

Sterol Metabolic Constraints as a Factor Contributing to the Maintenance of Diet Mixing in Grasshoppers (Orthoptera: Acrididae)

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ABSTRACT

Sterols are essential nutrients for all arthropods, including grasshoppers, but metabolic constraints may limit which sterols can support normal growth and development. In the first part of this study, a comparative experiment, which included five different species of grasshoppers (Orthoptera: Acrididae) representing three separate taxonomic groups, was performed to determine how widespread sterol metabolic constraints are within the Acrididae. Grasshoppers were reared on artificial diets containing sterols that differed in the position of double bonds within the sterol structure, and various life history traits were measured. Sterols with double bonds at position 7, within the sterol nucleus, and/or at position 22, on the cholestane side chain, failed to support development to the adult stage for any of the five species. In addition, grasshoppers reared on sterols with these configurations often had extended developmental times and reduced growth rates in the first and second stadium compared with grasshoppers reared on sitosterol or cholesterol diets. In the second half of this study, we examined how mixtures of suitable and unsuitable sterols influenced survival, growth, and development. Artificial foods containing mixtures of suitable and unsuitable sterols were fed to the highly polyphagous grasshopper *Schistocerca americana*. Results suggest that survival and performance of this grasshopper suffer as the concentration of unsuitable sterols increases and as the ratio of suitable to unsuitable sterols in the diet decreases. We review the literature to document variation in plant sterol profiles and propose that constraints on sterol metabolism may contribute to the maintenance of diet mixing in the Acrididae.

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Introduction

Grasshoppers are highly mobile relative to other phytophagous insects, and diet mixing by individuals appears to be a common phenomenon. Among the grasshoppers that have been critically investigated, almost 60% are polyphagous, and a further 25% are oligophagous on grasses (reviewed by Bernays and Chapman 1994). For individual grasshoppers, the benefits of feeding on a mixture of plants may be twofold. First, it allows the opportunity to select among foods with a suitable balance of nutrients (Simpson and Simpson 1990; Bernays et al. 1994; Simpson et al. 1995); this generally leads to enhanced growth rate (Kaufmann 1965; MacFarlane and Thorsteinson 1980). Second, it may be an effective strategy to dilute potentially poisonous plant chemicals (Freeland and Janzen 1974; Guglielmo et al. 1996).

Sterols, which are required in lipid biostructures and serve as precursors for steroid hormones, are essential nutrients for grasshoppers, as well as for all other arthropods because sterols cannot be metabolized *de novo* (Clayton 1964; Svoboda and Thompson 1985). Most herbivorous insects metabolize phytosterols to cholesterol to fulfill sterol requirements; cholesterol is the dominant sterol found in cell membranes and serves as the precursor for the insect-molting hormone 20-OH ecdysone (Grieneisen 1994). For some herbivorous insects, however, metabolic constraints limit which phytosterol structures can be metabolized to cholesterol. In grasshoppers, sterol metabolic constraints have so far been identified in three different species: *Schistocerca gregaria* (Forskål; subfamily Cyrtacanthacridinae), *Schistocerca americana* (Drury; subfamily Cyrtacanthacridinae), and *Locusta migratoria* (L.; subfamily Oedipodinae). All three of these species grow and complete development on cholesterol, sitosterol, and other Δ^5 sterols (Δ indicates position of double bonds in sterols), but none is able to complete development on sterols containing Δ^7 and Δ^{22} bonds (Dadd 1960; Behmer and Elias 1999a). A recent study has shown that in *S. americana* the failure to develop on Δ^7 and Δ^{22} sterols is likely linked to an inability to metabolize sterols containing these bonds to cholesterol (Behmer et al. 1999b).

At least one species of grasshopper can, however, detect the presence of unsuitable sterols in its food. When sixth-stadium

S. americana were presented with spinach, a plant that contains only Δ^7 and Δ^{22} sterols (all unsuitable), it was initially accepted (Lee and Bernays 1988; Champagne and Bernays 1991). With continued exposure, however, its acceptability declined until it was completely rejected. In the field, grasshoppers that exhibit rejection behavior of this magnitude typically switch to an alternative host plant (Raubenheimer and Bernays 1993; Chambers et al. 1996). That the aversion response shown by *S. americana* toward spinach was indeed triggered by the presence of unsuitable sterols was recently confirmed by using an artificial diet (Behmer and Elias 1999b). As in the experiment with spinach, the acceptability of artificial diets containing unsuitable sterols declined with experience until they were completely rejected.

Different plant species often have different sterol profiles, but whether this is of any general consequence for generalist grasshoppers depends on how widespread sterol metabolic constraints are within the Acrididae and in what amounts unsuitable phytoosterols can be ingested before they begin to exert negative effects. In the current study we ask two questions. First, how widespread are sterol metabolic constraints in grasshoppers? Second, how do different mixtures of suitable and unsuitable sterols influence survival and performance in a highly polyphagous grasshopper? To address the first question, we use a comparative approach and examine sterol use in five different acridids representing three separate taxonomic groups. The species selected come from diverse habitats and have distinct feeding ecologies. For each species we analyze survival on an artificial diet containing one of six different sterol treatments; developmental time and growth rate were also measured. To address the second question, we fed the generalist grasshopper *S. americana* artificial diets containing different mixtures of suitable and unsuitable sterols and then measured performance. Unlike many insect species in other phytophagous groups that can develop on a range of sterols (Dadd 1977), results from the current study indicate that constraints on sterol metabolism are widespread in the acridids. The results also suggest that there are costs associated with eating foods containing mixtures of suitable and unsuitable sterols, even though the amount of suitable sterol by itself permits normal growth and development. We conclude by reviewing the literature on plant sterol profiles. We propose that constraints on sterol metabolism in grasshoppers may be underestimated as a factor contributing to the maintenance of diet mixing in this group of insects.

Material and Methods

Grasshoppers

The five species selected for the comparative study come from three subfamilies within the Acrididae: one from the Oedipodinae, two from the Romaleinae, and two from the Melanoplinae. Phylogenetically, the oedipodines are the most prim-

itive subfamily within the Acrididae, followed next by the romaleines; the melanoplinae are the most derived of the three subfamilies in this study (C. H. F. Rowell, personal communication).

Trimerotropis pallidipennis (Burmeister) was selected as a representative from the Oedipodinae. It is a wide-ranging species distributed throughout much of the new world and typically inhabits dry grasslands, where it feeds on a wide variety of plant species but predominately grasses (Mulkern et al. 1969; Otte and Joern 1977; Otte 1984).

Romalea gutatta (Houttuyn) and *Taenipoda eques* (Burmeister) are two species from the subfamily Romaleinae. *Romalea gutatta* is found throughout the southeastern United States (Rehn and Grant 1959, 1961), whereas *T. eques* inhabits desert grass or brushlands from central Mexico to southern Arizona, New Mexico, and southwestern Texas (Hebard 1925; Rehn and Grant 1959, 1961). Both species are highly polyphagous and include plant vegetative and reproductive tissue as well as a high proportion of insect and other animal tissue in their diets (Ball et al. 1946; Whitman and Orsak 1985; Raubenheimer and Bernays 1993).

Finally, *Barytettix humphreysii* (Thomas) and *Melanoplus differentialis* (Thomas) were chosen as representative species of the subfamily Melanoplinae. *Barytettix humphreysii* is found throughout southern Arizona and is rather common in the Upper Sonoran Zone and the higher desert grassland of the Lower Sonoran (Ball et al. 1942), while *M. differentialis* is found throughout much of the United States (Capinera and Sechrist 1982). Characteristic of most other members belonging to this subfamily, both species are generalists feeding on forbs and grasses (Ball et al. 1946; Joern 1979, 1985).

We collected adult *T. pallidipennis*, *T. eques*, and *B. humphreysii* from the field in southern Arizona and returned them to the laboratory so that eggs could be collected. Adult *R. gutatta* were obtained from a colony at Illinois State University, and *M. differentialis* were obtained from a colony at the Rangeland Insect Lab in Bozeman, Montana. All of the grasshoppers were fed a mixed diet of romaine lettuce, kale, 7–10-d-old seedling wheat, and wheat bran and were maintained under standard laboratory conditions with photophase 16L : 8D and radiant heat L : D 24°–35°C : 19°–22°C. Radiant heat was supplied by a 150-W incandescent bulb during photophase, allowing the grasshoppers to regulate their body temperature; during scotophase, air temperature fell to 19°–22°C. Cups of moistened sand were placed in the adult cages for ovipositing females. After diapause, where present, eggs for all grasshoppers were transferred to a Percival incubator set at L : D 35° : 28°C until hatching.

For the second experiment, we used the grasshopper *Schistocerca americana*. This species is highly polyphagous and occurs throughout the eastern United States and Mexico (Harvey 1981). It is recorded as feeding on a wide range of cultivated and naturally occurring plant species (Kuitert and Connin

1952). Eggs for this species came from a laboratory colony at the University of Arizona that were reared on a diet of romaine lettuce, 7–10-d-old wheat seedlings, and wheat bran; they were maintained under standard laboratory conditions (as described earlier). This particular line was nondiapausing, so the eggs were directly transferred to a Percival incubator set at L : D 35° : 28°C. They were kept under these conditions until hatching occurred.

The Sterols

Six different sterols were used in this study (Fig. 1). Cholesterol is the dominant sterol found in animals, including insects. Lathosterol is a cholesterol analog with a Δ^7 rather than a Δ^5 configuration. It, like cholesterol, is mostly found in animals, but trace amounts have been reported in plants (Nes and McKean 1977). The remaining sterols used in this study are found in plants. Sitosterol differs from cholesterol only by the presence of an ethyl group at C24. It is the dominant sterol found in grasses and many other plants (Nes and McKean 1977). Stigmasterol is a $\Delta^{5,22}$ sterol with an ethyl group at C24. Next to sitosterol, it is one of the more common phytosterols (Nes 1977; Nes and McKean 1977). Spinasterol ($\Delta^{7,22}$) and dihydrospinasterol (Δ^7) each have a methyl group at C24; they are the dominant sterols in the plant families Chenopodiaceae and Amaranthaceae (Salt et al. 1991).

This selection of sterols represents the dominant sterols found in plants and includes all possible permutations of saturation at C5, C7, and C22; these sterols also meet the minimum structural requirements for use by insects (Svoboda and Thompson 1985). All sterols were purchased from Sigma Chemical except for spinasterol and dihydrospinasterol. These two sterols, which we collectively refer to as the spinach sterols, were isolated from spinach, *Spinacia oleracea*, by using standard lipid extraction techniques and thin-layer chromatography; the amount collected was quantified by using high-pressure liquid chromatography (Heupel 1989). Cholesterol, lathosterol, and stigmasterol were approximately 99% pure. Sitosterol was derived from soybean and was a mixture of 60% sitosterol, 27% campesterol (Δ^5), and 13% dihydrobrassicasterol (Δ^5).

The Diets

For all of the following experiments, grasshoppers were reared on an artificial diet similar to the one used by Simpson et al. (1988). All diets contained a 28% protein (a 3 : 1 : 1 mixture of low-fat, vitamin-free casein; bacteriological peptone; and egg albumen), 28% digestible carbohydrate (a 1 : 1 mixture of sucrose and white dextrin), 39.7% cellulose, 2.4% Wesson's salts, 0.5% linoleic acid, 0.5% linolenic acid, 0.3% ascorbic acid, 0.1% ferulic acid, 0.2% phenylalanine, and 0.2% vitamin cocktail (Dadd 1961). For the first experiment, sterols were added to the diet at 0.1% dry weight, an amount that is sufficient for

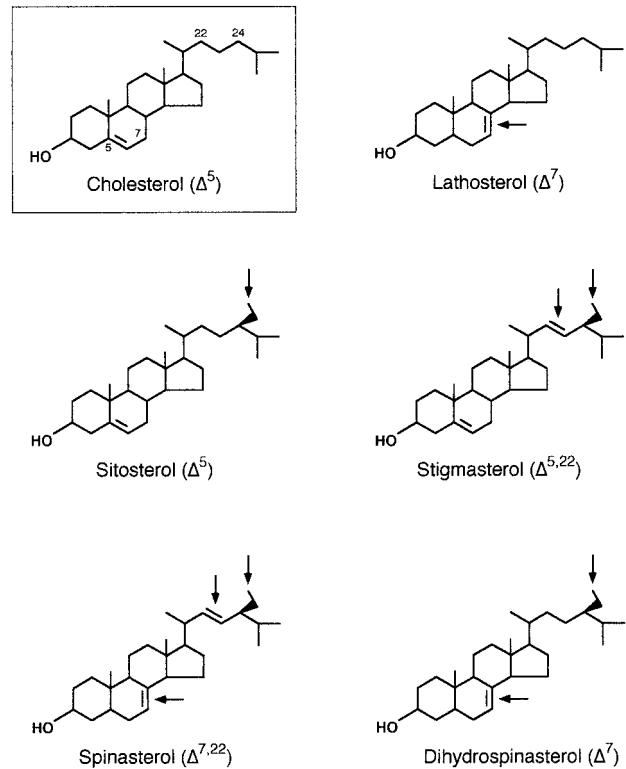


Figure 1. Sterol structures of interest. Cholesterol, with a double bond at position 5 (Δ^5), is the dominant tissue sterol found in insects. Lathosterol is a cholesterol analog with a Δ^7 configuration. The other four sterols are found in plants. The arrows indicate structural differences from cholesterol.

normal growth in other grasshopper species (Dadd 1960; Behmer 1998). To ensure complete development of *B. humphreysii* in the first experiment, a water-soluble factor extracted from romaine lettuce was added to the diet (Kreaky 1962). In the second experiment, different combinations of sitosterol (suitable) and stigmasterol (unsuitable) were added to the artificial diet. For both experiments, the artificial diet was suspended in a 1% agar solution in a 1 : 4 dry ingredients : water ratio and presented to grasshoppers as small cubes.

Experimental Protocol

The rearing procedures used in this study were as follows: on hatching, nymphs were weighed and transferred individually to 1-oz plastic cups (Solo Cup, Urbana, Ill.) that contained ventilation holes on both the sides and lids. In the first experiment, approximately 20 nymphs, taken from different egg pods, were started on each of the six different sterol treatments: cholesterol, sitosterol, lathosterol, stigmasterol, spinach sterols, and a control diet that was actually a trace sterol diet (the albumen [16.6 μg cholesterol/g] and casein [1.3 μg cholesterol/

g] were the source of the sterol contamination, but it was very minor at ~0.0018% dry weight). In the second experiment, up to 20 insects were started on each of the five different sterol treatments/mixtures (Table 1). For both experiments, grasshoppers were reared in a Percival incubator at L : D 35° : 28°C, without radiant heat, and with a photophase of 16L : 8D; they were given fresh diet cubes daily. They were monitored daily to record the duration of stadia, and the weight of the grasshopper was recorded immediately after each molt. When grasshoppers reached the fourth stadium, they were transferred to larger 6-oz plastic cups (Southwest Glassware, Tucson, Ariz.) with ventilation holes on both the sides and lids. A small strip of wire mesh, which provided a perch to better facilitate molting, was affixed to the underside of each lid. Experiments ran until all of the grasshoppers had died or molted to the adult stage (except for *M. differentialis*, which was ended prematurely because of time constraints). To ensure that grasshoppers had satisfactorily molted to the adult stage, they were monitored for one additional day after this final molt.

Statistical Analysis

In the first experiment, survival among the different treatments across stadia was analyzed separately for each species by using the nonparametric Lifereg Procedure SAS 6.12 (SAS Institute 1989); for the second experiment, survival across the different treatments was analyzed by using the same procedure. When significant differences among treatments were identified, a Tukey-type multiple comparison was used (Fox 1993). Performance on the different sterol treatments, including developmental time and growth rate within a stadium, was compared by using the nonparametric Kruskal-Wallis test. Comparisons were limited to the first two stadia in the first experiment because of low survivorship past the second stadium on some of the different sterol treatments. We used nonparametric tests throughout this study because sample sizes were often small and unequal. In addition, we did not have to be concerned with the underlying distributions of the data by using these analyses.

Results

Survival Analysis from the Comparative Study

Significant differences in survival among the six sterol treatments were observed for each species (Table 2), but among the different species there was a similar trend in survival on the different sterol treatments (Fig. 2). In all cases, survival was best on diets containing cholesterol or sitosterol, and only for *Melanoplus differentialis* was there a difference in survival among these two sterols; cholesterol was slightly superior to sitosterol ($Z = 2.667$, $P < 0.01$).

Survival on the other four sterol treatments was mixed among the different grasshopper species, but some patterns did emerge. For the species that were able to complete development to the adult stage, survival was always poorest on the trace sterol diet (Table 2; Fig. 2). For *Trimerotropis pallidipennis* and *Romalea gutatta*, survival was not significantly different among lathosterol, stigmasterol, or the spinach sterol diets; survival on each of these diets was, however, superior when compared with the trace sterol diet (Table 2; Fig. 2). For *Barytettix humphreysii*, survival on stigmasterol and the spinach sterols was superior to that on lathosterol; survival on all three of these diets was superior to that on the trace sterol diet (Table 2).

For the two species that failed to complete development to the adult stage, *Taenipoda eques* and *M. differentialis*, slightly different patterns emerge. Survival was equally poor for *T. eques* on the stigmasterol and trace sterol diet; it was intermediate on the lathosterol and spinach sterol diet compared with the other diets. For *M. differentialis*, survival was equally poor on the spinach sterol, stigmasterol, and trace sterol diet; it was intermediate on the lathosterol diet compared with the other sterol treatments.

Performance from the Comparative Study (the First Two Stadia Only)

Developmental time during the first stadium was significantly affected by sterols in four of the five species (Table 3). In most instances, development was fastest on the cholesterol and sitosterol diets; with the exception of *R. gutatta*, development on the lathosterol, stigmasterol, and spinach sterol diets was

Table 1: Sterol combinations for the second experiment

Treatment	Cholesterol	β -Sitosterol	Stigmasterol	Total
Sitosterol018	1.00	...	1.018
Stigmasterol018	...	1.00	1.018
Sit/Stig (1)018	1.00	.50	1.518
Sit/Stig (2)018	.75	.25	1.018
Sit/Stig (3)018	.50	.50	1.018

Note. All values are expressed as mg sterol/g diet. Trace levels of cholesterol were identified in both the albumen and peptone. The base components in the diet and experimental protocol are described in the "Material and Methods" section.

Table 2: Results from survival analysis using the Lifereg procedure

	Oedipodinae	Romaleinae		Melanoplinae	
	<i>Trimerotropis pallidipennis</i>	<i>Romalea gutatta</i>	<i>Taenipoda eques</i>	<i>Baryttix humphreysii</i>	<i>Melanoplus differentialis</i>
Lifereg procedure:					
df	5	5	5	5	5
χ^2	196.97	1023.75	108.49	209.91	94.87
P-value	$P < .001$	$P < .001$	$P < .001$	$P < .001$	$P < .001$
Multiple comparison:					
Cholesterol	A	A	A	A	A
Sitosterol	A	A	A	A	B
Lathosterol	B	B	B	C	C
Spinach sterols	B	B	BC	B	D
Stigmasterol	B	B	CD	B	D
Trace sterols	C	C	D	D	D

Note. Multiple comparisons were made among the different treatments using a Tukey-type test. Different letters indicate significant differences among the treatments ($\alpha = 0.05$).

not significantly different from the trace sterol diet. During the second stadium, developmental time was significantly longer on the lathosterol, stigmasterol, and spinach sterol treatments compared with the cholesterol and sitosterol diets (Table 3).

Significant differences in growth rate were only observed in the two romaleines during the first stadium (Table 4). For both *R. gutatta* and *T. eques*, growth rate was lowest on the spinach sterol and trace sterol diets compared with the cholesterol and sitosterol diets. During the second stadium, significant differences in growth rate were observed in *T. pallidipennis*, *T. eques*, and the two melanoplinae (Table 4); for *R. gutatta*, growth rate was only measured on the cholesterol and sitosterol diets because of high mortality on the other sterol treatments. For all species, growth rate was always highest on the sitosterol and cholesterol diets in the second stadium.

Survival Analysis from the Mixed Sterol Study

Significant differences in survival were observed when *Schistocerca americana* was reared on diets containing different mixtures of suitable and/or unsuitable sterols (Lifereg Procedure; $df = 4$, $\chi^2 = 87.63$, $P < 0.001$). Survival was equally best on treatments containing sitosterol or a mixture of sitosterol and stigmasterol at a concentration of 0.75 and 0.25 mg/g diet, respectively, and significantly better than on the other three treatments (Fig. 3). Among the latter three treatments, survival on the treatment containing sitosterol and stigmasterol at 1.0 and 0.5 mg/g diet, respectively, was significantly better compared with grasshoppers reared on the treatments containing equal concentrations of sitosterol and stigmasterol (each at 0.5 mg/g diet) or the stigmasterol-only diet. Finally, survival on the treatment containing sitosterol and stigmasterol in a 1 : 1 ratio

(each at 0.5 mg/g diet) was significantly better than on the stigmasterol-only treatment; on the treatment with sterols in a 1 : 1 ratio, however, only 10% of the grasshoppers completed development.

Performance from the Mixed Sterol Study

Developmental time was significantly affected by the combinations of sterols present in the food for all but the last two stadia (Table 5); large differences in developmental time across the first two stadia were not, however, recorded. During the third and fourth stadia, though, developmental time was significantly longer on diets containing equal concentrations of sitosterol and stigmasterol (0.5 mg/g diet each) compared with the other treatments. With regard to growth rate (mg wet mass/d) significant differences were observed across treatments for the first four stadia but not for the last two stadia (Table 5, Growth Rate). Typically it was much lower on the treatment containing sitosterol and stigmasterol in a 1 : 1 ratio. Surprisingly, growth rate on the sitosterol only diet was lowest across the first two stadia. By the third stadium, however, growth rate on this diet was similar to, or greater than, all the other treatments.

Discussion

Cholesterol and sitosterol were the only sterols on which grasshoppers were able to complete development. For the two species that never reached the adult stage, *Taenipoda eques* and *Melanoplus differentialis*, survival was significantly better on cholesterol and sitosterol than on lathosterol, stigmasterol, or the spinach sterols (for these two species, the failure to complete

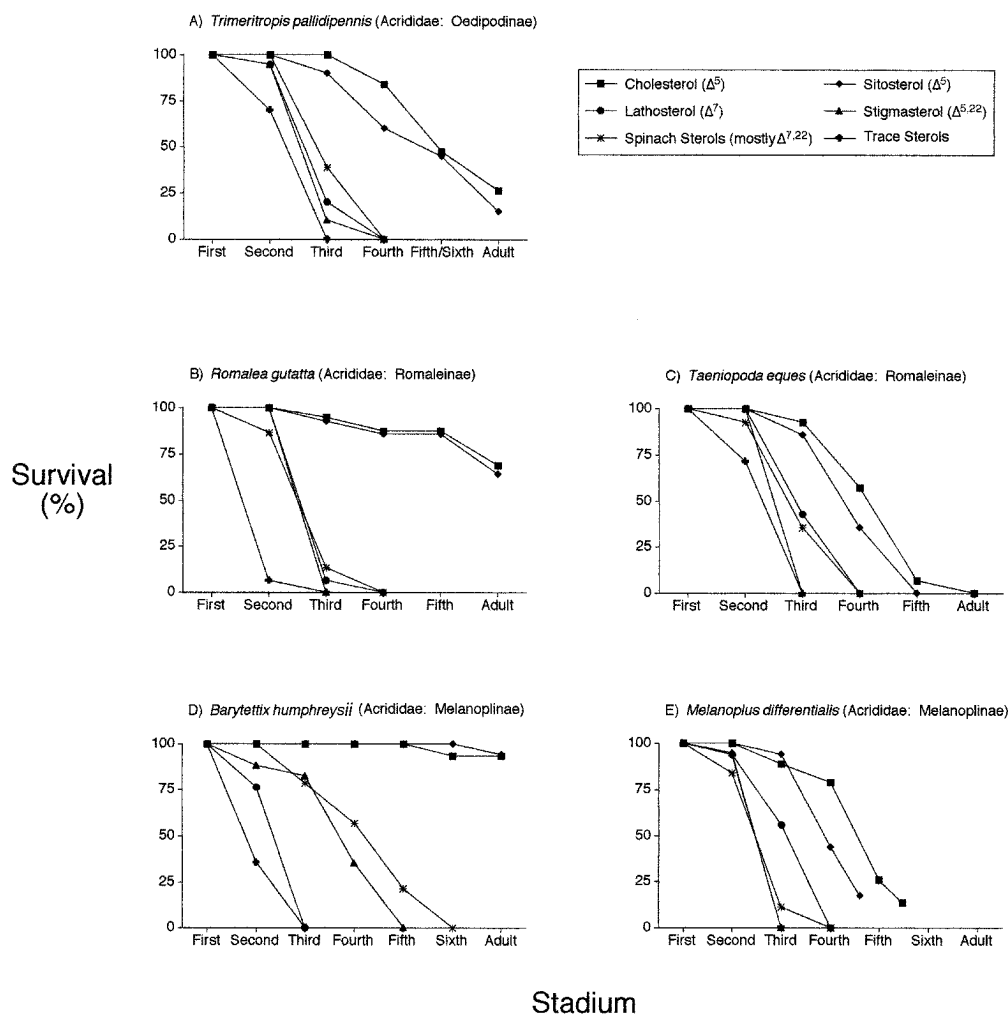


Figure 2. Survival curves for grasshoppers reared on artificial diets containing different dietary sterols. Points on the graphs indicate the percentage of the total individuals on a given treatment that were alive at the beginning of a particular stadium.

development to the adult stage likely reflects some missing micronutrient from the artificial diet rather than some missing sterol requirement, as both of these species will complete development to the adult stage in the laboratory when reared on plants that only contain sitosterol). The results from the current study, when combined with those previously obtained for the grasshoppers *Schistocerca gregaria* (subfamily Cyrtacanthacridinae), *Schistocerca americana* (subfamily Cyrtacanthacridinae), and *Locusta migratoria* (subfamily Oedipodinae; Dadd 1960; Behmer and Elias 1999a), strongly suggest that constraints on sterol metabolism are a shared characteristic within the Acrididae; sterols with a double bond at position 7, within the sterol ring structure, or at position 22, on the cholestane side chain, are unsuitable for normal growth and development. The Gomphocerinae, which are oligophagous on grasses, now stand alone as the only major subfamily within the Acrididae for

which sterol use has not been examined in at least one species. It seems unlikely that sterol use in this group would deviate from that observed for other acridids, as grasses tend to be sitosterol-rich (Nes and McKean 1977). The current study represents the most comprehensive study of sterol use in a single family of phytophagous insects, but it would be interesting to know if sterol metabolic constraints are common within other phylogenetically related groups of phytophagous insects.

Despite a specific sterol requirement to complete development to the adult stage, survival on diets with Δ^7 and/or Δ^{22} sterols was, in most instances, significantly better than on the trace sterol diet. This suggests two things: (1) Δ^7 and/or Δ^{22} sterols are not immediately toxic to grasshoppers, and (2) unmetabolized Δ^7 and/or Δ^{22} sterols might be used to partially fulfill the total sterol requirement in grasshoppers. A sparing mechanism, where these sterols are substituted for cholesterol

Table 3: Results from Kruskal-Wallis tests measuring days between molts for first and second stadium grasshoppers reared on diets containing different dietary sterols

	Oedipodinae	Romaleinae		Melanoplinae	
	<i>Trimerotropis pallidipennis</i>	<i>Romalea gutatta</i>	<i>Taenipoda eques</i>	<i>Barytettix humphreysii</i>	<i>Melanoplus differentialis</i>
First stadium:					
df	5	5	5	5	5
P value	$P < .001$	$P < .001$	$P < .001$	$P < .05$	ns
Cholesterol	8.0 (1.0) ^A	8.0 (0.0) ^A	7.0 (0.0) ^A	7.0 (1.0) ^A	6.0 (1.0) ^A
Sitosterol	10.0 (2.0) ^B	8.0 (1.0) ^A	7.0 (0.0) ^A	8.5 (1.5) ^{AB}	6.0 (1.0) ^A
Lathosterol	10.0 (1.0) ^B	9.5 (0.5) ^B	7.0 (1.0) ^A	9.0 (1.0) ^B	6.0 (0.5) ^A
Spinach sterols	10.0 (1.0) ^B	9.0 (1.0) ^B	9.0 (1.0) ^B	9.0 (1.0) ^B	6.0 (0.0) ^A
Stigmasterol	10.0 (1.0) ^B	9.0 (0.0) ^B	7.0 (0.0) ^A	9.0 (2.0) ^{AB}	6.0 (0.0) ^A
Trace sterols	10.0 (1.0) ^B	...	7.5 (0.5) ^{AB}	10.0 (1.0) ^B	6.0 (0.0) ^A
Second stadium: ^a					
df	4	1	3	3	3
P value	$P < .001$	ns	$P < .001$	$P < .001$	$P < .05$
Cholesterol	8.0 (0.0) ^A	9.5 (1.5) ^A	8.0 (1.0) ^A	7.0 (0.0) ^A	7.0 (1.0) ^A
Sitosterol	11.0 (2.0) ^B	9.0 (1.0) ^A	8.0 (1.0) ^A	8.0 (0.0) ^B	7.0 (0.0) ^A
Lathosterol	18.0 (2.5) ^C	...	12.5 (1.0) ^B	...	11.0 (1.5) ^B
Spinach sterols	19.0 (7.0) ^{BC}	...	14.0 (2.0) ^B	9.0 (1.0) ^B	13.0 (3.0) ^B
Stigmasterol	14.5 (1.5) ^{BC}	15.0 (3.0) ^C	...
Trace sterols

Note. Data are expressed as medians (\pm MAD). Different letters in columns indicate significant differences among the treatments (Tukey-type test with $\alpha = 0.05$). ns = not significantly different.

^a For *R. gutatta*, the statistical comparison among the two treatments was made using the Mann-Whitney *U*-test.

when it is not essential (i.e., cell membranes), could be a possibility (Clayton 1964). In the housefly, *Musca domestica* (L.), this substitution can be very dramatic; the essential cholesterol requirement was found to be only 0.5% of the total sterol requirement (Robbins 1963). If a sparing mechanism is operating in grasshoppers, however, it does not appear to be as efficient as that of the housefly. Cholesterol contamination from the albumen and peptone was, on a dry weight basis, equivalent to 4% of the minimal total sterol requirement; yet, in four of five species, mortality on diets with Δ^7 and/or Δ^{22} sterols exceeded or was near 50% by the start of the third stadium; for *Barytettix humphreysii*, mortality on these diets exceeded or was near 50% by the start of the fourth stadium. Recently, a more thorough investigation has revealed that sparing mechanisms only operate temporarily in grasshoppers (Behmer and Elias 1999a). It is interesting, however, that survival on the trace sterol diet was >75% during the first stadium in three of the five species. Cholesterol is one of the most common components of insect eggs (Hoffman and Lagueux 1985) and perhaps a proportion of the maternally allocated pool may have provided some of the essential cholesterol needed to fuel growth and to be the precursor in the production of the molting hormone 20-OH ecdysone.

The presence of Δ^7 and/or Δ^{22} sterols in the food also neg-

atively affected performance. These negative effects were fairly consistent among the different species and were often expressed quite early in development. For example, developmental time on these diets was typically extended compared with developmental time on the cholesterol and sitosterol diets. Interestingly, the differences were more pronounced in the second stadium than in the first. The presence of Δ^7 and/or Δ^{22} sterols in the food also influenced growth rate. When the negative effect was expressed, however, it was a reflection of species classification. In the first stadium, for example, significant differences in growth rate were only observed in the Romaleinae. The Romaleines are, compared to most other acridids, quite large, and the consequence of ingesting unsuitable sterols may be expressed much earlier in species from this subfamily, particularly if maternally inherited cholesterol reserves are quickly exhausted. By the second stadium, however, negative effects of Δ^7 and/or Δ^{22} sterols on growth rate were observed in species from all three subfamilies.

The negative effects of constraints on sterol metabolism were also observed in grasshoppers fed diets containing combinations of suitable and unsuitable sterols. For example, survival on treatments containing 0.5 mg stigmasterol/g diet, regardless of the sitosterol (suitable) concentration, was significantly reduced compared with the treatments containing only sitosterol;

Table 4: Results from Kruskal-Wallis tests measuring growth rate (mg wet mass/d) for first and second stadium grasshoppers reared on diets containing different dietary sterols

	Oedipodinae	Romaleinae		Melanoplinae	
	<i>Trimerotropis pallidipennis</i>	<i>Romalea gutatta</i>	<i>Taenipoda eques</i>	<i>Barytettix humphreysii</i>	<i>Melanoplus differentialis</i>
First stadium:					
df	5	5	5	5	5
P value	ns	$P < .001$	$P < .001$	ns	ns
Cholesterol54 (.17) ^A	5.80 (.84) ^A	5.62 (1.22) ^A	.74 (.14) ^A	.77 (.12) ^A
Sitosterol51 (.13) ^A	6.44 (.62) ^A	5.42 (.66) ^A	.46 (.19) ^A	.85 (.15) ^A
Lathosterol40 (.11) ^A	4.65 (.96) ^B	4.21 (1.12) ^B	.53 (.24) ^A	.80 (.07) ^A
Spinach sterols54 (.17) ^A	4.08 (.81) ^B	2.46 (.56) ^C	.38 (.17) ^A	.77 (.12) ^A
Stigmasterol43 (.08) ^A	4.27 (.99) ^B	5.44 (.88) ^A	.64 (.32) ^A	.78 (.11) ^A
Trace sterols41 (.10) ^A	...	3.40 (.42) ^B	.41 (.06) ^A	.72 (.16) ^A
Second stadium: ^a					
df	4	1	3	3	3
P-value	$P < .001$	ns	$P < .001$	$P < .001$	$P < .06$
Cholesterol	1.39 (.20) ^A	12.15 (2.12) ^A	11.02 (1.64) ^A	2.03 (.19) ^A	1.09 (.19) ^A
Sitosterol	1.13 (.21) ^A	12.64 (1.54) ^A	11.96 (1.46) ^A	1.77 (.23) ^A	1.04 (.13) ^A
Lathosterol71 (.09) ^B	...	4.70 (2.79) ^B86 (.28) ^A
Spinach sterols47 (.07) ^B	...	4.09 (.57) ^B	1.70 (.34) ^A	.41 (.09) ^A
Stigmasterol59 (.30) ^B	1.01 (.23) ^B	...
Trace sterols

Note. Data are expressed as medians (\pm MAD). Different letters in columns indicate significant differences among the treatments (Tukey-type test with $\alpha = 0.05$). ns = not significantly different.

^a For *R. gutatta*, the statistical comparison among the two treatments was made using the Mann-Whitney *U*-test.

performance on the treatment with sitosterol and stigmasterol in a 1 : 1 ratio (each at 0.5 mg/g diet) was also reduced compared with the sitosterol treatment. These results are particularly intriguing because a previous study had shown that sitosterol at 0.5 mg/g diet, which was the minimum amount present in both the treatments on which survival was low, is alone sufficient to permit normal growth and development in *S. americana* (Behmer and Elias 1999a). In contrast, survival and performance on treatments containing sitosterol and stigmasterol at 0.75 and 0.25 mg/g diet, respectively, were not reduced compared with the sitosterol-only diet. These results seem to suggest that grasshoppers can only tolerate a certain ratio of suitable to unsuitable sterols in their diets. We suggest that ratio, rather than the absolute concentration, of unsuitable sterol is important on the basis of the observed survival patterns. First, the percentage of individuals that completed development to the adult stage increased as the ratio of suitable to unsuitable sterols increased. Second, different final survival points were reached on the Sit/Stig (1) and Sit/Stig (3) treatments, even though the absolute concentration of stigmasterol (unsuitable) in these two treatments was identical. Uptake mechanisms of sterols in the gut would also point toward ratios being critical because absorption of sterols appears to be non-specific (Carey and Hernell 1992). If this is indeed the case,

the amounts of the different sterols that are eventually taken up should reflect the ratio at which they are ingested. Results do indicate, however, that variation exists among individuals as to what ratio of suitable to unsuitable sterols can be tolerated. On the treatment with sitosterol and stigmasterol at 1 and 0.5 mg/g diet, respectively, approximately half of the grasshoppers reached the adult stage; on the diet containing equal concentrations of sitosterol and stigmasterol (0.5 mg sterol/g diet each), though, only 10% of the grasshoppers completed development. This raises an interesting question of whether increased tolerance to unsuitable sterols might be selected for with continued exposure to diets with unfavorable ratios of suitable to unsuitable sterols.

The inability to metabolize Δ^7 and/or Δ^{22} sterols to cholesterol does not appear to prevent absorption across the midgut, as is suggested in a number of phytophagous insects (Ritter 1984; Svoboda and Thompson 1985; Behmer et al. 1999b), so at least two possible mechanisms may explain why survival and performance are reduced when grasshoppers are reared on diets containing these sterols. First, leaky membranes may occur if unmetabolized phytosterols are incorporated in a cell's phospholipid bilayer (Stein 1981). For example, if alkylated sterols are incorporated into new cells, the alkyl group on the cholesterol side chain can prevent phospholipids from packing

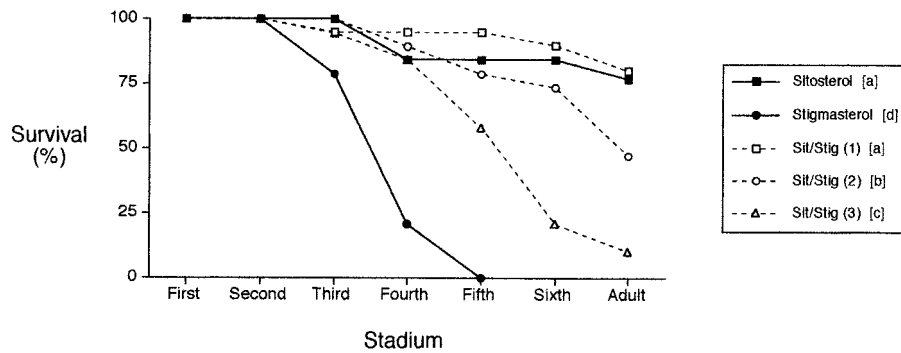


Figure 3. Survival curve for *Schistocerca americana* reared on artificial diets containing different combinations and amounts of suitable and/or unsuitable sterols. Points on the graph indicate the percent of the total individuals on a given treatment that were alive at the beginning of a particular stadium. Darkened symbols and solid lines indicate treatments with only one sterol, and open symbols and dashed lines indicate mixed sterol treatments. See Table 1 for treatment descriptions. Different letters adjacent to the treatment codes in the figure legend indicate significant differences in survival among the treatments ($\alpha = 0.05$).

tightly around sterol molecules. This could result in a decreased ability to regulate the movement of important ions and molecules across the cell membrane, which may lead to prolonged development, a decreased growth rate, or both. Second, the inability to metabolize sterols other than sitosterol to cholesterol might influence the production of 20-OH ecdysone, the insect-molting hormone. A low titer of cholesterol could reduce the amount of molting hormone produced or limit the rate at which it is produced. If this occurs, development may be extended. Perhaps this is the case with the corn earworm, *Helicoverpa zea* (Boddie), reared on a spinasterol diet. These earworms had tissue sterol concentrations of only 5% cholesterol (Ritter 1984) and always took longer to develop than those earworms reared on diets containing sterols (e.g., sitosterol) that are readily dealkylated to cholesterol (Ritter and Nes 1981).

Sterols have now been analyzed from hundreds of plants, and although there are bound to be exceptions, some prevailing patterns in sterol profile are emerging. First, there are two general variations that arise in phytosterol structure: (1) the presence of a double bond at position 5 and/or position 7, and (2) differences on the cholestane side chain, which can include the addition of a methyl or ethyl group at C24 and/or the presence or absence of a double bond at position 22. Second, plant species in a given family and especially a given genus often have very similar sterol profiles (Patterson 1994). For example, the Poaceae and Asteraceae have almost exclusively Δ^5 sterols, whereas the Curcubitaceae and Theaceae have almost exclusively Δ^7 sterols (Bergmann 1953). In the order Caryophyllales, seven of the 12 families possess major and sometimes dominant quantities of Δ^7 sterols; the other five families possess dominant quantities of Δ^5 sterols (Salt et al. 1991). Third, and last, most plants contain a mixture of different sterols. Where Δ^5 sterols are present, sitosterol (Δ^5), campesterol (Δ^5), and stigmasterol

($\Delta^{5,22}$) often occur together, although sitosterol is usually most abundant (Bergmann 1953; Nes and McKean 1977). Stigmasterol, which is unsuitable for grasshoppers, can sometimes be present to upward of 40% of the total sterol profile (Willuhn and Kostens 1974; Jewers et al. 1976). Where Δ^7 sterols are dominant, $\Delta^{7,22}$ sterols are also usually present (Nes and McKean 1977; Salt et al. 1991). It is possible, but still unproven, that dietary sterols are limiting nutrients for grasshoppers (Bernays 1992). For generalist grasshoppers such as those used in the current study, variation in plant sterol profile coupled with constraints on sterol metabolism make the likelihood of sterol shortage even greater.

Grasshoppers use a number of different mechanisms to evaluate the quality of their host plants, including contact chemoreceptors (Abisgold and Simpson 1988; Simpson et al. 1991), postingestive feedback (Lee and Bernays 1990; Simpson and Raubenheimer 1993), and associative learning (Lee and Bernays 1988; Simpson and White 1990; Champagne and Bernays 1991). Postingestive feedback and associative learning have recently been implicated as the mechanisms driving learned aversions to unsuitable sterols in the grasshopper *S. americana* (Behmer et al. 1999a). Although the effect that sterol metabolic constraints have on food-plant selection of grasshoppers in the field has yet to be critically examined, it is possible that plant sterol profile may influence the degree of diet mixing observed among different species. For example, *Trimerotropis pallidipennis* is polyphagous but feeds predominantly on grasses (Mulkern et al. 1969; Otte and Joern 1977). For this species, the degree that metabolic constraints impact food selection may be minimal because sitosterol, which is readily metabolized to cholesterol, is the dominant sterol found in grasses (Nes and McKean 1977). By contrast, sterol metabolic constraints may greatly impact food selection behavior in the romaleines and

Table 5: Results from Kruskal-Wallis tests measuring stadium duration (d) and growth rate (mg wet mass/d) for *Schistocerca americana* through the first six stadia

	Stadium					
	First	Second ^a	Third	Fourth	Fifth	Sixth
Developmental time (d):						
df	3	3	3	3	3	3
P value	$P < .001$	$P < .001$	$P < .001$	$P < .001$	ns	ns
Treatments:						
Sitosterol	8.0 ^B (1.0)	7.0 ^B (.5)	6.0 ^A (1.0)	7.0 ^A (.0)	9.0 ^A (.0)	12.5 ^A (.5)
Sit/Stig (1)	7.0 ^A (1.0)	6.0 ^A (.0)	6.0 ^A (1.0)	8.0 ^A (1.0)	9.5 ^A (1.0)	12.0 ^A (.5)
Sit/Stig (2)	7.0 ^A (1.0)	6.0 ^A (.0)	7.0 ^A (1.0)	8.0 ^A (1.0)	9.0 ^A (1.0)	12.0 ^A (1.0)
Sit/Stig (3)	7.0 ^A (.0)	7.0 ^B (1.0)	10.5 ^B (1.5)	11.0 ^B (2.0)	10.0 ^A (2.0)	17.5 ^A (.5)
Growth rate (mg wet mass/d):						
df	3	3	3	3	3
P value	$P < .05$	$P < .001$	$P < .001$	ns	ns
Treatments:						
Sitosterol	2.2 ^B (±.3)	9.7 ^A (±1.8)	13.8 ^A (±1.9)	27.2 ^A (±5.0)	29.1 ^A (±3.8)
Sit/Stig (1)	3.1 ^A (±.5)	9.1 ^A (±1.2)	14.0 ^A (±1.3)	22.8 ^A (±4.0)	30.5 ^A (±12.4)
Sit/Stig (2)	2.5 ^{AB} (±.4)	8.5 ^A (±1.1)	14.6 ^A (±1.5)	25.4 ^A (±3.4)	36.4 ^A (±7.1)
Sit/Stig (3)	2.7 ^{AB} (±.4)	4.6 ^B (±1.6)	5.5 ^B (±2.1)	23.8 ^A (±10.1)	20.8 ^A (±.7)

Note. Reared on diets with different concentrations of suitable and/or unsuitable sterols (data only shown for treatments on which grasshoppers completed development to the adult stage). Different letters indicate significant differences among the treatments (Tukey-type test with $\alpha = 0.05$). See Table 1 for treatment descriptions. ns = not significant.

^a Growth rate was calculated over the first two stadia.

melanoplines; grasshoppers in these two subfamilies tend to be extreme mixers.

Diet mixing in grasshoppers appears to be an effective strategy to obtain a suitable balance of nutrients that can maximize growth rates, but it may also be an effective strategy to reduce the intake of noxious chemicals including unsuitable sterols that, if allowed to accumulate, can be toxic (Behmer et al. 1999b). For grasshoppers, the degree of diet mixing in the field has multiple determinants, but results from this article, which show a widespread constraint on sterol metabolism and a limited ability to tolerate unsuitable sterols even when sufficient quantities of suitable sterols are present, lead us to suggest that plant sterol profiles may be a contributing factor that has been underestimated.

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