significantly in their proportions of the two species. While it is obviously not a strict rule, a higher proportion of *D. melanogaster* was found in indoor sites, and the reverse was true for *D. simulans*.

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The effect of *Drosophila* larvae on the pH of their resource.

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Introduction

A number of studies have examined the relationship between *Drosophila* performance and resource acidity. These include investigations into the success of *Drosophila* larvae (Burdick and Bell, 1954; Posch, 1971; Hodge *et al.*, 1996), developmental stability (Goldat and Beliaieva, 1935; Gordon and Sang, 1941), and the responses of adult *Drosophila* to acidic media (*e.g.*, Fluegel, 1981).

A point which is often overlooked is that the pH of the resource may change with time. Thus, the correlation of responses in *Drosophila* performance with the initial pH of the resource may be erroneous, as this is unrepresentative of the pH the animals actually encounter (see Hodge and Caslaw, 1998). This aspect of the system may have further significance as the changes in resource pH may be caused by the *Drosophila* themselves (Pearl and Penniman, 1926; Bridges and Darby, 1933); the common reference to *Drosophila* as 'vinegar flies' has long been testimony to their association with the acidification of fermenting substances (see Unwin, 1907). Interactions between *Drosophila* have sometimes been ascribed to modifications in the environment caused by larvae ('resource conditioning') (Weisbrot, 1966; Budnik and Brncic, 1975; Dolan and Robertson, 1975). Modification of resource pH is a potential mechanism via which the effects of conditioning may become manifest.

This paper describes the changes which occurred in the pH of *Drosophila* resources and established how these changes were affected by *Drosophila* larvae. The pH changes in artificial and natural resources were examined and the influence of the initial pH on subsequent pH modification was investigated.

Methods

General methods

Two species of wild-type *Drosophila* were used in this study: *D. melanogaster* ('Kaduna') Meigen and *D. hydei* Sturtevant. All experiments were carried out using standard glass vials (75mm × 25mm diameter), plugged with polyurethane foam bungs, as the experimental vessel. Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent) was used as the laboratory rearing resource. The pH of the resource was determined using an electronic pH meter [Jenway 3015, Jenway Ltd., Essex, England].

The effect of larval density on induced pH changes

Vials of resource were set up using 1.0g of IDM and 4.0ml of distilled water and the initial pH measured. Four replicates of seven densities of first instar *D. melanogaster* larvae (0, 2, 4, 8, 16, 32, 64) were then added to these vials. The pH of the resource was measured again when pupation of the larvae had ceased.

The effect of initial resource acidity on the extent of Drosophila-*induced pH changes*

A range of initial resource pH values was obtained by hydrating IDM with various concentrations of hydrochloric, citric and acetic acids (see Results for actual pH values). Distilled water (resource pH ≈ 6.2) was used as a control. To make up the media, 1.0g of IDM was hydrated with 4.0ml of liquid. For the HCl, 6 replicates of each acid concentration were set up for both *D. hydei* and *D. melanogaster* (35 1st instar larvae) and the controls. For acetic and citric acid, only 3 replicates were set up and only *D. melanogaster* larvae were used (25 1st instar larvae). The vials were placed in an incubator maintained at 25±1°C, relative humidity 60%. The pH of the medium was measured again when pupation had ceased.

The effect of Drosophila *larvae on pH changes in natural resources*

Ten types of fresh fruit (orange, lemon, grapefruit, banana, cucumber, melon, tomato, avocado, pear and apple) were peeled, chopped and puréed using a pestle and mortar. 5.0g of purée was placed into vials, with each fruit replicated 12 times. The pH was measured and 35 first instar *D. melanogaster* larvae introduced into six vials in each fruit treatment. The vials were maintained in an incubator at 26±1°C, relative humidity 45±5% and a light:dark cycle of 16:8 hours. After 20 days (corresponding to the time of last adult emergence) the pH of the fruit was measured a second time.

Results

The effect of larval density on induced pH changes

The presence of *D. melanogaster* larvae caused a significant reduction in the pH of the medium to around 4 compared to the pH of the control medium (with no larvae) which remained close to the initial value of 6.2 (Table 1; $F_{6,21} = 686.1$, $P < 0.0001$). There was no clear relationship with larval density and pH change and the presence of larvae appeared all that was required to lower the pH.

The effect of initial resource acidity on the extent of Drosophila-*induced pH changes*

For each combination of acid and *Drosophila* species, there was a significant interaction between the initial pH of the resource and the presence of larvae on the extent of the pH change (Table 2; interaction terms GLMs, all $P < 0.001$). The pattern of pH change was similar for larvae of *D. hydei* and *D. melanogaster* and for *D. melanogaster* larvae on different acids. When the initial pH of the resource was greater than 5.0, the presence of larvae caused the pH to drop to between 4 and 4.5, whereas the pH of IDM without larvae tended not to change. At low pH (< 4.5) the final pH of the resource tended to remain unchanged whether *Drosophila* larvae were present or not. The exception to this occurred when *D. melanogaster* larvae where introduced onto medium hydrated with citric acid, the larvae causing a slight increase in pH (Table 2d).

The effect of Drosophila *larvae on pH changes in natural resources*

The initial pH values of the fruit were significantly different ($F_{9,110} = 4764.7$, $P < 0.001$) and provided a good range of values (3.5 - 7) (Table 3). The extent of the pH change - usually an increase - differed between fruits and could be further modified by the presence of *D. melanogaster* larvae (Table 3; interaction term GLM, $F_{9,99} = 7.1$, $P < 0.001$). The pH of the resources modified by larvae could be higher, lower or the same as the controls and there was no obvious relationship between pH change and the initial pH of the resource.

Discussion

Pearl and Penniman (1926) and Bridges and Darby (1933) found that the addition of *Drosophila* larvae to different types of culture medium brought about a reduction in pH to values varying between 3.8 and 4.8, depending on which culture media was used. In the current investigation, the experiments using artificial media extended those results by demonstrating an even greater generality; the convergence of pH being apparent when larvae of different species were used, when different acids were used to alter the initial pH of the resource, and being independent of the original pH of the resource and larval density. The consistency of

Table 1. The effect of density of *D. melanogaster* larvae on reduction in pH of artificial culture media (initial pH was 6.2) (mean standard error was 0.025).

Density of larvae	0	2	4	8	16	32	64
Final pH of resource	6.2	3.8	3.8	4.2	4.1	4.1	4.2

Table 2. The change in the pH of artificial resource caused by *Drosophila* larvae. (a) *D. melanogaster* with HCl, (b) *D. hydei* larvae with HCl, (c) *D. melanogaster* with acetic acid and (d) *D. melanogaster* with citric acid (mean SE ≈ 0.02).

(a)

Original pH	6.2	5.2	3.7	2.8	2.4
No larvae	6.1	5.4	3.9	3.3	2.8
Larvae	4.4	4.4	3.9	3.4	2.9

(b)

Original pH	6.2	5.2	3.8	2.9	2.4
No larvae	6.3	5.4	3.6	2.9	2.4
Larvae	4.4	4.5	3.7	2.7	2.0

(c)

Original pH	6.2	5.4	4.5	4.0
No larvae	5.8	5.3	4.4	3.7
Larvae	4.2	4.2	4.4	3.7

(d)

Original pH	6.2	5.2	4.3	3.5
No larvae	5.8	5.2	4.4	3.5
Larvae	4.2	4.2	4.5	4.1

Table 3. The change in pH of natural resources after 20 days with and without the addition of *D. melanogaster* larvae (mean ± SE).

Resource	Initial pH	Final pH no larvae	Final pH with larvae
Lemon	2.4 ± 0.0	5.8 ± 0.7	4.1 ± 0.5
Grapefruit	3.0 ± 0.0	6.3 ± 0.2	7.3 ± 0.2
Orange	3.5 ± 0.0	6.6 ± 0.5	5.0 ± 0.1
Apple	3.7 ± 0.0	5.9 ± 0.7	2.8 ± 0.1
Tomato	4.1 ± 0.0	9.3 ± 0.3	9.5 ± 0.1
Banana	4.7 ± 0.1	7.6 ± 0.1	4.6 ± 0.7
Pear	4.8 ± 0.0	3.6 ± 0.1	4.4 ± 0.1
Cucumber	5.3 ± 0.0	9.5 ± 0.0	9.5 ± 0.1
Melon	5.9 ± 0.0	9.6 ± 0.0	7.5 ± 0.5
Avocado	6.8 ± 0.1	9.0 ± 0.3	8.7 ± 0.1

the final pH in the laboratory culturing media may be a result of the buffering qualities of this resource when confronted with a small quantity of weak acid.

A pH of around 4 is considered good for yeast populations (Darby, 1930) and it can be speculated that the *Drosophila* larvae benefited themselves by promoting the growth of their primary food source. It has previously been suggested that the positive effects caused by the 'conditioning' of resources by dipteran larvae may be due to increased levels of micro-organisms (*e.g.*, Weisbrot, 1966; Dolan and Robertson, 1975) and this lowering of resource pH could therefore influence the quality of the larval diet.

The mechanism by which the pH drop was effected remains unclear. Excretion is one mechanism by which pH change of a dipteran resource can occur. However, uric acid would tend to be too insoluble to cause a pH shift, urea tends to be pH neutral and the excretion of free ammonia into the resource would tend to cause a pH increase rather than drop. Only the presence of a few larvae were required to lower pH, suggesting that pH was altered by micro-organisms transferred to the resource via the larvae, rather than by the larvae themselves. Pearl and Penniman (1926) and Bridges and Darby (1933) both demonstrated that the addition of yeast to culture media effected a reduction in pH without the presence of *Drosophila* larvae, though both papers also reported that the further addition of *Drosophila* larvae accelerated this change, implying there was an interaction between the yeast and larvae. The lack of effect of the larvae at low pH may be due to the larvae - and the microorganisms forming their diet - being inhibited in acidic media (see Posch, 1971; Hodge *et al.*, 1996).

In natural resources the presence of larvae modified how the pH changed over time but did not consistently bring about an increase or decrease nor was there a convergence to a particular pH value. This result again suggests that an interaction between the *Drosophila* larvae, the different microorganisms communities present on different fruits and other resource variables (such as protein and sugar content) may be determining the final pH.

In conclusion, the addition of *Drosophila* larvae to laboratory culture media brought about a convergence in pH, to a value which could potentially facilitate the growth of their primary food resource. However, the clear patterns observed using artificial culture media were no longer found when natural resources were used. pH modification would appear to be a product of the interaction between the *Drosophila*

larvae, microorganisms and various properties of the resource. The precise mechanism of pH modification remains unclear.

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A parthenogenetic strain of *D. pallidosa*-like in the *D. ananassae* complex.

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In the *D. ananassae* complex, parthenogenetic strains of *D. pallidosa* and *D. ananassae* were already reported by Futch (1972). According to Futch (1973, 1979) this trait is genetically controlled and the mechanism of parthenogenesis of *pallidosa* and *ananassae* seems to be pronuclear duplication and partly terminal fusion. Now, we found parthenogenetic females from an iso-female strain (LAE 345) of *D. pallidosa*-like collected at Lae, Papua New Guinea in 1981, and established a new parthenogenetically reproducing strain. *D. pallidosa*-like distributing in Papua New Guinea was described by Tomimura *et al.* (1993). In addition to a "impaternate" strain of LAE 345, we established a bisexual "bridge" strain of LAE 345. Because F_1 virgin females between LAE 345-Im females and e^D/Sb *ananassae* males have any parthenogenetic ability (Table 1), genes controlling the parthenogenesis might be recessive. Dr. Futch kindly gave us a parthenogenetic strain marked with *yellow* of *ananassae*, and we made crosses between *ananassae* impaternate females and males from the "bridge" strain of LAE 345 of *pallidosa*-like. Because F_1 virgin females have also parthenogenetic ability the same as parental parthenogenetic strains (Table 1), the parthenogenetic ability of the two species might be controlled by the same genetic factors.

Futch (1972) showed that parthenogenetic females of *ananassae* and *pallidosa* were found in only South Pacific Island. Now we found the parthenogenetic strain of *pallidosa*-like in Papua New Guinea. But, the distribution of parthenogenetic strains was still restricted in the South Pacific Islands including Papua New Guinea in *ananassae* complex (Table 2, and Futch 1972). Some genetic factors controlling parthenogenesis might incorporate into the gene pool of *pallidosa*-like in Papua New Guinea from *pallidosa* and/or *ananassae* from South Pacific Islands by hybridization in nature as already suggested by Tomimura *et al.* (1993) based upon the components of chromosome rearrangements among *ananassae* complex.

Table 1. Parthenogenesis ability and productivity of impaternate adults.

Strains (range)	No. of mothers tested	No. of mothers produced adults	% of mothers produced adults	Impaternates / mother
pallidosa-like (LAE 345-Im)	61	59	96.7	12.6 (1-28)
ananassae - Im[y]	43	35	81.4	8.1 (1-28)
F_1 (LAE345-Im/*ana*[e^D])	75	0	0.0	0
F_1 (*ana*-Im/LAE345-Br)	13	13	100.0	13.7 (2-19)

Im: "impaternate" strain. Br: "bridge" strain.

F_1: (female parents / male parents)

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