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15. The eigenvalue/eigenvector decomposition of the matrix A (singular value decomposition) discussed by Lanczos (*7*) tells in a very illuminating way the origin in the conservation requirements of various features in the solution space. For example, the southward flow under the Gulf Stream is seen immediately to be a consequence of the need to conserve total transport and water between 2.5° and 4°C. Water masses undergoing real transformations—that is, not conserved—may be treated by imposing inequality constraints and solving by linear programming methods.
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17. I thank L. V. Worthington for suggesting the use of the R.V. *Atlantis* sections and for discussions of the problem, and B. Grant for computing help. Supported by NSF grant OCE 75003998 (POLYMODE). This is MODE contribution No. 85.

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Aldehyde Oxidase Compartmentalization in *Drosophila melanogaster* Wing Imaginal Disks

Abstract. *Distribution of the enzyme aldehyde oxidase in mature* Drosophila melanogaster *wing disks may allow visualization of known developmental compartments comprising (i) presumptive dorsal and ventral wing surfaces, and (ii) the presumptive anterior wing and the presumptive posterior wing.*

Our objectives in this report are (i) to present aldehyde oxidase histological staining patterns that support the concept of developmental compartments within presumptive wing tissue of the fruit fly *Drosophila melanogaster*, and (ii) to report an upset in the distribution of the enzyme in the presence of the mutation for engrailed (*en/en*). Rigidly defined developmental compartments that originate early in development have been demonstrated for the *Drosophila* wing (*1, 2*). By staining for aldehyde oxidase (E.C. 1.2.3.1) during larval development, it has been observed that compartmental boundaries delineate the presumptive anterior wing blade compartment from the posterior compartment of the wing blade, as well as the dorsal from the ventral surfaces of the adult wing.

Adult *Drosophila* structures are formed primarily from groups of cells that have limited or no function during larval development. These groups of cells are referred to as imaginal disks and histoblasts. Our report concerns a single pair of imaginal disks which form the wings and some dorsal parts on the thorax. The *Drosophila* wing disk derives from about seven preblastoderm cells (*3*). Cell division proceeds until about 24 hours after the larva secretes the pupal case, at which time some 52,000 cells are present (*4*). The adult wing forms from an unfolding (evagination) and folding and extension (eversion) of the disk, primarily through cell flattening and possibly by some cellular movement (*5*).

Recently it was discovered that the *Drosophila* wing disk can be subdivided early in development into several defined areas, referred to as compartments, that are made up of small groups of founder cells (*1*). The term polyclone (*6*) is applied to descendants of these founders. Once a compartmental polyclone is formed, it may be divided into subcompartments with their founder cells. Resultant compartments may then be studied in the adult wing. Crick and Lawrence (*6*) suggest that histological staining for various enzymes in the imaginal disks may uncover biochemical compartments since cells in one compartment must biochemically differ in some way from cells of neighboring compartments. The enzyme aldehyde oxidase was chosen for study because of the relatively simple histochemical techniques involved, the highly repeatable patterns of activity that can be attained, and because we had already successfully used this histochemical technique to show abnormal aldehyde oxidase patterns in the eye portion of the eye-antennal imaginal disks of larvae possessing the homoeotic tumorous-head mutation (*7*).

The Canton-S wild-type laboratory strain, 15 other *Drosophila melanogaster* laboratory strains, a strain of flies mutant for engrailed (*8*), and an engrailed strain possessing about 2 to 4 percent of the normal amounts of aldehyde oxidase were used during the course of this study (*8*). All flies were maintained at 25°C on standard *Drosophila* medium. Third instar larvae and prepupae (from 1 to 12 hours after puparium formation) were sources for wing disks and prepupal wings. Preparation of disks and staining procedures followed those described previously (*7*).

The pattern of aldehyde oxidase activity in wing disks of late-third instars of the strains Canton-S, Oregon-R-C, and vermilion is shown in Fig. 1. Other laboratory strains analyzed showed similar aldehyde oxidase distribution in their wing disks. Areas of disk differentiation into adult wing blade, within region 1 (which includes the wing pouch), are central to our report. In Bryant's fate map of the mature wing disk (*9*), two parallel lines are shown that arc across the central portion of region 1 separating presumptive dorsal from ventral wing surfaces. Figure 1, a to c, shows that aldehyde oxidase activity follows the wing margin as outlined in Bryant's fate map (*9*).

Figure 1 also shows the developmental fate of various areas within the wing disk, while Fig. 2 shows their corresponding adult derivatives. Aldehyde oxidase appears to be a biochemical marker showing where folding at the presumptive wing margin will occur during differentiation into the dorsal and ventral surfaces of the adult wing blade.

Perpendicular to the aldehyde oxidase arc, on the left side of the disk, is activity in the form of two dark bands with a lightly stained area between them (region 1 of Fig. 1a). The entire left side of region 1 shows staining for aldehyde oxidase in the vermilion (Fig. 1c) and other laboratory strains. However, the stain does not extend to the right of the center band in the more than 200 disks studied from 16 laboratory strains. A similar but overstained aldehyde oxidase pattern has been recorded by Janning (*10*) in the Canton-S strain.

Perhaps the straight line separating the two areas represents a compartmental boundary separating the presumptive anterior portion of the adult wing blade from the posterior portion. Such a boundary must exist since Garcia-Bellido *et al.* (*1, 11*) found such a boundary in the adult wing blade. Cells from a clone are shown to divide mitotically along either side of a straight line following closely the fourth wing vein (Fig. 2) (*1, 6*). We used the homoeotic mutant engrailed, which transforms the posterior compartment incompletely into an anterior compartment, to study this compartmental boundary. Aldehyde oxidase distribution in an *en/en* wing disk is shown in Fig. 3, while Fig. 4 shows the triple row of bristles in the posterior compartment of *en/en* homozygous flies that is normally characteristic of the anterior compartment. A posterior row of hairs is normally observed in the posterior compartment (Fig. 2). Morata and Lawrence (*12*) show that the homozygous condition for engrailed (*en/en*) results in a breakdown of the anteroposterior compartmental line.

Apparently, function of the normal en^+ gene is necessary for maintenance of the compartmental boundary (*12*), and this gene controls pattern of veins and bristles (*13*). Based upon these results

we postulated that mature wing disks from *en/en* late-third instar larvae should stain histologically for aldehyde oxidase in both the anterior and posterior compartments. The pattern in the anterior compartment should have the appearance of the wild type, while the posterior compartment should be aldehyde oxidase positive. The *en/en* wing disk (Fig. 3) shows aldehyde oxidase present and distributed normally in the anterior compartment of the wing disk. As predicted, the posterior compartment is aldehyde oxidase positive. The normal enzyme pattern in the anterior compartment is expected because Morata and Lawrence (*12*) found, through clonal analysis, that the engrailed mutation affects only the posterior compartment where it increases the size and changes the shape of the compartment (*13*). A complete mirror image of the aldehyde oxidase pattern observed in the anterior compartment is not expected in the posterior compartment because the homoeotic transformation is not complete (*12*).

In order to determine whether the aldehyde oxidase arc across the wing disk corresponds to the presumptive wing margin as outlined on Bryant's fate map (*9*), we studied evaginating wing disks of Canton-S prepupae (1 to 12 hours after pupation). During prepupal development, the wing pouch lengthens (evaginates) with the epithelium folding at the margin of the prepupal wing so that cells of the ventral compartment eventually come to lie adjacent to those of the dorsal compartment. If the aldehyde oxidase arc actually represents the position of the presumptive wing margin, and if this pattern is maintained during evagination, enzyme activity should trace the outer edges of the prepupal wing.

Figure 5, a to c, shows aldehyde oxidase activity patterns in evaginating prepupal wing disks (from 4- to 7-hour prepupae). In each photograph and in all 100 disks examined during this period of development, the aldehyde oxidase staining pattern follows the presumptive wing margin. After folding, the presumptive wing margin clearly shows a band of aldehyde oxidase with activity in the anterior compartment just proximal to the aldehyde oxidase marked wing margin. As folding proceeds, it appears that the anteroposterior compartmental boundary can be seen at the junction of two folds (see arrow in Fig. 5c). The pupal wing will not stain after 6 to 8 hours of prepupal development, possibly because the staining solution cannot penetrate the pupal cuticle. The relation between the aldehyde oxidase arc and the dorsoventral compartmental boundary is unclear. The dorsal posterior and ventral posterior compartments are aldehyde oxidase negative except for the arc across the wing margin. It is impossible, therefore, to predict which compartment, if either, directs synthesis of aldehyde oxidase in the arc.

Our results indicate that aldehyde oxidase is compartmentalized in the mature wing disk. Thus, developmental compartments previously observed only in the adult stage may now be detected during the third instar stage, presuming that aldehyde oxidase distribution conforms to the same compartments. We do not know when during development the enzyme distribution initially conforms to these areas. Although aldehyde oxidase reveals the possible locations of developmental compartments, the role of the enzyme during development is unknown. We have studied flies homozygous for the engrailed mutation that possess only 2 to 4 percent of the normal

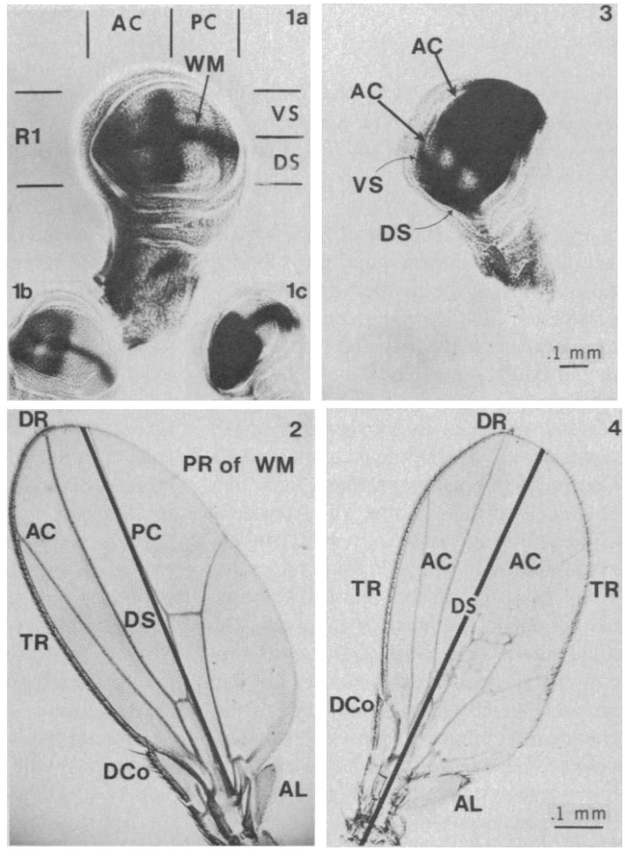

Fig. 1. Aldehyde oxidase distribution in a mature disk from a third instar larva showing the position of the presumptive wing margins. Nomenclature is based primarily on Bryant's fate map (*9*). Strains shown are (a) Canton-S, (b) Oregon-R-C, (c) vermilion. *AC*, anterior compartment; *PC*, posterior compartment; *VS*, ventral wing surface; *DS*, dorsal wing surface; and *WM*, wing margin. Fig. 2. Dorsal side of adult Canton-S wing with some labeled adult structures; *DCo*, distal costa; *TR*, triple row bristles; *DR*, double row bristles; *PR of WM*, posterior row of hairs of wing margin; *AL*, alar lobe; *DS*, dorsal wing surface. Other abbreviations as in Fig. 1. Fig. 3. Aldehyde oxidase distribution in a mature wing disk from an engrailed (*en/en*) third instar larva. Fig. 4. Dorsal side of adult engrailed (*en/en*) wing.

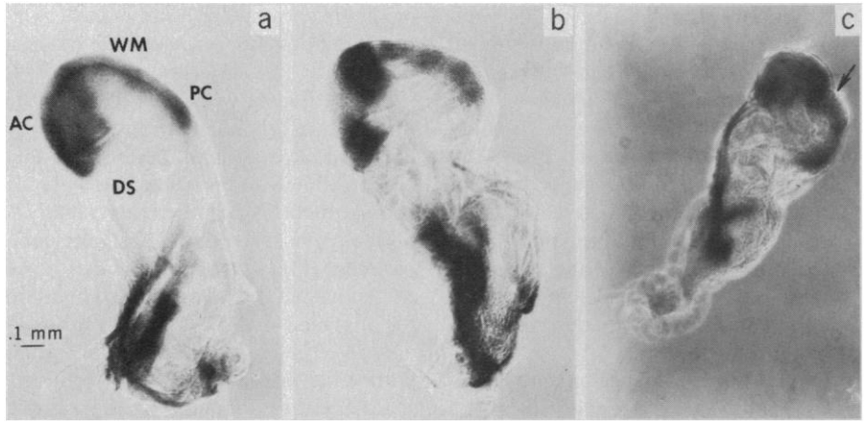

Fig. 5. Aldehyde oxidase distribution in three 4- to 7-hour Canton-S prepupal wings. In (c), an apparent meeting of two folds that could represent an anterior–posterior compartmental junction is indicated by the arrow; *AC*, anterior compartment; *PC*, posterior compartment; *DW*, dorsal wing surface; and *WM*, wing margin.

levels of aldehyde oxidase activity. All such flies display the engrailed phenotype. Aldehyde oxidase, therefore, is probably not responsible for the transformation of the posterior compartment to an incomplete anterior compartment. It is probably present in the transformed compartment because at compartment formation during embryogenesis (*1, 11*) in the absence of the en^+ posterior compartmental marking gene, instructions are given for an anterior compartment in which the aldehyde oxidase structural gene is or will be active. During the first instar stage of development the dorsoventral compartmental boundary is established and is apparently unaffected by the engrailed mutation (*12*). The wing margin, which is marked by aldehyde oxidase, carries instructions from the previously established anterior compartments so that bristle patterns in the adult wing will be characteristically anterior along the anterior and posterior wing margin.

Studies of enzymatic compartmentalization should lead to a better understanding of the logic behind gene deployment in pattern formation (*6*).

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Flavonoids and Other Chemical Constituents of Fossil Miocene *Zelkova* (Ulmaceae)

Abstract. *Organic solvent extractions of* Zelkova oregoniana, *a Miocene angiosperm compression fossil, indicate the chemical preservation of kaempferol, dihydrokaempferol, an* n*-alkane chain length range of 10 to 32 carbons, hydroxy acids, steranes, triterpenoids, and methyl pheophorbide a. This appears to be the oldest occurrence of flavonoids in fossil sediments reported.*

Green-colored angiosperm leaves have been reported by Weigelt and Noack (*1*), while Dilcher *et al.* (*2*) have isolated methyl pheophorbide a from middle Eocene sediments. We report here the organic chemical constituents isolated from the Succor Creek Flora from Oregon (*3*), which is Oligocene to Miocene in age (36 to 25 × 10^6 years old). In particular, data are presented from an analysis of *Zelkova oregoniana* compression fossils, which are vivid green and represent 90 percent of the megafossils seen on the fracture planes of fossiliferous sediments. A chemical investigation by means of paper chromatography and combined gas chromatography–mass spectroscopy (GS-MS) allowed for the detailed identification of associated organic constituents (*4*). The isolation of various fatty acids, aromatic compounds, steroids, and chlorophyll derivatives is precedented in the literature from older sediments [Green River Oil Shales of Eocene age, 53 × 10^6 years old (*5*)], as well as from Paleozoic plant debris (*4*). To our knowledge, material from the Succor Creek Flora represents the first case of the preservation of flavonoid compounds in comparably aged rock strata.

A portion of the rock containing fossil leaves of *Z. oregoniana* was pulverized and extracted for 72 hours in absolute methanol. The resultant pale yellow solution was concentrated under vacuum without heat and chromatographed on paper (Whatman, 3 mm) using standard techniques (*6*). Two spots were obtained on paper chromatography, the largest being bright fluorescent yellow, with an R_F value of 0.78 in solvent I and 0.06 in solvent II (*7*). Ultraviolet (UV) spectroscopy (*6*) showed the compound (*8*) to be the flavonol kaempferol. This identification was confirmed by cochromatography with authentic kaempferol (*9*) in additional solvents (*10*).

The second compound, occurring in much smaller amounts, appeared black under UV and UV with NH_3 and had R_F values of 0.85 and 0.56 in solvents I and II, respectively. A comparison of the UV spectra of this compound (*11*) with published data (*6*) show it to be dihydrokaempferol, the flavanonol form of kaempferol. The limited amounts of material precluded further analysis.

Previous work on wood of the extant, related taxon *Zelkova serrata* (*12*) also showed the presence of kaempferol and dihydrokaempferol, but as the 7-*O*-methyl and 6-*C*-glycosyl derivatives keyakinin and keyakinol, respectively. However, analysis of flavonoids in leaves of *Z. serrata, Z. sinica,* and *Z. verschaffeltii* available to us showed only the presence of kaempferol and quercetin 3-glycosides; that is, they lacked 7-*O*-methylation and 6-*C*-glycosylation. These data suggest that there may be considerable flavonoid variation in extant *Z. serrata* itself and, indirectly, that the flavonoids from the

Table 1. Carbon number data from gas chromatography (GC) (Apiezon L column).

GC peak number	Abundance (%)	Carbon number	Coinjected standards	Molecular formula
1	0.5	29.58	5β-Cholestane	$C_{27}H_{48}$
2	1.8	29.90	5α-Cholestane	$C_{27}H_{48}$
7	3.7	30.82		$C_{28}H_{50}$
8	1.5	30.94		$C_{30}H_{50}$
9	1.1	31.00		$C_{29}H_{52}$
12	1.3	31.23	Onocerane III	$C_{30}H_{54}$
13	1.5	31.35		$C_{30}H_{54}$
14	6.6	31.42	Lupane	$C_{30}H_{52}$
15	10.0	31.53	Stigmastane	$C_{29}H_{52}$
16	0.7	31.59		$C_{30}H_{54}$
17	11.2	31.98		$C_{30}H_{52}$
21	2.4	32.60		$C_{30}H_{52}$
24	1.3	33.02		$C_{30}H_{52}$
25	2.5	33.52		$C_{31}H_{54}$
26	3.6	33.72	Friedelane	$C_{30}H_{52}$
31	10.0	36.82	β-Carotene	$C_{40}H_{78}$