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Adaptations of *Drosophila* and Yeasts: their Interactions with the Volatile 2-propanol in the Cactus–Microorganism–*Drosophila* Model System

William T. Starmer,[A,B] *J. S. F. Barker,*[A] *Herman J. Phaff*[C] *and James C. Fogleman*[D]

[A] Department of Animal Science, University of New England, Armidale, N.S.W. 2351.
[B] Department of Biology, Syracuse University, Syracuse, New York 13210.
[C] Department of Food Science and Technology, University of California at Davis, California 95616.
[D] Department of Biological Sciences, University of Denver, Denver, Colorado 80208.

Abstract

The interactions of yeasts growing in decaying cactus tissue with and without 2-propanol were studied with respect to the costs and benefits provided to three cactophilic *Drosophila* species (*D. mojavensis*, *D. arizonensis* and *D. buzzatii*). Two common cactus yeasts, *Candida sonorensis* and *Cryptococcus cereanus*, which can tolerate and metabolize 2-propanol, provide benefits to the three *Drosophila* species in the presence of the alcohol, as compared with another common cactus yeast, *Pichia cactophila*, which has less tolerance and cannot metabolize 2-propanol.

Because 2-propanol is commonly found in decaying cactus tissue and *C. sonorensis* and *Cr. cereanus* are also frequently recovered from the rotting tissue being utilized by the *Drosophila* species, the interactions described here are viewed as a possible adaptation in which the yeast provides benefits to one of its vectors by metabolism of 2-propanol in the habitat.

Introduction

The association of yeasts with species of the genus *Drosophila* is well known (Begon 1982). The nutritional requirements of *D. melanogaster*, many of which are provided by yeasts, have been to a large extent determined and quantified (Sang 1978). The natural habitats of different species and species groupings of the family Drosophilidae have distinct yeast communities associated with them (Starmer 1981; Begon 1982). The metabolic and physiological abilities of these yeast communities show a pattern of similarity that parallels the evolutionary history of several separate *Drosophila* radiations (Starmer 1981). This correlation of physiological activity with *Drosophila* phylogeny suggests that yeasts are coadapted with the *Drosophila* species which are associated with them. However, almost any yeast is sufficient for growth and development of larvae of *Drosophila* species in the laboratory (Wagner 1944, 1949), and in some cases bacteria growing in the natural substrate also are nutritionally sufficient (i.e. *D. disticha*, Robertson *et al.* 1968; *D. buzzatii*, Vacek 1982; *D. mojavensis*, Starmer 1982). Therefore, if coadaptation has occurred, it may be manifested in terms of benefits to the *Drosophila* species other than nutritional sufficiency.

In general, metabolism and processing of decaying plant tissue by yeast and bacterial cells provide sources of essential nutrients to the *Drosophila* species, while the microorganisms rely on insects such as *Drosophila* species for transportation to new habitats (Phaff *et al.* 1978; Gilbert 1980). Investigations by Starmer *et al.* (1982) also provide evidence that cactophilic *Drosophila* species carry and vector

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yeasts which are found in the larval habitat. However, other cactophilic insects (e.g. syrphids) also carry and potentially vector yeasts (W. T. Starmer, H. J. Phaff and M. A. Lachance unpublished data) and thus the yeasts are not totally reliant on the adult *Drosophila* for dispersal. Even partial reliance of the yeast on *Drosophila* species should favour adaptations by the yeast which serves to increase the viability, dispersal ability and/or fecundity of the *Drosophila*. One example of how the physiological ability of a yeast might be coadapted with respect to *Drosophila* is the association of the lipolytic yeast *Candida ingens*, with *D. mojavensis* in decaying *Stenocereus thurberi* stems. In this case the yeast is capable of oxidizing toxic, medium-chain fatty acids which are found in the host tissue, thus providing survival, developmental and size benefits to *D. mojavensis* larvae and adults (Starmer 1982).

In this paper, we report investigations on the possible adaptations of cactophilic yeasts and larvae of three *Drosophila* species in terms of the volatile 2-propanol which is present in the decaying tissue of both columnar and opuntia cacti (Heed 1978; Vacek 1979; Fontdevila *et al.* 1980; Fogleman 1982). The metabolic fate of 2-propanol has not been well studied in any *Drosophila* species, but the presumed fate is oxidation by alcohol dehydrogenase to acetone which is then retained or excreted. Introduction of the volatile 2-propanol at moderate to high concentration is toxic to adults and larvae of several species of *Drosophila* (David and Bocquet 1975; Argues and Duarte 1980; Daggard 1981; Batterham *et al.* 1982). However, the effects of 2-propanol on larvae of cactophilic *Drosophila* have not been investigated.

The yeasts associated with cactus have been characterized for their ability to utilize 2-propanol and acetone as sole sources of carbon. These studies (H. J. Phaff, J. C. Fogleman and W. T. Starmer, unpublished data) show that of the 20 relatively common cactus-associated yeasts, 16 cannot utilize the volatiles 2-propanol or acetone. However, *Candida sonorensis*, *C. ingens*, *Pichia opuntiae* var. *opuntiae*, and some strains of *Cryptococcus cereanus* can utilize 2-propanol and acetone as carbon sources, when they are provided in solid medium. Hypothetically, the metabolism of 2-propanol by these yeasts could provide benefits to cactus-breeding *Drosophila* via decreased toxic effects and/or increased yeast growth. Here we compare the larval development and growth of the cactus-breeding *D. mojavensis*, *D. arizonensis* and *D. buzzatii* on decaying cactus tissue to which increasing levels of 2-propanol have been added, when in conjunction with yeasts that can and cannot metabolize 2-propanol.

Materials and Methods

The concentrations of volatiles in naturally occurring necrotic cactus tissue were determined by quantitative gas chromatography using a Varian 3700 gas chromatograph (with dual FID's) linked to an Apple IIe computer through an analog/digital interface (AI13, Interactive Structures). A 2 m by 2 mm i.d. by 6·35 mm o.d. glass column packed with 6·6% Carbowax 20M on 80/120 Carbopack B AW (Supelco, Cat. No. 2-3845) was used. Samples of necrotic tissue were collected in the field by placing approximately 2 g into a sterile, 10-ml Vacutainer. Samples were frozen immediately and returned to the laboratory for analysis. Gas chromatography involved thawing the samples, separating the tissue and rot liquid by centrifugation in a table-top, clinical centrifuge, and injecting 3–5 μl of the supernatant directly into the gas chromatograph. Peak identification was based on retention time as compared with retention times of known compounds, and quantification was based on empirically derived regression equations relating peak area and injection of standards of specific concentration.

The effects of yeasts and 2-propanol on the survival, size and developmental time of *D. mojavensis*, *D. arizonensis* and *D. buzzatii* were determined in three factorial experiments. Each experiment was conducted so that the *Drosophila* species was exposed to 2-propanol and yeasts on its natural cactus

substrate. For *D. mojavensis*, 40 larvae (0–2 h old) were obtained from sterilized eggs (Starmer and Gilbert 1982) and placed on 8 g of autoclaved pieces of *Stenocereus gummosus* (agria) stem tissue in a 30-ml glass vial containing 10 g of sterile sand. This tissue was inoculated with a suspension of the soft rot bacterium *Erwinia carnegieana* strain EC191 (50 μl inoculum of approximately 10^8 cells/ml). Yeast treatments consisted of 50 μl inocula of approximately 10^5 cells/ml suspensions of *Pichia cactophila* strain 83-862·1, *Candida sonorensis* strain 83-867·2 and *Cryptococcus cereanus* strain 83-860·3. The last two strains both showed positive growth on 2-propanol as the sole carbon source, while *P. cactophila* (83-862·1) could not utilize the volatile. All three strains had been isolated from decaying *Opuntia stricta* cladode tissue on Navassa Island (Caribbean Sea). Three concentrations of 2-propanol (0, 0·5 and 1·0% v/w of tissue) were added after seeding the larvae and inoculating the microorganisms.

Each yeast and volatile treatment was incubated at 23°C and replicated four times for 3 yeast species × 3 volatile levels × 4 replicates (36 vials). When larvae had started to pupate, samples of decaying tissue were checked microscopically for yeast purity. The percentage emergence, developmental time, sex and size of the animals were determined. Size was measured as thorax length (in millimeters), while developmental time was measured in days. Vial means were used as the observations of a two-way analysis of variance (yeast by volatile) for size, developmental time and percentage emergence. Sex was utilized as a covariate in the analysis of size since males are in general smaller than females.

The experimental design for *D. arizonensis* was the same as that for *D. mojavensis* except that *Opuntia ficus-indica* pad tissue was utilized instead of tissue of *S. gummosus*. The experimental design for *D. buzzatii* was also similar except that homogenized *Opuntia stricta* tissue was utilized, no bacterium was inoculated, and the *Cr. cereanus* yeast treatment was not carried out; three replicates were conducted and vials did not contain sand and were incubated at 25°C.

The experiments with *D. mojavensis* (from Rancho A. V. Bonfil, La Paz, Baja Calif., Mexico) and *D. arizonensis* (from Desemboque, Sonora, Mexico) were carried out in Syracuse, New York.

The experiment with *D. buzzatii* was carried out in Australia. The source of *D. buzzatii* larvae was a population founded from 54 isofemale lines originally collected from Hemmant, Queensland. The yeast strains, designated Y1 (*C. sonorensis*) and Y2 (*P. cactophila*) (Barker *et al.* 1981; Vacek 1982) were originally isolated from decaying *Opuntia stricta* cladodes in Australia and are considered typical representatives of their species.

Results

Table 1 lists the distribution of the most common (>10% in at least one plant type) cactus yeasts in terms of the frequency of isolation from a given number of plants sampled. This table is a compilation of various cactus yeast surveys and indicates the ability of strains of these species to grow on 2-propanol as a sole source of carbon.

Table 2 presents the concentrations of selected volatiles found in naturally occurring rots of six species of cactus. One-way analyses of variance (with replications) performed on the data for each volatile indicate that significant differences ($P < 0{\cdot}01$) exist between cactus species for the volatiles methanol, acetone, 2-propanol, 1-propanol, propionic acid, and n-butyric acid. No significant differences between cacti were found for ethanol, acetic acid, or 2,3-butanediol. Specifically considering 2-propanol, a Student–Newman–Keuls test of the equality of means demonstrated that the average concentration of this volatile in agria was significantly higher than in the rest of the cacti and there was no significant difference between organpipe, cina, saguaro, senita, or *Opuntia*. As expected, the average concentrations of 2-propanol and acetone are positively correlated ($r = 0{\cdot}886$; $P < 0{\cdot}05$; d.f. = 4).

Table 3 lists the results for the three *Drosophila* species. In all three analyses of the variable size, the effect of sex was highly significant, males being smaller than females. *D. mojavensis* shows main effects of yeast and 2-propanol for both size and time variables and a yeast by 2-propanol interaction for thorax size. In this species, increasing 2-propanol concentration results in increasing adult size with *C. sonorensis*

or *Cr. cereanus* present but not with *P. cactophila*. Average effects for yeast species on size show that *P. cactophila* and *C. sonorensis* were not significantly different, but that flies developing on *Cr. cereanus* were significantly larger. For developmental time, addition of 2-propanol generally caused an increase, with that on 1% (v/w) 2-propanol being significantly longer than those on 0 or 0·5%. Yeast species effects for developmental time contrasted with those for size, as the times on *P. cactophila* and *Cr. cereanus* were not significantly different, but both were significantly shorter than the developmental time on *C. sonorensis*.

Table 1. General distribution of common cactus yeasts over three cactus groups

Yeast	No. of isolates (%) per No. of plants sampled: *Opuntia*[A] (865 plants)	*Stenocereus*[B] (388 plants)	*Pachycereus*[C] (243 plants)	Ability to metabolize 2-propanol
Pichia cactophila	39·9	60·5	46·1	–
P. heedii	0	0·7	28·8	–
P. amethionina[D]	8·2	11·6	16·5	–
P. kluyveri	10·1	3·4	2·5	–
P. deserticola[E]	3·9	20·2	2·9	–
Clavispora sp. 'O'	11·9	3·1	0·8	–
Candida sonorensis	34·7	29·1	17·7	+
C. mucilagina	11·0	3·6	0·5	–
C. ingens	0·6	14·7	11·1	+
Cryptococcus cereanus	25·0	18·8	16·4	±

[A] These samples include *Opuntia* species from the south-western United States (W. T. Starmer and H. J. Phaff 1983), north-western Mexico (W. T. Starmer, unpublished data), Caribbean Islands (W. T. Starmer, H. J. Phaff and M. A. Lachance, unpublished data), Hawaiian Islands (Starmer, unpublished data), and Australia (Barker *et al.* 1983).

[B] These samples include species of the genera *Stenocereus* and *Myrtillocactus* from Mexico (W. T. Starmer and H. J. Phaff 1983) and Caribbean Islands (W. T. Starmer, H. J. Phaff and M. A. Lachance, unpublished data).

[C] These samples include species of the genera *Pachycereus*, *Carnegiea*, *Lophocereus* and *Cephalocereus* from Southern Arizona (W. T. Starmer and H. J. Phaff 1983; Starmer, unpublished data), Mexico and Caribbean Islands (W. T. Starmer, H. J. Phaff and M. A. Lachance, unpublished data).

[D] This category includes *P. amethionina* var. *amethionina*, var. *pachycereana* and an undescribed variety 'F'.

[E] This category includes isolates of *Candida deserticola*.

D. arizonensis did not show significant yeast or propanol effects nor a significant interaction for thorax size. However, the interaction shows the same pattern as the *D. mojavensis* experiment and when analysed together with *D. mojavensis* the interaction is significant ($F = 3·63$; d.f. = 108, 4; $P < 0·01$). Developmental time increased significantly with increasing 2-propanol concentration, the interaction ($P < 0·10$) being due to a greater increase when *P. cactophila* was present with 1% (v/w) 2-propanol. Average effects for yeast species show a significantly longer time on *P. cactophila* than on either of the other two yeasts.

The *D. buzzatii* experiment showed an effect ($P < 0·10$) for 2-propanol for the size variable. The interaction, although non-significant, shows the same trend as that seen in *D. mojavensis* and *D. arizonensis* and when the three species are analysed together with a general linear model for unbalanced designs the 2-propanol by

Table 2. Concentrations of selected[A] volatiles (in mM) in *Drosophila* substrates[B]
Number of samples given in parenthesis

Volatile compound	Subtribe Stenocereinae									Subtribe Pachycereinae								
	Agria (7)			Organpipe (36)			Cina (26)			Saguaro (18)			Senita (8)			Opuntia (30)		
	Av.	s.d.	Max.	Av.	s.d.	Max.	Av.	s.d.	Max.	Av.	s.d.	Max.	Av.	s.d.	Max.	Av.	s.d.	Max.
Methanol	9·6	8·3	33·1	3·6	5·9	30·6	0·9	0·9	2·3	6·7	5·7	20·9	6·0	8·1	22·9	2·4	2·1	9·7
Acetone	2·2	3·5	9·5	0·1	0·1	0·3	1·3	1·6	6·7	0·0	0·1	0·1	0·1	0·1	0·3	0·5	0·9	4·6
Ethanol	5·0	9·4	25·1	3·7	13·9	81·9	2·5	4·5	16·8	0·5	1·1	4·9	0·5	0·4	1·1	2·9	4·0	16·5
2-Propanol	15·9	27·8	75·4	0·1	0·3	1·2	1·9	2·0	8·3	0·2	0·5	1·7	0·8	1·3	3·5	1·4	3·5	13·1
1-Propanol	3·2	4·2	11·9	0·6	1·2	6·4	0·8	1·4	7·0	0·3	0·6	2·3	1·5	2·0	5·5	0·2	0·5	1·8
Acetic acid	35·4	23·7	82·1	10·5	9·2	39·3	15·2	28·0	145·0	19·6	28·0	115·4	31·8	34·9	98·6	14·7	18·6	63·4
Propionic acid	14·9	11·8	39·6	7·3	9·5	44·7	5·0	5·2	18·2	3·8	10·4	44·5	5·9	5·4	14·6	2·3	2·6	9·5
2,3-Butanediol	4·1	5·4	18·3	4·0	9·9	43·6	2·1	1·8	7·8	3·2	6·2	23·9	3·6	7·0	20·3	1·4	3·6	19·7
n-Butyric acid	3·2	2·6	8·8	0·6	0·9	3·6	3·8	3·9	16·6	1·4	3·8	15·4	5·3	7·0	20·6	2·2	4·0	16·9

[A] Volatiles detected but not listed: ethanal, methyl acetate, isopropyl acetate, n-propyl acetate, n-butanol, 3-pentanol, acetoin, n-pentanol, n-pentyl acetate and iso-butyric acid.

[B] Scientific names of cacti: *Stenocereus gummosus* (agria), *S. thurberi* (organpipe), *S. alamosensis* (cina), *Carnegiea gigantea* (saguaro), *Lophocereus schottii* (senita), and *Opuntia stricta* (opuntia).

yeast interaction is significant ($F = 4{\cdot}52$; d.f. = 154, 4; $P < 0{\cdot}01$). Analysis of developmental time indicates a significant yeast effect with larvae growing faster in vials containing *C. sonorensis*.

Analysis of percentage emergence per vial (transformed to the arcsin square root) showed no significant interaction or main effects of yeast or 2-propanol treatments in any of the three experiments. The average percent emergence was 37, 37 and 57 for *D. mojavensis*, *D. arizonensis* and *D. buzzatii* respectively. Vial means used as observations in the analyses of development time and thorax size were thus based on approximately 15 adults (the first two species) and 23 adults (*D. buzzatii*).

Table 3. Thorax size (mm) and developmental time (days) for three levels of 2-propanol, three yeasts *P. cactophila*, *C. sonorensis*, *Cr. cereanus* and the three *Drosophila* species

†$P < 0{\cdot}10$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$

Drosophila species	Yeast	Thorax size (mm) for 2-propanol levels (%) of			Developmental time (days) for 2-propanol levels (%) of		
		0	0·5	1·0	0	0·5	1·0
D. mojavensis	*P. cactophila*	0·936	0·914	0·938	17·27	16·99	17·18
	C. sonorensis	0·925	0·937	0·946	17·37	17·98	18·48
	Cr. cereanus	0·932	0·962	0·969	16·78	16·89	17·88
D. arizonensis	*P. cactophila*	1·012	1·014	1·011	16·81	17·19	18·39
	C. sonorensis	1·005	1·002	1·016	16·43	16·99	17·33
	Cr. cereanus	0·998	1·017	1·020	16·47	16·72	17·02
D. buzzatii	*P. cactophila*	0·978	0·952	0·981	27·35	28·94	28·19
	C. sonorensis	0·935	0·961	0·985	25·79	24·93	25·26
	Cr. cereanus	–	–	–	–	–	–

Analyses of variance

	D. mojavensis			*D. arizonensis*			*D. buzzatii*		
	d.f.	m.s.	*F*	d.f.	m.s.	*F*	d.f.	m.s.	*F*
Thorax size									
Yeast	2	0·00399	10·2***	2	0·00014	0·40	1	0·00090	0·80
Propanol	2	0·00242	6·18**	2	0·00068	1·93	2	0·00286	2·54†
Yeast × propanol	4	0·00147	3·76**	4	0·00046	1·32	2	0·00243	2·15
Sex	1	0·07227	185***	1	0·11712	375***	1	0·03006	26·6***
Error	62	0·00037		62	0·00033		29	0·00112	
Developmental time									
Yeast	2	2·41	8·17**	2	1·72	12·35***	2	0·20	0·04
Propanol	2	1·67	5·65**	2	3·10	22·27***	2	0·20	0·04
Yeast × propanol	4	0·56	1·91	4	0·37	2·64†	2	2·26	0·50
Error	27	0·29		27	0·14		12	4·53	

Discussion

The production of 2-propanol in the decaying tissue of cacti is likely to be carried out by anaerobic bacteria, such as species of the genus *Clostridium* (Vacek 1979; Gottschalk *et al.* 1981; Young *et al.* 1981; Fogleman 1982). The maximum concentration of 2-propanol that was measured in any necrotic tissue (Table 2) was 75·4 mM or about 0·58% vol./wt. This supports the contention that the

concentrations of 2-propanol used in these experiments are, in fact, ecologically realistic.

Since cactophilic *Drosophila* females lay their eggs early in the rotting process, the young larvae will be exposed to the initial production of volatiles. At natural concentrations, these volatiles may have no direct detrimental effect on the larvae. However, the effect on the microbiota, in particular the rate of yeast growth, may ultimately determine the larval survival, developmental time and adult size. It is well known that ethanol can serve as an energy source for *Drosophila* species (i.e. *D. melanogaster*, Van Herrewege and David 1974; *D. mojavensis*, Starmer *et al.* 1977), but the utilization of 2-propanol as an energy source is limited because the oxidation product, acetone, cannot be further metabolized (Van Herrewege *et al.* 1980; Daggard 1981). It is probable that alcohol dehydrogenase (ADH) in larvae converts 2-propanol to acetone via oxidation, cycling NAD to NADH, with ADH remaining bound to the ketone or the ketone being excreted. There is some evidence that the specific activity of ADH in *D. melanogaster* is reduced in the presence of acetone by post-translational modification (Papel *et al.* 1979; Anderson and McDonald 1981; McElfresh and McDonald 1983) and in the cactus-breeding *D. mojavensis* (Batterham *et al.* 1982). It is thus possible that the presence of 2-propanol will decrease the activity levels of ADH and thus make larvae more susceptible to toxic levels of other alcohols (Gelfant and McDonald 1980). In any event, there is little direct benefit expected from 2-propanol in the larval habitat.

Table 3 indicates that, relative to the control without 2-propanol, the cactus yeasts *C. sonorensis* and *Cr. cereanus* provide larger adults in cactus tissue containing 0·5–1% 2-propanol than does *Pichia cactophila*. The increase in thorax length (0·021–0·037 mm) for *D. mojavensis* can be converted into increase in potential fecundity. Utilizing Mangan's (1978) analysis of the relationship of thorax length and ovariole number for *D. mojavensis* breeding on agria cactus, the increase is equivalent to 1·5–2·5 ovarioles or a 7·5–12·5% increase in potential fecundity. It is thus likely that the presence of *C. sonorensis* or *Cr. cereanus* in natural rots of cactus is beneficial to the *Drosophila* which breed there. This is especially relevant for *D. mojavensis*, which shows the strongest interaction (Table 3) and whose substrate has a significantly greater concentration of 2-propanol (Table 2). However, Table 3 also shows that developmental time is, in general, increased in the presence of 2-propanol. This increase is significantly greater for the 1% 2-propanol–*P. cactophila* treatment in *D. arizonensis* and thus is consistent with the hypothesis that larvae growing in the presence of 2-propanol and a yeast which cannot metabolize 2-propanol are at a disadvantage. However, contrasting results were obtained for *D. mojavensis*, where the yeast × 2-propanol interaction was not significant for developmental time, and developmental time was significantly longer with *C. sonorensis* than with *P. cactophila* or *Cr. cereanus*. Such increase was not observed with *D. arizonensis* and *D. buzzatii*. Clearly there are differences among the *Drosophila* species, the basis of which remains to be elucidated.

As cactus rots are usually ephemeral, dispersal to new rots will at times be more important to survival and reproduction than will developmental time. At such times, the size advantage will outweigh the time cost. In any event, the ability of *C. sonorensis* and *Cr. cereanus* to metabolize 2-propanol and possibly other volatiles (e.g. methanol by *C. sonorensis*), and thereby to benefit one of their vectors, could help explain the widespread occurrence of these species in the cactus–microorganism–*Drosophila* system.

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References

Anderson, S. M., and McDonald, J. F. (1981). Effect of environmental alcohol on *in vivo* properties of *Drosophila* alcohol dehydrogenase. *Biochem. Genet.* **19**, 421–30.

Argues, L. V., and Duarte, R. G. (1980). Effect of ethanol and isopropanol on the activity of alcohol dehydrogenase, viability and life-span in *Drosophila melanogaster* and *D. funebris*. *Experientia* **36**, 828–30.

Barker, J. S. F., Parker, G. J., Toll, G. L., and Widders, P. R. (1981). Attraction of *Drosophila buzzatii* and *D. aldrichi* to species of yeasts isolated from their natural environment. I. Laboratory experiments. *Aust. J. Biol. Sci.* **34**, 593–612.

Barker, J. S. F., Toll, G. L., East, P. D., Miranda, M., and Phaff, H. J. (1983). Heterogeneity of the yeast flora in the breeding sites of cactophilic *Drosophila*. *Can. J. Microbiol.* **29**, 6–14.

Batterham, P., Starmer, W. T., and Sullivan, D. T. (1982). Biochemical genetics of the alcohol longevity response of *Drosophila mojavensis*. In 'Ecological Genetics and Evolution: The Cactus–Yeast–*Drosophila* Model System.' (Eds J. S. F. Barker and W. T. Starmer.) pp. 307–21. (Academic Press Australia: Sydney.)

Begon, M. (1982). Yeasts and *Drosophila*. In 'The Genetics and Biology of *Drosophila*'. Vol. 3B. (Eds M. Ashburner, H. L. Carson, and J. N. Thompson, Jr.) pp. 345–84. (Academic Press: New York.)

Daggard, G. E. (1981). Alcohol dehydrogenase, aldehyde oxidase and alcohol utilization in *Drosophila melanogaster*, *D. simulans*, *D. immigrans* and *D. busckii*. In 'Genetic Studies of *Drosophila* Populations'. [Proceedings of the Kioloa Conference.] (Eds J. B. Gibson and J. G. Oakeshott.) pp. 59–75. (Australian National University: Canberra.)

David, J. R., and Bocquet, C. (1975). Compared toxicities of different alcohols for two *Drosophila* sibling species: *D. melanogaster* and *D. simulans*. *Comp. Biochem. Physiol.* **54C**, 71–4.

Fogleman, J. C. (1982). The role of volatiles in the ecology of cactophilic *Drosophila*. In 'Ecological Genetics and Evolution: The Cactus–Yeast–*Drosophila* Model System.' (Eds J. S. F. Barker and W. T. Starmer.) pp. 191–206. (Academic Press Australia: Sydney.)

Fontdevila, A., Santos, M., and Gonzalez, R. (1980). Genotype-isopropanol interaction in the Adh locus of *Drosophila buzzatii*. *Experientia* **36**, 398–400.

Gelfant, L. J., and McDonald, J. F. (1980). Relationship between ADH activity and behavioral response to environmental alcohol in *Drosophila*. *Behav. Genet.* **10**, 237–49.

Gilbert, D. G. (1980). Dispersal of yeasts and bacteria by *Drosophila* in a temperate forest. *Oecologia (Berlin)* **46**, 135–7.

Gottschalk, G., Andreesen, J. R., and Hippe, H. (1981). The genus *Clostridium* (Nonmedical aspects). In 'The Prokaryotes'. (Eds M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and M. G. Schlegel.) pp. 1767–1803. (Springer-Verlag: Berlin, New York.)

Heed, W. B. (1978). Ecology and genetics of Sonoran Desert *Drosophila*. In 'Ecological Genetics: The Interface'. (Ed. P. F. Brussard.) pp. 109–26. (Springer-Verlag: New York.)

McElfresh, K. C., and McDonald, J. F. (1983). The effect of alcohol stress on nicotinamide adenine dinucleotide (NAD+) levels in *Drosophila*. *Biochem. Genet.* **21**, 365–74.

Mangan, L. M. (1978). Competitive interactions among host plant specific *Drosophila* species. Ph.D. Dissertation, University of Arizona.

Papel, I., Henderson, M., van Herrewege, J., David, J., and Sofer, W. (1979). *Drosophila* alcohol dehydrogenase activity *in vitro* and *in vivo*: effects of acetone feeding. *Biochem. Genet.* **17**, 553–63.

Phaff, H. J., Miller, M. W., and Mrak, E. M. (1978). 'The Life of Yeasts.' (Harvard University Press: Cambridge, Massachusetts.)

Robertson, F. W., Shook, M., Takei, G., and Gaines, H. (1968). Observations on the biology and nutrition of *Drosophila disticha* Hardy, an indigenous Hawaiian species. *Univ. Texas Publ.* **6818**, 279–99.

Sang, J. H. (1978). The nutritional requirements of *Drosophila*. In 'The Genetics and Biology of *Drosophila*'. Vol. 2. (Eds M. Ashburner and T. R. F. Wright.) pp. 159–92. (Academic Press: New York.)

Starmer, W. T. (1981). A comparison of *Drosophila* habitats according to the physiological attributes of the associated yeast communities. *Evolution* **35**, 38–52.

Starmer, W. T. (1982). Associations and interactions among yeasts, *Drosophila* and their habitats. In 'Ecological Genetics and Evolution: The Cactus–Yeast–*Drosophila* Model System'. (Eds J. S. F. Barker and W. T. Starmer.) pp. 159–74. (Academic Press Australia: Sydney.)

Starmer, W. T., Heed, W. B., and Rockwood-Sluss, E. S. (1977). Extension of longevity in *Drosophila mojavensis* by environmental ethanol: Differences between subraces. *Proc. Natl Acad. Sci. U.S.A.* **74**, 387–91.

Starmer, W. T., Phaff, H. J., Miranda, M., Miller, M. W., and Heed, W. B. (1982). The yeast flora associated with the decaying stems of columnar cactus and *Drosophila* in North America. *Evol. Biol.* **14**, 269–95.

Starmer, W. T., and Gilbert, D. G. (1982). A quick and reliable method for sterilizing eggs. *Drosophila Inf. Serv.* **58**, 170.

Starmer, W. T., and Phaff, H. J. (1983). Analysis of the community structure of yeasts associated with the decaying stems of cactus. II. *Opuntia* species. *Microb. Ecol.* **9**, 247–59.

Vacek, D. C. (1979). The microbial ecology of the host plants of *Drosophila mojavensis*. Ph.D. Dissertation, University of Arizona.

Vacek, D. C. (1982). Interactions between microorganisms and cactophilic *Drosophila* of Australia. In 'Ecological Genetics and Evolution: The Cactus–Yeast–*Drosophila* Model System'. (Eds J. S. F. Barker and W. T. Starmer.) pp. 175–90. (Academic Press Australia: Sydney.)

Van Herrewege, J., and David, J. (1974). Utilisation de l'alcohol éthylique dans le métabolisme énergétique d'un insecte: Influence sur la durée de survie des adultes de *Drosophila melanogaster*. *C.R. Acad. Sci.* (Ser. D.) **279**, 335–8.

Van Herrewege, J., David, J. R., and Grantham, R. (1980). Dietary utilization of aliphatic alcohols by *Drosophila*. *Experientia* **36**, 846–7.

Wagner, R. P. (1944). The nutrition of *Drosophila mulleri* and *D. aldrichi*. Growth of the larvae on a cactus extract and the microorganisms found in cactus. *Univ. Texas Publ.* **4445**, 104–28.

Wagner, R. P. (1949). Nutritional differences in the *mulleri* group. *Univ. Texas Publ.* **4920**, 39–41.

Young, D. J., Vacek, D. C., and Heed, W. B. (1981). The facultatively anaerobic bacteria as a source of alcohols in three breeding substrates of cactophilic *Drosophila*. *Drosophila Inf. Serv.* **56**, 165–66.

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