THE DEVELOPMENTAL GENETICS OF APTEROUS MUTANTS OF *DROSOPHILA MELANOGASTER*

F. M. BUTTERWORTH[1] AND R. C. KING

Department of Biological Sciences, Northwestern University, Evanston, Illinois

Received June 21, 1965

THE apterous (*ap*) mutant of *Drosophila melanogaster* was first found by MISS EDITH M. WALLACE in 1913 while working in the laboratory of DR. T. H. MORGAN. This mutant, which occurred spontaneously, was shown by C. W. METZ (1914) to be recessive and located on the second chromosome near the black (*b*) locus. Subsequently, other apterous mutants were found, such as ap^4, which was localized at 2–55.4 on the recombinational map and within the 41A or B region on the cytological map (see BRIDGES and BREHME 1944).

All the *ap* mutants studied in this paper possess abnormal wings and halteres and low numbers of thoracic bristles. Some of the mutants are also female-sterile, have lowered viabilities, and possess abnormal adipose tissue. The purpose of this paper is to explore these various phenotypic traits in order to gain insight into the cause of the *ap* syndrome and to get a better understanding of certain aspects of normal development. Adults of various *ap* genotypes will be categorized according to the length of their wings and halteres, the morphology of their wings and the distribution of their thoracic bristles. These flies will also be tested for viability and female fertility. The adipose tissue of some of the genotypes will be studied.

MATERIALS AND METHODS

Stocks used: The *ap* strains used are described in Table 1. The alleles ap^4, ap^6, ap^{49j} and ap^{T60} are female-sterile when homozygous, and are kept as balanced-lethal stocks (see Table 1). Since ap^{56f} homozygotes are viable and fertile, a homozygous stock was maintained.

Xasta is a mutation associated with a reciprocal translocation between 2R and 3R. According to BRIDGES the breaks are between 41F and 42A1 in chromosome 2 and just distal to 89D4 in 3 (MORGAN, BRIDGES and SCHULTZ 1936). Only translocation heterozygotes are found among adults in the stock, and their wings show a specific pattern of scalloping (Figure 5a). Flies homozygous for the nontranslocated third chromosome are inviable owing to the recessive lethal gene *l(3)XaR*. Rarely seen translocation homozygotes (Xasta/Xasta) have wings and halteres that are reduced in size (BRIDGES and BREHME 1944). The compound Xasta/ap^4 has reduced wings (Figure 5b) and halteres. The fact that ap^4 and Xasta do not complement one another to produce a wild-type phenotype indicates that they affect the same cistron. Xasta will therefore be symbolized ap^{Xa}.

Df*(2)MS4* is a deficiency between 55.1 and 55.4 on the chromosome 2 genetic map (MORGAN, BRIDGES and SCHULTZ 1938). It is kept as a balanced-lethal stock because homozygotes are inviable.

Stocks were reared at 22 to 24°C on a cornmeal, molasses, agar medium (recipe: KING 1965, p. 429) under a normal regime of daylight and darkness.

[1] Present address: Department of Biology, University of Virginia, Charlottesville.

TABLE 1

Information concerning the stocks used

Symbol	Discoverer	Date	Occurrence	Stock	Source	Reference*
ap⁴	N. N. MEDVEDEV	1932	Spontaneous	*ap⁴/SM5, al² Cy lt^v sp²*	Calif. Inst. Tech.	B&B
ap⁶	I. FAULHABER	1963	Spontaneous	*ap⁶/SM5, al² Cy lt^v sp²*	Univ. Bonn	DIS 37:48
ap^49j	R. K. RITTERHOFF	1949	Spontaneous	*ap^49j/Cy*	Johns Hopkins Univ.	DIS 25:76
ap^56f	P. E. THOMPSON	1956	Spontaneous	*ap^56f/ap^56f*	Purdue Univ.	DIS 30:69
ap^T60	S. THOMAS	1960	X ray	*fes ms b ap^T60 cn sp/ al² dp^1×1 Cy(In2LR) pr Bl cn² lt² L⁴ sp²*	Univ. Indiana	DIS 37:50
ap^Xa	A. S. SEREBROVSKY	1928	X ray	*T(2;3)Xa/l(3)XaR*	Calif. Inst. Tech.	B&B
Df(2)MS4	J. SCHULTZ	1933	X ray	*Df(2)MS4/SM1, al² Cy sp²*	Calif. Inst. Tech.	B&B; DIS 27:58

* B&B: BRIDGES and BREHME (1944). DIS: Drosophila Information Service.

Production of compounds: Adults having combined apterous genotypes (compounds) were produced by mating virgin females of one stock with males of another. In all tables and figures where data concerning compounds are presented, the chromosome of maternal origin is always written first (i.e. *ap^56f* female/*ap⁴* male). Approximately 50 virgin females and 50 males were used per cross. The parents mated, and eggs were laid, over a 10-day period. Progeny were classified according to sex and genotype each day for 4 days. These data were used to determine the relative preadult viability of the *ap* compounds; the compounds were also tested for longevity and fertility as will be described below.

Determination of relative preadult development time: The relative duration of preadult development was determined for homozygous and heterozygous males and females. This is expressed as E_{50}—the number of days after day zero when at least 50% of the total adult population in a given phenotypic class has emerged. Equal numbers of males and females about one week old from *ap⁴*, *ap⁶*, *ap^49j*, *ap^T60* and *ap^56f* stocks were placed in bottles of fresh medium for 24 hours, while the females laid eggs; then the parents were discarded. About 2 weeks later the first adults eclosed, and progeny continued to emerge during a 4 to 6 day period, depending on the stock. The day when the first progeny emerged is called day zero. The flies that emerged throughout this period, were classified daily according to sex and genotype. These data were used to determine the relative preadult viability of the *ap* homozygotes. Heterozygotes were discarded and the homozygotes were tested for longevity and fertility.

Determination of preadult viability: The relative preadult viability was determined for *ap* homozygotes and compounds in the following way. From a given mating the observed number of non-*ap* offspring was divided into the corrected number of *ap* offspring and multiplied by 100 to obtain the relative preadult viability expressed as percent. The corrected number of *ap* offspring was calculated by multiplying the observed number of *ap* offspring by the number of different non-*ap* genotypic classes found in the progeny of the given cross.

Determination of adult longevity and female fertility: Longevity was determined for the homozygous and compound *ap* flies of both sexes collected from the studies on preadult development. These *ap* flies initially 0–1 day old were maintained in shell vials containing medium. No more than 20 flies were put in one vial. Since the fecundity of mutant females was studied, wild-type males were included to insure insemination. The number of living flies and the presence of eggs were determined daily. Surviving flies were transferred to vials containing fresh medium every 5 days.

Studies of wing, haltere and bristle phenotypes: Adults were collected and preserved in 80% alcohol. Using a dissecting microscope and camera lucida, outline drawings were made of the dorsal aspects of the wings of five males and five females. For a given genotype the range of wing lengths was similar in both sexes. The drawings, when arranged in a linear array from longest to shortest, were divided into three major classes containing equal numbers of wings. The first class "strap" was 35 to 75% as long as an arbitrarily selected wild-type wing (Figure 3a); the second class "club" was 10 to 34%; and the third class "nub" was less than 10%.

Although the wing lengths of a given genotype varied to the extent that the range extended into all three classes, one length class predominated. In some genotypes, wing length varied only slightly, and only one class occurred. For photography wings were removed with jewelers forceps, dipped in xylene, and mounted in Permount on a microscope slide.

Camera lucida drawings were also made of halteres, which for apterous flies were divided into two major classes, 50 to 25% and less than 25% of the wild-type length.

Four sets of thoracic bristles (postalar, dorsocentral, scutellar and supraalar) were counted on five males and five females of each genotype. Certain genotypes characteristically lack certain bristles.

Cytological techniques: The abdomens of wild type and certain *ap* adult females were fixed in weak alcoholic Bouin's and embedded in paraffin, or fixed in osmium tetroxide and embedded in Epon according to the procedure of KING and KOCH (1963). Paraffin and plastic sections were stained with a 0.025% aqueous solution of azure B at pH 4 (KING 1960). The sections were stained for proteins with a 0.1% aqueous solution of fast green at pH 2 (KING 1960) or stained for glycogen by the periodic acid Schiff (PA/S) procedure (CASSELMAN 1959, p. 100). Abdomens were also fixed in formaldehyde-calcium, embedded in gelatin (BAKER 1946), and frozen sections from these blocks were stained for lipids with Sudan black B according to the method of BAKER (1956).

Studies on adipose tissue: The amount of abdominal adipose tissue of the adult type was estimated in wild type and various 2-day old *ap* adult females. Flies of various genotypes belong to one of two groups with respect to adult adipose tissue: those having relatively small amounts and those having normal amounts. In the first group the adult adipose tissue forms clusters of a few cells located peripherally in the abdomen; in the latter, the adipose tissue is well developed and fills a large part of the abdominal cavity. The amount of tissue in these two groups was estimated by counting the number of squares of an ocular grid which was superimposed on the image of the fat body present in each section. In both cases the analysis was carried out on 40 ten-micron sagittal sections, selected at random from at least three females of each genotype.

RESULTS AND CONCLUSIONS

Preadult development time (E_{50}): The relative preadult development time (E_{50}) for females and males homozygous or heterozygous for ap^4, ap^6, ap^{49j} and ap^{T60} or homozygous for ap^{56f} are presented in Table 2. The E_{50} values for certain homozygotes are one day longer than for the heterozygotes. ap^6 homozygotes develop at the same rate as heterozygotes. In most cases the E_{50} values of males and females are equal, but ap^4 is an exception. Although ap^{56f} homozygotes and heterozygotes were not compared, their preadult development rate is similar to the heterozygotes of the other stocks.

Preadult viability: The relative preadult viabilities of phenotypically *ap* flies of various genotypes are given in Table 3. The values presented are relative to the preadult viabilities of the non-*ap* progeny of each cross. There are one to three genotypic classes of non-*ap* progeny depending on the cross involved, and the viabilities of each class may be different. For this reason generalizations were made on only those compounds that differ from one another by relatively large amounts.

In most cases, ap^{56f} can be considered dominant over other alleles with respect to preadult viability, because the compounds of ap^{56f} and certain alleles have higher viabilities than the homozygotes of the latter. Generally hemizygotes are less viable than the homozygotes. Exceptions to this rule are ap^6 and ap^{T60}. The preadult viability of the ap^6 homozygotes and hemizygotes is similar, but ap^{T60}

TABLE 2

Relative duration of preadult development

Allele	Successive days of eclosion	Number of adults emerged on specific day			
		Homozygotes		Heterozygotes	
		male	female	male	female
ap^{4}	0	3	1	5	13
	1	30	69	146	188
	2	31	21	91	46
	3	16	8	6	1
	4	4	0	3	4
	E_{50}* =	2	1	1	1
ap^{6}	0	2	5	7	6
	1	28	34	157	164
	2	80	114	234	229
	3	40	37	98	87
	4	16	11	60	31
	5	6	1	15	15
	E_{50} =	2	2	2	2
ap^{49j}	0	0	2	37	30
	1	7	11	184	174
	2	35	29	12	17
	3	4	3	2	0
	4	0	0	2	0
	E_{50} =	2	2	1	1
ap^{T60}	0	2	0	11	30
	1	15	25	278	312
	2	29	27	198	172
	3	7	5	23	20
	E_{50} =	2	2	1	1
ap^{56f}	0	20	19	..	..
	1	109	120	..	..
	2	20	17	..	..
	E_{50} =	1	1	..	..

* E_{50} = Number of days after day zero when at least 50% of the total number of flies in each column have eclosed.

hemizygotes are more viable than homozygotes. Since the stocks are not isogenic, this anomalous behavior may be attributed to different genetic backgrounds. The fact that the relative preadult viability of ap^{T60}/ap^{T60} is lower than $ap^{T60}/MS4$ may be explained in the following way. Homozygotes of ap^{T60} have strap-like, sticky wings which hinder emergence from the puparium, whereas hemizygotes of ap^{T60} have nub wings, which might make emergence easier and result in relatively higher preadult viability.

Although all the genotypes show a small amount of sexual dimorphism, some genotypes exhibit considerably more than others. In ap^{56f}/ap^{Xa}, ap^{4}/ap^{6}, $ap^{T60}/MS4$ and $ap^{56f}/MS4$ the females are more viable, whereas in ap^{56f}/ap^{T60}, the males are more viable.

TABLE 3

Relative preadult viability

Genotypes of *ap* offspring	Sex	Observed* non-*ap* offspring (*y*)	Observed* *ap* offspring	Zygotic ratio†	Corrected *ap* offspring (*x*)	Relative viability (percent) (100 *x*/*y*)
ap^{56f}/ap^{Xa}	m	96	71	1:1	71	73.9
	f	105	119		119	100
$ap^{T60}/MS4$	m	287	61	2:1	122	42.5
	f	244	153		306	100
ap^{56f}/ap^{6}	m	93	77	1:1	77	82.7
	f	76	90		90	100
ap^{56f}/ap^{4}	m	93	95	1:1	95	100
	f	114	99		99	86.8
ap^{56f}/ap^{T60}	m	48	58	1:1	58	100
	f	66	43		43	65.2
ap^{4}/ap^{6}	m	190	72	2:1	144	75.8
	f	194	99		198	100
ap^{4}/ap^{T60}	m	243	92	2:1	184	75.7
	f	239	95		190	79.5
$ap^{6}/MS4$	m	133	47	2:1	94	70.7
	f	130	54		108	83.1
$MS4/ap^{Xa}$	m	211	57	3:1	171	81.0
	f	198	52		156	78.7
ap^{4}/ap^{4}	m	251	84	2:1	168	66.9
	f	260	91		182	70.0
ap^{6}/ap^{6}	m	571	172	2:1	344	60.2
	f	532	202		404	75.9
$ap^{56f}/MS4$	m	167	78	1:1	78	46.7
	f	133	100		100	75.2
ap^{4}/ap^{Xa}	m	238	50	3:1	150	63.0
	f	184	51			
ap^{49j}/ap^{49j}	m	237	46	2:1	92	38.8
	f	221	45		90	40.7
$ap^{4}/MS4$	m	353	82	2:1	164	46.5
	f	318	52		104	32.7
ap^{T60}/ap^{T60}	m	510	53	2:1	106	20.8
	f	534	57		114	21.4

* The values represent the total number of flies collected on successive days throughout the first week of emergence of the F_1 generation.

† Zygotic ratio= (expected number non-*ap* offspring)/(expected number *ap* offspring).

Adult longevity: Homozygotes and compounds of various *ap* alleles form three distinct classes with respect to adult longevity (Table 4). Both sexes of any given genotype, except ap^{56f}/ap^{Xa}, die at about the same rate, and therefore each point on the graphs represents an average between the values for each sex (Figure 1).

Adults belonging to the first group live for relatively short periods (Table 4a, Figure 1a). The observed half-times are 1 to 3 days, and no flies live longer than 4 days. The half-time is the number of days after emergence when half of the flies in a given population are dead. The second group (Table 4b, Figure 1b) has longevities that are similar to wild type (WILSON, KING and LOWRY 1955). All

TABLE 4

Longevity of various apterous adults

Group	Genotype	N*	Percent living longer than 5 days	Half-time† of total population
(a)	ap^{49j}/ap^{49j}	79	0	1–2
	ap^{4}/ap^{Xa}	135	0	1–2
	$ap^{4}/MS4$	144	0	1–2
	$ap^{6}/MS4$	101	0	2–3
	ap^{4}/ap^{4}	212	0	2–3
(b)	ap^{56f}/ap^{T60}	121	95	>15
	ap^{56f}/ap^{56f}	259	94	>15
	ap^{56f}/ap^{4}	194	87	>15
	ap^{56f}/ap^{6}	185	85	>15
(c)	ap^{56f}/ap^{Xa}	258	77	15
	$ap^{56f}/MS4$	166	53	5–6
	ap^{T60}/ap^{T60}	109	32	2–3
	ap^{6}/ap^{6}	426	6	1–2
	$MS4/ap^{Xa}$	114	5	1–2
	$ap^{T60}/MS4$	249	1	1–2
	ap^{4}/ap^{T60}	354	0.5	1–2
	ap^{4}/ap^{6}	287	0.3	1–2

* N = the total number of adult males and females tested.
† Half-time of population = the number of days after emergence when half of the flies in a given population are dead.

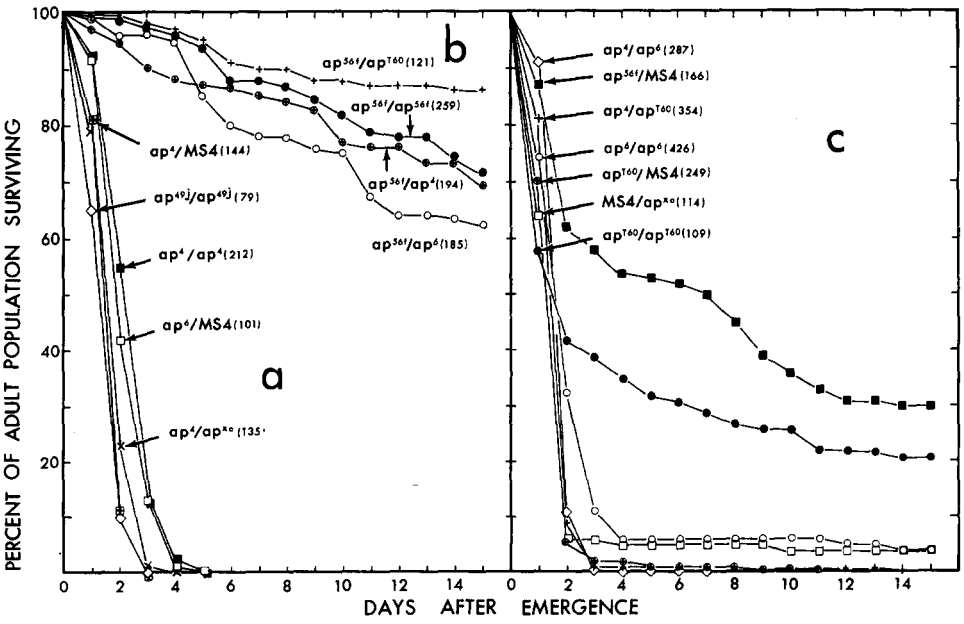

FIGURE 1.—Survival curves for adult flies of various genotypes. Representatives of three different phenotypic groups are shown: (a) the short-lived group, (b) the long-lived group and (c) the group composed of two subpopulations having different mortality rates. The number in parentheses after each genotype gives the total number of males and females studied.

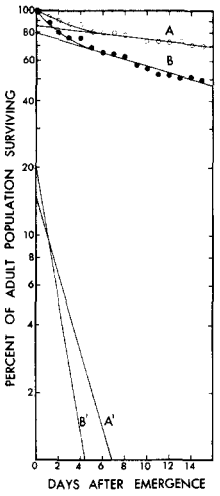

FIGURE 2.—Survival cures of ap^{56f}/ap^{Xa} females (A and A′) and males (B and B′) analyzed as two-component rate functions. The *Y*-intercept of each curve represents the percent of the total population of males or females made up by the respective subpopulation. The half-times in days of the four subpopulations are: A′ = 2, A = > 15, B′ = 1 and B = 15 days. A total of 100 males and 158 females were studied.

the genotypes in this category are compounded with ap^{56f}, and all have half-times greater than 15 days.

In the third group, the flies of a given genotype appear to be made up of two subpopulations: one relatively long-lived and the other short-lived (Table 4c, Figure 1c). In the case of ap^{56f}/ap^{Xa} the data for the two sexes have been plotted semilogarithmically (Figure 2) so that by using a method of analyzing two-component rate functions (SACKS 1953, p. 83) the relative size and half-time of each subpopulation can be determined. In both sexes the slow-dying subpopulations make up about 80% of the total (Y-intercept of curves A and B) and have half-times greater than 15 days. The fast dying subpopulations make up the remaining 20% (Y-intercept of curves A′ and B′) and have half-times of 1 to 2 days. The males have shorter half-times in both subpopulations. If a similar analysis is made for ap^{T60}/ap^{T60}, the slow and fast dying subpopulations are found to be equal in size and to have half-times of 12 days and 1 day, respectively. The fast dying subpopulation appears to make up 30% of the total population of $ap^{56f}/MS4$ and over 90% of the total in the remaining genotypes shown in Figure 1c.

Certain conclusions can be made on the effect of *ap* on adult longevity. (1) The allele ap^{56f} is apparently dominant over the other *ap* alleles because when other *ap* alleles, the homozygotes of which have low adult viabilities, are combined with ap^{56f} the resulting compounds have normal viabilities. (2) The alleles ap^{6} and ap^{T60} are hypomorphic because the homozygotes are more viable than the compounds containing ap^{4} and the hemizygotes. (3) ap^{4} and ap^{Xa} are hypomorphic in some combinations, but not in others. Both alleles have some activity because ap^{56f}/ap^{4} and ap^{56f}/ap^{Xa} are more viable than $ap^{56f}/MS4$. However, ap^{Xa} is more

TABLE 5

Female fertility of various apterous adults

Group	Genotype	N*	Fertile	Egg laying begins (days after eclosion)	Percent females alive when first eggs laid
(a)	ap^4/ap^4	99	no	0	0
	ap^{49j}/ap^{49j}	45	no	0	0
	$ap^4/MS4$	80	no	0	0
	ap^4/ap^{Xa}	100	no	0	0
(b)	ap^{56f}/ap^{56f}	141	yes	1–2	95
	ap^{56f}/ap^{T60}	67	yes	1–2	95
	ap^{56f}/ap^4	99	yes	1–2	95
	ap^{56f}/ap^6	123	yes	1–2	95
	ap^{56f}/ap^{Xa}	158	yes	1–2	95
	$ap^{56f}/MS4$	94	yes	2–3	70
(c)	ap^6/ap^6	202	yes	10–11	8.0
	ap^4/ap^{T60}	198	yes	5–6	0.5
	$MS4/ap^{Xa}$	55	yes	4–5	2.0
	$ap^{T60}/MS4$	184	yes	5–6	2.0
	ap^4/ap^6	206	yes	5–6	0.5
(d)	$ap^6/MS4$	54	yes	3–4	2.0

* N = total number of females.

active than ap^4 in one case ($MS4/ap^{Xa}$ is more viable than $ap^4/MS4$), and ap^4 is more active than ap^{Xa} in another case (ap^{56f}/ap^4 is more viable than ap^{56f}/ap^{Xa}). The anomalous behavior of ap^4 and ap^{Xa} might be due to the different genetic backgrounds modifying the expression of certain allelic combinations, or to interaction between the products of the alleles in question.

Female fertility: Homozygotes and compounds of various *ap* alleles form three major groups with respect to female fertility or the capacity to lay eggs(Table 5). All the females in Group a are short-lived and sterile. Group b is comprised of genotypes containing at least one ap^{56f} allele where a large percentage of the females are long-lived, fertile, and egg laying usually beings on the second day of adult life. In Group c only a low percentage of the females lay eggs, and these fertile females also form the long-lived subpopulation (Table 4c). Egg laying usually begins much later than in Group b. $ap^6/MS4$ females, Group d, do manage to lay a few eggs during their short lives.

Several generalizations can be made regarding the degree of dominance of various *ap* alleles on the capacity to lay eggs. For example, the fertile allele ap^{56f} masks other alleles that cause sterility and low adult longevity when homozygous. It is not known if ap^{T60}/ap^{T60} females are sterile solely because the female-sterile gene *fes* is on the same chromosome. However, ap^{T60} may be a fertile gene which behaves dominantly because a low percentage of ap^{T60}/ap^4 and $ap^{T60}/MS4$ females are fertile. Low percentages of females having the genotype ap^6/ap^6, ap^6/ap^4 and

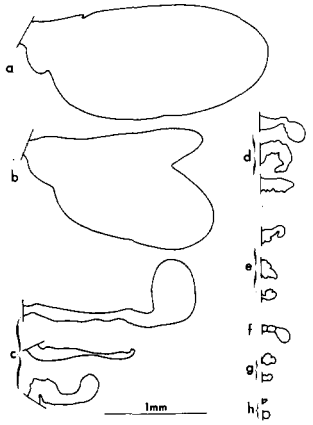

FIGURE 3.—Outline drawings of the wings and halteres of wild-type and *ap* flies. a = +/+ female wing, b = ap^{Xa}/+ female wing, c = strap, d = club, e = nub, f = +/+ female or ap^{Xa}/+ female haltere, g = medium and h = short. See MATERIALS and METHODS for explanation of terms.

ap^{6}/*MS4* lay eggs, whereas ap^{4}/ap^{4} and ap^{4}/*MS4* are sterile. From these facts it is concluded that ap^{6} is dominant over ap^{4} with respect to fertility and adult longevity.

Wing and haltere morphology: The outlines of wings in Figure 3 are characteristic of the normal and *ap* flies studied. The wing rudiments of these *ap* genotypes fall into three categories with respect to wing length: strap, club and nub. Three drawings are included in each category to demonstrate the variability of the length and shape. The halteres, outlines of which are also drawn, fall into three general categories with respect to length: normal, medium and short.

Although the wings of some genotypes fall into only one length-category, the wings of most genotypes vary enough in length to fall into two or even three categories (Table 6). Nevertheless, each genotype usually can be characterized by having wing lengths predominantly in one category. The wings of genotypes in Group a are predominantly strap but none are nub; and in Group d the wings are preponderantly nub, but none are strap. The wings of the remaining genotypes are predominantly club; however, those in Group b possess strap in addition to nub.

It is apparent from these observations that ap^{Xa} acts dominantly by increasing wing length when combined with other *ap* alleles. However, ap^{Xa} tends to decrease wing length when combined with the wild-type allele. ap^{6} and ap^{56f} are hypomorphic, since longer wings are formed when the alleles are present in double dose than in single (i.e. combined with *MS4*).

Wild-type and ap^{Xa}/+ halteres are composed of three segments (Figure 3). The halteres on other *ap* genotypes are at least half the length of wild-type halteres, and appear to be composed of only one or two segments. The genotypes in the first group have at least 50% of their halteres in the medium category (Table 7). The second group has greater than 90% of the halteres in the short category,

TABLE 6

*The distribution of wing lengths of various apterous adults**

Group	Genotype	Strap†	Club†	Nub†
(a)	$ap^{Xa}/+$	20	0	0
	ap^{4}/ap^{Xa}	18	2	0
	$MS4/ap^{Xa}$	14	6	0
	ap^{56f}/ap^{Xa}	15	5	0
(b)	ap^{56f}/ap^{T60}	3	14	3
	ap^{6}/ap^{6}	1	15	4
	ap^{56f}/ap^{6}	1	14	5
(c)	ap^{4}/ap^{T60}	0	18	2
	ap^{56f}/ap^{56f}	0	14	6
	ap^{4}/ap^{6}	0	14	6
(d)	ap^{56f}/ap^{4}	0	7	13
	$ap^{56f}/MS4$	0	6	14
	$ap^{T60}/MS4$	0	5	15
	$ap^{6}/MS4$	0	5	15
	ap^{4}/ap^{4}	0	2	18
	$ap^{4}/MS4$	0	0	20

* The values in each row total 20, since the wings of 5 males and 5 females were classified.
† Strap=75 to 35% of +/+ female wing length.
Club=34 to 10% of +/+ female wing length.
Nub=less than 10% of +/+ female wing length.

TABLE 7

*The distribution of haltere lengths of various apterous adults**

Group	Genotype	Medium†	Short†	Missing
(a)	ap^{4}/ap^{Xa}	20	0	0
	ap^{6}/ap^{6}	15	5	0
	ap^{56f}/ap^{Xa}	13	7	0
	$MS4/ap^{Xa}$	12	8	0
	ap^{4}/ap^{T60}	10	10	0
(b)	ap^{4}/ap^{6}	6	11	3
	ap^{56f}/ap^{56f}	2	18	0
	$ap^{T60}/MS4$	2	7	11
(c)	ap^{56f}/ap^{T60}	0	20	0
	ap^{56f}/ap^{6}	0	18	2
	ap^{4}/ap^{4}	0	11	9
	$ap^{56f}/MS4$	0	7	13
	$ap^{6}/MS4$	0	5	15
	ap^{56f}/ap^{4}	0	4	16
	$ap^{4}/MS4$	0	3	17

* The values in each row total 20, since the halteres of five males and five females were classified.
† Medium=50 to 25% of the length of +/+ female haltere.
Short=less than 25% of the length of +/+ female haltere.

and many flies in almost all genotypes are missing halteres. When combined with other *ap* alleles ap^{Xa} acts by increasing the haltere length. Some of the alleles are more active in certain combinations than in others. For example, ap^{6} is hypomorphic because medium length halteres are produced when homozygous or when combined with the amorphic allele ap^{4}. However, ap^{6} produced only short halteres when in single dose or when combined with ap^{s6f}. This variability in the expression of the phenotype may be due to the effect of different genetic backgrounds.

Wing development: The development of normal wings has been described by CHEN (1929), GOLDSCHMIDT (1935), AUERBACH (1936), ROBERTSON (1936), WADDINGTON (1940), and BODENSTEIN (1950). CHEN, GOLDSCHMIDT, AUERBACH and WADDINGTON have also studied the abnormal development of wings caused by certain mutations.

Normally, the primordial wing tissues arise in the embryo from thickenings of the hypodermis. This tissue invaginates from the hypodermis, forms a lumen, and grows in volume during the larval period. Shortly after the formation of the puparium the wing rudiments evaginate, broad veins begin to appear, and the blade portion becomes bloated and increases in area. About 30 hours after puparium formation, the wing rudiment flattens and contracts in area. About midway through metamorphosis the adult form of the wing in terms of venation pattern and silhouette is fully recognizable, cell division becomes infrequent, the wing begins to fold, adult chitin is deposited, and the wing cells begin to produce hairs. At emergence the wing unfolds and flattens. The cytoplasm of the wing cells at this time has largely disappeared.

The chaetotaxy of the Drosophila wing has been described by FERRIS (1950) and by HADORN, ANDERS and URSPRUNG (1959). Female wings possess at least five different types of hairs. Types 1 and 2 are shown in Figure 4b, type 3 in 4c, and types 4 and 5, together with dome organs (do), in 4d. Dome organs consist of a convexity in the cuticle attached to a single sense cell. There are 12 such organs located on specific veins in the wild-type wing (MILLER 1950, p. 501). Hair types 1, 2 and 4 are located along the anterior or leading edge of the wing, and type-2 hairs are also found along the distal and posterior or trailing edge of the wing. Type-5 hairs are located on the major portion of the wing blade, whereas type-3 hairs are confined only to the most proximal region, particularly along the proximal portion of the veins. There are about three times as many type-3 hairs per unit area as type-5 hairs.

The wings of the *ap* genotypes, ap^{4}/ap^{4}, ap^{s6f}/ap^{s6f}, ap^{6}/ap^{6}, ap^{4}/ap^{Xa} and $ap^{Xa}/+$ (Figure 5) represent the three categories of wing length. The *ap* wings, except for $ap^{Xa}/+$, lack veins and possess only two of the five types of hairs, a preponderance of type 3 and a few type 4. The wings are not flat and thin like +/+ wings, but are instead wrinkled, twisted, bag-like structures. The opaqueness in certain regions is due to the fact that the wing is either highly twisted or filled with an opaque substance in these regions.

The smallness of *ap* wings is not due to their failure to expand after emergence. When the thorax of ap^{4} homozygotes is squeezed gently the wings inflate, but their length does not change. The *ap* wing is small because it contains fewer cells. Since each of the cells that make up the surface of the wing produces a single hair

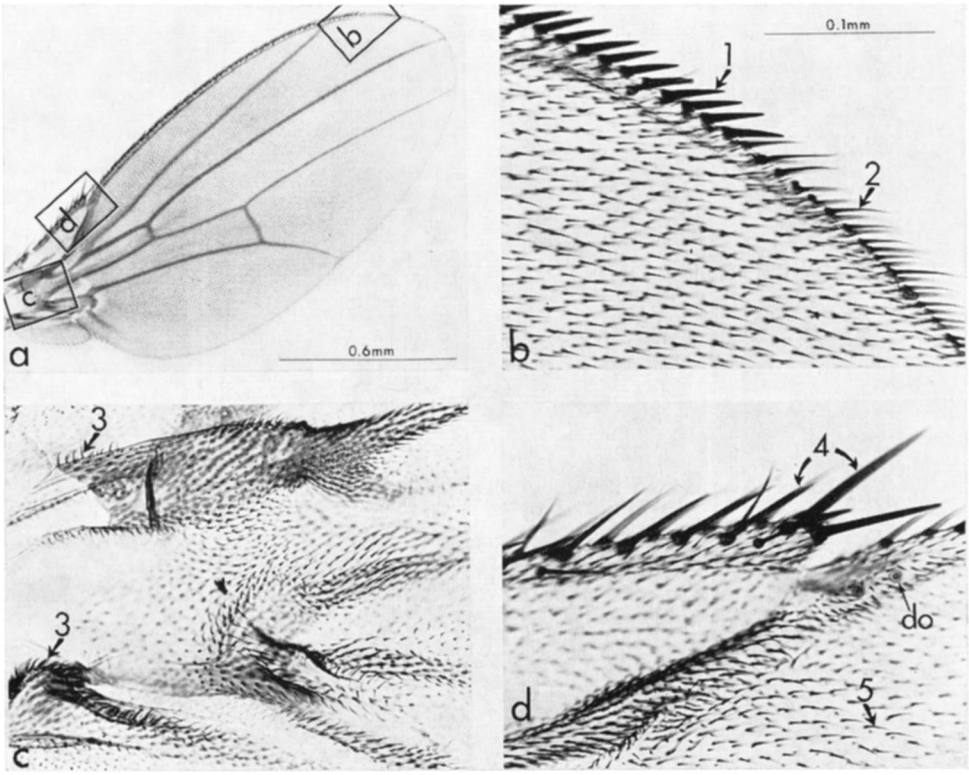

FIGURE 4.—Whole mount of the normal, female wing showing the five major hair-types. The areas enclosed by rectangles in (a) are shown at higher magnification in (b), (c) and (d). The different hair types (1 to 5) and the dome organs (do) are indicated. The magnification of (b), (c) and (d) is the same.

(DOBZHANSKY 1929), we were able to obtain an estimate of the relative number of cells of normal and *ap* wings by comparing the number of hairs in a given area on wings of both types. Both hair types observed in *ap* wings appeared in concentrations similar to those hair types found in wild type. The wings of ap^{Xa}/ap^{4} (Figure 5b) have a considerably larger blade portion and possess relatively greater numbers of type-3 hairs than the other three genotypes. The wings of $ap^{Xa}/+$ (Figure 5a) are normal in that they possess the five types of hairs, but are abnormal in that a specific region of the distal edge is missing, and the blade is broader than wild type.

The fact that type-4 hairs are normally found in the proximal portion, and that this hair type predominates in the *ap* wings studied, leads one to believe that only the basal portion of the wing forms in *ap*. Perhaps the cells which form the distal parts of the wing degenerate or are not produced in *ap* flies. More will be said about this in the discussion.

Normally dome organs are located in specific positions over specific wing veins. However, in ap^{Xa}/ap^{4} wings where veins are absent a few dome organs are

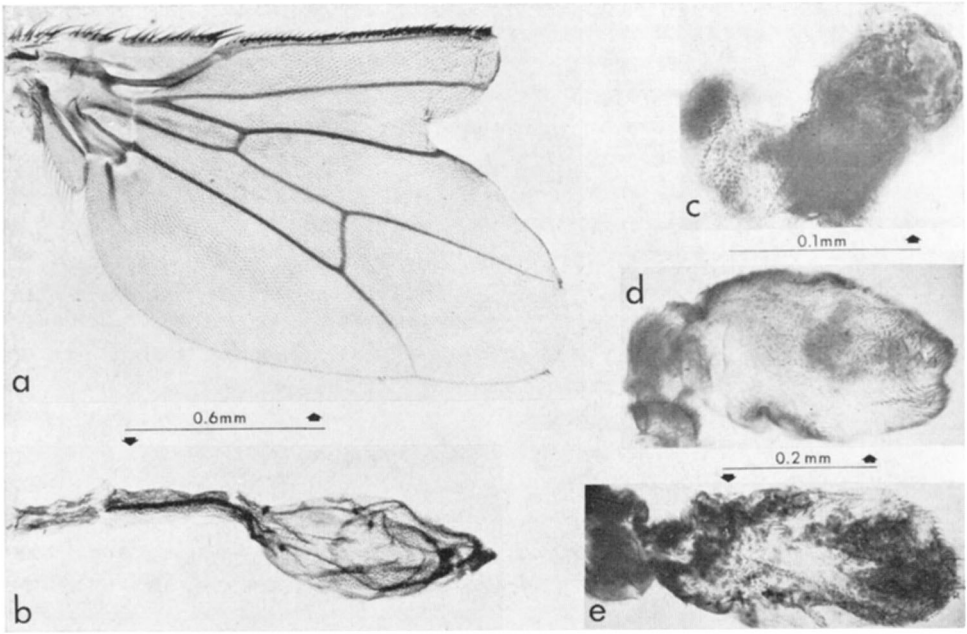

FIGURE 5.—Whole mounts of various *ap* wings. a = $ap^{Xa}/+$, b = ap^{Xa}/ap^{4}, c = ap^{4}/ap^{4}, d = ap^{56f}/ap^{56f} and e = ap^{6}/ap^{6}.

present, domonstrating that the formation of dome organs does not require the presence of an underlying vein. Dome organs were not seen in ap^{4}/ap^{4}, ap^{6}/ap^{6} and ap^{56f}/ap^{56f} wings, but these organs are present in the correct positions over the veins of $ap^{Xa}/+$ wings.

Thoracic bristle distribution: The normal development of bristles in Drosophila is described by LEES and WADDINGTON (1942).

The bristle is a chitinous structure secreted by a specific hypodermal cell, the trichogen, beneath which lies a tormogen cell which secretes the socket. Both cells, which are daughters, can be distinguished from other hypodermal cells at about 15 hours after the onset of metamorphosis. The bristle is secreted about 35 hours later. The trichogen becomes smaller during the process and eventually merges with the hypodermis.

The supraalar, postalar, dorsocentral, and scutellar bristles were scored as present or absent in various *ap* genotypes. Ten flies of each genotype were studied, and the average number of each type of bristle present (rounded off to the nearest whole number) is given in Table 8. Normally there are four bristles of each type present (FERRIS 1950, p. 398), but all the *ap* genotypes lack at least some bristles. The genotypes $ap^{T60}/MS4$, $ap^{56f}/MS4$, $ap^{6}/MS4$, ap^{4}/ap^{4}, $ap^{4}/MS4$ and $MS4\text{-}ap^{Xa}$ possess relatively normal numbers of post- and supraalar bristles, but the scutellar and dorsocentral bristles are reduced in number. The remaining genotypes completely lack the dorsocentral and scutellar bristles. Of these remaining genotypes ap^{6}/ap^{6} and ap^{4}/ap^{Xa} also lack postalar, and ap^{56f}/ap^{Xa} lacks supraalar

TABLE 8

*Number of certain thoracic bristles in normal and various apterous adults**

Group	Genotype	Postalar	Scutellar	Dorsocentral	Supraalar
(a)	+/+	4	4	4	4
	$ap^{Xa}/+$	4	4	4	4
	$ap^{6}/MS4$	4	4	3	4
	$ap^{4}/MS4$	4	4	3	4
	$ap^{56f}/MS4$	4	3	3	4
	$ap^{T60}/MS4$	4	3	3	4
	$MS4/ap^{Xa}$	3	2	1	4
	ap^{4}/ap^{4}	3	1	1	4
(b)	ap^{56f}/ap^{56f}	2	0	0	4
	ap^{56f}/ap^{4}	2	0	0	4
	ap^{4}/ap^{T60}	2	0	0	4
	ap^{4}/ap^{6}	2	0	0	4
	ap^{56f}/ap^{T60}	1	0	0	4
	ap^{56f}/ap^{6}	1	0	0	4
(c)	ap^{56f}/ap^{Xa}	4	0	0	0
(d)	ap^{6}/ap^{6}	0	0	0	3
	ap^{4}/ap^{Xa}	0	0	0	3

* Average values that are rounded off to the nearest whole number. Five females and five males of each genotype were analyzed.

bristles. $ap^{Xa}/+$ flies have a normal complement of bristles, and there are no differences between the sexes of any genotype.

ap alleles appear to inhibit the formation of thoracic bristles because any allele in single dose (i.e. when combined with *MS4*) tends to have a more normal complement of bristles. It is also possible that on the *MS4* chromosome there is a dominant gene which enhances bristle development.

Adipose tissue: The adipose tissue of *D. melanogaster*, is of two types: larval and adult (BUTTERWORTH, BODENSTEIN and KING 1965).

Normally, both are present simultaneously in the adult for two days after eclosion. During this period each type of tissue undergoes separate developmental changes. The larval adipose cells which have survived metamorphosis histolyze, while the adult cells grow. By the third day none of the larval cells remain, but the adult cells have increased in volume to such an extent that they fill most of the available space in the abdominal cavity. Adult adipose tissue persists throughout adult life. Both types of adipose tissue contain deposits of lipid and glycogen, but the larval adipose cells also contain proteinaceous globules, which are induced to form during the latter half of the third instar by a hormone from the larval ring gland.

In ap^{56f}/ap^{56f}, ap^{56f}/ap^{4} and ap^{56f}/ap^{49j} adult females, the adipose tissue develops normally. The larval cells that survive metamorphosis break down, and the adult adipose tissue increases in volume by the second day of adult life. Females of these genotypes have about the same amount of adult adipose tissue as wild-type females of the same age.

The adipose tissue of ap^4/ap^4, ap^{49j}/ap^{49j} and $ap^{49j}/MS4$ adult females develops abnormally. In females of these genotypes the larval cells do not break down by the second day of adult life. These cells in ap^4/ap^4 females are cytochemically normal in that they contain deposits of lipid, glycogen and protein. The cells of the other genotypes were not studied cytochemically. In addition, the adult adipose tissue in females of these three genotypes does not increase in volume. On the second day of adult life the cells are still morphologically and cytochemically similar to those of recently emerged *ap* and wild-type flies. In 2-day old flies the area of adult adipose tissue per section of abdomen is 50 times greater in ap^{56f}/ap^{56f} than in ap^4/ap^4 females.

We can conclude that the flies of the above genotypes belong to two groups: those with normal and those with abnormal adipose tissue. The allele ap^{56f} behaves dominantly over the other *ap* alleles since adult females heterozygous or homozygous for ap^{56f} have adipose tissue that develops normally. Adult females of the genotypes where the adipose tissue develops normally are also fertile and long-lived. Those where the adipose tissue develops abnormally are sterile and relatively short-lived. DOANE (1960) has described a strain of *D. melanogaster* which is of interest in this regard. Homozygotes are frail and live no more than one week. The adult adipose tissue fails to mature beyond rudimentary stages, but the surviving larval adipose cells do histolyze.

Peritrophic membrane: The ventriculus or midgut extends from the thorax into the abdomen, where the midgut coils several times before entering the hindgut (MILLER 1950, p. 424). A complex stomodaeal valve, the cardia, forms the junction of the oesophagus and the midgut. A ring of cells in the ventricular portion of the cardia secretes the peritrophic membrane. This membrane normally forms a cylindrical sheath around the ingested food and extends throughout the ventriculus (Figure 6a). The ventriculus just posterior to the cardia of ap^4/ap^4 females that are one-day old, is abnormally swollen. This region, when dissected open, is packed with a transparent material which appears to be disorganized peritrophic membrane (KING and SANG 1958). About one day later this disorganized membranous material can be seen in more posterior regions of the ventriculus (Figure 6b).

DISCUSSION

Wing development: The amount of genetic information needed for the formation of any organ must be immense. The insect wing is no exception. In Drosophila over 200 nonallelic mutations that affect wing morphology are known (KING 1965, p. 331). The gene under consideration presumably plays an important role in wing morphogenesis. When certain *ap* alleles are homozygous or in combination with other *ap* alleles, the resulting wings are extremely abnormal. They never attain the normal adult form and in some cases develop to only 5% the length of wild-type wings. The *ap* wings are small because they contain fewer cells. In addition, the wings fail to flatten, they lack veins and cells that produce certain specific hair types, and the wing margin is abnormal.

The experiments of WADDINGTON (1940) and LEES (1941) suggest that the

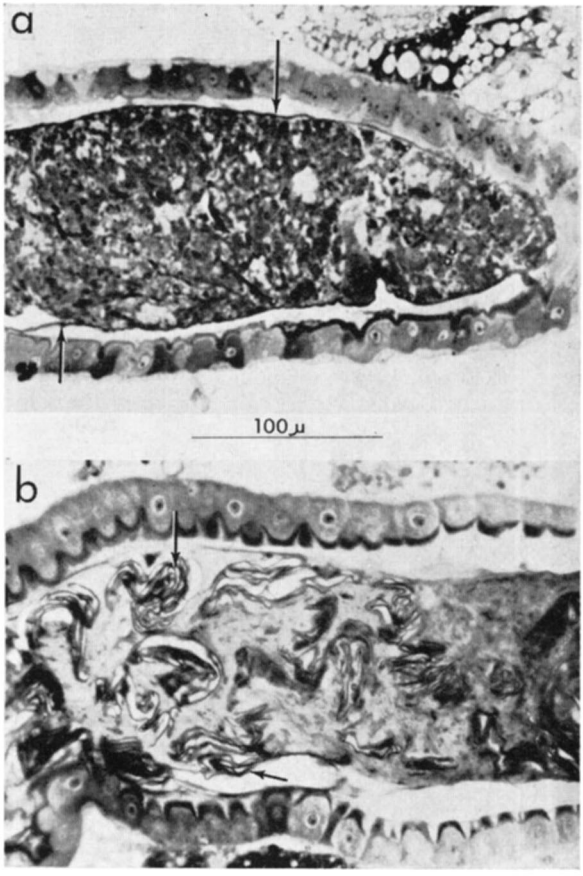

FIGURE 6.—Sections through ventriculi showing the peritrophic membrane (arrows) of (a) normal and (b) ap^4/ap^4 adult females after two days of adult life. The sections were stained by the PA/S procedure followed by azure B.

process of wing flattening and contraction that occurs just after the bloated stage, the forming of veins and the vein-pattern, and the forming of the wing margin are all morphogenetically determined before the time of puparium formation when the wing rudiment is beginning to evert. WADDINGTON compared wing development of wild type with the abnormal development of various mutants and LEES studied the development of wild-type wings that were wounded at given times during prepupal and pupal development. WADDINGTON argues that the wing margin is determined before the time of puparium formation because the scalloped effect of $ap^{Xa}/+$ and other scalloped mutants is first seen at this time. He also argues that since the venation is not adjusted to the change in shape of these mutant wings it is clear that the course of the veins must be determined before metamorphosis begins. LEES corroborates these findings by showing that

wing rudiments wounded at this stage will develop into phenocopies of $ap^{Xa}/+$ wings, and that some of the operated wings have various veins missing. Some of these wings are also blistered in the wounded areas indicating the potentiality of the tisssue to flatten out is lost in the injured region. This blistering phenomenon suggests that the ability of the wing to flatten out is determined before the time of puparium formation, since LEES claims there is no evidence that the blisters are caused by the trapping of haemolymph between the wing surfaces. According to WADDINGTON and LEES the beginning of pupation is the latest time when the process of the flattening of the wing, the forming of veins and venation pattern, and the forming of the wing margin are determined. Since the wing discs are first seen in recently hatched, first instar larvae, it is reasonable to assume that the *ap* locus exerts its effect sometime during the larval period.

GOLDSCHMIDT (1935) and AUERBACH (1936) have also compared normal and mutant wing development. The vestigial (*vg*) mutant which they both studied has a relatively normal, miniature wing whose margin is severely scalloped. These authors conclude that the mutant wing is reduced in size because the rudiment in the newly formed prepupa is smaller than that of wild type. They argue that scalloping occurs by the degeneration of the wing margin at about 6 hours after puparium formation, implying that the *vg* mutation affects two separate processes which occur in forming a wing. WADDINGTON (1940) has found no evidence for degeneration in *vg* or other scalloping mutants, and LEES (1941) was unable to produce phenocopies of *vg* by wounding normal wings at this later stage.

The *ap* wing in its most reduced form is similar to the basal or proximal portion of the wild-type wing. Perhaps early in development two cell-lines differentiate: one which will form the distal parts of the wing and one which will form the basal part of the wing. The cells of the distal line will produce hairs of types 1, 2, 4 and 5, and the cells of the basal line will produce type-3 and a few type-5 hairs. Such an allocation of cell lines fits the map of presumptive structures drawn up by HADORN and BUCK (1962) for the wing imaginal disc. Perhaps the growth or survival of cells of the distal line requires a substance which *ap* larvae cannot make or derive from the medium. For example, linoleic acid is required for the formation of wing scales (homologous to wing hairs) in various moth species belonging to the genera Ephestia and Plodia (FRAENKEL and BLEWETT 1946). The degree of scale formation is dependent on the concentration of linoleic acid fed to the larvae. If the concentration of acid is exceedingly low, the wings are completely scaleless, abnormally small and deformed, and lack certain veins. CHIPPENDALE, BECK and STRONG (1965) showed that when larvae of the cabbage looper *Trichoplusia ni* were not fed the saponifiable portion of wheat germ oil in their diet, the wings of the adults were stunted and deformed. Preadult viability was also lowered. A methyl linoleate supplement to the diet increased viability but did not improve wing development.

An alternative hypothesis is that growth of the *ap* wing rudiment is inhibited

by a substance to which the distal cell-line is sensitive. Genetically induced inhibition of growth in a developing limb rudiment has been demonstrated by ELMER and PIERRO (1964) in chickens. Most embryos that are homozygous for the gene Creeper die early in development, but those that live longer have malformed limbs. ELMER and PIERRO have shown that the malformation is due to an inhibition of growth in the limb, and that this inhibition is caused by a diffusible substance produced by the abnormal limb. It would be interesting to see if *ap* wing rudiments would exert an inhibiting effect on developing wild-type wings.

Female fertility, adult longevity and adipose tissue: Although flies of all the *ap* genotypes lack normal wings, flies of some *ap* genotypes suffer from defects even more critical for survival, such as female sterility and low adult longevity. Some of these genotypes such as ap^4/ap^4, ap^{4g^j}/ap^{4g^j} and $ap^{4g^j}/MS4$ also have abnormal adipose tissue. The other female-sterile, poorly viable genotypes may also have abnormal adipose tissue, but this remains to be investigated. It has been shown by KING and BURNETT (1957) that ap^4/ap^4 females cannot make yolky oocytes. However, the ovaries of these flies are normal because ap^4/ap^4 ovaries can synthesize yolk when implanted into the abdomens of wild-type females (KING and BODENSTEIN 1965). This finding indicates that the abdominal environment of ap^4 females is in some way abnormal and is unable to satisfy the requirements of vitellogenesis. Perhaps sterility in the other mutants is due to the same cause.

For several reasons it is unlikely that the abnormal abdominal environment and poor viability are caused by starvation. First, the adipose tissue of wild-type flies that are starved 1 to 2 days becomes devoid of all reserve substances (WIGGLESWORTH 1949), but adipose tissue of ap^4/ap^4 flies possess deposits of lipid and glycogen and the larval cells contain protein globules. Second, KING and SANG (1958) have shown that the ap^4/ap^4 flies can ingest food. Although the foreventriculus is packed with a transparent material (probably disorganized peritrophic membrane), more posterior regions of the gut contain recently ingested yeast. These workers demonstrated the pH of the lumen of the ventriculus to be abnormal. When both normal and ap^4/ap^4 female adults were fed dead yeast containing various pH indicators, they found that the ventriculus of day-old, wild-type females is generally pH 6, but that of ap^4/ap^4 females is only pH 5. The abnormality in pH suggested to KING and SANG that ap^4/ap^4 flies could not hydrolyze proteins. However, ap^4/ap^4 larvae when reared axenically on a minimal medium containing only those low molecular weight compounds required for normal oogenesis, including a casein hydrolysate, developed into adults that did not differ phenotypically from those raised on the usual cornmeal, molasses, yeast diet. Thus the abnormalities characteristic of the ap^4/ap^4 female adults cannot be ascribed simply to the inability of the organism to hydrolyze the complex macromolecules normally present in its diet.

ROBERTSON and SANG (1944) demonstrated that the fecundity of Drosophila adult females is influenced by the quantity and quality of food eaten during the

larval stages. SANG and KING (1961) have shown that adults of Drosophila reared on a protein-free medium produce a few normal eggs at first, but subsequently cease laying eggs. Normal vitellogenesis occurs only if the flies' diet is supplemented with protein or a mixture of certain amino acids. Since adipose cells of the adult type contain no protein deposits, it is reasonable to assume that the stores of protein in the larval adipose cells surviving metamorphosis normally supply the necessary amino acids for the production of the few normal eggs made during the period of protein deprivation. Perhaps the *ap* ovary cannot obtain the reserve substances because the larval adipose cells do not break down. Interestingly enough, DAY (1943) has shown that the corpus allatum must be present in the adult flies Lucilia and Sarcophaga, in order that the surviving larval adipose cells histolyze.

In Drosophila, it is clear that secretions of the corpus allatum are essential for vitellogenesis (VOGT 1943; BODENSTEIN 1947), and similar conclusions have been reached by a number of investigators for other muscoid flies [THOMSEN (1942) for *Calliphora erythrocephala*; DAY (1943) for *Lucilia sericata* and *Sarcophaga securifera*; ORR (1964) for *Phormia regina*]. All these studies have demonstrated that the ovaries of allatectomized females contain only oocytes in previtellogenic stages, provided the operation is performed early enough in adult life. ORR's studies suggest that the corpus allatum regulates the ovary indirectly through its action upon the lipid metabolism of the adult adipose cells. Drosophila yolk contains myriads of tiny lipid droplets and smaller numbers of large, lipoprotein α spheres (KING 1960), and it may well be that some essential precursors of the lipoidal components of yolk are synthesized by hormonally activated adipose cells and are subsequently transferred to the egg-nurse cell complexes during vitellogenesis.

One can assume from the studies of THOMSEN (1954) that the corpus allatum is activated by neurosecretory material manufactured by special clusters of cells in the pars intercerebralis region of the brain and transported to the corpus allatum in axons which pass through the corpus cardiacum. It is also known (MILLER 1950) that the cardia is innervated by nerves arising from the corpus cardiacum. Perhaps the abnormal secretion of peritrophic membrane and the paralysis which mark the extreme apterous syndrome are a manifestation of an underlying neurological disturbance. A malfunctioning of the intercerebralis-cardiacum-allatum system could well result in the manifold abnormalities described above, and consequently a cytological investigation of this system in ap^4/ap^4 homozygotes seems justified.

The authors gratefully acknowledge support from the Public Health Service (grant 2-F1-GM-15,795) and express their thanks to MR. E. JOHN PFIFFNER for skillfully inking and lettering the figures.

SUMMARY

Phenotypes of homozygotes and compounds of various alleles of apterous (*ap*) were compared. Adults of all *ap* genotypes studied have short, abnormal wings.

ap wings are small because they contain fewer cells than wild type. Adults having the allele ap^{Xa} have the longest wings, and hemizygotes usually have the shortest. The genetic effects on haltere length parallel those on wing length. Wings of *ap* flies lack three of the five hair types found in the wild-type wing. However, the hair types that are observed are present in the same concentration as in wild type and are those characteristically found on the basal portion of the wild-type wing. *ap* appears to inhibit thoracic bristle formation, since hemizygotes have more normal numbers of bristles. Preadult viability is generally greater with ap^{56f} than with other alleles. The *ap* genotypes fall into three adult longevity classes: (1) short-lived, (2) long-lived, and (3) having flies belonging to either a long-lived or a short-lived subpopulation. Females of (2) and the long-lived subpopulation of (3), lay eggs. Other females are sterile. Generally *ap* flies with ap^{56f} are long-lived, and the females lay eggs. The ventriculi of ap^4/ap^4 adult females are packed with what appears to be disorganized peritrophic membrane. During the first two days of adult life in *ap* flies possessing an ap^{56f} allele and in wild-type flies, those larval adipose cells which have survived metamorphosis histolyze, and the adult adipose tissue increases in volume. In the adults of certain *ap* genotypes, the larval adipose cells fail to histolyze and the adult adipose tissue remains immature. *ap* genotypes having abnormal adipose tissues are also female-sterile and short-lived. It is suggested that a malfunctioning of the intercerebralis-cardiacum-allatum system may underly the lowered fertility and longevity of these flies.

LITERATURE CITED

AUERBACH, C., 1936 The development of the legs, wings and halteres in wild-type and some mutant strains of *Drosophila melanogaster*. Trans. Roy. Soc. Edinburgh **58**: 787–815.

BAKER, J. R., 1946 The histochemical recognition of lipine. Quart. J. Microscop. Sci. **87**: 441–471. —— 1956 Improvement in the Sudan black technique. Quart. J. Microscop. Sci. **97**: 621–623.

BODENSTEIN, D., 1947 Investigations on the reproductive system in Drosophila. J. Exptl. Zool. **104**: 101–151. —— 1950 The postembryonic development of Drosophila. pp. 275–367. *Biology of Drosophila*. Edited by M. DEMEREC. Wiley, New York.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**.

BUTTERWORTH, F. M., D. BODENSTEIN, and R. C. KING 1965 Adipose tissue of *Drosophila melanogaster*. I. An experimental study of larval fat body. J. Exptl. Zool. **158**: 141–154.

CASSELMAN, W. G. B., 1959 *Histochemical Techniques*. Methuen, London.

CHEN, T-Y, 1929 On the development of imaginal buds in normal and mutant *Drosophila melanogaster*. J. Morphol. **47**: 135–199.

CHIPPENDALE, G. M., S. D. BECK, and F. M. STRONG, 1965 Nutrition of the cabbage looper, *Trichoplusia ni* (Hubner). I. Some requirements for larval growth and wing development. J. Insect Physiol. **11**: 211–223.

DAY, M. F., 1943 The function of the corpus allatum in muscoid Diptera. Biol. Bull. **84**: 127–140.

DOANE, W. W., 1960 Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. II. Effects of altered environment and residual genome on its expression. J. Exptl. Zool. **145**: 23–41.

DOBZHANSKY, TH., 1929 The influence of the quantity and quality of the chromosomal material on the size of the cells of *D. melanogaster*. Arch. Entwicklungsmech. Organ. **115**: 363–379.

ELMER, W. A., and L. J. PIERRO, 1964 The Creeper trait in chickens and growth of tibiotarsi *in vitro*. Am. Zoologist **4**: 381.

FERRIS, G. F., 1950 External morphology of the adult. pp. 368–419. *Biology of Drosophila*. Edited by M. DEMEREC. Wiley, New York.

FRAENKEL, G., and M. BLEWETT, 1946 Linoleic acid, vitamin E, and other fat soluble substances in the nutrition of certain insects, *Ephestia kuehniella*, *E. elutella*, *E. cautella*, and *Plodia interpunctella* (Lep.). J. Exptl. Biol. **22**: 172–190.

GOLDSCHMIDT, R., 1935 Gen und Aussencharacter. III. Biol. Zbl. **55**: 535–554.

HADORN, E., G. ANDERS, and H. URSPRUNG, 1959 Kombinate aus teilweise dissoziierten Imaginalscheiben verschiedener Mutanten und Arten von Drosophila. J. Exptl. Zool., **142**: 159–175.

HADORN, E., and D. BUCK, 1962 Ueber Entwicklungsleistungen transplantierter Teilstücke von Flügel-Imaginalscheiben von *Drosophila melanogaster*. Rev. Suisse Zool. **69**: 302–310.

KING, R. C., 1960 Oogenesis in adult *Drosophila melanogaster*. IX. Studies on the cytochemistry and ultrastructure of developing oocytes. Growth **24**: 265–323. —— 1965 *Genetics*. 2nd edition. Oxford, New York.

KING, R. C., and D. BODENSTEIN, 1965 The transplantation of ovaries between genetically sterile and wild-type *Drosophila melanogaster*. Z. Naturforsch. **20b**: 292–297.

KING, R. C., and R. G. BURNETT, 1957 Oogenesis in adult *Drosophila melanogaster*. V. Mutations which affect nurse cell nuclei. Growth **21**: 263–280.

KING, R. C., and E. A. KOCH, 1963 Studies on the ovarian follicle cells of Drosophila. Quart. J. Microscop. Sci. **104**: 297–320.

KING, R. C., and J. H. SANG, 1958 Additional description of *ap*^{4}. Drosophila Inform. Serv. **32**: 133.

LEES, A. D., 1941 Operations on the pupal wing of *Drosophila melanogaster*. J. Genet. **42**: 115–142.

LEES, A. D., and C. H. WADDINGTON, 1942 The development of the bristles in normal and some mutant types of *Drosophila melanogaster*. Proc. Roy. Soc. London B **131**: 87–110.

METZ, C. W., 1914 An apterous Drosophila and its genetic behavior. Am. Naturalist **48**: 675–692.

MILLER, A., 1950 The internal anatomy and histology of the imago of *Drosophila melanogaster*. pp. 420–534. *Biology of Drosophila*. Edited by M. DEMEREC. Wiley, New York.

MORGAN, T. H., C. B. BRIDGES, and J. SCHULTZ, 1936 The constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Yearbook **35**: 289–297. —— 1938 The constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Yearbook **37**: 304–309.

ORR, C. W. M., 1964 The influence of nutritional and hormonal factors on the chemistry of the fat body, blood, and ovaries of the blowfly, *Phormia regina* Meig. J. Insect Physiol. **10**: 103–119.

ROBERTSON, C. W., 1936 The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes. J. Morphol. **59:** 351–399.

ROBERTSON, F. W., and J. H. SANG, 1944 The ecological determinants of population growth in a Drosophila culture. I. Fecundity of adult flies. Proc. Roy. Soc. London B **132:** 258–277.

SACKS, J., 1953 *Isotopic Tracers in Biochemistry and Physiology*. McGraw-Hill, New York.

SANG, J. H., and R. C. KING, 1961 Nutritional requirements of axenically cultured *Drosophila melanogaster* adults. J. Exptl. Biol. **38:** 793–809.

THOMSEN, E., 1942 An experimental and anatomical study of the corpus allatum in the blow-fly *Calliphora erythrocephala* Meig. Videnskabelige Meddelelser Fra Dansk Naturhistorisk Forening **106:** 320–405.

THOMSEN, E., 1954 Studies on the transport of neurosecretory material in *Calliphora erythrocephala* by means of ligaturing experiments. J. Exptl. Biol. **31:** 322–330.

VOGT, M., 1943 Zur Produktion gonadotropin Hormones durch Ringdrüsen des ersten Larvenstadiums bei Drosophila. Biol. Zbl. **63:** 467–470.

WADDINGTON, C. H., 1940 The genetic control of wing development in Drosophila. J. Genet. **41:** 75–139.

WIGGLESWORTH, V. B., 1949 The utilization of reserve substances in Drosophila during flight. J. Exptl. Biol. **26:** 150–163.

WILSON, L. P., R. C. KING, and J. L. LOWRY, 1955 Studies on the tu^{w} strain of *Drosophila melanogaster*. I. Phenotypic and genotypic characterizations. Growth **19:** 215–244.