinstein and Gorlin, 1969; Sharma and Tandon, 1970; Pfeiffer *et al.*, 1972; Walbaum *et al.*, 1973; Sauk *et al.*, 1974; this report).

Assuming that the autosomal dominant and the autosomal recessive types are represented among the isolated cases in the same proportion as among the 7 families where recurrence was observed, it can be estimated that 5/7 of 15, or 11 of the isolated cases are of the dominant type (fresh mutation)

and 2/7 of 15, or 4 cases, are of the recessive type. All told, there would be 16 families with the dominant form and 6 families with the recessive type. Therefore, in the presence of a new isolated case, such as ours, counseling may be given as follows:

There is an a priori probability of 6/22 or 0.27 for the condition to be recessive and 0.73 for it to be dominant. Since the recurrende risk is 0.25 in the case of a recessive gene and zero in the case of a dominant gene, the overall recurrence risk becomes 0.27 x 0.25 = 0.07 for a new sib of the affected to exhibit the syndrome.

As for a future child of the affected, the risk is 0.5, if the gene is dominant, and practically zero (if he does not marry consanguineously),if it is recessive. Therefore, the overall risk is 0.73 x 0.05 = 0.365.

In conclusion, the risk for a sib of the affected is small (7 %), but the risk for a child of the affected is large (about 37 %).

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SEX DETERMINATION IN BEES. VIII. RELATIVE ACTION OF GENES *xa* AND *xb* ON SEX DETERMINATION IN *MELIPONA* BEES*

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ABSTRACT

Three hypotheses on the function of the *xa* and *xb* genes in *Melipona quadrifasciata* were tested: 1) *xa* and *xb* may act complementarily in the production of juvenile hormone (J.H.), while the remaining genes are quantitative. An increase in the percentage of queens would correspond to each increase in J.H. 2) *xa* and *xb* produce the same type of J.H. 3) *xa* and *xb* independently produce two types of J.H.; in this case, the J.H. may be chemically and/or quantitatively a) equal or b) different. By analyzing the data obtained with various doses and different types of juvenile hormone, we obtain a type of segregation which favors hypothesis 3b. This hypothesis was confirmed by an experiment of queen exchange between two subspecies (*anthidioides* and *quadrifasciata*). A scheme for a possible network of genic programming and control is presented.

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INTRODUCTION

The genetic and environmental factors which contribute to sex determination in bees have been extensively studied by our group in a series of recent publications (cf. Campos *et al.*, 1975, for paper VI in this series). The salient features of these results are: a) there is a series of maleness and femaleness-determining genes whose balance in haploids or diploids produces male or female phenotypes (Garófalo, 1973; Garófalo and Kerr, 1975; Chaud-Netto, 1975; Chaud-Netto and Duarte, 1975); b) these genes are divided into two sets: one that acts on the early embryonic stages and determines ovaries or testes (Kerr and Nielsen, 1967; Kerr, 1975), and another that acts on the prepupal stage and determines the femaleness or maleness of the imaginal discs and tegument (Kerr, 1974 a and b, 1975; Camargo, 1974, 1976); c) the genes that control the determination of ovaries or testes are different from, and independent of, those that determine female or male tegument and imaginal discs development (Camargo, 1976). The antennae and genitalia are determined by the same events that control formation of testes and ovaries (Camargo, 1977); a tentative model to explain the genic control, based on the hypothesis of Britten and Davidson (1969, 1971), was proposed by Kerr in 1973 (Kerr, 1975) and slightly modified in 1974 (Kerr, 1974 b). After the demonstration that juvenile hormone is produced by the cells of *corpora allata* under the control of *xa* and *xb* genes, the model was again modified (Kerr *et al.*, 1975). This model obtained important experimental support from the studies of Campos (1975) and Campos *et al.* (1975), who were able to obtain 100 % queens by externally treating whole prepupal larvae with juvenile hormone analogues.

This model for sex determination in bees (Kerr *et al.*, 1975) suggests that two *Integrator* (I) genes, *xa* and *xb*, activate only one *Receptor* (R) and only one *Producer* (P) gene.

The experiments described here were designed to test three different hypotheses concerning the action of genes *xa* and *xb*:

(1) There is no *Receptor*. Genes *xa* and *xb* act complementarily and the factors involved in the production of cells and juvenile hormone are multifactorial. Therefore, an increase in the percentage of queens will correspond to each increase in juvenile hormone.

(2) *xa* and *xb* control the same *Receptor*. Under this hypothesis, an

xa^1/xa^2; xb^1/xb^2 queen crossed with an xa^2; xb^2 male, for example, would produce the following offspring: 1) poorly fed: workers only; 2) well-fed: 3 workers (xa^1/xa^2; xb^2/xb^2, xa^2/xa^2; xb^1/xb^2, xa^2/xa^2; xb^2/xb^2) to 1 queen (xa^1/xa^2; xb^1/xb^2); 3) with increasing doses of juvenile hormone analogue (JHA): at a certain dose, 25% queens (xa^1/xa^2; xb^1/xb^2); after a certain threshold, 100 % queens.

(3) *xa* and *xb* control one *Receptor* each. We must consider 2 cases under this assumption: a) xa^1/xa^2 and xb^1/xb^2 are equally efficient in producing juvenile hormone. Considering the same cross mentioned above (xa^1/xa^2; xb^1/xb^2 X xa^2; xb^2), three different conditions may exist: 1) poorly fed: 0 % queens are obtained; 2) well-fed: 25 % queens are obtained; 3) with gradually increasing doses of juvenile hormone or juvenile hormone analogue (JHA) the following will happen: a peak corresponding to 25 % queens (xa^1/xa^2; xb^1/xb^2), another peak corresponding to 75 % queens (xa^1/xa^2; xb^1/xb^2, xa^1/xa^2; xb^2/xb^2, xa^2/xa^2; xb^1/xb^2) and one around 100 % (all four genotypes); b) xa^1/xa^2 is superior to xb^1/xb^2 either because it produces juvenile hormone (JH) in greater amounts than xb^1/xb^2, or because it produces a more efficient JH. Again, we would obtain different results in different circumstances: a) poorly fed: 0 % queens; b) well-fed: 25 % queens xa^1/xa^2; xb^1/xb^2, 50 % queens (xa^1/xa^2; xb^1/xb^2, and xa^1/xa^2; xb^2/xb^2), 75 % queens (xa^1/xa^2; xb^1/xb^2, xa^1/xa^2; xb^2/xb^2, and xa^2/xa^2; xb^1/xb^2), and finally 100 % queens. A summary of these hypotheses is shown in Table I.

MATERIAL AND METHODS

The species studied was the social Apidae stingless bee, *Melipona quadrifasciata* Lep.

Three approaches were followed: 1) Analysis of all published and unpublished data concerning pupae treated with JHA; 2) 8 groups of pupae were treated with the following regimen of juvenile hormone: 19 pupae with 0.043 μg ; 23 pupae with 0.054 μg ; 45 with 0.065 μg ; 23 with 0.081 μg ; 10 with 0.129 μg; 20 with 0.162 μg ;14 with 0.323 μg; and 110 pupae with 0.647 μg . The hormone used was *Hyalophora cecropia* juvenile hormone. 3) To check whether different subspecies produce different amounts of juvenile

Table I - Summary of experiments to test three hypotheses for sex determination in diploid bees. The asterisks represent the genotypes that will become queens. All offspring were treated with increasing quantities of JH (as indicated by the four arrows) and are the offspring of a normal $xa^1/xa^2; xb^1/xb^2$ queen mated with one $xa^2; xb^2$ drone.

GENOTYPE OF OFFSPRING	NO RECEPTOR (QUANTITATIVE EFFECT) Hypothesis Nº 1			One Receptor Hypothesis Nº 2			TWO INDEPENDENT RECEPTORS Hypothesis Nº 3								
							$xa^1/xa^2 = xb^1/xb^2$				$xa^1/xa^2 \neq xb^1/xb^2$				
$xa^1/xa^2 ; xb^1/xb^2$		*	more	*	*	*		*	*	*		*	*	*	*
$xa^1/xa^2 ; xb^2/xb^2$			queens			*			*	*			*	*	*
$xa^2/xa^2 ; xb^1/xb^2$			with			*			*	*				*	*
$xa^2/xa^2 ; xb^2/xb^2$			more JH			*				*					*
Percentages of queens expected	0	25%	25% - 100	0	25%	100%	0	25%	75%	100%	0	25%	50%	75%	100%
Increasing dosages of JH	→			→			→				→				

hormone two subspecies were brought to Ribeirão Preto, namely, *Melipona quadrifasciata quadrifasciata*, from Florianópolis, SC, Southern Brazil, and *Melipona quadrifasciata anthidioides* from Pocinhos do Rio Verde, Minas Gerais, Central Brazil. These sites are 800 km apart; the queens of the two colonies were exchanged, i.e. *quadrifasciata* workers received an *anthidioides* queen and vice-versa.

RESULTS

First Approach – Fig. 1 shows an analysis of previously published (Campos *et al.*, 1975) and unpublished (L.A.O. Campos, unpublished data) cases of treatment of female pupae with juvenile hormone and juvenile hormone analogues. Each square in the graph represents a different sample. The number in parentheses indicates the treatment received (see legend to Fig. 1). The top number on the right side is the number of workers produced, the middle number is the number of queens produced. The lower number represents dead and/or too defective individuals.

The abscissa is divided into 10 parts, and the samples entered according to the percentage of queens. The distribution of the percentage of queens produced by hormone treatment has four peaks: around 0, around 50 %, around 75 %, and around 100 %.

Second Approach – Treatment with different amounts of *Hyalophora cecropia* juvenile hormone. The results obtained are shown in Table II.

Table III was obtained by grouping the samples of each treatment.

The data in Table III were submitted to the following analysis:

Testing hypothesis 1 – For each increased dose of JH we should have an increase in the percentage of queens produced. We would then expect a strong correlation between these two variables. The correlation coefficient, $r = 0.68$, was not significant. The percentage of each sample was balanced with its total number (queens + workers). This result indicates that hypothesis 1 is not the mode of action of the genes controlling caste in these bees. If the correlation is estimated in two groups, lst to 4th dose and 5th to 9th dose, there is high correlation in the first groups and no correlation at higher doses.

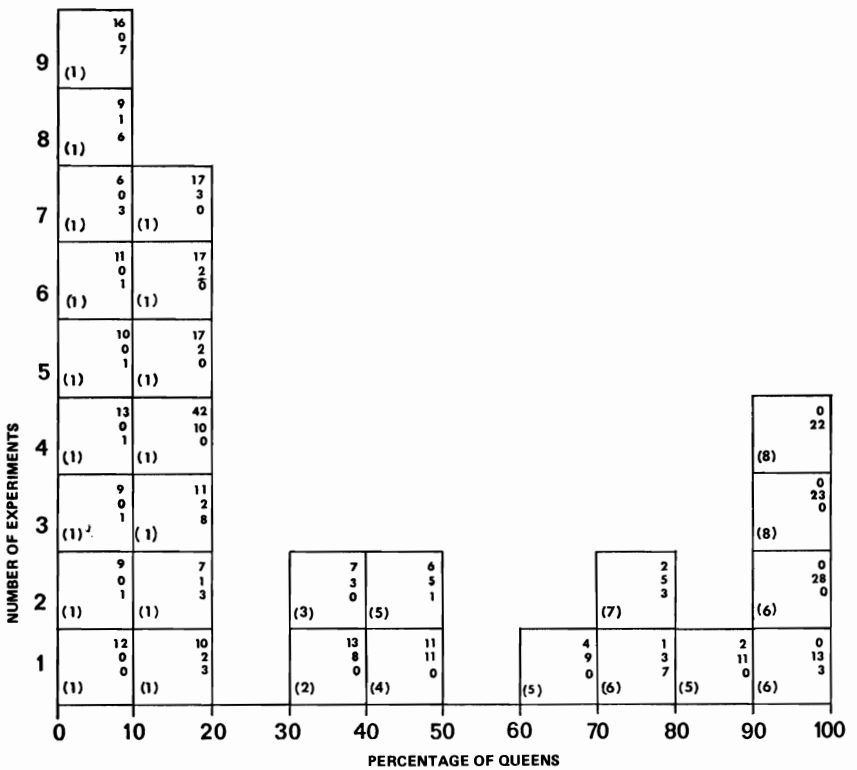
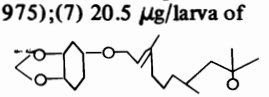


Figure 1 - Effect of juvenile hormone and related compounds on yield of queen bees. The data summarize 29 experiments reported in the literature (Velthuis and Velthuis-Kluppell, 1975; Velthuis, 1976; Campos *et al.*, 1975) and by Campos (unpublished results). Independent samples are given on the ordinate, and the abscissa gives the percentage of queens based on the total number of survivors. The three vertical numbers (on the right side of each individual square) indicate: number of workers (upper); number of queens (middle); number of dead or very defective bees (lower). The numbers in parentheses, on the left side of each square, indicate the following treatments: (1) Acetone (control); (2) 18 μg Altosid-R per larva; (3) 3.26 μg/larva of product 39 of Dr. Bowers; (4) 0.0000002 μg Hoffman mixture per larva (Campos *et al.*, 1975); 9 μg Altozar-R per larva (Campos *et al.*, 1975); (7) 20.5 μg/larva of



also given to us by Dr. W. Bowers; (8) 0.002 μg Hoffman mixture per larva (Campos *et al.*, 1975).

Table II - Larvae of *Melipona quadrifasciata* treated with *Hyalophora cecropia* juvenile hormone.

Samples	Treatment in μg of JH	Number of Treated Larvae	Number of Workers Produced	Number of Queens Bees	Dead or Deformed	% of Queens
1	0.054	8	7	0	1	(0.0)
2	0.065	22	12	3	7	(13.6)
3	0.043	23	15	4	4	(17.4)
4	0.065	10	6	2	2	(20.0)
5	0.081	23	5	8	10	(34.8)
6	0.129	17	4	6	7	(35.3)
7	0.161	20	2	8	10	(40.0)
8	0.054	22	7	9	6	(40.9)
9	0.647	20	1	9	10	(45.0)
10	0.647	30	5	14	11	(46.7)
11	0.065	17	1	8	8	(47.1)
12	0.647	8	3	4	1	(50.0)
13	0.323	20	4	10	6	(50.0)
14	0.647	17	5	9	3	(52.9)
15	0.647	22	7	12	3	(54.5)
16	0.054	14	3	10	1	(71.4)
17	0.647	38	1	28	9	(73.7)
18	0.647	14	1	11	2	(78.6)
19	0.081	11	1	9	1	(81.8)
20	0.161	11	0	10	1	(90.9)

Table III - Effect of applying different doses of *Hyalophora cecropia* juvenile hormone to *Melipona quadrifasciata* larvae

H. cecropia Juvenile Hormone dose in μg	Number of Workers Produced	Number of Queens Produced	Total Number of Larvae	% Production of Queens Survivals	% Production of Queens Based on Treated Larvae
0	123	7	164	5	4.2
0.043	15	4	23	21	17.3
0.054	14	9	30	39	30.0
0.065	22	23	63	51	36.5
0.081	6	17	34	78	50.0
0.129	4	6	17	60	35.3
0.161	2	18	31	90	58.1
0.323	4	10	20	71	50.0
0.647	23	87	149	79	58.4

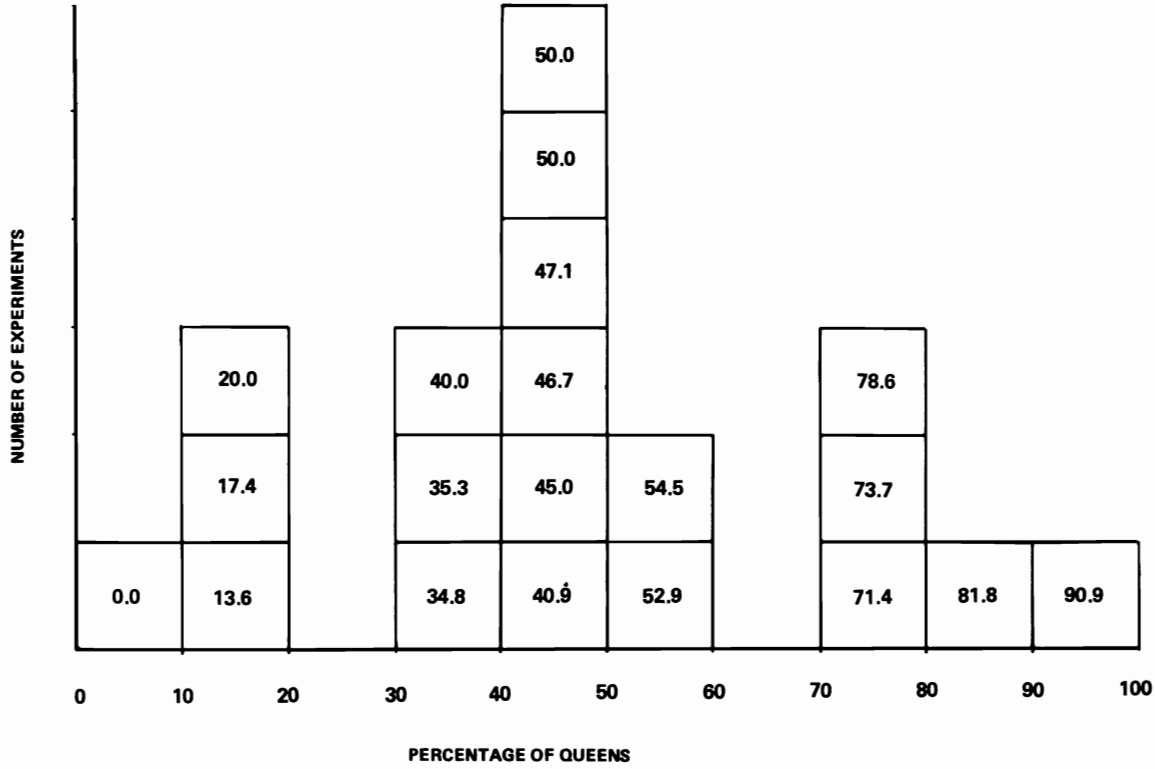


Figure 2. Distribution of the percentage of queens after treatment with *Hyalophora cecropia* juvenile hormone. The number in each box is the percentage of queens related to the total number of larvae treated. The data in the Figure describe 20 independent experiments.

Testing hypothesis 2 – According to this second hypothesis, two groupings should be found: one around 25 %, and another around 100 %. Any cluster of all the data around 25 % and 100 % would produce a highly significant result; therefore, hypothesis 2 is eliminated.

Testing hypothesis 3 – This hypothesis assumes two independent receptors. Each should be looked at from the point of view of two possible genetic effects: $xa^1/xa^2 = xb^1/xb^2$, and $xa^1/xa^2 \neq xb^1/xb^2$.

1)– Effect of xa^1/xa^2 equal to xb^1/xb^2. In this case the data should cluster around 2 peaks: 25 and 75 %. One possibility is: 29 (= 15 + 14): 13 (= 4 + 9) which, when tested to see whether it fits a 3:1 segregation, produces a $\chi^2 = 0.8$ (P = 0.37), and, if the rest of the data are tested against 1:3, we would have 61:161, which produces a $\chi^2 = 0.64$ (P = 0.42).

2)– xa^1/xa^2 has a different effect than xb^1/xb^2. Let us suppose that xa^1/xa^2 determines the production of either a greater amount of JH or a more efficient juvenile hormone. Peaks of queen percentages should be around 25, 50 and 75. The χ^2 is slightly lower than in the preceding case and there is one more degree of freedom. However, the data are insufficient to test wether or not xa^1/xa^2 has a different effect than xb^1/xb^2.

Third Approach – Exchange of queens between two subspecies of *Melipona quadrifasciata.* As stated in Methods, the queens of two colonies of subspecies, *quadrifasciata* (colony 65) and *anthidioides* (colony 63) (the first subtropical and the second tropical) were exchanged, with the *anthidioides* queen being treated by *quadrifasciata* workers and vice-versa. The idea behind this experiment is that each species and subspecies has its own production of juvenile hormone, with possibly wide differences due either to hypopharingeal glandular secretion or to the species of pollen utilized.

The results presented in Table IV, show that both controls gave the expected segregation: 3 workers to 1 queen. However, in spite of the fact that the weight of the prepupae was statistically similar for control 1 and experiment 1, experiment 1 shows a decrease in the production of queens unexplained by loss of weight.

Table IV - Number and mean weight of queens and workers obtained in the experiments where the queens of two colonies, one from *M. anthioides* and the other from *Melipona quadrifasciata quadrifasciata*, were exchanged.

Treatment	Number and Mean Weight of Queens	Number and Mean Weight of Workers	% of Queens
Control 1 - *anthidioides* workers and *anthidioides* queen	7 86.6 mg	18 86.2 mg	28.00
Control 2 - *quadrifasciata* workers and *quadrifasciata* queen	14 87.5 mg	39 87.9 mg	26.42
Exp.1 - *anthidioides* queen treated by *quadrifasciata* workers	30 92.7 mg	170 85.8 mg (29 workers heavier than 92.8)	15.00
Exp.2 - *quadrifasciata* queen treated by *anthidioides* workers.	95 92.6 mg	140 90.4 mg (43 workers heavier than 92.6)	47.74

Even more striking is the increase in the proportion of queens in experiment 2. These data were waiting for an explanation since May, 1971. Our question was: how could a segregation of 3:1, under these experimental conditions and with the same queen and the same male go to 1:1 ? It is our present understanding that the juvenile hormone naturally produced by the bees or found in the pollen of the plants in their environment was enough to raise the production of xa^1;xa^2 to the level necessary to activate the femaleness genes. Therefore, this experiment gives weight to the hypothesis that implies

two independent batteries of genes *xa* and *xb*, with two different and independent receptors, with xa^1/xa^2 being more effective (either quantitatively or qualitatively) than xb^1/xb^2.

CONCLUSION

Seven of the thirteen papers recently published by our group on sex determination in bees constitute an attempt to clarify the genic control of the sex and caste mechanism in bees, especially *Melipona* bees (Kerr, 1974 a and b, 1975; Campos, 1975; Campos *et al.*, 1975; Kerr *et al.*, 1975; Camargo *et al.*, 1976). They are primarily based on the observation made by Kerr in 1973 (in Kerr, 1974 a) that workers of stingless bees resemble more the males than the females in their external morphology, and that this implies a genic control that stops the action of (or does not activate) femaleness genes in the imaginal discs and tegument. Recent papers by Camargo (1976, 1977) showed that the *xo* genes which determine ovaries and the *xa* and *xb* genes are not alleles. All this demonstrates that two independent sets of genes are involved in sex control: one that acts on the embryo and another that acts on the prepupa. The present publication provides evidence that the producers of juvenile hormone in *Melipona quadrifasciata* belong to two different batteries and, according to previous results, especially those of Campos (1975, 1977), these juvenile hormones "open" the femaleness gene or genes. A likely mechanism, slightly modified from Kerr *et al.*, 1975, is shown in Fig. 3.

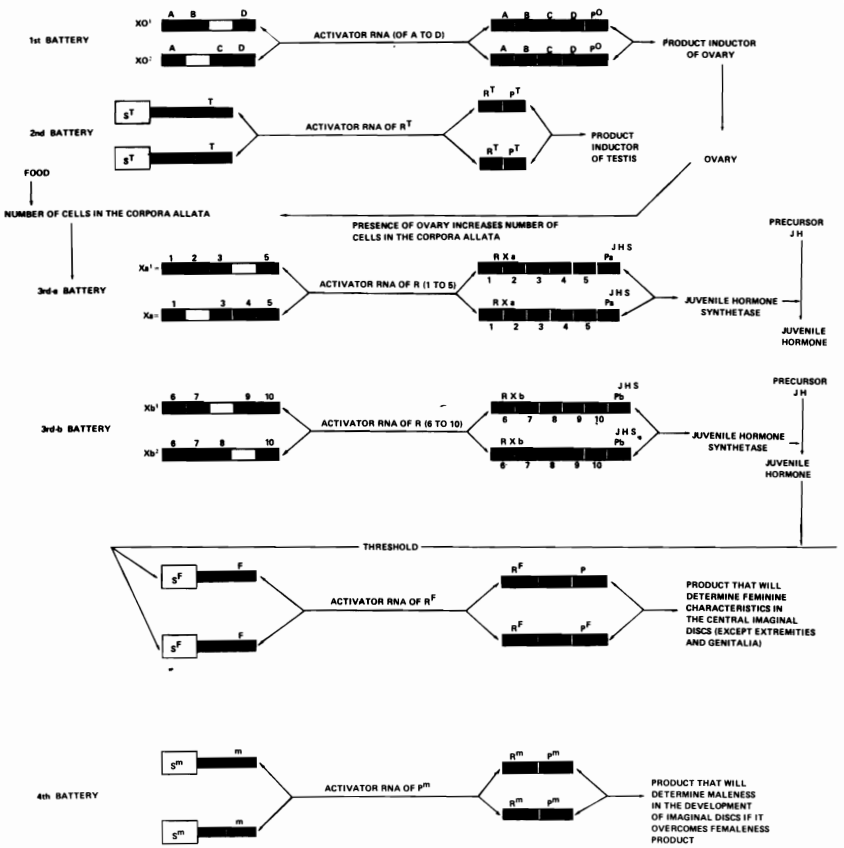


Figure 3. Sex determination in diploid bees. Batteries 1 and 2 show ovary determination (diploid) or testis determination (haploid) by gene *xo*, which in *Apis mellifera* has about 20 multiple alleles. Batteries 3 (a and b) and 4 indicate production of juvenile hormone and its activating action on femaleness genes.

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GENETIC EFFECTS ON REPRODUCTION IN CANCHIM CATTLE

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ABSTRACT

Individual records on the reproductive performance of a herd of Canchim Cattle (5/8 Charolais – 3/8 Zebu) at São Carlos, SP, Brazil, were evaluated for genetic and environmental influences on age at first calving, calving interval, pregnancy rate and calf survival through parturition. The mean for age at first calving was 1388 ± 14.5 days (or 45.7 ± 0.5 months) with an h^2 of 0.16 ± 0.13. Year and sire affected ($P < 0.01$) the age at first calving. The average calving interval was 616 ± 8.7 days, or 20.3 ± 0.3 months, with a negative estimate of h^2. Year and order of parturition affected calving interval ($P < 0.05$), but sire effect was not significant. The average pregnancy rate was 69 ± 1.1. %, being affected by age of cow and year of record ($P < 0.01$). Sires significantly influenced the pregnancy rate of their daughters ($P < 0.01$), with h^2, estimated directly on binomial data (0 or 1), equal to 0.10 ± 0.09. Probit transformation to normal distribution resulted in a heritability estimate of pregnancy rate of 0.17 ± 0.15. Mean calf survival from diagnosis of pregnancy through parturition was 95 ± 0.9 %. Year had a significant influence on calf survival ($P < 0.05$). Sire effects on calf survival were significant ($P < 0.05$),

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yielding an h^2 estimate of 0.27 ± 0.29, based directly on coded (0 or 1) observations. Probit transformation increased this value to an unrealistic estimate of 1.20 ± 1.29.

INTRODUCTION

Reproductive performance of cattle in the tropical and subtropical areas of the world generally is suboptimal with age at first calving, subsequent calving rate and calf survival all being involved. Age at first calving has been reported to be frequently above 40 months (Carneiro *et al.*, 1961; Guha *et al.*, 1968; Nagpal and Acharya, 1970; Prasad and Prasad, 1972; Lemka *et al.*, 1973; Oliveira Filho, 1974), with earlier first calving reported for some crossbreds (Kaul *et al.*, 1973; Madsen and Vinter, 1975). Subsequent annual calving rates usually are below 80 % (Wiltbank *et al.*, 1961; Donaldson, 1962; Tundisi *et al.*, 1962; Warnick *et al.*, 1969; Deese and Koger, 1967; Wagan and Nair, 1971; Linares *et al.*, 1974). Willis and Wilson (1974) reported higher rates from Cuba along with Villares (1975) from Brazil. Calf losses due to abortions, stillbirths and neonatal death losses vary widely with conditions. Mitchell (1966) reported these losses to average 8.0 % in experimental herds in Florida.

Limited studies only have been made to assess the opportunities for genetic improvement of reproductive performance of cattle under tropical and subtropical conditions. Reports from crossbreeding trials reviewed by Koger *et al.*, (1973) indicated that exploitation of hybrid vigor through crossbreeding is an effective procedure. The technology required to make sustained controlled matings, however, frequently limits the usefulness of this practice. Controlled studies assessing the effectiveness of selection within intermating composite populations in retaining this advantage are few and inconclusive (Franke, 1973). Heritability estimates for age at first calving in tropical cattle vary from .08 by Prasad and Prasad (1972) to .94 by Tomar and Arora (1972) with intermediate values predominating. Heritability estimates for calving rate based on calving intervals generally have been low (Lindley *et al.*, 1958; Davenport *et al.*, 1959; Dearborn *et al.*, 1973; Fabregue and Gastinel, 1975; Sularsassa, 1977). It should be noted, however, that utilizing calving intervals as an observation automatically eliminates data from

low-fertility cows with less than two calving records and that reproductive failures following last calving generate no records. This results in estimated reproduction rate being biased upward while heritability estimates would tend to be biased downward. Estimates of heritability for calving rate in Zebu and crossbred type cattle in Florida, based on annual records of one (1) or zero (0), have varied from .38, obtained from the regression of lifetime record of the daughter on that of the dam (Deese and Koger, 1967), to .63 from half-sib analysis of records from first exposure heifers.

The objective of the present study was to evaluate genetic and environmental influences on age at first calving, calving interval, pregnancy following exposure for a season of four months and survival of calf from pregnancy diagnosis through parturition.

MATERIAL AND METHODS

The data for this study were obtained from the Ministry of Agriculture Canchim Farm Research Unit, São Carlos, SP, Brazil, located at latitude 22°01'S, longitude 47°53'W, at an average altitude of 854 m. The climate is tropical with the dry season occurring during the winter months. The average annual rainfall is 1,520 mm, concentrated mostly during the months of November through February.

The Canchim breed was derived from a cross between Charolais (*Bos taurus*) and Brazilian Zebu (*Bos indicus*) cattle with breed proportions fixed at 5/8 and 3/8, respectively. The breed was developed by the Brazilian Ministry of Agriculture with the objective of obtaining more productive cattle for the region by combining the growth and carcass characteristics of the Charolais with the tropical adaptation and foraging ability of the Zebu.

The data presented here were collected from 1958 through 1974 during which time management procedures and pastures remained relatively stable. Pasture forages included *Digitaria decumbens* Stent and *Paspalum notatum* Fluge without fertilization. The cattle remained on the pastures throughout the year without supplement. The herd health program included vaccination against foot-and-mouth disease, symptomatic anthrax and brucellosis. Anthelmintics were administered sporadically to young animals. Ectoparasites were controlled by dipping all animals monthly.

The mating season for most of the herd extended from October through January during the flush of grass growth following the spring rains. This resulted in births being concentrated during the months of July through September. However, a small number of matings were also made during other seasons. All matings were made in single-sire herds of 20 to 30 cows to assure known paternity.

Calves were weighed and identified within 24 hours following birth. All cattle were weighed at 30-day intervals. Calves were weaned at 7 to 8 months of age. Complete individual records were maintained for each cow. Values for cow performance traits were computed from these records as follows:

(1) Age at first calving (days).
(2) Interval between successive calves (days).
(3) Pregnancy status following the mating season.
Codes of one (1) or zero (0) were assigned to designate pregnant and non-pregnant cows, respectively.
(4) Calf survival codes of one (1) were assigned for calves which successfully survived birth while codes of (0) were assigned for abortions, stillbirths and calves failing to survive parturition.

The data for these traits were analyzed by routine least squares techniques (Harvey, 1960, 1972). The models employed were determined following preliminary analyses, with most nonsignificant environmental effects being eliminated from the final models indicated in Tables I, III, IV and VI.

RESULTS AND DISCUSSION

Age at first calving

Records from 324 cows by 21 known sires were available for study. The least squares mean for age in days at first calving was 1388 ± 14.5, or 45.7 ± 5 months. The only environmental effect recorded that was significantly associated with this trait was that of year.

The effect for season was nonsignificant and was deleted. The effects for sires and years are summarized in Figure 1 and Table I.

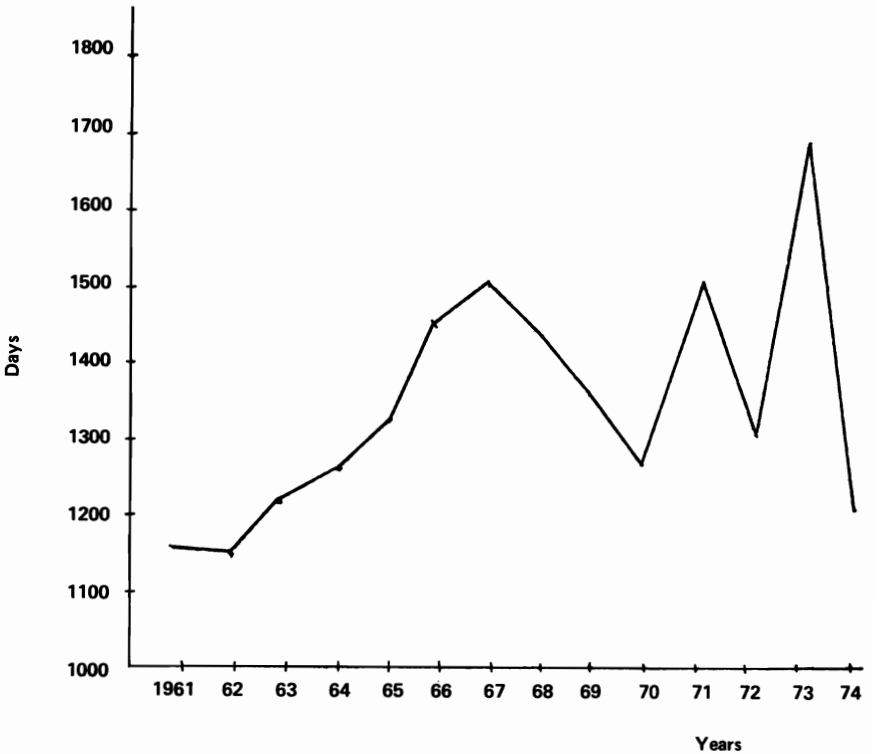

Figure 1 - Age at first calving according to the year of birth of the dam.

Table I - Analysis of variance for genetic and non genetic effects on age at first calving of Canchim heifers.

SOURCES OF VARIATION	D.F.	MEAN SQUARES	F
Year	13	364,992	7,9680*
Sire	20	95,894	2,0934*
Remainder	290	45,807	
Total	323		

*P > 0.05

The absence of within-year seasonal effects but positive effects for years are in general agreement with Guha *et al.* (1968), working with Hariana cattle in India, Nagpal and Acharya (1970), with Sahiwal cattle in India, and Lemka *et al.* (1973), reporting on cattle from both India and Colombia. Madsen and Vinther (1975), working with milking Zebu and their crosses in Thailand, reported significant effects for both year and season. Divergent results were reported by Oliveira Filho (1974) working with Nellore cattle in Brazil where seasonal effects were present ($P < .05$) but year effects were not. Variation in environmental effects from one situation to another are to be expected. In the present study there was an apparent tendency for favorable climatic conditions to be followed by a reduced age at calving and vice-versa.

Heritability of age at first calving estimated from the half-sib analysis in this study was .016 ± .13. Similar low estimates have been reported by Redy and Bhatnagar (1971), Dass *et al.* (1971), Dutt and Singh (1972) and Kaul *et al.* (1973). Lôbo (1976), working with 5/8 Red Poll – 3/8 Guzera (Pitangueiras) cattle in Brazil, reported h^2 of the trait to be .24 ± .21. Estimates above .40, however, were reported by Singh *et al.* (1968) Tomar and Arora (1972), Nagpal and Acharya (1970), Naidu and Desai (1970) and Kaul *et al.* (1973).

These and other results, along with common experience, show that age at first calving is a trait strongly influenced by nutrition and environmental effects which may mask genetic potential. Thus, it is not susprising that, under the harsh conditions of this trial, heritability of age at first calving would be relatively low. The response emphasizes the point that genetic improvement must be accompanied by conditions which permit the expression of genetic potential if maximum progress is to be achieved. That measurable heritability was exhibited under the conditions of this trial is encouraging evidence that some genetic progress can be made even under unfavorable conditions.

Calving interval

Calving interval frequently is used as a measure of reproductive rate. It is characterized by a continuous although not necessarily normal distribution. A total of 682 intervals computed from 1634 calving records were available for analysis in this study. This reduction in observations from converting single records to intervals emphasizes the point that the procedure eliminates the records of most low fertility cows and records of terminal reproductive failures from the data set.

Order of parturition was the most important variable influencing calving interval ($P < .01$). Mean values are shown in Table II. The first interval was the longest with 666 days, declining steadily to 534 days for interval number 4, then increasing slightly to 548 days.

Year effects resulted in wide fluctuations ($P < .05$) with long intervals tending to be associated with periods where conditions were unfavorable for forage production (Table III).

The half-sib analyses resulted in an estimated negative value for the sire component and heritability of calving interval. These results agree generally with those of other studies employing calving intervals as observations, including Brown *et al.* (1954), Lindley *et al.* (1958), Dunbar and Henderson (1953), Prasad and Prasad (1972), Alim (1972) and others.

Table II - Calving interval according to the order of parturition.

INTERVALS	NUMBER	%	MEAN (DAYS)	S.D.
1st - 2nd parturitions	287	42.1	666.2	238.4
2nd - 3 rd parturitions	172	25.2	618.3	222.8
3rd - 4th parturitions	102	15.0	561.5	212.5
4th - 5th parturitions	60	8.8	533.9	180.6
5th - 9th parturitions	61	8.9	548.4	205.7
TOTAL	682	100.0	616.3	228.3

Table III - Analysis of variance for genetic and non genetic factors affecting calving interval in Canchim cows.

SOURCES OF VARIATION	D.F.	MEAN SQUARES	F
Order	4	476,878	10.1802*
Year	11	141,508	3.0208*
Sire	20	26,746	0.5710
Error	512	46,843	
Total	557		

*P > 0.05

Pregnancy rate

The average pregnancy rate resulting from 1634 exposures to bulls in this study was 69.8 ± 1.09 %. This value is in the intermediate range of pregnancy rates reported from tropical areas. The analysis of variance for the data is shown in Table IV. Significant effects ($P < .01$) were found for sire of cow, cow within sire, year of record and age of cow.

Pregnancy rate by age of cow (Table V) was highest among first-exposure heifers (73.2 %) and declined linearly to a low of 46.1 % for cows 13 years of age. This is a departure from the pattern observed by Temple (1966) among cattle of the Southeastern United States where cows of intermediate ages had the highest reproductive rate. The reason for declines over age observed in the present study is not known but may have been the result of gradual depletion of nutrients from the bodies of producing cows under hard conditions.

The estimate for heritability of pregnancy rate computed directly from the binomial (0,1) data was .10 ± 09. Probit transformation increased these values to .17 ± .15. Deese and Koger (1967), utilizing similar procedures, reported h^2 estimates of .39 ± .21 for crossbred cattle. Cruz (1972) reported heritability estimates of calving rate in Brahman heifers to be .25 ± .17 and that for lactating cows in their second reproductive season to be .09 ± .14.

Table IV - Analysis of variance of conception rate in Canchim cows.

SOURCE	D. F.	MEAN SQUARES	F
Sire	53	.3646	1.65*
Dam/Sire	462	.2212	1.25*
Season	1	.6381	3.61
Year	16	.8445	4.78*
Breed of Bull	3	.2432	1.38
Age of Dam, linear	1	22.2502	12.74*
Age of Dam, quadratic	1	.1756	.99
Remainder	1096	.1757	

*$P > 0.05$

Table V - Conception rate of Canchim cows in different age groups

Age group	Nº of exposures	Nº of conceptions	Conception rate
Up to 3.9 years	438	321	73.2 %
4 to 6.9 years	636	456	71.7 %
7 to 9.9 years	359	241	67.1 %
10 to 12.9 years	174	110	63.2 %
13 years and over	27	13	46.1 %
Total	1,634	1,141	69.8 %

Calf survival

Survival of the calf during gestation, birth and the nursing period is an important variable influencing net reproductive rate. Of 1141 pregnancies recorded in the present study, 95 ± .9 % were completed with the delivery of a surviving calf. These losses included 1 % by abortions and 4 % by death at or near birth. They compare favorably with those reported by Mitchell (1966) for experimental herds in Florida.

The analysis of variance for calf survival is shown in Table VI. The variables significantly influencing survival were sire of cow ($P < .05$), cow within sire ($P < .01$) and year ($P < .05$).

Table VI - Analysis of variance for successful pregnancies of Canchim cows.

SOURCE	D. F.	MEAN SQUARE	F
Sire	51	.1157	2.31*
Dam/Sire	394	.0501	1.23*
Season	1	.0002	.00
Year	16	.0652	1.60*
Breed of Bull	3	.0517	1.27
Age of Dam, linear	1	.0905	2.05
Age of Dam, quadratic	1	.0915	2.25
Remainder	673	.0406	

*$P > 0.05$

Heritability of calf survival was 0.27 ± 0.29 from the records, becoming 1.20 ± 1.29 after probit transformation. Repeatability estimate was 0.28 ± 0.04 and, after correction, 1.28 ± 0.16. Sampling error and improper method of analysis could be the reason for the discrepant heritability and repeatability estimates, and further studies are needed to provide more information on the subject.

The overall results of this study on some reproductive characters of Canchim cattle suggest that this herd is greatly influenced by environmental factors. The excessive dependency of reproductive performance on favorable climatic conditions could be alleviated by an adequate nutritional program, with supplementation in the dry season. Selection programs are somewhat jeopardized by the low heritability estimates, unless a simultaneous effort to diminish environmental variance is carried out.

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