Structural organization of the *Amy* locus in seven strains of *Drosophila melanogaster*

R.M.Gemmill¹, P.E.Schwartz and W.W.Doane

Department of Zoology, Arizona State University, Tempe, AZ 85287 and ¹The Genetics Center, Southwest Biomedical Research Institute, Tempe, AZ 85281, USA

Received 15 January 1986; Revised 12 May 1986; Accepted 21 May 1986

ABSTRACT

Restriction maps were made by Southern blot analysis of the *Amy* (α -amylase) region in 7 strains of *D. melanogaster* using endonucleases *SalI*, *XhoI* and *EcoRI*. These were compared to the map of λ Dm65 which contains the cloned *Amy* region. Strains used produce either two amylase variants, a single variant, or no amylase, yet all 7 strains carry two *Amy* genes as inverted repeats at the *Amy* locus. This and the orientation of the repeats resembles the situation in λ Dm65. Most restriction sites mapped are conserved but two strains contain a large insertion which differs in size and position between strains. A complex anomaly, probably an inversion, exists at the *Amy* locus in a null strain. Maps for our *Amy*^{1,3} strain and the λ Dm65 clone are identical, the DNA of each having been derived from a Canton-S wild stock. Restriction and genetic maps of the *Amy* region were aligned and alleles assigned to the proximal and distal genes, *Amy-p* and *Amy-d*.

INTRODUCTION

In *Drosophila melanogaster*, the structural gene locus for alpha-amylase (*Amy*, 2-77.7) is in section 54A1-B1 (1) of the right arm of the second chromosome. Eight electrophoretic variants of the amylase protein have been described, and additional variation has been revealed on the basis of heat lability studies (reviewed in 2). Many strains homozygous for chromosome 2 express two amylase isozymes. This, together with a variety of other genetic and biochemical evidence, has been interpreted as indicative of a duplication of the *Amy* structural gene, with each gene copy represented by a number of allozymic forms (3-5). Whether or not the *Amy* gene is duplicated in strains characterized by a single amylase isozyme has been, until recently, a moot point.

The molecular cloning and characterization of sequences from the *Amy* locus of *D. melanogaster* (1, 6) provided direct

evidence that individuals from one strain which produces two electrophoretic amylase variants have two copies of the amylase structural gene at this locus. Flies of this strain, Canton-S, produce amylase isozymes numbered 1 and 3 which are products of the respective genes, Amy¹ and Amy³. The cloned Amy genes, in recombinant phage λ Dm65, encode the appropriate amylase isozymes and are oriented in opposite directions, separated by approximately 5 to 6 kb of DNA (6).

The present study was designed to answer several important questions about the nature of the Amy locus. First, is it typical for strains of D. melanogaster to contain duplicated structural genes at the Amy locus, even when characterized by a single amylase isozyme? Second, in strains that do contain the duplication, are the Amy genes oriented in the same directions as those observed for Canton-S and is the distance separating the two copies the same? Third, in the Amy "null" strain of Haj-Ahmed and Hickey (7) is there a structural rearrangement at the Amy locus which might be responsible for the lack of amylase expression? Fourth, what degree of variability is associated with various Amy strains in terms of specific restriction endonuclease sites within and flanking the Amy genes? Finally, what is the relative orientation of the Amy locus with respect to the genetic and cytological maps of chromosome 2R, i.e., which Amy gene is centromere proximal to the other?

To investigate the above questions, we examined the physical organization of the Amy locus in seven "isogenic" strains of D. melanogaster by means of Southern blot analysis.

MATERIALS AND METHODS

Fly Stocks.

Six of the seven "isogenic" Amy strains of D. melanogaster utilized in this study (Table 1) were originally homozygous for chromosomes 1, 2 and 3 (5, 8, 9) and have been maintained in this laboratory for many years by single pair matings. The exception to this is the "null" strain, which was derived from the Amy "null" strain of Haj-Ahmed and Hickey (7). This strain was made

Table 1. Isogenic Amy strains of D. melanogaster

Strain	Source of Chromosome 2	Amylase Isozymes Expressed	Reference
<u>Amy</u> ^{1c} *	Oregon-R	1	5, 8
<u>Amy</u> ¹ (CA 1)	Capetown 1	1	9
<u>Amy</u> ^{1,3}	Canton-S	1, 3	5, 8
<u>Amy</u> ^{1,6}	Suyama	1, 6	5, 8
<u>Amy</u> ²	Copenhagen	2	4, 5, 8
<u>Amy</u> ^{2,3}	Copenhagen	2, 3	4, 5, 8
<u>Amy</u> null**	Brownsville, Texas	none	7, 18

*The shortened symbol for Amy^{1-c} is used here as it will appear in the revised list of genetic mutants and strains of D. melanogaster being compiled by Lindsley and Zimm (11).

**Chromosome 2 of this strain was made homozygous by routine crosses employing the balancer chromosomes SM1,al² Cy cn² sp² and In(2LR)bw^{V1}, dp b ds^{33k}.

homozygous for chromosome 2R only, by using suitable balancer chromosomes and routine chromosome mechanics. All flies were reared at room temperature on a standard cornmeal-molasses-yeast food medium (10).

DNA.

High molecular weight chromosomal DNA was isolated as described by Gennill et al. (1). DNA was digested with restriction endonucleases according to supplier's specifications. Samples of digested DNA were resolved electrophoretically on gels containing 0.7 or 1.0% agarose and 90 mM Tris-borate, pH 8.3, 1 mM EDTA. Southern transfers (12) were prepared according to the Gene Screen manual (New England Nuclear).

Molecular probes used in Southern analyses were prepared from plasmid subclones of the λ Dm65 clone which spans the Amy locus and its flanking sequences. Isolation of λ Dm65 (1) from a *Drosophila* genomic library in Charon 4 (13) and construction of the plasmid subclones from it have been described (6). Plasmid DNA was isolated using the alkaline-SDS method of Birnboim and Doly (14) followed by purification on CsCl-ethidium bromide density gradients. DNA preparations were labeled to high specific activity with ³²P by nick translation (15). Hybridization and autoradiography of Southern transfers were performed as described

Figure 1. Restriction maps of the *Amy* region. The open bar at the top represents the DNA sequences present in the *Amy* locus clone, λ Dm65 (6). Restriction endonuclease cleavage sites are indicated above and below the bar (B = BamHI, E = EcoRI, H = HindIII, S = Sall, X = XhoI). Only those below the bar were analyzed for the present report. The approximate position of the *Amy*¹ and *Amy*³ genes is shown by shaded regions in the open bar. The four subcloned DNA fragments used as hybridization probes in Southern analyses are indicated below the map of λ Dm65.

Beneath the *Amy* probes are shown restriction maps of the *Amy* region in the 7 strains studied. Restriction sites labeled below the map for each strain are also present in λ Dm65 and are thus conserved. Sites above the lines are either different from those in λ Dm65 or located beyond its boundaries. Insertions are shown above the *Amy*^{1c} and *Amy*¹ (CA 1) maps with the minimal length of each indicated. The length of the double-ended arrow drawn below the triangle representing each insertion defines the limits of uncertainty in the precise position of the insertion. Restriction sites within insertions are not shown (see text). A short EcoRI fragment from λ Dm65 which was not used as a probe here is reflected in the short dotted line left of center in each map. We cannot be certain whether or not our 7 strains possess both EcoRI sites that flank this fragment in λ Dm65. In *Amy*² and *Amy*^{2,3} the fragment homologous to pDm2.8 was slightly smaller on Southern blots than the equivalent fragment from other strains.

This difference is not large enough to be resolved on the maps. Certain restriction sites were detected at a large distance from the main part of the map for two strains, Any^{1c} and Any². This is shown by a break in the map with the size of the fragment in question noted in parentheses at the right. One EcoRI site in the Any null map is marked with an asterisk to indicate that its position was determined by analysis of isolated recombinant DNA clones (23) rather than by genomic Southern blot analysis.

(1) except that the hybridization temperature was 42° C and final washes were at high stringency (0.1 x SSC, 0.1% SDS at 65° C).

RESULTS

The published (6) restriction map of the Any locus clone, λ Dm65, is shown at the top of Figure 1. Immediately below it are indicated the four EcoRI-generated fragments, subcloned into pBR325 (pDm2.8, pDm3.8, pDm1.4, pDm5.6), which were used as hybridization probes in this study. To construct the other maps shown in the figure, restriction fragments that hybridized with any particular probe sequence were compared for size with appropriate restriction fragments generated by digestion of λ Dm65 DNA.

Figure 2 shows an example of this analysis as performed using the endonuclease SalI. This enzyme cleaves twice within λ Dm65, generating an internal fragment 6.6 kb in size. Lambda Dm65 contains two copies of a DNA sequence, approximately 1.7 kb in size, which represent the protein coding regions of two Any structural genes present in this clone (6). These two regions are separated by 5 to 6 kb of DNA, are inverted with respect to each other, and possess conserved patterns of restriction sites. The two SalI cleavage sites present in λ Dm65 occur within these repeated sequences and are part of this conserved pattern of restriction sites (Fig. 1). Thus the generation by SalI-cleavage of a 6.6 kb DNA fragment with homology to the pDm3.8 or pDm5.6 Any probe (Fig. 1, top) is a sensitive indicator of the organization of the Any locus. The presence of this fragment in any strain will indicate that the Any gene is duplicated, that the separation distance between Any gene copies is maintained, and that one of the duplicated sequences is inverted with respect to the other. The following analysis for the presence of the 6.6 kb

SalI fragment (Fig. 2) provides evidence that indicates the *Amy* gene is duplicated in all of the strains examined.

Figure 2 demonstrates that probe pDm3.8 is homologous to two DNA fragments in *SalI*-digested chromosomal DNA from six of the seven strains studied. DNA from *Amy*¹ (CA 1), the exception to this, displayed three homologous *SalI* fragments. DNA from all strains, again excluding *Amy*¹ (CA 1), contained a 6.6 kb *SalI* fragment similar to that present in λ Dm65. These observations imply that *Amy* is duplicated in at least six of the strains examined and that the two gene copies have an overall organization identical to that found in the molecular clone, λ Dm65. This organization is similar, but somewhat modified, in the *Amy*¹ (CA 1) strain, as described below.

Our pDm3.8 probe hybridized strongly to an additional 9 kb *SalI* fragment in six of the *Amy* strains. This fragment extends past the left end of the restriction map for λ Dm65 and into flanking sequences of the *Drosophila* genome (Fig. 1). The band representing this fragment is visible in Figure 2 because the

Figure 2. Southern blot using *SalI*. DNA from the 7 *Amy* strains in Table 1 and from λ Dm65 were cleaved with *SalI* and resolved on an agarose gel. The resulting blot was hybridized with the pDm3.8 probe (Fig. 1). Samples run in each lane are indicated and sizes of the detected fragments are listed. The large fragment in the λ Dm65 lane represents the right arm of the lambda vector, Charon 4, attached to adjacent sequences from the *Amy* locus.

pDm3.8 probe used here was derived mainly from this sequence. These results indicate that in at least six strains a SalI site exists in the genome of *D. melanogaster* 9 kb to the left of the SalI site present in the left hand copy of *Amy*, as it is figured.

The pattern of DNA fragments generated by SalI cleavage and observed by hybridization with pDm3.8 (Fig. 2) is identical in each strain with two exceptions, *Amy*^{lc} and *Amy*¹ (CA 1). As already noted, the 6.6 kb SalI fragment is missing in the *Amy*¹ (CA 1) strain; it is replaced by two new fragments about 5.9 and 4.6 kb in size. Since the sizes of the two new fragments do not sum to that of the missing 6.6 kb SalI fragment, simple introduction of a new SalI site cannot be the explanation for their appearance. Rather, it seems likely that an insertion of DNA has further separated the two *Amy* genes (Fig. 1) and that the additional SalI site lies somewhere within this insertion. Further evidence for this comes from XhoI digests of DNA from *Amy*¹ (CA 1) (not shown). Most *Amy* strains contain a 12 kb XhoI fragment homologous to both the pDm3.8 and the pDm5.6 probes. However, *Amy*¹ (CA 1) has a 19 kb XhoI fragment instead. Both SalI and XhoI results can be explained by the same insertion. The minimum size for this insertion, predicted from the SalI results (4 kb) and the XhoI results (7 kb), is quite different. This suggests that the insert may contain at least two SalI sites. Resolution of this issue awaits characterization of cloned sequences from the *Amy*¹ (CA 1) locus. The position of the insertion on the map in Figure 1 was determined by examination of EcoRI fragments on Southern blots (not shown).

A similar analysis of DNA digested by SalI, XhoI and EcoRI identified a probable insertion in the *Amy* region of strain *Amy*^{lc} (Fig. 1). This insertion is at least 10 kb in size and is located between the two XhoI sites on the left side of the map.

Figure 3 presents a second example of Southern blot analysis. In this case, EcoRI-digested DNA from two strains, *Amy*^{1,6} and *Amy* null, was probed with radiolabeled subclones from λ Dm65. Probed filters were organized on the autoradiogram, from left to right, in the order in which the subcloned EcoRI fragments occur in

Figure 3. Southern blot of Amy^{1,6} and Amy null DNA using EcoRI. Parallel lanes of EcoRI-cleaved DNA from the Amy^{1,6} and Amy null strains were transferred to a filter and the filter was then cut into strips. Each strip was hybridized with a separate probe, as indicated, except for the strip on the far right which was hybridized with a mixture of the four probes shown in Figure 1. Sizes are given for the four detected fragments from λDm65.

λDm65. The resulting band patterns obtained from Amy^{1,6} DNA indicate that the majority of restriction sites tested were identical to those found in λDm65. Two exceptions included a new EcoRI site within the region homologous to the 2.8 kb probe and the loss of the EcoRI site which separates the 3.8 and 1.4 kb fragments, resulting in a fusion fragment (Fig. 1).

An inverse relationship in hybridization intensity was discovered between the two fragments which contained copies of the Amy gene. Subclone pDm3.8, containing an Amy¹ gene, hybridized to two EcoRI fragments from Amy^{1,6}, one showing greater homology to the probe than the other. Thus, the 5.8 kb EcoRI fragment from Amy^{1,6} appears less intense in Figure 3 than the 5.2 kb fragment and presumably has less homology to the probe than the 5.2 kb fragment. The reverse of this relationship was observed when the pDm5.6 probe was used. This probe, carrying an Amy³ gene, hybridized to the 5.8 kb fragment with greater intensity. These results indicate that both the 5.8 and 5.2 kb EcoRI frag-

ments in Amy^{1,6} carry a copy of the Amy coding sequence. The changes in hybridization intensity can be explained as follows: Weak signal intensity results when the Amy coding sequence is the only sequence shared by both probe and fragment, while strong intensity occurs when sequences surrounding the Amy copy are also homologous to the probe. Thus, the 5.8 kb fragment, which shows strong homology with the pDm5.6 probe, arises from the right side of the map of the Amy locus; the 5.2 kb fragment, which shows strong homology to the pDm3.8 probe, arises from the left side.

The second lane of each pair in Figure 3 contains DNA from the Amy null strain. Both the pDm3.8 and pDm5.6 probes hybridized to two EcoRI fragments, one of about 3.8 kb and the other of about 6 kb in size. However, in contrast to all other Amy strains examined, the 6 kb fragment from the Amy null strain showed strong hybridization intensity with both probes and the 3.8 kb fragment showed weak hybridization intensity with both probes. In addition, probe pDm1.4 hybridized to the 3.8 kb fragment.

These results demonstrate the presence of several anomalies in the Amy null strain. First, the inverse relationship between hybridization intensities of the two fragments which hybridized with the pDm3.8 and pDm5.6 probes is violated. Second, the 3.8 kb EcoRI fragment present in this strain must have arisen through a fusion between the 3.8 and 1.4 kb EcoRI fragments because both the pDm3.8 and pDm1.4 probes hybridized to it. However, the size of the fusion fragment suggests that a substantial deletion of sequences must have also occurred. In spite of this, previous analysis of this strain with SalI (Fig. 2) and with XhoI (not shown) revealed only normal band patterns, a result not consistent with the presence of a deletion.

The anomalies present in the DNA from the Amy null strain suggest that some complex rearrangement has occurred within the Amy region here. Preliminary restriction endonuclease analysis of several Amy null genomic DNA clones provides strong evidence that an inversion of sequences has occurred between the two Amy coding sequences (23). This inversion can explain the anomalous

banding patterns detected on our genomic Southern blots. The Amy null restriction map in Figure 1 shows one EcoRI site marked with an asterisk to indicate that this site was determined through analysis of genomic clones (23) rather than from the Southern blots reported here.

DISCUSSION

Analysis of a number of Southern blots similar to those shown in Figures 2 and 3 has allowed us to build restriction maps of the Amy region from seven different isogenic strains of D. melanogaster. The maps shown in Figure 1 provide information about cleavage sites for three restriction endonucleases, SalI, XhoI and EcoRI.

Four types of molecular changes at the Amy locus were potentially detectable by our analysis: insertions, deletions, inversions and single base changes that altered restriction endonuclease cleavage sites. Large insertions were detected in two of our strains, Amy^{1c} and Amy¹ (CA 1). The approximate position and size of these insertions were determined, as presented in Figure 1. Sequence variation that affected restriction sites were found in a number of strains. In some instances this caused fusion of two fragments and in others splitting of one fragment into two. The Amy^{1,6} strain provides examples of both types of variation. Fusion of fragments is illustrated by the appearance of a 5.2 kb EcoRI fragment which shares homology with both the pDm3.8 and pDm1.4 probes. Splitting of one fragment is exemplified by the two smaller EcoRI fragments produced by the extra EcoRI site shown on the left side of the restriction map for Amy^{1,6}. There were no clear-cut examples of deletions detected in the 7 strains. The Amy null strain showed evidence of a complex anomaly which is most easily explained by an inversion of sequences between the two Amy coding regions (23).

The 7 restriction maps in Figure 1 show a relatively high degree of conservation. In spite of this, certain EcoRI sites are quite variable. The EcoRI site which separates the 3.8 and 1.4 kb fragments in Amy^{1,3} is absent in 4 of the 7 strains tested. In addition, there is variability in EcoRI sites on the

right hand sides of the restriction maps, as figured.

The restriction map for the Amy^{1,3} (CS) strain matched the map for the Canton-S clone, λ Dm65, in every detail tested. This indicates that the arrangement of sequences in the λ Dm65 clone (6) accurately reflects the situation in the Canton-S strain, the source of chromosome 2 in our Amy^{1,3} strain.

Another significant conclusion from this work is that all seven *Drosophila* strains tested appear to possess two copies of the Amy gene at the Amy locus and that these copies are inverted with respect to each other. Thus, even those strains that express only a single amylase isozyme contain duplicate copies of the sequences encoding the enzyme. We do not know at present whether one or the other or both copies are functional in strains expressing a single amylase variant. However, we do know that the relative separation between the two copies is identical in each strain examined except one, the Amy¹ (CA 1) strain, which apparently contains an insertion with a minimal size of 4 kb between the Amy genes. It should be noted that this strain expresses very low levels of midgut amylase activity in flies reared on standard food medium and is unique among the strains we have examined in that the larval and adult midguts display similar patterns of amylase distribution (2, 9).

The Amy null strain does not express detectable levels of amylase activity (7, 16), nor of amylase protein (17, 18) and amylase messenger RNA was not detected in third instar larvae in experiments done in our laboratory (19). These findings suggest that the two copies of Amy coding sequences in this strain may be defective. However, a very low level of amylase-homologous RNA has been detected in the Amy null strain by another laboratory group (18). Our analysis indicates that an anomaly, probably an inversion (23), exists in the organization of the Amy locus in the null strain. We do not understand why this strain has lost all or most of the ability to express amylase mRNA and protein. However, the inversion could be responsible for loss of Amy gene function if its breakpoints were at nonhomologous positions and located within sequences critical for such function.

One pair of strains, Amy² and Amy^{2,3}, provided a unique opportunity to determine the left-right orientation of restriction maps of the Amy locus with respect to the genetic map of chromosome 2R. The Amy² strain was derived from the Amy^{2,3} strain through a recombination event with an Amy¹ strain (4). Presumably the cross-over point was between the duplicated amylase genes. The Amy² strain must have received both an Amy² gene and, probably, a nonfunctional distal Amy gene copy. The nonfunctional copy could have been inactive in the parental strain or it could have been rendered nonfunctional during recombination through a mutational event. In either case, it can be predicted that the restriction maps of the Amy locus in the Amy² and Amy^{2,3} strains are very likely to be identical on the Amy² side of the cross-over point, but different on the opposite side of this point. Examination of the restriction maps for the Amy locus in these two strains (Fig. 1) shows that the pattern of cleavage sites on the left side of the map, as drawn, are identical. In contrast, the EcoRI sites on the right hand side of these maps are clearly different. This would place the Amy² gene for these two strains on that side of their restriction maps showing identity, i.e. on the left side. The orientation of these maps with respect to the genetic and cytogenetic maps of chromosome 2R may then be deduced from the following considerations.

Bahn (4) concluded from recombination data using flanking markers that the Amy² and Amy³ genes in the Amy^{2,3} strain were centromere proximal and distal, respectively. This is the orientation given them in Figure 1, where the centromeric and telomeric directions are marked by arrows for maps of the 7 strains analyzed. The restriction map for the Amy² strain is aligned with that for Amy^{2,3} for reasons discussed earlier. Orientation of the maps for the remaining 5 strains is based on restriction site pattern similarities among themselves and with respect to the maps for Amy² and Amy^{2,3}. It should be noted that the orientation of the maps spanning the Amy locus shown in Figure 1 agrees with our earlier suggestion (6) regarding the orientation of the Amy¹ and Amy³ genes in the Canton-S strain.

It is now possible to assign a number of alleles to each of the amylase structural genes in *D. melanogaster* based on combined data from Bahn's recombination experiments (4) and from our Southern analyses. Thus, for strains in which the left-right relationship between the two genes is established, we propose that the structural genes be designated by distinguishable genetic symbols, the gene nearest the centromere by Amy-p (proximal) and the one farthest from it by Amy-d (distal). Alleles of each gene may then be indicated by superscripts which, as before, reflect allozymic variation in their respective amylase products. The upper half of Table 2 lists Amy strains for which alleles of the Amy-p and Amy-d genes may currently be designated. Tentative assignments for alleles in certain other strains are suggested in the lower half. Except for the first Amy¹ strain listed (which is not among our stocks) each of the amylases produced by the strains tabulated is relatively heat stable, but some more so than others (5). Thus, the former may be distinguished as thermostable and the latter, for contrast, as thermolabile. Alleles producing the latter type are indicated as such in Table 2, the remainder being associated with greater thermostability. Since amylases with similar electrophoretic mobilities may conceal additional variation, the source of each strain is also given.

Tentative assignments for amylase alleles in Table 2 are based on the following assumptions. First, in strains producing two amylases with different electrophoretic mobilities, different Amy alleles exist at the Amy-p and Amy-d loci. Arguments for this are self-evident and supporting evidence has been reviewed (2, 5, 6). Second, strains that produce an amylase isozyme of the no. 1 type carry an Amy¹ allele at the Amy-p locus and these alleles are related by descent from a common ancestral Amy¹ gene. This assumption hinges on the observation that all amylase no. 1 isozymes examined show similar thermostabilities (5) and that, in the three strains in Table 2 for which an Amy-p¹ allele could be assigned, it exists at the proximal location. Furthermore, the specific activities of purified amylase no. 1 isozymes from strains differing in total amylase activity are the same, e.g.

Table 2. Assignment of alleles to proximal and distal copies of the amylase structural genes in *D. melanogaster*

Strain	Source*	Alleles of Amylase Genes**	
		Amy-p	Amy-d
<u>Amy</u> ¹	c wt strain (4)	<u>Amy-p</u> ¹	inactive?
<u>Amy</u> ^{1,3}	Canton-S (5, 8)	<u>Amy-p</u> ¹	<u>Amy-d</u> ³
<u>Amy</u> ^{1,6}	Suyana (5, 8)	<u>Amy-p</u> ¹	<u>Amy-d</u> ⁶ , thermolabile
<u>Amy</u> ²	<u>Amy</u> ¹ / <u>Amy</u> ^{2,3} re-combinant (4, 5)	<u>Amy-p</u> ²	inactive
<u>Amy</u> ^{2,3}	Copenhagen (4, 5)	<u>Amy-p</u> ²	<u>Amy-d</u> ³
<u>Amy</u> ^{2,6}	<u>Amy</u> ^{2,3} / <u>Amy</u> ^{4,6} re-combinant (4, 5)	<u>Amy-p</u> ²	<u>Amy-d</u> ⁶ , thermolabile
<u>Amy</u> ^{4,3}	" " " " " "	<u>Amy-p</u> ⁴ , thermolabile	<u>Amy-d</u> ³
<u>Amy</u> ^{4,6}	<u>adp</u> ⁶⁰ strain, Kaduna (5, 8)	<u>Amy-p</u> ⁴ , thermolabile	<u>Amy-d</u> ⁶ , thermolabile
Tentative assignments:			
<u>Amy</u> ^{1a}	Hikone-AW (5, 8)	<u>Amy-p</u> ^{1a}	?
<u>Amy</u> ^{1c}	Oregon-R (5, 8)	<u>Amy-p</u> ^{1c}	inactive?
<u>Amy</u> ^{1,2}	<u>adp</u> ^{fs} strain, Kaduna (5, 8)	<u>Amy-p</u> ¹	<u>Amy-d</u> ² , thermolabile
<u>Amy</u> ^{2,6}	Hikone-AS (5, 8)	<u>Amy-p</u> ²	<u>Amy-d</u> ⁶ , thermolabile
<u>Amy</u> ^{3,6}	Kyoto City (5, 8)	<u>Amy-p</u> ³ , thermolabile	<u>Amy-d</u> ⁶ , thermolabile

*All strains except the first listed were made isogenic for chromosomes 1, 2 and 3 in this laboratory. Reference indicated in parentheses give details about strain origins.

**The proximal gene, Amy-p, lies closer to the centromere than Amy-d (distal). Strains differ in the thermostability of the amylase isozymes they produce (see text).

from Amy^{1a} and Amy^{1c} (20, 21). The latter is not true for all amylase variants from *D. melanogaster* (21). Finally, we propose that all strains in Table 2 characterized by the no. 6 amylase variant carry an Amy-d⁶ allele at the distal site which derived from a common ancestral Amy⁶ allele of this gene. This is consistent with the observation that the assigned Amy-d⁶ alleles (top of table) in strains from widely separated geographical locations produce no. 6 isozymes with similar thermostabilities and amylase activities (5, 8, 22).

Confirmation of the restriction maps presented here and further refinements in the characterization of the region of DNA

containing the Amy locus from the 7 strains we examined awaits the molecular cloning of these sequences and their eventual sequencing. Molecular clones with homology to some of the sequences within the Amy region have been isolated from two of these strains which express little or no amylase activity, i.e. the Amy¹ (CA 1) and Amy null strains (23).

ACKNOWLEDGEMENTS

We thank Dr. D.A. Hickey for providing the Amy null stock and Dr. Eric Bahn for his recombinant Amy strains. We are grateful to Susan Rhodes, John Schaefer, Patrick Sherrill and Timothy Cook for technical assistance, and Fred Florschutz for the art work. We thank Dr. Jack N. Levy for providing the DNA probes and for helpful discussions. This work was supported by National Institutes of Health research grant GM25255 to W.W.D.

REFERENCES

1. Gennill, R.M., Levy, J.N. and Doane, W.W. (1985) *Genetics* 110, 299-312.
2. Doane, W.W., Treat-Clemons, L.G., Gennill, R.M., Levy, J.N., Hawley, S.A., Buchberg, A.M. and Paigen, K. (1983) in *Isozymes: Current Topics in Biological and Medical Research*, Rattazzi, M.C., Scandalios, J.G. and Whitt, G.W., Eds., Vol 9, pp. 63-90, Alan R. Liss, New York.
3. Kikkawa, H. (1964) *Jpn. J. Genet.* 39, 401-411.
4. Bahn, E. (1967) *Hereditas* 58, 1-12.
5. Doane, W.W. (1969) in *RNA in Development*, Hanly, E.W., Ed., pp. 73-109, University of Utah Press, Salt Lake City.
6. Levy, J.N., Gennill, R.M. and Doane, W.W. (1985) *Genetics* 110, 313-324.
7. Haj-Ahmad, U. and Hickey, D.A. (1982) *Nature* 299, 350-352.
8. Doane, W.W. (1969) *J. Exp. Zool.* 171, 321-342.
9. Doane, W.W. (1980) *Drosophila Inform. Serv.* 55, 36-39.
10. Doane, W.W. (1967) in *Methods in Developmental Biology*, Wilt, F.H. and Wessells, N.K., Eds., pp. 219-244, Thomas Y. Crowell, New York.
11. Lindale, D. and Zimm, G. (1985) *Drosophila Inform. Serv.*, Vol 62, pp. 1-227.
12. Southern, E.M. (1975) *J. Mol. Biol.* 98, 503-517.
13. Maniatis, T., Hardison, R.C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G.K. and Efstratiadis, A. (1978) *Cell* 15, 687-701.
14. Birnboim, H.C. and Doly, J. (1979) *Nucl. Acids Res.* 7, 1513-1523.
15. Rigby, P.W.J., Dieckman, M., Rhodes, C. and Berg, P. (1977) *J. Mol. Biol.* 113, 237-251.
16. Echo, B.S. and Doane, W.W. (1984) *Genetics* 107, a28; and in preparation.

17. Dumapies, F.E. and Doane, W.W. (1984) *Genetics* 107, s26; and in preparation.
18. Hickey, D.A., personal communication.
19. Gemmill, R.M., unpublished data.
20. Hickey, D.A. (1981) *Biochem. Genet.*, 19, 783-796.
21. Treat-Clemons, L.G. and Doane, W.W. (1982) *Isozyme Bull.*, 15, 90-91.
22. Doane, W.W., unpublished data.
23. Schwartz, P.E., unpublished data.