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OBSERVATIONS SUR L'AVIFAUNE MIGRATRICE DANS LES SPORADES DU NORD

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Des observations effectuées au cours du printemps(1984,1986)et de l'automne(1984,1985)dans 9 iles et ilots,les plus distantes du conti-nent, des Sporades du Nord ont permis de recueillir une série d'infor-mations inédites sur les especes migrant, sur les iles et les biotopes fréquentés*.On a relevé 81 especes migrant au printemps -avec la pré-sence de 4 rapaces- et 32 en automne -avec la présence de 11 rapaces.

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I-IV4

THE IN VIVO EFFECTS OF PESTICIDES ON AMPHIBIAN ESTERASE ISOZYMES

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Non-specific esterases represent a complex major hydrolytic enzyme system cata-Non-specific esterases represent a complex major hydrolytic enzyme system cata-lysing the hydrolysis of carboxylic ester bonds. Esterase isozymes have been chara-cterized according to their inhibitor and substrate specificities. An extended clas-sification of esterase isozymes according to inhibition by disopropylfluorophosphate. (DFP), eserine sulfate and parahydroxymercuribenzoate (PHMB) has been reported (1). These esterase inhibitors also represent environmental pollutants. In a previous study of frog liver esterases by polyarylamide gel electrophoresis (PAGE) several isozymes were resolved, characterized as carboxylesterases and cholinesterases both inhibited by DFP (2). were resolv by DFP (2).

by DFP (2). In the present study 25 male and female specimens of Mertensiella luschani luschani ni were collected from Castellorizo an island of south eastern Aegean Sea. This spe-cies is a protected one in Greece. Tissues were homogenized with 0.1 M Tris-HC1 buffer pH 7.2, extracts were electrophoresed and gels stained for esterase activity in the presence and absence of inhibitors according to Haritos & Salamastrakis (3). Isoelectric focusing (I.F.) on gel slabs (pH 3.5-9.5) was performed according to Veini et al. (4) and Sephadex G-200 gel filtration according to Haritos & Rosemeyer (5). (5).

(5). Liver had the highest overall esterase activity as seen by the number of the bands and their staining intensities (Fig. 1). Four groups of esterase bands were distinguished according to their electrophoretic mobilities, phenotypic variation, tissue distribution and substrate and inhibitor specificities. Isozymes of groups II and III were observed only in the digestive system while groups I and IV were present in all tissues examined. No sex-specific esterases were observed. Esterase isozyme IV has an apparent molecular weight of 245,000 while for the rest of the isozymes the molecular weight was 75.000. By preincubating the gels in 0.1 mM DFP, 0.1 mM eserine sulphate and 1 mM PHMB all esterases were characterized as carboxylesterases. DFP inhibited all bands while genome and PHMB while eserine and PHMB inhibited only partially the bands of group III. All esterases showed comparable heat lability with complete inactivation at 50°C in 15 min. The staining intensity of the bands was inversely related to the length of

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Fig. 1. Electrophoretic patterns of soluble nonspecific esterases in Mertensiella luschani luschani. L, liver; S, stomach; I, intestine; M, muscle; T, testis; O, ovaries; B, brain. Or, origin and Bb, bromophenol blue band. Latin numerals stand for the four groups of esterase isozymes. a-Naphthyl acetate was used as substrate.

the organic acid moiety of the alpha-naphthyl esters with only group II bands stain-ing with alpha-naphthyl caprate (ClO). The isoelectric points of the esterases ranged from 4.60 to 5.65. In order to assess the effect of DFP in liver and brain, pairs of Mertensiella luschani uschani were put in 100 mi or 1 mM and 0.1 mM of this inhibitor for 20 min. All bands were reduced in intensity in liver while complete inhibition was ob-served in brain for both concentrations (Fig. 2). In 40 min at 1 mM the animals were dying and no esterase bands could be detected in liver extracts (results not shown).

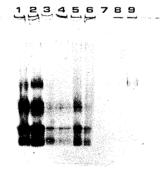


Fig. 2. The in vivo effect of DFP in Mertensiella luschani luschani liver and brain. Lanes 1-6, liver; lanes 7-9 brain. In lanes 1,2,9 tissue extracts of control animals. In lanes 3,4,7 exposed to 1 mM DFP and in lanes 5,6,8 to 0.1 mM DFP.

The evidence from this study suggests that the toxicity of organophosphates in amphibians could be directly related to the irreversible inhibition of serine este-rase isozymes (carboxylesterases) in at least two of their tissues. Moreover none of the several esterase isozymes revealed by PAGE or I.F. was found to be resistant to the in vitro and in vivo deactivation by DFP. This is in agreement to a similar study for frog esterases (2) while this is not the case for fish (1,3) or avian (4) estera-ses. Although further experiments are needed for the determination of the environ-mental impact of commonly used organophosphate pesticides on amphibia, this verte-brate class should be considered particularly vulnerable. study

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