GENETIC AND CYTOLOGICAL STUDIES OF *Drosophila nigrospiracula* IN THE SONORAN DESERT

by

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Date

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ABSTRACT

In testing flies of the species D. nigrospiracula from various localities in the Sonoran Desert for genetic divergences both in their chromosome morphology and genetic differences, it was found that the populations were homogeneous between the cultures tested. This homogeneity was determined through statistical analysis of data taken from the results of matings made between flies from the test localities, and the lack of inversions in the salivary chromosomes of the locality hybrids.

The conclusions drawn here try to relate this genetic homogeneity with the diversity noted in the ecological factors known about this species. It was found that certain stock cultures formed of individuals from various localities have different diet requirements from one another. Also, the populations differ in the type of cactus available for and used for larval development in the regions of the desert tested. The natural substrates for the larvae are the cacti, Carnigiea gigantea, Ferocactus wislizeni, and Pachycereus pringlei.

INTRODUCTION

The Problem

There are two approaches to the study of evolution, the historical and the experimental. The experimental approach is valuable for determining those mechanisms that operate on the level of the the population. Ecological genetics embraces the experimental approach and is useful in attempting to bridge the two diverse factors that control the fate of any species, the genotype and the environment. The Sonoran Desert of North America is an ideal habitat in which to study ecological relationships because of the simplicity of environmental conditions. Drosophila are valuable organisms with which to work because of the large number of species and their testability in the laboratory.

Axelrod (1950) has proposed that the deserts of the western North American continent are relatively new environments for organisms. He estimates their age to be between four and five million years old. If this is so, there may be as yet many unfilled niches for organisms to occupy. Isolation of fragment populations would seem probable if the organisms studied were dependent upon certain types of vegetation. Local peculiarities of elevation, soil, rainfall, drainage, and other factors tend to create island-like dispersions of certain plants throughout the Sonoran Desert. Drosophila nigrospiracula Patterson and Wheeler is a characteristic species of the Sonoran Desert. Its ecology

(larval feeding sites) is restricted almost entirely to the sahuaro, Carnegiea gigantea (Englemann) Britton and Rose, and the cardon cacti, Pachycereus pringlei S. Watson. The purpose of the present investigation is (1) to discover if there are any genetic differences in populations of D. nigrospiracula by hybridization tests and cytological observation and (2) to relate the differences to local ecological conditions and probable past history for each population.

Phylogeny and Related Species

D. nigrospiracula is a member of the melanopalpa subgroup in the desert-adapted repleta species group of the subgenus Drosophila (Figure 1). It has been placed in the group according to its anatomical and cytological characteristics. There are six other species in the subgroup, five of which Wasserman (1954) has shown to be closely related to one another. He has denoted D. nigrospiracula as an independent branch (Figure 2). Also the fact that D. nigrospiracula is the only species of the melanopalpa subgroup found in the Sonoran Desert indicates that its development from the ancestral population was certainly independent. Wasserman has been unable to work out the exact relationship of D. nigrospiracula to the remainder of the subgroup due to the large number of rearrangements on the second chromosome. At one point he even questions whether the species may not be more closely related to the hydei subgroup (Wasserman and Wilson, 1957). It is evident then that D. nigrospiracula has no very close relatives even though a sibling species has been discovered in the past few years.

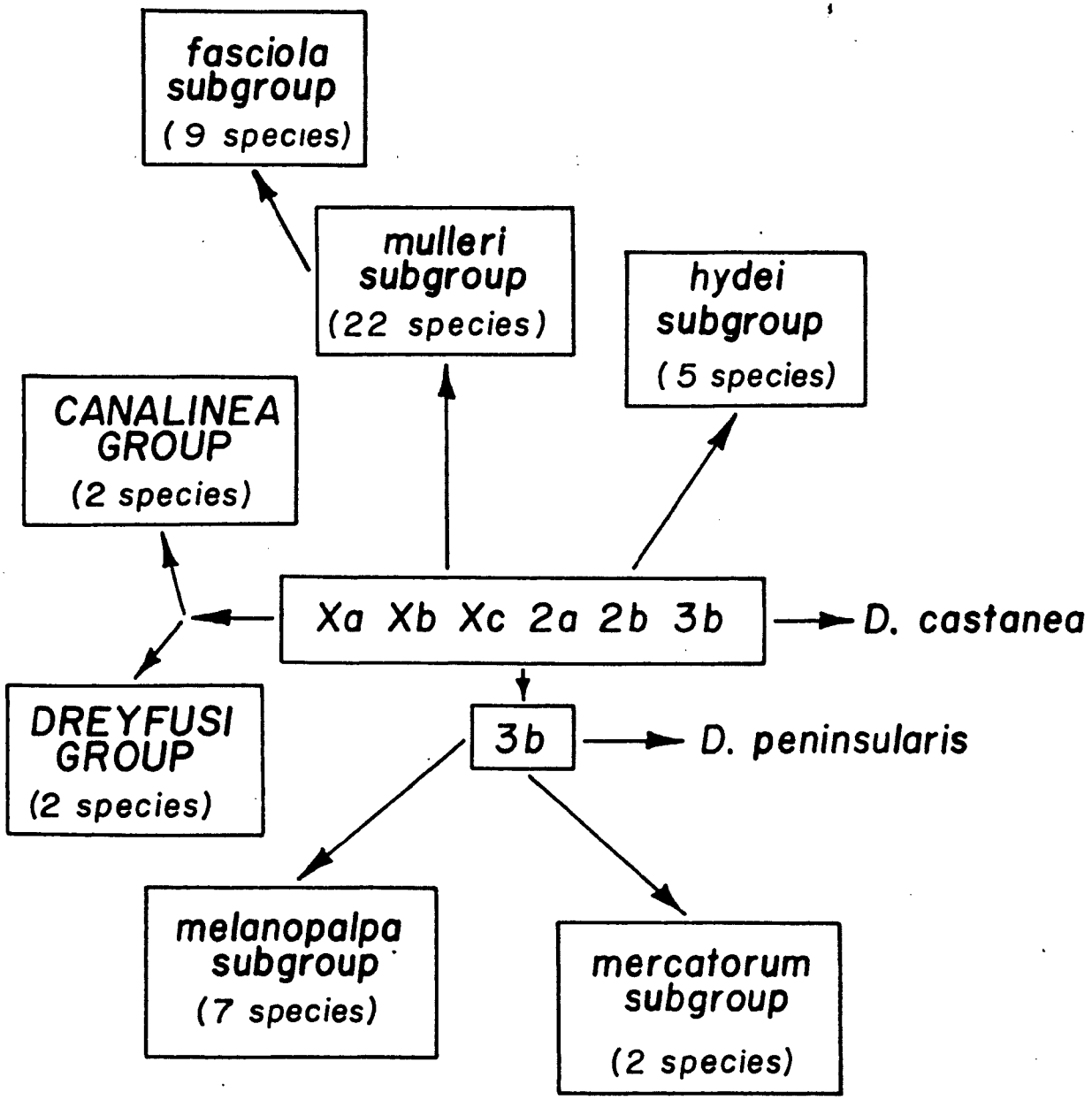


Figure 1. Phylogeny of the repleta group in the genus Drosophila. (from Wasserman, 1960)

This shows the phylogeny of the group as determined by the sequences of inversions between the species. The primitive hypothetical ancestor of the group is shown with the inversions Xa Xb X c, 2a 2b, and 3b.

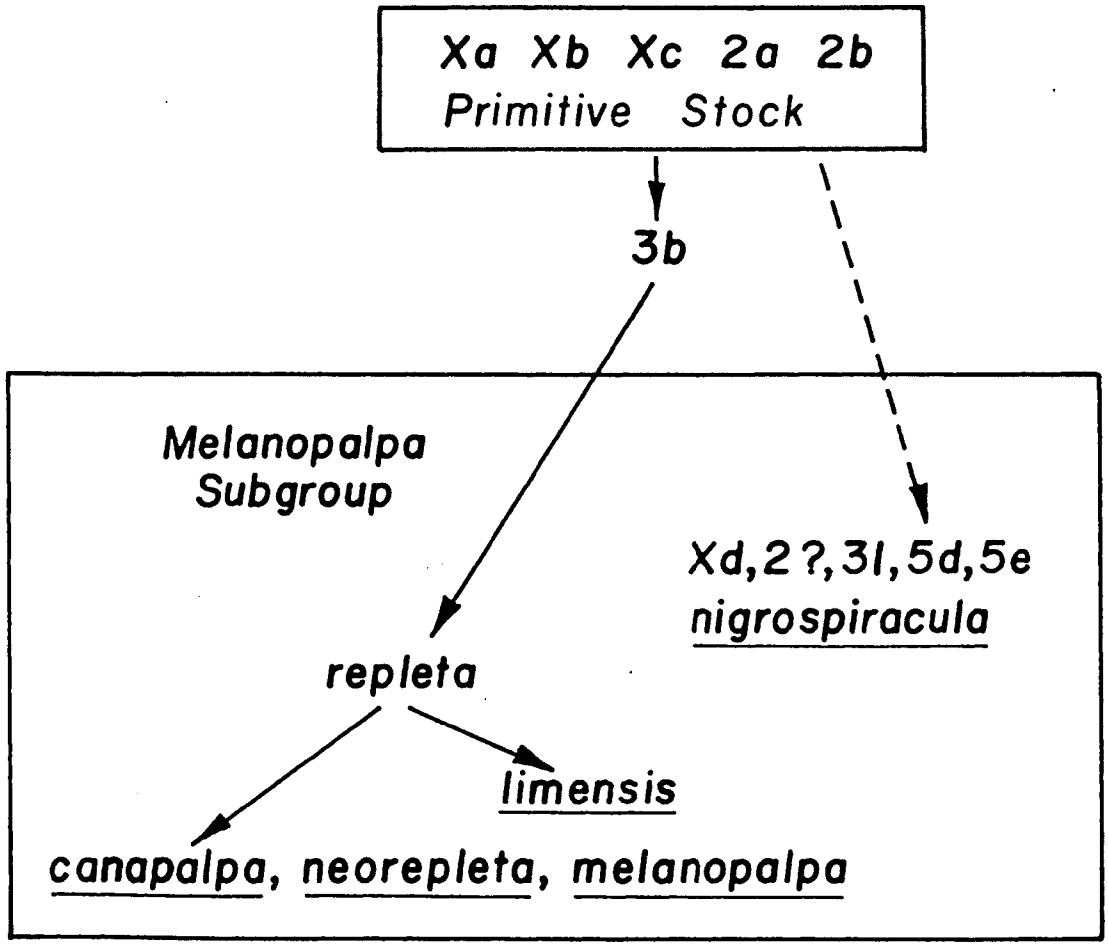


Figure 2. The relationship of D. nigrospiracula to the melanopalpa subgroup. (from Wasserman, 1954)

In 1961 in San Felipe, Baja California, Dr. William Heed and Dr. Lawrence Mettler noted that what had formerly been a single species, D. nigrospiracula, actually consisted of two species (the second species will be called Species M) which were almost phenotypically identical. Surprisingly enough the internal anatomy of the new species is more similar to the hydei subgroup than the melanopalpa subgroup which indicates that the external similarity is probably the result of parallel evolution. Species M is sympatric with D. nigrospiracula. Hybridization tests made by the present investigator show that the two species are reproductively isolated (Appendix). Although Species M has been captured feeding on sahuaros invariably in company with D. nigrospiracula, it has never been reared from rotting specimens of the cactus. The ecology and exact relationship of Species M to the remainder of the repleta group are unsolved problems, but the fact that both species are so similar indicates that the desert environment may be rather exacting in its requirements for survival.

Ecology

Collection records show that this species inhabits almost every region of the Sonoran Desert as described by Shreve (1951). The species has been trapped extensively throughout the range of the sahuaro cactus, in areas of cardon cactus and in mixed stands of the two cacti. In the laboratory D. nigrospiracula has been reared from larvae found in rotting specimen of sahuaro, cardon, and the Fishhook Barrel cactus, Ferocactus wislizeni (Englemann) Britton and Rose.

Adult flies have been collected feeding on the slime flux formed in the decayed tissue of dying sahuaros. This decay is caused by Erwinia carnegieana, a bacterial disease that possibly infects frost-damaged or otherwise damaged tissue of the sahuaro (Niering, Whittaker, and Lowe, 1963). Actively infected sahuaros are most prevalent during and soon after the desert's rainy seasons of the late summer and mid-winter. A build up of the D. nigrospiracula populations also occurs at these times for the soft, damaged flesh of the plant is an ideal substrate for the larvae to develop in.

The distribution of the sahuaro cactus depends greatly upon temperature and soil conditions. The heaviest stands of sahuaro are found in the coarse rocky soil of low mountain ranges and foothill regions. As the soil becomes finer with poorer drainage and the winter temperature extremes more rigorous, as in the bajada or basin floor, the density of the sahuaro population decreases until only solitary individuals are seen at widely scattered intervals (Shreve, 1951). Therefore the populations of D. nigrospiracula would appear to be at their highest density around mountain ranges in conjunction with the high sahuaro populations. This would help in setting up small disjunct populations; possibly isolated enough to show evidence of local adaptations and genetic divergence. The degree of migration between the areas of high densities is not known.

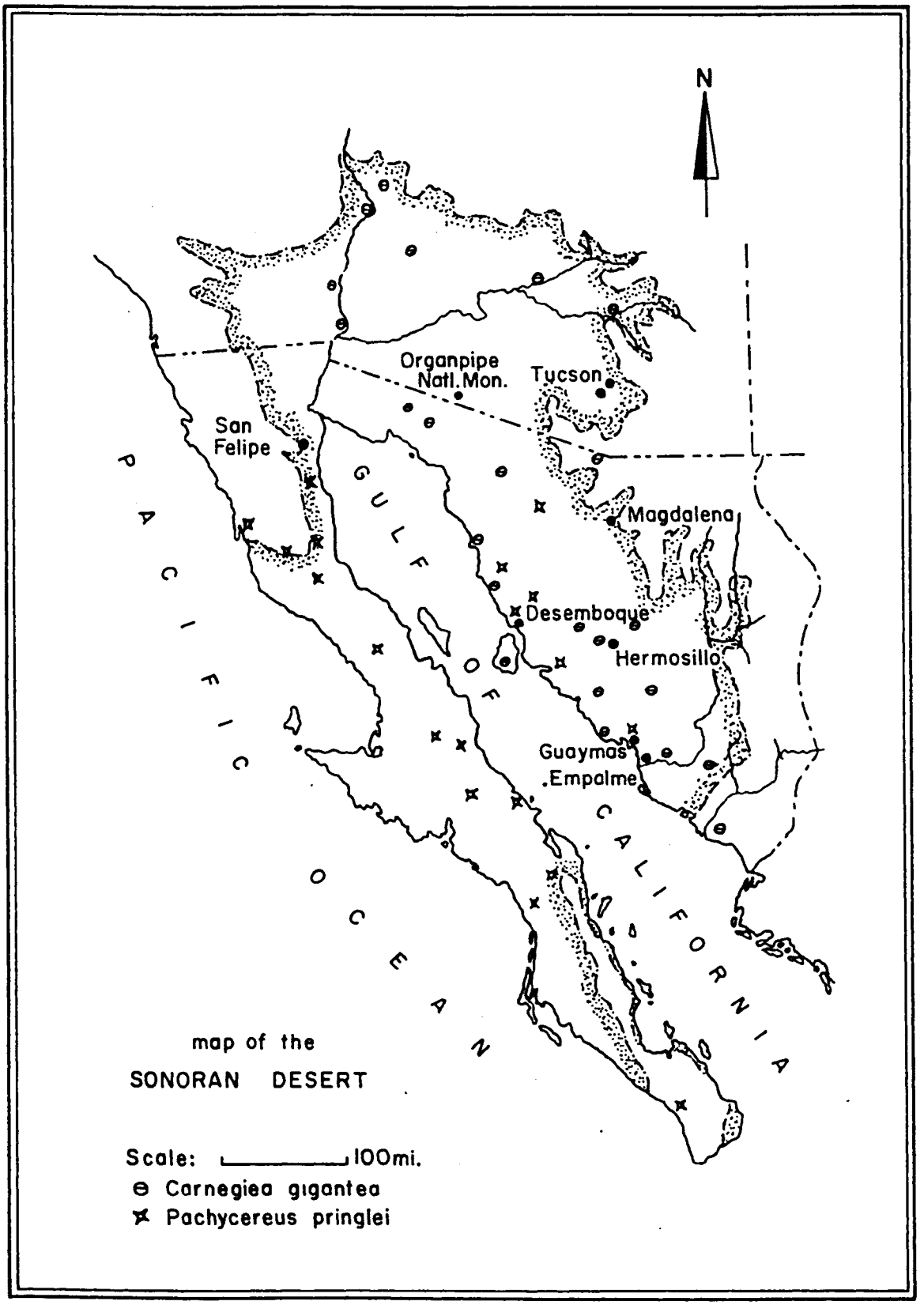
The investigator feels that the dependence of the fly on the Fishhook Barrel cactus is probably limited, for these plants tend to be widely scattered in the desert and the discovery of a decaying individual is a rare occurrence.

The ecological relationship of D. nigrospiracula to the cardon is not as well understood as that to the sahuaro. Only a single locality representing a 'cardon only' ecological area has been trapped for flies: San Felipe, Baja California, Mexico. The remainder of the cardon range that is unmixed with sahuaro extends down the east coast of Baja California until it crosses the peninsula at its southern tip (Figure 3). There are no collecting records available for this region.

Cardon cactus is tolerant of sandy soils and foothill area soils also, so its distribution is relatively even throughout much of its range (Shreve, 1951). Here the D. nigrospiracula populations are less likely to be disjunct. Cardon occurs in mixed stands with sahuaro along the western coast of Sonora, Mexico, as far south as Guaymas. The sahuaro distribution to the northwest ends at the Colorado River (Figure 3).

The dependence of D. nigrospiracula on two different genera of cactus that are located in three different ecological communities indicates that the species is ideal for this study.

Figure 3. A map showing the distribution of Carnegiea gigantea and Pachycereus pringlei in the Sonoran Desert (from Shreve, 1951).


N
Organpipe
Natl. Mon.
Tucson
San
Felipe
Magdalena
Desemboque
Hermosillo
Guaymas
Empalme
GULF OF CALIFORNIA
PACIFIC OCEAN
map of the
SONORAN DESERT
Scale: 100mi.
Carnegiea gigantea
Pachycereus pringlei

METHODS

Culture and Collection Records

Cultures of D. nigrospiracula were developed from individuals collected from many locations in the Sonoran Desert (Table 1). These cultures were kept on regular banana media with a tiny piece of canned sahuaro or opuntia cactus added to each of the breeding vials. This was required in the diet of all the stocks, except those which had been obtained from Hermosillo and Magdalena, Sonora, Mexico, and one stock of flies from Tucson. The generation length and fertility of the Magdalena and Mermosillo stocks is only slightly less on the non-cactus banana media than that of the other stocks on a cactus-banana media. However, the Tucson stock has shown itself to be of low viability and slow development when cultured without cactus. In fact, its numbers were consistently too low to be used in any of the mating tests.

Using the stocks maintained in the laboratory, the following mating, chromosome, and fitness tests were carried out.

Mating Tests

Early in the experimentation, test crosses between flies of each location were made with two females and one male paired in each vial. These tests were to check the interfertility of the flies of each geographical test location.

In order to test the comparable fitness of the flies, matings between flies from the different geographical locations were again

Table 1. Collection data for *D. nigrospiracula*

Location	Date	Method	Collection No.	Other Drosophila species collected	Vegetation Type
Hermosillo, Sonora, Mexico	10-61	Banana trap	A 45.2	*D. arizonensis* *D. gitora*	Organpipe (*Cereus thurberi*) cholla and opuntia cacti mesquite and paloverde shrub.
San Felipe Territory a Baja, California, Mexico	10-61	Banana trap	A 46.3		Cholla, organpipe, cardon (*Pachycereus pringlei*) cacti; ocotillo and mesquite shrubs.
Magdalena, Sonora, Mexico	9-61	Banana trap	A 42.2	*D. longicornis* *D. arizonensis*	Sahuaro (*Carnegiea gigantea*), organpipe cacti, area rich in shrub types of ironwood, mesquite, catclaw, and creosote.
Desemboque,	10-61		A 48.3		*Sahuaro*, *cardon*, organpipe and agria cacti, ironwood, paloverde and mesquite shrubs.
Tucson, Arizona (Mt. Lemmon Hgwy.)	11-61	Banana trap	A 50.1		*Sahuaro*, prickly pear (*Opuntia*), paloverde, mesquite.
Guaymas, Sonora, Mexico (San Carlos Bay)	11-62	Banana trap	A 81.1		50-50 *cardon* and *sahuaro* cacti, also hecho (*Pachycereus pectin aboriginum*) and organpipe cacti.
Organpipe National Monument Lukesville, Arizona	3-64	Picked from Sahuaro rot pocket.	A 120.2	Only species	Sahuaro cactus forest, Senita (*Cereus schotti*) and organpipe. (Not used in breeding tests)
Empalme, Sonora, Mexico	1-64	Banana trap	A 115.7		Cactus forest of *sahuaro*, hecho, senita, and organpipe.

(from the collection records of Dr. William B. Heed)

carried out. Since the earlier work had shown that simple pair matings of one male and one female did not result in successful reproductivity amongst the majority of females, these tests were carried out in small mass matings of three males and three females.

Virgin flies of both sexes were removed from the stock cultures and aged four days before placement in breeding vials. The matings between flies from each of the geographical locations were made in duplicate, with one group of matings being placed on plain banana media in the breeding vials and the other group placed on the banana-cactus media. The volume of media in each vial was approximately five milliliters. The control matings were made by using three males and three females from the same locality (e.g., Hermosillo ♀♀ x Hermosillo ♂♂). These matings were set up exactly as were the test matings.

The parent flies remained on the test media until the majority of larvae had reached the third instar and the vials were ready to be yeasted. If the adults are removed before this time, mold often would destroy a test. Yeasting consisted of dipping a third of a Kleenex tissue in a suspension of Fleischmann's yeast and water, wringing and inserting it into the media. This provided extra nourishment for the larvae, and the Kleenex is a base for the larvae to pupate on.

When the F_1 imagos emerged, they were etherized, counted, and sexed. The data was recorded. This total procedure was repeated two times, giving three replications for statistical analysis.

A laboratory infection in the stocks caused some concern about the validity of the tests. When the infection seemed at its height during the third replication of the tests, a brother-sister F_2 mating

of all hybrids and controls was made. A record was made of each vial which showed the infection by the brown color of the media and a Student's t Test was run on the reproduction results. It was found that the flies tested on the cactus-banana media were not significantly affected, but those on the plain banana media were affected by the infection. The t value for the disease versus undiseased banana media flies was 3.35 with 39 degrees of freedom, $t_{.05} = 2.121$; in the cactus-banana media raised flies the t value was 1.50, the $t_{.05}$ for 17 degrees of freedom was 2.11. Therefore in drawing conclusions from later tests and in using statistical analysis, only those tests run on a cactus-banana media were considered. However, the non-cactus media results are shown.

Chromosome Study

The study of chromosomes involved a check of both the salivary and ganglion metaphase chromosomes of the locality hybrids. The metaphase cells were prepared using a squash technique described by Lewis and Riles (1960), except that steps A and B involving colchicine were not used.

The salivary gland chromosomes were prepared by the squash technique using a 1% orcein, lactic acid and acetic acid solution as a stain. The larvae were dissected and the salivary glands removed in a physiological saline solution.

The prepared slides were then read for inversions and morphological structure of the chromosomes, respectively.

Statistical Analysis

When recognized sources of variation are present in an experiment, it is appropriate to portion that variation before comparisons are made. The method of randomized complete-block design of analysis of variance is advantageous when replication is included in the experimental design. The variability of the experiment is then separated into that due to the replicates or blocks, the treatment, and experimental error. In this manner situations in each replication, such as effects of food quality, temperature or handling which cannot be measured, are eliminated from the experimental error. Procedure and formulas for handling the sums of squares and degrees of freedom are in most texts on statistics (Steel and Torie, 1960). The F test for testing the null hypothesis and calculation of the coefficient of variability are carried out in the usual manner.

$$CV = \frac{\sqrt{MSE}}{\bar{X}}$$

(MSE = mean square error)

In determining the effect of mold on larvae viability, the t test for unpaired observations and unequal sample sizes was employed. It was assumed that the variance for the population was constant and a null hypothesis of no difference of sample means for diseased and non-diseased vials was used as a test criterion.

In order to compare the similarity of results between the F_1 and F_2 tests, a t value formula was used to compare a single replication with a sample was employed.

(Simpson, Roe, Lewontin, 1960)

$$t = \frac{(\bar{X} - X)\frac{N}{N-1}}{s} \qquad (df = 1+N-2 = N-1)$$

RESULTS

The Matings

All of the matings of flies from various localities were successful in producing fertile offspring. There seemed to be no isolating mechanisms of any sort formed between these geographical populations.

The statistical analysis of the mating tests confirmed the null hypothesis of no significant difference in the genetic makeup between the tested geographical populations.

An observation of the raw means of the test locality hybrids with the parental type controls in a manner similar to that used by Vetukhiv (1953) in his fitness tests reveals that in the F_1 cactus-media test all of the hybrid means have a value either intermediate to or below that of the controls. No superiority is revealed (Figure 4).

The F values obtained in the analysis of variance on each of these tests showed no significance at the 10% level (Table 2). The large block values seen when performing this analysis tended to show that there were other experimental factors that affected the results obtained. The coefficient of variability is so great in most cases that the dependability of this experimental data is to be questioned. From this data, if other supportive evidence were available, one could say that the tested populations are homogeneous.

The results of the non-cactus media tests show a varied trend. Particularly in the matings involving Guaymas males, Guaymas females,

Table 2. Statistical data from mating tests

Hybrid Tests	Cactus Media			Non-cactus Media			
	Means	F value	Coef. of variability	Means	F value	Coef. of variability	
H x H df=½	76	--	--	49.0	--	--	df=2/4
H x M	67	0.363	24.5%	56.7	0.255	54.7%	H - Hermosillo
H x T	65	0.088	24.2	43.0	0.259	41.7	M - Magdalena
H x G	73	2.440	20.1	68.3	1.07	41.0	T - Tucson
H x D	91.5	0.419	18.3	61.0	1.18	55.7	G - Guaymas
H x S	62	0.831	13.7	52.7	0.98	48.4	D - Desemboque
							S - San Felipe
M x H df=2/4	79.3	2.919	11.6	46.0	0.037	58.2	
M x M	93.3	--	--	52.3	--	--	$F_{.20}$ = 8.53 df ½
M x T	74.0	1.638	28.37	41.3	0.137	55.4	$F_{.10}$ = 6.94 df 2/4
M x G	80.7	0.374	32.2	74.7	2.27	33.1	$F_{.20}$ = 4.32
M x D	87.3	0.049	30.3	55.0	1.13	62.8	
M x S	83.0	0.109	35.0	48.3	1.32	36.2	
T x H	44.7	0.866	17.5	42.3	0.033	47.0	F values between locality controls
T x M	79.3	5.113*	15.5	24.3	0.469	83.7	
T x T	61.7	--	--	46.0	--	--	
T x G	84.7	0.611	53.5	60.7	0.764	60.9	Cactus
T x D	65.0	0.633	52.2	51.0	0.488	68.2	F = 0.36 df 5/10
T x S	73.7	0.426	40.0	87.7	6.55*	36.8	$F_{.10}$ = 2.52
G x H	83.7	1.250	19.4	81.0	1.69	35.1	Non-cactus
G x M	61.3	0.724	50.5	75.0	1.10	46.3	F = 1.353 df 5/10
G x T	91.3	0.744	48.8	74.3	0.638	60.4	
G x G	101.3	--	--	40.3	--	--	
G x D	68.0	0.439	52.2	83.0	1.32	71.8	
G x S	98.3	0.145	45.5	99.0	4.26	38.2	

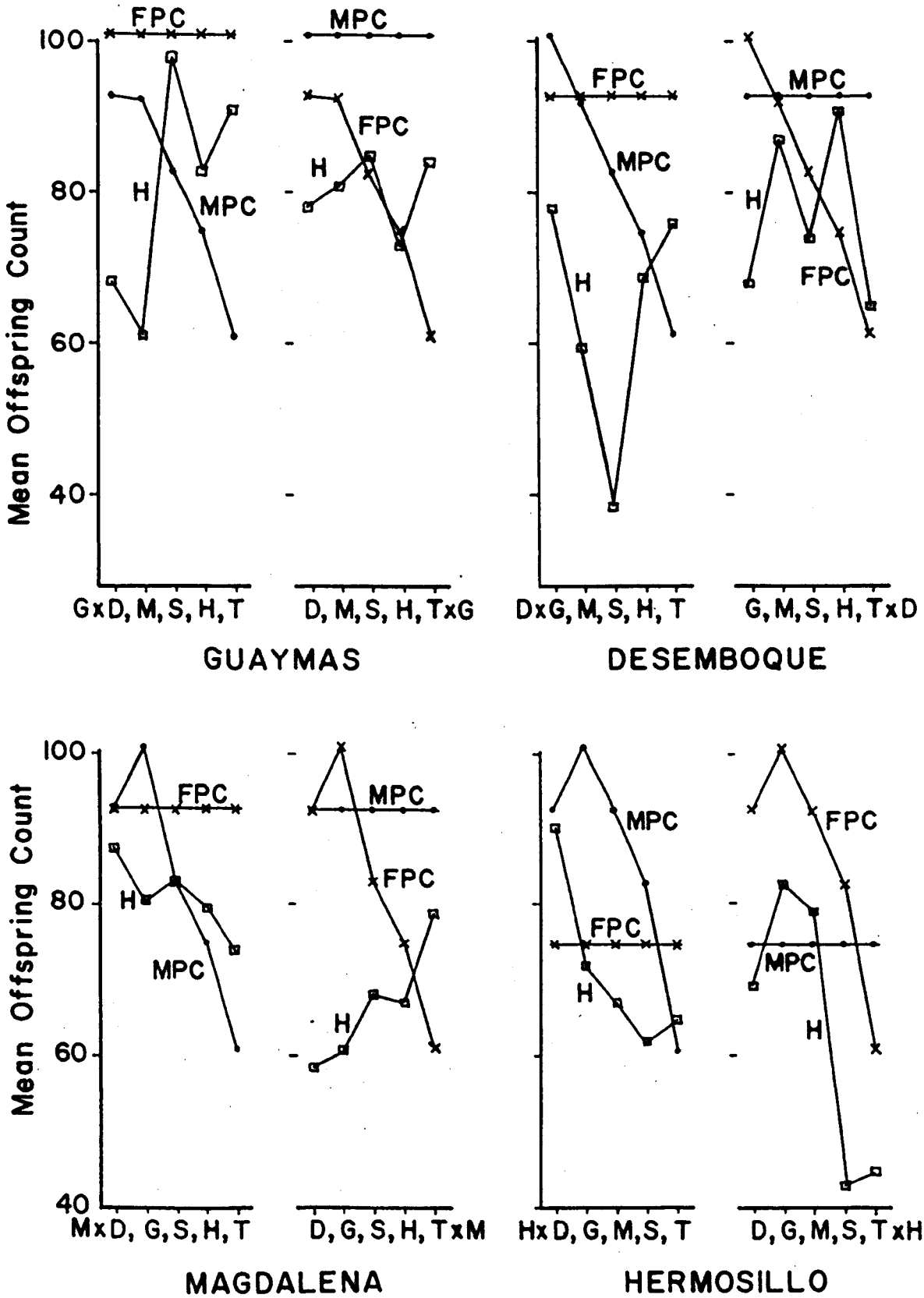
Table 2--Continued

Hybrid Tests	Cactus Media			Non-cactus Media		
	Means	F value	Coef. of variability	Means	F value	Coef. of variability
D x H	69.3	0.304	7.4%	39.0	0.454	65.8%
D x M	58.7	0.930	44.1	33.3	0.157	99.5
D x T	76.0	0.586	47.3	29.0	0.246	69.2
D x G	78.0	0.171	54.8	32.0	1.52	11.5
D x D	93.7	--	--	29.0	—	--
D x S	38.3	1.241	50.2	60.3	1.77	45.5
S x H	43.0	0.667	42.3	42.0	0.981	48.4
S x M	68.3	2.186	18.1	70.7	0.984	42.4
S x T	25.3	6.140*	36.7	10.3	6.470	36.7
S x G	85.3	0.200	41.7	58.0	4.450*	42.0
S x D	79.3	1.042	24.8	46.0	2.604	48.4
S x S	83.7	--	--	30.0	--	--

*Significant values

Figure 4. A comparison of the means between the locality hybrids and the parental controls from the test localities. These were bred on cactus-banana media.

FPC - Female parental control means, MPC - male parental control means, H - hybrid means. The graph to the left is of the matings with the females from the indicated test area, to the right is the reciprocal matings. G - Guaymas parent, D - Desemboque, M - Magdalena, H - Hermosillo, S - San Felipe, and T - Tucson.


Mean Offspring Count
100
80
60
40
FPC
MPC
H
GxD, M, S, H, T
D, M, S, H, TxG
GUAYMAS
DxG, M, S, H, T
G, M, S, H, TxD
DESEMBOQUE
MxD, G, S, H, T
D, G, S, H, TxM
MAGDALENA
HxD, G, M, S, T
D, G, M, S, TxH
HERMOSILLO

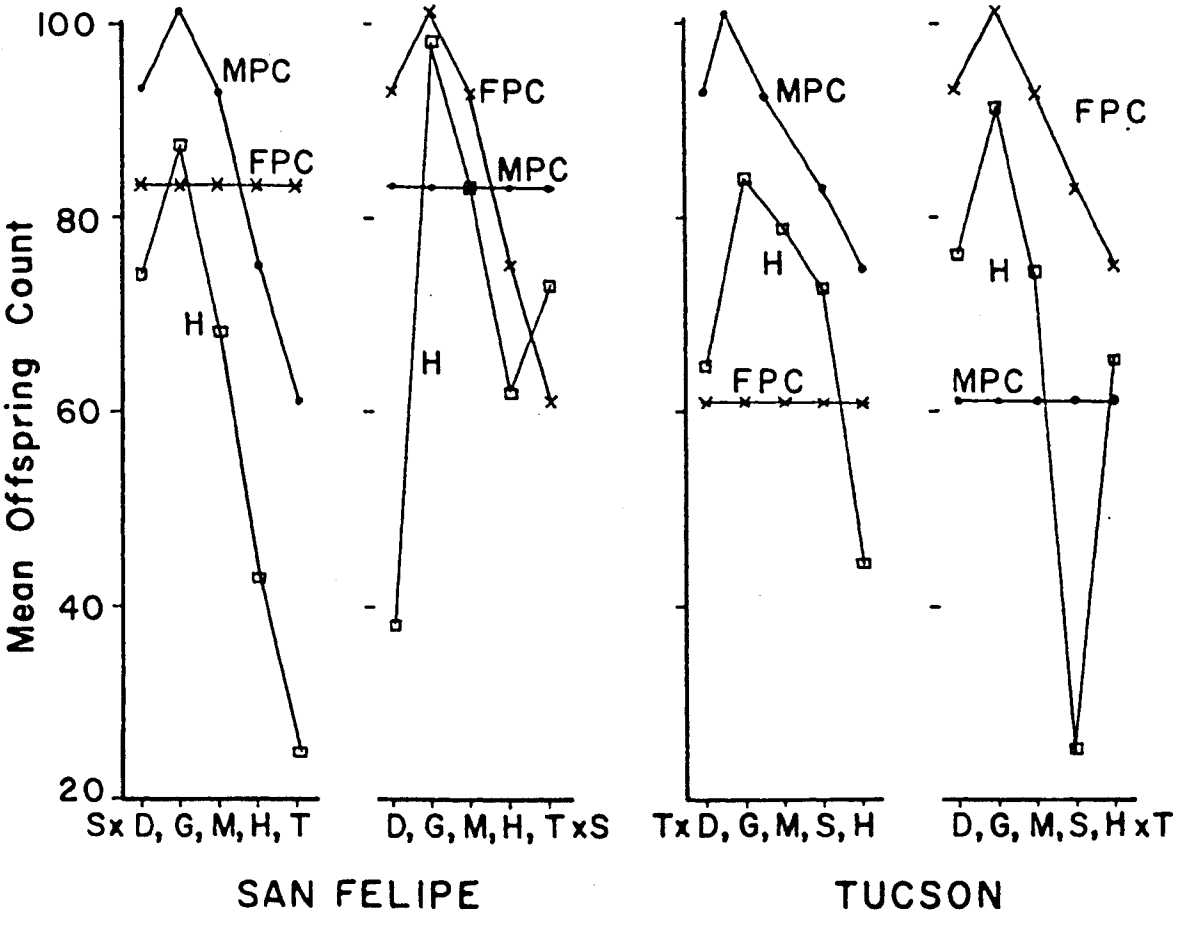
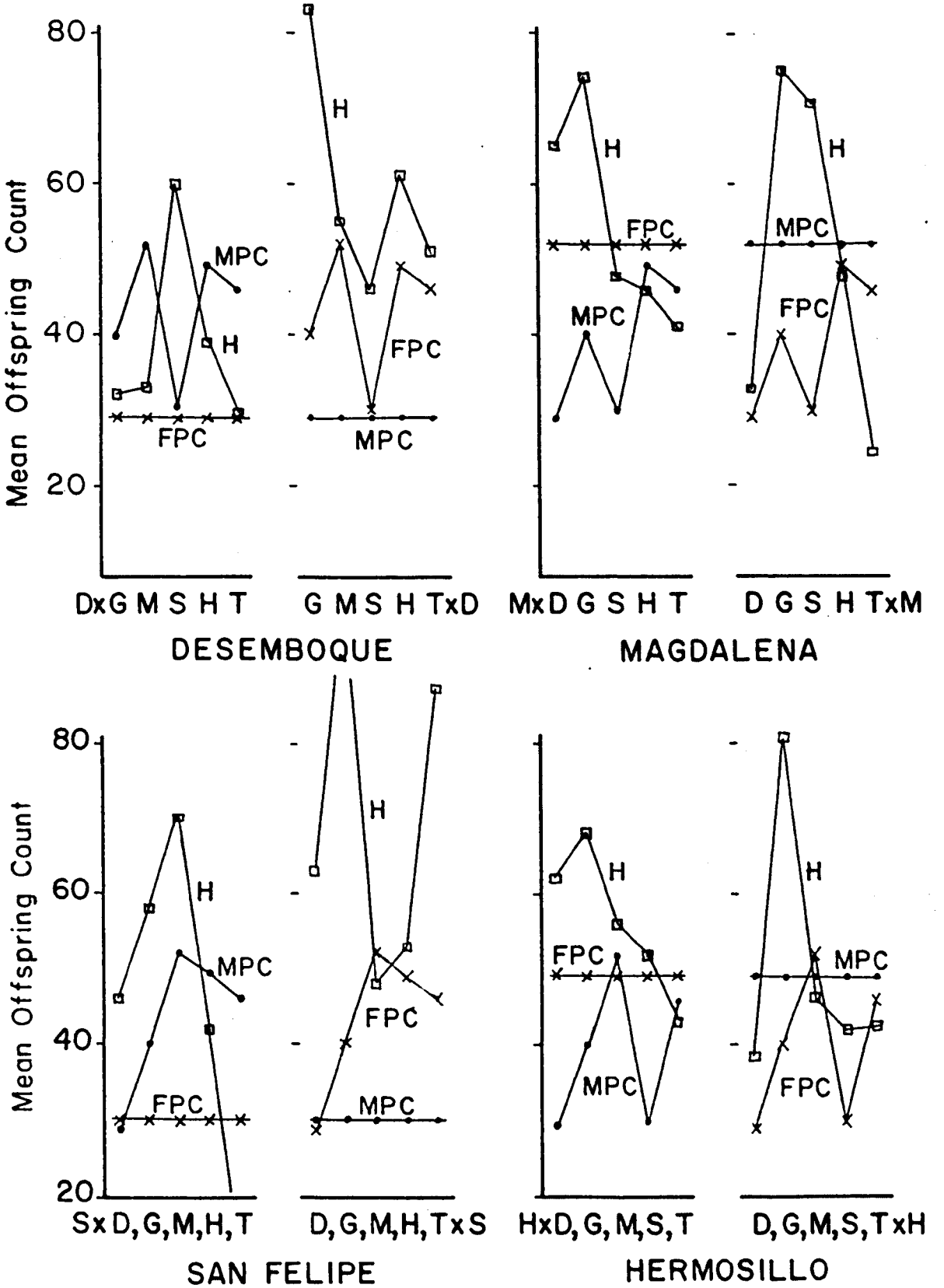


Figure 4. Continued

Figure 5. A comparison of the means of the F_1 matings carried out on a plain banana media.

This figure is similar to Figure 4. The symbols used are the same.


Mean Offspring Count
80
60
40
20
MPC
H
FPC
H
FPC
MPC
H
FPC
MPC
H
MPC
FPC
DxG M S H T
G M S H TxD
MxD G S H T
D G S H TxM
DESEMBOQUE
MAGDALENA
Mean Offspring Count
80
60
40
20
H
MPC
FPC
H
FPC
MPC
H
FPC
MPC
H
MPC
FPC
SxD,G,M,H,T
D,G,M,H,TxS
HxD,G,M,S,T
D,G,M,S,TxH
SAN FELIPE
HERMOSILLO

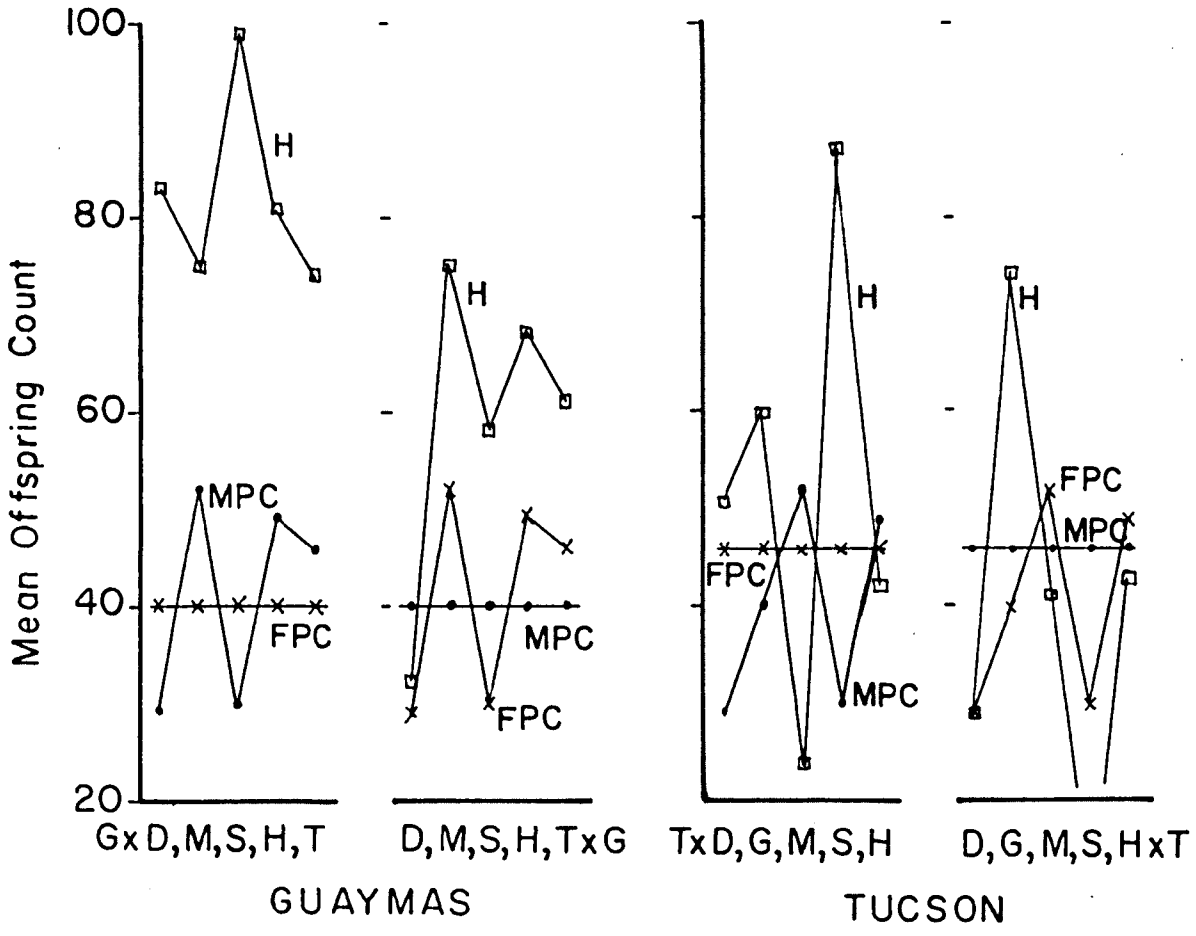


Figure 5. Continued.

and the Desemboque males. These means are consistently above the means of either parental type control (Figure 5). However, the F values obtained for this group of matings are not significant statistically, so heterosis is not indicated; also the coefficients of variability in these tests are somewhat higher than those in the cactus media tests. This would confirm evidence that the disease had a greater effect on this group.

The F_2 tests, when graphed according to order of decreasing mean value regardless of location of hybrid parents, show in some cases an overall raw value higher than the F_1 means; in other cases they vary from superior to inferior to the parental controls (Figure 6). When the single F_2 replications are compared to the results obtained in the F_1 using a t test for the cactus-media data, it is noted that in most cases the F_2 falls within the F_1 values and no differentiation is shown in the t values (Table 4).

Two of the five cases in which the t values obtained were significant were controls. Therefore, the comparison is probably poor.

Chromosome Study

The observation of the salivary chromosomes of all the hybrid tests showed that no inversions were represented in the populations of D. nigrospiracula.

The metaphase configuration for D. nigrospiracula as given by Wharten (1943) is 5 pairs of rods and a pair of dots. She indicates that all of the rods of the four autosomal pairs are of similar lengths. Measurements with an ocular micrometer by the present author

Table 3. F_2 imago counts.

F_2 imago counts on the differing media used in breeding of the P_1 and F_1. NC - plain banana media, C - cactus-banana media, D - disease destroyed vials, squares marked * also were affected by disease, and (--) no cross made.

Female parent	Media P_1	Media F_1	Male parent H	M	T	G	D	S	E
Hermosillo	C	C	112*	110*	100	145*	136*	82*	122
(H)	NC	NC	153	5	6*	D*	105	22*	D*
	C	NC	D*	72*	41*	158	219	D*	44*
	NC	C	98	98	91	55*	116	159	89
Magdalena	C	C	131*	100	113*	83*	119*	112*	116
(M)	NC	NC	65	45*	13*	--	108	23*	D*
	C	NC	106	63	98	10*	93*	94	94
	NC	C	67	--	73	12*	--	80	106
Tucson	C	C	85	182*	111	64	122*	114*	72*
(T)	NC	NC	52	85*	--	D*	131*	54	--
	C	NC	101	66	19*	74*	D*	108	--
	NC	C	155*	--	--	100	118*	125	--
Guaymas	C	C	98	3*	80*	13*	D*	135	125*
(G)	NC	NC	207	93	--	--	25*	50	56
	C	NC	123	162	94	113	113	D*	55
	NC	C	113	80	--	--	58*	95*	66
Desemboque	C	C	84	D*	90	--	86	0	D*
(D)	NC	NC	140	141	84	80*	118	117	141*
	C	NC	121	58	--	101	30*	0	D*
	NC	C	126*	113*	D*	72*	66*	152	123
San Felipe	C	C	58	132	6*	98*	36*	169*	D*
(S)	NC	NC	97*	124	31	152*	122	37*	D*
	C	NC	D*	10*	35	0	204*	102	D*
	NC	C	103	95*	128*	43*	123*	--	92*
Empalme	C	C	152*	81*	112	5*	74	70*	156
(E)	NC	NC	2*	77	D*	85	--	4*	--
	C	NC	89*	197	41	42*	D*	103	6*
	NC	C	0	94	2*	113*	--	27	--

Table 4. Comparison of F_2 result with the F_1.

t - test values from a single replication of the F_2 compared with the three F_1 replication values in the cactus media test.

Female parent	Male parent H	M	T	G	D	S
Hermosillo	-8.46*	-12.42*	-0.653	-8.32*	-0.904	-0.653
Magdalena	-0.704	-0.165	-2.56	-0.037	-1.32	-0.638
Tucson	-2.058	-2.58	-1.041	1.801	-2.462	-1.886
Guaymas	-0.767	1.28	0.96	1.42	------	-1.129
Desemboque	-0.489	-----	-0.708	------	0.18	------
San Felipe	-0.928	-4.14*	0.678	0.459	0.794	3.41*

*Significant values at the 10% level.

For Hermosillo female df value = 1, all other df values = 2.

at df = 2
$t_{.10} = 2.920$

at df = 1
$t_{.10} = 6.314$

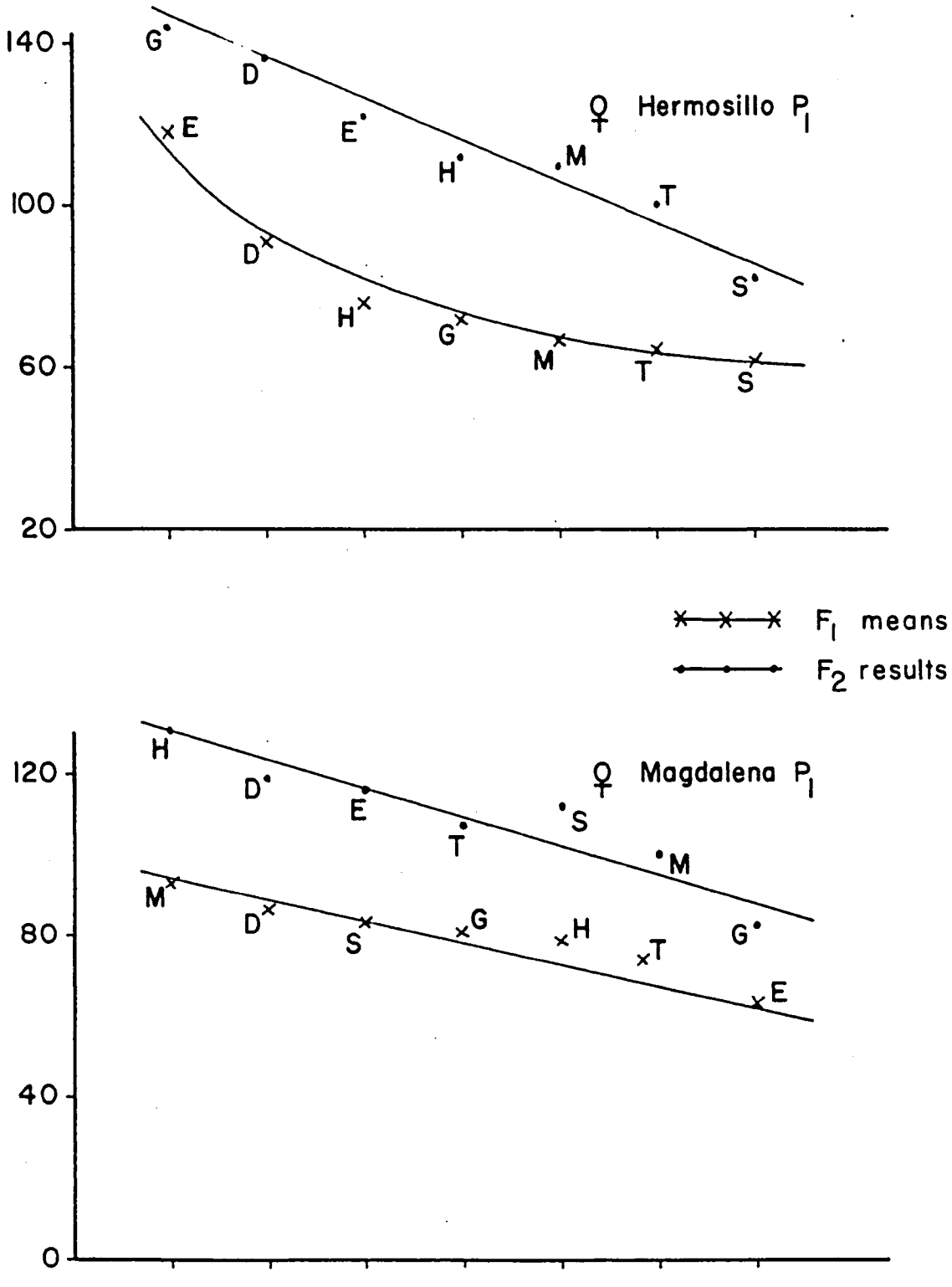


Figure 6. A comparison of the F_2 results and F_1 means for each locality female.

Numerical values of the matings are placed in decreasing order.

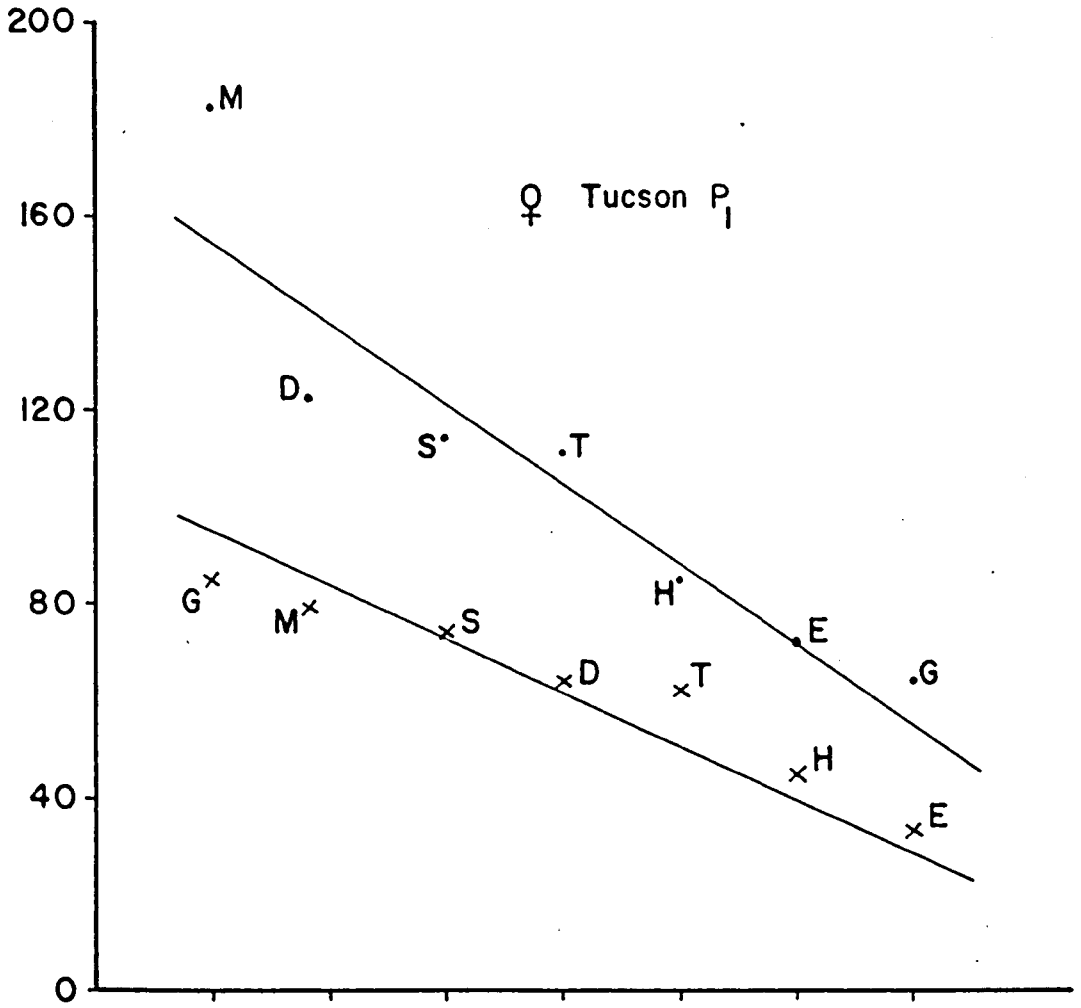


Figure 6. Continued.

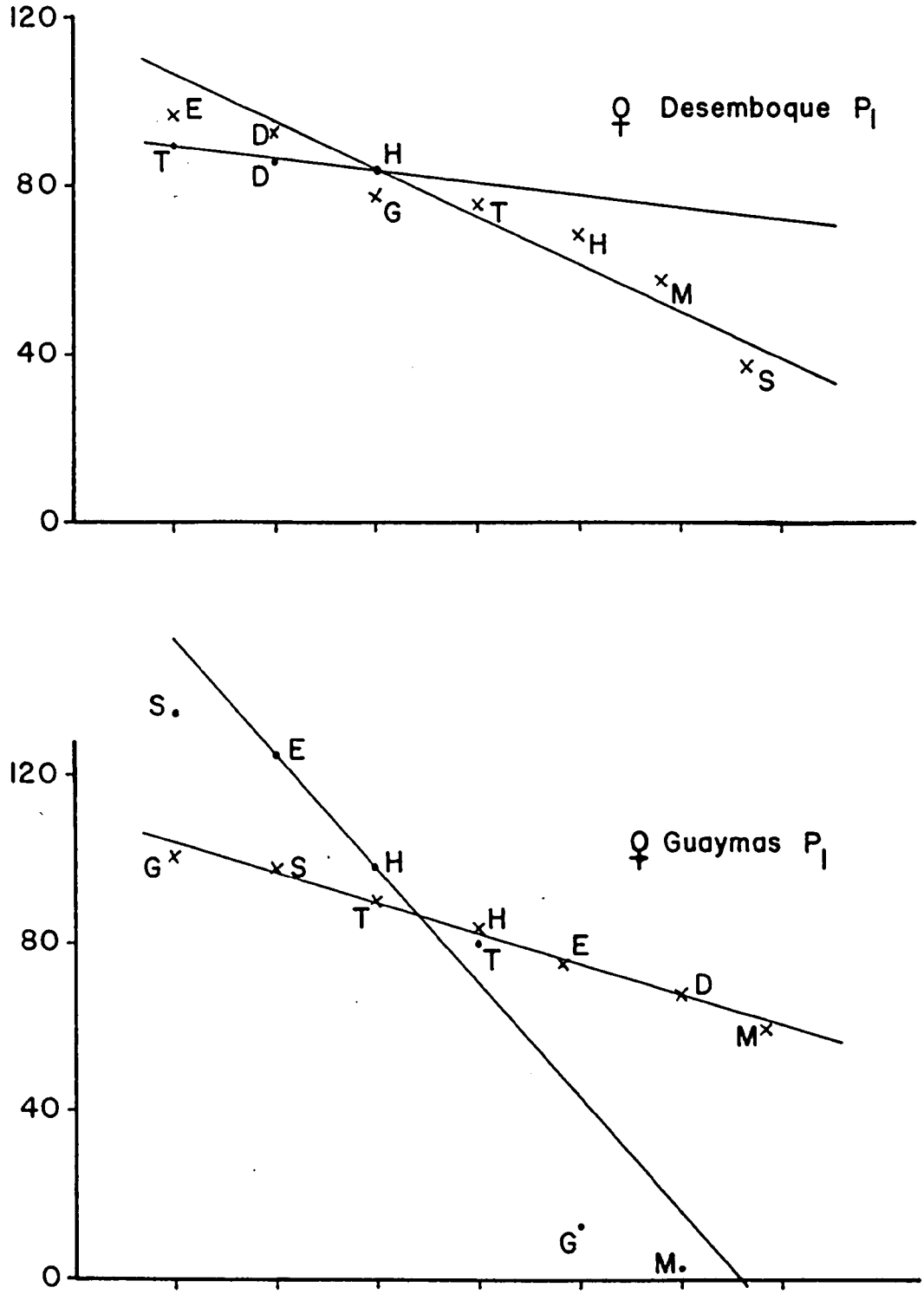


Figure 6. Continued.

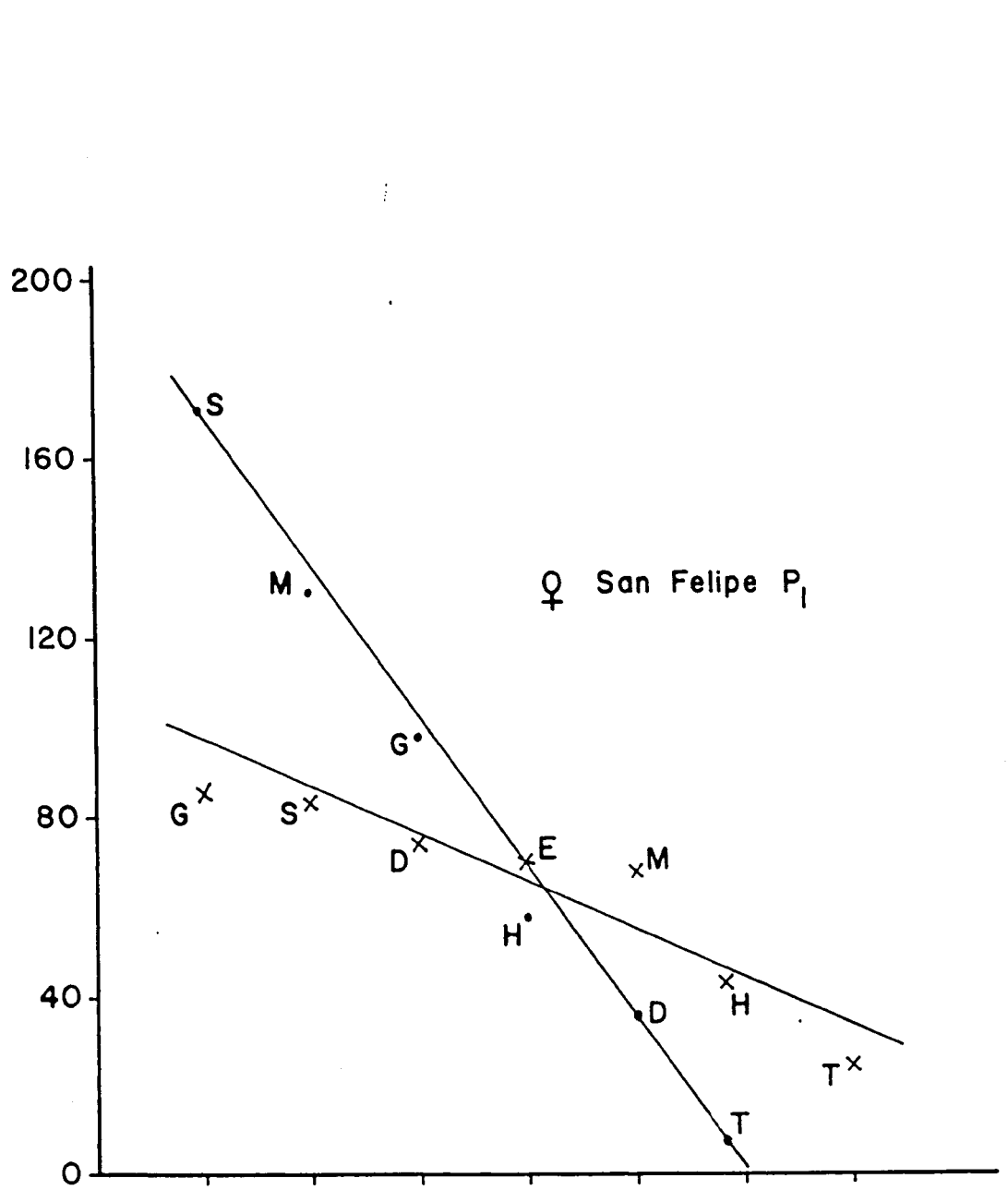


Figure 6. Continued.

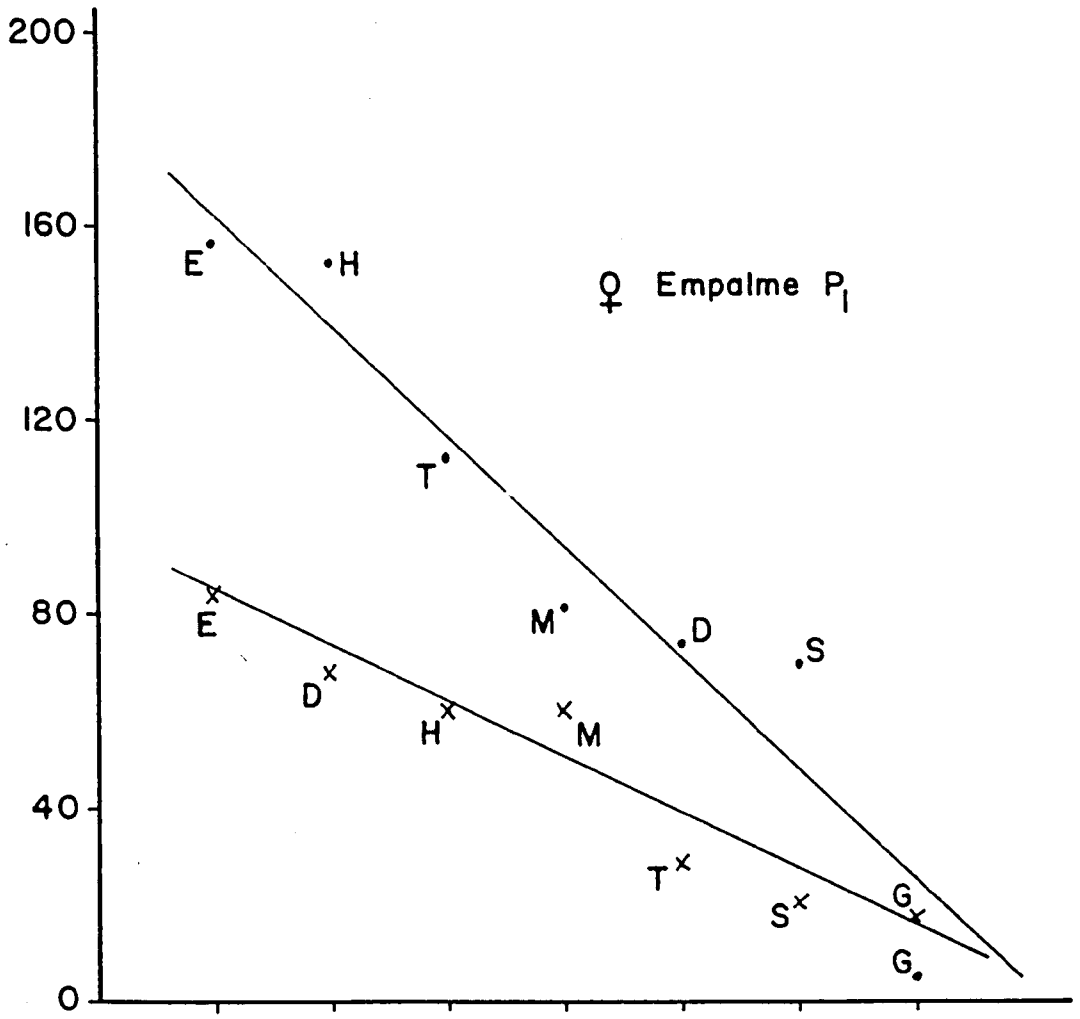


Figure. 6. Continued.

show that the pairs differ in length (Table 5). The X and Y chromosomes seem to vary in shape as seen in the various cells; however, difficulty in obtaining late prophase or early anaphase cells of sufficient clarity or numbers and a lack of accurate recording methods prevented determination of whether or not these morphological differences were consistent with locality or simply the inconsistencies often seen in sex chromosome material.

Table 5. Rough measurements of metaphase chromosomes made with an ocular micrometer

Measured at 970X with a micrometer reading of 1 - 0.010754. An analysis of variance was run on this data; the resulting values are shown. Some differences in lengths are due to the various stages in mitosis.

Mating	Rod chromosome lengths					Dot
	A	B	C	D	E	
D x D	0.50	0.45	0.40	0.40	0.30	0.15
D x D	0.40	0.30	0.30	0.25	0.25	0.15
D x D	0.35	0.35	0.30	0.30	0.30	0.10
G x G	0.35	0.35	0.35	0.30	0.25	0.12
M x M	0.30	0.30	0.30	0.25	0.25	0.10
M x H	0.40	0.30	--	0.25	0.25	0.15
M x H	0.40	0.35	0.30	--	--	0.15
T x G	0.30	--	0.25	0.25	0.25	0.075
S x G	0.30	0.27	0.27	0.25	--	0.10
E x T	0.40	0.40	0.35	0.35	0.30	0.15
$\bar{X}$ (means)	0.37	0.341	0.313	0.289	0.269	0.124

$F = 5.6538$ $F_{.005} = 4.08$

DISCUSSION

Speciation in the Repleta Group

Wasserman's work (1960) on the chromosome phylogeny of the repleta group in the genus Drosophila has helped indicate the type of speciation which has possibly taken place in this species group. He has confirmed the division of the repleta group into five subgroups, of which four - the hydei, melanopalpa, mercantorum and mulleri subgroups - have species members found mainly in the Nearctic and Neotropic deserts. The total number of species in the repleta group presently is about 68.

Wasserman asserts that the primitive ancestral stock of the mulleri subgroup was a desert inhabitant occupying a large diverse ecological area. The desert environment possibly forced dispersion of the population into many small semi-isolated units. Mutation and cytological variations arose within these units and selection took place at a local level. However, in the beginning of the process some exchange of genetic material between units did occur, but as local adaptations increased in perfection the effects of immigration decreased until all gene exchange was stopped and eventually new species were formed.

The lack of a substantial number of cytological variations in the repleta group in general, as especially shown in D. mulleri, D. aldrichi, and D. wheeleri of the mulleri subgroup, which have no inversions amongst them, indicates that variation at the genic level was the dominant factor in the speciation of the group.

D. nigrospiracula, being limited in its environment to a very few kinds of cacti and in population size to restricted numbers dependent upon the seasons when decaying cacti are available, would be expected to be a species that is relatively homozygous genetically as well as cytologically. The lack of heterosis as shown by the data indicates this, and also there is probably no selection for genes giving heterosis.

Population Structure and Heterosis

Carson (1959) has suggested that large populations have developed a genetic system that depends on heterozygosity between alleles or heteroselection. This type of selection makes it possible to store a great deal of variability within the population. Dobzhansky (1955) reviews the fact that a heterozygous population carries many lethals and semilethals, while a small homozygous population carries a much smaller percentage of these factors. The populations depending on heterselection must be large enough to withstand the loss of those individuals with deleterious homozygous traits that appear in heterozygous matings.

It is felt that these populations have selection for sets of genes that aid in the integration of heterozygous factors resulting in the overdominance of these heterozygous individuals.

Carson (1959) goes on to discuss those populations in which homoselection or homozygous selection occurs. He feels that they are populations which are small in size and in which a substantial amount of inbreeding occurs. The small populations cannot afford to lose 25 to 50 percent of their genetic material in each generation because of inferior alleles being carried in the population, as a large population

is supposed to be able to do. Instead these populations must have sets of genes that are integrated on the homozygous level.

Carson mentions the repleta group as a good example of one whose species generally show a strong tendency for homoselection. This type of selection would account for the carriage of the inversions found in this group in a fixed rather than in a heterozygous state, as pointed out by Wasserman (1960).

Rate of Speciation

Haldane (1957) demonstrates mathematically that the time needed to replace one allele with another within a population takes many generations even when the selective value for it is rather high. Many deaths of individuals with the old alleles are needed to substantially increase the percentage of the new allele within the populations' total genetic makeup. At the same time this new allele is being fit into a population, modifiers that increase the alleles' selective advantage and dominance are being selected. Using this basis Haldane states that the time needed to produce a real genetic divergence on a species level is approximately one million years for mammals in a stable environment.

However, very rapid selection for several loci at a time could occur under certain circumstances, as in an environment that may be favorable to colonization, and offers no competition from other organisms to the pioneering individuals. Here the death rate may be high but those individuals that do survive continue the change of the genotype until it eventually is well-adapted to the environment. This is possibly what happened when the repleta group moved into the desert

areas. Here was a new environment which offered many unfilled niches much as a deserted island would offer. Rapid genetic change and speciation could occur via homoselection of small populations. Once the new niches were filled the rate of genetic change and speciation would probably slow down to the normal extremely slow rate.

Heterosis

A species' population and genetic structure depends upon many environmental and chance factors. Vetukhiv (1953, 1954, 1956) in working with D. pseudoobscura, D. willistoni, and D. paulistorum has noted that heterosis existed between most of the geographical populations of the two former widely-distributed species, while it did not exist between populations in the latter species that build up smaller populations. Where heterosis is evident in his F_1 tests, a breakdown occurred in the F_2 to a level lower than that seen in either of the parental type controls. It was between these populations that differentiation had occurred. Each had sets of genes that aided its success in its environment. In the F_1 these sets of genes were maintained intact in a combination that gave the larvae a superior ability to survive. But in the F_2 the proven gene combinations were broken up by recombination during meiosis and the poor integration of function in the new sets of genes produced inferior offspring.

D. nigrospiracula

The tests with D. nigrospiracula may not be as critical as many of the tests used by Vetukhiv and others since the criterion for the

presence or absence of heterosis is the comparison of the total number of hybrid progeny with the total number of homogamic progeny. Table 6 shows a great deal of fluctuation between the control matings within the three F_1 replications. However, when the total number of treatments (30 within each replication) are considered, all of the hybrid mean values are intermediate or below the control mean values. Thus, it appears to be more than coincidence that no heterosis exists between the geographical strains of D. nigrospiracula.

The lack of any isolating mechanisms which would indicate a divergence between those flies living in areas of cardon and those living in sahuaro or a sahuaro-cardon mixture would demonstrate several possible conditions. The San Felipe cardon area is separated from the sahuaro and the mixed area by forty to sixty miles of water across the Gulfo de California and the Gran Desierto to the north of the Gulf. This would appear to prevent any gene flow between the two areas.

One explanation for the absence of any isolating mechanisms is that the ecological substrate which appear so different are not. Cardon and sahuaro have been placed in different genera, but Buxbaum (1958) suggests that both cacti belong in the same genera, Pachycereus, on the basis of morphology. Their appearance is certainly similar. If this is so, then the micro-environmental stresses may not be so dissimilar. Also the separation between these populations is probably too recent an event to produce noticeable biological barriers to gene exchange.

Table 6. Fluctuations seen in the control results

Raw data obtained in the replications of the locality parental controls are shown with the means. Ratios of this data show that little consistency is maintained between replications.

Mating	Replication number				Ratios from replications		
	1	2	3	$\bar{X}$	1	2	3
H x H	--	78	73	75.5	2.80	1.30	2.01
M x M	98	126	56	93.3	2.45	2.10	1.55
T x T	40	109	36	61.7	1.00	1.82	1.00
G x G	50	97	157	101.3	1.25	1.61	4.38
D x D	135	60	86	93.7	3.38	1.00	2.38
S x S	101	92	59	83.7	2.25	1.53	1.63

SUMMARY

The geographical populations of D. nigrospiracula studied did not show any evidence of genetic divergence in the hybridization and cytological tests carried out in this work.

As stated in the discussion this species probably conforms to earlier statements made concerning the species members of the repleta group. They arose recently (geological age) in the deserts of the western hemisphere in a manner which has favored homoselection rather than a heterotic form of selection.

Either the environmental pressures on the flies from the diverse ecological conditions studied are very similar and/or not enough time has yet passed for any major divergences to show in the genetic makeup of the populations as tested.

APPENDIX

In order to test the relationship between Species M and D. nigrospiracula, matings between the two species were carried out. Three females of Species M were placed with three males of D. nigrospiracula in a cactus-media vial. Matings of Species M with each of the D. nigrospiracula locality stocks were made in duplicate; also the reciprocal matings were made. Control matings on a plain banana and intraspecific matings were made up. No larvae were noted in the vials over the three weeks observation period. Observation at the beginning of the mating period showed no courtship activities taking place. The sexual isolation between the species seems to be complete.

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