that neither the epinephrine nor the norepinephrine content of the adrenal glands changed after central 6-OHDA administration. Further studies will be required to obtain more detailed information concerning the role of central catecholamines in the metabolism of adrenal catecholamines.

In summary, the present results indicate that under physiological conditions, central monoaminergic neurons have an inhibitory effect on the adrenergic neurons of the vas deferens. This inhibitory regulation, however, does not appear to be exerted on the adrenal glands. The roles of the central catecholamines in the regulation of catecholamine metabolism in the adrenal glands remain to be elucidated in further studies.

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Rapid isolation of mitochondrial DNA. Mitochondrial DNA from *Drosophila serrata*

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Abstract. A simple and rapid method for isolation of high quality mitochondrial DNA (mtDNA) is presented in this report. Using this method, isolation and restriction site maps for 10 enzymes of the mtDNA of *Drosophila serrata* were established.
Key words. Mitochondrial DNA; isolation; *Drosophila serrata.*

Analysis of mtDNA has proved to be a powerful tool in many fields of biology. Simple and rapid methods for mtDNA isolation are needed especially in population genetics where many samples have to be examined. High quality mtDNA is essential, so that digestion enzyme analyses and Southern blot hybridizations yield unequivocal results. We describe here a rapid and efficient two-step method of mtDNA isolation: a) the purification of the mitochondria, avoiding the nuclear DNA, and b) the isolation of the mtDNA. The reagents used in this protocol are very common in every laboratory [1].

Using the above method, the mtDNA of *Drosophila serrata*, a species belonging to the *montium* subgroup of the *melanogaster* species group [2, 3], was isolated and digested with several restriction enzymes. Both restriction enzyme analyses, and hybridizations mtDNA fragments either from *D. serrata* or from *D. melanogaster* as probes, result in a detailed map for 10 restriction endonucleases.

Materials and methods

Animals. Drosophila serrata, strain No. 3018.1 from the University of Texas Stock Center [3] and *D. melanogaster,* Canton/S (C/S) were used in the present study.

Isolation of the mtDNA. The method used to isolate the *Drosophila* mtDNA, is described below:

1) Frozen flies (20 min, −20 °C) (100 mg = 100 flies) were homogenized gently with a dounce homogenizer in 1 ml of buffer containing 0.3 M sucrose, 10 mM EDTA, 30 mM Tris-HCl (pH 7.5).
2) The homogenate was centrifuged, 2–3 times, (5 min, 1000 × g) until all particulate matter (nuclei, cellular debris, etc.) had been pelleted out.
3) The supernatant was centrifuged (20 min, 12,000 × g, +4 °C) and the mitochondrial pellet was resuspended in 100 µl of the homogenization buffer (step 1) and transferred into an eppendorf tube.
4) 300 µl of a buffer containing 5% sucrose, 10 mM EDTA, 300 mM Tris-CHl (pH 8) was added. 20 µl of 20% SDS was added and the mixture was incubated for 15 min at 65 °C.
5) 100 µl of 5 M potassium acetate, pH 5 was added, mixed gently, and incubated for 15 min, −20 °C.
6) Centrifugation at 13,000 × g for 5 min followed and then the supernatant was transferred into a new eppendorf tube.
7) Extraction and purification of the mtDNA was performed according to standard procedures[1].
8) The pellet was dissolved in 20 µl of 1 × TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

Agarose gel electrophoresis and Southern transfer. Isolated mtDNA was digested with several restriction enzymes (according to the instructions of the manufacturer), fractionated in 0.8% agarose gels and transferred onto nitrocellulose filters[4].

Labelling of the probes and hybridizations. MtDNA isolated by the above method was digested, loaded on a (0.5%) low melting point agarose gel, the appropriate band was isolated, and the DNA was extracted with phenol/chloroform: isoamylalcohol (50/48 : 2). 20 ng of this DNA was labelled using the random priming-method[5] with ^{32}P-dCTP as radioactive precursor. Hybridizations were performed according to Church and Gilbert[6] at 65 °C.

Results and discussion

MtDNA isolation. The protocol described in this report is based on two main steps: first, isolation of very pure mitochondria, and second, treatment of the mitochondria in a manner similar to that of bacteria and bacteriophages[1] in order to isolate the mtDNA. During the first step (1 to 4, see 'Materials and methods') intact nuclei have to be well separated so that no nuclear DNA contaminates the mitochondrial fraction. Afterwards, a heat-freeze step (5 and 6) destroys the mitochondria and the mtDNA is released. No alkali is used in this method, and it is here that our method differs from other methods previously described[7–9]. We avoided the alkaline denaturation, since this often leads to degradation of the mtDNA. The results of the described method are shown in figure 1. Isolated mtDNA from *D. melanogaster* (lane 2) and from *D. serrata* (lanes 3 to 14) are digested with

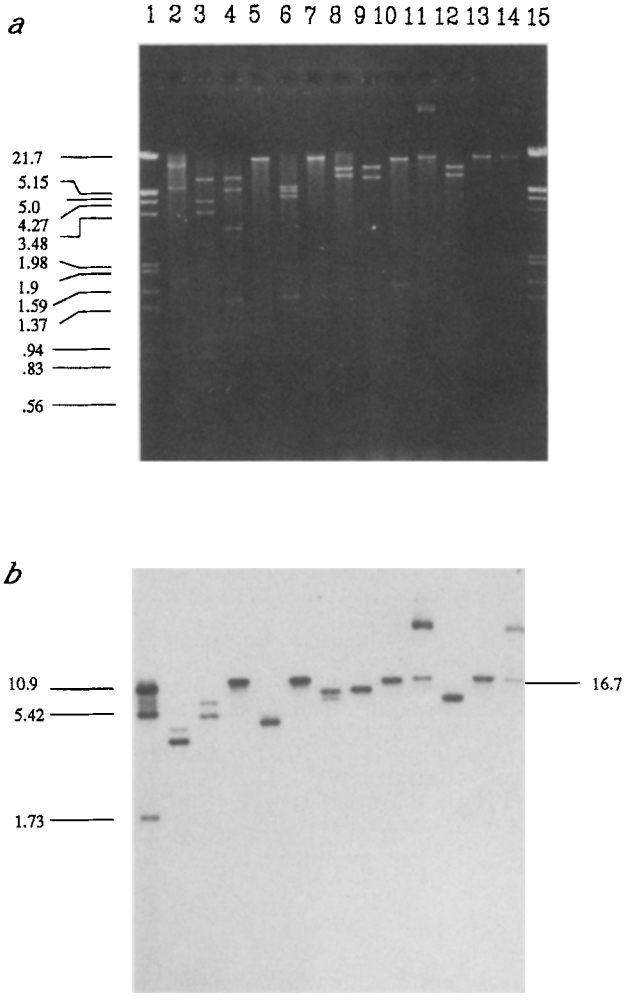

Figure 1. *a* Restriction enzyme digests of mitochondrial DNA (mtDNA) from *Drosophila serrata*. Lanes 3 to 14, mtDNA of *D. serrata* digested with: EcoRI (3), HindIII (4), BglII (5), ClaI (6), HpaI (7), XbaI (8), Hae III (9), EcoRV (10), PvuI (11), PstI (12), XhoI (13) and SalI (14). Lanes 1 and 15, λ III marker as a reference size marker. Lane 2, EcoRI digests of the mtDNA of *D. melanogaster* (C/S). *b* DNA from the above gel was transferred to a nylon membrane and hybridized with the isolated 6.18 kb HaeIII mtDNA fragment of *D. melanogaster* (see fig. 2). Numbers on the left indicate known fragments (λ III marker fragments in the case of *a*, and mtDNA fragments from an EcoRI digest of *D. melanogaster* in *b*). The number on the right indicates the estimated size of the mtDNA of *D. serrata*. The sizes are given in kilobases (kb).

different restriction endonucleases. Each lane contains the resulting mtDNA from approximately 12–15 flies and the yield can easily be compared with the λ III marker (fig. 1a). Undigested mtDNA, as shown in lanes 11 and 14 of figure 1, shows its obvious purity. Figure 1b shows the filter obtained by blotting the gel seen in figure 1a, and hybridizing it to the 6.18 kb HaeIII fragment of the mtDNA of *D. melanogadster* (see fig. 2, *D. mel*).

The mtDNA of Drosophila serrata. MtDNA from *D. serrata* was isolated and digested with 12 restriction endonucleases (figs. 1 and 2). Two of them, PvuI and SalI (lanes 11 and 14, respectively of fig. 1), are unable to digest the mtDNA of *D. serrata*. By measuring the

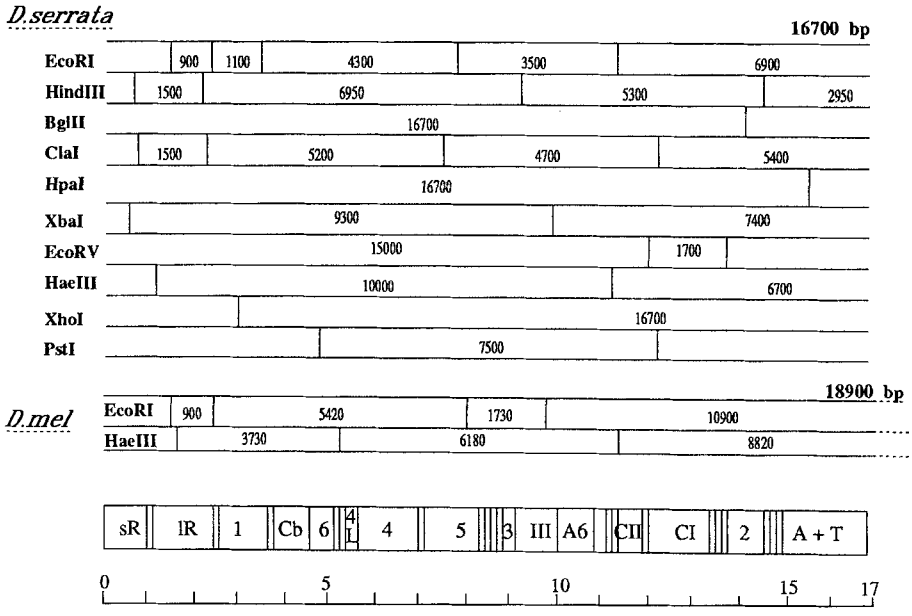

Figure 2. Mitochondrial DNA (mtDNA) site restriction maps of *Drosophila serrata*. *D. melanogaster* restriction maps for EcoRI and HaeIII (see also Solignac et al.). The equivalent mtDNA organization, at the bottom, is taken from Clary and Wolstenholme[10].

bands produced by the EcoRI and ClaI restriction endonucleases (fig. 1a), we estimated that the total length of the mtDNA of *D. serrata* is rather small, about 16.7 kilobases (kb). However, fragments longer than 6 kb could not be measured accurately. Bands corresponding to very small DNA fragments which may have not been visualized in our gels, or were not detected by hybridization experiments, are not included in our estimation.

Single and double, complete and partial digestions were performed in order to map the mtDNA of *D. serrata*. In addition, a number of mtDNA restriction fragments was isolated both from *D. serrata* and *D. melanogaster* and used as hybridization probes. An example of this procedure is shown in figure 1. The final restriction maps of the mtDNA of *D. serrata* for 10 different enzymes is shown in figure 2.

Taking into account all the restriction enzymes, a total of 24 different sites was detected, which correspond to a sample of 144 nucleotides, about 0.9% of the mtDNA of *D. serrata*. The 0.9 kb EcoRI fragment which has been found in several *Drosophila* groups studied[10-12] is also present in the mtDNA of *D. serrata*, indicating the conservation of the large rRNA gene (lR, fig. 2).

It has been proposed that the evolution of mtDNA in *Drosophila* species goes in parallel with the evolution of the genome[13] and the sizes of the mtDNA molecules fit with the phylogeny of the species[11,12]. If this is so, then the biochemical and cytogenetical data previously reported[3,14], suggesting a rather old origin of *D. serrata*, are in agreement with the present results concerning the size of the mtDNA of this species.

We consider that the advantage of the method described here is its simplicity and the quantity of high quality mtDNA obtained. By changing mainly the concentration of the sucrose, this method can be applied to many other organisms or cells as well (Pissios and Scouras, in preparation). Using this method, restriction site maps for 10 enzymes of the mtDNA of *D. serrata* have been established. As far as we know these are the first mtDNA data concerning the *montium* subgroup of the *melanogaster* species group.

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