31. The Genetics of *Drosophila virilis*

MARY L. ALEXANDER

Biology Department
Southwest Texas State University
San Marcos, Texas, U.S.A.

I. Introduction

The species, *D. virilis*, was described by Sturtevant in 1916 and taxonomically belongs in the Subgenus Drosophila Fallen. This species is one of a group which includes *D. littoralis* (Meigen, 1830), the subspecies *D.*

americana americana (Spencer, 1938) and *D. americana texana* (Patterson, Stone and Griffen, 1940), *D. novamexicana* (Patterson, 1941), *D. montana* (Patterson and Wheeler, 1942) and *D. imeretensis* (Sokolov, 1948). In this group, *D. virilis* was regarded as the modern form most like the proposed ancestral form by Patterson and Stone (1952). Although *virilis* has the widest distribution of any other member of the group and has been reported in collections from four zoogeographic realms, Nearctic, Neotropical, Paleoarctic, and Oriental, the species is probably native in the eastern Paleoarctic and Oriental realms. Genetically, *americana*, *texana*, and *novamexicana* are the species most closely related to *virilis*, and all three species occur in North America. The other North American forms, such as *montana* and *flavomontana*, are more distantly related. The European forms include *littoralis* and *imeretensis*. The extensive data on the genetic relationship of the various members of the virilis group have been discussed by Patterson and Stone (1952) and Stone (1962).

II. Morphological and Developmental Characteristics

Morphologically, *virilis* has a blackish body color with clouded posterior crossveins and sterno-index varying from 0·8 to 0·9. The comparison of *virilis* with the closely related species, *americana*, has been reported by Spencer (1938, 1940a, b). In addition to the darker body color and clouded posterior crossveins characteristic of *virilis*, the two species differ in the size of the eyes, the eye pile, carina shape and the number of branches of the arista. There are differences in the physiological adaptations in the two species. The pupae of *americana* are found on the surface or edge of the food, whereas the larvae of *virilis* move out of the food and up the side of containers before pupation. The pattern of pupation for *americana* is dominant to that of *virilis* and is expressed in hybrids between the two species. Another consistent physiological difference between *virilis* and *americana*, as well as other members of the group, is the ease with which all the members of the group, except *virilis*, can be etherized. While it is difficult to etherize *Drosophila virilis*, care must be taken not to over etherize other members of the group. Another difference is the red pupal case color observed for *americana* as compared to the black or gray pupal case color found for *virilis*. Stalker (1942) determined that the location of the main factor for red pupal case color was in the 5th chromosome with modifiers present in the 2–3 fusion of *americana*.

A. Life Cycle

The life cycle of *virilis* is approximately twice as long as that of *D.*

melanogaster. The period from egg to adult stage is approximately 18 days for *virilis* at 24°C. In addition, the young adults of *D. virilis* require an aging period of 6 days after emerging before consistent fertility for mating tests are obtained. A period of 6 days is usually sufficient for aging virgin females after hatching rather than the one day period for *D. melanogaster*. Young *virilis* males 1 or 2 days old contain non-motile sperm as the most mature germ cell type. The additional aging period is required for a sufficient supply of motile sperm to develop in the males (Clayton, 1962).

B. Histological Studies

The course of meiosis in *virilis* spermatogenesis and several other species of *Drosophila* was first reported by Metz (1926). The chromosome behavior during meiosis was essentially the same in all the species studied. However, the study did not report the details of germ cell development, such as the time each stage of spermatogenesis appears in the development of the organism. The histological study of *virilis* by Clayton (1957, 1962) gives a complete time table for spermatogenesis. The time when each type of germ cell appears and the relative and absolute number of each type of germ cell in larvae, pupae and adult development were reported.

The mean number of cells in each stage during germ cell development in larvae, pupae and adults of *D. virilis* is given in Table I. The relative

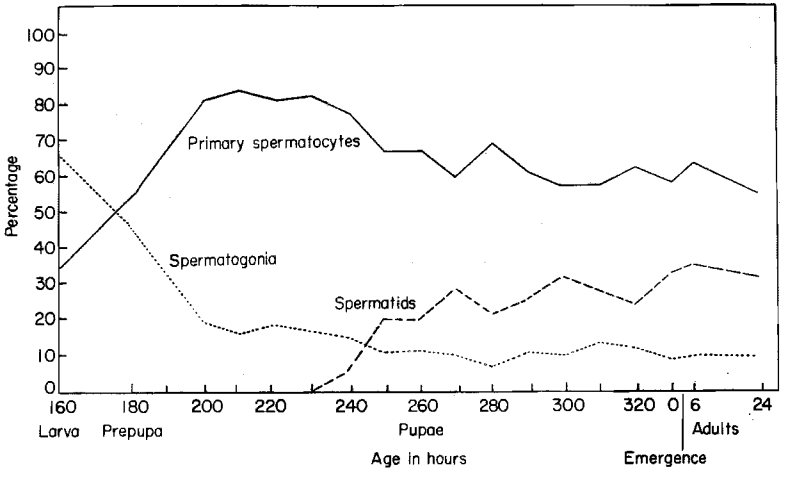

FIG. 1. Relative frequencies of cells in testes of *D. virilis* during development. (From Clayton, 1957.)

TABLE I. Mean Number of Meiotic Cells in Testes during Development in *Drosophila virilis*.[a]

	Larva	Prepupa	Pupae							
Age in hours	160	180	190	200	210	220	230	240	250	260
Number males scored	1	6	2	6	5	10	10	7	10	6
Total cells scored	1123	7621	3091	10,581	11,710	22,529	22,653	17,115	25,911	15,064
Mean Number Cells per male:										
Spermatogonia	739	585	492	345	387	423	349	359	284	280
Primary spermatocytes:										
Prophase I	384	685	1054	1418	1955	1830	1869	1880	1734	1659
Metaphase I							5	2	3	3
Anaphase I							4	3	1	1
Telophase I							11	8	5	15
TOTAL	384	685	1054	1418	1955	1830	1889	1893	1743	1678
Interphase							27	17	15	17
Secondary spermatocytes:										
Prophase II								6	2	3
Metaphase II								7	15	10
Anaphase II								5	6	6
Telophase II								9	4	16
TOTAL								27	27	35
Spermiogenesis:										
Early spermatid								37	76	80
Nuclear growth								66	167	114
Linear growth								31	112	137
Axial filament									167	168
TOTAL								134	522	499

[a] From Clayton (1957).

TABLE I—(*continued*)

	Pupae						Adults			
Age in hours	270	280	290	300	310	320	0	6	24	13 days
Number males scored	10	10	6	10	3	10	6	9	10	6
Total cells scored	25,515	26,051	15,112	24,914	7249	25,735	11,281	17,396	19,337	6903
Mean Number Cells per male:										
Spermatogonia	257	173	281	247	330	310	166	182	184	145
Primary spermatocytes:										
Prophase I	1496	1769	1537	1393	1375	1586	1090	1078	1084	772
Metaphase I	8	13	4	5		9	8	7	6	6
Anaphase I	2	6	3	2		6	4	5	2	2
Telophase I	10	10	10	9	7	8	4	11	7	2
TOTAL	1516	1798	1554	1409	1382	1609	1106	1101	1099	782
Interphase	17	31	12	15	9	13	4	14	11	6
Secondary spermatocytes:										
Prophase II	1	15	2	1	5	5	4	4	11	1
Metaphase II	16	13	15	12	12	11	10	14	6	2
Anaphase II	9	8	8	5	11	7	5	6	3	2
Telophase II	14	8	7	9	9	10	6	11	11	1
TOTAL	40	44	32	27	37	33	25	35	31	6
Spermiogenesis:										
Early spermatid	86	58	51	61	57	42	64	46	55	3
Nuclear growth	171	194	201	197	186	157	129	149	148	47
Linear growth	227	141	177	282	116	161	155	196	146	106
Axial filament	238	166	210	255	299	249	263	222	260	56
TOTAL	722	559	639	795	658	609	611	613	609	212

TABLE II. Chronological Sequence of Germ Cell Development in *Drosophila virilis*.

Time	Development	Germ Cells
Larvae		
1–160 hours	Larvae active, feeding	Spermatogonia
170 hours	Larvae inactive	Primary Spermatocytes
180 hours	Puparium formation starts	
180–190 hours	Prepupae	
190 hours	Pupation	
Pupae		
210 hours	Largest number of primary spermatocytes	
220 hours	First division metaphases	
230 hours	First appearance of secondary spermatocytes	Secondary Spermatocytes
240 hours	First spermatids appear	Spermatids
250 hours	Testes coiled—first sperm bundles	Sperm Bundles
280 hours	Sperm bundles frequent	
320 hours	Emergence of adult; large mass of sperm bundles	
Adults		
1–5 days	Spermatogonia, primary and secondary spermatocytes, spermatids, and large masses of sperm bundles	
5 days	First functional sperm (2% of males)	Mature Sperm
6 days	Functional, motile sperm	

frequencies of each type of cell are illustrated in Fig. 1. Both Table I and Fig. 1 are from Clayton (1957).

The mean number of cells (Table I) gives some measure of the total number of particular types of cells at any age of the larvae or pupae and some adult stages. Figure 1 illustrates the relative frequencies for the spermatogonial, primary spermatocyte and spermatid cells during the larval, pupal or adult periods. Both give the age at which any one type of cell appears. A more detailed time table of events and the relationship of the germ cell types with the morphological stage of development are given in Table II. This table also contains the data for sperm bundles and mature sperm as well as details on the time of appearance and development of earlier stages.

The meiotic process in *virilis* is essentially the same as that reported in *D. melanogaster* by Cooper (1950). One difference in the two species is the difference in the stage of morphological development which shows the development of particular meiotic stages. In *melanogaster* the first maturation division occurs in late larvae but in *virilis* first division metaphases are not observed until after pupation (Clayton, 1957). The appearance of mature, motile spermatozoa in *virilis* is also delayed in adult males. When the adult fly emerges, the testes contain large masses of sperm bundles but functional (motile) spermatozoa are not predominant for 6 days. In *melanogaster*, the adult male testes contain an ample supply of motile sperm within 24 hours after emergence.

C. Cytoplasmic Elements in Spermatogenesis

Detailed studies of mitochondrial behavior and time intervals in the development of the primary and secondary spermatocytes of *virilis* were reported by Momma (1962). In *Drosophila*, as in most insects, the mitochondria are rod or thread-like bodies extending between the two spindle poles in telophase. This was reported for *D. melanogaster* (Cooper, 1950), for *D. pseudoobscura* (Dobzhansky, 1934) and previously for *D. virilis* (Metz, 1926). The detailed study for *virilis* (Momma, 1962), showed that during the latter stages of the growth period of primary spermatocytes the cytoplasmic volume increased and the mitochondria appear as rod or thread-like shaped bodies. In late prophase, probably diakinesis, the mitochondria have a definite orientation in arrangement. As the asters form for first metaphase, the mitochondria lie nearly parallel to the astral rays and cover the surface of the nuclei. At the same time, a number of Golgi-like elements accumulate close to the core of each aster. The mitochondria appear to be united into long strings and during metaphase the strings are extended with some strings bending into right and acute

angles. From late metaphase to early anaphase, the angular points of the mitochondria run against the plasma membrane. The mitochondrial strings become arranged parallel to the polar axis on the surface of the spindle body during anaphase. At telophase they are stretched between the daughter nuclei which are being formed. As the cleavage furrow forms at the end of telophase, the mitochondrial bodies, as well as the cell body, are divided into two halves. After the cell body and mitochondria bridges have divided, the mitochondria become scattered around the reconstructed nucleus of each cell.

In the second division, the mitochondrial bundles are stretched directly between the poles along the spindles as the cell proceeds from anaphase II to telophase II. As the cleavage furrow appears in the cell body, the medial portion of the mitochondrial bundles decrease in diameter. During these stages the Golgi elements are granular in shape and surround each pole. As the furrow divides the cell body, the mitochondrial bundles are divided into approximately equal halves, and form masses in each daughter cell. The mitochondrial bodies condense into round masses which later fuse into a single body and come into contact at the base of the nucleus. This body becomes spherical in shape and forms the "nebenkern" in spermatids. Mitochondrial elements are distributed mechanically and passively by cell constriction but the active division of mitochondria is indicated by the work of Momma (1962). The mitochondria appear to be divided and separate into two essentially equivalent groups without regard to the cleavage furrow. Such active behavior of mitochondria was observed in living material and was more obvious for the second than the first division.

The duration of each meiotic phase of spermatocytes was also measured by Momma in this study. At 20°C, the successive series of divisions were followed in the same cell starting with prophase or metaphase and ending with telophase. The times in the first division were: metaphase I, 55 minutes; anaphase I, 30 minutes and telophase I, 45 minutes. The time from the formation of the furrow to the complete division of the cell body was 25 minutes. This gives a total of 155 minutes from metaphase through the telophase division. The comparable periods in the second divisions were: metaphase, 35 minutes; anaphase, 25 minutes and telophase, 30 minutes with a 20-minute period to complete the division into two cells. This gives a total of 110 minutes from metaphase until the completion of telophase. The 170 minutes reported for the second division would indicate an average of 60 minutes before metaphase.

TABLE III. Mutations of *Drosophila virilis*.[a]

	Symbol	Name	Linkage group–locus	Description	Reference
1.	*a*	approximated	5–75·5	Two crossveins close together	MMM–23
2.	*Ax*	Abruptex	1–103·3	Heterozygous female with shortened L veins; wings expanded and thin; few hairs and bristles on head and thorax. Homozygous female and male more extreme	C–41b
3.	*ac*	acute	6–0·0	Pointed wings	MMM–23
4.	*ap*	apricot	1–136·0	Yellowish pink eye	C–29
5.	*ap*^{32}	apricot-32	1–136·0	Darker than *ap*	D–U
6.	*ar*	abdomen-rotatum	6–0·3	Abdomen rotated	D–U
7.	*Aw*	Abnormal wing	1–1·4	Abnormal flies like supermales of *melanogaster*; lethal homozygous	C–U
8.	*B*	Branched	5–141·0	Extra veins; not dominant in all crosses	MMM–23
9.	*b*	broken	2–188·0	Both crossveins broken or missing	MMM–23 C–39
10.	*Ba*	Baroid	4–63·0	Small, rough eyes; lethal homozygous	C–36
11.	*ba*	bat	3–86·2	Wings down-curved	C–29
12.	*Bb*	Barb	2–80·0	Bristles with knot on tip	C–30
13.	*bb*	bobbed	1–170·5	In female, bristles small, sclerites scaly	C–U
14.	*Bd*	Beaded	5–148·5	Wing margins scalloped	C–U
15.	*Bh*	Barish	4–160·0	Eyes narrow and ovoid, rough texture; lethal homozygous	C–40
16.	*bk*	brick	2–248·0	Pink eye color	C–30 C–39
17.	*Bl*	Bristle	3–30·5	Wings slightly extended and curved; bristles short and stubby; eyes deformed; lethal homozygous	C–41b
18.	*bl*	black	4–195·0?	Blackish body color	C–U

TABLE III—(*continued*)

	Symbol	Name	Linkage group–locus	Description	Reference
19.	*Br*	Bar	2–64·5?	Eyes narrow; homozygous lethal; associated with *In2a*	C–29
20.	*br*	broad	1–96·0	Wings about 4/5 normal length, slightly broader; crossveins closer together	K–U
21.	*Bx*	Beadex	1–94·5	Wing margins scalloped	C–29
22.	*C*	Confluent	2–45·0	L2 veins spread at margin of wing; eyes small, rough	MMM–23
23.	*c* (*cv*)	crossveinless	1–25·0	Posterior crossvein missing	MMM–23
24.	*ca*	claret	2–216·1	Eye color dark, brownish purple	C–36
25.	*cc*	concave	2–214·0	Arista hairs curled	MMM–23
26.	*cd*	cardinal	4–32·2	Vermilion-like eyes	C–37a, b
27.	*cf*	coffee	3–132·8	Deep brown eye color	C–39
28.	*ch*	cherry	1–17·0	Deep red eye color	Me–U
29.	*cho*	chocolate	2–227·3	Darkish brown eye color	C–37a, b
30.	*Cl*	Clipped	4–68·5?	Cut off wings	L–33
31.	*cn*	cinnabar	3–9·5	Vermilion-like eye	C–29
32.	*ct*	cut	1–112·4	Short, narrow, notched wings	MMM–23
33.	*co*	complex	2–178·0	Extra veins	C–39
34.	*cu*	curved	4–64·0?	Wings down-curved	C–29
35.	*cv-d*	crossveinless-d	5–97·0	Anterior and posterior crossveins missing	C–37a, b
36.	*d*	droop	1–150·0	Wings droop at tip	MMM–23
37.	*dc*	dachsoid	5–76·0	Rounded wings; tarsus 4 jointed	C–29
38.	*de*	declined	1–34·4	Wavy wings	D–U
39.	*D-cv*	D-crossveinless	5–145·0	—	C–41a
40.	*dh*	dahlia	3–81·0	Dark purplish eye color	C–39
41.	*Dl*	Delta	2–45·0	L veins fused at wing margin; eyes small, rough; lethal homozygous; probably allele to *C*	L–U

42.	*dl*	dishevelled	3–145·0	All hairs irregularly directed	C–35
43.	*Ds*	Dominant-short	1–100·4	Distal one-half of L5 obliterated; homozygous lethal	C–29
44.	*ds*	dachsous	4–173·0	Rounded wings; crossveins closer together	C–29
45.	*dt*	detached	2–255·0	Longitudinal veins short	C–U
46.	*dw*	dwarfex	1–60·0	Body and wings small in size	C–36
47.	*dy*	dusky	1–78·1	Wings about 4/5 normal size; dusky color	C–30 C–41a
48.	*eb*	ebony	2–83·5	Very dark body color	C–35
49.	*ec*	echinus	1–8·7	Rough eyes	C–30
50.	*ep*	expanded	3–130·5	Wings broad, extended	C–37b
51.	*es*	eosinoid	5–91·4	Dark purplish eye color	C–29
52.	*ex*	extended	5–103·0	Spread wings	C–29
53.	*f*	forked	1–89·0	Bristles twisted and gnarled	MMM–23
54.	*ft*	fat	5–44·5	Short, broad wings; tarsal joints shortened	C–35
55.	*G*	Garnet	3–8·0	Eyes dark purplish color	MMM–23
56.	*Gl*	Glass	5–150·0?	May be the same as Glued (158·2); facets fused; eye color diluted	C–37a
57.	*gl*	glossy	6–1·0	Small, rough eyes	Mo–U
58.	*go*	golf	5–176·5	Bristles with knot at tip	C–U
59.	*Gp*	Gap	6–0·4	L5 obliterated in middle part; homozygous lethal	CK–33
60.	*gp-L2*	gap-L2	3–118·5	L2 vein gap	C–37b
61.	*hk*	hooked	4–62·0	Bristles barbed or hooked	C–37b
62.	*ho*	hooked-ocellar	1–128·2	Ocellar bristles hooked	C–29
63.	*hp*	hump	6–0·5	Shortened thorax; dark glossy body color	C–37a
64.	*hy*	humpy	2–135·5	Thorax humped and glossy; extra wing veins	C–37b
64a.	*h*	hunch	3–31	Hunch-back appearance	
65.	*i*	interrupted	5–95·5?	Posterior crossvein broken or missing	MMM–23
66.	*in*	incomplete	2–45·2	L veins, esp. L2, short	C–30
67.	*ir*	irregular	4–198·0	Irregular direction of hairs on abdomen and wings	C–29

TABLE III—(*continued*)

	Symbol	Name	Linkage group–locus	Description	Reference
68.	*ix* or *ix*^{m}	intersex intersex-maleness	3–15·5	Produces development of male system in homozygous females; has no effect on males	L–34 C–36 L–39
69.	*Ix*^{B}	Intersex-Blanco	2–?	Produces development of male system in homozygous females; has no effect on males	N–42 S–42
70.	*Lb*	Lobster	5–118·0	Wings and abdomen slightly downcurved; homozygous lethal	K–U
71.	*ll*	lanceolate	4–3·9	Narrow, pointed wings	C–35
72.	*M*	—	6–?	Increases rate of germinal reversions of *mt-3* and *mt-5*	D–30
73.	*M-1a*	Minute-1a	1–18·0±	Bristles small	K–U
74.	*M-2a*	Minute-2a	2–218·2	Minute bristles	K–U
75.	*M-2b*	Minute-2b	2–220·6	Minute bristles	K–U
76.	*M-2c*	Minute-2c	2–243·5	Minute bristles	K–U
77.	*M-2d*	Minute-2d	2–168·0	Minute bristles; homozygous lethal	C–40
78.	*M-2e*	Minute-2e	2–256·6	Minute bristles; homozygous lethal	C–40
79.	*M-2f*	Minute-2f	2–257·6	Minute bristles; homozygous lethal	C–40
80.	*M-3c*	Minute-3c	3–84·2	Minute bristles; homozygous lethal	C–40
81.	*M-4a*	Minute-4a	4–36·0?	Minute bristles	K–U
82.	*M-4b*	Minute-4b	4–161·0	Minute bristles	C–U
83.	*M-4c*	Minute-4c	4–156·0	Minute bristles	C–U
84.	*M-4d*	Minute-4d	4–22·6	Minute bristles	C–U
85.	*M-4e*	Minute-4e	4–158·8	Minute bristles	K–U
86.	*M-4f*	Minute-4f	4–22·9	Minute bristles	C–36
87.	*M-4g*	Minute-4g	4–82·5	Minute bristles; homozygous lethal	C–37a
88.	*M-4h*	Minute-4h	4–165·0	Minute bristles; homozygous lethal	C–37a

89.	*M-4i*	Minute-4i	4–25·1	Minute bristles; homozygous lethal; extra veins near tip of L2, L5	C–40
90.	*M-4j*	Minute-4j	4–153·2	Minute bristles; homozygous lethal	C–40
91.	*M-4k*	Minute-4k	4–17·3	Minute bristles; homozygous lethal	C–40
92.	*M-5a*	Minute-5a	5–101·0	Minute bristles	C–36
93.	*M-5b*	Minute-5b	5–144·2	Minute bristles	C–39
94.	*m*	magenta	1–83·5	Dark purplish eye color	MMM–23
95.	m^{a}	magenta-alpha	1–83·5	Unstable, reverts to normal	D–27 D–41
96.	*mh*	mahogany	5–140·4	Deep brown eye color	C–30
97.	*mi*	minus	2–?	One or more thoracic bristles	MMM–23
98.	*mo*	mottled	2–89·0	Eye color red with small dark spots; female sterile	C–41b
99.	*mr*	morula	5–?	Rough eye; bristles occasionally missing	K–U
100.	*Ms*	Missing	4–63·0	Bristles and hairs on head and thorax missing; males sterile	C–U
101.	*mt*	miniature	1–78·0	Miniature wings, very dusky in color, about 2/3 of normal size	D–26
102.	mt^{2}	miniature[2]	1–78·0	Wings as long as abdomen	Mo–U
103.	mt^{3}	miniature[3]	1–78·0	Unstable, reverts to normal; wings like mt^{1}	D–26 D–41
104.	mt^{3a}	miniature[3]-alpha	1–78·0	Unstable in both somatic and germinal cells	D–29b D–41
105.	mt^{3b}	miniature[3]-beta	1–78·0	Stable	D–29b
106.	mt^{3c}	miniature[3]-gamma	1–78·0	Unstable in soma only	D–29b
107.	mt^{4}	miniature[4]	1–78·0	Wings about 4/5 of normal size	C–29
108.	mt^{5}	miniature[5]	1–78·0	Unstable, reverts to normal; wings like mt^{2}	D–41
109.	mt^{5a}	miniature[5]-alpha	1–78·0	Unstable as mt^{3a}	D–41
110.	mt^{5b}	miniature[5]-beta	1–78·0	Unstable as mt^{3b}	D–41
111.	mt^{5c}	miniature[5]-gamma	1–78·0	Unstable as mt^{3c}	D–41
112.	mt^{6}	miniature[6]	1–78·0	Wings about ½ normal size	C–29
113.	mt^{7}	miniature[7]	1–78·0	Wings reduced in size as *mt*	K–U

TABLE III—(*continued*)

	Symbol	Name	Linkage group–locus	Description	Reference
114.	*N*	Notched	1–102·9	Tips of wings broken; deficiency involving white locus; homozygous lethal	C–U
115.	*N-3a*	Notched-3a	3–83·0	Tips of wings broken; homozygous lethal	C–U
116.	*na*	nail	2–35·7	All bristles short and hook or barb on tip	C–41b
117.	*nd*	needle	1–10·4	Prickly-like bristle	C–39
118.	*ne*	nebulous	2–227·0	Thin wings with glitter	K–U
119.	*ni*	nick	4–19·7	Tip of wings nicked	C–40
120.	*p*	pink	2–212·5	Pink eye	C–29
121.	*pc*	polychaeta	5–113·0	Extra bristles; eyes rough	C–40
122.	*pe*	peach	5–203·0	Yellowish pink eye	Mo–U
123.	*Pi*	Pincer	2–79·6	Wings short and bent inside; males more extreme; lethal when homozygous	C–39
124.	*pi*	pointed	6–1·0	Wings narrow and pointed	C–41b
125.	*pk*	prickly	5–27·0	Prickle-like bristles	C–U
126.	*pk-b*	prickly-b	5–0·0	Similar to *pk* but not allelic	C–39
127.	*pm*	plum	4–18·7	Eye darkish brown	C–37a
128.	*po*	port	5–151·0	Eye color deep pink	C–37b
129.	*ps*	persimmon	2–151·5	Eye color darkish purple	C–35
130.	*Pu*	Puffed	2–64·5	Rough small eye	F–U
131.	*pw*	pink-wing	5–136·0	Wings shorter; deep pinkish eye color	C–U
132.	*px*	plexus	4–0·0	Extra wing veins	C–29
133.	*R*	Rounded	2–214·0	Wings retracted in marginal portion between L3 and L5	D–28a; L–32 C–39
134.	*r*	rugose	1–127·0	Rough, slightly yellowish eye	MMM–23
135.	*ra*	raspberry	2–223·5	Dark, ruby-like eye color	C–40
136.	*rc*	reduced	4–176·5	Minute bristles; female sterile	C–U

137.	*rd*	rudimentary	1–122·6	Small wings	C–29
138.	*re*	red	3–63·0	Reddish eye color	C–29
139.	*rg*	ragged	1–160·0	Wings notched in margin	C–29
140.	*rl*	rolled	3–0·0	Wing edges rolled	C–35
141.	*ro*	rough	1–74·9	Small, rough eye; small marginal hairs on wings; female sterile	Mo–U
142.	*ro2a*	rough 2a	2–230·0	Eyes rough, slightly small in size	C–37a
143.	*ro2b*	rough 2b	2–210·0	Eyes small, rough	K–U
144.	*ro2c*	rough 2c	2–231·0	Eyes slightly rough	C–40
145.	*ro5a*	rough 5a	5–145·0	Eyes rough, slightly smaller	C–37a
146.	*rr*	retracted	2–243·3	Wings short and broad	C–37a
147.	*rs*	rose	3–40·5	Purplish, pink eye color; males sterile	C–U
148.	*rt*	russet	2–162·0	Dark eye color	C–35
149.	*ru*	ruffled	5–44·2	Dorso-central bristles and hairs are curled	MMM–23
150.	*s*	short	1–114·0	L5 is obliterated in distal part	MMM–23
151.	*S-1* (S_1)	—	2–?	Increases rate of somatic reversions of mt^3 and mt^5	D–29a
152.	*s-2*	—	5–?	Same as *S-1*	D–29a
153.	*S-3*	—	4 or 6–?	Same as *S-1*	D–29a
154.	*S-4*	—	3 or 5–?	Same as *S-1*	D–41
155.	*sa*	small wings	4–49·3	Wings about 80% normal size	C–36
156.	*sal*	salmon	3–19·6	Reddish eye color	C–37
157.	*Sb*	Stubble	5–44·5	Bristles cut off; lethal when homozygous	C–39
158.	*sb*	small bristles	1–131·5	Bristles and hairs small	Me–U
159.	*Sc*	Scutellar	4–27·0	Bristles, esp. scutellar, are missing	K–U
160.	*sc*	scute	1–3·8	Scutellar bristles are missing	Me–U
161.	*se*	sepia	1–0·1	Dark eye color	MMM–23
162.	*sh*	shaggy	5–62·5	Hairs on abdomen and tarsus are irregularly directed	C–29
163.	*si*	singed	1–50·0	All bristles and hairs twisted and gnarled	MMM–23
164.	*sk*	spike	4–8·2	Bristles short and stubby	C–37a
165.	*sl*	slender	2–183·5	Minute bristles	K–U; C–39

TABLE III—(*continued*)

	Symbol	Name	Linkage group–locus	Description	Reference
166.	*sl-b*	slender-b	2–0·0	Similar to *sl*	C–41
167.	*sm*	semiplexus	5–174·0	Extra veins	C–29
168.	*sp*	spread	3–106·5	Spread wings	MMM–23
169.	*sq*	squat	4–20·3	Small rounded wings; sterile homozygous	C–36
170.	*ss*	spineless	4–40·0	All bristles short	C–37
171.	*St*	Star	4–84·0	Small rough eyes; homozygous lethal	K–U
172.	*st*	scarlet	5–67·5	Vermilion-like eye	C–29
173.	*sv*	short veins	3–24·5	All veins except L1 are short	R–U
174.	*Sv-5*	Short vein 5th	5–127·5	L5 short	R–U
175.	*sw*	straw	5–147·0	Yellowish body color	C–U
176.	*sy*	stubby	6–0·8	Stubble-like bristles	CK–33
177.	*T*	Triangle	1–104·5	Veins thickened and knotted	MMM–23
178.	*t*	telescoped	3–57·5	Shortened thorax; small eyes	MMM–23
179.	*tb*	tiny bristles	3–104·0	Minute bristles	C–U
180.	*v*	vermilion	1–25·5	Bright eye color	MMM–23
181.	*va*	varnished	2–231·5	Small rough eye with reddish color	C–39
182.	*vg*	vestigial	5–131·0	Wings and balancers reduced to vestiges	C–U
183.	*ve*	veinlet	4–81·7	L2, 4 and 5 wing veins short	C–36
184.	*vl*	vestigial-like	4–116·0?	Rudimentary wings	C–29
185.	*vs*	vesiculated	1–27·0	Vesicles in wings	MMM–23
186.	*w*	white	1–105·0	White eye color	W–U
187.	*we*	wee	1–72·0	Wings thin, short and narrow	C–40
188.	*y*	yellow	1–2·9	Yellow body color; hairs and bristles gray	MMM–23
189.	y^{re-a}	reddish-alpha	—	Unstable; reverts to normal in heterozygous females. Body and hairs and bristles more yellowish than *y*	D–28b D–41

190.	y^{re-1}	reddish–1	—	Stable; color like y^{re-a}	D–28b D–41
191.	*Yh*	Yellowish	4–115·0	Pale yellowish body color; tip of bristles colorless; lethal homozygous	C–40

[a] Based on mutant list which appeared in *Drosophila Information Service* **2,** 1934, pages 37–43. Additional mutants appeared in *Drosophila Information Service* **3, 5, 7, 8, 11, 13,** and **14.** These references appear under Chino, 1935–1941. *References:* C29–C41b: Chino (1929 through 1941); D26–D41: Demerec (1926 through 1941); CK33: Chino and Kikkawa (1933); L32–L39: Lebedeff (1932 through 1939); MMM23: Metz *et al.* (1923); N–42: Newby (1942); S42: Stone (1942); C–U: Chino (unpublished observations); D–U: Demerec (unpublished observations); F–U: Farrow (unpublished observations); K–U: Kikkawa (unpublished observations); L–U: Lebedeff (unpublished observations); Me–U: Metz (unpublished observations); Mo–U: Moses (unpublished observations); R–U: Raleigh (unpublished observations); W–U: Weinstein (unpublished observations).

III. Gene Variation

A. Visible Mutations

The number of genes in *D. virilis* was estimated as between eight and 25 thousand but closer to eight thousand by Komai and Takaku (1949). This estimate is similar to the ten thousand genes estimated for *D. melanogaster* by Muller (1947). The visible mutations reported for *virilis* include changes in nearly all morphological characteristics such as the eye color, body color, bristles, wing and body size and shape as reported for *melanogaster* (Bridges and Brehme, 1944; Lindsley and Grell, 1968). The first extensive list of mutations for *D. virilis* was compiled by Chino, Kikkawa, and Lebedeff (1934) for the Drosophila Information Service. The list included the name of the mutation, the symbol, the linkage group and location of each mutation, the date of discovery, and any references to the published data concerning the mutation. A description of the mutation and the designation, as RK1, 2, 3, 4, or 5, were also included in this list. The list included the published data from the following authors: Chino (1929, 1930), Chino and Kikkawa (1933), Demerec (1926, 1927, 1928a, b, 1929a, b, 1930), Lebedeff (1932, 1933, 1934a), and Metz *et al.* (1923). The literature containing information for the described mutations of *virilis* is difficult to obtain and many of the mutant stocks have been lost. A list of the mutations of *D. virilis* was prepared and appears in Table III. This list is based on data which appeared in DIS No. 2 (1934) and subsequent issues. Additional mutations were added from the published results of Chino (1936a, b, 1937). The symbol and name were taken from DIS No. 2 (1934) but most of the linkage groups and locus designation were based upon the results of Chino (1935, 1936a, b, 1937a, b, 1940, 1941a, b).

The extensive list of mutations indicates the genetic variation in *virilis*. Some of the mutations were recovered from natural populations, some from laboratory populations and some were induced by X-radiation (see references of Chino and Metz *et al.*).

The natural populations of *virilis*, especially those in North America, are found in domestic habitats in fairly small population sizes. Visible and lethal mutations in six different populations were tested by Patterson *et al.* (1942). They report approximately two recessive visible mutations from each tested individual. The recessive lethals were not as frequent with approximately one-half the tested individuals carrying a lethal. The average number of visible mutations per tested individual agrees with the genetic variability measured as visible mutations in two other species of the virilis group, *D. americana* and *D. texana* (Alexander, 1952). From the

data of Patterson *et al.* (1942), the *virilis* populations appear to be similar to the *americana* and *texana* population structure in that all three species show widespread "species-wide" mutations in a large number of the different populations tested and "population-specific" ones which are recovered from only one or a few populations.

B. Genetic Isolating Factors

The most important types of genetic variability in natural populations are those which function to isolate the members of that population from other populations or other species. These appear to be more important in some species than others. They are important in *virilis* as compared to species which exhibit chromosome polymorphism such as *D. pseudoobscura* and *D. willistoni* (Dobzhansky, 1950; Da Cunha *et al.*, 1953). Genetic isolating factors are not the only important factors for preventing gene exchange between any two forms but the present discussion will be limited to this type of isolation because of its importance in *D. virilis*, where chromosome polymorphism has not been detected. Other ecological and genetic isolating mechanisms have been discussed by Patterson and Stone (1952).

In general, genetic isolating factors can be measured by mating geographical strains of one species to a closely related one and measuring the isolation by the fertility of the crosses, the number of F_1 offspring produced and the fertility of the F_1 and F_2 offspring. Spencer (1938) was the first to test for sexual isolation among members of the *virilis* group. These experiments detected the success of crosses using *virilis* females in crosses to *americana* males. Patterson *et al.* (1942) showed that the sperm become non-functional in crosses of *virilis* males to *americana* females. Patterson (1954) crossed four geographic strains of *virilis* to other members of the *virilis* group and found varying amounts of isolation. He concluded that many of the isolating factors are recessive, others were dominant, and that some factors produce an increased vigor when chromosomes from closely related species were heterozygous. There is also a gene-cytoplasm interaction which is sometimes important. The chromosomes of one species and the egg cytoplasm of another species may result in a high percent of sterility (isolation) whereas, other gene combinations are independent or counter the effect of the cytoplasm.

Several members of the *virilis* species group show Y-autosome complementary fertility factors. In males certain autosomes of the hybrid must be from the same species as the Y chromosomes for the males to be fertile. In *virilis* species hybrids the Y and chromosome 5 must be *virilis* for the males to be fertile (Alexander *et al.*, 1952). In hybrids involving *texana* and *americana* the Y chromosome requires the 2–3 fusion and the chromo-

some 5 from the same species, at least heterozygous, for the hybrid males to be fertile (Patterson *et al.*, 1940).

IV. Gene Homologies

A comparison of similar genes in different species offers a method for studying evolutionary changes in *Drosophila* populations. Such comparisons are much more precise when the species are closely related and will produce offspring when crossed. Comparisons of the chromosomes (or elements) and genes are more difficult in species which are intersterile. Then, one of the few possible methods is by a comparison of the types of gene mutations present in each of the chromosomal elements.

The difficulties encountered in this type of study have been discussed by Sturtevant and Novitski (1941) and Patterson and Stone (1952). These difficulties include mimic mutations at different loci. For example, the *cardinal* gene of *virilis* (chromosome 4) and the sex-linked vermilion gene of *melanogaster* are both concerned with the biosynthesis of the brown eye pigments and both will develop non-autonomously. Despite their phenotypic similarity they are not homologous. The cardinal gene of *virilis* has no known counterpart in *melanogaster*.

Comparisons of the mutations on the various chromosomes of *virilis* with those of *melanogaster* are given in Table IV. These comparisons are based on a system which was suggested by Muller (1940) and applied to a number of species by Sturtevant and Novitski (1941). The recognizable elements of *melanogaster* and *virilis* are lettered. The *melanogaster* sequence is used as the basic one for the comparisons to *virilis*. The X chromosome of *melanogaster* is Element A; 2L, B; 2R, C; 3L, D; 3R, E; 4, F. The correspondence of the *virilis* chromosome to particular elements is based on the system used by Chino (1936b). The X chromosome of *virilis* corresponds to Element A, the 4 to B, 5 to C, 3 to D, 2 to E, and 6 to F.

The gene homologies are based on Metz *et al.* (1923), Chino (1929, 1936, 1941a) and Sturtevant and Novitski (1941). The loci for the *melanogaster* mutations are based upon the revised volume of mutations of *D. melanogaster* by Lindsley and Grell (1967).

A. Element A

The X chromosomes of the two species show convincing parallel mutations in the cases of yellow, forked, singed, glazed (with lozenge), crossveinless, scute, white, Notch, miniature, dusky, rudimentary, Beadex and bobbed. The homologies of vermilion in *melanogaster*, *virilis*, *simulans* and *pseudoobscura* were established by eye disk transplants (Howland *et al.*, 1937).

The additional comparisons of echinus of *virilis* with echinus of *melanogaster*, magenta with ruby, vesiculated with vesiculated, decline with wavy, ragged with cut, apricot and garnet, and small bristles with tiny were suggested by Chino (1941a). These latter comparisons were considered doubtful homologies but served as corroborative evidence for the similarity of the X chromosome for the two species suggested by Sturtevant and Novitski (1941).

B. Element B

The similarity of chromosome 2L of *melanogaster* with chromosome 4 of *virilis* is based upon the similarity of dachsous, Star and reduced in the two species. Clipped of *virilis* is considered to be one of the truncate alleles of dumpy (Chino, 1941).

The comparisons by Chino of rough-4a of *virilis* with roughish of *melanogaster*, black with black, Squat with Squat, and flipper with pupal are considered more doubtful (Sturtevant and Novitski, 1941). There is also some question as to whether veinlet of *virilis* is homologous to veinlet of *melanogaster*. Plexus of *virilis* could correspond to either net of 2L or of plexus on 2R of *melanogaster*. Lanceolate of *virilis* has no comparable mutant in the 2L arm of *melanogaster* but is similar to lanceolate in the 2R arm.

C. Element C

Eosinoid (5) of *virilis* appears to be similar to brown (2R) of *melanogaster* in that a white eye color results when eosinoid is combined with either scarlet or cinnabar (Mori, 1937). The vestigial and straw mutations also appear to support the homologies of the *virilis* 5 chromosome with 2R of *melanogaster* (Chino, 1941). The scarlet gene of the 5 chromosome of *virilis* was suggested as being the same as cinnabar on 2R of *melanogaster* by Sturtevant and Novitski (1941) and was later shown to be autonomous by eye disk transplantation and therefore falls into the scarlet-cardinal group (Price, 1949a). The remaining suggestions of Chino (1941) that ruffled of *virilis* is homologous to intwined (3L of *melanogaster*), fat to fat (2L), dachsoid with four-jointed (2L), mahogony with clot (2L) and with sepia (3L), Beaded with Beaded (3R) and morula with morula (2R) have been questioned by Sturtevant and Novitski (1941). Branched might be the same as either net (2L) or plexus (2R) of *melanogaster*.

D. Element D

The best similarities of mutations to indicate the homology of chromosome

3 of *virilis* with 3L of *melanogaster* are short vein of *virilis* with veinlet of *melanogaster* (Chino, 1941); hunch with ascute (Metz *et al.*, 1923) and the dominant dark eye colors, Garnet of *virilis* with Henna of *melanogaster* (Sturtevant and Novitski, 1941). The suggested homology of cinnabar of

TABLE IV. Gene Homologies of *Drosophila melanogaster* and *Drosophila virilis*.[a]

Element A

melanogaster (X Chromosome) Mutant	Locus	*virilis* (X Chromosome) Mutant	Locus
yellow	0·0	yellow	2·9
scute	0·0	scute	3·8
white	1·5	white	105·0
Notch	3·0	Notch	102·9
echinus	5·5	echinus	8·7
ruby	7·5	magenta	83·5
crossveinless	13·7	crossveinless	25·0
vesiculated	16·3	vesiculated	27·0
cut	20·0	ragged	160·0
singed	21·0	singed	50·0
lozenge	27·7	glazed, rugose	127·0
vermilion	33·0	vermilion	25·5
miniature	36·1	miniature	78·0
dusky	36·2	dusky	78·1
wavy	40·7	decline	34·4
garnet	44·4	apricot	136·0
tiny	44·5	small bristles	131·5
rudimentary	54·5	rudimentary	122·6
forked	56·7	forked	89·0
Beadex	59·4	Beadex	94·5
bobbed	66·0	bobbed	170·5

Element B

melanogaster (Chromosome 2L) Mutant	Locus	*virilis* (Chromosome 4) Mutant	Locus
net	0·0	plexus	0·0
dachsous	0·3	dachsous	173·0
Star	1·3	Star	84·0
dumpy	13·0	Clipped	68·5
black	48·5	black	195·0
reduced	51·2	reduced	176·5

TABLE IV—(*continued*)

Element C

melanogaster (Chromosome 2R) Mutant	Locus	*virilis* (Chromosome 5) Mutant	Locus
straw	55·1	straw	147·0
cinnabar	57·5	scarlet	67·5
vestigial	67·0	vestigial	131·0
plexus	100·5	branched	141·0
brown	104·5	eosinoid	91·4

Element D

melanogaster (Chromosome 3L) Mutant	Locus	*virilis* (Chromosome 3) Mutant	Locus
veinlet	0·2	short veins	24·5
Henna	23·0	Garnet	8·0
scarlet	44·0	cinnabar	9·5
ascute	46·0	hunch	31·0

Element E

melanogaster (Chromosome 3R) Mutant	Locus	*virilis* (Chromosome 2) Mutant	Locus
glass	63·1	varnished	231·5
Delta	66·2	Confluent, Delta	45·0
crumpled	93·0	concave	214·0
claret	100·7	brick	248·0

Element F

melanogaster (Chromosome 4) Mutant	Locus	*virilis* (Chromosome 6) Mutant	Locus
abdomen rotatum	0·0	abdomen rotatum	0·3
shaven	0·1	stubby	0·8

[a] Based on the data of Sturtevant and Novitski (1941) and Chino (1941).

virilis (3) with scarlet of *melanogaster* (3L) by Sturtevant and Novitski (1941) was strengthened by the results of Price (1949a) with eye disk transplantations. The rose gene of *virilis* (3) was compared with rose of *melanogaster* (3L), spread with dihedral, and rolled with rolled by Chino, but these possible homologies were considered doubtful by Sturtevant and Novitski.

E. Element E

The possible gene homologies suggest that chromosome 2 of *virilis* is similar to 3R of *melanogaster*. Confluent (2) of *virilis* is considered homologous to Delta (3R) of *melanogaster* by Chino (1941). Concave of *virilis* appears to be similar to crumpled (3R) of *melanogaster* (Metz *et al.*, 1923) and varnished similar to glass (Sturtevant and Novitski, 1941). Other genes which are in the corresponding element of both species are ebony of *virilis* and ebony of *melanogaster*, and brick of *virilis* and claret of *melanogaster*. Broken may correspond to crossveinless-b or crossveinless-c (3R) of *virilis* (Chino, 1941). These latter genes are not considered as good genes for determining homologies but do appear in the correct elements (Sturtevant and Novitski, 1941).

F. Element F

The dot chromosomes of both *virilis* and *melanogaster* (4) appear to be homologous. The best comparisons are abdomen rotatum in both species and stubby of *virilis* to shaven of *melanogaster*. The homology of Gap of *virilis* with cubitus interruptus of *melanogaster* is doubtful.

V. Genetic Control of Sex Determination and Differentiation

The study of Bridges (1932) of *Drosophila* lead to the conclusion that sex is determined by a quantitative balance between female determining factors on the X chromosome and male determining factors on the autosomes. Several studies with *D. virilis* offered the possibilities of extending this analysis to the determination of sexual differentiation by specific genes.

There have been several mutants reported for *D. virilis* which disrupt the normal development of sexual characteristics. These factors differ from the genetic balance systems of male and female determining factors in that they are inherited as single factors (or several closely linked factors) and can be suppressed by specific genes. One intersex mutation, *ix*^{m}, is an autosomal recessive located on the third chromosome and its characteristics were reported in several papers by Lebedeff (1934b, 1938, 1939).

When homozygous, ix^m/ix^m led to a sterile intersex phenotype in genetic females (XX:2A) but showed no effect in males (XY:2A). The intersex condition varied from female-like individuals with undifferentiated ovaries to male-like individuals with underdeveloped testes. Between these two extreme expressions were a number of hermaphroditic individuals with some portions of both the male and female reproductive systems. Variations in expression of the intersex condition were dependent upon modifier genes. Without such modifying genes, the genetic females developed into sterile males when the ix^m was homozygous. The presence of recessive or semi-dominant modifier genes resulted in varying degrees of hermaphroditism when various intersex lines were crossed. Several dominant suppressor genes of ix^m were also reported. One suppressor, S_3, was linked with ix^m on the third autosome but the S_1 suppressor gene was located on one of the other autosomes. The suppressor genes were effective in suppressing the male-like development when heterozygous or homozygous in genetic females. They produced no suppression of normal sexual development in genetic males.

Another mutant, Ix^B (Blanco), acts as a dominant in zygotic females to produce intersexes but does not modify zygotic males (Newby, 1942). The external characteristics produced by Ix^B in zygotic females consist of the male-like characteristics of vertical genital valves and claspers and/or the female characteristic of small ventral plates. Internally the intersexes had one or more male-like structures but over one-half also had some female-like structures. The gonads, morphologically, resembled neither the ovaries or testes except for the presence of the yellow pigment characteristic of testes. Histologically, the gonads varied with ovarian testis-like cells. The studies on the development of the intersex individuals showed that not one but two genital imaginal disks were formed. The primary disk developed into the male system. The secondary disk was formed some five days later and developed into the female system. Generally the male system was better developed than the female system. The genetic tests by Stone (1942) located the Ix^B factor or factors on chromosome 2. The intersexes were not modified by replacement of any of the other autosomes by outcrossing. The lack of modification by other genes indicated that the Ix^B factor is just one gene, but the absence of crossing over in the male prevented the exact proof of this. Price (1949b) showed, by using X-ray induced recombinations in males, that the Ix^B factor is close to brick (248·0). The recombinations were not extensive enough to determine if one or several closely linked factors produce the Ix^B effect.

Several problems concerning sex determination, that of sexual differentiation of a reproductive system and the sexual determination of the male or female system have been discussed in relation to the intersex

genes in *virilis*. Both factors, the homozygous recessive ix^m/ix^m and the heterozygous dominant Ix^B, upset the balance in the diploid XXAA-female of *virilis* and produce some degree of maleness. Neither of the intersex genes change the normal development of males (XYAA). Lebedeff (1938) and Stone (1942) both consider that the intersex gene may act either to stimulate or fail to inhibit the additional system (male system) in genetic females. Lebedeff (1938) suggests that the normal allele (Ix^m) of ix^m is an autosomal male determining gene and that the mutant form is an intersex modifying gene which is involved in sex differentiation rather than sex determination. In the case of ix^m, a number of modifier genes were observed and are considered important in the hypothesis set forth by Lebedeff. In the absence of modifier genes, the genetic females developed into sterile males when ix^m was homozygous. However, the modifier genes act as suppressor of maleness and produce some form of hermaphrodite when present. Briefly, Lebedeff considers that the gene for maleness (Ix^m) in the autosome can be balanced by a gene for femaleness (F) in the X chromosome. These factors are equal in potency in determining whether the individual will be a male or female. The balance can be pushed toward femaleness when these factors are in balance by the inhibition or suppression of the activity of the male determining Ix^m gene by modifier genes. The F (Ix^mIx^m) genotype develops into normal males since the Ix^m genes are not balanced by F factors in the X chromosome and suppression by modifier genes does not occur.

Stone (1942) considered that the intersex genes were not genes for producing hermaphroditic individuals, thus not being neomorphs as indicated by Lebedeff (1938) or as suggested by Dobzhansky and Spassky (1941) for intersex mutants of *D. pseudoobscura*. Stone considered that these genes are mutations of normal alleles of genes which control sexual differentiation. Both ix^m and Ix^B affect the 2X:2A individuals and could be either genes for maleness which have a greater effect than normal alleles or genes for femaleness which have mutated to forms which are not effective in the suppression of the differentiation of the male system. Any unbalance in the male and female genes, either by mutation or aneuploidy (2X:3A) results in mixtures of varying parts of male and female systems being produced. A control of the system by modifier genes may be possible with the ix^m gene (Lebedeff, 1938) but the absence of such modifier genes with Ix^B (Stone, 1942) does not indicate a general, overall, control by modifier genes. However, the modifier genes may serve to produce an imbalance of male and female genes in the system to produce the hermaphroditic (or intersex) condition.

One other usually accepted characteristic of intersex development was questioned by Newby (1942) after histological studies on Ix^B. The "time-

law" of Goldschmidt (1934, 1938) suggested for the first interpretation of intersexes of *Lymantria*, indicates that an individual will develop according to its chromosomal sex up to a certain point and then change to development of the other sex. The main objection to such an interpretation of Ix^B, is the presence of not one but two genital primordia. The primary imaginal disk develops the male-like characteristics and the secondary disk forms the female-like characteristics. The turning point theory generally assumes that only one disk is present and the development of the disk should first be female and later be male. Even in this case where two imaginal disks are present the primary disk forms the male characteristics and not the female characteristics as would be expected since the chromosomal sex of the organism is female.

VI. Unstable Genes

The first case of unstable genes reported for *Drosophila* was in *D. virilis* by Demerec (1926) and extensive genetic studies of four cases were reported by Demerec (1941).

Unstable genes may mutate in several directions but in most of the cases studied by Demerec (1941) in *virilis* the unstable genes were recessive and mutated to the normal (+) condition. Having reverted they were then as stable as any other wild type allele. The four cases of unstable genes were all sex-linked. They are: reddish body color (y^{re}), magenta eye color (*m*) and two cases which show miniature wings (*mt-3* and *mt-5*). Two alleles of reddish were recorded; one was stable and one was an unstable allele. At the miniature locus, some thirteen alleles were detected. Six of the eleven were unstable and the remaining seven were stable. One stable and one unstable allele of magenta (*m*) were recovered.

The *re-a* allele (y^{re-a}) (reddish) showed a restricted period of instability (Demerec, 1928b). The instability of this gene was restricted to the maturation divisions of females heterozygous for *re-a* and y^+, *y* or y^{re-1}. The *re-a* allele was otherwise stable in heterozygous females. It was stable when homozygous in females and in males. The miniature genes which were unstable (the *mt-3* and *mt-5*) were phenotypically different but each appeared in three well-defined forms or sub-alleles. The miniature-alpha (*mt-a*) alleles were unstable in both germinal and somatic tissue; the miniature-beta (*mt-b*) alleles were stable in both types of tissues and the miniature-gamma (*mt-c*) genes were unstable only in somatic cells and stable in germinal tissue. The three forms also differed as to type of offspring produced. The *mt-a* gene produced miniature, mosaic and normal offspring. The *mt-b* form produced only miniature offspring and the *mt-c* allele produced both miniature and mosaic offspring. These miniature genes

appeared to be stable during the first eight cleavage divisions and probably during the early stages of somatic and germinal development. They become unstable only in the later stages of development.

The rates of reversions of the unstable loci were unaffected by such environmental agents as a 10°C variation in temperature, exposure to carbon dioxide or to X-rays (Demerec, 1932a). Particular factors were effective in changing the mutation rates for the various unstable genes. Reddish is unstable only in females, younger females showing a higher percent of reversions than older ones. For the *mt-c* allele the rate of reversions is twice as high in males than in females (Demerec, 1932b). Besides such sex differences, other genes may also influence the mutability rate (Demerec, 1928a, 1928b). Five autosomal genes increased the rate of reversion of miniature. The gene *M* only affected the mutability of the *mt-a* allele and increased reversions in germinal tissue but not in somatic tissue. The *M* gene is probably on the 6th chromosome. Four autosomal genes increased the reversions of both *mt-a* and *mt-c* in somatic cells. These were effective enough to increase the percentage of mosaic spots from 4% to 95% when one of the factors was present. Three of the factors were dominant. *S-1* was located on the second chromosome, *S-3* on either the third or fifth chromosome. A third gene, *s-2*, was recessive and was located on the fifth chromosome. The *S-4* factor appears to result in earlier mutability since larger mosaic areas were observed than with *S-1*, *s-2*, or *S-3*.

VII. Chromosome Variation and Gene Rearrangements

Salivary gland polytene chromosome maps of *D. virilis* have been published by Fujii (1936, 1942), Hughes (1939), Griffen (Patterson *et al.*, 1940) and Hsu (Figure 68, in Patterson and Stone, 1952). Fujii (1942) indicated the approximate cytological position of some genes and induced chromosomal rearrangements. Spontaneous inversions have not been reported for *virilis* but a spontaneous fusion of chromosomes 3 and 5 was reported by Chino and Kikkawa (1933). A cytological study of the various members of the subspecies group indicates that a number of fusions and inversions have occurred in the evolutionary history of the species. The gene orders in *virilis* and *americana* were compared by Hughes (1939) and comparisons of the gene order in *virilis*, *americana*, *texana*, and *novamexicana* were reported by Patterson *et al.* (1940, 1942). Warters (1944) studied the gene order in a number of strains in all these species. Hsu (1952) reported the rearrangements in gene order in the five elements in the various members of the *virilis* species group. These studies show that a number of different

gene arrangements have become fixed, as homozygous rearrangements, in the evolutionary history of the various members of this group. In addition, several fusions of chromosomal elements have been detected in the chromosome phylogeny (Patterson and Stone, 1952). The primitive gene order and metaphase configuration of five rods and one dot are found in *D. virilis*. A fusion of the second and third chromosomes appears in *texana* whereas the subspecies *americana* shows this fusion and an additional fusion between the X and fourth chromosome. None of the *montana*-like forms of this species group show either of these fusions but do have a J-shaped second chromosome resulting from a pericentric inversion. *D. littoralis* has a fusion between the third and fourth chromosomes.

D. virilis is chromosomally a monomorphic species, but the evolutionary history of the group shows the importance of chromosome variation in the formation of different species and subspecies. A detailed discussion of the numerous genetic tests and the complete cytological analyses of this group by J. T. Patterson, Wilson S. Stone and their many students and co-workers at the University of Texas Laboratory is neither possible nor proper in one section on the genetics of *virilis*. It is important that *virilis* is the most primitive living member of the group. A summary of the evolutionary history of the *virilis* species group is illustrated in Fig. 2 which appeared in the 1962 paper by Stone. The original data on the evolutionary history were presented in Patterson and Stone (1952). The *virilis* group phylogeny shown in Fig. 2 illustrates the diploid chromosome elements of the males of the species, subspecies, and necessary primitive forms. The X and the autosomes are numbered to distinguish the fusion of elements from pericentric inversions in one element. The paracentric inversions are indicated by capital letters where the inversions occurred and small letters in the descendent forms with the inversions. Italic letters appear when the inversions are heterozygous. Overlapping inversions were not distinguished from those which are not overlapping. The X chromosome of *D. ezoana* and *littoralis* contained so many inversion changes that they could not be analysed. The various inversions indicated were described by Hughes (1939); Griffen (Patterson *et al.*, 1942); Warters (1944); and Hsu (1952). According to this relationship Stone (1962) considers that *virilis* gave rise to a high evolutionary rate line in Primitive I although he also considers the possibility that the stable *virilis* line may have been produced from Primitive I. The two species differ by only one inversion in the second chromosome. The high evolutionary rate of Primitive I gave rise to several forms. The Primitive II species gave rise to the *texana–americana–novamexicana* complex. The Primitive III species may have been produced from either Primitive I or II. The species *ezoana* and *littoralis* and the several species of the *montana* group (which are not

illustrated) developed from a form similar to Primitive III. The phylogeny of the *virilis* group shows that several types of possible chromosome variations and gene rearrangements have been important in its evolutionary history.

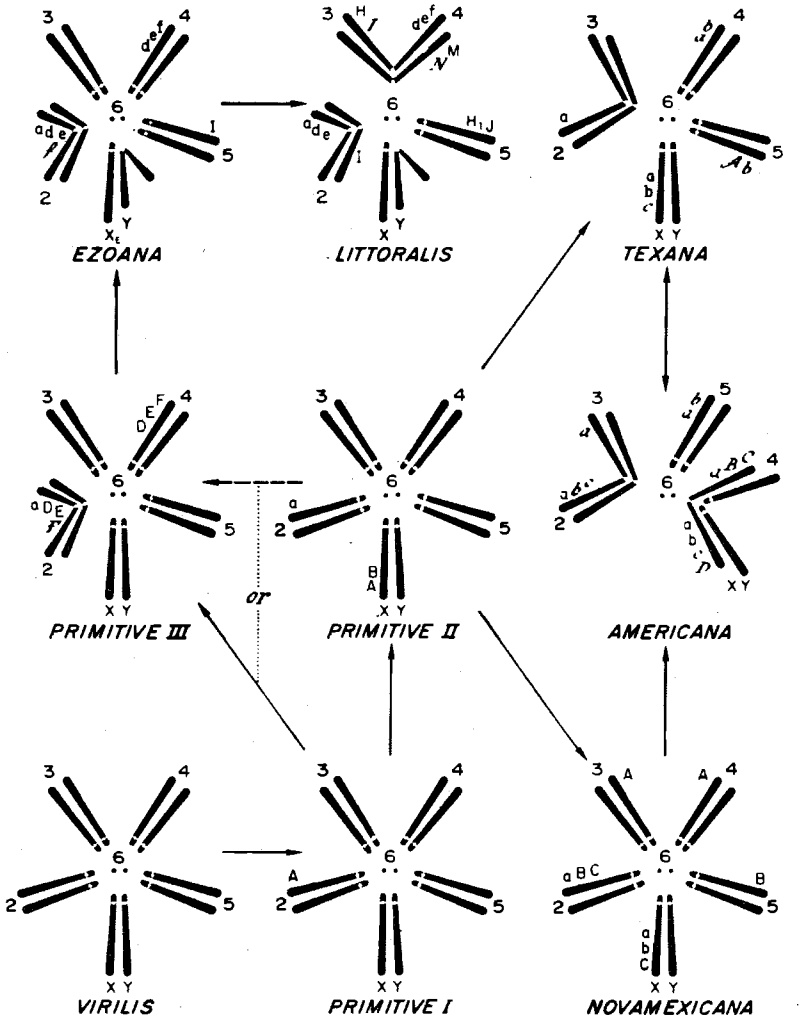

FIG. 2. The virilis group mitotic chromosome phylogeny. (From Stone, 1962.)

VIII. V-type Position Effects

Somatic variegations as the result of V-type position effects were analysed in *D. virilis* by Baker (1953) using a gene which is normally located in a heterochromatic region. The peach gene (*pe*) is located in the last four bands of heterochromatin at the base of chromosome 5—the 5 2H region. When the recessive peach mutant is heterozygous with rearrangements involving this heterochromatic region of chromosome 5, the eye color appears as a mosaic of peach and normal eye colors. The mottling patterns appear as patches of peach or normal pigment on the complementary background; mottling of different colored pigments in a salt and pepper pattern was not observed. The modification of the pigment distribution in the eye was recessive; variegation was produced when the chromosome rearrangement was heterozygous with the recessive peach mutant or when the rearrangement was homozygous.

Thirty-two X-ray-induced rearrangements were analysed by Baker and all showed at least one break in the basal heterochromatic region of the fifth chromosome. Among the rearrangements, variegations were produced when the rearrangements placed foreign heterochromatin either distal or proximal to the peach locus. Foreign heterochromatin originating from either the Y chromosome or the basal portion of the fourth chromosome produced variegation. Variegation also resulted when euchromatic regions of chromosomes were placed either proximal or distal to peach. Baker (1953) concluded that the appearance of variegation is dependent upon the movement of either euchromatic or heterochromatic regions near the peach locus and upon the interruption in the continuity of the heterochromatin.

Foreign heterochromatic regions are effective in producing variegation when they are involved in chromosome breakage and moved in close juxtaposition to the peach locus. Extra heterochromatin, such as an extra Y chromosome, does not enhance the variegation (Baker, 1953). The lack of enhancement with extra heterochromatic regions (extra Y chromosome) was also observed for variegation of genes normally euchromatically located (e.g. yellow-mottling; Girvin, 1949).

The basal heterochromatin region of the fifth chromosome occupies only a small region of the salivary gland chromosomes but in the metaphase ganglion at least two-fifths of the total chromosome length appears as a heterochromatic region (Baker, 1954). Baker studied the relative positions of the breakage points in the heterochromatin in ganglion metaphase chromosomes. A break in the long heterochromatic region can result in variegation even though it is not physically close to the peach locus. The important requirement appears to be a break in the heterochromatin

region which functions as a unit. Such breaks disrupt the continuity of the heterochromatin and result in the variegation.

Parental genotype affects the variegation of the offspring even though the components of the parental genotype are not transmitted to the offspring (Spofford, 1959). A number of various types of one-generation parental effects have been described by Spofford (1959, 1961), Baker and Spofford (1959), and Hessler (1961) for *melanogaster*. In *virilis*, Schneider (1962) tested the maternal effects of parents, homozygous or heterozygous, for a series of six translocations which produce variegated position effects. The parental genotype had an influence on the amount of pigmentation which appeared in the heterozygous variegated offspring. The drosopterins and other pteridines, some of which are intermediates in the formation of the red pigments, were measured quantitatively in the eyes and also in the testis sheath of males. The heterozygous variegated offspring were consistently more highly pigmented when the arrangement, *R* (*pe*+), was transmitted by a homozygous female parent than when transmitted by a heterozygous female or by a heterozygous male. Homozygous males produced offspring with more pigment than heterozygous males. There is also in some cases a suppression of pigmentation in offspring from mothers carrying a Y-5 translocation although the offspring themselves did not receive the Y chromosome. Non-pigment pteridines show no consistent pattern as far as the parental source of translocations are concerned.

IX. Crossing Over and Disjunction

A. Crossing Over

The first crossover studies in *D. virilis* were reported by Weinstein (1922) using the sex-linked genes of sepia, crossveinless, forked, Triangle, and rugose. In these recombination tests the coincidence of double exchanges varied from 0·68 to 0·89 with the average distance between exchange points from 12·7 to 65·9 crossover units. From these tests, and previous data, the most frequent distance between exchange points in double crossovers was 47; this estimate agrees with that of 46 calculated for *D. melanogaster*. Additional gene loci for crossover tests with the X-chromosome of *virilis* were used by Jennings (1923) and Kikkawa (1932a). They concluded that the strength of interference to double crossing over in *melanogaster* seems to be somewhat greater than in *virilis*. In both species, interference arises after one break has occurred and inhibits a second break for a certain distance. This inhibition then gradually disappears beyond a certain distance of the first break. Kikkawa (1932a) tested the

characteristics of interference with a series of curves based on the coincidence of crossing over with the crossover distance between genes on the X-chromosome of *virilis*. The shape of the curves and the absence of a secondary maximum or a symmetrical fall to the curve suggested that the "loop theory", which required interchanges at nodes where chromosomes overlap each other, was not adequate to explain the results observed. The synapsis of chromosomes was considered by Kikkawa (1932a) to be the important factor for the exchange between strands of different homologous chromosomes. He postulated that exchange can occur at any point on the chromosome where synapsis takes place.

The crossover studies in *virilis* contributed to the generally accepted characteristics of crossing over, such as interference, synapsis of homologous chromosomes for crossing over, an exchange of portions of strands of different homologous chromosomes, and non-crossover between sister strands of the same homologous chromosome. Other characteristics of crossing over, such as (1) the time of crossover (two-strand or four-strand stage); (2) that only two of the four strands are involved in crossing over at any one place on the chromosome and (3) that the first meiotic division is reductional and the second equational (for the centromeres), required the data obtained from the attached-X chromosome of *melanogaster* (Anderson, 1925).

An interrelationship between crossing over in the various chromosomes has been reported for *melanogaster*. The amount of crossing over has been reported to increase in one chromosome when inversions were present heterozygous in another chromosome (Steinberg, 1936). The same type of interrelationship was tested in *virilis* by Wilson (1950). Crossing over was tested in the X-chromosome in hybrids between *D. virilis* and *D. americana texana, D. a.americana* or *D. novamexicana*. Each hybrid offers a different number and types of inversions in the autosomes. An increase in the number of autosomes which contained heterozygous inversions appears to decrease the amount of crossing over in the X-chromosome rather than increase the amount of crossing over as in *melanogaster*.

Additional data for crossing over in *D. virilis* were reported by Kikkawa (1933, 1934, 1935a). In *virilis*, the males showed an almost complete reduction in crossing over as in *melanogaster*. The few cases of possible crossing over in males of *melanogaster* (Muller, 1916; Bridges and Morgan, 1919) were attributed to mutation, reversions, deficiencies, duplication or somatic crossing over. The second chromosome of *virilis* males was tested using four genes which give clear, consistent morphological characteristics. In addition, two of the genes, confluent (0·0) and incomplete (0·1), were some 111 units from the other two genes, Rounded (111·0) and varnished (128·0). The longest region is between incomplete and Rounded (Region 2) and should show the highest rate of crossing over. This was confirmed

by testing for crossing over in females (Kikkawa, 1935a). The possibilities of mutations and reversions were eliminated as explanations since the expected recombination would separate two of the genes from the other two. The recombination values in Region 2 in males were reduced from close to 50% in females to 0·0078% in males. Only three crossover individuals were observed in a sample of 38,598.

The data for crossing over in the second chromosome of males supplemented and supported the previous recombination tests between Gap and glossy on the dot-like chromosome 6 of *virilis* (Chino and Kikkawa, 1933). Tests in chromosomes 2 and 6 of *virilis* males indicated that the recombination in *virilis* was higher than in *melanogaster*. In addition to the higher rates in *virilis* males, the genetical maps of *virilis* females are longer than in *melanogaster* females for chromosomes of equal cytological size and length. The X-chromosomes of the two species are similar in cytological length, but the *virilis* X-chromosome is comprised of 2·8 times more crossover units than the X-chromosome of *melanogaster* (Chino and Kikkawa, 1933).

Several experiments on the modification of crossing over by environmental factors (or non-inherited variations) were reported for *D. virilis* females by Kikkawa (1934). Gene recombination in the distal, intermediate and proximal portion of the X-chromosome of *virilis* was tested at a temperature of 25°C and 30°C. At the higher temperature, crossover values were decreased in regions distal to the centromere and, to a lesser degree, in the middle (intermediate) region. However, recombination was increased in the region proximal to the centromere at the higher temperature. Similar results were observed for the X-chromosome with X-ray treatment (Fujii, 1933). The autosomes appear to respond the same way. The distal end of the fifth chromosome showed a reduction in crossing over with an increase in temperature and with X-ray treatment. These characteristics of recombination in *virilis* agree with those for *melanogaster* (Kikkawa, 1934).

B. Non-disjunction

The frequency of primary non-disjunction of the sex chromosomes in *virilis* differs somewhat between strains. The rate of primary non-disjunction of the X-chromosomes in *virilis* females varied from 1:3,459 (Arai, 1930) to 1:1,430 (Demerec and Farrow, 1930) to 1:912 (Kikkawa, 1932b). A rate of 1:1000 in *virilis* is considered to be the most representative for primary non-disjunction of the X-chromosome and is higher than the average rate of 1:2000 for *melanogaster* (Kikkawa, 1932b). The primary non-disjunction rate was increased to 1:174 (Arai, 1930) or 1:162 (Demerec and Farrow, 1930) after X-ray treatment. The exceptional

individuals, XXY (♀) and X0 (♂) resulting from primary non-disjunction in females were not recovered in equal numbers as expected. The XXY:X0 ratio was reported as 1:1·7 by Arai (1930), 1:11 by Demerec and Farrow (1930) and 1:15 by Kikkawa (1932b). The relative ratio of XXY:X0 individuals is distorted further by the use of X-rays. The relative number of X0 males was increased from 1:1·7 to 1:2·5 by X-rays in the strain used by Arai (1930) and from 1:11 to 1:20 by Demerec and Farrow (1930).

In contrast to the higher rate of primary non-disjunction in *virilis*, the rate of secondary non-disjunction appears to be lower in *virilis* females than in *melanogaster*. A rate of 1·3% secondary non-disjunction was reported by Weinstein (1922) but only 0·67% was reported by Demerec and Farrow (1930) and 0·53% by Kikkawa (1932b). These are lower than the 4·3% reported for *melanogaster* by Bridges (1916). The exceptional (non-disjunction) classes of XXY and X0 were approximately equal in the experiments of Weinstein (1922) and Demerec and Farrow (1930), but Kikkawa (1932b) reported an increase of the X0 exceptions with a ratio of 1:4·5 for the XXY:X0 types.

The non-disjunction studies in *D. virilis* were extended to include XXY and XXYY females and XYY and XYYY males. Kikkawa (1932b, 1935b) found that the XXY females gave a ratio of 5:3 (or 1·3:1) of XX:XXY daughters when a 1:1 ratio was expected from the *melanogaster* experiments (Bridges, 1916). Kikkawa (1932b) suggests that this results from the elimination of the Y-chromosome in the meiotic divisions. Several *virilis* strains were utilized by Kikkawa (1935b) and the number of XY and XYY males from XXY females was studied. The expected 1:1 ratio was found to be 1·3:1 for the XY:XYY males. Eggs containing an X-chromosome were much more frequent than those containing both an X and Y. Secondary non-disjunction was only 0·48%. All of the studies on secondary non-disjunction in XXY females by Kikkawa (1932b, 1935b), Weinstein (1922) and Demerec and Farrow (1930) show a ratio of 1:1·6 of exceptional female to male progeny instead of the expected 1:1 ratio.

The frequency of non-disjunction in the XXYY females was very low. The segregation was primarily XY–XY; very rarely XXY–Y or XYY–X segregations were observed. XX–YY segregation was not observed in the tests of Kikkawa (1935b). These results with *virilis* differ from the results of Stern (1929) for *melanogaster*.

In XYY males, XY–Y segregation is more frequent than X–YY in both *melanogaster* and *virilis*. In *melanogaster* the XY–Y segregation is about twice as frequent as X–YY segregation (Bridges, 1916; Stern, 1927, 1929) while in *virilis* the XY–Y segregation is about 3·8 times more frequent than X–YY. The disjunction in XYYY males was primarily of XY–YY. Only one individual in 479 resulted from the X–YYY segregation

(Kikkawa, 1935b). Baker (1956) measured non-disjunction in XYY and XYYY males of *virilis* with one or more of the Y chromosomes marked with the peach gene (Y^{pe+}) which produced somatic variegation. Cytological examination showed that the chromosomes associated as multivalent groups in metaphase I as in *melanogaster* (Cooper, 1949). The XYY and XYY^{pe+} *virilis* males showed a segregation pattern similar to that reported by Kikkawa (1935b). XY–Y segregation was the more frequent type and occurred 75–80% of the time in triploid males. The marked Y^{pe+} chromosome did not change the segregation pattern when present with the normal Y in triploid males. When both Y chromosomes were Y^{pe+}, disjunction of XY^{pe+} from Y^{pe+} occurred only with a frequency of 48%. This difference in segregation types appears to be dependent upon the presence of the two marked Y chromosomes. The presence of extra chromocentric material in the marked Y and the possibility of a closer association of these Y chromosomes than an association of the X and Y may explain the results with the triploid males. However, when tetraploid males with a complement of one normal Y and two Y^{pe+} chromosomes and an X chromosome were tested, the 2–2 segregation was random among the Y chromosomes. The two Y^{pe+} chromosomes did not show a higher rate of segregation to the same pole than the Y and Y^{pe+} chromosomes (Baker, 1956).

C. Crossing Over in Non-disjunction XXY Females

The rate of crossing over was compared in XX females and XXY females by Kikkawa (1932b). The regions *ec–cv*, *cv–sg* and *sg–mt* in the X-chromosome of *D. virilis* were tested in the normal XX female and non-disjunction XXY females. The frequency of crossing over in XXY females was not different from that in XX females. The Y-chromosome does not exert any influence upon crossing over in the X-chromosome of *virilis*. These results are similar to those reported for *melanogaster* by Anderson (1925) and Bridges and Olbrycht (1926).

X. Radiation Studies

Numerous studies of genetic damage resulting from radiation in *Drosophila*, as well as other genetic systems, have been reported since Muller's original report of the mutagenic effect of X-rays in 1927. Mandl (1964) has published an extensive review of radiation-induced genetic effects and factors which modify induced damage. Although a few of the older publications are cited, the review covers publications from 1957 to 1964. Conger (1960) reviewed some aspects of genetical protection against radiation exposures.

Reviews of earlier genetic work are included in Muller (1954) and in Chapter 26 of this volume.

In *Drosophila virilis*, translocations and dominant lethal damage have been utilized most often as measures of radiation damage. The karyotype of five rods and a dot chromosome offers a method to detect a larger number of chromosome breaks than in *melanogaster* with only the X, two autosomes and a dot chromosome. In *melanogaster*, a second break in the same chromosome may not be detected in translocation tests. Dominant lethal tests are based upon the failure of egg development. Failure of development due to genetic lethal effects or to a lack of egg fertilization by sperm can be distinguished in *virilis*. The method of detection of sperm in eggs was first used by Patterson *et al.* (1942) and a modified method was reported by Alexander (1963). This method of sperm detection in eggs is not possible in *melanogaster*.

A. Specific Loci

One of the first radiation studies using *Drosophila virilis* was reported by Girvin (1949) who tested mutation rates at seven specific gene loci on the X-chromosome. Gynandromorph and somatic mosaics were also scored. The loci of yellow (y^{40a}), crossveinless (*cv*), vermilion (*v*), singed (si^2), dusky (*dy*), white (*w*) and apricot (ap^{40a}) were used in these tests. The results are given in Table V. The mutation rate is expressed by the number of mutations per roentgen of X-radiation. For this rate, the number of mutations was divided by the total sample scored for the loci and the dose of 3047 R.

The singed locus gave the highest rate with apricot, vermilion and

TABLE V. Mutation Rates at Specific Loci on the X-Chromosome of *D. virilis*.[a]

Locus	Total sample	Total Mutations Determined	Total Mutations Including undetermined	Mutation rate $X10^{-8}$ per r
y	83,949	12	13	4·6– 5·0
cv	58,525	4	9	2·2– 5·1
v	56,525	8	12	4·6– 7·0
si	83,949	29	56	11·3–21·9
dy	83,949	9	16	3·5– 6·3
w	20,744	8	8	12·7–12·7
ap	66,525	28	38	13·8–18·7
AVERAGE				7·5–11·0

[a] Based on the data of Girvin (1949).

dusky showing the next highest rates. The yellow and crossveinless loci showed the lowest rates (Table V). Girvin (1949) reports that the mutation rates of these loci in *virilis* are higher than those of the homologous loci in *melanogaster*. These comparisons are based upon the data for *melanogaster* reported by Patterson and Muller (1930) and Patterson (1932, 1933). The rate for the white locus was approximately twice as high in *virilis* than in *melanogaster*. The two species also differed in the ratio of viable and lethal induced mutations. The data of Patterson (1932) for *melanogaster* showed only 9 viable and 45 lethal mutations at the yellow, white, crossveinless, vermilion, singed and garnet loci. This is approximately 17% viable mutations among the total. In *virilis*, 49 of 89 mutations were viable (55%). These comparisons are between homologous loci in the two species but the dosage used in the *melanogaster* experiments is not definitely stated by Patterson (1932) although Girvin (1949) states that the rates of mutations were compared with the same dose of X-rays.

If sex-linked *virilis* mutations are compared to mutations at autosomal loci in *melanogaster*, with a 3000 R dose, then *virilis* shows 55% viable sex-linked mutations and *melanogaster* only 34% viable autosomal mutations. The average rate for all seven sex-linked loci in *virilis* is $11{\cdot}0 \times 10^{-8}$/r as compared to $5{\cdot}98 \times 10^{-8}$/r for *melanogaster* (Alexander, 1954). This is based on the total number of mutations recorded. When the rates were based on only the mutations which were tested further, the *virilis* rate is $7{\cdot}5 \times 10^{-8}$/r as compared to the *melanogaster* rate of $5{\cdot}72 \times 10^{-8}$/r. These rates are not too different. However, the rates of three (*si*, *w*, *ap*) of the seven loci in *virilis* were as high or higher than the autosomal loci (Alexander, 1954; and Patterson, unpublished observations, included in Alexander, 1954). The other four loci gave rates of $2{\cdot}2$ or $4{\cdot}6 \times 10^{-8}$/r and were similar to the *melanogaster* rates. Based on the available data for comparisons, the *virilis* mutation rates appear to be higher than those in *melanogaster*. Only a comparison of homologous loci in both species treated at the same time with the same dose of irradiation will allow precise estimates of the difference or similarity in mutation rates in these two species.

B. Ionizing Radiations

A series of experiments on the modification of X-ray induced genetic damage by environmental change in *D. virilis* have been published. The response of mature sperm at 2 and 28°C temperature in air with increasing doses of X-radiation was reported by Baker (1949). A higher rate in translocation damage was observed at the lower temperature when the total dose was 2000 R or more. The temperature effect was also observed in

similar tests by Haas *et al.* (1954). The temperature effect is thought to depend upon the high solubility of oxygen, which is increased 1·6 times, at the lower temperature and possibly also by the reduced activity of other biological protective agents at the lower temperature (Baker and Edington, 1952; Baker and von Halle, 1953; Haas *et al.*, 1954).

Further tests by Haas *et al.* (1954) showed that an increase in the dose rate of X-radiation from 100r/minute to 1867r/minute also increased the translocation rate. This increase was observed when treatments were carried out in atmospheres of air, 99·5% O_2 and 96% N_2 + 4% O_2. The most significant differences were observed when X-ray treatments were done in a 96% N_2 + 4% O_2 atmosphere. The enhancement of translocation damage was observed at both the high and low temperature.

The enhancing effect of oxygen on X-radiation treatment was first tested in *virilis* by Baker and Edington (1952). With a dose of 2000 R, translocation damage increased as the concentration of oxygen was increased. The results for 100% nitrogen (0% oxygen) was 3·7%; for 95% nitrogen + 5% oxygen, 9·9%; for 79% nitrogen + 21% oxygen, 16·1%; and for 100% oxygen was 17·2%. Translocation damage increased approximately 1% for each 1% increase in oxygen up to a concentration of 21% oxygen. In mature sperm, an additional increase in oxygen from 21% to 100% oxygen only increased the translocation damage by an additional 2%.

In addition to a change in oxygen concentration, various physiological systems involved with oxygen were tested by Haas *et al.* (1954). The gases used were carbon monoxide, carbon dioxide and mixtures of these gases with oxygen. The physiological changes resulting from these gases often produced unexpected results. Carbon monoxide is known to inhibit the cytochrome oxidase system and at a high temperature and fast dose rates, higher rates of translocations were observed with X-ray treatment in a 95% carbon monoxide + 5% oxygen mixture than when a higher concentration of oxygen was present (80% carbon monoxide + 20% oxygen). With a lower temperature, similar amounts of translocation damage were seen in both gas mixtures. These results were explained by proposing that oxygen restored the protective activity of the cytochrome system at the higher temperature but not at the lower temperature (Haas *et al.*, 1954). The combination of 80% carbon monoxide + 20% oxygen did not show the usual increase in translocation damage with the faster rates of radiation. The slower rates of irradiation gave higher rates of translocations. Apparently in this particular atmosphere of gases, a slower rate of irradiation requires time for some cumulative action to affect breakage.

When 20% oxygen was combined with carbon dioxide, the translocation damage was higher than in air with a 20% oxygen concentration. The

same results were observed when 20% oxygen was combined with carbon monoxide. If carbon dioxide and carbon monoxide are used in the same mixture with oxygen, the radiation damage was reduced at most combinations of temperature and gas mixture. An increase in the activity of enzyme systems are indicated in the carbon dioxide–carbon monoxide mixture by the observation that the lowest translocation rate was observed with a fast dose rate and high temperature.

The comparisons of various physiological conditions and environmental changes were extended by use of the entire cycle of spermatogenesis. The presence of radiation sensitivity cycle in the germ cells of *D. melanogaster* was reported by Bonnier and Luning (1950). Auerbach (1954) used X-ray-induced crossing over in the males to separate the pre- and post-meiotic germ cells and establish the sensitive stage for sex-linked and autosomal recessive lethals.

The response of the germ cell cycle in *D. virilis* was reported by Alexander and Stone (1955). The characteristics and relative sensitivity of the various germ cells of *virilis* are similar to *melanogaster*. The relative rate of development of these organ systems are not the same in both species. In *melanogaster*, primary spermatocytes are present in 50 hour larvae and nearly mature sperm are present during the pupal stage—120 hours after the larvae hatch (Bodenstein, 1950). In *virilis*, primary spermatocytes are not present until the larvae are 170 hours old. Sperm bundles are formed in pupae (250 hours after the larvae hatch). Mature sperm (motile) are not present until the adults are five or six days old (Clayton, 1962). Either species may vary as to which stages of treated germ cells are tested in any particular mating period. Such variation may be produced by varying the length of each mating period, the numbers of males and females in each mating period and even the strain of the same species may differ in the rate of germ cell development. Any environmental change such as temperature and the type of foods may modify the rate of development. If the time that each germ cell stage appears in development and the relative number of each cell type is known, the type or types of germ cells represented in each mating period can be established. The characteristics of radiation damage to spermatogonia and mature sperm are the easiest to determine without mixture with other germ cell stages. Spermatogonial germ cell samples can be obtained by irradiating larvae at an age before spermatocytes are formed. Mature sperm can be sampled by limiting the mating time of treated adult males. The radiation damage in the intermediate stages such as spermatocytes and spermatids can be determined by treatment of the larva or pupae at a particular age. The number and type of germ cells present at the time of treatment can be determined from Clayton's data (1957).

In the *virilis*, translocations and dominant lethals are scored by mating each adult male (6 days old) to three females. Two of the females contain recessive marker genes on four of the autosomes (2, 3, 4, 5) for the translocation test and one normal female from a cross of two different strains for dominant lethal tests. The treated germ cells are sampled by remating the treated males every two days for eight two-day periods.

High rates of translocations were observed in postmeiotic cells. Translocations were rare from germ cells which were treated in meiotic and premeiotic stages. When the tests involve treating and sampling a number of different germ cell stages, some mixing of the various stages may occur. It is difficult to tell whether the few translocations recovered from meiotic and premeiotic mating periods are actually induced in these stages or result from mixing of a few postmeiotic cells with meiotic or premeiotic germ cells.

Translocations were not recovered in the specific loci studies in *melanogaster* when young larvae, containing only spermatogonia, were treated (Alexander, 1954, 1960). These data are not large enough to completely eliminate the possibility that translocations can be induced in premeiotic cells but the rate must be extremely low. However, when several types of germ cells are treated with radiation, there is no way to eliminate the possibility of some mixing of different germ cell types in the various mating periods. In the tests with *virilis* high rates of translocations were recovered only when spermatids were present, i.e. such as in treated pupae (Alexander and Stone, 1955).

Among postmeiotic cells, spermatids are more sensitive than mature sperm. A dose of 2000 R of X-rays (or gamma rays) is necessary to produce the same amount of genetic damage in mature sperm as 500 R produces in spermatids. There is a dose factor difference of 4 in the two types of postmeiotic cells with treatment in air (Alexander *et al.*, 1959). The same difference in sperm and spermatid sensitivity appears with translocations and dominant lethals. This increase in sensitivity in spermatids is dependent upon the amount of oxygen present at the time of treatment. The difference in sensitivity in sperm and spermatids is less in inert gases such as nitrogen and argon or in carbon monoxide (cytochrome inhibitor) than in gas mixtures containing oxygen. The genetic damage is about twice as high in spermatids than in mature sperm in the inert gases (Alexander and Stone, 1955; Chang *et al.*, 1959). In air (20% oxygen), spermatids show a translocation or dominant lethal rate some seven times higher than mature sperm (Alexander *et al.*, 1959).

Dominant lethals are recovered from treated meiotic, post- and premeiotic germ cells. At doses of 500 or 1000 R of X-radiation, in air, the highest rates of dominant lethals were recovered from young spermatids. At higher doses of 2000 R or more, the dominant lethal rates are high

in both the more mature spermatids and in meiotic cells. Dominant lethal damage in meiotic and possibly some premeiotic cells is due not only to an injury to the chromosomes (genetic lethals) but also results from the lack of egg fertilization due to the reduction in the number of mature sperm. All the dominant lethal damage in postmeiotic cells show a high percentage of eggs which contain mature sperm but fail to develop (genetic lethals). In meiotic and premeiotic cells, a proportion of the eggs which contain sperm do not develop and also a proportion do not develop because sperm are absent. With a dose of 2000 R of X-ray, in oxygen, most postmeiotic (spermatid), meiotic and premeiotic cells show 99·3 to 99·8% dominant lethals. In postmeiotic spermatids from 91·9 to 96·8% of the eggs were fertilized; however, in meiotic cells only 36·4% were fertilized and 81·3% eggs were fertilized in the spermatogonial cells (Alexander and Stone, 1955). A reduction in the mature sperm from treated meiotic stages results from the degeneration of a portion of these cells after irradiation. This produces a period of aspermy by reducing the number of mature sperm which would have been produced from these cells. An increase in the dose of irradiation decreases the number of mature sperm resulting from treated meiotic cells and increases the time period of aspermy. Cell degeneration appears to be more important at higher dose levels of X-rays (2000 R or more) than at lower levels. In the histological studies of *virilis* by Clayton (1962), there were no periods in which spermiogenesis was absent after irradiation with 1000 R of X-rays. This agrees with the histological results for *D. melanogaster* (Welshons and Russell, 1957). With a dose of 1000 R, the number of primary spermatocytes were reduced, but they were never completely absent, in the irradiated testes. With 4000 R, the number of spermatogonia was reduced within 55 hours after treatment. The spermatogonia which survived and continued to pass through spermatogenesis showed a complete absence of primary spermatocytes four days after treatment. The next stage of spermatogenesis which reflected radiation damage from the treatment of spermatogonia was the spermatogenic cyst. Spermatogenic cysts were absent from the germ cell population from day 6 to day 9 after X-ray treatment.

The high sensitivity of spermatids to oxygen is a consistent characteristic of the germ cell cycle with radiation treatment. The only quantitative tests in *virilis* on the increase of genetic damage in spermatids with increases in oxygen concentration are those of Rinehart (1963). An increase from 0% oxygen (helium) to 3% oxygen (in helium) increases the dominant lethal rate about twofold in postmeiotic germ cells if treatments are at 3–5°C. The results in premeiotic and possibly meiotic cells were more erratic, with some showing an increase in genetic damage and some showing a decrease. At the higher temperature of 23–25°C, an increase of 3% oxygen

did not increase the dominant lethal damage in postmeiotic cells. Only one stage of premeiotic cells showed an increase in dominant lethal with a 3% increase in oxygen. Capps (1961) observed the absence of an oxygen effect under the same conditions of oxygen concentration. The temperature at the time of irradiation is not stated but it is assumed that the higher temperature was used in these tests. When the results of Rinehart (1963) for spermatids are compared to the mature sperm tests at 0–5°C of Baker and Edington (1952), the increase from 0% oxygen to 3% oxygen and the increases in genetic damage agree well in the two types of cells. In spermatids, the dominant lethal damage is twice as high in 3% O_2 (at 0–5°C) and in mature sperm the translocation damage is increased from 3·7% in 0% oxygen (100% nitrogen) to 9·9% with 5% oxygen (in 95% nitrogen).

The increase in genetic damage in spermatids when oxygen is increased from 21% (air) to 100% (pure oxygen) is dependent upon the pressure of the gas at the time of irradiation. At 1 atm, an increase in genetic damage was observed when oxygen was increased from 21% to 100%. At 10 atm, air and oxygen produce the same amount of genetic damage. Exact comparisons of these increases are not available from the *virilis* data. In *melanogaster*, the rate of sex-linked recessive lethals is increased from 2·2% (air) to 4·1% (oxygen) in mature sperm and from 5·4% (air) to 12·8% (oxygen) in spermatids (Chang, 1962). The rate of genetic damage in both sperm and spermatids is twice as high in pure oxygen as compared to radiation treatments in air (21% oxygen) at 1 atm of gas pressure. Ebert *et al.* (1958) were the first to demonstrate that the gas pressure can modify the amount of induced radiation damage and that the presence of inert gases can counter the effectiveness of oxygen during irradiation. Inert gases in combination with oxygen, under certain pressures, were also found to reverse the genetic damage induced in *D. virilis* with X-rays (Chang *et al.*, 1959; Chang, 1962). The dominant lethal damage in *virilis* and sex-linked recessive lethals in *melanogaster* were used as genetic measures in these tests. One atmosphere of oxygen was sufficient to produce a maximum amount of genetic damage. An increase in oxygen from 1 to 10 atm produced only a very slight increase. However, an increase in the pressure of air (20% oxygen) from 1 to 10 doubled the genetic damage resulting from a treatment of 1000 R of X-rays. The effectiveness of 1 atm of oxygen was completely reduced or countered by the addition of 9 atm of either nitrogen, argon or methane. Genetic damage was only slightly higher than with radiation treatment in one of the inert gases (absence of oxygen). The combination of 8 atm of an inert gas with 2 atm of oxygen did not show a complete reduction in the oxygen effect. The addition of 9 atm of carbon monoxide to 1 atm of oxygen does not reduce the genetic damage as much as the other three gases but does show some reduction

in genetic damage. A more complex situation is presented by carbon monoxide since the efficiency of the cytochrome oxidase system is then reduced. Low concentrations of oxygen appear to be more effective when the cytochrome system is blocked with carbon monoxide (Kihlman, 1958; Chang *et al.*, 1959; Schmid, 1961).

Oxygen is necessary for the modification of genetic damage in the germ cell cycle with changes in temperature (Rinehart, 1963). With an anoxic atmosphere produced by helium, dominant lethal damage in *virilis* is not increased by reducing the temperature from 23–25°C to 0–5°C. With a 3% oxygen + 97% helium mixture, the lower temperature increased the dominant lethal damage to a rate approximately twice that observed at 23–25°C in most germ cell stages. Osmundson (1961a, 1961b) measured the rate of dominant lethals and translocations induced in the germ cell cycle of *virilis* at 3·5°C in one atmosphere of oxygen (99·5% + 0·5% argon). Osmundson (1961a) indicated that the rates were higher by comparing the data to those observed in air at 27°C (Alexander *et al.*, 1959). The difference in both oxygen concentration and temperature in the comparisons make it difficult to assign the modification in genetic damage to oxygen concentration or to temperature change.

Equal amounts of oxygen and nitric oxide have been reported to increase X-ray damage in approximately the same proportions in several biological systems (Howard-Flanders, 1957; Kihlman, 1958; Gray *et al.*, 1958). The equivalence of oxygen and nitric oxide in producing genetic damage was tested in *D. virilis* by Capps (1961) and Rinehart (1963). In the tests of Capps (1961) a 3% concentration of nitric oxide (in helium) was compared to a 3% oxygen concentration (in helium). This concentration of nitric oxide delayed the development of the germ cells and produced an absence of germ cells for several mating periods. The increase in genetic damage with 3% nitric oxide and the absence of an increase with an increase of 3% oxygen in these tests probably result from the absence of an oxygen effect at temperatures of 23–25°C. Rinehart (1963) observed similar results and found also that a temperature of 0–5°C does produce an increase in genetic damage with an increase of 3% oxygen. By adjusting the physiological conditions to reduce the metabolic activity of the cell with low temperatures, the oxygen concentration can be retained and can be compared to the nitric oxide concentration. Then equal frequencies of dominant lethals were produced. A mixture of both gases reverses, to some extent, the effectiveness of either gas to produce genetic damage. This reduction in effectiveness of the gases is explained as a reaction of oxygen and nitric oxide to produce nitrous or nitric acid. With irradiation of the gas mixture, nitrogen dioxide (NO_2) is formed and is not effective for producing lethal changes.

Equal concentrations of nitric oxide and oxygen give equal amounts of radiation damage under certain physiological conditions. Howard-Flanders (1957) and Howard-Flanders and Moore (1958) found that in biological systems which have water present, such as *Shigella flexneri*, the two gases were equivalent. Sparrman *et al.* (1959) found an equivalent action in barley seeds which have a minimum water content of 12%. Powers *et al.* (1959a, b) found that in dry bacterial spores, nitric oxide protects the spores against radiation but oxygen increases radiation damage and they suggested that nitric oxide inactivates long-lived free radicals with which oxygen can react to produce a toxic condition for the cell. The separation of the action of nitric oxide and oxygen was indicated in *virilis* experiments (Rinehart, 1963) by a reversal in dominant lethal damage with an immediate post-treatment in oxygen after radiation treatment in nitric oxide. Previous tests in *virilis* have not indicated an effectiveness of post-treatments for modifying genetic damage as is the case in *melanogaster*. In *virilis*, post-treatments in oxygen and carbon monoxide did not modify the genetic damage induced with X-ray treatments in oxygen (Alexander and Stone, 1955; Schmid, 1961; Alexander and Bergendahl, 1962).

C. Neutrons

Analysis of the response of germ cells to neutron exposure is based upon studies utilizing neutrons from nuclear detonations (Stone *et al.*, 1954), from the Brookhaven Reactor (Alexander, 1958a) and from a 250-keV Cockcroft-Walton Accelerator at the Biology Division, Oak Ridge National Laboratories (Alexander, 1958b, 1959). The linear increase in translocation frequency with neutron dose showed that only one hit per cell was necessary for multiple chromosome breakage. At least two breaks must be produced within a fairly short time for the productions of translocation. A linear relationship was found in mature sperm over a wide range of neutron doses from a nuclear detonation. Neutron doses in rep (roentgen equivalent physical) ranged from 94 rep to 1700 rep. Rough equivalences of rep and roentgen, based upon biological data, are 100 rep and 750 R, 500 rep and 2000 R and 1300 rep and 4000 R. The proportional increase in translocation frequency with increase in neutron dose agrees with other biological tests resulting from the same nuclear detonations. These include dominant lethal damage in *melanogaster* (Baker and Von Halle, 1954) and chromosome breakage and rearrangement in *Tradescantia* (Conger, 1954; Kirby-Smith and Swanson, 1954). Previous experiments, reviewed by Lea (1946), Catcheside (1948) and Muller (1954), gave similar results.

In the nuclear detonation tests, the males exposed to high doses of neutrons (3500 rep) were completely sterile. This sterility, which resulted

from treatment of mature sperm, was due to genetic damage rather than to cell degeneration. The lack of development was not due to the absence of sperm or to any reduction in sperm motility. Motile sperm were present both in the females fertilized by the treated males and in the eggs which failed to develop. Approximately 33 % of the exposed males were sterilized with a dose of 1000 rep. Only 15 % of males exposed to the lower dose of 510 rep of neutrons were sterile.

One other characteristic of the translocation damage observed in these neutron tests was the non-random rejoining of broken chromosome ends. According to the calculations of Lea and Catcheside (1945), Haldane and Lea (1947) and Catcheside (1948), the random attachment of broken chromosome ends would produce translocations involving four chromosomes (T_4) twice as frequently as cases of two translocations which involve two chromosomes (T_{2+2}). Neutron exposure produced 43 T_{2+2} and 32 T_4 translocation types rather than the ratio of 1 : 2 expected. X-ray treatment also produced the same disproportional ratio of the two translocation types (Baker, 1949; Stone *et al.*, 1954).

The developing germ cells responded to fast neutrons from the Brookhaven reactor in both a quantitative and qualitative way (Alexander, 1958a). Translocations were recovered from postmeiotic but not premeiotic germ cells. There was an increase in the number of translocations observed in spermatids when compared to mature sperm. Approximately twice as many translocations were produced in postmeiotic spermatids than in mature sperm exposed to the same neutron dose. The increase in translocation damage was proportional to the increase in dose in all postmeiotic germ cell stages (mating periods A–D). Dominant lethals were recovered from all stages of pre- and postmeiotic germ cells. Dominant lethals induced in postmeiotic cells sampled the first 8 days from remating mature males were classified as genetic lethals since a majority of the eggs contained sperm. In some stages of meiotic or premeiotic cells, dominant lethals, that is, absence of egg development, resulted from the lack of sperm in the egg. The lack of mature sperm from treated males appeared in the 13th and 14th days (period G) of remating. Treatment of younger, premeiotic, stages produced dominant lethals and a majority of the eggs contained motile sperm.

The increase in dominant lethal damage was proportional to doses of 500, 1200, or 2400 $\times$ 10^8 n^f/cm^2 in both postmeiotic and premeiotic germ cells. Although the response to an increase in dose is similar in all germ cell stages, the sensitivities of the various stages are different. The premeiotic stage (period H, 15–16 days) was the most resistant in that lower percentages of dominant lethals were observed than in any other stage. A dose of 2400 $\times$ 10^8 n^f/cm^2 produced approximately 40 % dominant lethal

damage in mating period H (spermatogonia). The relative sensitivities of the other stages were calculated by comparing the dose necessary to produce the same amount of lethal damage in the most resistant cells. The second most resistant cell type was in the meiotic stage (period F, 11–12 days) with a dose of 1500 $\times$ 10^8 n^f/cm^2 being required for 40% dominant lethal damage. The ratio of $\frac{2400}{1500}$ gives a relative sensitivity of 1·6. All stages of postmeiotic germ cells were more sensitive than meiotic or premeiotic germ cells. Approximately one-half the dose, 1200 $\times$ 10^8 n^f/cm^2, was necessary to produce 40% dominant lethal damage in the most mature sperm cells $\left(\frac{2400}{1200}\right)$, thus giving a sensitivity 2 times greater than premeiotic cells. Younger postmeiotic cells (spermatids) from mating periods B and C were quite similar in sensitivity; doses of 1300 or 1320 $\times$ 10^8 n^f/cm^2 produced 40% dominant lethal damage to give nearly twice the sensitivity of premeiotic cells $\left(\frac{2400}{1300}\right)$. Young spermatids (mating period D, 5th–6th day of mating) were the most sensitive cells of the entire germ cell cycle. A dose of 900 $\times$ 10^8 n^f/cm^2 was necessary for 40% dominant lethal damage $\left(\frac{2400}{900}\right)$ to give a relative sensitivity of 2·7.

The sensitivity difference of 2·7 between the most sensitive spermatids

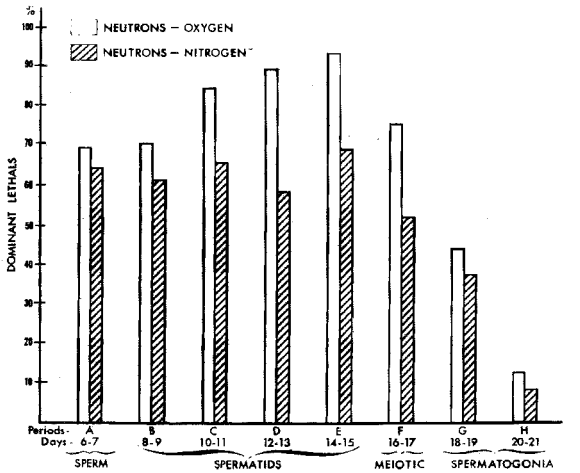

FIG. 3. Dominant lethal damage in oxygen and nitrogen with 24 meV neutrons. (From Alexander, 1959.)

and most resistant cells is based upon the number of dominant lethals induced in these cells and sampled in mature sperm. Radiation injury which produces cell degeneration in treated premeiotic cells as they enter cell division is indicated in neutron treatment as well as X-rays (Alexander and Stone, 1955). Certain mating periods, period G in these tests, show a reduction of mature sperm in males and this reduction can be detected by the absence of mature sperm in eggs from females used for these mating periods. The frequency of dominant lethals recovered from this period is reduced as a consequence of the additional cell degeneration.

The entire germ cell cycle was treated with 14 Mev neutrons in an atmosphere of nitrogen or oxygen to compare the direct and indirect effect of neutron radiation. The results for dominant lethals (Fig. 3) show that spermatids are more sensitive than mature sperm in oxygen, but not in nitrogen. Both series were treated at the same time and therefore received exactly the same dose. In these same tests the translocation studies showed a higher rate of translocation induction in all postmeiotic stages when neutron exposure was in oxygen. Treatments in nitrogen gave significant increases in translocation rates but all the rates were lower than treatments in oxygen. In both oxygen and nitrogen, twice the amount of translocation damage was produced in spermatids as in sperm treated with the same dose of neutrons. The translocation tests differed from the dominant lethal results in that nitrogen did not eliminate the higher sensitivity of spermatids for translocation damage (Alexander, 1958b).

The ion density of 14 Mev neutrons is 400 ion pairs/μ. This is considered to be too dense for the usual type of chemical reactions which result from X-ray treatment (Hollaender *et al.*, 1952; Gray *et al.*, 1953; Ehrenberg, 1954; Stone, 1956). Free radical formation is predominant for radiation with ion densities less than 200 ion pairs/μ (X-rays). The high yield of H_2 and H_2O_2 with radiation of ion densities over 200 indicates the predominance of the molecular decomposition of H_2O. Free radical formation may result from irregular spacing of radicals along the ion column and to the side tracks and ends of electronic trajectories (Allen, 1961; Ehrenberg, 1954; Ehrenberg *et al.*, 1953). The oxygen effect with 14 Mev neutrons may be due to this molecular decomposition of water or to a direct action of oxygen as indicated by P. Alexander (1957).

D. Relative Biological Efficiency of Radiations of Different Ion Densities

The translocation and dominant lethal damage induced with ionizing radiations of different ion densities have been tested in the germ cell cycle with 200 KV X-ray, 1·17–1·33 MeV gamma rays and 22 MV Betatron

X-rays (Alexander *et al.*, 1959). The roentgen units for the three radiations were converted to the relative absorbed dose (rads) of irradiation. The conversion factor of roentgen unit to rads was 0·96 rads = 1 R for X-rays or gamma-rays and 0·87 rads = 1 R for Betatron X-rays (Sinclair *et al.*, 1958). The physical values of the radiations may be expressed as ion density or Linear Energy Transfer (LET) and the calculations were based upon those of Gray (1947). According to Gray's calculations, 200 KV/ X-rays have a value of 80 ion pairs/μ or a LET value of 2·6 keV/μ; gamma rays from Cobalt-60 have a value of 11 ion pairs/μ or 0·36 keV/μ LET and 22 MeV Betatrons have a value of 8·5 ion pairs/μ or 0·28 keV/μ LET. The radiation sources were all located at the M. D. Anderson Hospital and Tumor Institute, The University of Texas at Houston and the physical source and dosimetry have been described by Sinclair and Blackwell (1958).

In the testing with 200 KV therapy X-rays and gamma rays, doses of 480, 960 and 1920 rads were used. For Betatron X-rays, doses of 435, 870, 1000, 1740 and 2000 rads were used. All treatments were in air at 27°C. The results observed for dominant lethals with X-ray treatments are given in Fig. 4. The sensitivity peak in spermatids is obvious with either 480 or 960 rads. With the highest dose, the maximum amount of lethal damage

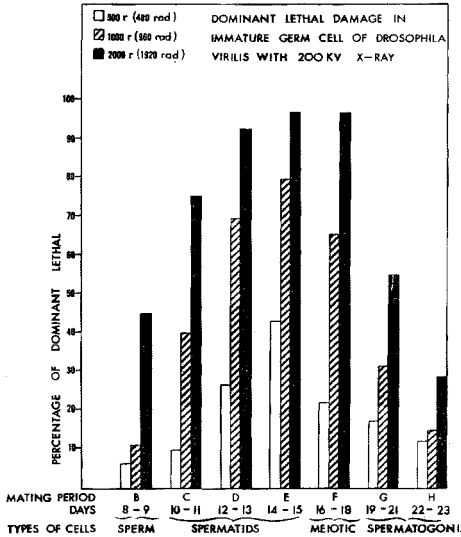

FIG. 4. Dominant lethal damage in immature germ cells of *D. virilis* with 200 KV X-rays. (From Alexander *et al.*, 1959.)

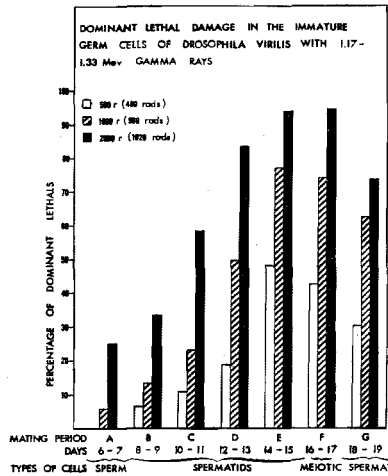

FIG. 5. Dominant lethal damage in the immature germ cells of *D. virilis* with 1·17–1·33 MeV Gamma rays. (From Alexander *et al.*, 1959.)

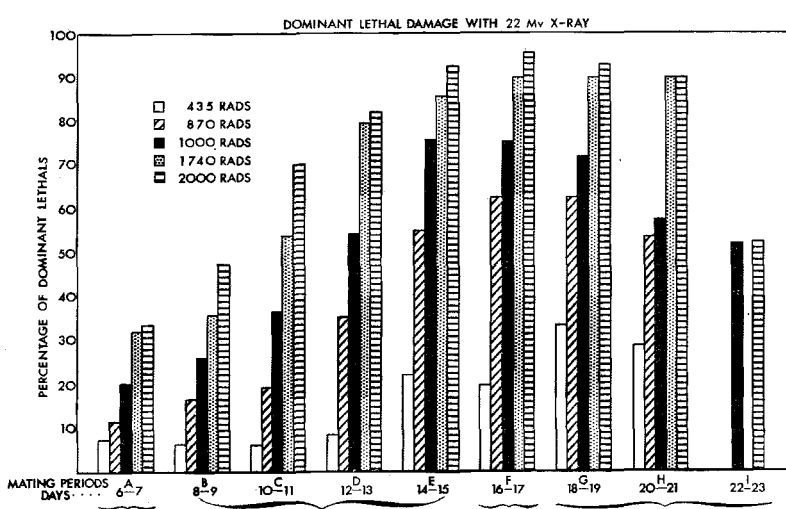

FIG. 6. Dominant lethal damage in the immature germ cells of *D. virilis* with 22 mV Betatron X-rays. (From Alexander *et al.*, 1959.)

is observed in both spermatids and spermatocytes. Comparisons of the relative sensitivity of the germ cells show a variation from the least sensitive mature sperm, to spermatogonia, to spermatocytes to the most sensitive type spermatids. The dominant lethal damage in spermatids is approximately 8 times higher than in sperm. The same doses of gamma rays produce a quite different pattern. The dominant lethal damage in meiotic and spermatogonia remains quite high as compared to the results with X-rays (Fig. 5). The relative sensitivity of the germ cells is similar to X-rays with sperm being the least sensitive followed by spermatogonia, spermatocytes and the most sensitive types being spermatid cells. The difference in the lethal damage between the most sensitive postmeiotic spermatids is 8 times higher than the most resistant sperm cells.

Betatron X-rays produce higher or equal percentages of detectable dominant lethals in meiotic and/or spermatogonial cells when compared to spermatids (Fig. 6). The lethal difference can be measured more accurately at lower doses. Sperm cells are the most resistant type of cell followed by spermatids, spermatocytes with spermatogonia being the most sensitive and producing the highest rate of dominant lethals. The dominant lethal damage in spermatids is from three to five times higher than in sperm cells. Betatron X-rays differ from both therapy X-rays and gamma-rays in that the dominant lethal damage does not decrease at all in meiotic and premeiotic germ cell stages. In fact, the dominant lethal damage increases approximately 10% in meiotic and late spermatogonial stages. If the germ cell sampling is continued until early spermatogonial cells are tested (period I) then the dominant lethal damage shows a plateau at 50% lethal damage with doses of both 1000 and 2000 rads of Betatron X-rays.

The differences in the response of post- and premeiotic germ cells with the various radiations are illustrated in Table VI by comparing the dose of each type of radiation necessary to produce 50% lethal damage in the various germ cell types. The relative biological efficiency for percent lethal damage =

$$\frac{\text{Dose in rads of 200 KV X-rays}}{\text{Dose in rads of gamma rays (or 22 MV X-rays)}}.$$

200 KV X-rays are more efficient—that is, require a lower dose—than either gamma rays or 22 MV X-rays in postmeiotic cells (periods C–D). The RBE for these two types of irradiations is approximately 0·7 as compared to X-rays in postmeiotic cells. In meiotic cells (period F) all three types of irradiations produce 50% lethal damage with approximately the same dose to give RBE values of 1·34 or 1·05. In the premeiotic spermatogonial cells, a lower dose of gamma rays or 22 MV Betatron X-rays was required for producing 50% lethal damage. In spermatogonia, the

TABLE VI. Relative Biological Efficiency of 200 KV X-ray, 1·17 to 1·33 MeV Gamma Rays and 22 MV X-ray.[a]

Type of radiation		Post meiotic C	Post meiotic D	Meiotic E	Spermatogonial F	Spermatogonial G
200 KV X-ray	Dose in rads 50% lethal damage	1250	750	570	790	1720
Gamma rays 1·17 to 1·33 MeV	Dose in rads 50% lethal damage	1720	990	510	590	770
	Relative biological efficiency	0·73	0·75	1·11	1·34	2·23
22 MV X-ray	Dose in rads 50% lethal damage	1650	1170	810	760	690
	Relative biological efficiency	0·76	0·64	0·70	1·05	2·39

[a] From Alexander (1959).

RBE values are 2·23 for gamma rays and 2·39 for 22 Mv X-rays. The postmeiotic cells responded to the irradiations in relation to their ion density. X-rays (80 ion pairs/μ) are more efficient than gamma rays (10 ions pairs/μ) or Betatron X-rays (8 ion pairs/μ). However, the amount of biological damage induced with X-rays as compared to gamma-rays or Betatron is not as great as the difference in the ion density. The ion density of X-rays is approximately ten times higher than that of gamma rays or Betatron X-rays—but an increase in the dose necessary for gamma rays to produce the same amount of lethal damage is only 0·3. The increase in ionizations with X-rays of 80 ion pairs/μ is not effective per increase in each ion pair. An ion density of 8 or 10 ion pairs/μ is sufficient to produce a particular rate of lethals, and additional hits in the cell produced by radiations of higher ion density are in excess and are often not detected. The response of premeiotic cells, as measured by the number of dominant lethals recovered, is exactly the reverse of that of postmeiotic cells. Lower rates of dominant lethals are recovered with the same dose of X-rays than with gamma rays or Betatron X-rays which have a lower ion density. The most plausible, although as yet unproven, explanation for the high rate of dominant lethals with radiations of low ion density is the difference in the efficiency of the radiations for producing cell degeneration. Radiations of higher ion density produce cell degeneration which reduce the number of cells surviving the meiotic divisions to produce mature sperm cells which carry dominant lethals.

Radiations with ion densities higher than X-rays are more efficient for producing genetic damage but the increase in biological efficiency is not as great as the increase in ion density. In mature sperm, 14 MeV neutrons, produced by a 250 KeV Cockcroft-Walton accelerator have an average of 12–14 KeV/μ which is equivalent to 400 ion pairs/μ. The ion density is approximately five times higher than the 80 ion pairs/μ for X-rays but 14 MeV neutrons are only two or three times more effective for producing dominant lethals in mature sperm than X-rays (Alexander, 1958b). Fission neutrons from the Brookhaven Reactor with ion densities of 1200 ion pairs/μ were tested (see Handloser and Delihas, 1955, for details of the dosimetry of the Brookhaven Reactor). A comparison of the radiation doses (in rads) necessary for 50% dominant lethal damage with X-rays as compared to neutrons gave the following RBE values when the doses of $\frac{\text{X-rays}}{\text{Neutrons}}$ were compared: for sperm 2880/432 = 6·6; spermatids (mating period C), 1250/410 = 3·1; spermatids (mating period D), 750/233 = 3·2; spermatids (mating period E), 570/250 = 2·3 and meiotic cells (mating period F), 790/487 = 1·6 (Alexander, 1958a). The fission neutrons have an ion density 15 times higher than X-rays but the RBE comparison of biological damage for mature sperm is only 6·6. Although the physical characteristic of the ion density of radiations is an important characteristic which determines the amount of biological damage recovered, it is not the only one. The indirect or chemical actions of radiations which lead to genetic damage also contribute to the total genetic damage. The difference in the response of various germ cells to indirect effects is reflected in the difference in the RBE values. The spermatids are much more sensitive to indirect effects than mature sperm and require a lower dose of radiations for 50% lethal damage than mature sperm. The difference in the doses necessary for 50% lethal damage in sperm and spermatids depend, in part, upon the amount of indirect effect produced by the radiation. The indirect effect is important with X-rays and the difference in dose is four times higher in mature sperm than in spermatids. The ion density of neutrons is high enough to reduce the importance of such indirect effects and with neutrons a dose only twice as high is necessary in mature sperm to produce the same amount of genetic damage as in spermatids. A comparison of the doses necessary for 50% lethal damage in spermatids with X-rays and neutrons will give a RBE value approximately one-half that for mature sperm. When the RBE values of fission neutrons and X-rays are determined, the value for mature sperm is 6·6 but only 3·1 or 3·2 for spermatids (mating period C or D).

The effectiveness of radiations with high ion density to produce cell degeneration in meiotic and premeiotic cells is again shown when the

RBE values of neutrons and X-rays are compared. The RBE for dominant lethals is only 1·6 for fission neutrons with meiotic cells but 6·6 for mature sperm. The low RBE values result from the high effectiveness of neutrons to produce cell degeneration and thus eliminate cells, carrying dominant lethals, from the mature cell population. This is obvious with both types of neutrons. A high rate of dominant lethals are recovered from postmeiotic spermatids but mating periods immediately following the spermatid cells (meiotic) show a lower rate which is very similar to the control rate for dominant lethals (Alexander, 1958b).

E. Radiation Damage in Oogenesis

A comparative study of oogenesis in four species of *Drosophila* was reported by King and Wolfsberg (1957). The species included in the study were *virilis*, *melanogaster*, *gibberosa* and *pseudoobscura*. The following time intervals were determined for *D. virilis* Japan at a temperature of 25°C. The average minimum time from fertilization to eclosion was 12 days and four days were necessary after eclosion before the females reached maturity. The average weight of mature adult females was 2·9 mg, and 11·1 μg was the average weight of mature eggs. There was an average of 17 ovarioles per ovary and each ovary contained an average of 6·5 primary oocytes which will give an average of 220 primary oocytes per mature adult female. The *virilis* females laid an average of 130 eggs per day which is more than twice the average number of eggs laid by females of any one of the other three species. When the adult females of *virilis* emerge, the most mature ovarioles in the posterior chamber of the ovary are in Stage 4 or 5 of development according to the description and designation of egg stages by King *et al.* (1956). There may be some variation in the rate of development of various stages in different strains. Dickerman (1963) reported that *D. virilis* Texmelucan 1801·1 females contained Stage 7 as the most mature type of oocyte in newly emerged flies. By seven days, females of both *virilis* strains contained mature oocytes (Stage 14).

Modifications of genetic damage by environmental changes in gases and gas pressures in Stage 7 and Stage 14 oocytes of oogenesis in *D. virilis* Texmelucan were reported by Dickerman (1963). X-ray treatments of 0–3 hour-old females were tested for Stage 7 oocytes and 5 day-old females were treated for the more mature Stage 14. All treatments were at 22–24°C and at a dose rate of approximately 300 R/minute with 250 KV X-rays. An increase in dominant lethal damage was quite definite when X-ray treatments in inert gases (helium or argon; 0% oxygen) were compared to those in air (21% oxygen). There was an increase in lethal damage from 1 to 10 atm pressure. The induced dominant lethal damage was from

2 to 4 times higher than in air. An increase in oxygen concentration from air (21 % oxygen) to pure oxygen produced an additional, but smaller, increase in genetic damage in all experiments except in Stage 7 oocytes in 10 atm pressures of the gases where the lethal damage was similar in both concentrations of oxygen.

The two stages of oocytes show an increase in dominant lethals in the presence of oxygen. The large increases in lethal damage between 0 % and 21 % oxygen and a smaller enhancement between 21 % and 100 % oxygen agree with the results for most germ cell stages of males (see previous section on "Ionizing Radiations"). Male germ cells also showed an increase in the genetic damage with an increase in gas pressure from one to ten atmospheres in air, but not oxygen. The Stage 7 oocytes gave the same results but Stage 14 oocytes showed an increase in genetic damage with an increase in gas pressure in both air and oxygen. Inert gases, argon or helium, countered the effect of oxygen to some extent but not as completely as in male germ cells. In 9 atm of argon (or helium) + 1 atm of oxygen the dominant lethal damage was reduced by 15–20 % below the 1 atm oxygen test in both Stage 7 and 14 oocytes. In male germ cells, 9 atm of inert gases reduced the oxygen effect completely and the genetic damage in the combination of 9 atm of argon with one atmosphere of oxygen was no higher than in 10 atm of the inert gas (Chang *et al.*, 1959). A lipid soluble gas, methane, was found to counter the effect of oxygen as effectively as the inert gases. This agrees with the theory of Ebert *et al.* (1958) that specific oxygen sites in the cells are lipid fractions.

The Stage 14 oocytes were more sensitive to radiation than Stage 7 in the tests. This agrees, in general, with the relative sensitivity in the two oocyte stages of females in *melanogaster* (Parker, 1959). The results for repair in Stage 7 oocytes, also appear to agree with the *melanogaster* data (Parker and Hammond, 1958). When the total dose was fractionated into two doses, there is a reduction in the dominant lethal damage when the two doses are fractionated for 15 minutes or longer. This indicates that in *virilis* oocytes, as in *melanogaster*, a number of breaks can rejoin within the 15 minutes between the first and the second dose.

References

ALEXANDER, MARY L. (1952). Gene variability in the americana–texana–novamexicana complex of the virilis group of *Drosophila*. *Univ. Texas Publ.* **5204**, 73–105.

ALEXANDER, MARY L. (1954). Mutation rates at specific autosomal loci in the mature and immature germ cells of *Drosophila melanogaster*. *Genetics* **39**, 409–428.

ALEXANDER, MARY L. (1958a). Radiation damage in the developing germ cells of *Drosophila virilis* from fast neutron treatment. *Genetics* **43**, 458–469.

ALEXANDER, MARY L. (1958b). Biological damage in developing germ cells of *Drosophila virilis* in oxygen and nitrogen with 14 Mev neutrons. *Proc. Natl. Acad. Sci. U.S.A.* **44**, 1217–1228.

ALEXANDER, MARY L. (1959). The effect of radiations of different ion density on the germ cells of *Drosophila virilis*. *In:* "Radiation Biology and Cancer", pp. 51–70. University of Texas Press, Austin, Texas.

ALEXANDER, MARY L. (1960). Radiosensitivity at specific autosomal loci in mature sperm and spermatogonial cells of *Drosophila melanogaster*. *Genetics* **45**, 1019–1022.

ALEXANDER, MARY L. (1963). The detection of sperm in eggs of *D. virilis*. *Dros. Inf. Serv.* **37**, 137.

ALEXANDER, MARY L. and BERGENDAHL, J. (1962). Biological damage in the mature sperm of *Drosophila virilis* in oxygen and nitrogen with different dose intensities of gamma rays. *Genetics* **47**, 71–84.

ALEXANDER, MARY L., LEA, R. B. and STONE, W. S. (1952). Interspecific gene variability in the virilis species group. *Univ. Texas Publ.* **5204**, 106–113.

ALEXANDER, MARY L. and STONE, WILSON S. (1955). Radiation damage in the developing germ cells of *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **41**, 1046–1057.

ALEXANDER, MARY L., BERGENDAHL, JANET and BRITTAIN, MADELEINE (1959). Biological damage in mature and immature germ cells of *Drosophila virilis* with ionizing radiations. *Genetics* **44**, 979–999.

ALEXANDER, P. (1957). Effect of oxygen on inactivation of trypsin by the direct actions of electrons and alpha-particles. *Rad. Res.* **6**, 653–660.

ALLEN, A. O. (1961). "The Radiation Chemistry of Water and Aqueous Solution", 204 pp. Van Nostrand, New York.

ANDERSON, E. G. (1925). The proportion of exceptions in the offspring of exceptional females from X-ray treatment in *Drosophila*. Michigan Acad. Sci. Arts and Letters **5**, 355–366.

ARAI, Y. (1930). The production of non-disjunction by X-rays in *Drosophila virilis*. *Jap. Jour. Genet.* **6**, 178–179.

AUERBACH, C. (1954). Sensitivity of the *Drosophila* testis to the mutagenic action of X-rays. *Z. Indukt. Abstamm. Vererblehre* **86**, 113–125.

BAKER, W. K. (1949). The production of chromosome interchanges in *Drosophila virilis*. *Genetics* **34**, 167–193.

BAKER, W. K. (1953). V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin. *Genetics* **38**, 238–344.

BAKER, W. K. (1954). The anatomy of the heterochromatin. *J. Hered.* **45**, 65–68.

BAKER, W. K. (1956). Chromosome segregation in *Drosophila virilis* males with multiple sex chromosomes. *Genetics* **41**, 907–914.

BAKER, W. K. and EDINGTON, C. W. (1952). The induction of translocations and recessive lethals in *Drosophila* under various oxygen concentrations. *Genetics* **37**, 665–677.

BAKER, W. K. and SPOFFORD, J. B. (1959). Heterochromatic control of position-effect variegation in *Drosophila*. *Univ. Texas Publ.* **5914**, 135–154.

BAKER, W. K. and VON HALLE, E. (1953). The basis of the oxygen effect on X-irradiated sperm. *Proc. Natl. Acad. Sci. U.S.A.* **39**, 125–161.

BAKER, W. K. and VON HALLE, E. (1954). The production of dominant lethals

in *Drosophila* by fast neutrons from cyclotron irradiation and nuclear detonation. *Science, N.Y.* **119,** 46–49.

BODENSTEIN, D. (1950). The postembryonic development of *Drosophila*. "Biology of Drosophila" (M. Demerec, ed.) pp. 275–367. John Wiley and Sons, New York.

BONNIER, G. and LUNING, K. G. (1950). X-ray induced dominant lethals in *Drosophila melanogaster*. *Hereditas* **36,** 445–456.

BRIDGES, C. B. (1916). Non-disjunction as proof of the chromosome theory of heredity. *Genetics* **1,** 1–52, 107–163.

BRIDGES, C. B. (1932). "Cytological and Genetic Basis of Sex." Sex and Internal Secretion, Chpt. III, pp. 55–93, Williams and Wilkins, Baltimore.

BRIDGES, C. B. and BREHME, K. S. (1944). "The Mutants of *Drosophila melanogaster*", 257 pp. Carnegie Inst. Wash. Publ. 552, Washington, D.C.

BRIDGES, C. B. and MORGAN, T. H. (1919). The second chromosome group of mutant characters. Carnegie Inst. Wash. Publ. 278, 188–197.

BRIDGES, C. B. and OLBRYCHT, T. M. (1926). The multiple stock "x-ple" and its use. *Genetics* **11,** 41–56.

CAPPS, A. S. (1961). The effects of nitric oxide on radiation damage in *Drosophila virilis* and *Drosophila melanogaster*. *Genetics* **46,** 123–127.

CATCHESIDE, D. G. (1948). Genetic effects of radiations. *In:* "Advances in Genetics" Vol. 2, pp. 271–358. Academic Press, New York, London and San Francisco.

CHANG, T. (1962). Further observations on the relation between gas pressure and X-ray damage in *Drosophila melanogaster*. *Univ. Texas Publ.* **6205,** 385–393.

CHANG, T., WILSON, F. D. and STONE, W. S. (1959). Genetic radiation damage reversal by nitrogen, methane and argon. *Proc. Natl. Acad. Sci. U.S.A.* **45,** 1397–1404.

CHINO, M. (1929). Genetic studies on the Japanese stock of *Drosophila virilis* I. *Jap. J. Genet.* **4,** 117–131.

CHINO, M. (1930). Genetic studies on the Japanese stock of *Drosophila virilis* II. *Jap. J. Genet.* **5,** 190–197.

CHINO, M. (1935). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **3,** 41.

CHINO, M. (1936a). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **5,** 22.

CHINO, M. (1936b). Genetic studies on the Japanese stock of *Drosophila virilis*. *Jap. J. Genet.* **12,** 187–210.

CHINO, M. (1937a). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **7,** 74–75.

CHINO, M. (1937b). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **8,** 61.

CHINO, M. (1939). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **11,** 32.

CHINO, M. (1940). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **13,** 62–63.

CHINO, M. (1941a). New mutants in *Drosophila virilis*. *Jap. J. Genet.* **17,** 185–206.

CHINO, M. (1941b). *Drosophila virilis*, report of new mutations. *Dros. Inf. Serv.* **14,** 44.

CHINO, M. and KIKKAWA, H. (1933). Mutants and crossing over in the dot-like chromosome of *Drosophila virilis*. *Genetics* **18,** 111–116.

CHINO, M., KIKKAWA, H. and LEBEDEFF, G. A. (1934). *D. virilis*, legends for symbols, mutants, valuation. *Dros. Inf. Serv.* **2**, 37–44.

CLAYTON, FRANCES E. (1957). Absolute and relative frequencies of spermatogenic stages at different pupal periods in *Drosophila virilis*. *J. Morphol.* **101**, 457–476.

CLAYTON, FRANCES E. (1962). Effects of X-ray irradiation in *Drosophila virilis* at different stages of spermatogenesis. *Univ. Texas Publ.* **6205**, 345–373.

CONGER, ALAN D. (1954). The relative biological effectiveness of radiation from a nuclear detonation on *Tradescantia* spores. *Science, N.Y.* **119**, 36–42.

CONGER, ALAN D. (1960). Genetical protection. *In:* "Radiation Protection and Recovery", pp. 212–241. Pergamon Press, New York.

COOPER, K. W. (1949). The cytogenetics of meiosis in Drosophila. Mitotic and meiotic autosomal chiasmata without crossing over in the male. *J. Morphol.* **84**, 81–122.

COOPER, K. W. (1950). Normal spermatogensis in Drosophila. *In:* "Biology of Drosophila" (M. Demerec, ed.), pp. 1–61. John Wiley and Sons, New York.

DA CUNHA, A. B., BURLA, H. and DOBZHANSKY, TH. (1950). Adaptive chromosomal polymorphism in *Drosophila willistoni*. *Evolution* **4**, 212–235.

DEMEREC, M. (1926). "Miniature-alpha", a second frequently mutating character in *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **12**, 687–690.

DEMEREC, M. (1927). "Magenta"—a third frequently mutating character in *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **13**, 249–253.

DEMEREC, M. (1928a). The behavior of mutable genes. *Int. Kong. Vererb. Supple-Bd.* **1**, 183–193.

DEMEREC, M. (1928b). Mutable characters of *Drosophila virilis*, I. Reddish-alpha body color. *Genetics* **13**, 359–388.

DEMEREC, M. (1929a). Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **15**, 834–838.

DEMEREC, M. (1929b). Changes in the rate of mutability of the mutable miniature gene of *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **15**, 870–876.

DEMEREC, M. (1930). A genetic factor affecting germinal mutability of miniature-alpha wing character of *Drosophila virilis*. *In:* "The Laws of Life", 45–56, Prague.

DEMEREC, M. (1932a). Effect of temperature on the rate of change of the unstable miniature-3 gamma gene of *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **18**, 430–434.

DEMEREC, M. (1932b). Rate of instability of miniature-3 gamma gene of *Drosophila virilis* in the males in the homozygous and in the heterozygous females. *Proc. Natl. Acad. Sci. U.S.A.* **18**, 656–658.

DEMEREC, M. (1941). Unstable genes in Drosophila. *Cold Spring Harb. Symp. Quant. Biol.* **9**, 145–150.

DEMEREC, M. and FARROW, J. G. (1930). Non-disjunction of the X-chromosome in *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **16**, 707–711.

DICKERMAN, R. C. (1963). The induction of dominant lethal mutations in X-irradiated *Drosophila virilis* oocytes. *Genetics* **48**, 311–319.

DOBZHANSKY, TH. (1934). Studies on hybrid sterility I. Spermatogensis in pure and hybrid *Drosophila pseudoobscura*. *Z. Zellforsch. Mikroskop. Anat.* **21**, 169–223.

DOBZHANSKY, TH. (1950). Genetics of natural populations. XIX Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. *Genetics* **35**, 288–302.

DOBZHANSKY, TH. and SPASSKY, B. (1941). Intersexes in *Drosophila pseudoobscura*. *Proc. Natl. Acad. Sci. U.S.A.* **27**, 556–562.

EBERT, M., HORNSEY, S. and HOWARD, A. (1958). Effect on radiosensitivity of inert gases. *Nature, Lond.* **181**, 613–616.

EHRENBERG, L. (1954): Radiation: Mechanism of action and dosimetry. *Acta Agr. Scand.* **4**, 365–395.

EHRENBERG, L., GUSTAFSSON, A., LUNQUIST, V. and NYBOM, N. (1953). Irradiation effects, seed soaking and oxygen pressure in barley. *Hereditas* **39**, 493–504.

FUJII, S. (1933). The effect of X-rays on the linkage of Mendelian characters in the first chromosome of *Drosophila virilis*. *Jap. J. Genet.* **8**, 137–149.

FUJII, S. (1936). Salivary chromosomes of *D. virilis*. *Cytologia* **7**, 272–275.

FUJII, S. (1942). Further studies on the salivary chromosomes of *D. virilis*. *Cytologia* **12**, 435–459.

GIRVIN, E. C. (1949). X-ray produced mutations, deletions and mosaics in *Drosophila virilis*. *Univ. Texas Publ.* **4920**, 42–62.

GOLDSCHMIDT, R. B. (1934). Lymantria. *Bibliographica Genetica* **11**, 1–186.

GOLDSCHMIDT, R. B. (1938). The time law of intersexuality. *Genetica* **20**, 1–50.

GRAY, L. H. (1947). The distribution of the ions resulting from the irradiations of living cells. *Br. J. Rad. Suppl.* **1**, 7–15.

GRAY, L. H., CONGER, A. D., EBERT, M., HORNSEY, S. and SCOTT, O. C. A. (1953). The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br. J. Rad.* **26**, 638–648.

GRAY, L. H., GREEN, F. O. and HAWES, C. A. (1958). The effect of nitric oxide on the radio-sensitivity to tumor cells. *Nature, Lond.* **182**, 952–953.

HAAS, F. L., DUDGEON, EDNA, CLAYTON, FRANCES E. and STONE, W. S. (1954). Measurement and control of some direct and indirect effects of X-radiation. *Genetics* **39**, 453–471.

HALDANE, J. B. S. and LEA, D. E. (1947). A mathematical theory of chromosomal rearrangements. *J. Genet.* **48**, 1–10.

HANDLOSER, J. S. and DELIHAS, N. (1955). Gamma-ray neutron dosimetry of the Biology Neutron facility. *Brookhaven Nat. Lab.* **386**, T-72.

HESSLER, A. Y. (1961). A study of parental modification of variegated position effects. *Genetics* **46**, 463–484.

HOLLAENDER, A., BAKER, W. K. and ANDERSON, E. H. (1951). Effect of oxygen tension and certain chemicals on the X-ray sensitivity of mutation production and survival. *Cold Spring Harb. Symp. Quant. Biol.* **16**, 315–326.

HOWARD-FLANDERS, P. (1957). Effect of nitric oxide on the radiosensitivity of bacteria. *Nature, Lond.* **180**, 1191–1192.

HOWARD-FLANDERS, P. and MOORE, D. (1958). The time interval after pulsed irradiation within which injury to bacteria can be modified by dissolved oxygen. *Rad. Res.* **9**, 422–437.

HOWLAND, R. B., GLANCY, E. A. and SONNENBLICK, B. P. (1937). Transplantation of wild type and vermilion eye disk among four species of *Drosophila*. *Genetics* **22**, 434–442.

HSU, T. C. (1952). Chromosomal variation and evolution in the virilis group of *Drosophila*. *Univ. Texas Publ.* **5204**, 35–72.

HUGHES, R. D. (1939). An analysis of the chromosomes of two subspecies *Drosophila virilis virilis* and *Drosophila virilis americana*. *Genetics* **24**, 811–834.

JENNINGS, H. S. (1923). The numerical relations in the crossingover of the genes, with a critical examination of the theory that the genes are arranged in a linear series. *Genetics* **8**, 393–456.

KIHLMAN, B. A. (1958). The effect of oxygen, nitric oxide, and respiratory inhibitors on the production of chromosome aberrations by X-rays. *Expl. Cell Res.* 639–642.

KIKKAWA, H. (1932a). Studies on the mechanism of crossing-over in *Drosophila*, with special reference to the phenomenon of interference. *Cytologia* **3**, 285–332.

KIKKAWA, H. (1932b). Contributions in the knowledge of non-disjunction of the sex chromosomes in *Drosophila*. *Cytologia* **3**, 340–349.

KIKKAWA, H. (1933). Crossing-over in the males of *Drosophila virilis*. *Proc. Imperial Academy Japan* **9**, 535–536.

KIKKAWA, H. (1934). Studies on non-inherited variation in crossing-over in *Drosophila*. *J. Genet.* **28**, 329–348.

KIKKAWA, H. (1935a). Crossing-over in the male of *Drosophila virilis*. *Cytologia* **6**, 190–194.

KIKKAWA, H. (1935b). Contributions to the knowledge on non-disjunction of the sex-chromosome in *Drosophila virilis*. II. The mode of reduction of the heterochromosomes. *Cytologia* **6**, 177–189.

KING, R. C. and WOLFSBERG, M. F. (1957). Oogensis in adult *Drosophila melanogaster*. IV. A comparison of oogensis among *Drosophila melanogaster*, *virilis*, *pseudoobscura* and *gibberosa*. *Growth* **21**, 281–285.

KING, R. C., RUBINSON, A. C. and SMITH, R. F. (1956). Oogensis in adult *Drosophila melanogaster*. *Growth* **20**, 121–157.

KIRBY-SMITH, J. S. and SWANSON, C. P. (1954). The effects of fast neutrons from a nuclear detonation on chromosome breakage in *Tradescantia*. *Science, N.Y.* **119**, 42–45.

KOMAI, T. and TAKAKU, T. (1949). A study of closely linked genes, Miniature and Dusky, in *Drosophila virilis*, with considerations of the allelism of genes. *Cytologia* **14**, 87–97.

LEA, D. E. (1946). "Actions of Radiations of Living Cells", Cambridge University Press, The Macmillan Co., New York.

LEA, D. E. and CATCHESIDE, D. G. (1945). Recessive lethals, dominant lethals and chromosome aberrations in *Drosophila*. *J. Genet.* **47**, 10.

LEBEDEFF, G. A. (1932). Interaction of ruffled and rounded genes of *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **18**, 343–349.

LEBEDEFF, G. A. (1933). Studies on factor interaction in *Drosophila virilis*. *Am. Nat.* **67**, 69–70.

LEBEDEFF, G. A. (1934a). A gene for intersexuality in *Drosophila virilis*. *Am. Nat.* **68**, 68–69.

LEBEDEFF, G. A. (1934b). Genetics of hermaphroditism in *Drosophila virilis*. *Proc. Natl. Acad. Sci. U.S.A.* **20**, 613–616.

LEBEDEFF, G. A. (1938). Intersexuality in *Drosophila virilis* and its bearing on sex determination. *Proc. Natl. Acad. Sci. U.S.A.* **24**, 165–172.

LEBEDEFF, G. A. (1939). A study of intersexuality in *Drosophila virilis*. *Genetics* **24**, 553–586.

LINDSLEY, D. L. and GRELL, E. H. (1967). "Genetic Variations of *Drosophila melanogaster*", 471 pp. Carnegie Inst. Wash. Publ. No. 627.

MANDL, A. M. (1964). The radiosensitivity of germ cells. *Biol. Rev.* **39**, 288–371.
MEIGEN, J. W. (1830). Systematische Beschreibung der bekannten europäischen zwei-flugeligen Insekten **6**, 81. Aachen and Hamm, Schulz.
METZ, C. W. (1926). Observations on spermatogensis in *Drosophila*. *Z. Zellforsch. Mikrosk. Anat.* **4**, 1–28.
METZ, C. W., MOSES, M. S. and MASON, E. D. (1923). Genetic studies on *Drosophila virilis* with considerations on the genetics of other species of Drosophila. Carnegie Inst. Wash. Publ. 328, 1–94.
MOMMA, E. (1962). Behavior of mitochondria in living cells during meiosis in *Drosophila virilis*. *Univ. Texas Publ.* **6205**, 375–383.
MORI, K. (1937). A study of development of pigment in various eye color mutants of *Drosophila*. *Jap. J. Genet.* **13**, 18–99.
MULLER, H. J. (1916). The mechanism of crossing-over. *Am. Nat.* **50**, 193–221, 284–305, 350–366, 421–434.
MULLER, H. J. (1927). Artificial transmutation of the gene. *Science, N.Y.* **66**, 84–87.
MULLER, H. J. (1940). Bearings of the Drosophila work on systematics. *In:* New Systematics, 185–268. (Ed. J. Huxley) Oxford, Clarendon Press.
MULLER, H. J. (1947). The gene. *Proc. Royal Soc. B.* **134**, 1–37.
MULLER, H. J. (1954). The nature of the genetic effects produced by radiation and the manner of production of mutations by radiation. *In:* "Radiation Biology", Vol. 1, Part 1, Chapters 7 and 8 (Alexander Hollaender, ed.), McGraw-Hill Book Co. Inc., New York.
NEWBY, W. W. (1942). A study of intersexes produced by a dominant mutation in *Drosophila virilis*, Blanco stock. *Univ. Texas Publ.* **4228**, 113–145.
OMUNDSON, G. E. (1961a). A study of radiation-induced dominant lethals in *Drosophila virilis* in an oxygen atmosphere at 4°C. *Univ. Texas Publ.* **6918**, 13–23.
OMUNDSON, G. E. (1961b). A study of radiation-induced translocations in *Drosophila virilis* in an oxygen atmosphere at 4°C. *Univ. Texas Publ.* **6918**, 25–38.
PARKER, D. R. (1959). Dominant lethal mutation in irradiated oocytes. Univ. Texas Publ. 5914, 113–127.
PARKER, D. R. and HAMMOND, A. E. (1958). The production of translocations in *Drosophila* oocytes. *Genetics* **43**, 92–100.
PATTERSON, J. T. (1932). Lethal mutations and deficiencies produced in the X chromosome of *Drosophila melanogaster* by X-radiation. *Am. Nat.* **66**, 132–206.
PATTERSON, J. T. (1933). The mechanism of mosaic formation in *Drosophila*. *Genetics* **18**, 32–52.
PATTERSON, J. T. (1941). The virilis group of *Drosophila* in Texas. *Am. Nat.* **75**, 523–539.
PATTERSON, J. T. (1954). Genetic variability in geographic strains of *Drosophila virilis*. *Univ. Texas Publ.* **5422**, 7–18.
PATTERSON, J. T. and MULLER, H. J. (1930). Are "progressive" mutations produced by X-rays? *Genetics* **15**, 495–578.
PATTERSON, J. T. and STONE, W. S. (1952). "Evolution in the Genus Drosophila", 610 pp. The Macmillan Co., New York.
PATTERSON, J. T. and WHEELER, M. (1942). Description of new species of the subgenera Hirtodrosophila and Drosophila. *Univ. Texas Publ.* **4213**, 67–109.

PATTERSON, J. T., STONE, W. S. and GRIFFEN, A. B. (1940). Evolution of the virilis group in Drosophila. *Univ. Texas Publ.* **4032,** 218–250.

PATTERSON, J. T., STONE, W. S. and GRIFFEN, A. B. (1942). Genetic and cytological analysis of the virilis species group. *Univ. Texas Publ.* **4228,** 162–200.

POWERS, E. L., KALATA, B. F. and WEBB, R. S. (1959a). Nitric oxide protection against radiation damage (Abstr.). *Rad. Res.* **11,** 461.

POWERS, E. L., WEBB, R. B. and EHRET, C. F. (1959b). An oxygen effect in dry bacterial spores and its temperature dependence. *Expl. Cell Res.* **17,** 550–554.

PRICE, J. B. (1949a). Transplantation experiments in *Drosophila virilis*: The formation of brown pigment. *Univ. Texas Publ.* **4920,** 24–30.

PRICE, J. B. (1949b). The location of a sex-limited factor, Ix^B, in *Drosophila virilis*. *Univ. Texas Publ.* **4920,** 22–23.

RINEHARDT, ROBERT R. (1963). Some effects of nitric oxide and oxygen on dominant lethal production in X-irradiated *Drosophila virilis* males. *Genetics* **48,** 1673–1682.

SCHMID, WERNER (1961). The effect of carbon monoxide as a respiration inhibitor on the production of dominant lethal mutations by X-rays in Drosophila. *Genetics* **46,** 663–670.

SCHNEIDER, I. (1962). Modification of V-type position effect in *Drosophila virilis*. *Genetics* **47,** 25–44.

SINCLAIR, W. K. and BLACKWELL, L. H. (1958). The relative biological effectiveness of 22 Mvp X-rays, cobalt-60 and 200 Kvp X-rays determined from the LD-50 in mice. *In:* "Radiation Biology and Cancer", pp. 103–120. Univ. of Texas Press, Austin, Texas.

SINCLAIR, W. K., LAUGHLIN, S. S., ROSSI, H. H., TERPOGOSSIAN, M. and MOOS, W. S. (1958). Intercomparison of X-ray exposure dose using victoreen dose meters at various energies, particularly 22 Mevp. *Radiology* **70,** 736–744.

SOKOLOV, N. N. (1948). A new species of Drosophila—*Drosophila imeretensis*. *C.R. (Dokl.) Acad. Sci. U.S.S.R.* **59(5),** 1007–1008.

SPARRMAN, B., EHRENBERG, L. and EHRENBERG, A. (1959). Scavenging of free radicals and radiation protection by nitric oxide in plant seeds. *Acta Chem. Scand.* **13,** 199–200.

SPENCER, W. P. (1938). *Drosophila virilis americana*, a new subspecies. *Genetics* **23,** 169–170.

SPENCER, W. P. (1940a). Subspecies hybrids and speciation in *Drosophila hydei* and *Drosophila virilis*. *Am. Nat.* **74,** 157–179.

SPENCER, W. P. (1940b). Levels of divergence in Drosophila speciation. *Am. Nat.* **74,** 299–311.

SPOFFORD, J. B. (1959). Parental control of position-effect variegation. I. Parental heterochromatin and expression of the white locus in compound-X *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **45,** 1003–1007.

SPOFFORD, J. B. (1961). Parental control of position-effect variegation. II. Effect of sex of parent contributing white-mottled rearrangements in *Drosophila melanogaster*. *Genetics* **46,** 1151–1167.

STALKER, H. D. (1942). The inheritance of a subspecific character in the *virilis* complex of Drosophila. *Am. Nat.* **76,** 426–431.

STEINBERG, A. G. (1936). The effect of autosomal inversions on crossing over in the X-chromosome of *Drosophila melanogaster*. *Genetics* **21,** 615–624.

STERN, C. (1927). Ein genetischer und zytologischer Beweis für Vererbung im

Y-chromosom von *Drosophila melanogaster*. *Z. Indukt. Abstamm. Vererblehre* **44**, 188–231.

STERN, C. (1929). Über Reduktionstypen der Heterochromosomen von *Drosophila melanogaster*. *Biol. Zbl.* **49**, 718–735.

STONE, W. S. (1942). The Ix^B factor and sex determination. *Univ. Texas Publ.* **4228**, 146–152.

STONE, W. S. (1956). Indirect effects of radiation on genetic material. *Brookhaven Symp. in Biol.* **8**, 171–190.

STONE, W. S. (1962). The dominance of natural selection and the reality of superspecies (species groups) in the evolution of Drosophila. *Univ. Texas Publ.* **6205**, 507–537.

STONE, WILSON S., ALEXANDER, MARY L., CLAYTON, FRANCIS E. and DUDGEON, EDNA (1954). The production of translocations in *Drosophila virilis* by fast neutrons from a nuclear detonation. *Am. Nat.* **88**, 287–293.

STONE, W. S., HAAS, F., ALEXANDER, M. L. and CLAYTON, F. E. (1954). Comments on the mechanism of action of radiations on living systems. *Univ. Texas Publ.* **5422**, 244–271.

STURTEVANT, A. H. (1916). Notes on the North American Drosophilidae with descriptions of twenty-three new species. *Ann. Ent. Soc. Amer.* **9**, 323–343.

STURTEVANT, A. H. and NOVITSKI, E. (1941). The homologies of the chromosome elements in the genus Drosophila. *Genetics* **26**, 517–541.

WARTERS, MARY (1944). Chromosomal aberrations in wild populations of Drosophila. *Univ. Texas Publ.* **4445**, 129–174.

WEINSTEIN, A. (1922). Crossing over, non-disjunction and mutation in *Drosophila virilis*. *Am. Sci.* **10**, 45–53.

WELSHONS, W. J. and RUSSELL, W. L. (1957). The effect of X-rays on the *Drosophila* testis and a method for obtaining spermatogonial mutation rates. *Proc. Natl. Acad. Sci. U.S.A.* **43**, 608–613.

WILSON, JOYCE (1950). Recombination in the X-chromosome of virilis species hybrids. Master of Arts Thesis, University of Texas, Austin, Texas.