0.20%) to day-6 cultures induces progressive retraction of the cell margin. Figures g and h show the same living cells before (fig. g) and after (fig. h) ethanol treatment). In addition to inducing the retraction of cell processes, the single exposure to ethanol alters existing cell-to-cell contacts. When cells are removed from ethanol for 22 h, effected cells remain unable to develop normal processes whereas uneffected cells in the same cultures continue to differentiate. Moreover, autoradiographs reveal that ethanol-effected cells (2 h 20 min) remain capable of incorporating tritiated thymidine or leucine and synthesize them into DNA and protein respectively. Tritiated thymidine is exclusively incorporated above the nuclei whereas the leucine label is found over the entire cell. The fluorescent actin pattern following short-term ethanol treatment is similar to that observed following long-term ethanol treatment (figs d and f). Using Nomarski optics, we determined that ethanol-induced changes are not accompanied by cell lysis.

Cell shape changes are triggered by ethanol in a number of systems[6–10], and this uniform cellular response underscores the likelihood that actin is a teratogenic target. In particular, ethanol induces changes in the dendritic arborization of neurons in the cerebral cortex[9] and neurite elongation of chick embryo sensory and spinal cord neurons[7]. Our findings corroborate and extend these studies. After ethanol treatment, many neural crest cells do not undergo dendritic branching, a condition we show to be correlated with a disorganized actin cytoskeleton. These effects in our culture system are due to ethanol alone because there is no enzymatic conversion to acetaldehyde or acetate in isolated neural tubes and crest cells. However, it is important to stress that in situ the ethanol byproducts acetaldehyde and acetate may pass from the maternal to the fetal circulation and therefore possibly play a role in morphogenetic alteration[1].

The known ability of cells to develop a metabolic and functional tolerance to continuous ethanol exposure may account for the presence of some cells with a normal morphology[1]. At present, we do not know whether ethanol's effect upon actin is direct or indirect. Consideration of ethanol's insult is made difficult by actin's intimate association with the cell membrane, which is known to become more fluid in the presence of ethanol[13]. Specifically, the ethanol permeation may affect a reconfiguration of proteins and lipoproteins of the plasmalemma[14]. Membrane proteins play an important role as select linkers of the actin cytoskeleton to the membrane and probably participate in the restructuring of the cytoplasmic matrix during morphological differentiation[15]. Also, an ethanol-induced change in membrane proteins and lipoproteins may induce altered ionic fluxes[14]. There is evidence from brain studies that ethanol reduces membrane-bound Ca^{2+}[16]. This reaction to ethanol could have important morphogenetic consequences because the Ca^{2+} pool, as well as its related fluxes, controls actin-assisted contraction and relaxation. Moreover, it is possible that the functional association of actin microfilaments (5–8 nM) and microtubules (tubulin, 25 nM) is somehow affected[17].

Morphogenetic dysfunction of the neural crest in situ could conceivably be expressed at the level of cell migration and/or morphological differentiation[3]. While in situ, ethanol may cause subtle alterations in the migratory pattern of neural crest cells, it is clear from the present study that even after continuous treatment, neural crest cells remain capable of migrating in vitro and that these cells maintain their ability to synthesize both DNA and protein. What is shown for the first time in this study is that ethanol treatment markedly disrupts the organization of the actin cytoskeleton and prevents normal dendritic branching in many cells. Even when applied in the short-term to well-differentiated neural crest cells, ethanol rapidly disrupts the actin pattern, promotes cell retraction, and alters cell-to-cell filopodial contacts.

During morphogenesis, cellular interactions are dependent upon precise contact at cellular interfaces. We suggest, therefore, that ethanol-induced malformations may result from the inability of aberrently formed neural crest cells (i.e., mesenchyme) to interact normally with the primordial tissues that form craniofacial structures. Our findings may provide a basis for further investigations on how ethanol causes the morphogenetic anomalies manifested in FAS.

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Differential expression and dosage compensation of the α-amylase gene in *Drosphila miranda*[1]

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Summary. The α-amylase gene of *Drosophila miranda* is located on the X^2- and on the neo-Y-chromosome, both developing sex chromosomes. Crosses between strains carrying different electrophoretically distinguishable alleles of the α-amylase gene were performed. Females of the F_1 offspring showed the expected heterozygosity, while the males proved to be hemizygous for this locus. Only the gene on the X^2-chromosome is expressed, whereas the corresponding gene on the neo-Y-chromosome is not. Estimates of the α-amylase activity in crude homogenates of male and female flies suggest strongly that the α-amylase gene is dosage compensated in *D. miranda*. In contrast to this situation, in all other *Drosophila* species the α-amylase allele is autosomal and hence not dosage compensated.

Key words. Differential gene expression; *Drosophila miranda;* dosage compensation; α-amylase.

α-Amylase activity in male and female flies of *D. miranda*. Results from four independent series of determinations were pooled. The mean values with the standard deviations are given

Drosophila miranda	Maltose/protein (nmoles/μg)
Male	9.03 ± 1.49
Female	9.25 ± 1.70
Male/female	0.98

The translocation of one of the two autosomes from the C element pair to the Y-chromosome in an ancestor of the *Drosophila miranda* species led to an extraordinary *Drosophila* karyotype: male metaphase plates consist of 2n = 9 chromosomes only, while the females show the expected chromosome number 2n = 10. Due to this chromosome rearrangement a monosomic X^2-chromosome occurs in the male karyotype besides the X^1-chromosome and the autosome-Y-translocation (designated neo-Y-chromosome[3], see fig. 1). This neo-Y-chromosome shows the phenomenon of genetic degeneration[4], while the X^2-chromosome appears to be on the way becoming a sex chromosome with partial dosage compensation[5]. Dosage compensation is a regulatory mechanism for X-chromosomal genes to overcome the dose differences between the XX and the XY chromosome constitution in females and males respectively. According to the hypothesis of Muller[6,7] the Y-chromosome has lost its genetic activity owing to a slow 'degeneration' as a consequence of its permanent heterozygosity. This phenomenon is thought to be associated with an accumulation of mutations.

We are, for this reason, interested in genes located on the X^2- or the neo-Y-chromosome and their regulation. One of these genes is the α-amylase locus. The location of this gene in the X^2- and the neo-Y-chromosome is deduced from chromosome homologies[8] with other *Drosophila* species. In *D. melanogaster*[9] the α-amylase gene lies on the right arm of chromosome 2 (2R) section 54A and 55 and in *D. pseudoobscura*, a sibling species of *D. miranda*, on chromosome 3. Both chromosome elements are homologous to the X^2-chromosome and the translocated section of the neo-Y-chromosome of *D. miranda*.

Material and methods. *D. miranda* flies were cultured on standard *Drosophila* food at 18 °C. The *D. miranda* strains 32/33, 36/3, 44/94, 45/91, 46/46 are isolines from single females collected in the wild. Strain Mir/100 is a combination of the two isolines, 21/70 and 28/34. These strains were kindly provided by Betty C. Moore. Strain *D. miranda* 14011-0101 was obtained from the National Drosophila Species Resource Center, Bowling Green, Ohio, U.S.A. *D. miranda* MPI is a strain from our laboratory which is of unknown origin. For preparation of crude fly homogenates and for details on electrophoresis the protocol of W. Pinsker (personal communication) was followed. Flies, frozen in solid CO_2, were homogenized in 25 μl sample buffer. A stock solution of the sample buffer was prepared as follows: 1 ml of a 0.47 M Tris solution, adjusted with H_3PO_4 to pH 6.9; 4 ml 40% sucrose solution; 3 ml H_2O and 40 μl of a 1% bromphenol blue solution. The homogenate was centrifuged for 30 min at 4 °C in an Eppendorf minifuge. To 20 μl of the supernatant 3 μl of 100% glycerol were added. After vortexing, the sample was applied directly to a gel. The polyacrylamide gelelectrophoresis was performed according to Davis[10]. We used a 3.75% stacking and a 7.5% resolving gel and the multiphasic buffer system A (cf. Davis Table 3.1). The gel was run for 3.5 h, 200 Volt and 20 mA. To make the α-amylase bands visible the gel was equilibrated for 10 min in 0.5 M Tris-HCl, pH 7.1 and then incubated for 90 min in a starch solution (1 g soluble starch and 220 mg $CaCl_2$, dissolved in 100 ml of boiling 0.1 M Tris-HCl, pH 7.5). The gel was washed twice in distilled water and stained for 2–5 min in a staining solution[11] (300 mg KI and 130

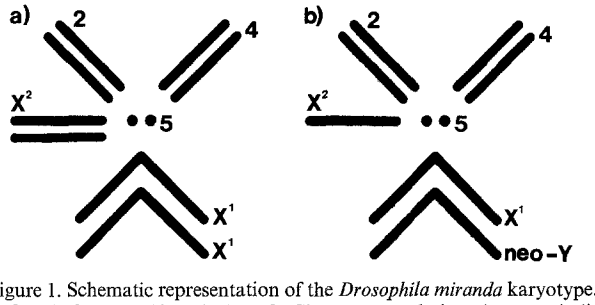

Figure 1. Schematic representation of the *Drosophila miranda* karyotype. a) female 2n = 10, b) male 2n = 9. Chromosome designations are indicated. Beside the two X-chromosomes, designated X^1, the female karyotype shows a pair of X^2-chromosomes (corresponding to element C), while in the male karyotype only a single X^2-chromosome (monosome) can be identified. The homologous chromosome element is translocated to the Y-chromosome, now designated neo-Y-chromosome.

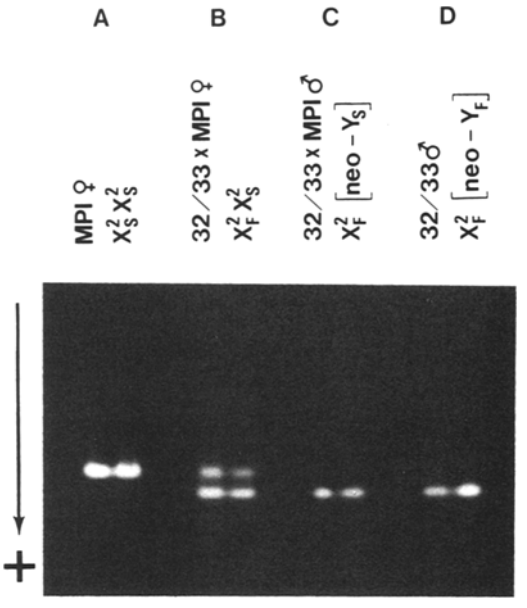

Figure 2. The two electrophoretic variants of α-amylase of *D. miranda*. Strain MPI (slow, S), lane A, B and strain 32/33 (fast, F), lane C, D. In each gel slot the homogenate of 4 flies was applied.

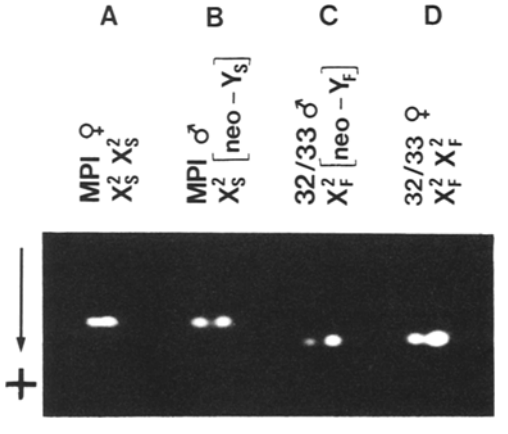

Figure 3. α-Amylase pattern in the crosses between strain 32/33 (F) and MPI (S). Lane A: MPI ♀♀ (parental strain used as mobility reference); lane B: F_1-♀♀ from the cross 32/33 × MPI; lane C: F_1-♂♂ from the cross 32/33 × MPI; lane D: 32/33♂♂ (parental strain used as mobility reference). While the expected heterozygosity (2 bands) is seen for the F_1-♀♀ hemizygosity (1 band) occurs in the F_1-♂♂. Only the allele of the X^2-chromosome is expressed.

mg iodine dissolved in 100 ml distilled water). For documentation the gel was photographed with an AGFA Ortho, 25 ASA, film.

In order to determine whether the α-amylase allele on the X^2-chromosome is dosage compensated in males or not, the α-amylase activities in the homogenates of female and male flies were estimated in relation to the total protein content. To avoid dietary effects on the α-amylase activity all flies of one series were taken from the same bottle[9]. In four independent series using 2 males or females respectively, flies were homogenized in 25 µl phosphate buffer (20 mM $Na_2HPO_4 \times 2\ H_2O$, 20 mM KH_2PO_4, 25 mM NaCl, pH 7.4). The homogenate was adjusted with the same buffer to a final volume of 50 µl and centrifuged for 30 min, 4 °C. From the supernatant two aliquots of 12.5 µl each were used to determine the α-amylase activity by the modified 3,5-dinitrosalicylic acid reduction assay[9] and simultaneously the protein concentration measured according to Bradford[12]. For the measurement of the α-amylase activity the samples were incubated for 30 min at 25 °C.

Results. Eight different strains of *D. miranda* were screened for electrophoretic α-amylase variants. Only one, strain 32/33, showed a different, faster moving allozyme (designated F), while all the other seven strains showed the same slow allozyme (designated S). The mobility differences between strain 32/33 and MPI are illustrated in figure 2. Both strains show a single band. Crosses were made in both directions between the F- and the various S-strains. The electrophoretic pattern of the α-amylase variants appearing in one of these crosses is exemplified in figure 3. The heterozygote F_1 females show the expected double band pattern, while the F_1 males prove to be hemizygous, showing only one band.

Since only one α-amylase gene is expressed in the *D. miranda* male genome, it appeared to be of interest whether this gene is dosage compensated. We determined the α-amylase activity in crude homogenates of male and female flies[9] in relation to a defined protein amount (table). The obtained ratio male/female = 0.98 is close to 1, as is to be expected of the α-amylase genes in males and females produce the same amount of enzyme. As a consequence the only active α-amylase gene on the X^2-chromosome must be dosage compensated in males.

Discussion. The electrophoretic analysis of the α-amylase variants demonstrates that only the gene on the X^2-chromosome is expressed in males whereas the gene on the neo-Y is silent. At present we do not know the molecular basis of the inactivation of the gene on the neo-Y but we assume that this is due to the general degeneration of the neo-Y-chromosome[4]. Because in the male karyotype only the gene of the X^2-chromosome is expressed it seemed interesting to look at the dosage compensation. It is known from other investigations that about 70% of the X^2-chromosome are dosage compensated, 30% are not[5]. To compensate the differences in body size between males and females the α-amylase activity was determined in relation to the protein content in the crude homogenates. The obtained male/female ratio of 0.98 strongly suggests that the α-amylase allele in the male is dosage compensated and must be located in the 70% area of the X^2-chromosome. In other *Drosophila* species the α-amylase gene is autosomal. In order to learn more about the regulation of this gene experiments are under way to isolate the α-amylase gene from a genomic *D. miranda* phage library.

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Chromosomes of three *Brachymeria* species (Hymenoptera: Chalcididae)[1]

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Summary. Brachymeria intermedia, a pupal parasite of the gypsy moth *Lymantria dispar,* has a karyotype of K = 2SM+1M which is the lowest number (n = 3) in Hymenoptera. *Brachymeria lasus* has K = 3M+1SM+1A (n = 5), and *B. ovata* has K = 1M+2SM+2A (n = 5).

Key words. Brachymeria; Hymenoptera; Chalcididae; karyotype.

Haploid chromosome numbers in the order Hymenoptera have been reported ranging from 3 to 42[2]. The lowest number of 3 is found in the ant *Ponera scabra,* although 2n = 7 (female) and n = 4 (male) were reported for this species in the original paper[3]. As this species involves a complicated translocation polymorphisms and no males having n = 3 were observed (H. T. Imai, personal communication, 3/23/85), the case of *P. scabra* should not be considered the lowest number in Hymenoptera in the strict sense. Here, I report the karyotype of 2n = 6 and n = 3 in *Brachymeria intermedia,* a chalcid pupal parasite of the gypsy moth *(Lymantria dispar),* and 2n = 10 or n = 5 in two other species of *Brachymeria.*

Material and methods. Cerebral ganglia of early instar larvae, testes and ovaries of white pupae from laboratory cultures of *B. intermedia* (6 males, 5 females), *B. lasus* (6 males, 1 female), and *B. ovata* (6 males, 8 females, and 2 larvae) were used. *Brachymeria intermedia* was introduced from Europe as early as 1905 and has been established in North America since 1942[4]. The *B. lasus* culture originated from Japan. *Brachymeria ovata* is the only native species and the culture originally came from Ari-