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Development of *Drosophila* on sterol mutants of the yeast *Saccharomyces cerevisiae*

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SUMMARY

Four species of *Drosophila* (*melanogaster*, *simulans*, *mauritiana* and *virilis*) were tested for their ability to survive on *erg* strains of *Saccharomyces cerevisiae* each having a mutant block at a different reaction in the synthesis of ergosterol. Species capable of completing development on a given yeast mutant strain were tested for egg production and viability, and also for their ability to survive on the mutant yeast for five complete generations. Sterol analyses using gas–liquid chromatography show that the sterol composition of flies closely resembles that of the yeasts on which they are grown, confirming that the steric criteria for sterols capable of a structural role in lipoprotein membranes are relatively broad. Even so, different sterols are not equally efficient in this respect and there are differences between species in their tolerance to the sterols available from each of the yeast mutants. The range of sterols capable of satisfying the micronutrient, as distinct from the macronutrient, requirement is narrower. Growth on two of the yeast mutants (*erg-2* and *erg-6*) leads to developmental arrest in the larval stage due, it is suggested, to a block in the synthesis of ecdysone. The four *Drosophila* species lack an effective level of Δ^8–Δ^7 isomerase which is necessary for utilization of zymosterol and other Δ^8 sterol precursors of ergosterol. Ergosta-5,7-diene-3β-ol appears to be capable of substituting for cholesterol in the metabolism of *Drosophila*.

1. INTRODUCTION

A dietary supply of sterols is indispensable for insects because they lack the ability to synthesize them from simple precursors. *Drosophila melanogaster* larvae obtain preformed sterols from the yeasts on which they feed and to some extent also from the vegetable material on which the yeasts grow. Certain other *Drosophila* species, notably those in Hawaii which breed on rotting leaves of *Cheirodendron gaudichaudii*, obtain their dietary sterol supplies as phytosterols from the leaf tissue, since yeasts are normally absent, and the bacteria associated with the rotting leaf tissue produce no sterols (Robertson *et al.* 1968; Kircher, 1969). Sterols are used as structural components of cell membranes and as the precursors

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for synthesis of the moulting hormone ecdysone. Nutritional studies using germ-free larva cultured on aseptic chemically defined medium show that ergosterol, the characteristic sterol of *Saccharomyces cerevisiae*, readily satisfies the growth requirement of *D. melanogaster* as also do cholesterol and certain phytosterols

Table 1. *The major sterol components of wild-type and* erg *mutant strains of* Saccharomyces cerevisiae (*Barton* et al. 1974; *Woods* et al. 1974).

Strain	Sterols present	Enzyme block
Wild-type	Cholesta-8,24-dien-3β-ol (zymosterol)* Ergosta-8,24(28)-dien-3β-ol (fecosterol) Ergosta-7,24(28)-dien-3β-ol (episterol) Ergosta-5,7,22,24(28)-tetraen-3β-ol Ergosta-5,7,22-trien-3β-ol (ergosterol)*	
erg-6	Cholesta-8,24-dien-3β-ol (zymosterol)* Cholesta-5,7,24-trien-3β-ol Cholesta-5,7,22,24-tetraen-3β-ol	C-24 Methyl transferase
erg-2	Ergosta-8,24(28)-dien-3β-ol (fecosterol) Ergosta-8-en-3β-ol Ergosta-8,22-dien-3β-ol Ergosta-5-8,22-trien-3β-ol	$\Delta^8 \rightarrow \Delta^7$ Isomerase
erg-3	Ergosta-8,24(28)-dien-3β-ol (fecosterol) Ergosta-8,22-dien-3β-ol Ergosta-8,22,24(28)-trien-3β-ol Ergosta-7,24(28)-dien-3β-ol (episterol) Ergosta-7,22-dien-3β-ol* Ergosta-7,22,24(28)-trien-3β-ol*	5,6-Dehydrogenase
erg-5	Cholesta-8,24-dien-3β-ol (zymosterol) Ergosta-7,24$_1$(28)-dien-3β-ol (episterol) Ergosta-5,7,24(28)-trien-3β-ol Ergosta-5,7-dien-3β-ol*	22,23-Dehydrogenase

* Denotes the most abundant sterol in a given strain.

such as β-sitosterol (Cooke & Sang, 1970). Different species of *Drosophila* differ quantitatively in their requirements for purified sterols. The optimum level of cholesterol for larval growth in *D. melanogaster* is approximately four times greater than in its sibling species *D. simulans* (Erk & Sang, 1966) and appreciably higher than in *D. immigrans* (Royes & Robertson, 1964). The availability of particular sterols can be an important component in the adaptation of certain species to specific ecological niches. For example, *D. pachea*, which breeds only in the stems of the Senita cactus, has a requirement for an unusual sterol, schottenol (Δ^7-stigmasten-3β-ol), which is present in the tissues of this plant (Heed & Kircher, 1965).

The major sterols present in *Saccharomyces cerevisiae* and other species of yeasts are ergosterol and 24(28)dehydroergosterol (Breivik & Owades, 1957). Molzahn & Woods (1972) isolated four sterol mutants in a wild-type strain of *S. cerevisiae* by screening for induced mutations conferring resistance to polyene

antibiotics. The mutants are located at four unlinked loci *erg-2*, *3*, *5* and *6*. They affect the sterol composition of the yeast cell.

Essentially there are five reactions in the conversion of zymosterol to ergosterol. Each step in the modification of the structure of the sterol molecule is independent of the others so that a mutant block at one step does not interfere with completion of the other four, which is to say that the mutant blocks are parallel rather than sequential. The consequence of this is that there is some accumulation of precursor preceding the blocked reaction, but in addition a number of unusual sterol derivatives are synthesized. The major sterol components of the mutant strains are listed in Table 1. These yeasts provide us with a system to compare the capability of different species of *Drosophila* to utilize biological mixtures of sterols resulting from a block at one of four separate reactions in the biosynthesis of ergosterol.

2. MATERIALS AND METHODS

(i) *Yeast strains*

The yeasts used in these experiments were a wild-type strain of *Saccharomyces cerevisiae* (A184A) and four sterol mutants derived from this stock (*erg-2*, *erg-3*, *erg-5* and *erg-6*). These mutants were formerly named *pol-2*, *pol-3*, *pol-5* and *pol-1* respectively (Molzahn & Woods, 1972). The yeast stocks are isogenic with wild type except at the relevant *erg* locus. They were regularly crossed into, and reisolated from, the parent strain and rigorously screened for purity.

(ii) Drosophila *stocks*

The fly stocks used were as follows:

(*a*) Four highly inbred strains of *D. melanogaster* of different geographical origin: Novosibirsk (Novo.), Moscow (Mosc.), Oregon-K (Ore.) and Canton (Can.).

(*b*) A mass bred stock of *D. melanogaster*, Egypt, from a wild isolate collected less than a year before being used in these experiments, and kindly supplied by Professor Forbes Robertson.

(*c*) Mass bred laboratory stocks of *D. simulans*, *D. mauritiana* and *D. virilis*.

(iii) *Experimental procedures*

Yeast strains were grown in 400 ml yeast complete medium (Molzahn & Woods, 1972) in 1 litre Erlenmeyer flasks on a rotary shaker at 28 °C. Cultures were harvested by centrifugation after 48 hr and either used immediately or stored frozen until required. All yeast preparations were routinely assayed for sterol composition before use by UV absorption spectrophotometry.

Growth tests for *Drosophila* larvae were made using $8 \times 2{\cdot}5$ cm glass vials each containing plain 2 % water agar gel overlaid with a layer of 0·5 g of live yeast. Each culture was inoculated with 50 newly hatched first instar larvae. Ten replicate vials were used for each growth test. After the adults hatched all the flies from five vials were transferred to 1 % glucose-agar culture medium without yeast in order to allow them to empty their crop and guts of yeast cells. They were

changed to fresh glucose-agar vials on each of three consecutive days and then stored at −6 °C for sterol analysis. The flies in the remaining five vials were transferred to fresh live yeast cultures containing the same strain of yeast as the larvae had been grown on. The flies were transferred again to fresh yeast after 24 h and used subsequently for tests on egg production.

UV absorption spectra and gas–liquid chromatography were used to examine the sterol composition of yeast and fly extracts using the following method. Samples were saponified in alcoholic KOH as described by Molzahn & Woods (1972). The non-saponifiable fraction was extracted into *n*-heptane, its UV spectrum checked, and then dried down in a rotary evaporator. The sterols were taken up into acetone and chromatographed on a 7′ × ¼ in. column of SE–30 on Diatomite CLQ (100–120 mesh) using a Pye 104 gas chromatograph with a flame ionization detector. Column temperature was 256 °C and the carrier gas Argon at 60 ml/min. Cholesterol was added to some samples to standardize retention times.

For measurements of egg production, females were allowed an oviposition period of 6 h on watch glasses filled with 2% water agar containing 1% acetic acid. Following an incubation period of 24 h after oviposition the viability of the eggs was scored.

Measurements of larval development rate and survival were made using twice-daily counts of pupae and subsequently of hatching adult flies. Thorax length measurements were made on live flies by the method described by Robertson & Reeve (1952).

All experiments were carried out at 25 ± 1 °C.

3. RESULTS

(i) *Development of* Drosophila *on sterol mutant yeasts*

The ability of four species of *Drosophila* to complete their growth and development using each of five strains of *S. cerevisiae* as their sole dietary source was tested by inoculating newly hatched first-instar larvae into vials, containing an *ad libitum* level of live yeast. A summary of the developmental stage finally reached by each species of fly in the nutritional environment provided by each strain of yeast is given in Table 2. Wild-type yeast and two of the mutant strains *erg-3* and *erg-5* supported development to the imaginal stage in all four *Drosophila* species. Three species (*simulans*, *mauritiana* and *virilis*) reached the adult stage on *erg-2* but the development of *D. melanogaster* proceeded no further than the late third larval instar or early pupal stage on this yeast strain. None of the *Drosophila* species completed its life-cycle on *erg-6* mutant yeast.

Larvae from the massbred stocks of the four *Drosophila* species grown on *erg-6* yeast failed to proceed beyond the third larval instar. In each inbred stock of *D. melanogaster* the larvae showed some growth and continued to feed for about 4–7 days, after which they died. Examination of the mouth hooks of these larvae showed that they had all remained in the first instar, none having gone through ecdysis, although their final body size was 10–20% greater than that reached by

normal larvae at the time that ecdysis normally occurs. These results suggest that the sterols provided by *erg-6* yeast may satisfy the bulk sterol requirement for larval growth but cannot satisfy the molecular requirement for synthesis of moulting hormone.

Table 2. *The developmental stage to which* Drosophila *survive when grown on sterol mutants of* Saccharomyces cerevisiae

	Yeasts				
	erg-6	*erg*-2	*erg*-3	*erg*-5	Wild-type
D. melanogaster					
Inbred strain					
Novosibirsk	L-1	L-3/P	A	A	A
Moscow	L-1	L-3/P	A	A	A
Canton	L-1	L-3/P	A	A	A
Oregon	L-1	L-3/P	A	A	A
Outbred strain					
Egypt	L-1/L-3	L-3/P	A	A	A
D. simulans	L-3	P/A	A	A	A
D. mauritiana	L-1/L-3	A	A	A	A
D. virilis	L-3	A	A	A	A

(A = adult fly, P = pupa, L-1 first instar and L-3 third instar larva, respectively.)

A comparison of the proportion of larvae surviving to the imaginal stage on each yeast is shown in Table 3. Mean survival is expressed in angles (degrees). Quite striking differences emerge between the abilities of larvae of the different species to utilize *erg-2* yeast for growth. *D. melanogaster* cannot survive on this yeast, and although some *D. simulans* adults were obtained these proved to consist in every culture entirely of males. Survival rate for *D. mauritiana* on *erg-2* approaches the level reached on wild-type yeast, whilst for *D. virilis* the *erg-2* cultures produced more adults than the wild-type controls. The three yeast strains *erg-3*, *erg-5* and wild-type, on which all four species of *Drosophila* can survive, are clearly not equivalent in their suitability for different species. *D. mauritiana* is less able to use *erg-5* than *erg-3*, whereas *D. virilis* has a better survival rate on *erg-3* than on wild-type yeast. The inbred strains of *D. melanogaster* seem to be a more sensitive indicator than the massbred of the relative suitability of different yeast strains for this species. In all four inbred strains fewer larvae survive to the adult stage on *erg-3* than on wild-type yeast. For three inbred lines, Novosibirsk, Moscow and Canton, *erg-5* yeast provides a better nutritional environment for survival than the wild-type yeast.

These facts suggest the following tentative biological order in the biosynthesis of sterols which can be utilized for development by *Drosophila*: erg-6 < erg-2 < erg-3 < erg-5 = wild type.

D. melanogaster and *D. simulans* raised aseptically on chemically defined media are able to utilize either cholesterol or ergosterol as their sole dietary sterol source. Deficiency levels of either of these sterols cause a significant reduction in adult

body size, which may thus be used as an additional criterion for assessing the adequacy for growth of the sterols supplied in mutant yeasts. The results in Table 4 show that, even where they are statistically significant, differences in mean body size between yeast environments are surprisingly small. Adult males of *D. simulans* hatching from *erg-2* yeast cultures have a mean thorax length which is only 1·3 %

Table 3. *Survival to adult of* Drosophila, *grown on sterol mutants of the yeast* Saccharomyces cerevisiae (*mean* ± S.E. (*angles*))

	Yeasts			
	erg-2	*erg*-3	*erg*-5	Wild-type
D. melanogaster				
Inbred strains				
Novosibirsk	0	50·5 ± 6·5	80·2 ± 2·6	73·2 ± 2·9
Moscow	0	55·9 ± 4·6	74·1 ± 2·5	59·6 ± 2·4
Canton	0·9	48·3 ± 1·9	58·8 ± 2·6	50·6 ± 1·8
Oregon	0	37·2 ± 3·6	58·0 ± 2·4	61·6 ± 1·8
Outbred strain				
Egypt	0	57·8 ± 2·2	49·1 ± 2·8	43·7 ± 2·5
D. simulans	20·5 ± 1·6*	58·8 ± 2·0	58·1 ± 1·0	55·3 ± 2·0
D. mauritiana	54·3 ± 2·4	47·1 ± 1·4	39·8 ± 2·4	57·1 ± 1·9
D. virilis	68·6 ± 1·2	71·0 ± 1·3	63·5 ± 1·8	65·3 ± 1·2

* Surviving adults consisted entirely of male *D. simulans* in *erg-2* cultures.

Table 4. *Thorax length* (*in 0·01 mm*) *of* Drosophila *males, grown on sterol mutants of the yeast* Saccharomyces cerevisiae

	Yeasts			
	erg-2	*erg*-3	*erg*-5	Wild-type
D. melanogaster				
Inbred strains				
Novosibirsk	—	87·4 ± 0·3	90·1 ± 0·3	89·7 ± 0·3
Moscow	—	87·1 ± 0·5	87·6 ± 0·3	87·1 ± 0·5
Canton	—	88·6 ± 0·3	89·4 ± 0·5	87·1 ± 0·8
Oregon	—	89·6 ± 0·3	91·4 ± 0·4	92·0 ± 0·3
Outbred strain				
Egypt	—	82·7 ± 0·5	87·6 ± 0·3	83·4 ± 0·5
D. simulans	88·3 ± 0·4	86·0 ± 0·3	88·7 ± 0·3	89·5 ± 0·3
D. mauritiana	92·2 ± 0·5	89·5 ± 0·5	92·7 ± 0·5	90·2 ± 0·5
D. virilis	124·0 ± 0·5	120·3 ± 0·7	123·8 ± 0·3	125·5 ± 0·3

less than in the corresponding live yeast controls. In *D. mauritiana* body size is increased on *erg-2* and *erg-5* yeast even though the survival rate is lower on the latter. Body size in the inbred strains of *D. melanogaster* tends to be reduced on *erg-3* yeast but not on *erg-5* yeast, thus tending to confirm the previous indication based on survival data that the sterols provided by *erg-3* are less well able to satisfy the growth requirement of *D. melanogaster*.

(ii) *Sterol composition of flies and yeast*

The sterol composition of wild-type yeast as revealed by gas–liquid chromatography (G.L.C.) is shown in Fig. 1. The major sterol is ergosterol together with zymosterol, 24(28)dehydroergosterol, episterol and lanosterol. Cholesterol, not present in yeast, was added to the extract before chromatography as a standard marker.

Adult flies were removed at eclosion from live yeast cultures and cleared of gut contents as described in the Materials and Methods section. The sterols present in such flies are consequently derived nutritionally from the yeasts on which the flies were grown together with whatever may have been transferred through the egg. Adult *D. melanogaster* which completed their development as larvae on wild-type yeast show a pattern of sterol composition which is closely similar to that shown by wild-type yeast. Ergosterol is present as the major sterol component together with zymosterol, 24(28)dehydroergosterol (ergosta-5,7,22,24(28)-tetraen-3β-ol), episterol and lanosterol. A minor peak appears in the fly extracts with a retention time corresponding to the cholesterol marker. A similar picture was found for *D. simulans*, *D. mauritiana* and *D. virilis*, the pattern of sterol composition in each case closely resembling that in wild-type yeast with the additional presence of a minor component corresponding to cholesterol.

The composition of *erg-5* yeast, which lacks ergosterol, is shown in Fig. 1. The major peak consists of a mixture of episterol and ergosta-5-7-dien-3β-ol. An identical major peak at this position appears in the *D. melanogaster* adults grown on this yeast, but it is not presently known whether this consists of only one, or both, of the two sterols present in the yeast. The flies contain no ergosterol detectable by G.L.C. procedures although they do contain zymosterol and a minor constituent coincident with the cholesterol marker.

The major sterol components of *erg-3* yeast form two major peaks, the first ergosta-7,22-diene-3β-ol, and the second ergosta-7,22,24(28)-triene-3β-ol together with ergosta-8,24(28)-diene-3β-ol. There are two additional minor components ergosta-8,22-dien-3β-ol and episterol. Adult flies of each *Drosophila* species which complete their larval development on *erg-3* yeast show a similar pattern of sterol composition to the mutant yeast. In *D. melanogaster* there is some variation between inbred strains in the relative height of the two major peaks, indicative of quantitative differences in sterol composition which are genotype-dependent. There is also between-strain variation in the amount of ergosta-8,22-dien-3β-ol which appears as a well-defined shoulder to the first major peak in some inbred strains but not in the Canton strain which was used for the G.L.C. analysis illustrated in Fig. 1. The flies also contain a minor peak appearing at the same position as cholesterol.

No adult *D. melanogaster* can be obtained from larvae grown on *erg-2* yeast, so that sterol extracts were made from third-instar larvae and their composition analysed using the same procedure as before. The gut contents of the larvae were completely cleared before extracts were made. The sterol composition of these

larvae as shown in Fig. 1 is similar to that of the *erg-2* yeast on which they were grown, with major peaks corresponding to ergosta-5,8,22-trien-3β-ol and fecosterol, together with a minor lanosterol peak and another corresponding to the cholesterol marker. A similar pattern of sterol composition was found in the adult flies of the other species grown on *erg-2* yeast.

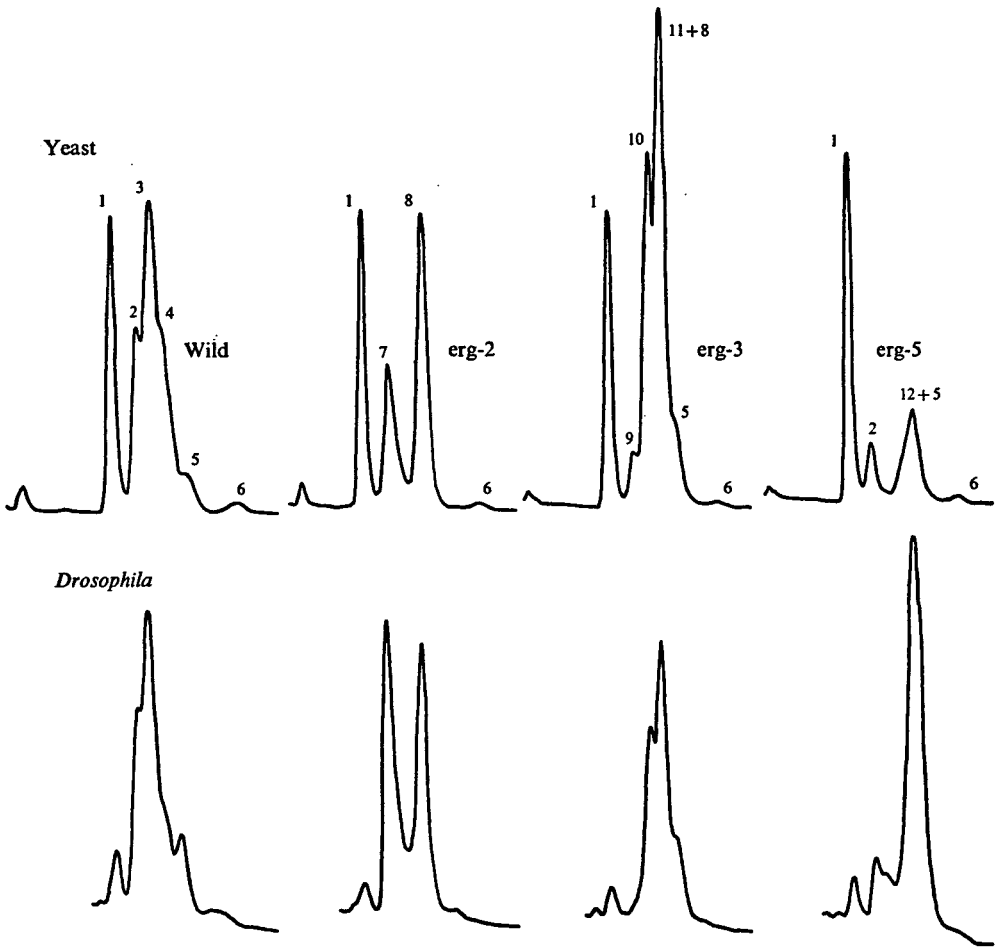

Fig. 1. Sterol composition of wild type and *erg* mutant strains of *Saccharomyces cerevisiae* and of *Drosophila melanogaster*, as revealed by gas–liquid- chromatography. 1, Cholesterol,* 2, zymosterol; 3, ergosterol; 4, 24(28)dehydroergosterol; 5, episterol; 6, lanosterol; 7, ergosta-5,8,22-trien-3β-ol; 8, fecosterol; 9, ergosta-8,22-dien-3β-ol; 10, ergosta-7,22-diene-3β-ol; 11, ergosta-7,22-24(28)-trien-3β-ol; 12, ergosta-5,7-dien-3β-ol. In 1, cholesterol was added to the yeast extracts as a reference sterol.

These results show that the sterol composition of adult flies and larvae is qualitatively similar to that of the yeasts on which they are cultured. Differences in the relative size of different peaks in the G.L.C. records as between flies and yeasts indicate that there are differences in the rate of uptake, or of utilization by *Drosophila*, of the different sterol components. The G.L.C. analyses show which

sterol components are present in the adult flies but we do not yet know to what extent they represent an accumulated store of non-utilized sterol such as might be present in fat body and to what extent they reflect the structural sterol components present in cell membranes and organelles.

(iv) *Bulk sterol and molecular requirements*

None of the strains of *D. melanogaster* completed their development to the imaginal stage on *erg-2* yeast. Development tended to case in late third instar or at the time of pupariation. Third-instar larvae grown on *erg-2* yeast up to 168 h of age pupated successfully to give normal imagoes if they were then transferred to wild-type yeast. Consequently it can safely be concluded that the sterols present in *erg-2* yeast are able to satisfy the bulk or macro-sterol requirement for larval development and the normal differentiation and growth of the imaginal disc tissues. Failure of development to proceed beyond the third instar appears to be due to the failure of *erg-2* sterols to satisfy a molecular requirement which may well be for synthesis of the moulting hormone ecdysone, although it is of course possible that there could be some other requirement for larval development which only becomes limiting in the third instar.

On *erg-6* yeast the development of *D. melanogaster* is arrested at an earlier stage, the larvae usually being unable to proceed beyond the first larval instar. First-instar larvae grown aseptically on Sang's medium C containing cholesterol as the sole sterol source and then transferred before 24 h of age to *erg-6* yeast complete their development to give normal adults. Larvae grown initially on *erg-6* yeast and transferred to wild-type yeast were found to be capable of completing development to the adult stage provided transfer was made before 48 h of age. Survival declined sharply in larvae transferred after longer periods on the mutant yeast. At least two separate processes are indicated here. The failure of larvae to go through ecdysis to the second larval instar may indicate a block in the availability of moulting hormone which can be bypassed by transferring larvae to wild-type yeast. However, larvae transferred after 48 h cannot be rescued in this way. It seems that some non-reversible change in larval tissues has occurred as a result of exposure to the sterols present in *erg-6* yeast.

(iv) *Egg production*

Sang & King (1961) have shown that the sterol requirement for normal daily egg production in *D. melanogaster* females must be very small compared with the larval growth requirement. However, larvae reared on dihydrocholesterol produce small adult females with rudimentary ovaries which produce sterile eggs (Sang, 1972), indicating that an adequate dietary provision of sterol is necessary for the long-term survival of the species.

Table 5 shows that egg production and egg viability are greatly reduced in two of the three species capable of surviving to the imaginal stage on *erg-2* yeast. *D. simulans* females could not be tested because only males survive on *erg-2* yeast. Egg production and egg viability in *D. melanogaster* tend to be lower on *erg-3* and

Table 5. *Egg production for 3-day-old fertilized females measured over a 6 h oviposition period, and egg hatchability (angles) in* Drosophila, *grown on sterol mutants of the yeast* Saccharomyces cerevisiae

	Egg production				Egg hatchability			
	erg-2	*erg*-3	*erg*-5	Wild type	*erg*-2	*erg*-3	*erg*-5	Wild type
D. melanogaster								
Inbred strains								
Novosibirsk	—	1·6 ± 0·7	5·4 ± 2·4	9·1 ± 3·5	—	21·4 ± 8·8	42·6 ± 4·2	55·8 ± 2·4
Moscow	—	8·3 ± 2·8	10·9 ± 1·7	12·4 ± 3·1	—	33·7 ± 15·2	67·1 ± 1·4	78·1 ± 1·8
Canton	—	5·0 ± 0·6	5·6 ± 1·7	13·3 ± 0·4	—	45·4 ± 16·2	57·2 ± 2·9	74·8 ± 1·9
Oregon	—	2·6 ± 1·0	2·1 ± 1·1	11·3 ± 1·5	—	32·9 ± 10·7	40·2 ± 11·6	58·1 ± 2·6
Outbred strain								
Egypt	—	7·4 ± 0·1	13·2 ± 2·1	13·1 ± 2·3	—	61·6 ± 1·8	76·5 ± 1·1	71·4 ± 0·9
D. simulans	—	9·6 ± 2·3	13·1 ± 1·6	15·8 ± 1·5	—	48·4 ± 6·6	61·3 ± 1·5	77·4 ± 1·0
D. mauritiana	0·3	3·0 ± 2·3	4·2 ± 2·5	1·9 ± 0·2	26·6	52·9 ± 7·9	69·8 ± 7·9	77·5 ± 12·6
D. virilis	1·3 ± 0·4	1·7 ± 0·6	9·3 ± 1·9	8·9 ± 1·9	0·0	42·5 ± 4·9	56·8 ± 2·4	63·5 ± 0·9

erg-5 than on wild-type yeast. In the other species (*simulans*, *mauritiana* and *virilis*) egg production is low on *erg-3* but not significantly different from wild type on *erg-5* yeast. However, egg viability, on both mutant yeasts, is significantly reduced in these *Drosophila* species. These results show that the efficiency with which the different *Drosophila* species utilize the sterols present in *erg* mutant yeasts for viable egg production parallels their ability to use these sterols for larval growth.

(v) *Long-term growth on mutant yeasts*

The ability of different *Drosophila* species to colonize and maintain themselves in alternative nutritional environments represented by the *erg* mutant yeasts was examined by inoculating a founder population of approximately 250 individuals

Table 6. *Ability of four species of* Drosophila *to colonize different environmental niches, measured by their ability to maintain themselves for five successive generations on different* erg *mutant yeasts*

	erg-2	*erg*-3	*erg*-5	Wild-type
D. melanogaster	−(0)	−(1)	+	+
D. simulans	−(1)	+	+	+
D. mauritiana	−(1)	+	+	+
D. virilis	−(1)	−(2)	+	+

(Figures in parentheses give the number of complete generations completed if less than five.)

into culture bottles containing *ad libitum* provision live yeast. A random sample of the progeny of these parents was then used as the parents of the next generation and so on. Ability to complete five successive generations was taken as the criterion for deciding that a given species has the capability for successfully colonizing and maintaining itself in the absence of competitors in a niche containing solely a given strain of yeast. As shown in Table 6, no species successfully maintained itself on *erg-2* beyond one generation. *D. simulans* and *D. mauritiana* were able to survive successfully on both *erg-3* and *erg-5* in addition to wild-type yeast. *Erg-5* was the only mutant yeast capable of supporting the long-term survival of *D. melanogaster* and *D. virilis*.

4. DISCUSSION

Cooke & Sang (1970) have shown that *Drosophila melanogaster* has a pattern of sterol utilization similar to that for other phytophagous insects. Their results suggest that sterols serve a bulk requirement for which the steric criteria for utilization are relatively broad, and a micronutrient requirement of narrow steric specificity which may only be satisfied by sterols closely related in structure to cholesterol. Our results are generally consistent with this view.

Erg-5 yeast alone supported normal development of all four *Drosophila* species over a period of five consecutive generations. The major sterol present in this yeast, ergosta-5,7-diene-3β-ol, is closer in structure to cholesterol than is ergosterol, being $\Delta^{5,7}$ but lacking $\Delta^{22(23)}$. The sterols present in *erg-3* yeast are Δ^{7} and Δ^{8} ergostanes with a predominance of Δ^{7}. These sterols support the growth and

development of all four *Drosophila* species for at least one generation, and in two species for five consecutive generations. In those insect species able to use phytosterols, an ability to convert them to cholesterol may be a prerequisite for utilization (Clayton, 1964; Thompson *et al.* 1972). Our results using G.L.C. analysis show that the sterol composition of the adult flies is qualitatively similar to that of the yeast on which they were cultured as larvae and this suggests that the yeast sterols are used to perform a structural role directly and with limited metabolic alteration. The implication of this is that the sterol structure of lipoprotein membranes may be varied within certain limits without deleterious effects to the insect. This appears to be borne out by the results obtained using *erg-5* sterols. However, *erg-3* sterols cause a reduction in reproductive rate, especially in *melanogaster* and *simulans*, brought about by a lowering of egg production and egg viability. Evidently the Δ^7 sterols present in *erg-3* yeast fulfil the structural requirement for utilization, but they do so less efficiently than the $\Delta^{5,7}$ sterols in *erg-5* and wild-type yeast.

If the steric criteria for a structural role are broad, the question remains as to whether conversion of an *erg-3* sterol component to cholesterol, or some closely related sterol, is necessary to satisfy a molecular requirement for the synthesis of ecdysone? This cannot be answered on the basis of presently available evidence. Flies cultured for one generation on *erg-5* or on *erg-3* yeast show a small peak coincident with the cholesterol standard in the G.L.C. analyses. The amounts involved are very small but positive chemical characterization may be possible by mass spectrometry.

The sterols present in *erg-2* yeast are Δ^8 ergostanes. None of the four species of *Drosophila* survived beyond a single generation on this yeast mutant, and in the two species (*virilis* and *mauritiana*) in which individuals of both sexes survived there was a drastic reduction in egg production and egg viability. From this we may conclude that the Δ^8–Δ^7 isomerization is necessary for sterol utilization, and that these *Drosophila* species lack an effective level of the relevant enzyme necessary for this step. In *D. melanogaster* development is arrested at the larval–pupal boundary. The fact that these larvae can be rescued by a short exposure to ergosterol at this point and are then able to complete their development to the adult stage strongly suggests that developmental arrest is caused by a block in the synthesis of ecdysone. Differences between species with respect to their ability to reach the imaginal stage on *erg-2* yeast may be due to differences in the extent to which the sterol requirement for hormone synthesis can be satisfied by egg sterols carried over from the mother. The G.L.C. traces for *melanogaster* larvae arrested on *erg-2* yeast reveal the presence of a small peak with the same retention time as cholesterol. If this peak is cholesterol derived from the egg it may be membrane bound in the larva and unavailable as a hormone precursor. The possibility that *melanogaster* larvae cultured on *erg-2* sterols are selectively blocked in ecdysone synthesis merits more detailed investigation with regard to the potential of this system for studies on the relationship between hormones and gene action.

The major sterol fraction present in *erg-6* yeast is zymosterol which as we have

noted above may not be utilized because flies lack Δ^8–Δ^7 isomerase. Also present are two cholestanes: cholesta-5,7,24-trien-3β-ol and cholesta-5,7,22,24-tetraen-3β-ol. That none of the *Drosophila* species appear capable of utilizing these sterols is surprising in view of their structural similarity to cholesterol. Both sterols have a double bond at C24, and it is interesting that Cooke & Sang (1970) have found that desmosterol, which differs from cholesterol only by possession of this double bond, is very poorly utilized. These findings imply a low level of efficiency of the Δ^{24}-reductase in *D. melanogaster*.

Developmental arrest in the inbred *melanogaster* strains cultured on *erg-6* yeast always occurred at the boundary between the first and second larval instars, indicating a hormone block. But the fact that these larvae could not be rescued by transfer to a nutritional source of ergosterol after 48 hrs seems to suggest either that the *erg-6* sterols cause an irreversible block in the ecdysone pathway, or that incorporation of these sterols in sufficient quantity into lipoprotein membranes may make the tissues less capable of responding to the moulting hormone.

Present knowledge concerning sterol nutrition in *Drosophila* is based upon studies using single sterols. This is, of course, artificial since in the natural situation sterols are available as mixtures. The approach taken in the present study which exploits mutant blocks in yeast gets round the difficulty of deciding, out of the many possible permutations and combinations, what is a biologically realistic mixture of sterols to test. The approach has the obvious limitation that sterols may be present in the yeast cells in quantities too small for exact chemical characterization, so that a complete inventory is difficult to achieve. There is also an additional source of contamination, common to all studies on sterol utilization, caused by the transmission of small but metabolically significant quantities of sterols through the egg. Moreover it may not be immediately apparent which are the relevant sterol components of the yeast cells actually responsible for a given effect on the growth and development of *Drosophila*. Consequently the method can only be used as a preliminary screen. The next step will be to refine the approach, firstly by the use of double mutants which give a much narrower spectrum of sterols in the yeast cells (Barton *et al.* 1975), secondly by chemical purification of individual 'mutant sterols' in sufficient quantities that they can be used in nutritional tests on chemically defined media.

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