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Studies on Laboratory Populations of *Drosophila americana americana* and *Drosophila americana texana*

by

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Studies on Laboratory Populations of *Drosophila americana americana* and *Drosophila americana texana*

by

H. Michael LeFever*

INTRODUCTION

Creation of an experimental Drosophila population even remotely comparable to a free-living one is quite difficult. The first attempts made to solve this problem were to increase the size of the container in which the flies were raised and bred. However, this approach was unsuccessful due to the fact that no matter how much food it contained, sooner or later the flies had to be transferred to fresh medium. This continual transfer of a population presents overwhelming difficulties in sampling technique (Wright and Dobzhansky, 1946). The nearest approach to a successful solution is that of L'Heritier and Teissier (1933), who were the first to utilize the population cage. This cage type and modifications of it, have been used on studies of *Drosophila pseudoobscura* by Dobzhansky and fellow workers (Dobzhansky, 1945, 1947, 1951; Dobzhansky and Levene, 1951; and Wright and Dobzhansky, 1946), by Stone, Alexander, and Clayton (1954) in a study of heterosis in *Drosophila hydei* and *Drosophila novamexicana,* and by Mettler (1956) and Bruneau (unpublished).

Other methods of producing Drosophila populations have been presented in the literature. Reed and Reed (1948, 1950) made use of a population chamber in which fresh food was introduced into the population by changing one of two half-pint milk bottles. These milk bottles were connected by a three-inch long section of automobile radiator tubing. Merrell (1953) used a modified version of the population chamber designed and used by Reed and Reed (1948, 1950). Merrell used two small homeopathic bottles with a combined volume of 32 cubic centimeters, which were held together by cotton bound with scotch tape. Reed's population chamber was also modified and used by Ludwin (1951). Epling, Mitchell, and Mattoni (1953) used three types of cages. The first cage was constructed of galvanized iron and 50-mesh screen in such a way that pint Kerr jars could be screwed into the bottom and serve as receptacles. This cage, being large in size, was primarily designed for out-of-doors experiments.

*Dr. LeFever is an Associate Professor of Biology at Kansas State Teachers College, Emporia, Kansas. This study originated as a partial fulfillment of the requirements for the degree of Master of Science at Oklahoma State University.

However, this type of cage was also used in the laboratory. The second cage, designed by Mitchell, was made of plywood with 50-mesh screens on the sides and so devised that a series of plastic trays could be introduced and removed at regular intervals. These experimenters also made use of Fernbach flasks in which only liquid food was employed in such a way as to simulate a slime flux.

The evolution and species relationships of the virilis species group has been studied and worked out by Patterson and Stone (1952). Hsu (1952) reviewed the chromosomal variation and evolution in the virilis group.

The virilis species group was divided by Patterson and Stone (1952) into four subgroups: (1) *Drosophila virilis,* which is native in the eastern Palaeartic and Oriental regions; (2) *Drosophila americana americana, Drosophila americana texana,* and *Drosophila novamexicana,* which occur in North America; (3) *Drosophila montana, Drosophila flavomontana, Drosophila borealis,* and *Drosophila lacicola,* which are more distantly related North American forms; (4) *Drosophila littoralis* and *Drosophila imeretensis,* which are European forms. Two subspecies were chosen for this study: *Drosophila americana americana* and *Drosophila americana texana.* These are two closely related forms from the second group.

The purpose of this study was to investigate the subspecific relations between specific stocks of the two subspecies. The two subspecies, *americana* and *texana,* are known to have a zone of overlap in which they will hybridize (Patterson and Stone, 1952). The two stocks used in this study were selected from two far removed locations in order to insure that hybridization had not occurred. Two cage populations were used in this study, one started with *americana* females and *texana* males and the second, *texana* females and *americana* males. It was hoped, by sampling these cages at given intervals, that data would be gathered by which two hypotheses could be tested. The first hypotheses was that selection would have an effect on a given genotype in the laboratory population. The second hypotheses was that there might possibly be an interaction effect between some of the genotypes. In other words, the effect on one given genotype may influence another genotype in some manner. The overall purpose of this study then, was to test the relative adaptibility of a given genotype in a laboratory population.

MATERIALS AND METHODS

The cages used were modified versions of these designed by L'Heritier and Teissier (1933) and L'Heritier (1937). The cages were designed so that fresh food could be introduced and the worked-out food removed. This arrangement enables the size and age distribution in the population cage to remain approximately stationary (Wright and Dobzhansky, 1946).

The cages had the inside measurement of 14 x 10 x 6 inches. Three sides of the cage, the two long sides and the back were covered with fine copper mesh screens. In these cages, the two long sides were covered with aluminum foil to prevent the loss of moisture in the

cages. The screened back was left open for ventilation purposes. The front was enclosed entirely by wood except for a funnel which was closed by a cork. The funnel allowed for the addition of a yeast solution to the food while the flies were breeding in the cages. The bottom of each cage had 15 circular openings 2¼ inches in diameter, closed by tightly fitting tapered corks. The top of each cage had a glass window through which the flies and the condition of the food cups could be observed.

The food used throughout the population study was a banana-agar medium. The medium consisted of water, yeast, agar, molasses, karo syrup, crushed bananas, and propionic acid. The medium was mixed and brought to a boil and then poured into one-half pint milk bottles for storage in a refrigerator. For use in the cage, the medium was transferred to crystallizing dishes, which were securely taped to corks. The medium was diced to facilitate egg laying by the females. A weak water-yeast suspension was added daily to prevent the medium from drying and also to provide extra nourishment for the larvae (Wright and Dobzhansky, 1946).

The cages were sampled at 15 days from origin and at 30-day intervals after the initial sampling. A sample was taken over a 5-day period. This was done to minimize sampling error (Wright and Dobzhansky, 1946). On the first day of sampling, a fresh food cup was introduced into the cage. Twenty-four hours later, a second day cup was added; the first day cup was removed and the eggs transferred to a culture bottle. The third, fourth, and fifth day sample was each taken using the same procedure. The third day was designated as the actual sample date. The culture bottles were stored at approximately 25°C.

Slides were made when the larvae reached the third instar stage. The third instar stage was reached usually on or about the eighth day after the sample was taken. The procedure for making the slides was as follows: (1) The larvae were placed in Drosophila saline (0.67 gm NaCl/100 ml. of H_2O). The salivary glands were dissected and immediately placed in 1N HCl for one minute. (2) The glands were removed from the HCl and placed in aceto-orcein stain for approximately 12 minutes. The time in stain was not controlled precisely because staining time had proved not to be critical. (3) The glands were removed from the stain and placed on a slide in one drop of 45% acetic acid. (4) The glands were covered with a cover slip and squashed by pressing on the cover slip with a wooden dowel. This step was critical because if the pressure placed on the cover slip was too hard, the chromosomes were shattered and analysis was impossible. Analysis was also impossible when the pressure applied was too light. The light pressure prevented the chromosomes from being spread enough to allow critical observation. (5) The cover slips were ringed with a mixture of resin, lanolin, and Canada balsam. The method described produced excellent slides, nearly all of which were suitable for analysis. The slides were of a temporary nature and therefore stored in a refrigerator to prevent drying due to evaporation of the acetic acid, and to prevent destaining of the chromosomes.

The two subspecies used in this study were members of the virilis group of the subgenus Drosophila. The stocks used, with the University of Texas stock numbers and collection localities were:

Drosophila americana americana
2515.3 Nebraska
Drosophila americana texana
252.2a Jamestown, South Carolina

The two stocks were homozygous for chromosome inversions. Each inversion could be located and recognized by the sequence of the bands on each of the salivary chromosomes. Heterozygous inversions could be recognized by the characteristic inversion loops which were formed. Each stock was crossed to *Drosophila virilis,* because *virilis* had been taken as a standard for the species group and had no inversions in either the homozygous or the heterozygous condition. Therefore, all the progeny in the F_1 from this cross would show, in the heterozygous condition, the inversions present in each of the subspecies stocks. Specific inversions are designated by letters of the alphabet as shown in the following table.

Chromosome Number	*virilis* and *texana*	*virilis* and *americana*
X	overlapping A and B	-
2	A	A
3	A	A
4	-	A
5	A	B

From this table it can be seen that in a cross between *texana* and *americana* that the X, fourth, and fifth chromosomes could be followed. In this study only the X and the fifth chromosomes were considered, due to the extreme difficulty in recognizing the presence of the "A" inversion on the fourth chromosome in the homozygous state. In all instances, the sixth chromosome could not be analyzed.

Two cages were run in the study. These were set up as follows:

Cage I – *americana* females and *texana* males
Cage II – *texana* females and *americana* males

The cages were started on September 22 and September 27, 1961. Throughout the study, the cages were referred to as Cage I and Cage II. The cages were begun by introducing 200 males and 200 females into each cage. The flies were virgin, and five days old when introduced into the cages. One food cup was also introduced into the cages at this time. A new food cup was added every third day for the remainder of the study.

RESULTS

In this population study, two chromosomes were analyzed. These were the X and the fifth. The inversions used were: overlapping A and B on the X; and inversions A and B on the fifth. The first sample was taken on the fifteenth day. Samples were taken at 30-day intervals after the initial sample until the study was completed. Each sample consisted of 75 individuals except where noted under each cage result.

The X chromosome data are based only on females from the samples. This would mean that the number of X chromosomes analyzed was less than that for the autosomal chromosomes. The number of X chromosomes analyzed is given in table 5.

Cage I

Cage I was set up on September 22, 1961. The initial population consisted of 400 virgin flies of equal numbers of *texana* males and *americana* females. The first emergence was observed on October tenth. More than 50 percent of the initial population was dead at the time the first emergence was noted.

In table 1, it can be seen that the percentage of *texana* chromosomes dropped in the X chromosome samples. Equilibrium for the X chromosome was apparently reached when the *americana* X chromosome reached approximately 60 percent. Chromosome 5 apparently reached equilibrium when the *texana* fifth chromosome reached between 50 and 54 percent.

Data in table 2 shows that in the X chromosome, the two homozygous forms were found more frequently than the heterozygous form. Homozygous *americana* was found more frequently than was homozygous *texana*. Data in table 2 also shows that in chromosome 5, the heterozygous form was more frequent than either homozygous *americana* or *texana*. At the 135-day level, the two homozygous forms were found to be nearly equal in number.

In this cage, samples three and four did not consist of 75 individuals. Sample three consisted of 41 individuals and sample four consisted of 58 individuals. The low number in sample three was attributed to some unknown factor which caused a high egg mortality. There was not any way of definitely pinpointing this factor. The low number in sample four was caused by improper technique of the author. A new batch of stain used in this sample produced slides which were unable to be analyzed.

Cage II

Cage II was set up on September 27, 1961. The initial population consisted of 400 virgin flies of equal numbers of *americana* males and *texana* females. The first emergence was observed on October 15. As in Cage I, the initial population was more than 50 percent depleted at the time the first emergence was noted.

Data in table 3 shows that in both the X chromosome and the fifth chromosome, the *americana* chromosomes were more frequent. Equilibrium was reach in the X chromosome when the *americana* X chromosome reached approximately 56 percent. Equilibrium was reached in the fifth chromosome when the *americana* fifth chromosome reached approximately 53 percent.

Data in table 4 shows that in both the X chromosome and the fifth chromosome, the heterozygous combinations were found more frequently. Table 4 shows that homozygous *americana* occurred more frequently than homozygous *texana*.

DISCUSSION

Patterson and Stone (1952) have recognized ten forms in the virilis species group. Two of these forms, both North American species, have been used in this study. The two are *Drosophila americana americana* and *Drosophila americana texana*. These two subspecies were separated in this study by means of chromosome inversions, which can be identified and analyzed in the salivary gland chromosomes. The inversions in the group have been intensively studied by Hsu (1952).

The karyotype of *americana* differs from the basic karyotype of the genus, which has five pairs of rod-shaped chromosomes and one pair of dot chromosomes. The difference lies in that *americana* has a fusion of the second and third chromosomes and also a fusion of the X and the fourth chromosomes, forming metacentric elements. The karyotype of *texana* also differs from the basic karyotype in that the second and third chromosomes are fused. In the *americana* and *texana* populations, the possibilities of any effects on recombination of the fusion of the chromosomes were not analyzed as the second, third, and fourth chromosomes could not be followed in this study.

Reciprocal crosses between *americana* and *texana* are fertile and produce fertile offspring (Patterson, Stone, and Griffen, 1940; Patterson and Stone, 1952). The two cages indicated reasonable fertility in that the F_1 populations were quite large. Patterson and Stone (1952) state that the percentage of cultures when *americana* was used as the female parent was higher than in the reciprocal cross. This statement did not seem to be true in this instance as there did not seem to be any appreciable difference between Cage I and Cage II in the number of F_1 offspring.

The number of flies in the cages did not seem to vary to any great extent from generation to generation. Even though there was not any method of counting the number of flies in the cages, estimates of the population present could be made by observation through the glass window. The number of flies in each cage was estimated to be between 10,000-15,000. However, there did seem to be a trend toward fewer flies in the cages toward the end of the study. These results differ from those found by Bruneau (unpublished). He found that there was a regular alternating cycle of large and small populations in any given

cage. The number of adults produced must have been quite small when compared with the number of eggs laid. When each used food cup was removed at the end of 45 days, the author noticed that there were hundreds of dead larvae found under the remains of dried food. A lack of food was probably the cause for this larvae mortality, although lethal genes could have also caused the same result.

In the reciprocal crosses between *americana* and *texana*, *americana* chromosomes were predominant except for one chromosome in Cage I. As shown in tables 2 and 4, the analysis of the chromosome combinations usually showed the heterozygous combinations to be more frequent than either homozygous class. These results, in general, agree with those of Dobzhansky and fellow workers with the third chromosome of *pseudoobscura* both in natural and laboratory populations (Dobzhansky, 1945, 1947, 1948, 1949, 1951; Dobzhansky and Epling, 1944; Dobzhansky and Levene, 1951; Dobzhansky and Pavlosky, 1953, 1958; Wright and Dobzhansky, 1946). Equilibrium of the chromosome types within the population is reached because the value of the heterozygote exceeds the adaptive value of either homozygote. The adaptive superiority of the heterozygotes is called heterosis. When equilibrium is reached in a cage due to the presence of heterosis, natural selection prevents the elimination of any of the gene arrangments from the population. If elimination of a gene arrangement occurred, the adaptive plasticity of the population would be reduced (Dobzhansky, 1948).

In one case in this study, the data in table 2 shows that one of the homozygous combinations may be adaptively superior to the heterozygote. This result was also found by Bruneau (unpublished) and Epling, Mitchell, and Mattoni (1953). Epling and fellow workers in working with *pseudoobscura* indicated that seasonal differences may result in the heterozgotes not being superior to the homozygotes in a natural population. In a cage population, differences in adaptability may exist between different samples. Dobzhansky and Levene (1951) and Dobzhansky and Pavlosky (1953) show with their data that the adaptiveness of a chromosome combination is a changing factor during the course of cage experiments with *pseudoobscura*. There are two possible reasons why the heterozygotes are not heterotic under the given cage conditions. If heterosis is not present, then the heterozygotes are not adaptively superior to the homozygotes. Random mating in the cage may have been disturbed and there would be a possibility of an over-production of homozygotes (Bruneau, unpublished). There was not any evidence obtainable from the data to indicate which of the two possibilities caused the homozygotes to be more frequent than the heterozygotes. Both of the conditions could have been present in this study.

Environmental changes have been shown to be significant in population cage studies (Wright and Dobzhansky, 1946). These changes or variables include temperature, light, and food. In order to minimize the effects of these variables, efforts were made to maintain the cages in a static or stable environment. Temperature was the most difficult

to control. A room was used for this study in which the temperature varied from 21.5°C. to 26.5°C. As can be seen, this fluctuation of several degrees could have had an effect on the populations. However, the two cages were subject to the same fluctuations. Light and food were controlled to a minimum fluctuation for the entire time of the study.

In regard to the literature, only two previous studies have been done which are comparable to this study. These were done by Bruneau (1956) and Mettler (1956). However, neither of these studies utilized subspecies, and hence are not directly comparable.

This study cannot be directly compared to studies of Dobzhansky, in that he has worked with a single chromosome, the third of *pseudo-obscura*. This study of *americana* and *texana* not only considers the main effects of two chromosomes in a population, but also the possibility of interaction between the two main effects.

The data from this study take the form of a 3X3 matrix as shown in table 7. The rows are associated with the fifth chromosome combinations and the columns are associated with the X chromosome combinations. The observed number of individuals having the i^{th} fifth and the j^{th} X chromosomal types is denoted as n_{ij}; $n_{i.}$ ($i = 1, 2,$ and 3) are the row marginal totals and $n_{.j}$ ($j = 1, 2,$ and 3) are the column marginal totals and n is the total. The expected row proportions are denoted by P_i and the column proportions by q_j. In this study, tables 8 through 15 indicate the observed number of females, with deviations from expectation, calculated from the marginal totals. The expected n_{ij} values are derived by multiplying each row total by each column total and then dividing by n (the total number of observations). The observed values minus the expected values give the deviations.

As stated by White (1957):

> In any given test the actual number of degrees of freedom associated with the total x^2 for the 3X3 table is determined by the number of parameters estimated from the data. It is conceivable that certain hypotheses to be tested give theoretical marginal frequencies a priori. In such cases all eight degrees of freedom are available and each contrast yields an independent x^2_1. However, if the hypothesis requires that a single parameter be estimated from the row marginal totals and one from the column marginal totals, then a single degree of freedom is lost from each of the main effects x^2's and for each classification the linear and quadratic components are pooled to give a combined x^2 with one degree of freedom. Finally, if the hypothesis requires that the observed marginal frequencies be used to estimate the expected marginal frequencies, then all "main effects" contrasts equal zero and the total x^2 collapses into the interaction x^2 with four degrees of freedom.

In this study, four degrees of freedom were used to compute the significance of the interaction as the analysis used followed that of White (1957).

In Cage I, a negative interaction existed between the X chromosome and the fifth chromosome at both the heterozygous level (TT, TA) and the homozygous level (TT, AA). Another negative interaction existed where *americana* chromosomes coexisted in the homozygous condition with the X chromosome (TA, AA and AA, TT).

In Cage II, a negative interaction existed between the X chromosome and the fifth chromosome at the homozygous level. Another negative interaction existed where *americana* coexisted in either the heterozygous or the homozygous condition with the X chromosome.

The lack of consistency between the samples was due to the considerable sampling errors in some of the small samples.

SUMMARY

1. Two subspecies of flies, *Drosophila americana americana* and *Drosophila americana texana* were maintained in laboratory populations.

2. Two cages were set up as follows: Cage I – *americana* females and *texana* males; Cage II – *texana* females and *americana* males.

3. In nearly all cases, *Drosophila americna americana* chromosomes were more frequent in the final samples of the two cages.

4. In all instances but one, the heterozygous combinations were superior to the homozygous combinations.

5. A discussion is presented, including a statistical analysis of the data, giving the relationship of this study to others of a similar and related nature.

TABLE 1

Chromosome frequencies for Cage I

Sample No.		0	1	2	3	4	5
Days from Origin		0	15	45	75	105	135
Chromosome Species							
X	T	50.0	50.0	39.0	41.1	42.3	39.4
	A	50.0	50.0	61.0	58.9	57.7	60.6
5	T	50.0	50.0	52.0	54.8	53.0	50.7
	A	50.0	50.0	48.0	45.2	47.0	49.3

The symbols T and A used above refer to the subspecies *texana* and *americana* respectively.

TABLE 2

Frequency of homozygous and heterozygous chromosomes for Cage I

Sample No.		0	1	2	3	4	5
Days from Origin		0	15	45	75	105	135
Chromosome Combination							
X	TT	50.0	0.0	19.7	25.7	30.4	33.9
	TA	0.0	100.0	36.1	28.6	23.9	13.6
	AA	50.0	0.0	44.2	45.7	45.4	52.4
5	TT	50.0	0.0	25.3	21.9	18.9	18.7
	TA	0.0	100.0	53.3	63.4	67.2	64.0
	AA	50.0	0.0	21.4	14.7	13.9	17.3

The symbols TT, TA, AA, used above, refer to homozygous *texana*, heterozygous *texana* and *americana*, and homozygous *americana* chromosomes, respectively.

TABLE 3

Chromosome frequencies for Cage II

Sample No.		0	1	2	3	4	5
Days from Origin		0	15	45	75	105	135
Chromosome Species							
X	T	50.0	50.0	45.0	43.1	41.7	43.5
	A	50.0	50.0	55.0	56.9	58.3	56.5
5	T	50.0	50.0	48.0	46.0	47.3	45.7
	A	50.0	50.0	52.0	54.0	52.7	54.3

The symbols T and A used above refer to the subspecies *texana* and *americana* respectively.

TABLE 4

Frequency of homozygous and heterozygous chromosomes for Cage II

Sample No.		0	1	2	3	4	5
Days from Origin		0	15	45	75	105	135
Chromosome Combination							
X	TT	50.0	0.0	27.4	23.6	19.3	26.1
	TA	0.0	100.0	37.1	40.0	45.6	39.1
	AA	50.0	0.0	35.5	36.4	35.1	34.8
5	TT	50.0	0,0	21.3	13.3	10.7	6.9
	TA	0.0	100.0	53.3	65.3	73.3	77.6
	AA	50.0	0.0	25.4	22.4	16.0	15.5

The symbols TT, TA, AA, used above, refer to homozygous *texana*, heterozygous *texana* and *americana*, and homozygous *americana* chromosomes, respectively.

TABLE 5

Number of X chromosomes analyzed in Cage I and Cage II

Sample No.	1	2	3	4	5
Cage I					
X Chromosome	75	61	42	46	59
Cage II					
X Chromosome	75	62	55	57	46

TABLE 6

Relationship of the recovered number of genotypes of days from origin

Cage I

Days from	(X)	TT	TT	TT	TA	TA	TA	AA	AA	AA
Origin	(5)	TT	TA	AA	TT	TA	AA	TT	TA	AA
15						75				
45		5	6	1	3	13	6	5	15	7
75		5	3	1	-	9	1	2	11	3
105		4	8	2	1	8	2	5	15	1
135		3	12	5	1	6	1	7	17	7

Cage II

Days from	(X)	TT	TT	TT	TA	TA	TA	AA	AA	AA
Origin	(5)	TT	TA	AA	TT	TA	AA	TT	TA	AA
15						75				
45		5	8	4	6	13	4	2	13	7
75		3	8	3	3	16	6	2	10	4
105		-	11	-	2	17	7	2	15	3
135		-	10	2	1	15	2	1	10	5

TABLE 7

Representation of the two-way classification of the data involving the X chromosome and the fifth chromosome

Chromosome 5	Chromosome X			Row Total	Theoretical Proportion
	TT	TA	AA		
TT	n_{11}	n_{12}	n_{13}	$n_{1.}$	P_1
TA	n_{21}	n_{22}	n_{23}	$n_{2.}$	P_2
AA	n_{31}	n_{32}	n_{33}	$n_{3.}$	P_3
Column Total	$n_{.1}$	$n_{.2}$	$n_{.3}$	n	
Theoretical Proportion	q_1	q_2	q_3		1

TABLE 8

THE "INTERACTION EFFECTS" OF SAMPLE 2 FROM CAGE I

Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	5(+2.443)	3(−1.688)	5(−0.754)	13
TA	6(−0.688)	13(+0.738)	15(−0.049)	34
AA	1(−1.754)	6(+0.951)	7(+0.803)	14
Totals	12	22	27	61

$X_4^2 = 4.556$ (.30–.50)

TABLE 9

THE "INTERACTION EFFECTS" OF SAMPLE 3 FROM CAGE I

Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	5(−3.200)	0(−2.000)	2(−1.200)	7
TA	3(−2.914)	9(+2.429)	11(+0.486)	23
AA	1(−0.286)	1(−0.428)	3(+0.714)	5
Totals	9	10	16	35

$X_4^2 = 10.910$ (.02–.05)

TABLE 10

THE "INTERACTION EFFECTS" OF SAMPLE 4 FROM CAGE I
Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	4(+0.957)	1(−1.391)	5(+0.435)	10
TA	8(−1.435)	8(+0.587)	15(+0.848)	31
AA	2(+0.478)	2(+0.804)	1(−1.283)	5
Totals	14	11	21	46

$X_i^2 = 2.877$ (.50–.70)

TABLE 11

THE "INTERACTION EFFECTS" OF SAMPLE 5 FROM CAGE I
Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	3(−0.729)	1(−0.492)	7(+1.220)	11
TA	12(+0.136)	6(+1.254)	17(−1.390)	35
AA	5(+0.593)	1(−0.763)	7(+0.170)	13
Totals	20	8	31	59

$X_i^2 = 1.414$ (.80–.90)

TABLE 12

THE "INTERACTION EFFECTS" OF SAMPLE 2 FROM CAGE II
Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	5(+1.436)	6(+1.178)	2(−2.613)	13
TA	8(−1.322)	13(+0.387)	13(+0.036)	34
AA	4(−0.113)	4(−1.564)	7(+1.678)	15
Totals	17	23	22	62

$X_i^2 = 5.579$ (.20–.30)

TABLE 13

THE "INTERACTION EFFECTS" OF SAMPLE 3 FROM CAGE II

Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	3(+0.964)	3(−0.636)	2(−0.327)	8
TA	8(−0.654)	16(+0.546)	10(+0.109)	34
AA	3(−0.309)	6(+0.091)	4(+0.218)	13
Totals	14	25	16	55

$X_4^2 = 0.724$ (.90–.95)

TABLE 14

THE "INTERACTION EFFECTS" OF SAMPLE 4 FROM CAGE II

Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	0(−0.772)	2(+0.176)	2(+0.596)	4
TA	11(+2.702)	17(−2.614)	15(−0.088)	43
AA	0(−1.930)	7(+2.439)	3(−0.509)	10
Totals	11	26	20	57

$X_4^2 = 5.579$ (.20–.30)

TABLE 15

THE "INTERACTION EFFECTS" OF SAMPLE 5 FROM CAGE II

Observed numbers of females individuals, with deviations from expectation calculated from marginal totals

Chromosome 5	Chromosome X			Totals
	TT	TA	AA	
TT	0(−0.526)	1(+0.217)	1(+0.304)	2
TA	10(+0.870)	15(+1.304)	10(−2.174)	35
AA	2(−0.348)	2(−1.526)	5(+1.870)	9
Totals	12	18	16	46

$X_4^2 = 3.143$ (.50–.70)

TABLE 16

TOTAL DEVIATION FROM EXPECTATION FOR CAGE I

Chromosome 5	Sample Date	Chromosome X		
		TT	TA	AA
TT	45	+2.443	−1.688	−0.754
	75	+3.200	−2.000	−1.200
	105	+0.957	−1.391	+0.435
	135	−0.729	−0.492	+1.220
		+5.871	−5.571	−0.319
TA	45	−0.688	+0.738	−0.049
	75	−2.914	+2.429	+0.486
	105	−1.435	+0.587	+0.848
	135	+0.136	+1.254	−1.390
		−4.901	+5.008	−0.105
AA	45	−1.754	+0.951	+0.803
	75	−0.286	−0.428	+0.714
	105	+0.478	+0.804	−1.283
	135	+0.593	−0.763	+0.170
		−0.969	+0.564	+0.404

TABLE 17

TOTAL DEVIATIONS FROM EXPECTATION FOR CAGE II

Chromosome 5	Sample Date	Chromosome X		
		TT	TA	AA
TT	45	+1.436	+1.178	−2.613
	75	+0.964	−0.636	−0.327
	105	−0.722	+0.176	+0.596
	135	−0.526	+0.217	+0.304
		+1.102	+0.935	−2.040
TA	45	−1.322	+0.387	+0.936
	75	−0.654	+0.546	+0.109
	105	+2.702	−2.614	−0.088
	135	+0.870	+1.304	−2.174
		+1.596	−0.377	−1.217
AA	45	−0.113	−1.564	+1.678
	75	−0.309	+0.091	+0.218
	105	−1.930	+2.439	−0.509
	135	−0.348	−1.526	+1.870
		−2.700	−0.560	+3.257

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