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The *mei* mutations intensify rDNA mobility in polytene nuclei of *Drosophila melanogaster*.

In a *Drosophila melanogaster* polytene nucleus, all chromosomes are gathered in a structure called a chromocenter. Two nucleolus organizers (NOs) of the nucleus, located either on the X or on the Y chromosome, are united together and form a single nucleolus. Nucleoli non-associated with the NOs, but connected to various chromosomal sites, have also been observed to occur with definite frequency in the wild type polytene nuclei (Semionov *et al.*, 1978). The ribosomal DNA (rDNA) of such mobile nucleoli contains all types of the ribosomal gene repeats (Semionov and Kirov, 1986) and displays active replication and transcription (Ananiev *et al.*, 1981).

Our previous data (Semionov and Kirov, 1986; Toshev and Semionov, 1987) show that the frequency of formation of mobile nucleoli is substantially increased under conditions of rDNA dose compensation, which provoke intensive recombination in rDNA (Tartof, 1971). The results presented here (Table 1) reveal that conditions of repair deficiency lead to the same effect. The formation of mobile nucleoli is enhanced by genotypes deficient either in the excision repair (alleles *mei-9* and *mei-9^a*) or in the post-replicative repair (alleles *mei-41* and *mei-41^{195}*), as compared to the wild-type (Canton-S males and females). The combinations of the repair deficiency and the rDNA dose compensation condition (genotypes *Xmei-41^{195}/O* and *Xmei-41/Y^{bb-}*) do not show cumulative effect on the feature analysed (Table 1).

Table 1. Frequency of the salivary gland cells with mobile nucleoli in wild-type and in mutant genotypes.

Genetic constitution	Number of nuclei analysed	Frequency of nuclei with mobile nucleoli (% ± 2 SEM)
X/Y	931	40.3 ± 3.2
X/X	608	39.3 ± 4.0
Xmei-9/Y	829	70.8 ± 3.2
Xmei-9^a/Y	768	68.9 ± 3.3
Xmei-41/Y	771	70.7 ± 3.3
Xmei-41/Xmei-41	500	67.8 ± 4.2
Xmei-41^{195}/Y	638	65.8 ± 3.8
Xmei-41^{195}/O	419	67.5 ± 4.6
Xmei-41/Y^{bb}	574	53.8 ± 4.2

The distribution of the rDNA-specific insertion sequence type 1 (see Glover, 1981), revealed by the *in situ* hybridization within polytene nuclei of the mutant genotypes, was very similar to that found earlier in the compensating *Drosophila* (Semionov and Kirov, 1986). In particular, numerous labeled inter- and intrachromosomal ectopic fibers, asterisk-like shaped nucleoli scattered throughout the genome sited, where only a part of the chromosome diameter is labelled, were observed.

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Preliminary results from an allozyme survey of *Drosophila phalerata* using cellulose acetate electrophoresis.

This paper reports early results obtained from screening three distinct laboratory stocks of *Drosophila phalerata* for allozymic variation across nineteen enzyme systems. Some preliminary data from F1 rearings of individuals from natural populations are also presented. This is the first allozymic study of this species, and the first reported use of cellulose acetate (CA) electrophoresis with a *quinaria*-group species

Since their initial use in the 1960s (*e.g.*, Lewontin and Hubby, 1966) electrophoretic variation in allozymes has proved to be a valuable tool for studying genetic population structure. Starch gels and polyacrylamide gels have been the most widely used media, although more recently cellulose acetate membranes have been gaining in popularity, due to their easier preparation and reduced run-times (Easteal and Boussy, 1987).

Stocks of *D. phalerata*, of three separate origins, were maintained in the laboratory for at least nine months by continuous culture on cereal based media (Shorrocks, 1972). The first strain originated from wild flies collected in

Table 1. Mobility and staining characteristics of allozymes of *D. phalerata*.

enzyme	buffer[a]	run-time[b] (mins)	stain intensity [c]	resolution of bands	No. of alleles detected
ACP	C	120	3-5	high	2+
AD	TG	25	5	med-low	1
ADH	TG	50	1	med	1
AO	TG	25	5	med-low	1
EST	TG	65	0-5	med	3+
FUM	TG	60	3-4	med-high	2
GLC	TG	?	0	—	0
α-GPDH	TG/C	60	5	high	2
GPI	C/TG	100	5	low	1
HEX	TG/C	?	0	—	0
LAP	C	100	0-3	low	1
LDH	C/TG	30	2-3	med	1
MDH1	C	110	4	high	2
MDH2	C/TG	110	4	high	2
ME	C/TG	110	4	high	2
ODH	TG	40	0-1	med	1
PEP1	TG	50	3-4	med-low	2
PEP2	TG	25	3-4	med-low	2+
PGM	TG	70	3-5	high	5
SOD	TG	?	0-3	low	1+
XDH	TG/C	40	3-5	med-high	1

[a]Buffer systems: TG = Tris glycine, pH 8.5; C = CAAPM, pH 7.0. (see Hebert & Beaton, 1993) Where two buffers give good results, the preferred buffer is given first.
[b]Run times required with the preferred buffer to achieve a migration of 2-4 cm. TG buffer run at 200v, CAAPM at 130v. (All allozymes migrate anodally with the exception of MDH1 with CAAPM buffer.)
[c]Stain intensity: subjective assessment from 5 (high intensity) to 0 (no staining).

suburban gardens near the centre of Leeds, England, National Grid reference SE 27-35-, (=Leeds strain). The second strain originated from larvae reared from mushroom baits placed in Arthington wood, ten kilometres north of Leeds, SE 27-45-, (= Arthington strain); and the final strain was a laboratory stock supplied from Leiden University, Netherlands, originating from locally caught, wild flies (Sevenster, pers. comm.) (= Leiden strain). One would expect each strain to be highly inbred, since laboratory populations are relatively small, but due to their separate origins they would not necessarily be fixed for the same electromorphs (alleles). To investigate levels of polymorphism in natural populations, first generation wild-type flies were obtained by rearing larvae from baits of domestic mushroom, *Agaricus bisporus*. The baits had been exposed for fourteen days in various woodlands within 30km of Leeds, using a method similar to Bingley and Shorrocks (1995).

As a prelude to future genetic population studies, the three laboratory strains were screened for allozymic variation across nineteen enzyme systems (21 putative loci) using cellulose acetate membranes (Helena cat. # 3023 and 3024) and the methods and staining recipes outlined in Hebert and Beaton (1993). Additional recipes were adapted from starch gel electrophoresis (Pasteur 1988) by increasing the concentrations and reducing the volumes of the necessary reagents.

Flies were removed from a -70 °C freezer, defrosted, and had their wings removed, before being ground in 15µl of distilled water. The homogenate was centrifuged at 13000 rpm for 30 sec and then 8µl of the supernatant was pipetted into the sample wells of a Helena sample well plate (Helena cat. # 4085 or 4095). A fixed quantity (approx. 1µl) of supernatant was transferred onto the cellulose acetate membrane using a Z-8 or Z-12 applicator (Helena cat. # 4084 or 4090). Occasionally the volumes or concentrations of some of the reagents were varied to achieve stronger or weaker staining as required. The mobility, variation and general performance of the different enzyme systems and staining recipes are detailed in Table 1.

Migration was slightly slower when run in a cold room at 4 °C rather than at room temperature, but for certain enzymes, such as α-GPDH, this produced neater, more even bands.

Further details for each enzyme system are as follows:

ACP: Up to three distinct bands of activity may appear for inbred individuals, decreasing in intensity from slow to fast. However, usually only the slowest migrating band is visible for females, and the slowest two for males; the fastest band always being very faint, if at all visible. Parkash *et al.* (1992) repeatedly observed 3 bands in inbred laboratory stocks of *D. busckii* from India, which they interpreted as fixed alleles at two separate loci producing compatible subunits for a dimeric enzyme. In one cross between two different laboratory strains of *D. phalerata*, a heterozygote banding pattern (three bands of relative intensity 1:2:1) appeared at the slowest zone, whilst the faster two banding zones were too faint to score. One possible explanation for the patterns currently observed in *D. phalerata* is that there are two independent loci, the faster of which has lost most of its activity, and is only regularly expressed in males. Nonetheless, the faster, "null" allele can still interact with the active slow allele to form a heterodimer of reduced activity.

AD and AO: Produced streaky and indistinct bands that could not be scored

ADH: Compared to most fruit-breeding *Drosophila*, *D. phalerata* has extremely low levels of ADH activity (Jacobs, 1994). Consistent with the findings of Jacobs (1994), staining is slightly stronger using iso-propanol as a substrate rather than ethanol, but still not sufficient for reliable scoring.
EST: Extremely variable in the number and quality of bands that appear. There is certainly some polymorphism at this locus/loci, but interpretation is problematic.
FUM: Is a tetrameric enzyme as revealed by a 5-banded heterozygote. The rare electromorph has a slower relative mobility (approx. 60%).
GLC: As of yet no activity has been detected, but may be worth further experimentation.
α-GPDH: The quality of the bands is susceptible to the temperature at which the plate is run, better results being obtained at lower temperatures. A rare electromorph with approximately 120% relative mobility was detected from natural populations.
GPI: Produces streaky bands with both standard buffers.
HEX: No activity detected, the reasons for this are unclear.
LAP: Weak, indistinct bands, not suitable for analysis.
LDH: Fairly weak bands, but could be useful, no polymorphism detected yet.
MDH: MDH1, the mitochondrial form migrates cathodally in CAAPM buffer. MDH2, the supernatant form, migrates anodally. Alternative (slower) electromorphs have been detected occasionally at both loci, from natural populations.
ME: Can be run in combination with MDH. A rare allele (slower electromorph) was detected from natural populations.
ODH: Stains very weakly, often shows up ADH bands as well.
PEP: Stains more strongly when using *leu-ala* rather than *leu-gly* as the dipeptide substrate. Two independent loci, both of which seem to harbour considerable polymorphism, however, the bands are often wide and of similar mobility and consequently difficult to score reliably.
PGM: Inter-strain crosses confirmed that this is a monomeric enzyme with simple Mendelian inheritance. Five readily distinguishable electromorphs were recovered from F1 rearings, originating at Arthington Wood. The three commonest electromorphs were present in the laboratory strains.
SOD: This enzyme does not stain well on cellulose acetate membranes (see Hebert and Beaton, 1993), and is often very streaky and sporadic.
XDH: Stains reasonably well with TG or CAAPM buffers and may be run in conjunction with MDH and/or LDH. No polymorphism detected in *D. phalerata* to date.

Discussion: The closest phylogenetic relative to *D. phalerata* for which allozyme information exists is *D. falleni* and, with the exception of LDH, all of the above have been found to be polymorphic in *D. falleni* when assayed with starch-gel electrophoresis (Lacy, 1982a,b, 1983; Shoemaker and Jaenike, 1997). Shoemaker and Jaenike (1997) also found EST, LAP, MDH1, MDH2, PEP1 and PGM consistently polymorphic for two other *quinaria*-group species. Offenberger and Klarenberg (1993) found no polymorphism in alpha-amylase, when comparing eight German lines of *D. phalerata*.

The low levels of variation observed in this study relative to Lacy (1982) or Shoemaker and Jaenike (1997) may be due to three main reasons. Firstly, by chance, all three laboratory strains may have been fixed for the same alleles. This is most likely where one allele is much more frequent than the others in natural populations. Nonetheless, one might expect the Dutch stocks to differ more substantially from the two stocks originating near Leeds, England. Secondly, CA may detect less variation than starch gel electrophoresis, a possibility that has been suggested by Jaenike (pers. comm.) although Easteal and Boussy (1987) suggest the opposite, namely, that CA electrophoresis often results in superior separation. Thirdly, it may indeed be the case that, relative to the studies on North American *quinaria*-group species, *D. phalerata* does possess low levels of genetic variation.

Conclusion: Of the enzyme systems tested to date PGM shows by far the greatest amount of allozymic variation using CA electrophoresis, and could profitably be used in studies of genetic population structure of *D. phalerata*.

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609; Parkash, R., J.P. Yadav, and Shamina 1992, Korean J. Genetics. 14: 107-118; Pasteur, N., G. Pasteur, F. Bonhomme, J. Catalan, and J. Britton-Davidson 1988, *Practical Isozyme Genetics*. Ellis Horwood Ltd, Chichester; Shoemaker, D.D., and J. Jaenike 1997, Evolution 51: 1326-1332; Shorrocks, B., 1972, *Drosophila*. Ginn and Company Ltd, London.

Oliver, Brian, and Daniel Pauli. Department of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland. Correspondence to: Brian Oliver, LCDB NIDDK NIH, 6 Center Dr. MSC 2715, Building 6 Room B1-13, Bethesda MD 20892. Email: oliver@helix.nih.gov. Duplications of *ovo*$^+$ do not result in XY germline death or sex transformation.

The *ovo* locus encodes C_2H_2 sequence-specific DNA-binding proteins (Mével-Ninio *et al.*, 1991; Garfinkel *et al.*, 1992; Lü *et al.*, 1998) that have been implicated in germline sex determination based on XX-specific germline death and germline sex transformation phenotypes; and on genetic interactions with mutations in *Sxl*, *snf*, *otu* and *stil* (Oliver *et al.*, 1987, 1990; Pauli *et al.*, 1993; Staab and Steinmann-Zwicky, 1996; Penetta and Pauli, 1997). At the molecular level, the *ovo*$^+$ locus is required for female-specific splicing of *Sxl*$^+$ mRNAs in the germline (Oliver *et al.*, 1993) and for high level expression of *otu* and *ovo* reporter genes (Oliver *et al.*, 1994; Mével-Ninio *et al.*, 1995; Lü *et al.*, 1998).

Given that decreased *ovo*$^+$ activity results in XX germline death and the differentiation of some surviving germ cells as spermatocytes (Oliver *et al.*, 1990, 1993), we were interested in determining if, conversely, increased *ovo*$^+$ activity would result in XY germline death and male to female transformation. This is especially pertinent given that *ovo* is an X-linked gene and is, therefore, present in two copies in females, but only one copy in males. The dose of *ovo*$^+$ was elevated using *ovo*$^+$ transgenes that fully rescue the ovo$^-$ germline phenotype in females and result in functional OVO proteins in male germ cells (Andrews *et al.*, 1998; Lü *et al.*, 1998). The most striking phenotype seen in females homozygous for strong loss-of-function *ovo* alleles is the extreme reduction of germ cell number (usually none) in XX flies (Oliver *et al.*, 1987, 1994; Staab and Steinmann-Zwicky, 1996). We did not see a corresponding germ-cell-death phenotype in males bearing three to five copies of *ovo*$^+$. There were rare individuals (less than 1%) with few or no germ cells, but germ cell death was not characteristic. Most testes had wildtype gross morphology, and flies with up to five copies of *ovo*$^+$ were at least semi-fertile. At the cellular level, the morphology of the germ cells appears to be male in hundreds of examined testes.

However, keeping *ovo*$^+$ expression levels low may be important for late steps in spermatogenesis (Figure 1). Nearly all XY male flies with five copies of *ovo*$^+$ showed spermatid cysts with defects and other spermatid cysts that appeared wildtype. The most common defect was cells with a single large mitochondrial derivative surrounded by four spermatid nuclei. This phenotype is similar to that found in a number of male-sterile mutations and is believed to be due to defects in the completion of cytokinesis during meiosis (reviewed by Fuller, 1993). More rarely we saw altered spermatid nuclei sizes. Because nuclear size is dependent on chromosome content, non-uniform nuclei could be due to non-disjunction (Gonzalez *et al.*, 1989). If so, defective sperm were not successfully transmitted, as we found little XY or 2nd chromosome non-disjunction (few *+/+/B^{s}Y, +/0*, or *2^2/0* progeny). Meiosis in *Drosophila* is sexually dimorphic (reviewed by Hawley, 1993) and a female meiosis in a male germ cell might well be expected to result in gross defects in spermatogenesis. Indeed, *ovo* mutations disrupt recombination in female germ cells, which indicates that *ovo* is required for proper sex-specific meiosis (Cook, 1993). However, we do not know if the defects we saw in XY males with increased copies of *ovo*$^+$ were due to reciprocal sex-specific defects in meiosis. The salient point for this work is that high *ovo*$^+$ copy number was not sufficient for overt of XY germ cells. Absence of *ovo*$^+$ in females and extra copies of *ovo*$^+$ in males do not result in reciprocal phenotypes.