THE MOLECULAR THROUGH ECOLOGICAL GENETICS OF ABNORMAL ABDOMEN IN *DROSOPHILA MERCATORUM*. I. BASIC GENETICS

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ABSTRACT

The abnormal abdomen syndrome (*aa*) in *Drosophila mercatorum* is characterized by the persistence of juvenilized cuticle on the adult abdomen. The *aa* phenotype is shown to depend on at least two *X*-linked genetic elements that are about one map unit apart near the centromeric end of the *X* chromosome. These two genetic elements are necessary for *aa* expression; one behaves as a dominant element and the other as a recessive. Overlaying these genetic studies upon molecular work reported elsewhere, it is argued that the dominant element is the presence of a 5 kb insertion in a majority of the *X*-linked repeats coding for the 28S ribosomal RNA. The recessive element appears to be a locus controlling differential replication of noninserted over inserted 28S genes during polytenization. The *aa* syndrome requires both the presence of the inserted repeats and the failure to preferentially amplify noninserted repeats. Given the necessary *X*-linked elements for *aa*, a variety of modifiers are revealed. First, *aa* expression in males is *Y*-linked, apparently corresponding to a deletion of the 18S/28S rDNA gene cluster normally found on the *Y*. Moreover, all major autosomes can modify the penetrance of *aa*.

THE abnormal abdomen syndrome in *Drosophila mercatorum* is characterized by the persistence of juvenilized cuticle on the adult abdomen, thereby causing an irregular absence of distinct tergites and sternites and of the bristle and pigment patterns normally found on the abdomen. In addition, the abnormal abdomen (*aa*) flies tend to have a longer egg-to-adult developmental time, earlier ovarian maturation and onset of oviposition measured from eclosion, an overall increase in fecundity and a decrease in adult longevity (TEMPLETON and RANKIN 1978; TEMPLETON 1982, 1983). The syndrome was first discovered while doing experiments on stocks derived from a screening of a natural population of *D. mercatorum* for parthenogenetic capacity (note, all natural populations are sexually reproducing). This screening resulted in the establishment of a parthenogenetic strain, designated by K28-0-Im (TEMPLETON, CARSON and SING 1976). This strain was phenotypically normal, but when it was crossed to another phenotypically normal stock, *aa* flies appeared in some of the parthenogenetic progeny of the resulting hybrid females (TEMPLETON 1979). A more detailed examination of older laboratory stocks and

recently established isofemale lines indicated that this syndrome was common and widespread (TEMPLETON and RANKIN 1978).

A series of studies have been performed on the *aa* syndrome, ranging from the molecular through the ecological. This paper is the first in a series that will present these studies, with the focus of this paper being the basic Mendelian and quantitative genetics of *aa* scored as a morphological trait. Later papers in this series will investigate the molecular basis of *aa*, the physiological consequences of *aa* on juvenile hormone metabolism, the developmental and life history consequences of the altered juvenile hormone metabolism and, finally, the adaptive significance of these life-history alterations in a natural population.

MATERIALS AND METHODS

Modes of reproduction: In the genetic experiments to be described, ordinary sexual reproduction is generally used, but in some cases, parthenogenetic reproduction occurs. Under sexual reproduction, *mercatorum* is very similar to *melanogaster* in its basic genetic attributes, including the absence of crossing over in males. Under parthenogenetic reproduction, an unfertilized egg undergoes normal meiosis, including the genetic phenomena of segregation, independent assortment and recombination. After the production of a haploid egg nucleus, diploidy is almost always restored by fusion of two cleavage nuclei derived by mitosis from the haploid egg nucleus (gamete duplication). This results in total homozygosity in the parthenogenetic strains. Other types of fusion occasionally restore diploidy in unfertilized eggs, particularly fusion of the pronuclei that separate at meiosis I (central fusion). More details about parthenogenesis in *D. mercatorum* can be found in TEMPLETON (1983).

Strains: The two primary strains in these experiments are the parthenogenetic strain K28-0-Im (hereafter abbreviated by K) and the stock S-sl v pm vl-Br14 (hereafter abbreviated by S). Although the S stock is sexual, it was obtained by repeated backcrosses to the parthenogenetic strain S-1-Im in order to introduce visible markers on a totally homozygous background [see TEMPLETON, SING and BROKAW (1976) for details]. The S-1-Im strain was already homozygous for the *X*-linked recessive trait spotless (*sl*), which is the absence of the pigment spot at the base of the middle orbital bristle. In addition, the following autosomal recessive alleles were introduced by backcrossing: vermillion eyes (*v*) on the metacentric, plum eyes (*pm*) on the acrocentric *I*, and veinless wings (*vl*) on the acrocentric *II* chromosome. Only the dot chromosome lacks a visible marker in this strain. The K strain was established from collections made near Kamuela, Hawaii, whereas the S strain's ancestors came from San Salvador.

The *X*-linked visible markers white eyes (*w*) and yellow body (*y*) were introduced onto the S autosomal background by hybridizing with a *w y* stock, followed by backcrossing to S. The *y* phenotype is also spotless (*sl*), and indeed, these two phenotypes have never been separated. Hence, *y* is either at the same locus as *sl* or is very tightly linked to *sl*. Moreover, the *X* chromosome from the *w y* stock traces its origin primarily to the S-1-Im strain and has never been associated with the expression of *aa*. A parthenogenetic strain bearing all these visible markers was established and is designated by S-y w v pm vl-Im.

Various tester strains displaying the morphological effects of *aa* with high penetrance are also used. The first of these is designated by 50% F aa. This strain was formed by artificial selection for extreme *aa* phenotypes among the progeny of an isofemale line established in 1974 from the natural population near Kamuela, Hawaii. After four generations, the penetrance of *aa* plateaued at about 50% of the females showing some morphological disruption of abdominal cuticle, with males being normal (further details can be found in TEMPLETON and RANKIN 1978). The 100% F aa strain was etablished by crossing a male from the 50% F aa strain with a K28-0-Im female, followed by additional generations of artificial selection. This strain shows 100% penetrance of *aa* in females under standard laboratory conditions (25°, no larval crowding). Further details can be

found in TEMPLETON and RANKIN (1978). Both the 50 and 100% aa strains normally have expression of *aa* limited to females.

Additional strains were bred from the strains described above as part of the genetic analysis of *aa*, but these strains will be described in the RESULTS section.

Scoring of aa: The amount of abdominal cuticle affected in *aa* flies can vary from just a tiny patch to one covering the entire abdomen. To measure the extent of the abnormality, the following scoring conventions were employed. If only a small patch of cuticle amounting to less than 5% of the total abdominal cuticle were affected, the fly was assigned a score of 1. If between 5% and 50% were affected, the fly was designated by a 2, and if more than 50% were abnormal, a score of 3 was assigned. The penetrance of *aa* is operationally defined in this paper as the total number of flies showing *aa* (regardless of score) divided by the total number of flies examined. The expressivity of *aa* is defined as the average score of those flies showing any degree of *aa*. The total expressivity (P × E) is the product of the penetrance with the expressivity and can be regarded as the average score of all flies examined with the convention that non-*aa* flies are assigned a score of 0.

RESULTS

The inheritance of male expression: The expression of *aa* was limited to females in all the *aa* stocks initially isolated. All *aa* strains occasionally produce *aa* males (up to 2%), but these males usually turn out to be sterile and are presumably *XO*. However, one of the *aa* males produced by the 100% F *aa* strain turned out to be fertile, and matings of this male with his sisters led to the establishment of the 100% MF *aa* strain in which *aa* is expressed in both males and females. The penetrance of *aa* in males was initially around 70% in this strain, but artificial selection subsequently raised it to close to 100%. Reciprocal crosses were made between the 100% F and MF strains when the male penetrance was 70%, followed by both types of reciprocal backcrosses for each of the two original reciprocal crosses. The results are shown in Table 1. As can be seen from that table, a high penetrance of *aa* in males is paternally inherited. Whenever the Y chromosome came from the MF strain, *aa* was expressed at high levels in the male progeny; whenever the Y chromosome came from the F strain, *aa* was expressed only at the low levels typical of the original F strain. Hence, the expression of *aa* in males is controlled by a Y-linked element, and hereafter we will refer to the Y chromosome from the 100% MF aa stock as *aa*-Y. It is also important to note that in all the subsequent crosses with this strain to other strains, that male expression of *aa* only occurs when the males inherit both the *aa*-Y and an X chromosome that allows expression of *aa* when homozygous in females. The *aa-Y* chromosome when coupled with a non-*aa* *X* chromosome has no observable phenotypic effects in males.

The genetics of aa-X *chromosomes*: Because *aa* was first discovered in the recombinant progeny of hybrids between the K and S stocks, TEMPLETON and RANKIN (1978) bred males with all possible combinations of K and S major chromosomes and testcrossed these males to the 50% F aa stock. Their results indicated that *aa* expression was only observed when the males bore a K-type *X* chromosome, and no autosomal combination in the absence of a K-derived *X* ever expressed *aa*. Moreover, given a K-type *X*, *aa* expression was observed in all autosomal combinations, although the penetrance of *aa* in these flies was

TABLE 1

Phenotypes of male and female progeny used to examine the genetics of male expression of aa

	Cross					
	F × MF		F_1 (F × MF) × MF		MF × F_1 (F × MF)	
Progeny	♀	♂	♀	♂	♀	♂
+	1	68	13	14	1	16
aa(1)	43	46	23	25	5	20
aa(2)	56	71	27	48	29	20
aa(3)	5	6	0	1	0	0
P	0.99	0.64	0.79	0.84	0.97	0.71
E	1.63	1.67	1.54	1.68	1.85	1.50
	Cross					
	MF × F		F_1 (MF × F) × MF		MF × F_1 (MF × F)	
Progeny	♀	♂	♀	♂	♀	♂
+	0	123	9	19	0	58
aa(1)	29	1	17	11	2	4
aa(2)	41	1	60	58	28	0
aa(3)	9	0	1	16	12	0
P	1.00	0.02	0.90	0.82	1.00	0.07
E	1.75	1.50	1.79	2.06	2.24	1.00

The two primary strains are the 100% F aa strain for which the males did not display *aa* and for which the abbreviation "F" is used in the table) and the 100% MF aa strain (for which the males displayed *aa* with a 70% penetrance at the time of these crosses and for which the abbreviation "MF" is used). In all cross designations, the stock origin of the female parent is given first. The progeny were scored for the presence and degre of *aa* (on a scale of 1–3), and the penetrance (P) and expressivity (E) were calculated as explained in the text.

influenced by the autosomes, with the acrocentric *II* autosome (marked by *vl* in the S stock) being the major enhancer of *aa*. Thus, the major and necessary locus or loci for *aa* resides on the *X* chromosome.

To further confirm this hypothesis, various strains were bred to examine the genetic control of *aa* expression in the recombinants between K and S. Neither the K nor S strains display the aa phenotype. However, when K and S are crossed and the resulting F_1 females are allowed to reproduce parthenogenetically, 0.4% of the resulting parthenogenetic F_2 (two of 522) displayed an aa phenotype in the original experiments of TEMPLETON (1979). Although this incidence is low, it is replicable with 0.8% (seven of 829) displaying aa phenotypes in a subsequent repeat of this experiment, for a combined incidence of 0.7%.

Since the testcross results of TEMPLETON and RANKIN (1978) indicated that the K stocks provided the necessary elements for *aa*, a set of crosses was initiated that would put a single K-derived chromosome upon an otherwise S background. For the *X* chromosome, a K × S F_1 male was backcrossed to an

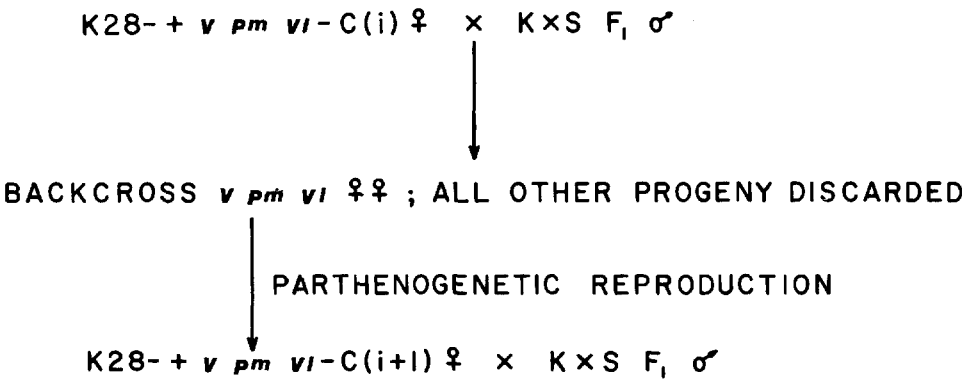

FIGURE 1.—The breeding scheme used to place a K28-0-Im *X* chromosome on a S-sl v pm vl-Br14 autosomal background. K × S F_1 males were generated by crossing a K28-0-Im female to S-sl v pm vl-Br14 males. These F_1 males were backcrossed to S-sl v pm vl-Br14 females, and the resulting females that were homozygous for the visible autosomal markers were designated as K28- + v pm vl-C1. These females were then used to initiate the breeding scheme illustrated in this figure.

S female, and the v pm vl female progeny were retained. These females would be heterozygous for the K and S type *X* chromosomes, and homozygous for all the major S autosomes. They were allowed to reproduce parthenogenetically, resulting in totally homozygous daughters. The daughters were scored for *sl* and *aa*, and those that were spotted (homozygous for the K allele) were backcrossed to another K × S F_1 male. This basic breeding cycle is illustrated in Figure 1 and was continued for a total of ten cycles. Obviously, with each cycle, more and more of the *X* chromosome should be of the K type. By the end of the cycle, the resulting females should be homozygous for a K-type *X* chromosome and homozygous for an S-type set of major autosomes. Similar breeding cycles were also carried out for each of the major autosomes, with the primary difference being that selected backcross males were used instead of K × S F_1 males. These backcross males were produced as follows. First, a K × S F_1 male was backcrossed to an S female. The resulting backcross males would be hemizygous for the S-type *X* chromosome and would be either homozygous S or K/S heterozygous for any particular autosome. Backcross males that were homozygous for two of the S markers and heterozygous for the third were chosen for the next breeding cycle. By selecting against the visible marker at the autosomal locus for which the males are heterozygous among the parthenogenetic females produced at each cycle, one obtains flies that are homozygous S for the *X* and two of the autosomes, while more and more homozygous K for the remaining major autosome. These breeding schemes were continued for 12 cycles for the metacentric autosome marked by *v*, ten cycles for the acrocentric *I* autosome marked by *pm*, and eight cycles for the acrocentric *II* autosome marked by *vl*.

During these breeding cycles, neither the parthenogenetic nor sexual progeny of any of the three autosomal contrasts ever produced an *aa* fly. However, *aa* did appear at a very low frequency among some of the parthenogenetic

TABLE 2

The incidence of aa *phenotypes among the parthenogenetic females produced at each breeding cycle during the production of the K28- + v pm vl-Br10 strain*

	Parthenogenetic female phenotypes	
Cycle no.	Normal abdomen	Abnormal abdomen
1	23	0
2	18	0
3	35	0
4	249	1
5	65	0
6	55	0
7	205	0
8	237	0
9	37	0
10	48	0

and sexual progeny in the contrast involving the *X* chromosome. Two *aa* flies appeared at the third backcross cycle among the sexual progeny, and one appeared during the fourth cycle of parthenogenetic reproduction (Table 2). This single *aa* fly was allowed to reproduce parthenogenetically to establish the stock designated K28-aa v pm vl-C4, and the penetrance of *aa* in this strain is 0.54, with an expressivity of 1.93 (based on a sample of 111 females). Note from Table 2 that, after the fourth cycle, *aa* never reappeared, nor has *aa* ever been observed in the stock K28 + v pm vl-C10 in over 4 yr of stock maintenance. Hence, homozygosity for a K-type *X* chromosome does not allow the expression of *aa* in either a K or S autosomal background. However, as mentioned earlier, when recombination is allowed between K and S genomes, 0.7% of the resulting parthenogenetic F_2 progeny show *aa*. In this regard, it should be kept in mind that, during the early cycles, there is much potential heterozygosity for K and S *X*-chromosomal sections, and hence there is potential for genetically meaningful recombination of the *X* chromosomes, but not of the autosomes. This potential for recombination between K and S *X*-chromosomal sections is progressively lost with each increasing cycle number.

The results of these chromosomal contrast strains clearly localize the occurrence of *aa* in S × K recombinants to the *X* chromosome because *aa* appeared only in the strain allowing for *X*-chromosomal recombination and in none of the strains at risk for autosomal recombination. Moreover, the incidence of *aa* among the parthenogenetic progeny at cycle 4 was 0.4%, a result compatible with the previous incidences measured when recombination was allowed between the entire K and S genomes. The absence of *aa* after the fourth cycle implies that heterozygosity for the appropriate S-type section of the *X* chromosome was lost after the fourth cycle. Moreover, the fact that the *X* chromosome was homozygous for the K + allele at the *sl* locus from the first cycle on implies that the section of the *X* chromosome involved with *aa* is not closely

linked to *sl*. Otherwise, there would be no opportunity for K/S heterozygosity in the early cycles.

To map the *aa* section of the *X* chromosome more precisely, S-y w v pm vl-Im females were crossed to 100% MF aa males. (The *w* locus had previously been mapped to one end of the *mercatorum X* chromosome, with *y* and *sl* being in the middle.) Some of the resulting F_1 males were then backcrossed to S-y w v pm vl-Im females, and the backcross males displaying the y w vl phenotype were then testcrossed to S-sl v pm vl-Br14 females. Only those males that were homozygous for *v* and *pm* were retained (note, the white-eyed phenotype of the *w* locus epistatically masks the eye-color phenotypes associated with the *v* and *pm* loci). The *Y* chromosome of these males was derived from the 100% MF aa stock (which by this time was showing 100% male penetrance), but all other major chromosomes come from the S marker stock. Recall that this *Y* chromosome allows expression of *aa* in males hemizygous for an *aa-X*. These males were designated by the symbols y w v pm vl aa-Y.

The remainder of the F_1 males from the S-y w v pm vl-Im by 100% MF aa cross were backcrossed to 100% MF aa females. The resulting backcross females will be heterozygous at the *X* chromosome, with one *X* bearing the alleles *y*, *w* and + (*i.e.*, non-*aa*), and the other *X* having +, + and *aa*. The autosomes of these backcross females will be either homozygous for the 100% MF *aa* type or *aa*/S heterozygous, with all major S autosomes marked by a visible marker. These backcross females were then crossed to y w v pm vl aa-Y males. The incidence of *aa* was then recorded in the males, as well as the relevant phenotypes at the *y* and *w* loci. The data on the *y*, *w* and *aa* phenotypes in the males constitute a standard three-point testcross. Because of potential reductions in *aa* penetrance due to autosomal background, the testcross progeny were scored separately for each individual backcross female. From the phenotypes of both her male and female progeny, the exact autosomal genotype of the backcross female could be determined, and hence the effects of this background on *aa* penetrance is the testcross male progeny. There were no significant effects of female autosomal genotype on *aa* penetrance in the testcross male progeny, so Table 3 gives the results pooled over all females. As can be seen from Table 3, the section of the *X* chromosome responsible for *aa* maps to the end of the *X* chromosome opposite the *sl* locus, with *y* being in the middle, as expected from the results obtained in breeding the K and S chromosomal contrast strains.

The data given in Table 3 can also be used to localize the *aa* region specifically to the centric end of the *X* chromosome when combined with linkage data on the isozyme locus glucose-6-phosphate dehydrogenase (*G6PD*). Table 4 shows the various male genotypes scored from a three-point testcross of the *X*-linked markers *w*, *y*, and *G6PD*. The distance between *w* and *y* is not statistically significantly different from that given in Table 3, and the data in Table 4 imply that *G6PD* is close to *w* and even more distant than *w* from *y*. Hence, *G6PD* is at the opposite end from *aa* in the mapped portion of the *mercatorum X*. The advantage of knowing where *G6PD* is relative to *aa* is that females can be easily scored for heterozygosity at the *G6PD* locus through standard elec-

TABLE 3

Results of a three-point testcross and the inferred linkage map of the X-linked loci white eyes (w), yellow body (y), and abnormal abdomen (aa)

Phenotype of son	No. of flies
w y +	153
+ + aa	332
w y aa	157
+ + +	142
w + aa	158
+ y +	135
w + +	85
+ y aa	114
Total	1276

Inferred Map: w — 38.4 — y — 39.0 — aa

TABLE 4

Results of a three-point testcross and the inferred linkage map of the X-linked loci white eyes (w), yellow body (y), and glucose-6-phosphate dehydrogenase (G6PD)

Phenotype of son	No. of flies
S w y	45
F + +	48
F w y	3
S + +	2
F + y	33
S w +	19
F w +	0
S + y	0
Total	150

Inferred Map: G6PD — 3.3 — w — 34.7 — y

Female flies with the genotypes *w y S/+ + F*, where *S* and *F* are alleles at the *G6PD* locus reproduced, and the phenotypes of their male progeny were scored for all three *X*-linked loci.

trophoretic techniques (TEMPLETON, SING and BROKAW 1976). Being able to assay heterozygosity allows centromere mapping to be done under parthenogenetic reproduction. For details of how parthenogenesis can be used for centromere mapping, see TEMPLETON (1983), and particularly figure 5 of that paper.

Basically, a parthenogenetic strain that restores diploidy through gamete duplication 96% of the time and through central fusion 4% of the time was made heterozygous at the *G6PD* locus (TEMPLETON, SING and BROKAW 1976). As discussed in TEMPLETON (1983), if a locus is absolutely linked to its centromere, then all cases of central fusion will preserve heterozygosity at that locus under parthenogenetic reproduction. However, if the locus is randomly recombining with its centromere, the heterozygosity should be two-thirds the central fusion rate. Using unpublished data from the parthenogenetic progeny of *G6PD* heterozygous virgin females described in TEMPLETON, SING and BROKAW (1976), 20 heterozygotes for *G6PD* were detected in 950 parthenogenetic progeny of heterozygous virgin females, giving a heterozygote frequency of 0.021. This value is significantly different from the value of 0.040 that is expected under close linkage to the centromere, but it is not significantly different from the expected value of 0.027 obtained under the hypothesis of random recombination with the centromere. Hence, *G6PD* is located very far away from the centromere, which, in turn, implies that *aa* is located at the centric end of the *X* chromosome.

The genetics of the autosomal modifiers: Although the necessary genetic elements for *aa* reside on the *X* chromosome, the results of TEMPLETON and RANKIN (1978) clearly show that the penetrance and expressivity of *aa* can be greatly influenced by autosomal modifiers. To investigate these autosomal modifiers, additional crosses were made in which the *X* chromosome state was fixed but various autosomal contrasts were generated. The first such contrast was a straightforward extension of the chromosomal contrasts given in TEMPLETON and RANKIN (1978). These chromosomal contrasts were generated by first crossing K females with S males and by then crossing the resulting F_1 males to K28- + v pm vl-C5 females. The male progeny of this cross should be hemizygous for a K-type *X* chromosome and either heterozygous K/S or homozygous S for any particular major autosome. Moreover, the autosomal state of any male can be inferred directly by its phenotype for *v*, *pm* and *vl*. These males of known genetic composition were testcrossed to 50% F aa females, and the incidence of *aa* in their female progeny was scored. It was these scores that TEMPLETON and RANKIN (1978) used to infer that the major enhancer of *aa* is on the K acrocentric *II* chromosome (marked by *vl* in S). Unfortunately, due to the fact that *v*, *pm* and *vl* are recessive alleles, the autosomal genotype of the female progeny scored for *aa* is not directly observable in this testcross for *aa*. To overcome this deficiency, the experiment was repeated, and the females from the *aa* testcross were scored for *aa* and then were mated with S males as a testcross for *v*, *pm* and *vl*. In this way, the actual autosomal genotype of the *aa* testcross females could be determined. Of the eight possible autosomal genotypes, two can be produced more directly. First, the female genotype having all autosomes derived from K can be produced by simply crossing K females with 50% F aa males. Second, the female genotype having all autosomes derived from S can be produced by crossing K28- + v pm vl-C5 females with 50% F aa males. The total data set obtained

TABLE 5

The incidence of abnormal abdomen phenotypes among the female offspring of 50% F aa *females crossed with males hemizygous for the* X *chromosome from the stock K28- + v pm vl-C5 and heterozygous K/S for the three major autosomes*

Genotype	Phenotype +	1	2	3	P	E	P × E
K K K / *aa aa aa*	41	12	34	27	0.64	2.21	1.41
v K K / *aa aa aa*	7	2	10	5	0.71	2.18	1.54
K *pm* K / *aa aa aa*	5	2	3	7	0.71	2.42	1.71
K K *vl* / *aa aa aa*	14	7	7	1	0.52	1.60	0.80
v pm K / *aa aa aa*	21	4	6	7	0.45	2.18	0.97
v K *vl* / *aa aa aa*	26	2	3	0	0.16	1.60	0.26
K *pm vl* / *aa aa aa*	18	9	7	2	0.50	1.61	0.81
v pm vl / *aa aa aa*	206	14	16	5	0.15	1.74	0.25

After the female offspring were scored for *aa*, they were crossed to the S strain as a testcross to determine their autosomal genotype. The *aa* scoring results are given for each of the eight possible autosomal genotypes. Autosomes derived from the K strain are designated by "K," those from 50% F aa by "*aa*," and those from S by the appropriate visible marker ("*v*" for the metacentric, "*pm*" for the acrocentric *I* and "*vl*" for the acrocentric *II*). Flies showing no *aa* are designated by a "+," and flies showing *aa* are designated by a number, as explained in the text. The penetrance (P) and expressivity (E) are calculated as described in the text, as well as their product (P × E).

by the testcrosses and these two supplementary crosses are presented in Table 5.

Three well-defined phenotypic classes of *aa* emerge from these results. First, there is a group of three genotypes with high penetrance (0.64 to 0.71). Let "K" designate autosomes derived from the K stock, and let the appropriate allelic marker symbol designate an S-derived autosome. Presenting the chromosomal genotypes in the following order: metacentric, acrocentric *I* and acrocentric *II*, the females in this high penetrance/expressivity category inherited the autosomal genomes K K K, v K K and K pm K from their father. A second phenotypic category is characterized by intermediate penetrance (0.45 to 0.52) and contains females with the following paternally derived genomes: K K vl, v pm K, and K pm vl. Finally, females inheriting v K vl or v pm vl genomes from their fathers showed low penetrance (0.15 to 0.16) for *aa*. The role of the K acrocentric *II* is unambiguous; on all four autosomal backgrounds in which a K acrocentric *II* is substituted for a *vl* marked autosome, the penetrance of *aa* is greatly enhanced. Hence, as concluded by TEMPLETON and RANKIN (1978), the K acrocentric *II* is clearly a major enhancer of *aa*. The K

TABLE 6

The incidence of abnormal abdomen phenotypes among the female offspring when F_1 males are backcrossed to the 100% F aa *strain*

Genotype	Phenotype				P	E	P × E
	+	1	2	3			
aa aa aa / aa aa aa	1	3	7	0	0.91	1.70	1.55
v aa aa / aa aa aa	0	0	4	2	1.00	2.33	2.33
aa pm aa / aa aa aa	12	10	2	0	0.50	1.17	0.58
aa aa vl / aa aa aa	3	8	7	0	0.83	1.47	1.22
v pm aa / aa aa aa	1	2	13	5	0.95	2.15	2.05
v aa vl / aa aa aa	1	0	12	2	0.93	2.14	2.00
aa pm vl / aa aa aa	9	3	5	0	0.47	1.62	0.76
v pm vl / aa aa aa	1	4	11	1	0.94	1.81	1.71

The F_1 males were produced by crossing 100% F *aa* females to S males. Hence, the F_1 males all bear an *aa-X*. After the female offspring were scored for *aa*, they were crossed to the S strain as a testcross to determine their autosomal genotype. The *aa* scoring results are then given for each of the eight possible autosomal genotypes. Autosomes derived from the 100% F aa are designated by "*aa*," and those from S by the appropriate visible marker ("*v*" for the metacentric, "*pm*" for the acrocentric *I* and "*vl*" for the acrocentric *II*). Flies showing no *aa* are designated by a "+," and flies showing *aa* are designated by a number, as explained in the text. The penetrance (P) and expressivity (E) are calculated as described in the text, as well as their product (P × E).

metacentric and acrocentric *I* also behave as enhancers relative to their S homologues, although they are not nearly as strong as the acrocentric *II*. There also seems to be a saturation phenomenon in terms of the *aa* enhancement. If a fly inherited the K acrocentric *II* (the major enhancer) and one other K autosome, the third autosome has no additional enhancement effect. Moreover, the acrocentric *I* autosome seems to be capable of enhancing *aa* expression only if the fly also inherited a K acrocentric *II*. As a result of these autosomal interactions, the K metacentric enhances in three of the four possible genetic backgrounds on which metacentric substitutions occur, but the acrocentric *I* only enhances in one of the four combinations of the other autosomes.

Another set of contrast experiments was designed to study the effects of S *vs.* 100% F aa autosomes on *aa* expression. The design was very similar to the experiment described above. The 100% F aa females were crossed to S males, the resulting F_1 males (all F_1 individuals of both sexes had normal abdomens) were backcrossed to 100% F aa females. The female progeny stemming from this backcross were scored for *aa* and then were crossed to S males as a testcross for *v*, *pm* and *vl*. The results are shown in Table 6. Here, there are two major phenotypic categories for *aa*: (1) a high penetrant group (0.83 to

1.00) consisting of females that inherited paternal genomes of the compositions *aa aa aa* (letting *aa* designate an autosome derived from the 100% F aa strain), *v aa aa, aa aa vl, v pm aa, v aa vl* and *v pm vl* and (2) an intermediate penetrant group (0.47 to 0.50) consisting of *aa pm aa* and *aa pm vl*. Hence, in this genetic background, the only autosomal variation affecting *aa* penetrance is variation at the acrocentric *I* autosome on a background fixed for the *aa* derived metacentric.

DISCUSSION

The experiments described here support the initial conclusion of TEMPLETON and RANKIN (1978) that the necessary genetic elements for *aa* expression are located on the *X* chromosome. Moreover, the experiments on recombinants of K and S, and more specifically of K and S *X* chromosomes, indicate that a minimum of two genetic elements are needed for *aa* expression. In addition, the fact that hybrids between S and the *aa* tester stocks (100% F, 50% F or MF aa stocks) are wild type, whereas hybrids between K and the *aa* tester stocks are *aa*, implies that one of these necessary elements is dominant and the other is recessive. The frequency of *aa* in S × K recombinants of between 0.4 and 0.7%, a figure that, when coupled with a penetrance of about 50% in these stocks, implies that these two *aa* loci are on the order of one map unit apart from one another. In addition, the mapping experiments show that a terminal section of the *X* chromosome is responsible for *aa*.

These genetic results overlay very well upon recent studies on the molecular basis of *aa* (R. DESALLE, J. SLIGHTOM and E. ZIMMER, unpublished results; R. DESALLE and A. R. TEMPLETON, unpublished results). DESALLE and TEMPLETON have shown that the expression of *aa* depends on two molecular events, both involving the ribosomal DNA (rDNA) tandem gene cluster that is found near one end of the *X* chromosome. First, *aa* requires that a majority of the 28S rDNA cistrons be interrupted by a 5 kb insertion. Second, *aa* requires that there be no differential replication of interrupted *vs.* noninterrupted 28S cistrons during the formation of polytene tissues, such as the fat body. The preferential replication of noninterrupted repeats suppresses *aa*, and preferential replication occurs both interchromosomally and intrachromosomally (R. DESALLE and A. R. TEMPLETON, unpublished results). Furthermore, R. DESALLE, J. SLIGHTOM and E. ZIMMER (unpublished results) examined the K strain in some detail, as well as K28-ger, a spontaneous mutant stock derived from K that displays *aa* with a penetrance of 0.50 and an expressivity of 1.46 (based on a sample of 113 parthenogenetic females). The original K strain has very few of its 28S cistrons bearing insertions and, hence, lacks one of the necessary elements for *aa* expression. However, the spontaneous mutant showing *aa* has a large number of interrupted 28S genes, but shows no differential replication during polytenization (R. DESALLE and A. R. TEMPLETON, unpublished results). The results reported in this paper and in TEMPLETON and RANKIN (1978) show that the K *X* chromosome allows expression of *aa* when heterozygous with *X* chromosomes coming from an *aa* tester stock. These *aa X* chromosomes have the majority of their 28S genes bearing insertions and

display no preferential replication of noninserted cistrons during polytenization (R. DESALLE and A. R. TEMPLETON, unpublished results). The combined molecular and genetic data imply that the recessive genetic element carried on the K *X* chromosome needed for *aa* expression is the allele causing a failure of differential replication of noninserted repeats during polytenization. The dominant element needed for *aa* expression that is lacking on the K *X* chromosome is the occurrence of inserted 28S cistrons in a large fraction of the rDNA repeats. This type of dominance pattern is to be expected, given the molecular observation that the differential replication can be both interchromosomal as well as intrachromosomal. Under this interpretation, the genetic results reported in this paper indicate that the locus controlling differential replication is located about one map unit from the 18S/28S rDNA tandem cluster.

The work of R. DESALLE, J. SLIGHTOM and E. ZIMMER (unpublished results) also provides a straightforward explanation for the *Y*-linked inheritance of *aa* expression in males. Their work has shown that most *Y* chromosomes bear a cluster of 18S/28S genes that is distinguishable from the cluster on the *X* chromosome due to a difference in nontranscribed spacer length. R. DESALLE and A. R. TEMPLETON (unpublished results) have shown that when these normal *Y* chromosomes are present in a male hemizygous for an *aa X* chromosome, there is a differential replication of the *Y*-specific repeats, thus accounting for the *Y*-linked suppression of *aa* in males. Their results then showed that *Y* chromosomes allowing expression of *aa* in males are characterized by a total absence of the *Y*-specific rDNA restriction pattern. Hence, the molecular basis for the *Y*-linked inheritance of male expression is simply explained as a deletion of the rDNA gene cluster normally found on the *Y*.

The molecular studies, unfortunately, offer no clues at present as to the molecular basis of the modifiers of *aa*. The genetic studies reveal that the expression of *aa* can be greatly influenced by autosomal genes that epistatically interact not only with the major *aa* gene complex on the *X* chromosome but also with each other. The genetic studies further imply that the major modifiers of *aa* expression are located on the acrocentric *II* chromosome, with the metacentric being the next in importance and the acrocentric *I* being the least important. In the terminology of TEMPLETON (1981), the *aa* syndrome has a type II genetic architecture consisting of two major segregating elements—the 18S/28S rDNA cluster and the locus controlling differential replication of noninserted *vs.* inserted repeats—coupled with many epistatic modifiers.

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