

The utilization of naturally occurring yeasts by *Drosophila* species, using chemically defined substrates

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The ability of three *Drosophila* species; *Drosophila obscura*, *D. subobscura* and *D. phalerata*, to utilize a range of naturally occurring yeasts as food sources was determined. The yeasts used were the dominant species of two commonly used breeding sites, *Sorbus aucuparia* fruits and the fruiting bodies of the fungus *Phallus impudicus*. The range of yeast species used was found to reflect the degree of substrate specialization exhibited by these three species, with *D. subobscura* being broader niched than the other two species. There was an indication that *D. phalerata* was adapted to those yeasts normally found on its natural breeding site, *Phallus impudicus*. Many factors were found to affect the nutritional adequacy of the yeasts, including their growth period, the larval density, the presence of competitors and particularly the chemical composition of the basal growth medium.

KEY WORDS:— yeasts – breeding sites – competition – *Drosophila*.

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INTRODUCTION

Drosophila breed in substrates rich in microorganisms, particularly yeasts. With the exception of a few specialized species which feed on bacterial rots of plant

materials, such as *D. pachea* Patterson and Wheeler (Heed & Kircher, 1965), and the Hawaiian leaf breeders (Robertson, Shook, Takei & Gaines, 1968), the majority of *Drosophila* species probably have an absolute requirement for yeasts. The reason for this is that *Drosophila*, and insects in general, are unable to synthesize sterols which are important components of membranes and which are also required for the production of ecdysones. Although yeasts are rich in sterols, particularly ergosterol, bacteria are not and therefore bacterial feeders require an alternative source. Many leaf breeders are able to use the phytosterols present in plant material (Kircher, 1969).

Yeast species differ in their distribution patterns. Species such as *Aureobasidium pullulans* Arnaud appear to be ubiquitous, occurring in soils, on leaves, in fruits, etc., whereas other species are more limited in distribution. *Kloeckera apiculata* (Reess emend. Klöcker) Janke, for example, is restricted to the initial stages of fermentation of substrates rich in sugars.

Drosophila species also vary in their degree of specialization with regard to breeding sites. In Europe, for example, *D. subobscura* Collin uses fruits, fungi and a variety of other rotting substrates (Shorrocks, 1980), whereas *D. phalerata* Meigen is restricted to certain species of fungi (Shorrocks & Charlesworth, 1980). The extent to which the distributions of the species of *Drosophila* and yeasts are related is not known; however, a number of possibilities are apparent. The wide distribution of species like *D. subobscura* may be a result of specializing on species of yeasts which are themselves ubiquitous. Alternatively, if the yeast flora on each breeding site was different, the *Drosophila* species would require a generalist strategy for the utilization of yeasts. Similarly, breeding site specialists like *D. phalerata* may have also specialized on one or a few of the microorganisms occurring at these sites. Alternatively, theoreticians (MacArthur & Levins, 1964) have predicted that a habitat specialist should use resources within the habitat in the proportions in which they exist.

The possibilities were examined by testing the performance of larvae of *D. subobscura* and *D. phalerata* reared on the naturally occurring yeasts obtained from the fruits of *Sorbus aucuparia* L. and the fruiting bodies of the fungus, *Phallus impudicus* Persoon. In the Leeds area these are known to be the main breeding sites for these two species of *Drosophila* (Begon, 1975; Kearney, 1979; Shorrocks & Charlesworth, 1980). *Drosophila obscura* Fallen larvae were also used as this species does not use either of these substrates to any extent. It was therefore of interest to assess the ability of this species to use what is possibly an alien microflora, particularly in view of its close phylogenetic relationship with *D. subobscura*.

In addition to the yeasts obtained from *Sorbus* and *Phallus*, *Saccharomyces cerevisiae* Hansen was also tested. This species is routinely used for culturing the adult and larval stages of *Drosophila* in the laboratory, even though under natural conditions, fermentative species of this genus are typical of adult yeasts but are not found on breeding sites (Carson, Knapp & Phaff, 1956). It was therefore of interest to compare this species with naturally occurring larval yeasts.

In addition to determining the effects of yeast species it was also necessary to know whether the predictions of the laboratory model were influenced by other factors. These include the type of basal medium, the density of larvae and the quantity of yeast. If the model was insufficiently robust, then any conclusions may apply only to the laboratory environment and not to any field situation.

MATERIALS AND METHODS

Collection and sterilization of Drosophila eggs

Large populations of flies were maintained in cages (Shorrocks, 1972) on a malt-cornmeal-agar medium (Lakovaara, 1969), with the addition of live baker's yeast. Eggs for experimental purposes were collected from newly matured flies, three days after the appearance of eggs within the cage. The medium used for the collection of eggs consisted of a 2% agar gel, 10 ml aliquots of which were poured into $3 \times 1\frac{1}{2}$ " tubes. To stimulate oviposition, traces of acetic acid (0.1%) and ethanol (0.2%) were incorporated into the medium used for *D. subobscura* and *D. obscura*, and pieces of mushroom in the medium used for *D. phalerata*. A yeast paste was deposited on top of the gel and this was allowed to dry overnight. The tubes were then inserted into the population cages for a 6 h period, after which they were removed and the eggs and yeast cells were washed free of the agar gel with water. The eggs were separated from the yeast cells by repeated washing in water and subsequent filtration.

The eggs were sterilized by chemical de-chorionation (Sang, 1956). Sterile eggs were transferred under aseptic conditions, to sterile agar plates containing chloramphenicol (0.05 g/l) and calcium propionate (0.25 g/l) to inhibit the growth of any remaining bacterial or mould spores. These plates were incubated overnight at 18°C to allow the eggs to hatch. The resulting larvae were transferred to the experimental tubes using sterile, detachable paper spoons (Sang, 1956). Plates showing any signs of microbial growth were discarded.

Basal media and yeasts

The experimental yeasts were grown on two different basal media in order to assess any interaction between yeast species and medium on the performance of the *Drosophila* larvae. The media acted primarily as substrates for the growth of the yeasts, and one criterion for their choice was that they should not support the growth of *Drosophila* larvae in the absence of yeasts; however they undoubtedly contributed some nutrients to the larvae. Other criteria were that they should support the growth of all species of yeasts used, and that one medium should contain only a minimal subset of the nutrients of the other. The chemical composition of both media is given in Table 1.

Twelve species of yeasts were tested for their nutritional adequacy (Table 2). Eleven of these species were isolated from *Sorbus* fruits and *Phallus* fruiting bodies (Kearney, 1980), the twelfth yeast was *Saccharomyces cerevisiae* (baker's yeast). Live yeast cultures were used in all of the experiments. The reason for this was that under natural conditions the by-products of yeast metabolism are likely to be important in determining the nutritional suitability of a substrate and should therefore be included in the laboratory model.

Experimental procedure

The experiment consisted of a factorial design to determine the effects of a number of parameters on the growth of *Drosophila* larvae. These included *Drosophila* species; yeast species; media; amount of yeast present; density of

Table 1. The chemical constituents of the two basal media

	Bacto-Nitrogen- Base (B-N-B) Difco Laboratories plus added glucose (20 g)	Minimal Yeast (M-Y)
Nitrogen source (g)		
Ammonium sulphate	5	5
Carbon source (g)		
Glucose	20	20
Amino acids (mg)		
L-Histidine monohydrochloride	10	
DL-Methionine	20	
DL-Tryptophane	20	
Vitamins (µg)		
Biotin	20	
Calcium pantothenate	2000	
Folic acid	2	
Inositol	10 000	
Niacin	400	
p-aminobenzoic acid	200	
Pyridoxine hydrochloride	400	
Riboflavin	200	
Thiamine hydrochloride	400	
Compounds supplying trace elements (µg)		
Boric acid	500	
Copper sulphate	40	
Potassium iodide	100	
Ferric chloride	200	
Manganese sulphate	400	
Sodium molybdate	200	
Zinc sulphate	400	
Salts (g)		
Potassium phosphate monobasic	0.85	
Potassium phosphate dibasic	0.15	1
Magnesium sulphate	0.5	0.5
Sodium chloride	0.1	
Calcium chloride	0.1	
Agar	20	20

larvae and single or mixed *Drosophila* species cultures. It was not possible to carry out all the combinations of the various factors. Those combinations that were investigated were chosen on the basis of convenience, past experience, and from the results obtained as the work progressed.

Details of the experimental procedure were as follows:

- (1) As many replicate tubes as possible were set up for each treatment. This usually varied between three and five tubes and was dependent upon the number of eggs laid during an oviposition period.
- (2) An equal number of replicate tubes were held without yeast to check that the flies were unable to use the basal medium as a sole source of food. This was found to be the case in all experiments.
- (3) The required number of sterile larvae were transferred to each experimental tube, using sterile paper spoons (Sang, 1956).
- (4) Development time was measured as the time at which 50% of the surviving larvae pupated (Dudgeon, 1954). Although highly correlated with mean

Table 2. The species of yeast used as food sources for *Drosophila* larvae*Dominant yeast flora isolated from *Phallus impudicus*

1. *Pichia toletana* (Socias, Ramirez et Pelaez) Kreger-Van Rij
2. *Trichosporan pullulans* (Lindner) Diddens et Lodder
3. *Candida humicola* (Daszewska) Diddens et Lodder
4. *Rhodospiridium infirmominiatum* (*Cryptococcus infirmo-miniatius*) (Okunuki) Phaff et Fell
5. *Hansenula bimundalis* Wickerham et Santa Maria

Dominant yeast flora isolated from *Sorbus aucuparia*

6. *Aureobasidium pullulans* Arnaud
7. *Rhodospiridium infirmominiatum* (*Cryptococcus infirmo-miniatius*) (Okunuki) Phaff et Fell
8. *Cryptococcus albidus* var. *diffuens* (Saito) Skinner, (Zach) Phaff et Fell
9. *Cryptococcus albidus* var. *albidus* (Saito) Skinner
10. *Metschnikowia pulcherrima* Pitt et Miller
11. *Metschnikowia reukaufii* Pitt et Miller

Sac. *Saccharomyces cerevisiae* Hansen

* In all subsequent Tables and Appendices the yeast reference number is used for convenience in place of the specific name. The reference numbers of those species isolated from *Phallus impudicus* are italic, whereas those from *Sorbus aucuparia* are roman. Note that *Rh. infirmo-miniatium* was isolated from both breeding sites (ref. 4 & 7).

development time (Kearney, 1979), this parameter is easier to measure and is insensitive to the occasional larva with an extremely long development time.

- (5) The percentage of larvae pupating was used as a measure of survival.
- (6) All tubes showing signs of microbial contamination were discarded.
- (7) One tube of yeast was examined after emergence of the adult flies, by plating out the contents onto malt extract agar (Oxoid—CN59) and nutrient agar (Difco 0001), to ensure that the experimental yeast was the only microorganism present.
- (8) All tubes were maintained at $18^{\circ}\text{C} \pm 1^{\circ}\text{C}$, in constant illumination.
- (9) Control tubes containing *Drosophila* medium (Lakovaara, 1969) were set up to monitor any differences in survival caused by de-chorionation. No differences between successive experiments were detected.
- (10) The emerging adult flies were transferred to tubes of *Drosophila* medium and in all cases produced viable larvae. Whenever possible, the winglength of emerging adults was measured.

RESULTS

For each experiment the results are analysed using analysis of variance. A Student-Newman-Keuls (SNK) test (Sokal & Rohlf, 1969) was applied to the means in each case and these are presented in the appendices. Sets of values that are not significantly different (5% level) are underlined.

The comparative effects of yeast quantity and quality

Tubes of M-Y medium were inoculated with the five species of yeast obtained from *P. impudicus* (yeasts 1–5) and with *S. cerevisiae*. Inoculation took place either at the same time as the tubes were seeded with *D. subobscura* larvae or one week previously. Five replicates were used for each treatment.

The analysis of 'survivorship' showed that there was no overall effect of yeast species on the survival of *D. subobscura* larvae, whereas allowing the yeasts an initial growth period was highly significant ($F_{1,48} = 12.4$, $P < 0.01$). The reverse effect was observed for development time, with only 'species of yeast' having a significant effect ($F_{5,48} = 34.7$, $P < 0.01$). This reversal would result if the initial food shortage which caused early larval mortality, rapidly became a food excess during the yeasts exponential growth phase.

In both instances there was a significant interaction between the effects of inoculation time and yeast species, which can be seen in greater detail in Appendix 1. A Spearman's rank correlation calculated between the two yeast growth treatments was significant for development time ($r = 0.94$, $P < 0.05$) but not significant for percentage survival ($r = -0.2$).

The effects of yeast species on the development of Drosophila species

Tubes of B-N-B medium were inoculated with yeasts one week before they were seeded with larvae. The results obtained for *D. obscura* were analysed separately, as this species was not cultured on all yeast species because of a shortage of larvae.

Yeast species affected both the development time and the survival of *D. subobscura* and *D. phalerata* (Table 3) but no overall differences were detected between the two *Drosophila* species. However, the interaction between yeast species and *Drosophila* species for both these fitness parameters proved to be highly significant. The results of SNK tests were compared with those obtained for *D. obscura* (Appendices 4a, 5a).

The effect of yeast species on survival appears to be minimal. The ability of each *Drosophila* species to survive satisfactorily on each of the yeasts suggests that there was a sufficient quantity of food available. The only exception appears to be yeast 6 on which *D. phalerata* suffered high mortality and on which *D. subobscura* also exhibited its highest mortality. Development time of the two species was also longest on this species of yeast. Yeast 6, which is *Aureobasidium pullulans*, is not classified as a yeast by Lodder (1970) but is regarded by her to be a mould with an occasional yeast phase in its life cycle. Asexual reproduction usually results in the production of abundant mycelia which gives the culture a dry, tough, fibrous consistency which may be responsible for its low nutritional value.

Table 3. Analysis of variance carried out on the survivorship and development time of *D. subobscura* and *D. phalerata* on yeasts 1-11 and *S. cerevisiae*

Source of variation	d.f.	Survivorship			Development time		
		M.S.	F	P	M.S.	F	P
<i>Drosophila</i> species	1	17.3	0.1	N.S.	0.2	0.6	N.S.
Yeast species	11	1567.6	8.2	<0.01	6.7	20.2	<0.01
<i>Drosophila</i> × Yeasts	11	646.4	3.4	<0.01	3.6	11.0	<0.01
Residual	96	190.6			0.3		

Turning to the effect on development time it is apparent that *D. subobscura* is far less sensitive to yeast type than is either *D. phalerata* or *D. obscura*. For *D. subobscura* the majority of yeasts (yeast 11 to yeast 7) produced no more than a 0.8 day difference in development time, which was far less than was seen in the other two species. For *D. subobscura*, no pattern could be seen with respect to the substrate from which the species of yeast originated. However, in *D. phalerata*, of the six yeasts producing the shortest development time, only *S. cerevisiae* was not found on *P. impudicus* (yeast 4 and yeast 7 being identical). The only yeast isolated from *Phallus* that did not produce an increased development rate as compared to the *Sorbus* yeasts was 5.

Effect of basal medium

The previous experiment was repeated, this time using M-Y medium. Only three replicates per yeast were used in this case and therefore in order to facilitate comparison between the two media, three replicates were chosen at random from the five for each yeast of the previous experiment.

For percentage survival, all three main effects, media, yeasts and *Drosophila* species, were significant (Table 4). The SNK tests (Appendices 2a, 4a) indicate that the M-Y medium had very little effect on the survival of both *D. subobscura* and *D. obscura* on each species of yeast, but that there was a marked change in the survival of *D. phalerata* compared with the BNB medium. Survival decreased most markedly on those yeasts isolated from *Sorbus* (with the exception of yeast 3). This effect was unlikely to be due to a microbial food shortage as both types of medium produced similar quantities of yeast. It was therefore probably a consequence of M-Y medium containing fewer nutrients.

Table 4 indicates that both media and yeasts affect development time but that there was no difference between *Drosophila* species. From the mean values presented in Appendices 3a & 5a, it is clear that the overall development time of each *Drosophila* species appears to be longer on the M-Y medium.

It seems that the composition of the medium as well as the species of yeast can have a dramatic effect on the development of the *Drosophila* larvae. This suggests that the larval food should be regarded as a yeast/medium complex.

Table 4 Analysis of variance carried out on the survivorship and development time of *D. subobscura* and *D. phalerata*, using 12 yeast species and two different basal media

Source of variation	d.f.	Survivorship			Development time		
		M.S.	F	P	M.S.	F	P
Media (M-Y and B-N-B)	1	4975.3	27.4	<0.01	111.7	72.3	<0.01
<i>Drosophila</i> species	1	7559.9	41.7	<0.01	0.3	0.2	N.S.
Yeast species	11	1382.4	7.6	<0.01	18.7	12.1	<0.01
Media $\times$ <i>Drosophila</i>	1	9133.8	50.3	<0.01	1.2	0.8	N.S.
Media $\times$ Yeasts	11	477.9	2.6	<0.01	3.6	2.3	<0.05
<i>Drosophila</i> $\times$ Yeasts	11	320.2	1.8	N.S.	2.5	1.6	N.S.
Media $\times$ <i>Drosophila</i> $\times$ Yeasts	11	2055.6	11.3	<0.01	8.4	5.5	<0.01
Residual	96	181.4			1.5		

Effect of density

Fifty larvae were seeded into tubes of M-Y medium prepared exactly as in the previous experiments. An analysis of variance for survivorship (Table 5) revealed that all main effects and all interactions were significant.

For each species of *Drosophila*, mortality increased with an increase in density (Appendix 2a,b). The only exception was for *D. phalerata* where yeasts 10 and 6 produced a greater survivorship at the higher density. This is interesting in the case of yeast 6, *Aureobasidium pullulans*, which, as has already been described, is a tough mycelial yeast. The greater density of larvae may help to break down the mycelia thus providing more suitable conditions.

All main effects and interactions on development time were significant except for the density $\times$ *Drosophila* interaction (Table 5). The effect of higher density was to increase the development time (Appendix 3a,b).

This increased mortality and delay in development with higher density is a general phenomenon and has been observed for *Sitophilus* (MacLagan & Dunn, 1935), *Tribolium* (Park, 1938), *Callosobruchus* (Utida, 1941), as well as for many *Drosophila* species (Sang, 1949a; Barker & Podger, 1970; Sokoloff, 1955).

A comparison of the rank order of yeasts at the two densities, using Spearman's rank correlation (r_s) was significant only in the case of *D. obscura* ($r_s=0.94$, $P<0.05$) and then only for the development time.

Interspecific effects

One week-old (B-N-B medium) yeast cultures were seeded with five *D. subobscura* larvae and five *D. phalerata* larvae, and these mixed cultures were compared with the single species cultures described previously. All main effects and interactions on survivorship were significant (Table 6). The individual SNK analyses revealed that mixing the two species generally increased mortality in *D. phalerata* whereas *D. subobscura* was unaffected (Appendix 4a,b).

In contrast there were no interspecific effects on development time in either species (Table 6). Here the rank order of yeasts was similar under both types of culture in *D. subobscura*, although to a lesser extent in *D. phalerata* (Appendix 5a,b).

Table 5. Analysis of variance carried out on the survivorship and development time of *D. subobscura* and *D. phalerata* using 12 yeast species and two larval densities

Source of variation	d.f.	Survivorship			Development time		
		M.S.	F	P	M.S.	F	P
Density (10 & 50 larvae)	1	19 696.7	154.7	<0.01	135.2	73.1	<0.01
<i>Drosophila</i> species	1	3153.0	24.8	<0.01	8.9	4.8	<0.01
Yeast species	11	589.0	4.63	<0.01	17.9	9.7	<0.01
Density $\times$ <i>Drosophila</i>	1	15 968.5	125.4	<0.01	1.8	1.0	N.S.
Density $\times$ Yeasts	11	1035.9	8.1	<0.01	13.8	7.4	<0.01
<i>Drosophila</i> $\times$ Yeasts	11	369.8	2.9	<0.01	7.7	4.2	<0.01
Density $\times$ <i>Drosophila</i> $\times$ Yeasts	11	1319.9	10.4	<0.01	19.8	10.7	<0.01
Residual	96	127.34			1.8		

Table 6. Analysis of variance carried out on the survivorship and development time of *D. subobscura* and *D. phalerata* in single and mixed species cultures, and using 12 species of yeast

Source of variation	d.f.	Survivorship			Development time		
		M.S.	F	P	M.S.	F	P
Cultures (single and mixed species)	1	7075.0	26.1	<0.01	0.6	1.1	N.S.
<i>Drosophila</i> species	1	15 941.9	58.7	<0.01	0.2	0.5	N.S.
Yeast species	11	1704.1	6.3	<0.01	10.2	20.3	<0.01
Cultures $\times$ <i>Drosophila</i>	1	14 490.0	53.4	<0.01	0.02	0.04	N.S.
Cultures $\times$ Yeasts	11	639.1	2.4	<0.01	2.4	4.8	<0.01
<i>Drosophila</i> $\times$ Yeasts	11	665.0	2.4	<0.01	2.4	4.8	<0.01
Cultures $\times$ <i>Drosophila</i> $\times$ Yeasts	11	5172.1	19.1	<0.01	11.3	22.5	<0.01
Residual	192	271.4			0.5		

Winglength

The effect of yeasts on the winglength of *D. obscura* and *D. phalerata* is presented in Appendix 6a. The results for *D. subobscura* include also the effects of different media and densities (Appendix 6b). *D. obscura* is the only species for which yeasts significantly effect winglength when grown on M-Y medium ($F_{5,20} = 11.8$, $P < 0.01$). However, the winglength of *D. subobscura* is affected when the yeasts are grown on BNB medium, at both larval densities (10 larvae/tube, $F_{11,48} = 7.9$, $P < 0.01$; 50 larvae/tube, $F_{11,24} = 3.7$, $P < 0.01$). The range of wing size of *D. subobscura* is smaller than that seen in the other two species, indicating increased developmental homeostasis for this species under these nutritional conditions.

The reduction in the winglength of *D. subobscura* in response to increased density and poor basal medium can be clearly seen (Appendix 6b). However, a comparison of the rank order of the yeasts under these different conditions shows that this is not correlated.

DISCUSSION

The results indicate that the fitness of all three species of *Drosophila* was markedly affected by the species of yeast on which the larvae were grown, although there were differences in the magnitude of this effect. In general, the larval viability, development time and adult winglength of *D. subobscura* were less sensitive to changes in yeast species than the same fitness parameters measured in *D. phalerata* and *D. obscura*. The survivorship of *D. subobscura* and *D. phalerata* was similarly high over all species, when the basal medium was a particularly nutritious one (B-N-B medium). In maintaining this high rate of survival, *D. phalerata* was forced to extend its development time on the less favourable yeasts, whereas this prolongation was not seen in *D. subobscura*. However, the development time of *D. phalerata* on its preferred yeasts was very much faster than for *D. subobscura*. This suggests that *D. phalerata* has developed the ability to exploit certain yeasts, those found on its natural breeding site, very efficiently. These differences were further emphasized by growing the yeasts on a less

nutritious medium (M-Y medium) in which case the effect was seen as a lowered survival of *D. phalerata* on the *Sorbus* yeasts. This is presumably due to the limited degree to which the development period can be extended to compensate for poor food quality. As Sang (1949b) has shown, the yeast/medium quality gradually declines with time until the larvae are no longer even able to maintain their own biomass. If the critical pupation mass has not been reached at this point then death ensues, and hence survivorship will better reflect the nutritional conditions. Therefore, although development time is the recommended method for assessing the quality of nutrients, the use of more than one fitness parameter provides a more complete picture.

In contrast, the effect of the second, nutrient poor, medium on *D. subobscura* was to cause a slight increase in the development time on certain of the yeasts. However, its effect on a third parameter, wing size, was more marked. The sensitivity of the size of flies to food, presumably reflects the amount of time after reaching the critical mass that the larvae continue to feed, and on the quality of the food. Differences in wing size also resulted from the effect of different species of yeast, in *D. obscura*.

The results of these experiments, however, require careful interpretation. They do not reflect the effect of yeasts on the flies, but the effects of the yeast/medium complex. The ability to utilize certain yeasts has presumably evolved, in natural populations of *Drosophila*, when the yeasts have similarly been growing on a nutrient medium. Therefore, the more dissimilar the artificial media are from the natural substrates, the less likely will the results reflect any true nutritional adaptations. That there is such a large interaction between the effects of yeast species and the two artificial media used, on the fitness parameters of each *Drosophila* species, casts doubt on the generality of the results. This interaction may arise in two ways. Firstly, certain yeast species may provide all of the nutrients required for growth, whereas others may be lacking in one or more essential nutrients. Any change in the basal medium with respect to the missing foods may drastically alter the apparent suitability of these yeasts. Alternatively, the nutritive value of the yeast species may not be constant, but may depend on the growth medium used (Park, 1976). Differences in the quality of fungi due to their growth on different media have been shown to affect the growth of collembolans, which were fed on the washed fungal cells only (Booth & Anderson, 1979). This suggests that, despite the difficulties in obtaining sterile tissue and medium constancy, natural substrates should be used as basal media in testing the nutritional adequacy of yeast species (Kearney, 1979).

The results obtained when the density of larvae was increased showed the expected reduction in survival, lengthening of development period, and reduction in size of the flies. However, they were unusual, if only in the fact that the comparative performance of *D. subobscura* and *D. phalerata* was not, in this case, very different. Shorrocks & Charlesworth (1980) have shown that under natural conditions neither of these species appear to reach densities on fungi at which competition occurs. In fact, winglength was bigger for those flies emerging from the fungal bodies yielding greater numbers of flies. The density of flies on fruits has likewise not reached the levels imposed in these experiments (Kearney, 1979) and this may explain the lack of adaptation of both species to this level of crowding.

The relative improvement of *D. phalerata* could also be explained with

reference to the generalist versus specialist strategies of the two species. For a specialist species with few distinct breeding sites, the likelihood of many flies finding and using the same site must be quite high. It would be expected therefore, that a larva of this specialist species would be more likely to meet another of its own species than of a different species. Field observations on the occurrence of *D. phalerata* on fungi (Shorrocks & Charlesworth, 1980) suggest that it is indeed numerically dominant on *Phallus*. *Drosophila phalerata* is therefore likely to have become adapted to the presence of members of its own species as well as being physiologically and morphologically adapted to its specialized substrate. A generalist species, on the other hand, would be more likely to encounter larvae of different species and should therefore have evolved either increased competitive ability or mechanisms for the avoidance of competition. From the results of the experiments using mixed cultures, it appears that *D. subobscura* inhibits *D. phalerata* when they are grown together under the nutritional conditions imposed here.

The nutritional status of *Saccharomyces cerevisiae* was not consistent in these experiments, although it was never the best yeast and was very often near to being the worst. There was an indication that *D. subobscura* was better able to use this yeast than the other two species of *Drosophila*, but this probably reflects on the one hand the generalist abilities of *D. subobscura* and, on the other hand, the absence of baker's yeast from naturally occurring toadstools and probably also the breeding sites of *D. obscura*.

Turning to *D. obscura*, it appears that this species was also markedly affected by the type of yeast on which it was cultured, although interpretation is difficult in view of the fact that its breeding sites are not known, and the yeasts used in these experiments may not actually occur on them. However, this species did do consistently well on yeast species 2, which is *Trichosporon pullulans*. Of the strains of this yeast studied by Lodder (1970), those isolated from natural environments were found:

- (a) in birch sap, Finland: 2 strains;
- (b) in wood pulp, Sweden: 1 strain;
- (c) in *Acer* species slime flux, the Netherlands: 1 strain.

Also Stautz (1931) isolated it from the slime flux of a chestnut tree and Sheneman & Costilow (1959) reported that *Tr. pullulans* represented 20.4% of the yeasts present in maple tree tapholes. The association of this species of yeast with sap flows, together with its nutritional supremacy for *D. obscura* larvae, may be regarded as circumstantial evidence of an association between this *Drosophila* species and sap flows. Begon & Shorrocks (1978) found no evidence of this species or any other *Drosophila* breeding on sap flows in the woodlands around Leeds. On the other hand, Gordon (1942) did report the occurrence of *D. obscura* larvae in sap flows. It is possible that *D. obscura* usually or historically used sap flows and became adapted to the dominant microflora found at these sites; but that locally around Leeds, or in recent times, an alternative site has been exploited. More field work is required before the feeding relationships of *D. obscura* can be fully understood.

ACKNOWLEDGEMENTS

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APPENDICES

Appendix 1. SNK tests applied to the survivorship and development time of *D. subobscura*, after 0 and 7 days growth of yeasts

Yeast Growth 1 (0 days): % survival						
Yeast	2	4	Sac	5	1	3
Mean %	95.2	78.9	78.5	67.3	64.4	49.9
SNK						
Yeast Growth 2 (7 days): % survival						
Yeast	Sac	5	3	2	1	4
Mean %	96.6	92.0	91.2	87.7	87.2	80.6
SNK						
Yeast Growth 1: development time						
Yeast	2	1	4	5	3	Sac
Mean (days)	8.0	8.6	9.9	10.2	10.5	11.1
SNK						
Yeast Growth 2: development time						
Yeast	2	1	4	Sac	3	5
Mean (days)	8.2	8.3	8.4	9.1	10.9	12.1
SNK						

Appendix 5. SNK tests applied to the development time of *Drosophila* species on different yeasts and in single and mixed species cultures (B-N-B medium, 10/tube)

	(a) Single species culture												(b) Mixed Species culture											
<i>D. subobscura</i>																								
Yeast	11	5	1	4	2	8	9	3	Sac	7	10	6	11	5	1	9	8	4	Sac	2	3	7	10	6
Mean(days)	7.8	7.9	8.0	8.2	8.3	8.3	8.6	8.6	8.6	8.6	9.5	11.1	7.4	7.9	8.2	8.3	8.3	8.3	8.5	8.6	8.7	8.8	8.9	10.0
SNK																								
<i>D. phalerata</i>																								
Yeast	1	2	Sac	4	7	3	8	9	10	11	6	5	1	3	11	5	7	8	2	9	4	Sac	10	6
Mean(days)	6.7	6.8	7.5	7.8	8.6	8.7	8.9	8.9	9.2	9.3	9.7	10.3	6.8	7.5	7.6	7.8	7.9	8.2	8.6	8.6	8.7	9.4	9.6	10.6
SNK																								
<i>D. obscura</i>																								
Yeast	1	2	3	4	5																			
Mean(days)	10.8	11.6	11.8	12.4	13.9																			
SNK																								

Appendix 6. SNK tests applied to the winglength of (a) *D. obscura* and *D. phalerata* on different species of yeasts (M-Y medium) and (b) *D. subobscura* on different yeasts, on different media and at different densities

<i>D. phalerata</i>																																																																							
(a) <i>D. obscura</i>																																																																							
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