Fate map of the eye–antennal imaginal disc of the *tumorous-head* mutant of *Drosophila melanogaster*

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Summary

In specific genetic backgrounds, a mutation in the *tuh-3* gene results in the homeotic transformation of head structures to either leg disc derivatives or structures normally found in the extreme posterior end of wild-type animals. The origins of the homeotic structures were mapped to defined positions in the eye–antennal imaginal disc by transplanting abnormal regions of discs isolated from *tuh-3* mutants into host *mwh;e* larvae. These metamorphosed implants were removed and differentiated structures were identified. Of 211 successfully recovered implants, 157 gave rise to homeotic tissue: abdominal tergite, male or female external genitalia and/or leg tissue. Transformations to abdominal tergite occurred primarily in cells taken from the eye region of the compound disc. Male and female genitalia arose most often in implants taken from the antennal portion of the disc, although some tissue taken from the lateral region of the eye disc also gave rise to external genitalia. Leg structures came exclusively from implants from the antennal region of the imaginal disc. These results suggest that cells from within specific regions of the eye–antennal compound disc are constrained in their developmental potential. An obvious constraint observed with this mutation is a dorsal/ventral one: Cells from the eye disc, a dorsal structure, primarily gave rise to other dorsal structures, abdominal tergite tissue. Cells from the antennal disc, a ventrally derived structure, primarily gave rise to other ventral structures including genital tissue and distal leg.

Key words: fate map, *Drosophila melanogaster*, eye, antennal disc, mutant, *tumorous-head*, *tuh-3*, bithorax complex, homeotic transformation, graft, transplantation.

Introduction

Studies of the group of genes in *Drosophila melanogaster* known as the homeotics are contributing substantial insights into the mechanisms by which cell fate is determined during development. Mutations in homeotic genes result in the replacement of particular body structures with those normally found elsewhere in the animal (Bateson, 1894). Many of these homeotic genes are found organized into two major gene complexes: the Antennapedia complex (ANT-C) which controls development of anterior thoracic segments (Denell, 1973; Duncan & Kaufman, 1975; Sinclair, 1977; Kaufman, 1978; Lewis, Kaufman, Denell & Tallerico, 1980a; Lewis, Wakimoto, Denell & Kaufman, 1980b; Denell, Hummels, Wakimoto & Kaufman, 1981; Wakimoto & Kaufman, 1981) and the bithorax complex (BX-C) which controls development of thoracic and abdominal segments (Lewis, 1954, 1978, 1981a,b; Kerridge & Morata, 1982; Lawrence & Morata, 1983). Recently, genetic studies have provided evidence that genes in the bithorax complex are divided into two transcomplementing regions: one region controlling thoracic and anterior abdominal development, the *Ubx–bxd* region, and the other controlling posterior abdominal development, the *abd A–Abd B* region (Struhl, 1984; Casanova, Sanchez-Herrero & Morata, 1985; Sanchez-Herrero, Vernos, Marco & Morata, 1985; Teugels & Ghysen, 1985; Tiong, Bone & Whittle, 1985). Although genes affecting the developmental fate of the thoracic segments have been studied extensively, those genes affecting more posterior segments have not been as well analysed. Here I report studies on the role of a gene, *tuh-3*, in the bithorax complex that normally controls the developmental fates of the most posterior segments of the fly.
The tuh-3 gene has been localized by recombination mapping and deletion analysis to the distal end of the bithorax complex, 3-58.9, salivary region 89E4,5 (Gardner & Woolf, 1949; Kuhn, Woods & Andrew, 1981). The tuh-3 mutation is interesting in that there are two different phenotypes associated with this lesion. In zygotes carrying tuh-3, the phenotype expressed is dependent upon the maternal genotype at the X-chromosomal locus, tuh-1 (1-65.3). There are two naturally occurring alleles at this locus, tuh-1s and tuh-1h. These are found both in laboratory strains and in wild-type strains (Woolf & Passage, 1980). In offspring of females homozygous for the recessive allele, tuh-1s, tuh-3 acts as a semidominant mutation which causes transformation of head tissue to structures normally found in the posterior end of the animal, as well as causing antennal transformations to leg tissue (tumorous-head effect) (Woolf, 1965; Postlethwait, Bryant & Schubiger, 1972; Kuhn & Dorgan, 1975; Bournais-Vardiabasis & Bownes, 1978; Woolf & Passage, 1980). In offspring of females heterozygous or homozygous for the dominant allele, tuh-1h, tuh-3 acts as a recessive hypomorph causing both the loss of external genital structures and the improper elaboration of internal genitalia (sac testis effect) (Woolf, 1966, 1968; Woolf & Passage, 1980). Additionally, in tuh-3/Df offspring of females carrying the tuh-1s allele, sixth and seventh tergite tissue is partially transformed toward a more anterior tergite, probably tergite five (Kuhn et al. 1981). Thus the maternal effect of the tuh-1h allele causes tuh-3 to act zygotically as a dominant gain-of-function mutation in the anterior end of the animal while the maternal effect of the tuh-1s allele causes tuh-3 to act zygotically as a recessive loss-of-function mutation in the posterior end of the animal. The two effects of tuh-3 have never been separated genetically (Kuhn et al. 1981). It is therefore likely that the two effects are due to a single lesion within a single gene.

The tuh-3 gene has been cloned in a chromosomal walk through the right half of the bithorax complex. The DNA from the tuh-3 mutant stock has been isolated and a moderately repeated newly identified 7 kb element, called 'Delta 88', has been found inserted into the tuh-3 gene (Karch, Weifenbach, Peifer, Bender, Duncan, Celniker, Crosby & Lewis, 1985). The inserted element could be responsible for both tuh-3 mutant phenotypes, but since the chromosome in which the tuh-3 mutation occurred is not available, this cannot be easily tested.

Regardless of the exact molecular mechanisms, it seems likely that the 'tumorous-head' defect may result from the improper activation of the tuh-3 gene in the anterior end of the animal. If this were true, there are at least two models which would explain the variety of homeotic structures seen in the tuh-3 mutant flies. Under one model the types of homeotic transformations seen in the heads of these animals could be simply responses to varying levels of the tuh-3 gene product. For example only very low levels of tuh-3 product may be necessary for homeosis to some structures, such as abdominal tergite, but higher levels of tuh-3 product may be necessary for transformations to less frequently seen structures, such as those to genital disc derivatives. One argument against this model is that in some tuh-3 stocks, transformations to genital disc derivatives occur at a much higher frequency than transformations to abdominal tergite (Postlethwait et al. 1972; Bournais-Vardiabasis & Bownes, 1978). However, the variance in frequencies of types of homeotic structures found amongst different stocks could nonetheless be due to different 'stock-specific' levels of mutant product. Alternatively, homeosis to particular structures could be limited by the developmental potential of the tissues affected, i.e. genital tissue may be only able to develop from particular regions of the eye–antennal disc, while leg structures or abdominal tergite may be only able to develop from other regions. The work reported in this paper attempts to distinguish between these two possibilities by directly determining whether there is any pattern as to where in the eye–antennal discs of 'tumorous-head' animals the various types of homeotic transformations occur. To do this, I have transplanted abnormalities from specific regions of the eye–antennal discs of tuh-3 animals into host larvae and examined differentiated cuticle following its metamorphosis.

Materials and methods

The tuh(UCF) stock of Drosophila melanogaster maintained at the University of Central Florida for the past 10 years, is homozygous for both the tumorous-head maternal effect allele (tuh-1h) and the tumorous-head mutant gene (tuh-3). Flies with head abnormalities are selected every generation to ensure high penetrance and expressivity of the 'tumorous-head' phenotype. tuh(UCF) has the highest penetrance and expressivity (extent of homeotic transformations) amongst tuh-3 stocks currently available (Kuhn & Dorgan, 1975).

Eye–antennal imaginal discs from tuh(UCF) third instar larvae were examined for morphological abnormalities. These abnormalities were then cut away from the discs and implanted into the abdomens of late third instar mwh, e4 larvae (see Lindsley & Grell (1968) for descriptions of mutant phenotypes). Following metamorphosis, implants were removed and mounted on slides in Faure's mounting medium. Structures were identified based on the size, shape and texture of bristles, on the size and arrangement of trichomes, on the presence of morphologically distinct structures and on the colour of the cuticle. See Kuhn, Zust
Results

A total of 211 implants were recovered from portions of tumorous-head eye–antennal disc abnormalities injected into the abdomens of *mwh;e* larvae. Disc abnormalities selected for implantation were easily distinguishable through a dissecting microscope and included lobes arising from various regions of the eye and antennal discs, as well as deletions or interruptions from the expected wild-type eye–antennal disc pattern. Abnormalities were categorized on the basis of the region of the eye–antennal disc from which they arose (see Fig. 1) (Kuhn & Walker, 1978). One limitation with this procedure is that it is sometimes difficult specifically to assign gross abnormalities to particular eye–antennal regions. Normal head cuticle found with homeotic tissue in differentiated implants was, therefore, used as a verification of the assigned region of origin in the imaginal disc. By far the most common type abnormalities seen were lobes extending from areas of the eye imaginal disc that would normally be expected to give rise to the ventral head structures and the compound eye (Bryant, 1978). These are eye regions 1 and 2 (R1 and R2) indicated in the left-hand column of Table 1. A large number of discs was found in which almost all of the eye portion of the imaginal disc was missing. When the remaining tissue was transplanted, a high percentage gave rise primarily to homeotic tissue (Eye R123, Table 1). Given that no adult tuh(UCF) flies have been observed with abnormalities as extensive as these (Cook, 1981), it is likely that these animals would never have survived to eclose as adults. As this study attempts to localize homeotic transformations to specific regions of the eye–antennal imaginal disc, samples for injection were selected in order to obtain representatives of all abnormalities seen regardless of relative frequency. Hence, the number of implants recovered and examined in this study does not reflect the actual percentage of occurrence of each type disruption. The frequencies of different types of homeotic transformations in tuh(UCF) have been examined in other work (Cook, 1981).

Of the 211 implants recovered, 204 gave rise to cuticular structures that were easily identifiable. 29 of the implants examined contained only the structures that would have been expected from implantations from those regions from a wild-type eye–antennal disc (column A, Table 1). An additional 18 implants gave rise to head tissue only, but bristles and/or cuticle were extensively duplicated (column B, Table 1). The remaining 157 implants recovered contained homeotically transformed cuticle: either distal leg, abdominal tergite, or male or female external genitalia. Most (>92%) of these 157 implants also contained head cuticle which served to verify their region of origin in the imaginal disc. Some, however,
contained only homeotically derived tissue and, thus, their region of origin could not be independently verified.

The large number of implants with homeotic transformations made it possible to map their regions of origin to specific areas within the eye–antennal disc. For example, abdominal tergite tissue arose predominantly from the eye portion of the compound disc: 89 of 109 implants giving rise to female sixth or seventh tergite bristles arose from the eye disc. Abdominal tergite tissue is easily distinguishable from cuticle from other regions of the body by the pigmentation patterns of the cuticle, the arrangement of trichomes and the presence of uniformly sized striated bristles.

15 of 17 implants giving rise to male sixth tergite, with its characteristic darkly pigmented cuticle, were derived from the eye portion of the disc (combined totals of columns C, E, H, I, and J, Table 1). Fig. 2A,B shows typical homeotic transformations of presumptive eye region cuticle to abdominal tergite. Tergite primordia did not seem to be localized to a specific region of the eye portion of the disc, since tergite bristles arose from region 1, 2, and 3 (R1, R2 and R3) implants. However, as mentioned earlier, region 1 and 2 abnormalities are much more frequently observed than region 3 abnormalities. Whether these different frequencies of transformation result from variations in the sensitivity of these primordial regions to the tuh-3 defect or represent limits on the domain of expression of the tuh-3 mutation cannot be distinguished.

Female genital disc derivatives with the exception of eighth tergite bristles arose predominantly from the antennal portion of the imaginal disc. 14 of 20 implants that contain some female genital disc derivatives besides eighth tergite bristles (i.e. vaginal plate with thorn bristles, vulva and anal plate) arose from the antennal disc (combined data from columns F, H, and J, Table 1). Fig. 2C,D shows homeotic transformations to female seventh and eighth tergite, vaginal teeth and vulvar tissue. Similarly, 5 of 7 implants giving rise to male genital structures also came from abnormalities in the antennal portion of the disc (columns F, H, and J, Table 1). Fig. 2E,F shows typical homeones to male external genitalia including male lateral plate and male clasper teeth. However, with regards to female genitalia, 20 of 22 implants giving rise to eighth tergite bristles without other female genital disc derivatives came from the

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**Table 1. Results from implantations of region-specific abnormalities from 'tumorous-head' eye–antennal imaginal discs**

<table>
<thead>
<tr>
<th>Region of origin</th>
<th>Normal head</th>
<th>Duplicated head</th>
<th>6th, 7th Tergite</th>
<th>8th Tergite</th>
<th>6th, 7th, 8th Tergite</th>
<th>Genitalia Leg</th>
<th>Tergite genitalia</th>
<th>Tergite leg</th>
<th>Genitalia tergite leg</th>
<th>Undifferentiated</th>
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F, female; M, male.
Fig. 2. Representative homeotic implants recovered following injection of abnormal fragments of tumorous-head eye-antennal imaginal discs into host mwh<sup>a</sup> larvae. A, B show abdominal tergite and normal eye disc derivatives. C–F show homeotic transformations to female (C, D) and male (E, F) genitalia from implants from the antennal region of the disc. G, H show homeotic transformation of antennal disc derivatives to mesothoracic leg. a<sub>2</sub>, a<sub>3</sub>, second and third antennal segments; ar, arista; cl, claw; ct, male clasper teeth; lp, male lateral plate; om, ommatidia; t<sub>7</sub>, 7th abdominal tergite; ts, tarsal segments; vi, vibrissae; vt, female vaginal teeth; vu, vulva. X200.
eye disc (combined data from columns D and E, Table 1). Most of these (17/22) were found with sixth and seventh tergite (column E, Table 1). Moreover, genital structures derived from the eye disc were invariably found associated with abdominal tergite tissue (columns H and J, Table 1). It should be noted that within the 'tumorous-head' eye-antennal disc as a whole, there were cells capable of transforming to any part of the external genitalia since representatives of all identified external genital disc derivatives were seen in at least one implant.

Leg transformations mapped exclusively to the antennal disc. All 24 implants with distal leg tissue, tarsal segments and claw, were from abnormalities in the antennal disc (columns G, I, and J, Table 1; Fig. 2G,H).

Discussion

The tuh(UCF) stock, which is homozygous for both tuh-3 and tuh-lb, exhibits homeotic transformations of eye—antennal disc derivatives to abdominal tergite, genitalia and distal leg. Here I have mapped these homeotic transformations to defined positions in the tumorous-head eye—antennal imaginal disc by transplanting regions of discs containing specific abnormalities into host larvae and examining the cuticular structures derived from them after differentiation. Abdominal tergite mapped predominantly to the eye region of the disc whereas almost all genital disc derivatives arose in the antennal region of the disc. The exception was female eighth tergite bristles. In this study, these bristles occurred as frequently in implants from the eye portion as from the antennal portion of the disc. Homeotic transformations to distal leg mapped exclusively to the antennal disc.

Homeotic transformations to distal leg seem unusual in the light of the other homeotic transformations observed in this mutation. Transformation of head to posterior abdomen and genitalia suggests that these defects are due to the inappropriate expression of bithorax complex functions, whereas, transformations of antenna to leg suggests the inappropriate expression of Antennapedia complex functions. The abnormalities observed in 'tumorous-head' flies could be the product of two distinct types of homeotic transformations. The primary homeotic events would be the transformations of eye—antennal derivatives to structures normally found in the posterior end of the organism, i.e. sixth and seventh abdominal tergite and male or female genitalia and analia. The homeotic transformations of antennae to distal leg might be secondary events due to an 'environmental' effect of the primary disturbance causing 'indirect homeoses' (Strub, 1980). In long-term culture of imaginal tissue in the abdomens of adult females a change in the state of determination or 'transdetermination' occurs at predictable frequencies (Hadorn, 1976). In this situation, transdetermination of antenna to leg is a common occurrence. It has been suggested that transdetermination occurs as a result of the apposition of cells carrying widely disparate positional information (Strub, 1980). By analogy, many 'indirect' homeoses may be caused by events that result in the juxtaposition of cells carrying different positional information, for example, events such as cell death (which can be looked upon as in situ amputations) or other direct homeotic transformations of some cells in an imaginal disc. Given the large number of mutations that result in antenna-to-leg transformations and the fact that transdetermination of antenna to leg in long-term cultures is a frequent occurrence, the conditions necessary for this type of homeosis must be met quite easily. Thus, in 'tumorous-head' flies, transformation of antenna to leg could result as a consequence of cells destined to become terminalia growing in contact with cells destined to become head structures. The duplications of normal head tissues, which are also seen, could be secondary effects of the primary homeoses as well. It is known that when wild-type imaginal tissue is cut and incubated in either the abdomens of adult females or very young larvae to allow for growth, that when this tissue is forced through metamorphosis, large structural duplications are frequently observed. These duplications are believed to occur as a result of cells with different positional information being adjacent to one another. It is thought that these duplications are attempts to fill in those positional values that are no longer present (French, Bryant & Bryant, 1976; Bryant, French & Bryant, 1981). Alternatively, the inappropriate expression of BX-C functions may be directly interfering with the normal control of ANT-C functions in the anterior end.

Various stocks containing the same tuh-3 mutant allele exhibit highly variant frequencies and types of homeotic events (Woolf, 1965). The tuh-3 stock, tuh(CT), used by Bournias-Vardiabasis & Bownes in 1978 showed only homeosis to distal leg and genitalia. However, this stock originally exhibited homeosis to abdominal tergite when examined by Postlethwait et al. in 1972. In tuh(UCF) flies, transformations to abdominal tergite are by far the most common homeotic event observed (Kuhn & Walker, 1978; Cook, 1981). Although Bournias-Vardiabasis & Bownes (1978) did not report results on any differentiated implants containing abdominal tergite, their mapping of genital structures and leg tissue is consistent with results in this study. Overall, these results suggest that the variability between and within tuh-3 stocks may be due to regional differences (due to the
defect. The fact that particular homeotic transformations fate map to specific regions of the eye–antennal disc suggests that transformations to certain segmental structures is limited by the developmental potential of the tissue that would normally be present in wild type. Therefore in 'tumorous-head' flies, it might not be that homeosis to genitalia will occur only if some critical level of tuh-3 product is expressed, but that only certain cells are capable of making genital tissue in the presence of the tuh-3 gene product. The same principles might govern the transformations to abdominal tergite and distal leg. One developmental constraint on the homeotic transformations of cells could be their limited potential to form only dorsal or only ventral structures. Certainly this type of constraint fits with the data in this study: eye discs, dorsal structures, gave rise predominantly to abdominal tergite, other dorsal structures; whereas antennal discs, ventrally derived structures, gave rise predominantly to ventral structures, leg tissue and genital tissue. This is consistent with the demonstration that homeotically transformed tissues in studies of Antennapedia mutations (Postlethwait & Schneiderman, 1971; Schmid, 1985) and in studies of double mutant combinations between engrailed (en) and postbithorax (pbx) (Garcia-Bellido & Santamaria, 1972) do rely on underlying positional information.

**Model for mutant tuh-3 gene action**

It has been demonstrated that the tuh-3 gene maps to the distal end of the bithorax complex both by recombination mapping and deletion analysis (Kuhn et al. 1981). It has further been shown that mutations in this gene lead to two distinct phenotypes: In offspring of females heterozygous or homozygous for tuh-1<sup>8</sup>, tuh-3 acts like a loss-of-function mutation causing gonadal dysgenesis and abnormal development of the most posterior abdominal segments ('sac testis' effect). In offspring of females homozygous for tuh-1<sup>9</sup>, tuh-3 acts like a dominant gain-of-function mutation causing homeotic transformations of head structures to abdominal and genital structures ('tumorous-head' effect) (Wooff, 1966, 1968; Woolf & Passage, 1980). Both of the defects seen with this mutation affect expression of functions controlled by the Abd-B region of the bithorax complex (Struhl, 1984; Casanova et al. 1985; Sanchez-Herrero et al. 1985). I would like to propose that the primary defects seen with this mutation are caused by reduced expression of the Abd-B gene in animals with the 'sac testis' defect and the inappropriate expression of the Abd-B gene in animals with the 'tumorous-head' defect.

As was mentioned earlier, the DNA from the bithorax region has been isolated, including the DNA encoding the most posterior genetic functions in the complex, the Abd-B region (Bender et al. 1983a,6; Karch et al. 1985). Transcripts from the Ubx region of the complex are initiated distally and extend proximally. A DNA sequence known as a 'homeobox' is found in the 3' end of these transcripts. Homeoboxes are regions of transcripts that encode the protein domains of regulatory genes which are believed to bind DNA. They are found in the 3' ends of many segmentation- and segment-identity genes (McGinnis, Levine, Hafen, Kuroiwa & Gehring, 1984; Scott & Weiner, 1984). Two other 'homeo-boxes' are found in the bithorax complex. One maps proximally within the Abd-A region and the other maps proximally within the Abd-B region (Karch et al. 1985). Assuming that homeoboxes are always found in the 3' ends of those genes that contain them would suggest that the major transcripts from both the Abd-A and Abd-B genes are initiated distally and extend proximally. This would place the insertion element 'Delta 88', believed to cause the tuh-3 defects, at or near the 5' end of the presumptive Abd-B transcript. Insertion of this element at the 5' end of the Abd-B gene could cause disturbances in the expression of that gene. I would like to suggest that the products of the tuh-1 gene, supplied maternally, can act to either suppress or allow the inappropriate expression of Abd-B. With tuh-1<sup>8</sup>, the mutant tuh-3 allele containing the 'Delta 88' insertion reduces expression of the Abd-B gene to below some critical level to cause the 'sac testes' defect. tuh-1<sup>8</sup> would, therefore, be acting as a 'Suppressor of 'Delta 88". With tuh-1<sup>9</sup>, Abd-B transcription would not be suppressed but instead would be under the control of 'Delta 88' expression, which is presumably not regulated in the same segment-specific manner as is wild-type Abd-B. It is interesting to note that the tuh-1 gene maps on the X chromosome at 1-65.3, intriguingly close to a known insertion element suppressor, 'suppressor of forked', at 1-65.9. su(f) is known to suppress f and other mutations caused by 'gypsy' insertions. It is possible that tuh-1 may in fact be an allele of su(f) and may suppress mutations caused by other insertions elements, such as 'Delta 88'.

The above model would explain both the dominant effect of the tuh-1<sup>8</sup> allele over the tuh-1<sup>9</sup> allele and the fact that in the 'sac testes' defect, tuh-3 acts as a simple recessive allele and in the 'tumorous-head' defect, it acts as a semidominant allele. It is also consistent that the tuh-1 'suppressor' is maternal, given that genes in the bithorax complex function very early in development. This model explains most of the defects seen in both of the tuh-3 phenotypes. What remains unclear is the improper expression of
what are presumably Antennapedia complex functions in the 'tumorous-head' defect, i.e. the transformations of antenna to distal leg. Possible mechanisms for this type of homeosis were discussed earlier.

This model makes a number of predictions which can be easily tested. Since the DNA for the bithorax region has been cloned, transcripational data should soon be available. I have predicted that the major transcript in the Abd-B region should initiate distally and extend proximally with the 5' end mapping near the 'Delta 88' insertion and the 3' end containing the homeobox region. Probes for this transcript used in in situ hybridization to tissue sections of early embryos should indicate an extreme posterior distribution of mRNA in wild-type embryos with reduced levels in 'sac testes' embryos. Additionally, transcripts from Abd-B should be found in the head region of 'tumorous-head' animals while showing only a posterior distribution in wild-type animals. Finally, it would be interesting to test if the maternal effect gene, tuh-1, is, in fact, suppressor of f. One test would be to determine if alleles of tuh-1 can act to suppress f or other mutations caused by insertion elements. More specifically, one may need to look for tuh-1 maternal suppression of mutations in early acting genes caused by 'Delta 88' or 'gypsy' insertion elements.

One of the most important features of this model is that it suggests that under normal circumstances, i.e. when tuh-3 is wild type, the tuh-1 gene has no role in the regulation of tuh-3.

Special thanks are extended to Dr D. T. Kuhn in whose laboratory all of this work was carried out. For help in preparing the figures, I thank Dr J. G. Connolly. For their helpful suggestions and criticisms in the preparation of this manuscript, I thank Dr B. S. Baker, Dr M. B. McKeown, Dr J. A. Posakony and my colleagues at UCSD. This work was supported by NIH Grant No. AG 1846 awarded to DTK.

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(Accepted 13 February 1987)