IV. GENE VARIABILITY IN THE AMERICANA-TEXANA-NOVA-MEXICANA COMPLEX OF THE VIRILIS GROUP OF DROSOPHILA

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INTRODUCTION

Mutation studies are necessary for a more complete understanding of the process of evolution. The only proven, constant method for a continuation of variation is that of mutation. Such a basic material of evolution is not, however, independent of such factors as isolation, selection and environmental conditions. These are interacting, interdependent factors and any one or combination of these can vary in importance in different situations.

The study of evolution by use of Drosophila, as any other living form, demands a consideration of the characteristics of the genus and even the species used. Distribution, physiological peculiarities, the length of the life cycle, food supply and preference, fluctuation of population size and resistance to environmental changes cannot be disregarded.

The importance of mutations and geographical variation in evolution has been reviewed by Timofeeff-Ressovsky (1940). General consideration of gene mutation in Drosophila can be found in a review by Spencer (1947a). Studies of the evolution within the genus Drosophila and especially in the virilis group have been published in the University of Texas Publications (see references to Patterson and Stone).

The present investigation is designed to measure that gene variability which lies within the range of morphological detection found in natural populations of three members of the virilis group of Drosophila. Two of these, *Drosophila americana americana* Spencer (1938) and *Drosophila a. texana* Patterson, Stone and Griffen (1940), are closely related genetically since natural hybrids have been found in the overlap zone of the distribution ranges (Stone and Patterson, 1947). These two members of the virilis complex have reached a level of divergence of subspecies but the third member of this division, *Drosophila novamexicana* Patterson (1941), has been designated as a species (Patterson and Stone, 1949). For convenience of discussion "species" will be used to refer to any one of these three members of the group.

The use of these three closely related species allows a comparative study of the gene variability and general mutation structure of the natural populations of each. The populations of *americana* and *texana* are usually termed "medium-sized" thus being smaller than those of such species as *D. melanogaster* or *D. hydei* but larger than *D. limpiensis* populations. The *novamexicana* populations are much smaller than those of *americana* or *texana*.

Studies of morphological variation which exist within natural populations of Drosophila are rather incomplete and fragmentary at the present

time. This type of study measures the variability present in the form of relatively minor changes.

MATERIAL AND METHODS

The stocks tested for this investigation were obtained by field collections of natural populations. The three forms used, *americana, texana* and *novamexicana,* occur as "wild" populations as opposed to the "domestic" *virilis.* All of the laboratory and wild strains used are listed in Table 1.

TABLE 1

Laboratory and wild strains of *americana, texana* and *novamexicana* used in this study

Species	Stock number	Date of collection	Place of collection	Remarks
americana	2069.7	8-22-50	Hastings, Nebraska	wild strain
americana	1773.4e	8-8-47	Chadron, Nebraska	lab. strain
americana	2067.1	8-20-50	Chadron, Nebraska	wild strain
americana	2068.6	8-21-50	Oakdale, Nebraska	wild strain
americana	1760.8f	7-25-47	Poplar, Montana	lab. strain
texana	1128.10	6-15-41	New Orleans, La.	lethal free
texana	2007.6	6-12-50	Tallahassee, Fla.	wild strain
texana	2012.4	6-19-50	Keystone Heights, Fla.	wild strain
texana	2013.3	6-20-50	Lake Butler, Fla.	wild strain
texana	2014.1	6-20-50	Twin Lakes, Georgia	wild strain
texana	2015.4	6-21-50	Indian Springs, Ga.	wild strain
texana	2016.7	6-22-50	Acworth, Georgia	wild strain
texana	2017.4	6-23-50	Smokemont Camp, N. C.	wild strain
texana	2018.7	6-25-50	Trenton, Georgia	wild strain
texana	2019.1	6-25-50	Guntersville, Ala.	wild strain
texana	2020.1	6-26-50	Tupelo, Mississippi	wild strain
texana	2021.6	6-27-50	Hollandale, Miss.	wild strain
novamexicana	1714.4	6-16-47	San Antonio, N. M.	lethal free
novamexicana	2075.8	8-29-50	Cliff, New Mexico	wild strain

The procedure of testing individual flies from natural populations for morphological mutants necessarily varied somewhat with the different populations of the three species of Drosophila tested. Since a majority of the mutations carried in such populations are recessive, the general procedure consisted in obtaining F_1 offspring from the P_1 crosses and inbreeding the F_1's in pairs or in mass matings depending upon the number available for such test. The F_1, F_2 and subsequent generations were checked for morphological mutations.

For paired matings of F_1 individuals, virgin females were used; mass matings usually consisted of from ten to twenty pairs of virgin or non virgin flies. Most of the larvae produced from P_1 and F_1 matings were spread to fresh food to eliminate overcrowding of the vials and thus prevent selection against homozygous mutants if these were less viable than normal.

Three methods of obtaining F_1 offspring were used. The test males and females of the same population were inbred in pairs or outcrossed individually to a standard laboratory strain. In some cases offspring were collected from females which had been fertilized in nature previous to collection. The latter were designated as "iso" females.

Each test male and female was arbitrarily given a different letter, as a, b, c, etc., at the beginning of the experiment. The number and type of P_1 individuals tested from each population of the three species has been included in Tables 2, 3, 4, and 5. The number of F_1 paired matings which were checked for each tested individual or individuals has also been indicated.

RESULTS

The morphological mutations detected in *americana, texana* and *novamexicana* have been assigned descriptive names and are arranged in alphabetical order. This list, which also contains a brief description of the more interesting mutants, has been placed in the Appendix. Mutants which were not tested and were not particularly important in the present analysis are sometimes included under a general name as *rough*. The allelic mutants are designated as a, b, c, etc., in the distribution tables. When numbers as 1, 2, 3, etc., follow the mutant name, no allele tests were possible or have not been completed. Specific names as *roughoid* were assigned to those mutants which have been tested to other morphological similar mutants of the same or different populations.

The names which were used for mutants were based on the terminology used for the mutants of the better known species of this group, *D. virilis* Sturtevant. Since crosses between *virilis* and *americana, texana,* or *novamexicana* are possible, the linkage group and allelism of some mutants of these species have been established. When it was not possible to check allelism to *virilis* descriptive names based upon morphological similarity to mutants of this species were used. If the recovered mutants did not resemble any known *virilis* mutants, the names of phenotypically similar melanogaster mutants were used.

The distribution of mutants in populations of *americana, novamexicana* and *texana* are found in Tables 2, 3, 4 and 5. Table 2 is composed of three *americana* populations and one *novamexicana* population. The more extensive data from *texana* populations are recorded in Tables 3, 4 and 5. Females which were fertilized in nature previous to collection are designated as "iso" females in the tables and are treated in the final analysis as a mating of one male and one female of the same population. Although multiple fertilization by several males can occur, it is justifiable to assume only one male since the rate of replacement of the sperm from pervious inseminations by the sperm of a male which fertilized the female last is very rapid and very complete. The efficiency and rate of sperm replacement in crosses between *texana, americana* and *virilis* have been reported by Patterson, Stone and Griffen (1940). Additional tests for the rate of replacement in *americana* and *texana* were carried out by use of the *cinnabar* mutants. The *cinnabar* females from pure mutant stocks were isolated individually in vials. After larvae appeared in the first vial, the mutant female was crossed to a normal male of the standard strain. A *cinnabar* female and normal male were left in the second vial for four days and then transferred to a third vial. In all cases the first vial which was a control

TABLE 2: MUTANT DISTRIBUTION IN POPULATIONS OF DROSOPHILA AMERICANA AMERICANA AND DROSOPHILA NOVAMEXICANA

Stock Number	F1's Tested	Number of F1 Pairs Checked	Mutants Recovered						
			Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Other Body Structures	Multiple Effects
AMERICANA 2067.1	a (iso ♀)	11		Light-1			Extreme-like: Hairless		Everted-1	Rough-cut
	b (iso ♀)	12		Light-2	Tapering	Interrupted-1		Varnished		Slight-1
	c (iso ♀)	2		Light-3			Double			
	e (iso ♀)	8		Light-4		Interrupted-2				
	h (iso ♀)	7		Light-5		Interrupted-3			Everted-2	
	i (iso ♀)	Mass		Light-6						
	k (iso ♀)	11	Red	Light-7		Interrupted-4				
	m (iso ♀)	2	Cinnabar	Light-8					Downcast-sterile	
AMERICANA 2068.6	b (iso ♀)	16				Interrupted-5	Extreme-1: Small bristles-1: Strand-1			Displaced
	d (iso ♀)	7		Light-9	Constricted like-1	Interrupted-6			Spread-1	Slight-2
	e (iso ♀)	4			Constricted like-2: Arched		Small bristles-2			
	f (♀ x S♂●)	Mass			Wide					
	g (♀ x S♂●)	Mass		Light-10						
	h (iso ♀)	8	Orange			Interrupted-7	Strand-2		Tinted	
	l (♀ x S♂●)	7		Light-11						Weak Mosaic-1
	o (iso ♀)	7	Wine-1	Yellow	Parallel					
	p (iso ♀)	5	Wine-2			Interrupted-8	Blunt		Grooveless-like: Swollen	
	j♀ x a♂	13		Light-12	Narrow					Abruptex: Mosaic-2
AMERICANA 2069.7	a (iso ♀)	3		Light-13		Interrupted-9				
	b (iso ♀)	Mass							Increased	
	c (iso ♀)	Mass							Immature: Spread-2	
	f (iso ♀)	Mass							Aristapedia-like	
	g (iso ♀)	8	Wine-3	Light-14				Rough	Everted-3	
	n (iso ♀)	4		Light-15					Swollen	
	o (iso ♀)	6		Light-16					Bubble	Rough-spread
	d♀ x d♂	9		Light-17						
	i♀ x e♂	9		Light-18	Constricted	Abrupt	Extreme-2			
	j♀ x b♂	12			Pointed			Roughex		
NOVAMEXICANA 2075.8	a♀ x a♂	12				Broken-1				
	b(♀ x S♂●●)	8				Broken-2			Curved	
	c(♀ x S♂●●)	9								Shaggy
	d(♀ x S♂●●)	14								
	e (iso ♀)	16								
	f (iso ♀)	7					Sparse			
	g(♀ x S♂●●)	12								
	h(♀ x S♂●●)	19			Shortened					

● Laboratory Strain of D. americana, 1760.8f
●● Laboratory Strain of D. novamexicana, 1714.4

TABLE 3: MUTANT DISTRIBUTION IN DROSOPHILA AMERICANA TEXANA: INDIAN SPRINGS, GEORGIA

Pl's Tested	Number of Pl Pairs Checked	Mutants Recovered								
		Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Wing Structure	Other Body Structures	Multiple Effects
~~1a~~ (iso ♀)	3					Absent-5	Rougher			
1c (iso ♀)	7									
1g (iso ♀)	4									Rough-cut
1h (iso ♀)	Mass									
1k (iso ♀)	2					Small bristles-1				
1a (♂ x S♀*)	1			Pointed-1		Small bristles-3	Rough-7			
1b (♂ x S♀)	10			Pointed-2	Short 4th Gapped		Roughoid-2	Spread-2		
1c (♂ x S♀)	13				Short veins-b	Small-absent-1				
1d (♂ x S♀)	16									
1e (♂ x S♀)	9							Ragged-7	Grooveless-1	
1f (♂ x S♀)	5									
1g (♂ x S♀)	2									Dishavelled: Small-extra: Rough-grooveless
1h (♂ x S♀)	15									
1i (♂ x S♀)	8				Short 5th-5		Roughened			
1j (♂ x S♀)	8					Small bristles-4: Absent-6				Dumpy-2
1k (♂ x S♀)	4									
1m (♂ x S♀)	9									
1n (♂ x S♀)	5							Ragged-5		Absent-semi lethal
1o (♂ x S♀)	3									
1p (♂ x S♀)	5							Downcast-1		
1q (♂ x S♀)	12								Abnormal Abdomen-1	
1s (♂ x S♀)	7			Pointed-1						
1t (♂ x S♀)	4								Abnormal Abdomen-2	
1u (♂ x S♀)	17									
1w (♂ x S♀)	8									
1x (♂ x S♀)	6	Mahogany-3			Short veins-c	Small-absent-2		Ragged-6		
1y (♂ x S♀)	8								Haltere	
1z (♂ x S♀)	12					Scutullar-like: Hooked				
2a (♂ x S♀)	11					Small bristles-5	Rough-8	Downcast-2		
2c (♂ x S♀)	12							Downcast-3		
2e (♂ x S♀)	7									
2h (♂ x S♀)	7			Diminished-a			Rough-9			
2i (♂ x S♀)	3				Short 5th-6					
2z (♂ x S♀)	4			Small wing-1						
1e ♀ x 1b ♂	5			Pointed-like			Roughoid-1			
1k ♀ x 1k ♂	5			Narrow		Small bristles-6				Dumpy-2
1n ♀ x 1d ♂	14	Brilliant			Short veins-a	Small bristles-2		Extended-2 Spread-1		
1o ♀ x 1p ♂	3									

* Laboratory Strain of D. texana, 1128.10

TABLE 4: MUTANT DISTRIBUTION IN DROSOPHILA AMERICANA TEXANA

P_1's Tested	Number of F_1 Pairs Checked	Mutants Recovered								
		Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Wing Structure	Other Body Structures	Multiple Effects
TALLAHASSEE, FLORIDA										
1a (♂ x S♀*)	6					Small bristles-a				
1b (♂ x S♀)	10				Short 5th-1		Roughest			
1c (♂ x S♀)	5	Mahogany-1					Rough-1			
1d (♂ x S♀)	4				Veinlet	Absent-1	Rough-2			Bithorax
1e (♂ x S♀)	4									Rough-extreme
1f (♂ x S♀)	5					Absent-2: extreme				
1g (♂ x S♀)	9				Short 5th-2		Rough-3	Ragged-1	Mottled	
1n (♂ x S♀)	9	Mahogany-2			Thickened	Missing-like: Small bristles-b		Extended-1		
1b♀ x 1b♂	6	Scarlet-like					Rough-4		Closed	
1j♀ x 1k♂	3									
TUPELO, MISSISSIPPI										
1a (iso ♀)	4	Bright-1								
1b (iso ♀)	3									Spread-semilethal
1f (iso ♀)	-				Plexus					
1g (iso ♀)	4					Small bristles-7			Abnormal Abdomen-3	
1a (♂ x S♀)	12								Stocky-1	Rough-mottled
1b (♂ x S♀)	10								Stubby-1	
1c (♂ x S♀)	4									
1d (♂ x S♀)	9			Pointed-4						
1f (♂ x S♀)	6		Ebony							
1g (♂ x S♀)	8				Short 5th-7	Small bristles-8	Rough-10			
1d♀ x 1f♂	9		Ebony	Fan	Abruptoid					Rough-broad
1h♀ x 1b♂	5								Stubby-2	Rough-short
1h♀ x 1d♂	6	Cinnabar-c				Irregular				
1e♀ x 1e♂	1	Bright-2								
1i♀ x 1a♂	7						Fused		Stocky-2	

* Laboratory Strain of D. texana, 1128.10

TABLE 5: MUTANT DISTRIBUTION IN DROSOPHILA AMERICANA TEXANA

Stock Number	P_1's Tested	Number of F_1 Pairs Checked	Mutants Recovered								
			Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Wing Structure	Other Body Structures	Multiple Effects
Keystone Heights, Fla. 2012.4	a (♂ x S♀*)	5							Strap		Rough-absent
	b (♂ x S♀)	2					Small: Absent-3		Ragged-2		
	c (♂ x S♀)	4									Narrow-broken: Rough-vestigial
Lake Butler, Fla. 2013.3	a (♂ x S♀)	Mass			Wide			Rough-5	Blister		
Twin Lakes, Ga. 2014.1	a (♂ x S♀)	10	Cinnabar-a			Short 5th-3			Ragged-3		
	b (♂ x S♀)	11				Short 5th-4	Absent-4	Rough-6	Ragged-4		
Acworth, Ga. 2016.7	a (iso ♀)	5			Diminished-b						Mottled-localized
Smokemont, N.C. 2017.4	a (♂ x S♀)	Mass			Diminished-c						
	c (♂ x S♀)	17			Diminished-d	Short veins	Absent-6			Grooveless-2	
Trenton, Ga. 2018.7	a (♀ x S♂*)	12	Mahogany-4: Lustrous		Small Wing-2					Blister-3D	
Guntersville, Ala. 2019.1	a (iso ♀)	14	Cinnabar-b								Rough-missing
Hollandale, Mississippi 2021.6	a (iso ♀)	5				Semiplexus					Rough-nicked
	b (iso ♀)	2									
	c (iso ♀)	5						Rough-11			
	a (♂ x S♀)	10									Short
	b (♂ x S♀)	7	Translucent		Diminished-e						
	c (♂ x S♀)	9				Short 5th-8					
	e (♂ x S♀)	3									
	f (♂ x S♀)	3									
	g (♂ x S♀)	6			Shortened						

* Laboratory Strain of D. texana, 1128.10

vial produced *cinnabar* offspring. Five of the seven pairs of *americana* and six of the seven pairs of *texana* showed complete replacement of the mutant sperm in the second vial. The remaining pairs tested produced a few *cinnabar* individuals. These mutant individuals probably hatched from eggs which were deposited by the female before insemination by the normal male. The third vials contained only normal individuals in all cases.

The most prevalent type of mutations discovered in the *americana* populations was autosomal recessives. In addition two sex linked recessive mutants and one dominant mutation which is probably sex linked were recovered. The *roughex* mutant (2069.7, j female × b male), a sex linked recessive, was first detected among the F_1 male offspring. Crosses between F_1 females which were heterozygous for *roughex* and *roughex* males produce normal and *roughex* males and females. If heterozygous females were crossed to normal males all the female offspring and one-half the male offspring were normal. The remaining males were *roughex*. Although phenotypically similar to *echinus* of *D. virilis,* allele tests proved that *roughex* is non allelic to this mutant.

The second sex linked mutant, *yellow* (2068.6o female), is recessive and was not recovered in the F_1 generation. Allele tests to *yellow* of *virilis* have not been completed. The dominant mutant, *Abruptex* (2068.6j female × 2068.6a male), was detected in the F_3 or F_4 generation. About twelve affected females were recovered and crossed to normal males in mass matings. The ratio of *Abruptex* females to normal females to *Abruptex* males to normal males among the F_1 offspring was 1:1:0:1. The *Abruptex* male class was lethal and only fifty percent of the expected number of males was recovered. A sex linked type of inheritance or a less probable fourth chromosome linkage are indicated by the data.

The variability of the widely separated *americana* populations consisted of two mutants common to all of the tested populations and "population-specific" mutants which were found in only one or in some cases two localities. One of the widespread mutants, *light,* was recovered from every individual tested from the 2067.1 sample. The expression of this mutant was not as extreme or as frequent in the other two populations. Of the total number of 80 mutants recovered from *americana* populations, 18 were *light.*

The light mutation is probably widespread throughout the western distribution area of *americana.* The stocks retained in the laboratory from different localities of the western area have been examined and a majority of these contained lighter forms. Stocks from the eastern populations of *americana* do not exhibit such a variation of body color.

The fixation of the *light* form in at least one western population is probable. A mixture of individuals which showed light and dark body color was noticed in a stock, 1773.4e, which was established from an isolated female collected at Chadron, Nebraska, August 8, 1947. Isolation of dark and light strains was possible and further test proved that the light form was recessive. The light strains isolated, however, usually contain a low percent of intermediate gray forms. A dark strain which does not occa-

sionally produce light colored individuals has not been established. Paired matings of dark forms from the unselected stock do not produce lighter forms consistently enough or in a high enough frequency to indicate that the homozygous dark form is lethal. Cytological examination of the metaphase configuration of brain cells of dark and light strains by C. Ward showed the typical americana chromosome configuration. Further genetic test of these strains can be found in another paper in this publication.

Crosses of *light-4* (2067.1e) to *light* individuals of 1773.4e proved that the two are allelic and that the mutant has remained in the natural population at least from 1947 to 1950. These two collections, although made three years apart, were from the same locality. The first collection, 1773.4e, was made August 8, 1947 and the more recent one, 2067.1e, on August 20, 1950.

An exact determination of the frequency of *light* in the Chadron population in these two years is not possible. However, the fact that each of the eight isolated females was either heterozygous or had been fertilized by a male which carried the recessive gene heterozygous, and that two iso females (2067.1e, 2067.1h) produced F_1 individuals which were *light* suggests that the mutant reached a high frequency in the 1950 season. Stocks which have been retained in the laboratory from the 1947 collection were examined. Five of the seven stocks contained the extreme light expression and the other two showed the less extreme forms.

The expression of the light form is probably not due to a simple recessive but may depend, at least in some cases, upon modifiers or a multiple factor type of inheritance. If a multiple factor type of inheritance is involved, all of the genes for *light* are recessive to those for the dark present in the western and eastern populations. In backcross experiments which allow recombination the ratio of light to dark forms and intermediate expressions produce data beyond analysis until marker stocks can be established.

The second widespread mutant, *interrupted,* was not as frequent as *light* but the chance of morphological detection is reduced by a variation in expression. In some cases the entire posterior crossvein is missing but there are less extreme expressions, including some which approach normal. Crosses between *interrupted-8* (2068.6p) and *interrupted-9* (2069.7) produced 27 percent phenotypically affected and 73 percent normal F_1's. Test crosses between *interrupted-1* (2067.1) and *interrupted-9* gave from 22 to 80 percent affected F_1 individuals. When *interrupted-1* and *interrupted-8* were crossed, all of the F_1 offspring were normal even though each of these produced affected individuals when tested to *interrupted-9.*

The *interrupted* mutants have a very characteristic morphological expression and were recovered from three rather distant populations, the Chadron, Oakdale and Hastings. The Chadron (2067.1) and Oakdale (2068.6) samples came from areas some 240 miles apart. Hastings (2069.7) and Oakdale are about 80 miles apart; the Chadron area is separated from the Hastings sample by 260 miles. These facts suggest the widespread distribution of this mutant throughout the western *americana* populations. There are no data available for the eastern distribution range.

The "population specific" mutants of the Chadron sample are interesting in that two, *cinnabar* and *varnished*, are allelic to known mutants in the more primitive member of the group, *D. virilis*. The *cinnabar* mutant is also allelic to *cinnabar* mutants recovered in this investigation from *texana* populations. Of the remaining ten population-specific mutants recovered from the Chadron sample, five were either sterile or so abnormal in body structure that survival of homozygotes in natural populations would be impossible.

About one-half of the variations in the Chadron population were *light* or *interrupted*. The remaining variability was determined by less frequent occurring population specific mutants. An average of 1.5 mutations per fly was obtained for this sample.

The Oakdale sample of *americana* has a rather high average of 2.0 mutations per fly. The variability of this population was not limited to a concentration of one or two mutants but consisted of a low frequency of a large number of mutants. The expression of *light* mutants in this population was a low frequency of the extreme yellow form and a higher frequency of the gray variant.

A rather unusual population-specific mutant was recovered from the Oakdale population. A high percentage of individuals which showed unilateral morphological effects were recovered from two tested P_1's. The mutant, *mosaic*, was detected first by the expression of unilateral spreading of the wings. By a more thorough examination of mosaic strains, other structures such as the eyes and bristles were also found to have an abnormal, unilateral modification. There was not only a rather high percent of individuals which showed a recognizable morphological expression of the mutant but also there was a variation in the time of gene action as reflected by an effect upon different structures.

The Hastings population has the low average of 1.1 mutation per fly. About one-third of the total number of mutations recovered were *light*. The remaining population-specific mutants were autosomal recessive except for the one sex linked *roughex*.

The general structure of the western *americana* populations was composed of two consistent, widespread mutants, *light* and *interrupted*, in rather high frequency and population-specific mutants which were usually rare in occurrence and characteristic for each population. The average number of mutations per fly for the three populations was 1.69 (Table 6) which is a minimum average because a few more mutations have been confirmed since these determinations were made.

The sample of *novamexicana* was obtained from one locality, Cliff, New Mexico, and numbered eleven individuals. Gene variability of this population was very low, being 0.55 mutations per fly as shown in Table 6. The mutant distribution is shown in Table 2. All mutations were autosomal recessive and only two phenotypically similar mutants, *broken-1* and *broken-2* were recovered more than once. The *novamexicana* sample

not only differs from the *americana* and *texana* populations in the frequency of mutations but also by the absence of any mutant which effects a noticeable reduction in viability.

TABLE 6

Comparative Mutation Frequency in *Drosophila americana americana*, *Drosophila americana texana* and *Drosophila novamexicana*

Species	Total Number of Individuals Tested	Total Number of Mutations Recovered	Average Number of Mutations per Fly
Drosophila americana americana	53	80	1.69
Drosophila americana texana	107	141	1.32
Drosophila novamexicana	11	6	0.55

Samples of *texana* were taken from a greater geographic range than those of the other two species, but a great number of these populations were represented by only one or two individuals. Such small samples have been included in the study, however, since they contribute to an analysis of the population structure as a whole. The mutant distribution in *texana* populations is shown in Tables 3, 4 and 5.

The gene variability in *texana* was composed almost exclusively of autosomal recessive mutations. Only one of the 141 recovered was sex linked. This mutant, *abruptoid,* was detected in the F_1 generation of an iso female (2020.1d, Tupelo). The female was remated to a male from the same locality and again about one-half of the male offspring were *abruptoid.* About one hundred of these affected males were tested and all proved to be sterile. Dissection showed that the sterility was due to degenerate testicular development and to the absence of motile sperm.

Three species-wide mutations were found in *texana* but none of these was present in a frequency as high as those characteristic of the *americana* populations. The mutant *cinnabar,* which is a bright, scarlet-colored eye, was present in three populations which are separated from one another by a distance of from one hundred to three hundred miles. Two of the localities, Twin Lakes, Georgia, and Guntersville, Alabama, were represented by only a few individuals and the comparative gene frequencies could not be determined. A larger sample of 24 individuals collected at the third locality, Tupelo, Mississippi, did not show any concentration of

the mutant since the mutant was recoverel from one female. Crosses between the mutant from each of the three localities proved that all were allelic. These mutants were also allelic to *cinnabar* of *D. virilis.*

A second species-wide mutant, *diminished,* has several different expressions which is possibly due to an interaction of isoalleles. The extreme form is morphologically similar to dusky of *D. virilis* but it is not sex linked. The wings are about one-half the normal size with a reduction in width and length together with a darkening of the color. In the less extreme form the wings are larger and may or may not be dusky in color. In addition to these two manifestations, a third type occurs in which the ends of the wings are arched down over the end of the abdomen. Some F_1 pairs from the same P_1 parent produced one or several of these types although one type was usually more prevalent. In one case, 2015.2h, Indian Springs, the extreme form has been isolated from a strain which contained several expressions of the mutant.

Allele tests between the five *diminished* mutants, one from Indian Springs, Georgia (2015.2h), one from Acworth, Georgia (2015), one from Hollandale, Mississippi (2021) and two from Smokemont, North Carolina (2017), produced F_1 offspring which varied in the expression of the mutant. In some crosses all offspring could easily be classified as *diminished* whereas in other matings the expression varied from the extreme form to normal. In the latter cases, however, the normal individuals occurred as a low percentage of individuals.

The number of *diminished* forms detected in *texana* samples was small. In the large Indian Springs sample of 47, only one *diminished* mutant was recovered. Both of the two males in the Smokemont sample, however, produced morphologically detectable forms. The detection of this mutant was probably complicated by the interaction of iso-alleles since recovery of a pure *diminished* strain usually required selection for several generations.

A rather large number of morphologically similar mutants, *small bristles,* were recovered in three of the larger samples, the Indian Springs, Tallahassee and Tupelo localities of *texana* populations. The classification of this mutant as a species-wide mutation was complicated by the lack of allele tests since all but two of the ten recovered were completely sterile. These two, *small bristles-a* and *small bristles-b,* were allelic but were both recovered from the Tallahassee sample and thus do not furnish adequate information of the occurrence over the entire distribution range. The variation in expression of the mutant recovered from the same P_1 parent, the relatively high frequency in the Indian Springs sample and allelism of the two present in the Tallahassee sample suggest that a diverse series of alleles of this mutant exist throughout the *texana* distribution range.

The exact relation of a fourth mutant, *ragged,* has not yet been established. This mutant was not recovered in the F_2 generation but appeared in subsequent generations. Since in all but a very few cases only affected males were recovered, sex linkage was suspected and the mutant named *ragged* upon the basis of the linkage group and morphological similarity

to *ragged* of *virilis*. Further tests have established the fact that affected males are more prevalent than affected females. Whether this is due to a sexual dimorphism of the mutant, in which case an autosomal recessive type of inheritance is possible, or to a sex linked inheritance with or without an expression of sexual dimorphism has not been clarified.

Although one or two *ragged* males have been detected in a number of cases only seven mutants have been positively determined to be mutations and included in the results. These were found in the southern sector of the distribution range—Indian Springs, Georgia, Twin Lakes, Georgia, Keystone Heights, Florida, and Tallahassee, Florida. The classification of *ragged* as a species-wide mutation is still doubtful.

The gene variability of *texana* samples was composed of autosomal recessive mutations except for one sex linked mutant. There was no high concentration of a particular mutation in any of the large samples shown in Tables 3 and 4. The Indian Springs population, Table 3, showed that three-fourths of the individuals produced two, three or four mutations. No mutation was recovered from the remaining one-fourth of the sample. The number of mutations recovered in some strains may have been affected by bacterial infections.

The general structure of *texana* populations consisted of two or possibly three species-wide mutants in a low concentration and population-specific ones which were characteristic for each population. The average number of mutations per fly varied from 0.66 to 4.0 for the different samples. Some of the samples were small and the general average of 1.32 shown in Table 6 probably corresponds more nearly to the actual frequencies.

The population structure of the subspecies *americana* and *texana* is similar in that both contain widespread species-wide mutants which occur throughout the distribution range and population-specific ones which occur only in one or two samples. The gene variability of both species is primarily composed of autosomal recessive mutations. One mutation, *cinnabar*, was found to be present in both populations but occurred as a species-wide mutant in *texana* and was recovered only once in the *americana* samples.

Although species-wide mutants were recovered in both species, the concentration of these in the populations was different. The two mutants, *light* and *interrupted*, were common to all of the *americana* samples and accounted for a fairly large portion of the variability. There appears to be no particular reduction in the viability of the homozygous expression of these mutants. The *texana* populations contained no such high concentration of the characteristic widespread mutants, *cinnabar*, *diminished* and *small bristles*. Most of the *small bristles* mutants were sterile in a homozygous condition.

A comparison of *novamexicana* populations to the other two species is limited since a sample from only one locality was studied. The gene variability of this sample was lower than that for any population of the other

two species and none of the mutants recovered was noticeably below normal in viability. The only mutant similarity possible is between the *interrupted* of *americana* and *broken* of *novamexicana*. Allele tests for these two mutants have not been completed.

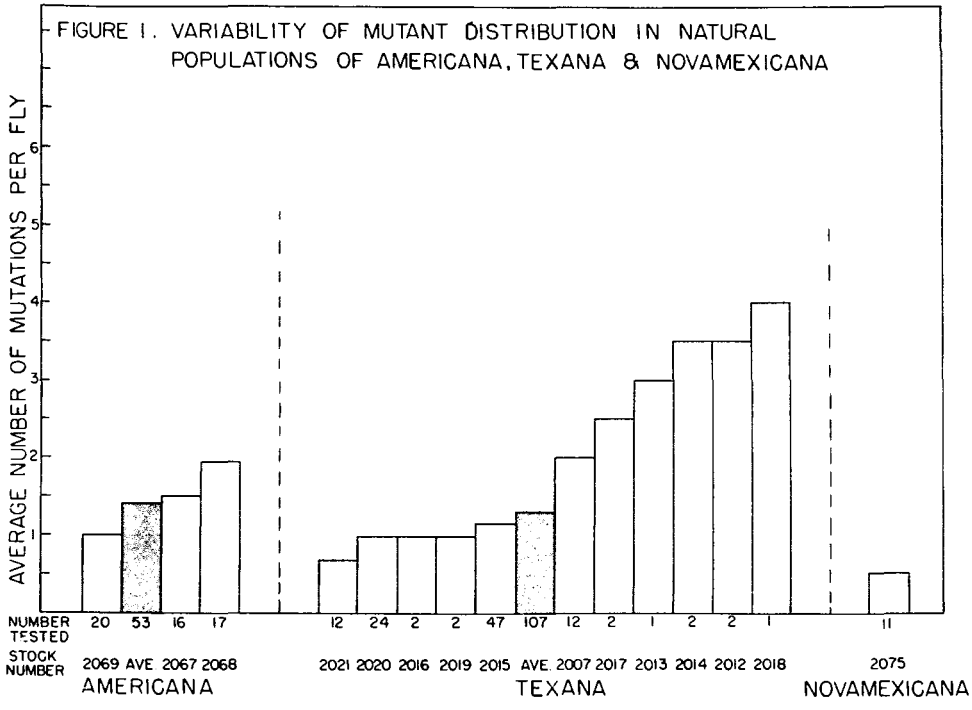


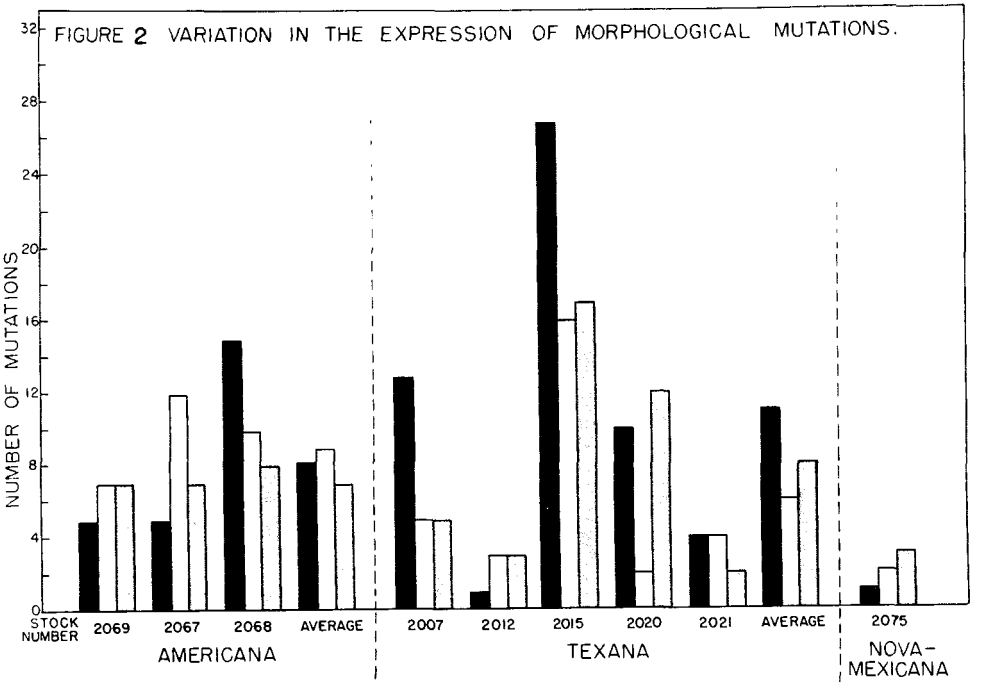
Figure 1 shows the variability for each sample of the *americana, texana* and *novamexicana* populations. The samples of each species were arranged from the less variable ones on the left to more variable ones on the right. The average for all the samples appears as a crosshatched bar and is placed in a relative position to the samples depending upon the value obtained. The number of individuals used for each determination appears under the respective sample. The average for all samples was determined from the total number of individuals tested and the total number of mutants recovered. The collection number of each sample appears below the number of tested individuals.

The *americana* samples fall slightly above and below the average but do not deviate more than about 0.5 mutations per fly. The *texana* samples show more variation but those which deviate the most are the smaller samples. The larger samples, 2007, 2015, 2020 and 2021 do not show as much relative difference. The *novamexicana* sample shows a lower value of variability than the lowest of the *texana* samples. The total averages for *americana* and *texana* samples are very similar.

The total number of individuals tested for each species, the total number of mutations recovered and the average number of mutations per

fly are given in Table 6. The averages are a measure of the minimum variability which occurred in these populations since no mutant was included unless either a stock was established, a recurrence from heterozygous mating was obtained or a relatively high percentage of morphological expression existed. The *americana* average is especially low because these samples were obtained from more recent collections and fewer generations have been obtained for thorough analysis. The *americana* populations, however, show the highest average of 1.69 mutations per fly which is fairly close to the 1.32 average obtained for *texana* populations. The *novamexicana* sample shows a low average of 0.55 mutations per fly.

A measure of more minor morphological variations as produced by multiple alleles, modifiers and possibly cumulative action of several genes is shown in Figure 2. The mutations of each sample were placed in one



of three categories. The first contains those mutants which showed a constant effect upon only one morphological structure such as bristles or wing veins. This class appears in the left column. The center column represents those mutations which had only one morphological structure affected but there was a variation in the expression of the trait. The third group consists of polymorphic mutants which show an effect upon two or more morphological structures. So few of this type of mutant showed any consistency in expression that further division of this group is impracticable. The determination of the category in which each mutant was placed was dependent upon the morphological characteristic of each

mutation. Those which had one morphological expression and were reduced in viability or sterile were placed in the first category rather than in the polymorphic one.

The *americana* populations showed a variation in the number of mutants in each category and a different pattern was produced for each sample. This is partially dependent upon the concentration of *light* and *interrupted* in the different samples. The three largest samples of *texana* populations showed a similar pattern of a high concentration of mutants in the first category and a drop in the number of those which vary in the expression of one morphological structure.

The most significant fact that is shown from this treatment of the recovered mutations is that, in all but one case (Tallahassee, 2007), the totals of the middle and right columns are greater than the first column. More than fifty percent of the mutations recovered showed either a qualitative or quantitative variation in expression.

MORPHOLOGICAL SIMILARITIES OF MUTANTS TO THE MUTANTS OF *VIRILIS* AND *MELANOGASTER*

The mutants of *americana* and *texana* have not been thoroughly studied and any definite gene homology with *virilis* is not possible except in one or two cases. The *varnished* and *cinnabar* mutants of *americana* and the *cinnabar* mutants of *texana* are allelic to the same mutants of *virilis*. The *yellow* of *americana* is sex linked, recessive, and is probably the same as *yellow* of *virilis* and other species. The *abrupt* mutant of *americana* is morphologically similar to the same mutant in *virilis* (Chino, 1934: D.I.S. No. 2) and the males are sterile in both cases. The dominant *Abruptex* mutants of *virilis* (Chino, 1941) and of *americana* are identical morphologically except in the latter case two additional bristles are missing. The widespread *interrupted* mutants of *americana* closely resemble the description of *interrupted* of *virilis* (Metz, Moses and Mason, 1923). However, the linkage of this mutant has not been determined.

In *texana* the *cinnabar* mutant is allelic to that of *virilis* and *scutellar-like* is similar morphologically to *scutellar* of *virilis* (Chino, 1936-b). The linkage of *ebony* has not been determined but the darkening of the pupal case, although not as extreme as in *virilis*, suggests that these mutants are homologous. The *bithorax, dumpy* and *grooveless* mutants of *texana* are morphologically similar to the same mutants of *melanogaster* and have not been reported in *virilis*.

DISCUSSION

Previous morphological mutation studies of natural populations of the virilis group have been limited to *Drosophila virilis* (Chino, 1936a, 1936b, 1937; Patterson, Stone and Griffen, 1942; Metz, Moses and Mason, 1923). This more primitive member differs from all other species of this group in two ways—nearly cosmopolitan distribution and domestic habitat. All

other species are limited to one zoogeographical realm and are not associated with man as are the domestic populations of *virilis*.

Cytological and genetic evidence shows that the three species, *Drosophila americana americana*, *Drosophila americana texana* and *Drosophila novamexicana*, are closely related and places them in one division of the virilis complex (Patterson and Stone, 1949), which is restricted to the Nearctic realm (Patterson, 1942).

Drosophila texana occurs in the southeastern portion of the United States extending as far west as central Texas and Oklahoma. The northern limit extends from North Carolina through Tennessee, Arkansas and Oklahoma. This distribution range is characterized by heavy rainfall, some swampy areas, rather heavy natural vegetation and mild to warm climate throughout the year. These conditions allow a prolonged breeding season and rather large populations are built up in some localities toward the end of the main breeding season (Patterson, 1942). The data on population size suggests that the density of *texana* populations is intermediate between the large breeding units of most *hydei* and the small populations of *limpiensis* or *novamexicana*. The yearly fluctuation of population size is probably not as extreme as that of *americana*.

The known distribution range of western *americana* includes the states of Montana, South Dakota, Nebraska and Kansas. The eastern population has a concentration in the state of Ohio with specimens reported as far west as Indiana and Arkansas. A southern extension of this range forms an overlap zone with the northern portion of the *texana* populations. Isolation between these two forms is incomplete since the occurrence of natural hybrids and of gene exchange has been proven by Stone and Patterson (1947). The short, cool summers and severe winters of this more northern distribution range probably shortens the breeding season of this species and produces greater fluctuation in population size.

Drosophila novamexicana has been found in small numbers in a distribution area which is characterized by small populations of most species. The localities from which *novamexicana* have been collected are San Antonio and Cliff, New Mexico, Whitewater, Colorado and Cave Creek, Arizona.

The basic types of morphological variation present within *americana* and *texana* populations are similar to those found in the Russian populations of *Drosophila melanogaster* by Dubinin and collaborators (Dubinin, Romashov, Heptner and Demidova, 1937); for *Drosophila immigrans* by Spencer (1940); for *Drosophila hydei* (Spencer, 1947a; Alexander, 1949); for *Drosophila limpiensis* (Alexander, 1949); and possibly for *Drosophila subobscura* (Gordon, Spurway and Street, 1939).

In each of these species two general types of morphological mutations occur. One type can be found rather widespread throughout the whole distribution range and has been designated as species-wide mutations. The second type, population-specific, includes a wide range of morphological mutants which are characteristic of one or a few localities of the distribution range.

The yet incomplete investigation of species-wide mutants of these forms makes any discussion of the general type of inheritance difficult. The *bobbed* mutants of *hydei* (Spencer, 1938), *diminished* of *texana, interrupted* of *americana* and probably *net* of *immigrans* (Spencer, 1940) exist as a complex series of iso-alleles within natural populations. One species-wide mutant present in populations of *subobscura* (Gordon, Spurway and Street, 1939), *striped* in *hydei* and *Mottled* in *limpiensis* (Alexander, 1949) showed a weak dominance often affected by gene modifiers. The *Mottled* mutant type may depend upon multiple factors. The widespread *light* mutants which were found in the western *americana* populations are recessive with different intensities of expression in the three different populations. The *cinnabar* mutants of *texana* are recessive with no apparent variation in expression.

Population studies reflect the success of certain mutants to become widespread and thus the more important in evolution as an extension of the genetic variability. About one-half of the wide-spread mutants which have been reported exist as a complex series of iso-alleles within natural populations. The *bobbed* mutants of *hydei* and *net* mutants of *immigrans* have been reported to be iso-alleles by Spencer (1938, 1940). The morphological expression of the species-wide mutant, *trident,* which has been found in Russian populations of *melanogaster* (Dubinin and collaborators, 1937) suggest the possibility that a series of iso-alleles exist. The *diminished* mutants of *texana* and *interrupted* mutants of *americana* also exist as a series of multiple alleles within natural populations.

The efficiency of this type of inheritance should not be overemphasized since other types of mutants have most certainly become widespread and fixed in populations. The *light* mutant has been up to the present time more successful, if success be based upon frequency, in the Chadron sample than the *interrupted* mutants.

Apparently the attainment of widespread distribution of any mutant throughout a species range is not dependent upon any one type of inheritance. A fairly mutable locus and a slight or high selective advantage are characteristics which will allow such genes to become widespread. The retention and concentration of these mutants in different populations probably depend more upon population dynamics and fluctuations.

A difference in the concentration of *light* (species-wide) in the Chadron population of *americana* and the other two localities exemplifies the results of such population dynamics. A moderately high level of this mutant has apparently been stabilized in this population whereas the other populations tested contained a lower frequency of the mutant and less extreme morphological forms. This particular population is rather isolated, thus preventing much interbreeding with other populations and most likely undergoes a sharp bottleneck in population size during the winter. Several general paths by which such a frequency could be attained lie within the realm of probability. Linkage with genes with a selective advantage or genetic drift and chance fixation seem to be the more likely possibilities in this case.

Spencer (1944) tested two populations of *hydei* for the frequency of a species-wide mutant, *bobbed*. One locality, Azusa, Southern California, contained a large population (estimated to be composed of 100,000,000 individuals) of *hydei* breeding in a citrus dump. The other population tested was from Wooster, Ohio, and was estimated to be of a magnitude of not less than 500,000 in size. By crossing phenotypically normal females of these populations to a standard stock, Wooster 20, Spencer obtained a high concentration of a few grades of bristle sizes and less spread in the allelic series from the Wooster sample than from the Azusa population. The population size and year-round pattern was used as an explanation for the difference in the *bobbed* frequency in these two populations.

Apparently not only species-wide mutants but also population-specific ones may attain a high frequency within a population. Spencer (1947b) reported a high frequency of the mutant, *stubble*, in a *D. immigrans* population. There was no apparent concentration of population-specific mutants in the *texana*, *americana* or *novamexicana* populations. In general the population-specific mutants had a lower frequency than species-wide ones in the *americana* populations. The *texana* samples showed no discrepancy in the frequency of population-specific and species-wide mutations.

There was such a tremendous amount of diversity in the category of population-specific mutations recovered from the populations studied in this investigation that a discussion of each would be impossible. However, a reduction in viability or fertility was characteristic for most mutant types.

The unusual action of one mutant, *mosaic*, which was recovered from the Oakdale collection of *americana* warrants discussion. The expression of this mutant is usually unilateral with a more frequent action upon the wings, eyes and bistles. A rather high percentage of individuals with detectable morphological changes in isolated strains of the mutant, and the absence of such phenotypic variation in other strains tested from this and the other two populations, strongly suggest that environmental conditions such as temperature and moisture are not responsible for the morphological changes. The detection of two sex-linked mutants, the recessive *yellow* and dominant *Abruptex*, as well as the high mutation average of 2.0 for this population may be entirely independent of the presence of the *mosaic* mutant. The effect of *mosaic* upon different structures suggest, however, a variation in the time of gene action. Such variation in the time of gene action need not be restricted to a period which produces the individuals classified as *mosaic*.

Sex-linked mutations in natural populations are rather rare when compared to those which show autosomal linkage. The action of selection is not as slow since the homozygous condition of the sex chromosome in the male allows the same selective action as the homozygous, autosomal condition. One exception to such a system of selection is the sex-linked mutants at the *bobbed* locus which have normal or less extreme alleles in the Y chromosome. In this case selective action is reduced even below that of autosomal recessives if the Y chromosomes contain equally normal alleles. Since

selection usually operates more efficiently upon a homozygous condition, the action is thus limited to females which are further protected in this particular case by a series of interacting iso-alleles. The presence of iso-alleles of *bobbed* in natural populations of *hydei* has been investigated by Spencer (1938, 1944). He found that this mutant reached such a distribution maximum that it can be considered a species-wide mutant, as it is common to most populations over the whole distribution range. Different populations were found to contain different concentrations and frequencies of this complex series of iso-alleles. Parallel mutations have been recorded in *D. melanogaster* (see Bridges-Brehme, 1944); in *D. simulans* (Sturtevant, 1929); in *pseudoobscura* (Sturtevant and Tan, 1937); in *affinis* (Sturtevant, 1940); in *ananassae* Moriwaki, 1935: Kikkawa, 1938); in *subobscura* (Jermyn, Philip, Rendel and Spurway, 1943, D.I.S. No. 17); in *funebris* (Timofeeff-Ressovsky, 1931; Spencer, 1934, D.I.S. No. 2); and in *virilis* (Chino, 1936b).

Although this sex-linked mutation has been found rather wide-spread throughout the genus Drosophila including *virilis*, no proven case was found in *americana, texana,* or *novamexicana.* Nevertheless, three and possibly four mutations, which showed a simple sex linkage, were recovered from *americana* and *texana.*

The sex-linked, recessive mutation, *abruptoid,* was carried by an iso female collected from the Tupelo population of *texana.* The F_1 male offspring were normal or *abruptoid* which is expressed morphologically as a shortening of the longitudinal veins of the wings. A strict 1:1 ratio was not obtained because of the reduced viability of the mutant. The fact that all affected males were completely sterile suggests a recent origin for this mutant since selective action against such a mutation should rapidly eliminate it from a population.

In *americana* two sex-linked recessives and one dominant mutation which is probably sex-linked were recovered. The dominant mutant, *Abruptex* (2069, Oakdale collection), either occurred as a spontaneous mutation in a phenotypically normal fly or was masked by some type of supressor gene or genes. Affected females crossed to normal males produce one-half normal females and one-half *Abruptex* females. All the F_1 males are normal and occur in about one-half the expected male frequency, thus proving a lethal action of the gene in males. Such lethal expression in males suggests a sex-linkage since the homozygous condition of the sex chromosome is more apt to allow a detrimental action than an autosomal heterozygous condition. The possibility of the presence of the gene in the male without expression is reduced by the recovery of only one-half of the expected number of males. Such reduction in number could occur if an unrelated sex-linked lethal was present in an *Abruptex* female but the test matings were made in mass and not in pairs. If one or even two of the six *Abruptex* females carried a lethal in one of the sex chromosomes, there would have been a decrease in the number of males, but not as definite a ratio in the reduction would have been obtained. Another possible but less probable explanation is the assumption of a fourth chromosome linkage.

In this particular species an X-4 chromosome fusion (Patterson, Stone and Griffen, 1940) permits recessive mutations in the fourth linkage group to show sexual dimorphism since a free fourth chromosome is present in the males and is inherited from male to male in the same way as the Y chromosome. In this case the lethal effect in the males would require the further assumption that the *Abruptex* gene in the free fourth chromosome will always interact with the allele carried by the female to produce a lethal action, or that it exhibited a special type of sex limited lethality which is improbable.

A rough eye mutant, *roughex*, which is a sex-linked recessive was detected in the F_1 male offspring of a cross between the j female and b male of the Hastings collection of *americana*. The comparative lack of a reduction in the viability of this mutant, as could be measured by the ratio of *roughex* to normal males, does not necessitate an assumption of recent origin for this mutant. Such a possibility can not be eliminated entirely since only one female of the twenty tested individuals carried this mutant.

The second sex-linked recessive mutation, *yellow*, was recovered from the Oakdale population. This mutation could have occurred as a spontaneous mutation in some subsequent generation after collection of the parent, since none of the F_1 offspring of the iso female was affected. In this case, however, the presence of a sex-linked lethal or semi-lethal would reduce the number of affected males and delay detection of the mutant until crossing over and recombination allowed a more suitable combination of genes. The allelism to *yellow* of *virilis* has not yet been tested but the phenotypic expression of this mutant is very similar to *yellow-40* of *virilis*.

Among the sex-linked mutations recovered from populations of Drosophila species, *yellow* has been one of the more common types. Different frequencies of *yellow* in Russian populations of *melanogaster* have been reported by Dubinin and collaborators (1937) and Berg (1942a, 1942b). The recovery of yellow individuals in American strains of *melanogaster* has been reported by Spencer (1944). Metz found one yellow male in a collection of *simulans* (Sturtevant, 1929). A sex-linked, recessive *yellow* mutant in *immigrans* was first reported by Stella (1936) and *yellow* individuals among wild specimens of *immigrans* have been found by Spencer (1944).

The amount of morphological variability concentrated as sex-linked mutations is very small in *americana* and *texana* populations as in other Drosophila species which have been investigated. The detection of few mutants with a simple sex-linked type of inheritance in natural populations is important since the occurrence of spontaneous mutations in the sex chromosome can be proven and the reduction in number conforms to the general theory of selection. Such a mutation as *bobbed* is a special case in which two adaptive mechanisms have been developed.

Major morphological mutants form only a small portion of the total variability of natural populations. Detectable lethal mutations have been estimated to be five to ten times more frequent than visibles by Spencer (1947a).

Dobzhansky and Wright (1941) found that the lethal mutation rate in the third chromosomes of *pseudoobscura* which were collected from Guatemala, Mexico and Death Valley were substantially the same (Death Valley 0.0027 ± 0.00032, Mexico 0.00359 ± 0.00062, Guatemala 0.00284 ± 0.00061), but 15.29 ± 0.83 percent of the chromosomes tested from Death Valley carried one or more lethals. In similar samples from Mexico and Guatemala, values of 28.1 ± 3.3 percent and 34.2 ± 5.2 percent were obtained. The difference of lethal concentration in this case was apparently not due to mutation rate but to some other factor or factors, presumably population size and selection pressure.

Differences between the Russian and American populations of *melanogaster* have been shown by the work of Dubinin (1946) and Ives (1945). Dubinin found mutation rates of 0.33 ± 0.007, 0.44 ± 0.08, 0.45 ± 0.1 percent for the second chromosome extracted from three different Russian populations. Ives obtained lethal mutation rates of from 0.49 to 6.20 percent for the same chromosome in American populations from different localities. One explanation for such discrepancy in the mutation rates is offered by the presence of genes which increase the mutation rate. Some Florida strains of *melanogaster* have been found to contain such "mutators" by Ives (1950). If homozygous, the action of this gene increases the mutations in many genes more than ten times the normal. The effect upon the rate of different genes varies, however. The mutation rate of the *folded* mutant was increased more than any other gene tested whereas *yellow* is not increased.

Additional data on the frequency of allelic mutations in American and Russian populations show that the American *melanogaster* breeds in comparatively large populations, and that these populations are continuous from year to year in the tropical, sub-tropical and temperate zones of the United States (Ives, 1945). The high concentration of a few mutants, both visibles and lethals, in Russian populations seems to indicate that these populations underwent a sharp reduction at one or more seasons of the year and then expanded in size (Spencer, 1947a).

The difference of genetic structure of populations of this and other species may be real or only superficial. Discrepancy may be obtained by the interpretation of different workers and different methods of procedure. Sampling at different times in the seasonal cycle as reflected in population size could certainly give quite different estimates. The male to female ratio in the samples of *americana* and *texana* suggests that these populations might have been at a different point in such a seasonal cycle. The *texana* samples were collected in June and were composed primarily of males. The *americana* collections were made in August and contained a predominance of females. A difference in the frequency of species-wide mutants within the populations of these two species may have been partially due to sampling at some point in the cycle. The pattern of the seasonal cycle—that is the time and extent of population peak and reduction —could, however, explain such differences. We do not know if the males

and females of the two species differ in their response to bait or in motility.

Certain physiological features of a species can possibly also determine the amount of gene variability which can be detected. Physiological differences, as reflected by the number of phenocopies obtained, exist between *hydei* and *limpiensis* at 22 degrees Centigrade (Alexander, 1949). The increased amount of crossing over in *virilis* and apparent inefficiency of simple inversions as balancers would allow more gene recombination and genotypes for the detection of morphological mutants than in *melanogaster*.

The treatment of morphological variations which are present within natural populations is difficult in that a graded series of intermediates between an easily classified mutant type and normal exist. This characteristic is inherent within the genetic system and it would indeed be surprising not to find a series of variations of different levels of divergence.

The complexity of morphological expression has been ably demonstrated by Timofeeff-Ressovsky (1934a, 1934b). The effect of more minor cumulative differences which have no particular morphological expression has been tested in *D. pseudoobscura* (Dobzhansky and Spassky, 1944 and Dobzhansky, Holz and Spassky, 1942) and in *D. Funebris* (Timofeeff-Ressovsky, 1935). All these data show not only a complex reaction between the genetic system and the external environment, such as temperature, but also an interaction between minor changes within the genetic system itself. Figure 2 shows the variation in the expression of morphological mutations in the *texana, americana* and *novamexicana* populations tested.

Natural mutation studies of this type measure the results of a number of interacting factors in the general process of evolution. The assignment of any one factor or factors in any one case is difficult and requires extensive study of the particular mutant, the species concerned, environmental conditions, population size and fluctation. Population studies have revealed, however, that a great amount of variability exists . This variation may be in the form of chromosome aberrations (fusions and inversions), gene mutation and minor variants. It is obvious that such variation is not incidental nor independent of the general process of evolution but is a part of that process.

SUMMARY

1. Natural populations of three closely related species, *Drosophila americana americana, Drosophila americana texana* and *Drosophila novamexicana*, were tested for morphological variation. Populations of *americana* from three localities, populations of *texana* from eleven localities and one *novamexicana* population were used.

2. The medium-sized populations of *americana* and *texana* showed a 1.69 and 1.32, average of mutations per fly, respectively. A low average of 0.55 was obtained for the *novamexicana* population.

3. The basic mutation structure of *texana* and *americana* were similar in that both contained two general types of mutation, species-wide and population-specific. The *cinnabar* and *diminished* mutants were found in three or more widely separated populations of *texana*. Different alleles of *diminished* interact to give a variation in expression and are therefore classified as iso-alleles. The *cinnabar* mutants give only one detectable expression. Two wide-spread mutants, *interrupted* and *light*, were found in all three populations of western *americana*. The *interrupted* mutants act as iso-alleles while *light* is recessive with modifiers. The two species-wide mutations of *americana* showed a higher gene frequency in the tested populations than *cinnabar* and *diminished* of *texana*.

4. The *cinnabar* mutant was recovered from *americana* and *texana* populations but occurred as a species-wide mutant in the latter and only once in the former. The *varnished* mutant of *americana* and *cinnabar* of *texana* are allelic to similar mutations in *virilis*.

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APPENDIX

The mutations which were recovered from natural populations of *americana*, *texana* and *novamexicana* have been given names and listed alphabetically. The mutants for the three species are included as separate lists. The mutant name is followed by the collection number of the stock, the type of inheritance and a brief description of the mutant. Double names, although usually not desirable, were assigned to those mutants which would be of no further use in genetic work. This conserves the more specific names.

Drosophila americana americana

1. *abrupt;* 2069; autosomal recessive. All of the longitudinal wing veins are shortened and the ocellar and/or orbital bristles are missing. Males sterile.

2. *Abruptex;* 2068; dominant, probably sex linked, lethal in the male. All of the longitudinal veins are shortened to about one-fourth the normal length. The posterior crossvein or a part of the vein is always present. The wings are wide across the center portion and fold under along the edges. There is a reduction in the size of the eye and the normal curvature is absent. The facets show complete abnormal arrangement and the pile is short. An absence of the orbital, ocellar, postvertical, presutural, posterior notopleural and supra-alar bristles is characteristic. Females are fertile. Morphologically similar to *Abruptex* of *virilis* (Chino, 1941).

3. *arched;* 2068; autosomal recessive. The wings are rolled under along the edges and arched down over the abdomen.

4. *aristapedia-like;* 2069; autosomal recessive. The aristae are thickened and often segmented. The aristal hairs are always present although reduced in number. Claw-like structures are sometimes present on the distal end of the aristae. There is no reduction in the length of the bristles to insure that this is a *spineless-aristapedia* allele.

5. *blunt;* 2068; autosomal recessive. The bristles are reduced in diameter and slightly increased in length. In older flies the distal half of the bristles breaks off thus appearing short and stubby.

6. *bubble;* 2069; autosomal recessive. Thin, puffed spots of different sizes occur in the marginal or submarginal wing cell. The wings are brown and curled but when expanded are narrow in width and pointed on the ends.

7. *cinnabar;* 2067; 3rd chromosome; autosomal recessive. The eye is a bright orange which is retained after aging. Allelic to *cinnabar* of *texana* and *virilis*.

8. *constricted;* 2069; autosomal recessive. The wings are short and narrow with rounded ends.

9. *constricted-like;* 2068; autosomal recessive. Phenotypically similar to *constricted.*

10. *displaced;* 2068; autosomal recessive. The eyes have displaced, swollen and occasionally fused facets. The scutellar, dorso-central, humeral and ocellar bristles are missing. The wings contain enlarged cells and patches of abnormally arranged cells which give it a pebbly appearance.

11. *double;* 2067; autosomal recessive. The anterior scutellar bristles are doubled. The expression is usually unilateral.

12. *downcast-sterile;* 2067; type of inheritance not determined. The wings are folded downward parallel to the thorax and are often folded under the thorax. Only affected females which showed no ovary development were recovered.

13. *everted;* 2067, 2069; autosomal recessive. A large mass of undifferentiated tissue extends from the external opening of the digestive tract. In the males the external genitalia may be normal in appearance, rotated to any extent and in any direction or missing entirely. The males have only rudimentary testes which contain sperm bundles but no motile sperm. The spermathecae are present in the females. No other normal structures of the reproductive system are present. The digestive tract of both males and females end within the abdominal cavity as an unattached gut.

14. *extreme-1;* 2068; autosomal recessive. The bristles are reduced in length and diameter. Any of the bristles of the head or thorax may be missing. Extra wing veins from the second longitudinal wing vein to the marginal vein sometime occur.

15. *extreme-2;* 2069; type of inheritance not determined. All of the bristles are reduced in length and diameter and any one or more may be missing entirely. The eyes contained fused and displaced facets. Only affected females recovered.

16. *extreme-like;* 2067; autosomal recessive. The bristles are reduced in length and diameter. In the females there is no hair growth or pigmentation on the abdomen; the males show almost normal pigmentation with sparse hair growth.

17. *hairless;* 2067; autosomal recessive. The number of hairs on the thorax is reduced to about one-half the normal number. Occasionally the bristles may also be missing.

18. *grooveless-like;* 2068; autosomal recessive. The line of demarcation between the thorax and scutellum is obliterated.

19. *immature;* 2069; autosomal recessive. The abdomen is immature in appearance.

20. *increased;* 2069; autosomal recessive. The eye facets are somewhat larger than normal and show abnormal arrangement.

21. *interrupted;* 2067, 2068, 2069; autosomal recessive. The posterior crossvein may have a small gap in the center, one-half of the vein or the entire vein may be missing. One or both posterior crossveins may be affected.

22. *light;* 2067, 2068, 2069; autosomal recessive with modifiers. The body pigmentation is yellow or gray as compared to the darker pigmentation of normal flies. The hairs and bristles are normal in color. There is a variation in expression of the mutant in different strains.

23. *mosaic;* 2068; type of inheritance not determined. The gene expression is a unilateral one affecting several different structures of the body. One wing is usually spread in a horizontal plane. Other unilateral effects are rough eye, extra wing veins, cut wings and missing bristles.

24. *narrow;* 2068; autosomal recessive. The wings are narrow and pointed. The wing cells are larger than normal.

25. *orange;* 2068; autosomal recessive. The eye is translucent and orange in color. The color is bright upon emergence and darkens upon aging.

26. *parallel;* 2068; autosomal recessive. The body pigmentation is a straw yellow. The wings are narrow, straight along the edges and taper to a point.

27. *pointed;* 2069; autosomal recessive. The wings are narrow, slightly longer than normal and pointed. Complete separation from normal is difficult.

28. *red;* 2067; autosomal recessive. The eye is translucent and an orange-red in color.

29. *rough;* 2069; type of inheritance not determined. The eye is rough due to abnormal pile development. Only affected female recovered.

30. *roughex;* 2069; sex linked recessive. The eye facets show slight disarrangement but the rough appearance is produced primarily by abnormal pile arrangement. Non-allelic to *echinus* of *virilis.*

31. *rough-cut;* 2067; type of inheritance not determined. The eye facets are abnormal in arrangement, fused and swollen. The longitudinal fourth and fifth veins are constricted through the middle portion to form a thickened junction with the posterior crossvein. The wings are small, short and notched. The wings are sometimes spread and some of the thoracic bristles may be missing. Only females were recovered which suggest either a lethal or balanced condition in the males.

32. *rough-spread;* 2069; autosomal recessive. The eyes are rough and one or both wings may be spread from 45 to 90 degrees. The wings have a brownish tinge and extra wing veins extend from the second and third ligitudinal wing veins. A vesiculated area in the distal half of the wing occurs. Males and females show rudimentary internal reproductive organs.

33. *slight;* 2067, 2068; autosomal recessive. The bristles are reduced in length and diameter. The eyes have abnormal facet arrangement and the pile is reduced in length thus giving the eye a smooth appearance. The entire fly is smaller in size and lighter in color.

34. *small bristles;* 2068; autosomal recessive. The bristles are reduced in length and occasionally in diameter. Sterility of this mutant is due to the degenerate ovary development in the female and absence of motile sperm in the male.

35. *spread;* 2068, 2069; autosomal recessive. Both wings are spread from 45 to 90 degrees in a horizontal plane.

36. *strand;* 2068; autosomal recessive. The scutellar and dorso-central bristles are reduced to a small strand. Variation in expression and overlaps normal.

37. *swollen;* 2068; autosomal recessive. The tarsal joints are shortened and a swollen knot appears on the first or second tarsal segment.

38. *tapering;* 2067; autosomal recessive. The wing edges are somewhat parallel and taper to a point on the end.

39. *tinted;* 2068; autosomal recessive. The wings are wide with rounded ends and slightly dusky in color. Males and females are sterile.

40. *varnished;* 2067.1b; autosomal recessive, Chromosome 2. The eye is almond-shaped and the facets are fused in such a way to produce a glossy appearance. The eye color is orange but a large colorless spot in the center of the eye is sometimes present. The ocelli are pale yellow. This mutant is allelic to *varnished* of *virilis.*

41. *weak;* 2068; autosomal recessive. Pleiotropic mutant which produces abnormal phenotypic expression of the wings, eyes, legs and bristles. The fly is weak and often dies almost immediately after emergence from the pupal case.

42. *wide;* 2068; autosomal recessive. The 3rd posterior wing cell is wider than normal and the outer edge constricts at the distal termination of the fifth longitudinal wing vein. Complete separation from normal is difficult.

43. *wine;* (1–3); 2068, 2069; autosomal recessive. The eye is opaque and a deep ruby-red in color. No allele tests.

44. *yellow;* 2068; sex linked, recessive. The body pigmentation is yellow being about the same color as *yellow 40a* of *virilis.* All the microchaetae of the body and wings are yellow as is the pile of the eye. The distal half of the bristles and elements of the aristae contain yellow pigmentation.

Drosophila novamexicana

1. *broken* (1–2); 2075a, 2075b; autosomal recessive. The posterior crossvein may be missing entirely or only a small section of the vein lost.

2. *curved;* 2075.8b; autosomal recessive. The wing edges are curved under along the entire length of the wing and the ends are arched down over the end of the abdomen.

3. *shaggy;* 2075.8c; autosomal recessive. The eyes have a rough appearance and most of the hairs of the body are irregular in the direction of growth. They may be directed dextral, sinistral or perpendicular to the normal hair growth direction. The tarsal joints are shortened and appear to have a heavier growth than normal.

4. *shortened;* 2075.8h; autosomal recessive. The wings are small, short and taper to a point. The posterior crossvein may be gapped or missing entirely.

5. *sparse;* 2075.8f; autosomal recessive. Areas occur on the thorax, eyes and wings which are completely denuded of microchaetae and macrochaetae. Other variations as extra wing veins and notched wings may occur.

Drosophila americana texana

1. *abnormal abdomen-1,* -2; 2015; autosomal recessive. The tergite formation is abnormal although there is no reduction in the number of abdominal segments present. The whole abdomen shows an almost complete loss of pigmentation and only a few hairs are present.

2. *abnormal abdomen-3;* 2020; autosomal recessive. The abdomen shows some loss of hairs, bristles and pigmentation. The eyes show some abnormal arrangement of facets and the pile is missing in spots.

3. *abruptoid;* 2020.1d; sex linked, recessive. All the longitudinal wing veins are shortened. Males are sterile.

4. *absent* (1–7); 2007, 2012, 2014, 2015, 2017; probably autosomal recessive. Any bristles of the head or thorax may be missing. The scutellar bristles are most often affected. No allele tests have been completed.

5. *absent-semi-lethal;* 2015; autosomal recessive. Various bristles of the head and thorax are missing. The sclerite formation is abnormal with a mosaic pattern of nonpigmented patches. Extra wing veins may branch from the longitudinal veins or appear as isolated fragments. Late emergence and low viability.

6. *bithorax;* 2007.1d; autosomal recessive. An extra growth of tissue is present between the thorax and abdomen. The halteres are enlarged, abnormal in shape and bent downward. The tibia is knotty and the tarsal segments are shortened. One wing is sometimes spread.

7. *blister;* 2013.1a; autosomal recessive. A thin area of the wing at, or near, the region of the anterior crossvein produces a blistered spot.

8. *blister-3d;* 2018.1a; autosomal recessive. A small area of thin wing tissue in the 3rd posterior wing cell produces a bubble-like structure. The expression is limited to this area.

9. *bright-1;* 2020.1a; autosomal recessive. The eye is bright orange upon emergence but darkens to nearly normal in two or three days.

10. *bright-2;* 2020.1e; autosomal recessive. The eye is translucent and bright orange-red in color. Darkens to nearly normal upon aging.

11. *brilliant;* 2015; autosomal recessive. The eyes are translucent and bright orange-red. There is some slight darkening when aged. Non allelic to *scarlet, cinnabar* and *cardinal* of *virilis.*

12. *cinnabar;* 2014, 2019, 2020; autosomal recessive, Chromosome 3. The eye is a bright orange in color. Allelic to *cinnabar* of *virilis.*

13. *closed;* 2007; autosomal recessive. The external genitalia and the external openings to the reproductive and digestive tracts are absent in both male-like and female-like individuals.

14. *diminished;* 2015, 2016, 2017, 2021; autosomal recessive. These mutants have three expressions which are probably dependent upon multiple iso-alleles. The extreme form is similar to *miniature* in that the wings are reduced in size and dusky in color. In the less extreme form the wings are not as small and may or may not be dusky in color. In the third form the wings are arched down over the end of the abdomen. A pure strain showing only one expression has been isolated from a mixed culture which showed several. All five *diminished* mutants are allelic.

15. *dishevelled;* 2015; autosomal recessive. Some eye facets are swollen and/or show abnormal arrangement. One or both wings may be spread in a horizontal plane. The tarsal joints are shortened and extremely long hairs and bristles are present in this region. The macrochaetae and microchaetae of the thorax are abnormal in growth arrangement.

16. *downcast;* 2015; autosomal recessive. The wings are sagged downward at a 25–45 degree angle to the body. The male, female or both are sterile.

17. *dumpy;* 2015; autosomal recessive. The wings are about one-half the normal length and broad. The posterior half of the wing is missing. Rather dark, heavy hair growth occurs along the cut edges. All veins of the remaining portion of the wing are normal. Whorls of hair on each side of the thorax sometimes occur. Males and females reduced in viability and probably sterile.

18. *ebony;* 2020; autosomal recessive. The entire body is darkened by black pigment. The eyes are also darker than normal. The wings are shaded with black pigment and all the longitudinal veins are cloudy, showing much darker shading than the rest of the wing. The pupal case has an area, about one-eighth of an inch on the anterior end, which is darkened to black. Mutant expressed in heterozygote.

19. *extended-1;* 2007; autosomal recessive. One or both wings are spread. Expression varies.

20. *extended-2;* 2015; autosomal recessive. One or both wings are spread. Viability and fertility are reduced.

21. *extreme;* 2007; autosomal recessive. All the hairs of the body are reduced to a minute size and some are missing. Males sterile.

22. *fan;* 2020; autosomal recessive. The wings are fan-shaped with cut ends. There is heavy dark hair growth along the distal edges. Similar to *dumpy* (2015).

23. *fused;* 2020; autosomal recessive. In the females the eyes contain large blister-like facets; the pile may be absent or abnormal in arrangement. The abdomen shows

abnormal tergite formation and some absence of pigment. The males do not show as an extreme expression.

24. *gapped;* 2015; autosomal recessive. The second longitudinal wing vein is shortened from one-half to one-fourth the normal length.

25. *grooveless;* 2015, 2017; autosomal recessive. The line of demarcation between the scutellum and thorax is obliterated by tissue growth.

26. *haltere;* 2015; probably autosomal recessive. The halteres are curved downward under the abdomen.

27. *hooked;* 2015; autosomal recessive. The bristles are reduced in diameter and length. The ends of the bristles are blunt in young flies and sometimes branched near the ends. Aged flies have only short blunt bristles since the ends are easily broken off.

28. *irregular;* 2020; autosomal recessive. The hairs and bristles are irregular in the direction of growth. One or both wings may be spread.

29. *lustrous;* 2018; autosomal recessive. The eye is bright red and darkens somewhat in color when aged.

30. *mahogany* (1–4); 2007, 2015, 2018; autosomal recessive. The eye color is a deep, dark red. No allele test completed.

31. *missing-like;* 2007; autosomal recessive. In the females all of the long bristles of the head and thorax are absent. The wings are abnormal in texture and curved over the end of the abdomen. The eyes are rough in texture. The males are less extreme. Males and females are reduced in viability and completely sterile.

32. *mottled;* 2007; autosomal recessive. The eye color is a translucent, light orange with a rather dense, deep layer of dark pigment specks. Viability and fertility are reduced.

33. *mottled-localized;* 2016; autosomal recessive. An area involving from ten to fifteen facets shows dark pigmentation. This area is localized in the anterior medial part of the eye. Late emerging and sterile.

34. *narrow;* 2015; autosomal recessive. The wings are more narrow than normal and have pointed ends. Other pleiomorphic effects are abnormal facet arrangement, shortening of tarsal joints and wing modifications.

35. *narrow-broken;* 2012; autosomal recessive. The wings are shorter and more narrow than normal. The posterior crossvein may be present, gapped or missing entirely.

36. *plexus;* 2020; autosomal recessive. A plexus of extra wing veins occurs between the second longitudinal wing vein and marginal vein.

37. *pointed* (1–4); 2015, 2020; autosomal recessive. The wings are more narrow and slightly shorter than normal. The posterior crossvein may be broken or missing completely. No allele test completed.

38. *pointed-like;* 2015; autosomal recessive. The wings are short and slant to a point on the ends. The center portion is wide and the ends curve downward over the end of the abdomen.

39. *ragged;* (1–7); 2007, 2012, 2014, 2015; type of inheritance not determined. Irregular notched places from the wing occur around the entire edge. No affected females have been recovered and the males show about 50% expression. No allele tests.

40. *rough* (1–11); 2007, 2013, 2014, 2015, 2020, 2021; autosomal recessive. This classification includes a large number of morphologically rough eyed mutants. When allele tests have been completed specific names will be assigned to the different mutants.

41. *roughened;* 2015; autosomal recessive. The facets are abnormal in arrangement and occasionally swollen. Abnormal pile arrangement coincides with facet anomalies. This mutant is nonallelic to *roughoid-2* (2015) and *roughest* (2007).

42. *rougher;* 2015; autosomal recessive. The facets of the eye are abnormal in arrangement with an irregular pattern of blister-like facets. The wings are small in size with large wing cells. Small notched places in the wing sometimes occur.

43. *roughest;* 2007; autosomal recessive. The facets are swollen, blister-like and abnormal in arrangement. Non-allelic to *roughened* (2015) and *roughoid-2* (2015).

44. *roughoid-1;* 2015; autosomal recessive. The eye facets have a slight abnormal arrangement and the pile is missing in spots.

45. *roughoid-2;* 2015; autosomal recessive. The eye facets are abnormally arranged with a fusion of several facets and blister-like facets occurring only rarely. Non-allelic to *roughened* (2015) and *roughest* (2007).

46. *rough-absent;* 2012; autosomal recessive. The eye facets are abnormally arranged and certain bristles of the head and thorax are missing. One or both sexes sterile.

47. *rough-broad;* 2020; autosomal recessive. The eye facets are abnormal in arrangement and blister-like. The wings are usually short, broad and very rounded on the distal end. Small notched places occur around the edge of the wing and extra wing veins extend from the second longitudinal vein to the marginal vein. Shortening of the leg joints and abnormal leg development occur occasionally. Viability and fertility are reduced.

48. *rough-cut;* 2015; autosomal recessive. In the males the entire surface of the eye is covered with swollen, abnormal facets. The wings are sometimes slightly spread and large notched places occur along the edge of the wing. The females are less extreme.

49. *rough-extreme;* 2007; autosomal recessive. Mosaic patches of irregular facets which have dark pigmentation are scattered over the entire eye. The wings are rough in texture and contain a plexus of extra wing veins from the posterior crossvein and longitudinal veins. Certain bristles of the head and thorax are absent.

50. *rough-grooveless;* 2015.1h; autosomal recessive. The facets are misplaced and/or swollen and may show dark pigmentation. The wings and halteres are bent downward, parallel to the body. The line of demarcation between the thorax and scutellum is obliterated.

51. *rough-missing;* 2019; autosomal recessive. The eye facets are irregular and the wings are shorter and more narrow than normal. The wing cells are larger in size than normal. A few or nearly all the long bristles of the thorax may be missing. Viability is low.

52. *rough-mottled;* 2020.1a; autosomal recessive. The facets which may be swollen, blistered, fused and darkly pigmented give a mottled appearance to the eye. The ocellar, humeral, dorso-central, postvertical and/or sternopleural bristles may be missing.

53. *rough-nicked;* 2021.1a; autosomal recessive. The eye facets are irregular and swollen. Small nicked places occur along the edge of the wing.

54. *rough-short;* 2020; autosomal recessive. The facets are irregular and the wings are narrow and short. The abdomen is somewhat short in proportion to the thorax and sometimes shows an absence of pigment and hairs. The orbital, scutellar and ocellar bristles may be missing.

55. *rough-vestigial;* 2012.1c; autosomal recessive. The eye facets are swollen and blister-like. The wing is fan-shaped and heavy hair growth occurs along the distal edge. The expression is variable and extreme forms are sterile.

56. *scarlet-like;* 2007; autosomal recessive. The eye is translucent and bright orange upon emergence but darkens to almost normal in two or three days. Suppressors are probably present in some strains.

57. *scutellar-like;* 2015; type of inheritance not determined. Certain bristles of the thorax are missing but the basal discs are not removed. The absence of the posterior scutellars is the most constant expression. About 75% expression at 25 degrees Centigrade.

58. *semiplexus;* 2021; autosomal recessive. Extra wing vein fragments extend from the 2nd and 4th veins and/or posterior crossvein.

59. *short;* 2021; autosomal recessive. All the tarsal segments, except the first, are shortened and a swollen mass is present on the distal end of each affected segment. The posterior crossvein is sometimes gapped but is never missing entirely. The wings may be narrow or wide and rounded.

60. *short 4th;* 2015.1b; autosomal recessive. The fourth longitudinal vein is shortened.

61. *short 5th-1,* -2; 2007; autosomal recessive. The longitudinal five wing vein is shortened. The expression varies and some strains overlap normal.

62. *short 5th*-3, -4; 2014; autosomal recessive. The 5th longitudinal wing vein is shortened to about two-thirds the normal length. The posterior crossvein is sometimes missing.

63. *short 5th* (5–7); 2015; 2020; autosomal recessive. The 5th longitudinal wing vein is shortened.

64. *short 5th-8;* 2021; autosomal recessive. The fifth longitudinal vein is short. Non-allelic to *short veins* (2015).

65. *shortened;* 2021; autosomal recessive. The wings are more narrow and shorter than normal. Complete separation from normal is difficult.

66. *short veins* (a–c); 2015; autosomal recessive. All the longitudinal wing veins are shortened with the fifth vein being the most extreme in expression and most often affected. In some strains certain bristles of the head, especially the ocellar, and of the thorax are missing. All three mutants are allelic. They give a slight expression with *veinlet* (2007.1d). Non-allelic to *short-5th* (2021).

67. *short veins;* 2017; autosomal recessive. The longitudinal veins, especially the second and fifth, are shortened.

68. *small;* 2012; autosomal recessive. The bristles are reduced in diameter.

69. *small-absent-1* -2; 2015; autosomal recessive. The bristles and hairs of the body are reduced in length and diameter. Certain bristles of the head and thorax are missing. The abdomen sometimes shows abnormal sternite and tergite arrangement and the absence of pigment. Late emergence with low viability and fertility.

70. *small bristles-a,* -b; 2007; autosomal recessive. The bristles are reduced in length and diameter.

71. *small bristles* (1–8); 2015, 2020; autosomal recessive. These eight mutants show a reduction in the bristle size. There is a reduction in the diameter in all cases and in the length except in one case. Other than a quantitative difference, minor qualitative differences as the absence of bristles also occur. In most cases either the male, female or both are sterile.

72. *small extra;* 2015; autosomal recessive. The wings are small in size but the wing cells are much larger than normal. The wing veins are wider than normal and extra veins branch from the fourth longitudinal vein. The abdomen is short with abnormal sternite and tergite formation. The microchaetae of the abdomen are irregular in arrangement.

73. *small wing-1;* 2015; autosomal recessive. The wings are normal in shape but are reduced in size and shortened in length.

74. *small wing-2;* 2018; autosomal recessive. The wings are short and dusky in color.

75. *spread-1;* 2015; autosomal recessive. The wings are spread in a horizontal plane or folded downward and curved under the thorax. Developmental modifications as duplicated portions of the dorsal side of the thorax and shortened tarsal joints also occur. Fertility is low.

76. *spread-2;* 2015; autosomal recessive. Both wings are spread from 45–90 degrees in a horizontal plane or folded under the thorax.

77. *spread-semilethal;* 2020; type of inheritance not determined. One or both wings may be spread. The wings have a pebbly appearance with irregular vesiculate spots occurring at times. The bristles may or may not be reduced in size. Only affected males were recovered.

78. *stocky-1,* -2; 2020; autosomal recessive. The femur and tibia are shortened, enlarged and knotty. Some strains show an absence of bristles and abnormal mounds of tissue on the femur. Late emergence and low viability.

79. *strap;* 2012; autosomal recessive. About two-thirds of the 3rd posterior wing cell, all of the 2nd posterior wing cell and about one-half of the marginal wing cell are

missing. The cut edges are smooth with no hair growth. There is some variation in expression but the mutant usually does not overlap normal. The viability is somewhat reduced.

80. *stubby-1*, -2; 2020; autosomal recessive. The tibia and tarsus joints of the second and third legs are shortened and knotty.

81. *stubby-3;* 2021; autosomal recessive. The second and third legs show abnormal development of the femur, tibia and tarsal segments. Most affected individuals emerge from the pupal case with very abnormal body development and die very young. Not included in Tables.

82. *thickened;* 2007; autosomal recessive. Irregular thickened areas occur along the longitudinal wing veins two, four and five.

83. *translucent;* 2021; autosomal recessive. The eye is translucent and bright red-orange.

84. *veinlet;* 2007; autosomal recessive. The fourth and fifth longitudinal wing veins are shortened. The wing is more narrow than normal and pointed. There is a slight expression with *short veins-b* and *-c* (2015).

85. *wide;* 2013; autosomal recessive. The wings are increased in width across the median part and the wing cells are larger than normal. The entire edge of the wing is usually rolled under to produce a slight arc.