Yolk protein differences between species of *Drosophila*[1]

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Summary. 1-3 major proteins considered as vitellins or yolk proteins are detected in mature eggs from different *Drosophila* species by gel electrophoresis on gradient slab gels. Qualitative and quantiative differences are found even between closely related species. In heteroplastic transplantations, no correlation was found between the similarity or dissimilarity of the protein pattern on the one hand, and success of failure of egg development and vitellogenesis on the other hand.

1-3 yolk proteins or vitellins have been described for *D. melanogaster*[2-5]. The number of such major proteins observed depends largely upon the type of polyacrylamide used for the separation. An investigation using different *Drosophila* species[T] has shown that the immunoreaction of antibody obtained from ovary extracts is, to some degree, specific for each species. On the other hand, it has been demonstrated that the adult immature ovary of Nematoceran Diptera develops normally and forms mature eggs, both in homo- and heteroplastic transplantations under normal nutritional conditions[7]. Such an independence of normal organic integration, at the same time as of transplant-host conspecificity, has been demonstrated even in combinations between species belonging to different genera, as *Aedes* and *Culex*. Heteroplastic transplantations of larval ovaries between *Drosophila melanogaster* and *D. funebris* gave, however, no positive results, except in those cases where ring glands of the donor species were at the same time injected into the host[8]. From this work it was concluded that the gonadotropic hormone is qualitatively different among the species used.

We have separated the major proteins from stage 14 eggs[9] by polyacrylamide gel electrophoresis on gradient gels (Pharmacia, 4–30%). Eggs were dissected from the ovaries of mature females of different species of *Drosophila*[10,11] and of *Zaprionus vittiger*, a representative of another genus belonging to the same family. The *Drosophila* species chosen are in part closely related and in part belong to taxonomically distant categories of the genus. The results of a typical gel slab are presented in figure 1. With respect to the major proteins, a rather artificial classification of the species would be obtained, if both the number of the proteins and their mol. weights were taken as taxonomical values. Thus *D. funebris* and *D. mercatorum* show a single major band. *D. lebanonensis, D. busckii, D. hydei* and *D. immigrans* have 2 bands in the implicated region, while in *Zaprionus vittiger, D. melanogaster, D. subobscura* and *D. virilis* 3 major egg proteins are revealed.

From a taxonomical point of view, important differences exist between the genera (*Zaprionus* vs *Drosophila*) as well as between the subgenera of *Drosophila (Pholadoris, Dorsilopha, Sophophora* and *Drosophila,* see figure 2). In the subgenus *Sophophora,* the proteins are rather homogeneous, whereas in the subgenus *Drosophila* considerable heterogeneity is observed.

The important differences in the major proteins from different species promised an ideal experimental material to answer many questions concerning vitellogenesis in *Drosophila,* provided that heteroplastic transplantations were as successful as indicated by earlier work on *Culicidae*[7]. We have done a series of transplantation experiments using the species mentioned above, and will show that the situation in *Drosophila* is quite different from the one in *Culicidae.*

The results of both homoplastic (between individuals belonging to the same species) and heteroplastic (between species) transplantations are shown in figure 2. In homoplastic transplantation, morphologically normal stage 14 eggs are obtained, both in female and male hosts. Heteroplastic transplantations were first performed with immature ovaries from adult *D. melanogaster.* These were injected into different species to see whether normal egg development (implying vitellogenesis) up to stage 14 will occur. Apparently, normal egg morphogenesis occurs only in *D. simulans* and in *D. subobscura,* while in all other species which do not belong to the subgenus *Sophophora,* the ovary remains immature. The situation is different for ovaries from *D. mercatorum.* These mature not only in *D. hydei,* which belongs to the same species group, i.e. the *repleta* group, but equally well in species belonging to 3 other groups, and even in a representative of a different genus,

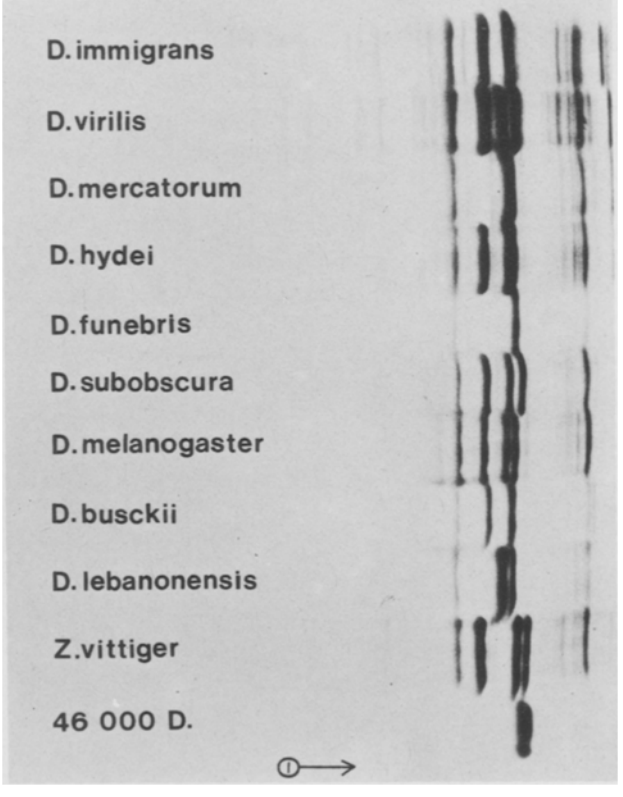

Fig. 1. Gel electrophoresis on gradient slab gel (Pharmacia, 4–30% polyacrylamide concentration) of proteins from stage 14 eggs of different *Drosophila* species and of 1 species belonging to a related genus. According to the number of major bands near the position of the ovalbumin marker (46,000 daltons), 3 species groups can be distinguished: *D. mercatorum* and *D. funebris* with a single protein, *D. immigrans, D. hydei, D. busckii* and *D. lebanonensis* with 2 bands and finally *D. virilis, D. subobscura, D. melanogaster* and *Zaprionus vittiger* with 3 major bands. Electrophoresis was performed according to the prescriptions of Pharmacia, but with Glycine-buffer pH 8.3, containing 1% SDS. Samples were prepared from 25 stage-14-eggs according to Weber et al.[12]. Gels are stained with Coomassie Brilliant Blue R250.

Zaprionus vittiger. In *D. funebris* and *D. virilis* by contrast, egg maturation was observed in homoplastic transplantations only.

It would seem justified to suppose that species producing 3 major proteins do not support normal vitellogenesis (characterized here by morphological criteria only) in heterospecific implants from species which have but 1 or 2 of them (e.g. *D. subobscura* in *D. funebris* or in *D. hydei*, see figure 2). But vitellogenesis appears equally defective when the reciprocal situation is considered (e.g. *D. funebris* in *D. hydei* or *D. simulans*). Even in transplantation experiments between species which exhibit the same number of major proteins (*D. virilis* in *D. melanogaster* or *D. subobscura; D. immigrans* in *D. hydei*), no egg maturation occurs. By contrast, *D. hydei*, a species with 2 major proteins, supports normal maturation of *D. mercatorum* eggs which contain only 1 band.

In all cases of heteroplastic transplantations where no egg maturation is observed, the injected ovary, when examined after a period judged to be sufficient for allowing stage 6 follicles to reach full maturity, viz. 4–7 days depending on the species, had remained perfectly viable but retained the same immature aspect as at the moment of injection. Thus the donor ovary is tolerated by all hosts, but its further development is inhibited or not favoured by the host. When such ovaries from *D. melanogaster* remained for 6 days in *D. mercatorum* and were then reimplanted into *D. melanogaster* females, these ovaries developed normal eggs.

The heteroplastic transplantations show that normal vitellogenesis is generally not possible in another species (4 cases out of 6). On the one hand, the heteroplastic injections with *D. melanogaster* indicate a parallelism between the taxonomic distance and success or failure of vitellogenesis, as exemplified by positive results within the *Sophophora* subgroup (figure 2). But this conclusion seems to be invalidated by the reverse experiments (*D. subobscura* ovaries in *D. melanogaster* or *D. simulans*) where vitellogenesis does not take place. On the other hand, the experiments with *D. mercatorum* do not seem to follow any rule, since the immature ovaries of this species produce mature eggs even in the genus *Zaprionus*, but not in certain species of *Drosophila*.

Gingeras et al.[3] have shown that the highly specific immunological reaction of the vitellogenic proteins in different *Drosophila* species parallels the phylogenetic distances to some degree, although these authors obtained positive immunoreactions also between taxonomically distant species (*D. melanogaster–D. hydei, D. virilis–D. cardini*), and in addition negative results with *D. melanogaster* and *D. subobscura*, which are taxonomically closely related species and which give positive results in our transplantation tests. It is possible that the immunoreactions are not specific enough, since the purification procedure employed by these authors does not exclude the presence of other, non-vitellogenic and widely occurring proteins.

Apparently the negative results in most heteroplastic transplantations are not directly correlated with the number and mol. weights of the major proteins to be incorporated into the egg. Thus *D. mercatorum* ovaries undergo vitellogenesis in several species, although some of the hosts show a

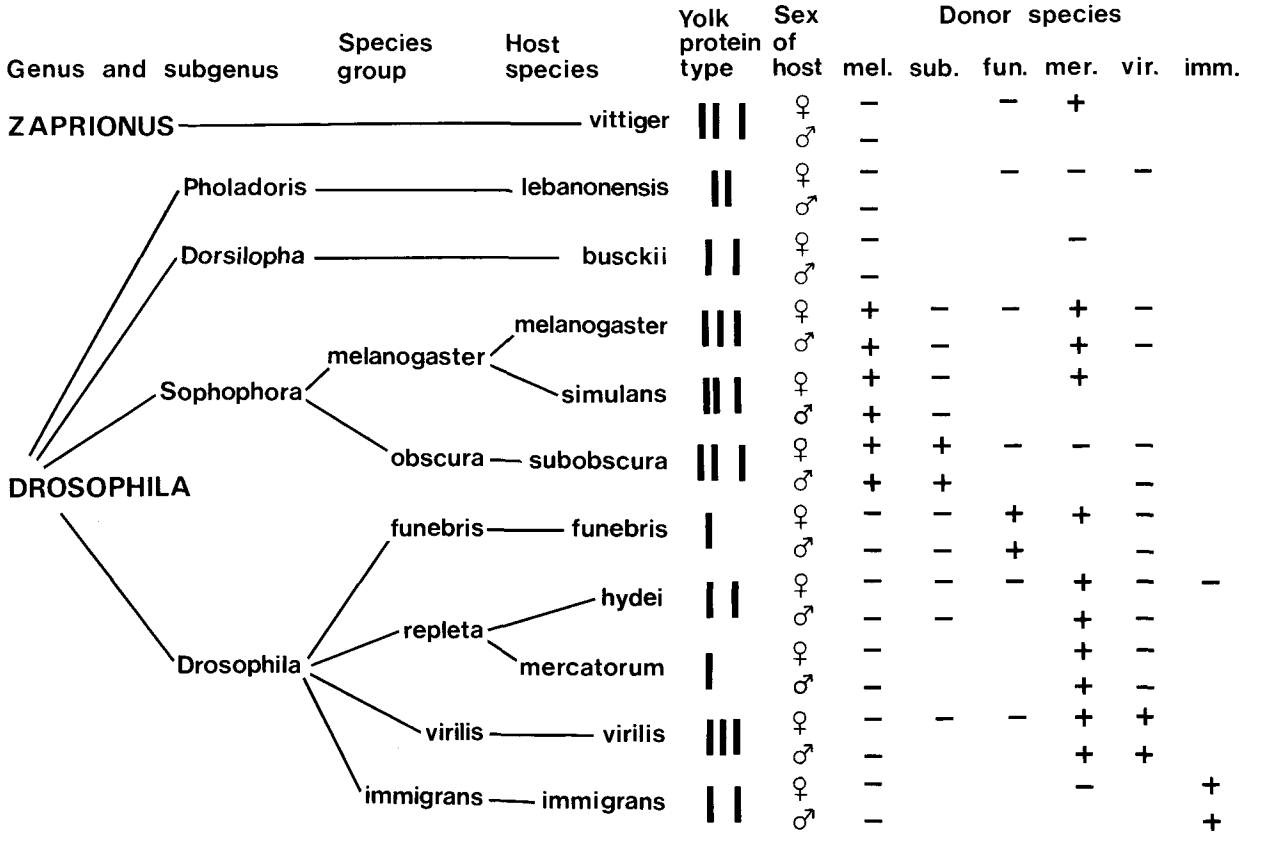

Fig. 2. Taxonomical relationships, and the results of homo- and heteroplastic transplantations of immature ovaries between different *Drosophila* species, including 1 representative of a related genus. Immature ovaries were injected into young adult (1–2-day-old) hosts, and inspected approximately 1 week later for the presence of mature (stage 14) eggs. The + and − signs indicate the presence or absence of mature eggs; absence of a sign means that the corresponding combination has not been tested. Each test is based on 10 transplantations. Mature eggs were either totally absent or else present in relative abundance in all or most of the ovaries. Donor species: mel. = *melanogaster;* sub. = *subobscura;* fun. = *funebris;* mer. = *mercatorum;* vir. = *virilis*; imm. = *immigrans.*

pattern of egg proteins quite different from that of the donor species. The same is true in transplantations between *D. melanogaster* and *D. subobscura*, where differences in the mobility of the major proteins are found. We suggest that the presumed factor which inhibits vitellogenesis, or alternatively a promoting factor whose absence precludes it, is to be searched in the quality of the gonadotropic hormone, as proposed by Vogt[8], or in the carrier system for this hormone. The case of *D. mercatorum* as a donor adds some further problems to the understanding of the process of vitellin formation in another species. Even if we assume that the donor ovary performs vitellogenesis according to its own intrinsic qualities (Postlethwait, personal communication), the quality of the surrounding medium of the host seems to play an important role, since vitellogenesis of *D. mercatorum* immature ovaries is observed in *Z. vittiger* but not in certain *Drosophila* species.

It will be interesting to see whether *D. mercatorum* eggs, developing in other species, elaborate a protein pattern which coincides with that of the donor or with that of the host species. Such studies are presently under way.

1 We thank Mr B. Barandun for excellent technical assistance. This investigation was supported by the Swiss National Science Foundation, grant No. 3.792.76.
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Injectable benzimidazole anthelmintics effective against liver flukes, tapeworms, lungworms and gastrointestinal roundworms

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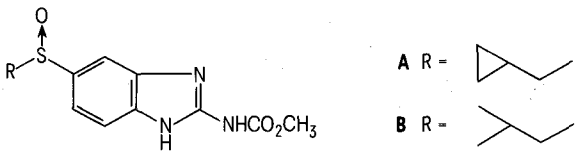
Summary. A series of orally active benzimidazole anthelmintics has been discovered to be active by injection against nematode, cestode and trematode species.

The use of benzimidazole derivatives as orally administered anthelmintics is well established. We wish to report the discovery that certain orally active benzimidazole anthelmintics have excellent injectable potency and spectrum of activity against liver flukes, tapeworms, lungworms and gastrointestinal roundworm infections. 2 of the more potent compounds are [5-[(cyclopropylmethyl)sulfinyl]-1H-benzimidazol-2-yl] carbamic acid, methyl ester (**A**) and [5-[(2-methylpropyl)sulfinyl]-1H-benzimidazol-2-yl] carbamic acid, methyl ester (**B**). The methylpropylsulfinyl benzimidazole **B** is synthesized from 4-thiocyano-2-nitroaniline in 35% overall yield. Sodium 4-amino-3-nitro-thiophenolate, prepared in situ by sodium borohydride reduction of 4-thiocyano-2-nitroaniline in dimethyl formamide is alkylated with isobutyl chloride and oxidized with sodium periodate to give 4-(2-methylpropyl)sulfinyl-2-nitroaniline. The nitroaniline derivative is reduced with sodium hydrosulfite to 4-(2-methylpropyl) sulfinyl-1,2-diaminobenzene which is treated with 1,3-bis-(methoxycarbonyl)-S-methyl isothiourea to yield **B**, m.p. 198–201 °C (decomposition). Benzimidazole **A** is prepared in analogous fashion, m.p. 223–224 °C (decomposition).

A single s.c. injection of **A** at 10 mg/kg to artificially infected sheep eliminated 91–100% of *Haemonchus, Ostertagia, Trichostrongylus* in the abomasum, and *Nematodirus, Trichostrongylus, Oesophagostomum* and *Chabertia* in the small and large intestines. A single oral dose of **A** at 2.5–10 mg/kg eliminated 85–100% of the above listed genera. Preliminary experiments indicate that compound **B** has a similar spectrum of activity in sheep.



In controlled experiments with sheep naturally infected with the lungworm *Dictyocaulus filaria*, a single s.c. injection of **A** at 5 mg/kg was 100% effective against mature and immature worms; **A** was orally active against sheep lungworm at 1 mg/kg. Compound **B** was 94% effective against *D. viviparus* in cattle when given as a single s.c. injection of 10 mg/kg and **B** was 100% effective against *D. filaria* in sheep s.c. at 10 mg/kg.

In sheep naturally infected with tapeworms of the genus *Moniezia* a single s.c. injection of **A** at 5 mg/kg reduced the worm burden 100%; **A** was orally effective against *Moniezia* at 2.5 mg/kg.

Compound **B** administered orally at 20 mg/kg to sheep artificially infected with metacercariae of *Fasciola hepatica* was 96% effective against patent infections. Compound **A** was not effective against adult *Fasciola* when given as a single s.c. infection of 20 mg/kg, whereas compound **B** was 50% effective. Compound **B** given s.c. at 20 mg/kg for 2 consecutive days was 100% effective against *Fasciola* in sheep.

Compound **A** applied dermally in DMSO/amyl alcohol at 10 mg/kg reduced the fecal egg count in sheep 100%.

Preliminary studies indicate that compound **B** at comparable low doses is effective both orally and injectably against small and large strongyles, *Oxyuris* and *Parascaris* in horses and orally against *Ascaridia* and *Heterakis* in chickens.

These are the first reported anthelmintics which demonstrate useful injectable activity against the 3 major helminth classes parasitizing domestic animals. Studies are underway to delineate the full spectrum of activity of these and other closely related compounds.