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INDUCTION OF MALE STERILITY THROUGH MANIPULATION OF GENETIC MECHANISMS PRESENT IN VECTOR SPECIES OF CHAGAS DISEASE (REMARKS ON INTEGRATING STERILE-MALE RELEASE WITH INSECTICIDAL CONTROL MEASURES AGAINST VECTORS OF CHAGAS DISEASE)

Alina PERLOWAGORA-SZUMLEWICZ (1) with the technical assistance of
Minervina Vieira CORREIA (2)

SUMMARY

The fragmentary data from interspecific crosses clearly indicate that sterility induction through genetic manipulation of vector species of Chagas Disease is possible. Though the picture of induced sterility is still incomplete, it offers the possibility of distinguishing two different types of interspecific crossability that contribute to the sterility phenomenon. If sterility produced in reciprocal crosses between *T. maculata* and *T. sordida* follows the pattern described for the *Anopheles gambiae* complex it may be chromosomal and genic in nature. If the situation of sterility induced through unilateral crosses between *T. maculata* and *T. infestans*, between *T. maculata* and *T. brasiliensis*, and between *R. neglectus* and *R. prolixus* is the same as in *Culex pipiens* and in *Aedes scutellaris*, we may expect the sterility phenomenon to be due to cytoplasmic incompatibility. The plausibility of these hypotheses requires basic genetic analysis not available at present.

Information gained from hybridization experiments herein described, may also be of value in clarifying the position of morphologically distinct species with regard to the degree of reproductive isolation and in revealing interspecific phenotypic dominance relationships. Thus, successful reciprocal crosses between *T. maculata* and *T. sordida* argues for a close relationship, while the unilateral cross between *T. maculata* and each of the other *Triatoma* species suggests a more distant relationship. It was therefore concluded that gene flow between *T. maculata* and the other species was only reduced and not absent, thus indicating that none of the three species achieved full reproductive isolation from *T. maculata*, though they are reproductively isolated among themselves. All interspecific hybrids resemble more one species than the other as judged by color and size, thus indicating a dominance phenomenon, behaving similarly in reciprocal hybrids when both occurred. Information gained from these studies, when analyzed together with information from other studies on the biology of these insects, allows certain speculations on the practicability of integrating sterile-male release with insecticidal methods of control against vector species of Chagas Disease.

Núcleo Central de Pesquisas do Instituto de Endemias Rurais, FIOCRUZ. This study received financial assistance from the Conselho Nacional de Pesquisas. Some financial support was received from WHO.

(1) Head of the Laboratory of Insect Biology and Control, Núcleo Central de Pesquisas. Estrada da Covanca 56. Jacarepaguá, GB, Brazil.

(2) Laboratory of Insect Biology and Control, Núcleo Central de Pesquisas.

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INTRODUCTION

The campaigns promoted against Chagas Disease during the last two decades have relied heavily upon the use of residual insecticides, which, when properly applied, were highly efficient in reducing the vector populations. However, the continuing costly efforts, which are necessary to maintain the population at the low level attained by the initial sprayings, are not economically feasible at present, thus being the chief obstacle to successful control.

There are also valid reasons for assuming that insecticide resistance will eventually be established, blocking the present control measures, as it did in campaigns against other vectors of medical importance.

In search for a more desirable economical way of control, an intriguing question is whether these insects possess the natural genetic mechanisms by manipulation of which they may be controlled.

Examples of such potential genetic means have been thus far shown to exist in the *Culex pipiens* complex, *Aedes scutellaris* complex and *Anopheles gambiae* complex. These mechanisms are being used in the development of integrated control measures, which involve sterile-male release to supplement the conventional insecticidal techniques.

So far, the vector of Chagas Disease has not been investigated in this respect. However, hypothetical models for integrated control methods designed by KNIPLING [11, 12] suggest that this insect may prove a good candidate for the practical application of sterile-male release integrated with the present control measures.

Reasons for neglect of this type of studies with respect to the vector of Chagas Disease include: first, the tendency to regard an insect that exhibits a long life cycle inconvenient for genetic manipulations and second, the general concept that a monogamous mating habit was necessary for successful control by sterile-male release. The female of this insect is capable of multiple matings and, as shown in experiments to be published, a single male was capable to inseminate 13 females when they were held together for five weeks. This runs counter to the Authors earlier hypothesis on the incapability of *Triatoma* males to inseminate more than one female (20). This divergence will be explained elsewhere.

Although the long life-cycle is well documented, it should not contribute to the neglect of the necessity of information in this area. As for the second problem, polygamy has not been an adverse factor in erradication of the melon-fly by the release of sterilized males, as demonstrated by STEINER [26].

To lay a foundation toward such an approach, hybridization among some of the vectors of Chagas Disease was undertaken at the end of 1970.

At present, the data are still far from complete, and the results obtained raised several questions, the answers to which are not yet available. Conclusions put forward here may be modified as further data become available. Nevertheless, the results herein summarized do permit some speculations concerning the degree of genetic affinities among the vector species studied, and this may be of interest to the taxonomist involved in problems of speciation.

The data with respect to genetical control means are too incomplete at present to permit even preliminary judgement, but their potential value makes further investigation seem worthwhile.

MATERIAL AND METHODS

Insects utilized in this study were derived from laboratory colonies. These were maintained since 1969, when they were established for studies of the biological parameters of various vector species including mating habits, fertility, fecundity, life cycle, sex ratio, longevity, feeding habits, resistance to lack of food and other factors of importance in planning control campaigns.

Triatoma infestans (Klug, 1834) and *Triatoma sordida* (Stal, 1859) originated from the State of São Paulo, where the former is mainly domestic and invades native huts and the latter is peridomestic, being mainly confined to primitively constructed chicken houses.

Triatoma brasiliensis (Neiva, 1911) originated from the State of Ceara, where it invades human habitations sporadically but is usually found in burrows of small rodents and between stones.

Triatoma maculata (Erickson, 1848) has been brought in from the State of Pernambuco where it is mainly peridomestic. The status of this vector relative to *maculata* Erickson 1848 has been in question for some time by Corrêa & Espinola[3] and by Galvão & Moura Carvalho[8]. Therefore, a definite and precise identification of this insect as *maculata* or *pseudomaculata* will have to await the results of breeding experiments.

Rhodnius neglectus (Lent) and *Rhodnius prolixus* (Stal, 1859) originated from the State of São Paulo. *Prolixus* had not been previously reported to exist in this state. However, specimens of this species were recovered in material collected in a bird-nest


MAP of BRAZIL
showing the geographic
position of its 22 states.
SCALE: 1:23.000.000

Fig. 1

and brought in as a group of immature forms. They were originally confused with *neglectus*, but upon emergence of adults they were readily distinguished by one of our technicians [27] and established as a colony in the laboratory.

The geographic position of the states from which the mentioned insects originated are shown in the map (Fig. 1).

The colonies of vector species utilized in these experiments and several others existing in the laboratory have been reared in wide-mouth battery jars, kept at room temperature and fed on chickens. The rearing method used is essentially the same as that described previously by the Author [20].

The material which was utilized in the determinations of biological parameters and throughout the experiments herein described was kept in laboratory incubators at 28°C and at 65-70% humidity.

Virgin females and virgin males were isolated during the fourth instar when they were readily separable by the pattern of the ninth abdominal sternite, as reported by PERLOWAGORA-SZUMLEWICZ & NIGRI DA CRUZ [21].

All crosses were made by placing one virgin female of one species with one virgin male of another species but of similar age. The resulting adult hybrids were tested for fertility; from five to 15 hybrid females were backcrossed to parental males of each species and males were similary backcrossed to parental females. As soon as newly emerged nymphs were found in the multiple cultures, single pairs were separated to study the fertility of individual females. Hatchability recorded in multiple cultures are given in column 3, and the results from individual pairs are summarized in column 5 of Table II.

In evaluating the results, we have considered lack of progeny in crosses between different species as evidence of interspecific incompatibility. Similarly, if no offspring resulted from backcrosses of male hybrids to parental females, the F_1 males were recorded as sterile. These criteria may be changed as information on embryo development and on inviability of zygotes become available.

At present, however, investigations were conducted primarily to obtain basic practical information on the crosses which result in living progeny.

EXPERIMENTS

The following bilateral crosses have been attempted:

T. infestans X *T. sordida*
T. infestans X *T. maculata* *
T. infestans X *T. brasiliensis*
T. infestans X *R. neglectus*
T. infestans X *R. prolixus*

T. sordida X *T. maculata* *
T. sordida X *T. brasiliensis*
T. sordida X *R. neglectus*
T. sordida X *R. prolixus*

T. maculata X *T. brasiliensis* *
T. maculata X *R. neglectus*
T. maculata X *R. prolixus*

R. neglectus X *R. prolixus* *

With exception of the crosses marked with an asterisk, there was no evidence of hybridization between species, as based on the arbitrarily adopted criterium of lack of progeny for interspecific and intergeneric incompatibility.

Results obtained in the crosses indicated by asterisk are shown in Table I and may be summarized as follows:

1) *T. maculata* X *T. sordida*

Crosses successful in both directions, with the hatchability of eggs greater and the percentage of adult progeny slightly lower when *maculata* was used as the female parent.

Before attempting to demonstrate the differences in results in reciprocal crosses, it will be useful to mention some information concerning the biological parameters for the pure species obtained in our laboratory.

In species *maculata* there was a hatching percentage of 77% with 3978 eggs scored. From these, 72% reached the adult stage under proper rearing conditions, with a sex

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TABLE I

Records of hybridization between vector-species of Chagas Disease

Female	Male	Number fertile females	Per cent eggs hatched	Number adult progeny	
				Females	Males
T. maculata	*T. sordida*	8 (10) a	76.2 (1806) b	164	166
T. maculata	*T. infestans*	2 (10)	53.5 (373)	79	41
T. maculata	*T. brasiliensis*	2 (15)	9.6 (344)	13	5
T. sordida	*T. maculata*	7 (10)	59.3 (1457)	169	158
T. infestans	*T. maculata*	0 (10)	0	0	0
T. brasiliensis	*T. maculata*	0 (15)	0	0	0
R. prolixus	*R. neglectus*	2 (10)	5.0 (577)	11	10
R. neglectus	*R. prolixus*	0 (10)	0	0	0

a — Total females in parenthesis
b — Total eggs in parenthesis

TABLE II

Hybrid females backcrossed to parental males for proofs of fertility

Source of hybrid-female	Male	Number eggs hatched	Number fertile F_1 females	Number eggs hatched	Developmental stage of F_2[d]
T. maculata	*T. maculata*	22 (1079)[a]	8 (13)[b]	27 (1455)[c]	5th inst. nymphs
x					
T. sordida	*T. sordida*	86 (1007)	2 (4)	5 (67)	adults & 5th inst. nymphs
T. sordida	*T. sordida*	27 (760)	8 (12)	42 (1068)	4th & 5th inst. nymphs
x					
T. maculata	*T. maculata*	43 (956)	9 (15)	18 (803)	3rd & 4th inst. nymphs
T. maculata	*T. infestans*	7 (1633)	1 (7)	6 (290)	adults & 5th inst. nymphs
x					
T. infestans	*T. maculata*	8 (1434)	5 (5)	17 (1355)	3rd & 4th inst. nymphs
T. maculata	*T. brasiliensis*	7 (240)	2 (5)	4 (162)	3rd & 4th inst. nymphs
x					
T. brasiliensis	*T. maculata*	3 (233)	1 (5)	2 (85)	3rd inst. nymphs
R. prolixus	*R. prolixus*	63 (420)	4 (9)	120 (549)	2nd & 3rd inst. nymphs
x					
R. neglectus	—	—	—	—	

a — Total eggs in parenthesis
b — Total females in parenthesis
c — Total eggs in parenthesis
d — April, 1972
— Not done

ratio of, 0.9:1.0 in favor of females. In crowded cultures the percentage of adult progeny was reduced to 58%.

In *sordida* there was a hatching percentage of 88% with 4319 eggs scored. From these approximately 69% reached the adult stage with a sex ratio of 0.8:1.0, indicating a surplus of females. Here again adult progeny was reduced to 34% by crowding among newly emerged nymphs in the cultures.

The crowding effect was also one of the reasons why the number of adult progeny was low in crosses between these two species, in spite of the relatively high degree of fertility revealed by the eggs (76% hatchability when *maculata* was used as the female, and 59% when *sordida* was the mother). Of these only 18% developed to adults in the former and 22% in the reciprocal cross, with females and males in nearly normal proportions. These figures were rather low, even compared with those of pure strains under crowded conditions.

The data do not permit evaluation of hybrid weakness, but if such exists, it seems to be confined to the very young nymphs. It certainly was not evidenced in adults, which as a rule demonstrated hybrid vigor.

Only a very few of the morphological characters have as yet been studied, but the adults obtained from all of the bilateral crosses closely approximated the *sordida* parent. All adults were lighter in color and larger in size than *maculata*, thus indicating that these two characters from *sordida* are dominant over the characters from *maculata*. The pronotum, however, with two or four light bands and the relatively short head resemble *maculata*.

No mating between hybrids yielded offspring, but fertility verification by backcrossing to parental members revealed that all F_1 males were sterile, as judged by lack of progeny. The F_1 females, however, were capable of producing living progeny, although usually in very low numbers, as seen in Table II.

When F_1 females (*maculata* ♀ X *sordida* ♂) were backcrossed to both parental males, 22 eggs hatched when *maculata* was used as male, and 86 hatched when *sordida* was the male (in cultures of 15 F_1 females confined with an equal number of pure parent males, Table II, column 3).

Fertility verification of individual F_1 females in single mating pairs showed that eight of the 13 surviving F_1 females confined with *maculata* males gave living progeny. In backcrosses to *sordida* male, many F_1 females died and only two of the four survivors gave living progeny (Table II, column 5). High mortality rates among F_1 females occurred only after confinement with parental males, no deaths occurred in cultures containing solely female hybrids.

In backcrosses of hybrid F_1 females which originated from the reciprocal cross (*sordida* ♀ X *maculata* ♂) to either of parental males, the survival of the females was greater. Eight of 12 F_1 females produced living progeny when *sordida* was used as the male and nine of 15 were fertile when *maculata* male was used.

Most of the nymphs hatched from backcrosses of hybrid females to parental males were weak and dying as they emerged. Survivors, however, succeeded in reaching advanced developmental stages. At present there are available 5th instar nymphs and adults which are being tested for fertility. Of the 16 F_2 adults available from the backcrosses to *sordida* males, there are nine females and seven males. This does not seem to be abnormal, since in the pure species females develop and mature several days earlier than males, and the first emerging adults are, as a rule, females.

The F_2 adults retained the morphological characters seen in the F_1, thus resembling, closely the *sordida* species with respect to color and size.

2) *T. maculata* X *T. infestans*

In the pure species of *T. infestans* there was a hatching percentage of 92% with 5576 eggs scored. From these 88% developed into adults with a male-female ratio of 0.8:1.0. The biological parameters run higher in *T. infestans* than in *T. maculata*, which showed only 77% hatchability from which 72% reached the adult stage under proper rearing conditions.

Vigorous male and female hybrids resulted from crossing male *infestans* with female *maculata*, but the crosses in the opposite direction failed.

In the successful unilateral cross only two of ten *maculata* females produced living progeny. There was a hatching percentage of 53%, of these 32% developed into 120 vigorous adults with an abnormal sex ratio of 0.5:1.0 in favour of females (Table I).

All F_1 adults from this cross phenotypically resembled *infestans* species. Here again the color and size of the hybrids indicated that these two characters of *infestans* were dominant over the characters from *maculata*. The pronotum, however, resembled that of *maculata*.

The F_1 females were backcrossed to males from the paternal and maternal species and the fertility or sterility of the F_1 males were tested similarly. Only the former produced living progeny. Eggs of backcrosses revealed low hatchability, as shown in column 3 of Table II. One F_1 female out of seven exposed individually to *infestans* males produced living nymphs. These developed into 5th instar and five adults. Of the five F_1 females backcrossed with *maculata* males all produced living progeny though in a very low number. Seventeen of 1355 eggs hatched and have at present reached the 4th instar.

3) *T. maculata* X *T. brasiliensis*

In the pure species of *T. brasiliensis* there was a hatching percentage of 92%. From these approximately 86% reached the adult stage with the usual for *Triatoma* predominance of females. The male/female ratio was 0.8:1.0.

Vigorous hybrids resulted from crossing male *brasiliensis* with female *maculata*. The reciprocal cross, however, never succeeded.

In the successful unidirectional cross only two of 15 *maculata* females confined with *brasiliensis* males in individual pairs produced living progeny. There was a hatching percentage of 9.6% with 344 eggs scored. Of these only 0.5% developed into adults with a strongly disproportionate sex ratio of 0.4:1.0 in favour of females (Table I).

The adult progeny more closely approximated the large, light-colored *brasiliensis* than the small and dark *maculata*. The pronotum and head resembled *maculata*.

Paternal and maternal fertility verification experiments subsequent to hybridization revealed that F_1 females, backcrossed to either of parental males, produced living progeny, though in extremely low numbers. Only two of five F_1 females mated with *brasiliensis* males and one of those mated with *maculata* males produced living offspring. These have currently reached the 3rd and 4th nymphal stage.

4) *R. prolixus* X *R. neglectus*

The two species of *Rhodnius* have recently been colonized in the laboratory, and the scanty data permit few conclusions and only with regard to *neglectus*. There was a hatching percentage of 84.5% in 3658 eggs scored. From these, 54% reached the adult stage with a 20% excess of females.

Male and female hybrids resulted from crossing female *prolixus* with male *neglectus*, but the reciprocal crosses failed. Only two of 10 females used in the former cross produced adult progeny. There was a hatching percentage of 5%. From these 21 developed into adults with a normal sex ratio of 0.9:1.0 in favour of females (Table I).

The hybrid progeny more closely approximated the paternal *prolixus*. The head, however, showed a mosaic of characteristics in favour of females (Table I).

Hybrid fertility verification by backcrossing to parental members of *prolixus* species gave living progeny only in backcrosses of F_1 females to males. Of 420 eggs collected in a culture of nine F_1 females confined with nine pure *prolixus* males, 63 hatched and have to date developed into 3rd instar nymphs (Table II). Of the nine hybrid females, only four produced living progeny. Of 549 eggs, collected in the four individual mating pairs, 120 hatched and exhibited normal development (Table II).

DISCUSSION

It seems likely that results obtained in the experiments on male sterility induction in vector species of Chagas Disease can be explained by two different mechanisms; one chromosomal or genic in nature and the other, cytoplasmic in origin. Any conclusion, however, is preliminary and may be changed.

Crosses between *T. maculata* and *T. sordida* in either direction resulted in an F_1 population with fertile females and sterile males in normal proportions.

If hybrid sterility in these reciprocal cross follows the pattern of genetic sterility in the male hybrid from the crosses between members of *Anopheles gambiae* complex, described by DAVIDSON [4, 5], PATTERSON [16, 17] and by others, the phenomenon in *Triatoma* may be explained by the presence of chromosomal aberrations in hybrids, resulting from a rearrangement of the autosomes in the parents, as explained by MASON [15].

If, however, it follows the analogous sterility phenomenon observed in *Drosophila permisilis* or in *Drosophila pseudoobscura*, in which no crossing-over at meiosis occurred, the male sterility may be attributable to genic effects, as stated by SINNOTT-DUNN-DOBZHANSKY [24].

Either hypothesis will of course have to wait until necessary cytological information is obtained.

Crossing experiments between *T. maculata* and *T. infestans*, and between *T. maculata* and *T. brasiliensis* showed the existence of a different type of crossability, while crosses between *maculata* and *sordida* could be made using females of either species, the others could only be made when *maculata* was the female.

Nevertheless, the non-reciprocal crosses succeeded in producing living progeny that developed into vigorous adults but with strongly disproportionate sex ratios. The records show that this was not due to differential mortality among emerging adults.

The sex ratio as exhibited in the adult population from crosses between *Rhodnius neglectus* and *Rhodnius prolixus* presents us with a third type of crossability; the living progeny from this non-reciprocal cross developed into sterile males and fertile females in normal proportions.

With regard to the sex ratio distortion in favor of females from the former unidirectional crosses, the distortion appears to act according to Haldane's rule, which holds that in organisms in which the male is the heterogametic sex, the predominant sex, when the sex ratio is abnormal, will be the female. The karyotype of all species utilized in the crosses is made up of 10 pairs of autosomes and XY in the male, and XX in the female, as reported by SCHREIBER & PELLEGRINO [23] and BARTH [2].

In backcrosses, hybrids from nonreciprocal crosses behaved like their maternal parent in that only female hybrids were able to produce progeny.

The fact that all F_1 males were unable to engender living offspring from parental females would seem to indicate an effective barrier between these species that restricts gene flow.

There is no indication whether the failure of the reciprocal cross to produce living progeny was due to mechanical isolation, prevention of copulation, or to genetic factors, post-copulation incompatibility either prezygotic or postzygotic.

It is unknown whether the unilateral expression of incompatibility in some of the vector-species has the same basis as in *Culex pipiens* and in *Aedes scutellaris.* If however such a possibility exists, cytoplasmic inheritance, as suggested by LAVEN [14] for *Culex* and by KITZMILLER & LAVEN [10] and SMITH-WHITE & WOODHILL [25] for *Aedes scutellaris*, might also be responsible for restriction of gene flow between these species. But the blockage of incompatible sperm after penetration the egg has still to be shown. Whether the factor responsible for death of the embryo is the incompatible RNA as suggested by LAVEN, or some viral or bacterial infection, a suggestion rejected for some time but taken up again by YEN & BARR [28] is also unknown.

Analysis of these crossing results permits an insight into the degree of genetic affinity between the species studied and also reveals interspecific relationships of phenotypic dominance.

It should be remembered that the three species of *Triatoma, infestans, sordida* and *brasiliensis* showed isolation among each other, but they all can be crossed with *maculata* which is related to all of them though in different degrees.

It is usually believed that crosses between morphologically distinct species which permit the most advanced development of progeny are those indicating close relationships. On this ground, evidence of adult progeny in all crosses herein described would seem to point to a close relationship between *maculata* and the other three *Triatoma* species. However, the fact that bidirectional crosses were possible between *maculata* and *sordida*, while only unidirectional occurred between *maculata* and the other two species, argues for a more distant relationship between *maculata* and the latter. Moreover, on the ground of egg fertility, *infestans* would to be located in an intermediate position in the scale of relationship, leaving *brasiliensis* at a more distant point, and *sordida* as the closest to *maculata*.

Nevertheless, reproductive isolation barriers exist between all of them and *maculata* as evidenced by two facts: first, that hybrid males were unable to produce offspring when mated with parental females and second, that fertility of females was rather low. Therefore, it may be concluded that gene flow was reduced but not absent, thus indicating that none of the four morphologically distinct *Triatoma* species achieved full reproductive isolation. RYCKMANN [22] reported on unsuccessful crossings between *T. barberi* and *T. infestans*, *T. sordida* and *T. brasiliensis*. Similarly, ESPINOLA [7] stated that *T. brasiliensis* is reproductively isolated from *T. petrochii* (PINTO & BARRETO, 1925).

Mating types showed a strict species inheritance. The interspecific hybrids more closely resembled one species than the other, indicating a dominance phenomenon, behaving similarly in reciprocal hybrids when both occurred. Two characters, color and size of *sordida*, *infestans* and *brasiliensis* were dominant over the characters derived from *maculata*. These two dominant characters were retained in the F_2 adults produced in backcrosses between F_1 hybrids and parental members of the three species.

All F_1 hybrids from crosses between *Rhodnius neglectus* and *Rhodnius prolixus* more closely resembled *prolixus*.

It is well known that species capable of laboratory hybridization may never cross in nature due to ecological, geographical and other effective barriers. The chances, therefore, of observing natural hybrids are low, but if hybridization can be achieved in the laboratory, the possibility of finding hybrids in nature exists.

ABALOS [1] described hybrids from *T. infestans* and *T. plantensis* (Neiva, 1913) found in nature in Argentina. The Author also obtained hybrids from crosses between *T. infestans* and *T. rubrovaria* (Blanchard, 1843).

The fact that *Triatoma* hybrids were found in nature and that *Triatoma* species are able to cross in the laboratory, argues for the necessity of hybridization experiments with any new species sharing a common ecological niche with other species of the same genus.

The fragmentary information that has been summarized affords the opportunity of considering further studies in this area.

Immediate attention should be given to the different grades of incompatibility introduced by genetic manipulation, starting with the lowest grade in which sperm failed to reach the eggs.

Mating ability and competitiveness of sterile males is being ascertained in laboratory cultures, leaving for the next step similar observations in artificially infested mud huts build in the neighborhood of the laboratory.

Experiments are being performed to determine the proportion of incompatible males necessary to reduce progeny emergence in laboratory populations.

Since female insects emerge and mature earlier than males, conditions that would permit rapid separation of the sexes are being considered. The method used at present, though accurate and satisfactory in laboratory "mini-mass" cultures, will be impractical for sexing large numbers of hybrids.

The immediate question that arises is whether research to develop the sterile-male technique is justified. Although the general tendency is to regard this method as impractical for control of the vector of Chagas Disease, it may be promising when integrated with the insecticidal technics.

The advantages of integrating sterile-male release with other methods of control against house-flies has already been shown by LA BRECQUE & WEIDHAAS [13].

The information available on vector biology, though of laboratory origin, when analized in the light of the hypothetical models of integrated control measures described by KNIPLING, suggest that this insect might be vulnerable to the new approach of control.

Several arguments might be given to support this suggestion: laboratory [18, 19] and field reports [6, 9] indicate that either HCH or Dieldrin when properly applied, can drastically reduce the natural population to one to three percent of pre-treatment levels. Based on our experience from field trials, we are justified in saying that after a single application of HCH, there are always very few survivors. Moreover, these insects are short-range migrants, thus indicating that the treated area is not subject so much to natural reinvasion as to accidental introduction of insects. After the population has been reduced to a low level, the release of sterile males, that would compete with the survivors and those that emerge from immature stages, appears feasible and more economical in preventing repopulation of the area than the insecticidal sprayings year after year, as pointed out by KNIPLING with regard to control of insect pests.

Furthermore, the vector of Chagas Disease is a long-lived insect. The mean life span for laboratory reared females is 421.7 ± 22.2% days in *T. infestans,* 348.3 ± 43.5% days in *T. brasiliensis,* 530 ± 32.6% in *T. sordida,* 507 ± 12.5% in *T. maculata* and 484.4 ± 34.2% days in *R. neglectus.* Males usually live longer than females. Thus these insects live long enough to span from 2-3 generations, as evidenced by data obtained in laboratory studies on the life cycle of each species.

KNIPLING stated that when organisms live long enough to span several generations, this is favorable for survival and increase of population when subjected to the killing effect of insecticides. When, however, the population is subjected to sterile-male release, the long life span, overlaping more than one generation, is disadvantageous to the insect.

It will, however, be anticipated that sterile-male release to prevent repopulation has no chances of success if introduction of sterile males creates hazards to man, if induced sterility destroys mating competitiveness and sperm competitiveness, and if laboratory rearing conditions affects the ability of insects to survive and compete with the wild population in the environment where release has to be made.

Lack of adaptability of laboratory cultivated insects to the environment was demonstrated by the Author in seminatural experiments on the efficiency of HCH against *T. infestans* [18]. The laboratory reared insects died abundantly when released in mud huts built in the neighborhood of the laboratory; mortality was 70% in summer months and 50% in winter months.

Whether these problems can be overcome remains to be seen.

RESUMO

Indução de esterilidade em machos através da manipulação de mecanismos genéticos presentes em espécies vectoras da doença de Chagas (especulações sobre a conjugação de medidas de controle através de inseticidas e do lançamento de machos estéreis).

Embora fragmentários, os dados de cruzamentos interespecíficos disponíveis indicam, obviamente, que é possível induzir a esterilidade, usando — como em nosso trabalho — a manipulação genética de espécies vectoras da doença de Chagas.

Confirmando o que foi dito, o quadro ainda incompleto da esterilidade induzida permite distinguir dois tipos diferentes de cruzamentos interespecíficos que contribuem para o estudo do fenômeno da esterilidade.

Se a esterilidade observada nos cruzamentos recíprocos entre *T. maculata* e *T. sordida* coincide com o tipo descrito para o complexo *Anopheles gambiae,* esse tipo de esterilidade poderia ser de origem cromossômica e gênica.

Por outro lado, se a condição de esterilidade induzida nos cruzamentos unilaterais em *T. maculata* X *T. infestans, T. maculata* X *T. brasiliensis* e *R. neglectus* X *R. prolixus* coincide com o tipo observado nos complexos de *Culex pipiens* e *Aedes scutellaris,* é de

esperar-se que tal fenômeno de esterilidade seja devido à incompatibilidade citoplásmica.

Entretanto, a confirmação dessa hipótese requer análise genética básica, ainda não disponível.

A informação proveniente das experiências de hibridação aqui descritas pode ser valiosa para esclarecer o grau de isolamento reprodutivo de espécies morfologicamente distintas e revelar as relações de dominância interespecífica.

Desse modo, a exeqüibilidade dos cruzamentos recíprocos entre *T. maculata* e *T. sordida* demonstra uma relação mais íntima, enquanto o cruzamento unilateral entre *T. maculata* e as outras espécies aqui consideradas sugere relação mais remota.

Conclui-se, portanto, que o fluxo genético entre *T. maculata* e aquelas espécies foi somente reduzida mas não eliminada, indicando dessa maneira que nenhuma das três espécies conseguiu isolamento reprodutivo completo de *T. maculata*, apesar de serem, entre si, reprodutivamente isolados.

Todos os híbridos interespecíficos, na côr como no tamanho, assemelham-se mais a uma espécie que à outra, revelando assim o fenômeno de dominância, o que ocorre também com os híbridos recíprocos. Cumpre registrar que os caracteres dominantes foram conservados na geração F_2, proveniente do cruzamento de fêmeas F_1 com machos paternos.

A informação conseguida desses estudos, quando analisada em conjunto com a informação proveniente de estudos sobre a biologia desses insetos, permite-nos certas especulações sobre a praticabilidade de integrar a libertação de machos estéreis com os métodos atuais de controle com inseticidas, contra as espécies vectoras da doença de Chagas.

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ADDENDUM

After this paper had been accepted for publication, further experiments on the mechanism involved in the induced male sterility revealed that lack of progeny has been due to unsuccessful transmission of sperm by the hybrid male. Evidence to be presented in a later paper will demonstrate that immature and mature spermatozoa were found when hybrid males were dissected. Spermatophores were discharged when hybrid males were exposed to parental females, indicating that copulation had occurred. The spermathecae, however, were found to be empty, when females were dissected within 24 hours after the detection of spermatophores. On a few occasions mature and immature spermatozoa were present in the female vaginal tract. The failure of the sperm to be transfered to the spermathecae indicates that the described method of achieving irreversible male sterility destroys mating competitiveness on the sperm level, thus making these males useless in limiting the growth of natural populations into which they are released. Whether the presence of sperm in the vaginal tract of females mated to hybrid males represents an adverse factor in subsequent mating to normal males remains to be seen.

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