appearance of the egg shell. This finding can be used to recognize populations easily, without having recourse to more specific cytological analyses. The two populations of *M. pseudohufelandi*, however, have very similar sclerified structures, as is found in all the other known eutardigrades, and mean chromosome DNA content. These similarities suggest that the present parthenogenetic populations (in which the genetic flow is interrupted) must have differentiated from the bisexual strain in a not very remote period.

Within the animal model studies here, a strict correlation appears between different types of data: mechanisms of reproduction, presence or absence of males, number of chromosomes, and shape of the egg shell.

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Residual influences on fecundity in drosophilid species

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Summary. A residual influence of males and females on oviposition has been examined in 7 drosophilids. There was evidence for oviposition deterrence in *Drosophila funebris*, with males as well as females producing the inhibitory effect. In contrast, male residues stimulated oviposition in *Zaprionus tuberculatus*. Male residues also stimulated oviposition and appeared to serve as an aggregation cue in *D. melanogaster*.
Key words. Drosophila; reproductive behavior; oviposition; pheromones.

There is evidence for a non-contact, residual influence on fecundity in *Drosophila*. Mainardi[1,2] reported that fly medium previously exposed to *Drosophila melanogaster* males received more eggs than unexposed medium. In a recent study[3] we suggested that Mainardi's results may have been due to the transmission of microbes onto medium. The growth of microorganisms on food could attract inseminated females and stimulate oviposition. However, we found evidence for a male effect on fecundity in *D. melanogaster* by confining males on non-nutritive agar. Residues from these males increased oviposition on an adjoining medium surface. These experiments were conducted without live yeast on the surface of the agar or medium and consequently fecundity was low.

The goal of the present study is to explore non-contact effects of residues on fecundity in other drosophilids. We have conducted a survey of male and female residual effects in species of *Drosophila* and one species of a related genus, *Zaprionus*. Within the constraints of a limited study it was not feasible to examine many species or multiple lines from a species. Tests were conducted on several species closely related to the previously studied *Drosophila melanogaster*[3], and several more distantly related taxa. We have also retested *Drosophila melanogaster* for residual male influence when yeast is added to the agar surface, a treatment which results in more eggs laid than in a previous experiment[3].

Materials and methods. The experiments employed the testing method of Hoffmann and Harshman[3]. Briefly, the open end of a 30-ml glass scintillation bottle was inserted into a 40-ml glass vial. The bottle contained 10 ml of 1% agar and the vial had 12 ml of fly medium (6.2% cornmeal, 3.1% semolina, 3.6% sucrose, 7.1% dextrose, 1.1% agar, 1.5% dead yeast, 0.5% propionic acid). In order to provide a smooth surface for oviposition, 2 ml of molten medium was added to an initial aliquot of 10 ml of medium in each vial. The scintillation bottles were used to collect adult residues. Twenty males or females were held in a bottle for 24 h before discarding the flies. A new set of flies was used to expose bottles for the next day. Unexposed bottles served as controls. Three inseminated females of the same species were aspirated into the assembled vials and bottles.

Species survey. There are inherent difficulties in using a variety of species with different rates of sexual maturation. In order to standardize the experiments and ensure that females were inseminated the following procedure was employed. Individuals that had emerged within the previous 16 h were placed, 50 of each sex, in a bottle with live yeast on the surface of the medium. When larval activity was observed the males were removed using CO_2 anesthesia and the females used in the testing devices. They were placed horizontally on shaded shelves with a light intensity of approximately 30 lx and a dark period of 7–10 h. Experiments were conducted at room temperature (22–24 °C). After 24 h females were transferred to a fresh bottle-vial and the number of eggs in vials from the previous day were counted. Typically, few or no eggs were laid in bottles on the agar surface. The experiments were run for 7 days which is an arbitrary period with respect to variation in reproductive characteristics of the species tested. Nevertheless, these experiments consistently evaluate the early phase of reproduction of each species.

Table 1 shows the drosophilids employed, where they were collected and approximately how long they have been in laboratory culture. All flies were maintained at Davis by mass transfer for at least 6 months before being tested. Residues were obtained from males and females that had been separated 2–7 days earlier under CO_2 anesthesia. For each species there were 20 replicates per treatment. Repeats with 30 replicates were carried out for those species which had statistically significant results in the first experiments. The data were analyzed by single classification analysis of variance on the total number of eggs, the number laid from days 1–3 and days 4–7, and the time taken for half the eggs to be deposited ($T_{½}$).

Added yeast. This part of the study used the same stock of *D. melanogaster* previously employed[3], 2½ years after it was initiated from the field. Male residues were introduced to the bottles with agar as previously described[3]. After males were removed one or two grains of live yeast were added to control bottles and bottles with male residues. The yeast granules rapidly absorb moisture, becoming soft and swollen. As a result, females oviposit in the yeast and on the agar as well as in the vial with fly medium. Fly transfers and egg counts were made every

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Table 1. Species surveyed

Species	Subgenus	Source	Years in culture
Drosophila funebris	*Drosophila*	Espanoza, New Mexico, USA	32
Drosophila mercatorum	*Drosophila*	Rochester, New York, USA	25
Drosophila mauritiana	*Sophophora*	Mauritius	> 10
Drosophila simulans	*Sophophora*	Riverside, California, USA	2½
Drosophila pseudoobscura	*Sophophora*	Death Valley, California, USA	2
Zaprionus tuberculatus	–	South Africa	> 10

day for 7 days in the 20 replicate bottles and vials. The analysis consisted of t-tests on the total number of eggs laid and on the proportion of eggs laid in the bottle.

Results. Species survey. The total egg counts and statistical analyses are given in table 2. The counts for days 1–3, days 4–7 and $T_{½}$ are not reported and are only mentioned in those cases where statistically significant differences were observed.

There were no significant overall treatment differences for *D. mauritiana* nor *D. simulans*. These species are closely related to *D. melanogaster* where male residues increased fecundity to 14.7% greater than control[3]. Thus, there is no indication that patterns of residual response are consistently expressed in a set of related species. There was also no overall effect of residues in *D. mercatorum* and *D. pseudoobscura*. Turner and Anderson[4] found when no live yeast was added that *D. pseudoobscura* females kept with males were more fecund than inseminated females kept alone. Our study indicates that residual substances deposited by males do not play a role in their results.

Zaprionus tuberculatus females exposed to male residues laid more eggs than those exposed to female residues or control bottles. This result is repeatable despite the 2.5-fold increase in the number of eggs laid in the repeat experiment. This is the same pattern of non-contact influence as found in *D. melanogaster*. Residues clearly affected fecundity in *D. funebris* with significant heterogeneity in total counts in both experiments. Residues from males as well as females inhibited egg-laying. The effect was quite strong with a reduction in fecundity up to 30% of the control.

Added yeast. Means and standard deviations for total fecundity and the proportion of eggs laid in the bottle are shown in table 3. Fecundity was 5 times that observed in a previous experiment[3], and the increase was probably attributable to the yeast. In this study there was a marginally significant difference in the total number of eggs laid ($t = 1.774$, $0.10 < p < 0.05$), and a more convincing difference for days 4–7 ($t = 2.224$, $p < 0.05$). Inseminated females produced 6% more eggs when exposed to male residues compared to 14.7% in the earlier study. More eggs were laid in the vials (with medium) than the bottles (with yeast). The proportion of eggs laid in bottles was significantly higher ($t = 2.266$, $p<0.05$) when male residues were present.

Table 2. Total egg counts for drosophilids exposed to male residues, female residues, and controls: Means (x̄) and standard deviations (SD) based on twenty or thirty (repeat experiment, rpt) replicate vials per treatment

Species	Male residues		Controls		Female residues		
	x̄	SD	x̄	SD	x̄	SD	p* (ANOVA)
D. mauritiana	164.0	36.69	158.8	35.0	169.2	39.81	0.6786
D. simulans	150.9	40.50	149.1	31.03	166.3	30.55	0.2291
D. pseudoobscura	242.2	77.16	245.6	50.04	238.4	47.12	0.9289
D. mercatorum	86.0	31.86	84.3	29.02	69.6	36.54	0.2268
D. funebris	74.8	20.45	89.4	12.06	78.7	20.59	0.0370
(rpt)	85.8	44.38	104.3	53.29	70.6	37.26	0.0212
Z. tuberculatus	78.0	18.30	64.0	23.27	66.60	16.78	0.0647
(rpt)	167.6	25.26	144.1	23.56	153.5	35.92	0.0085

*Probability level from the analysis of variance for heterogeneity among treatments.

Table 3. *Drosophila melanogaster*: total egg counts and location of eggs when live yeast was added to bottles with agar

	Eggs		Proportion of eggs in bottle*	
	x̄	SD	x̄	SD
Male residues	878.6	81.54	0.321	0.085
Control	825.8	105.22	0.254	0.101

*Number of eggs in bottle/number of eggs in bottle + vial.

Discussion. The tendency of species to aggregate or disperse undoubtedly depends on a variety of factors. Aggregation may result in reduction of interspecific competition, the location of suitable mates and mutually beneficial exploitation of resources[5,6]. One of the consequences of aggregation is that it can promote coexistence between closely related species[7].

Aggregation pheromones are known in several species of *Drosophila*[8,9]. In *Drosophila virilis* an aggregation compound is abundantly produced by mature males[9]. This pheromone acts to attract both males and females and the response depends on age and nutritional state of the flies. Bartelt and Jackson[9] suggest that the pheromone serves to attract conspecifics to a feeding site which also becomes a focus for mating and oviposition. In our study it is possible that *D. melanogaster* females are attracted to the bottles with male residues and as a result are more likely to lay eggs in the same location. A consequence of attraction to male odors may be that females spend additional time feeding on male residues and added yeast. Thus, they may acquire more nutrients for the production of eggs than the treatment without male residues. There may be a similar explanation for the residual response seen in *Zaprionus tuberculatus*. It is also possible that there may be other effects of male residues on female reproduction. In rodents, for instance, male pheromones can directly stimulate or inhibit different aspects of female reproduction[10,11].

Epideictic pheromones as dispersal cues are well known and the ecological consequences of this chemically mediated behavior has been reviewed by Prokopy[6]. He discusses dispersal to avoid intra-specific competition for larval or adult food sources, and avoidance of competition for mates and oviposition sites. A reduction in competition based on chemical cues may be more efficient and safe than alternatives such as physical conflict.

Epideictic pheromones affecting egg-laying behavior have been observed in a variety of insects. Salt[12] originally discovered that a hymenopteran parasitoid will avoid ovipositing in an occupied host that was marked by the first female to oviposit. Oviposition deterence is known in a variety of tephritids[13] and the apple maggot fly *(Rhagoletis pomenella)* is particularly well-studied[14]. A female of this species will drag its abdomen across a host fruit leaving a residue that deters subsequent oviposition. The ecological consequences of oviposition reduction in response to epideictic pheromones has also been examined in bean weevils. In one species, *Callosobruchus maculatus*, the pheromonal response is responsible for a reduction of competition on the larval resource[15,16]. In *Callosobruchus*, males as well as females can produce these pheromones[17,18]. Females can mark a resource during oviposition, whereas the odor from males could serve as a general indicator of density and reproductive activity at a particular site.

Our study reveals the presence of residues from both female and male *Drosophila funebris* substantially reduces oviposition. This is the first evidence for residual suppression of egg-laying in this genus of well-studied flies. *D. funebris* is a cosmopolitan species of *Drosophila* which tends to be more common at high latitudes. In general, we do not know enough about the ecology of *D. funebris* to speculate on why the observed residual effects are found in this species.

Future studies could assess whether females aggregate around male residues in *D. melanogaster* and *Z. tuberculatus* and avoid male and female residues in *D. funebris* in various laboratory paradigms. It would also be valuable to study these responses in the field where a natural range of behaviors could be expressed. There is a need to test more lines from each species and perhaps test additional species, as well as evaluate the possibility of cross-species responses to residual odors. Finally, observations at the whole organism level will lead to study of the chemical basis of these behaviors.

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Eicosapentaenoic acid in tissue lipids of *Pieris brassicae*

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Summary. Larvae of the cabbage white butterfly, *Pieris brassicae,* have a dietary requirement for linolenic acid (C18:3n3) and were found to accumulate two other members of the n-3 family, C20:3n3 and C20:5n3 (eicosapentaenoic acid) especially in testicular phospholipids. Arachidonic acid was observed in trace amounts only. During diapause the relative titer of eicosapentaenoic acid increased in testicular phospholipids to about 4.2% of the fatty acids. Eicosapentaenoic acid is a possible precursor of prostaglandins, suggesting that prostaglandins of the 3-series predominate in this insect.
Key words. Pieris brassicae; eicosapentaenoic acid; testis; diapause; prostaglandins.

The requirement for dietary C18 polyunsaturated fatty acids (PUFA), linoleic (C18:2n6) or linolenic (C18:3n3) or both, in insects suggests two different pathways of subsequent fatty acid elongation and desaturation to C20 PUFA for the possible synthesis of prostaglandins. In common with some vertebrate animals, e.g. several fish, many Lepidopteran species have been shown to require C18:3n3 as a major essential fatty acid. *Pieris brassicae* is one such species[1,2]. Dadd[3] discusses the PUFA requirement of insects and suggests that, with the exception of several species of mosquitoes which need dietary arachidonic acid (C20:4n6) or related PUFA, insects in general elongate and desaturate C18 PUFA to C20 and C22 acids, which are of common occurrence in insects just as they are in vertebrate animals.

If the C20 PUFA are converted to prostaglandins, they can be expected to be preferentially incorporated into membrane phospholipids. Data from some insect species support this[4]. Specifically, arachidonic acid is present in the reproductive tissues of the Australian field cricket *Teleogryllus commodus* and the American cockroach *Periplaneta americana*[5,6], and testicular phospholipids of *T. commodus* were recently shown to accumulate labeled arachidonic acid injected into the hemolymph[7]. Among the retinal phospholipids of the butterfly *Deilephila elpenor,* an unusually high concentration of eicosapentaenoic acid (C20:5n3) has been found[8].

The present study examines the possibility that *P. brassicae,* specifically requiring C18:3n3 in its diet for normal adult emergence, contains long-chain members of the n-3 family of fatty acids. If the essential C18:3n3 is used in part as a precursor of prostaglandins, C20 precursor fatty acids of the prostaglandin 3 series should be present in tissue lipids.

Material and methods. Our experimental stock of *P. brassicae* originated from insects obtained in 1984 from the Glasshouse Crops Research Institute, Littlehampton, England. Larvae were reared for these experiments at 23 °C, 16L:8D and about 65% relative humidity. Insects destined for diapause were transferred to 12–13 °C and 12L:12D at the beginning of the first larval instar and, upon pupation, to 2 °C in constant darkness. Larval food was *Brassica oleracea.*

Fat bodies, testes, heads and adult flight muscles were extirpated under a saline solution to rinse off any remaining hemolymph, and lipids were extracted with a modification of the procedure of Folch et al.[9] and separated into fractions in a column (1.5 × 8.0 cm) of activated silicic acid (Unisil)[10]. Lipids were transesterified for gas-liquid chromatography (GLC) using a methanolic-base (0.5 N) reagent (Supelco).

GLC of methyl esters was performed with a Hewlett-Packard 5890A instrument in a 50-m capillary column of 0.2 mm i.d. using CP SIL 88 and OV 275 as liquid phase and hydrogen or helium as carrier. Standard methyl esters used were C18:3n3, C18:3n6, C20:3n3, C20:4n6, C20:5n3 and C22:6n3 (Sigma) which were run individually between samples and subsequently each co-chromatographed with sample for peak identification. Peak areas were quantified with an electronic integrator (Hewlett-Packard 3392A). Mass spectra of testicular fatty acid methyl esters and of eicosapentaenoic acid methyl ester standard were determined with a Nermag R-10-10 C spectrometer (EI+ detection, 70 eV) and Varian 3400 gas chromatograph (30 m column, DB-1701, He as carrier).

Results. Only male insects were examined in this study. Rearing larvae at a low, diapause-inducing temperature and a short day-length results in an increased accumulation of the dietary