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Inhibition of *Drosophila melanogaster* and *D. hydei* by *Aspergillus niger*.

Introduction: In the laboratory, moulds can be a pest of *Drosophila* cultures, requiring the use of fungicides or the maintenance of high larval density to restrain fungal growth (Ashburner and Thompson, 1978). One common contaminant of laboratory *Drosophila* cultures is *Aspergillus niger*, which is also often found on natural *Drosophila* resources (Ashburner and Thompson, 1978; Sinha and Saxena, 1988; Hodge, 1996). In this paper we compare the effect of *A. niger* on the larvae of *D. melanogaster* and *D. hydei* and examine to what extent the observed effects can be explained by fungal 'metabolites' leached into the larval resource.

Methods: The *Drosophila* species used in the experiments were wild-type strains of *D. melanogaster* Meigen and *D. hydei* Sturtevant. The environment for all experiments described was an incubator maintained at 25±1°C and a 16:8 hour light:dark cycle. The relative humidity was not controlled but was generally between 45-55%.

To examine the effect of *A. niger* on *Drosophila*, forty first instar larvae were transferred to glass vials (75 x 25mm diameter). 0.5 g of Instant Drosophila Medium (IDM) hydrated with 3ml of distilled water was used as a resource. Half of the vials were then inoculated with *Aspergillus* by direct transfer of spores from a pure culture using a sterilized needle.

To prepare fungal 'extract' 6g of IDM were placed into each of six Petri dishes and hydrated using 36ml of distilled water. Three of the Petri dishes were inoculated with spores of *A. niger* from a pure culture and all six Petri dishes incubated for two weeks. After this time all three of the fungus-covered plates were emptied into a glass beaker with 200ml of distilled water and mixed thoroughly. The mixture was filtered twice using Buckner apparatus (10 μm pore size) to remove any fungal material and solid residues and the filtrate ('fungal extract') placed into sterile glass bottles for later use. The above procedure was repeated for the non-inoculated Petri dishes to obtain an 'IDM extract'. This was used to distinguish between the effects of fungal metabolites and the effects of other substances that may occur on two week-old IDM.

To examine the effect of the extracts on larval survival, 0.5g of IDM was placed into glass vials with 3ml of one of three liquids: (I) fungal extract, (ii) IDM extract or (iii) distilled water. Twenty-five first instar larvae were introduced into each vial.

To examine whether the larvae showed a behavioral response to the presence of fungal substances in the medium, the height from the base of the vial at which the larvae pupated was measured and a mean pupal height for each vial was calculated. For all three experiments replicate numbers are given in the results.

Results: *D. melanogaster* survival was not affected by the presence of *Aspergillus* fungus in cultures, whereas the survival of *D. hydei* larvae was significantly reduced (Table 1).

Table 1. Survival of *Drosophila* larvae (%; mean + SE (N)) in absence and presence of *Aspergillus niger*. Data were arcsine-root transformed before analysis.

	Aspergillus absent	*Aspergillus* present	F	P
D. melanogaster	40.5 ± 6.0 (8)	32.8 ± 9.2 (9)	0.88	> 0.35
D. hydei	56.0 ± 3.0 (8)	14.0 ± 10.0 (8)	18.3	< 0.001

Table 2. Survival of *Drosophila* larvae (%; mean + SE (N)) when using different extracts to hydrate the resource. Data were arcsine-root transformed before analysis.

	Distilled water	IDM extract	*A. niger* extract	F	P
D. melanogaster	90.0 ± 3.1 (12)	96.0 ± 1.2 (7)	88.0 ± 3.0 (12)	0.89	> 0.40
D. hydei	81.6 ± 2.4 (5)	56.0 ± 5.1 (5)	29.6 ± 5.7 (5)	29.3	< 0.001

Table 3. Pupation heights of *Drosophila* larvae (mm; mean ± SE, N = 5 for all treatments) when using different extracts to hydrate the resource.

	Distilled water	IDM extract	*A. niger* extract	F	P
D. melanogaster	21.4 ± 0.8	19.0 ± 1.2	25.9 ± 1.3	10.2	< 0.005
D. hydei	13.7 ± 0.8	3.8 ± 0.3	36.3 ± 1.6	324.1	< 0.001

With regard to 'extract' used to hydrate the medium, the liquid used had no effect on the survival of *D. melanogaster* larvae (Table 2). However, the number of *D. hydei* emergents was significantly affected, with larval survival being lowest when *Aspergillus* extract was used (Table 2).

The larvae of both species exhibited significant responses to the liquid used to hydrate the medium with regard to height

of pupation (Table 3). Both species pupated farther away from medium hydrated with *Aspergillus* extract, the response being much more extreme in *D. hydei*. (Tukey tests isolated fungus extract treatment for both *Drosophila* species; *D. mel.* $P < 0.05$, *D. hyd.* $P < 0.005$).

Discussion: Similar to the findings of Atkinson (1981) when investigating the effects of *Penicillium* on *D. immigrans* and *D. melanogaster*, we found that the inhibitory effect of *A. niger* on *Drosophila* was species-specific: survival of *D. hydei* larvae was reduced if *A. niger* or its extract were present on the resource whereas *D. melanogaster* larvae were unaffected. Many fungi, including species of *Aspergillus*, produced complexes of mycotoxins. This includes aflatoxin which is produced by *A. flavus* and has been shown to inhibit all parts of the *D. melanogaster* life-cycle (Matsumura and Knight, 1967). Other toxins produced by the *Aspergillus* group include ochratoxin A and sterigmatocystin and *A. niger* produces a number of bioactive substances, including enzymes such as amylases, invertases, pectinases and lipases (Jay, 1992). It appears that *A. niger* produces some water-soluble metabolite which significantly reduces the viability of *D. hydei* larvae. *A. niger* is used commercially to produce citric and oxalic acids (Collins *et al.*, 1989) and pH of the resource can affect *Drosophila* performance (Hodge *et al.*, 1996). However, the IDM buffered the pH of the *Aspergillus* extract from ~4.0 to ~6.0 so it is unlikely that pH *per se* produced the observed effects.

Differences in the life-history of the two *Drosophila* species may play a role in the specificity of the fungal effects. For example, the development time of *D. hydei* is longer than that of *D. melanogaster*, so *D. hydei* would tend to be exposed to toxins for a longer period. Also, *D. hydei* larvae tend to feed deeper in the medium than *D. melanogaster* which could influence the effects experienced if the distribution of the toxin(s) is not uniform.

In general, it is believed that dipteran larvae pupate further away from environments which may prove harmful to the developing pupae (Casares and Carracedo, 1987). In this experiment, the larvae of both species of *Drosophila* pupated at a greater distance from the medium if fungal extract was present. This suggests that they may be able to detect and respond to some potentially deleterious chemical in the resource.

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Joly, Dominique[1], **Christophe Bressac**[2] **and Daniel Lachaise.**[1] [1]Laboratoire Populations, Génétique et Evolution, C.N.R.S., 91198 Gif sur Yvette Cedex, France. [2] IBEAS, URA CNRS 1298, Faculté des Sciences et Techniques, Université F. Rabelais, Parc Grandmont, 37 200 Tours, France. Gradually Elongating Testis and 'Sperm Roller' in *Drosophila bifurca*.

The evolution of sperm of inordinate length has sporadically occurred in the arthropod phylogenetic tree: ostracods in crustaceans, *Scutigera* in millipedes, and waterbugs, ptiliid beetles, and fruitflies in insects (Sivinski, 1984). Within the Drosophilidae family, this trend has seemingly been magnified uniquely in the subgenus *Drosophila* (Joly *et al.*, 1989; Pitnick *et al.*, 1995a) and the most impressive lengths occur in the *hydei* species subgroup including *Drosophila hydei* and *D. bifurca*, of which sperm lengths are on average 16.9 (Joly and Bressac, 1994) and 58.37 mm (Joly *et al.*, 1995), respectively. The sperm of this last species was unambiguously determined both by direct measurements using a dissection technique described elsewhere (Pitnick *et al.*, 1995b) and by indirect measurements using the correlation curve between sperm and testis lengths established previously (Joly and Bressac, 1994).

During the pre-reproductive life, *D. bifurca* testes were shown to elongate gradually, growing two-fold every 5 days until the 20th day post-emergence (Figure 1). As a comparison, *D. melanogaster* testes reach their maximum size as soon as the first day after hatching. Testis and receptacle were measured from males or females at different ages from 24 hour until the age of sexual maturity in *D. melanogaster* Canton S and *D. bifurca* (from Bowling Green Stock Center, number 15085-1621.0). Flies were reared on a standard corn-meal medium at room temperature. In each case (that is 0, 1, 2 ,5, 10, 15 and 20 day-old flies), 25 testes and receptacles were dissected and spread out of the abdomen in a drop of saline solution on a microscope slide. Slides were then let dry at room temperature and organs from reproductive tracts were mounted in a drop of glycerol. The measurements were realized using a camera (Hitachi, model KP-C551) connected to a Macintosh 660 AV with the NIH-Image Program (written by W. Rasband at the U.S. National Institute of