

tachinid had turned up in the laboratory. As the butterfly eggs were obtained in muslin sleeves and transferred thence to insect-proof cages prior to hatching, the infection must have resulted from fly eggs deposited on the food-plant outdoors and subsequently ingested when fed to the caged caterpillars. The tachinid larva emerges from the butterfly pupal case, after biting an escape hole, to form its puparium elsewhere. Thus, both pupal mortality and fly numbers are easily scored.

38 broods of larvae from single females were reared from April to August 1975 (table) and fed on 1 of 5 different food-plants. Mortality was very significantly higher on *Tylophora stenoloba*, which contains no cardiac glycosides³, than on *Calotropis gigantea* which is a rich source⁴ ($d = 11.4$; $p < 0.001$). 2 broods were split equally at hatching between *Tylophora* and *C. gigantea*, the main alternative food-plants of the local wild population: pupal mortality on the former was 100% ($n = 23$) and on the latter 54.2%

Proportions of *D. chrysippus* pupae parasitized by tachinid flies while feeding as larvae on various Asclepiadaceae

Food-plants	No. of pupae	No. parasitized	Percent parasitized
<i>Calotropis gigantea</i> ^{a,d}	191	46	24.1
<i>Calotropis procera</i> ^{b,e}	45	15	33.3
<i>Asclepias curassavica</i> ^{b,f}	87	81	93.1
<i>Gomphocarpus fruticosus</i> ^{b,g}	29	2	6.9
<i>Tylophora stenoloba</i> ^{c,d}	160	136	85.0
Totals	511	277	54.2

^a This strain contains cardenolides⁴. ^b This species contains cardenolides⁴ but strain not tested. ^c Cardenolides absent³. ^d Plants naturally established at Dar es Salaam. ^e Seeds collected from Same, Pare District, Tanzania. ^f Seeds collected from Marangu, Kilimanjaro District, Tanzania. ^g Seeds obtained from Auckland, New Zealand by Dr W.B. Rudman.

($n = 24$) ($X^2 = 11.3$; $p < 0.001$). The data for the 3 introduced toxic food-plants, which were grown intermingled in the same flowerbed, are less straightforward. On both *C. procera* and *Gomphocarpus*, which are rich in cardenolides⁴, mortality was low. The very high mortality on *Asclepias curassavica*, normally a toxic species⁴, might imply that this Kilimanjaro strain, which has not been tested, is poor in cardenolides. This interpretation is supported by the small size of the 6 surviving butterflies (\bar{x} for forewing length = 37.0 mm), scarcely larger than the *Tylophora* specimens ($\bar{x} = 35.4$ mm)⁵ and very significantly smaller ($t_{34} = 4.7$; $p < 0.001$) than others raised on the Munich strain of *A. curassavica* ($\bar{x} = 41.1$ mm)⁵ which is known to be toxic⁴.

The association between mortality and food-plant is due either to differences in the number of eggs deposited on the leaves by the flies or to the caterpillars being protected in varying degrees according to the quantity of cardenolide sequestered from the food-plant. Whichever cause is the true one, and they are not mutually exclusive, wild butterflies laying upon *C. gigantea* and other poisonous species, with the exception of *Asclepias* which grows very poorly in Dar es Salaam, must have a strong selective advantage over those using *Tylophora* during a tachinid outbreak. My results show that enhanced protection against parasites may be an important selective attribute to be won by danaiids which feed on toxic plants. This would be emphatically so if, as has been suggested², parasites are more important than predators in regulating the butterfly population.

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The effect of cardiac glycoside storage on growth rate and adult size in the butterfly *Danaus chrysippus* (L.)

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Summary. *Danaus chrysippus* grows faster and attains significantly greater size when fed as a larva on several species of Asclepiadaceae (milkweeds) containing cardiac glycosides than on one which does not.

Danaus chrysippus (L.) (Danaiidae) is an abundant African, Asian and Australasian tropical butterfly² which is aposematic, frequently distasteful³ and a model for numerous mimics, both Batesian and Müllerian, from at least 5 families of Lepidoptera^{3,4}. Most of its numerous food-plants belong to the Asclepiadaceae (milkweeds) and contain heart poisons (cardiac glycosides or cardenolides), substances which have toxic (cardioactive), noxious and emetic properties^{3,5}. Cardenolides are sequestered by the larvae and often stored in the pupae, adults and eggs^{3,6}, all of which may thereby acquire protection from predators which not only find them distasteful but also often experience emesis^{7,8}. I now report that larvae of *D. chrysippus* fed upon toxic species of milkweed produce an adult of significantly greater body size compared with those reared on a nontoxic asclepiad, *Tylophora stenoloba*. Similar results have been reported for *D. plexippus*⁹. Eggs were obtained from *D. chrysippus* at Dar es Salaam, Tanzania, by sleeving females outdoors on the appropriate

food-plant. Subsequently, the larvae were reared indoors on the same species collected either from the natural habitat (*Calotropis gigantea* and *T. stenoloba*) or from a cultivated plot (*Calotropis procera*, *Asclepias curassavica* and *Gomphocarpus fruticosus*).

My results (table), based on forewing length, which correlates well with body mass in both sexes, show that males are larger than females by 0.8 mm ($t_a = 6.6$; $p < 0.001$). Variance analyses indicate that the size differences between butterflies reared on the various plant species are highly significant for both males ($F = 34.8$, d.f. $3/428$; $p < 0.001$) and females ($F = 21.2$, d.f. $4/547$; $p < 0.001$). The only individually significant comparisons in t-tests (all giving $p < 0.001$) are for butterflies fed on each of the toxic species³ on the one hand and on *Tylophora*, which contains no cardenolides¹⁰, on the other. Comparing means of the combined toxic plant samples with the *Tylophora* sample, the size advantage to the former is 5.9 mm ($t_a = 15.5$; $p < 0.001$) for males

Wing lengths of *D. chrysippus* reared as larvae on various Asclepiadaceae between September 1974 and August 1975 at Dar es Salaam

Food-plants	Approx. cardenolide content as percent dry weight ³	Length of forewing (mm)			Females		
		Males n	\bar{x}	s^2	n	\bar{x}	s^2
<i>Calotropis gigantea</i> ^{a,c}	0.08	396	42.0	3.202	481	41.1	5.439
<i>Calotropis procera</i> ^{b,f}	0.03	16	41.4	1.333	13	41.2	2.833
<i>Asclepias curassavica</i> ^a	0.03	—	—	—	30 ^d	41.1	4.345
<i>Gomphocarpus fruticosus</i> ^{b,g}	0.07	11	41.2	2.400	16	40.6	1.600
<i>Tylophora stenoloba</i> ^{a,c}	nil	9	36.0	1.250	12	35.0	4.727
Totals	—	432	41.8	3.060	552	41.0	5.165

^a This strain contains cardenolides³. ^b This species contains cardenolides³ but strain not tested. ^c Cardenolides absent¹⁰. ^d Butterflies from Kenya stock reared in the Federal Republic of Germany on the Munich strain by Prof. D. Schneider and Dr M. Boppré. ^e Plants naturally established at Dar es Salaam. ^f Seeds collected from Same, Pare District, Tanzania. ^g Seeds obtained from Auckland, New Zealand, by Dr W. B. Rudman.

and 6.1 mm ($t_0 = 9.6$; $p < 0.001$) for females, corresponding to a body mass ratio, toxic:nontoxic, of approximately 1.6:1.

As the generation time on the various food-plants is identical, though temperature dependent, the growth rate of larvae on cardenolide plants is substantially superior. My results do not therefore support a suggestion¹¹ that sequestration of cardenolides by danaid larvae, and hence protection from predation, involves a physiological cost. Indeed, the reverse is the case. The wild population from which the experimental butterflies were taken uses as food-plants^{3,4,12} mainly *C. gigantea*, which is rich in cardenolides including the types known to be most physiologically active¹³, and *Tylophora* which is negative¹⁰. Therefore, distasteful and relatively edible butterflies fly together, the latter being effectively Batesian mimics of the former. As they are presumably less well-protected from predators, it may be to their advantage to be smaller and hence less readily detected.

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Satellite DNA (II) from sea urchin (*Lytechinus variegatus*) sperm¹

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Summary. When DNA isolated from freshly collected sperm of sea urchin (*Lytechinus variegatus*) is centrifuged to equilibrium in CsCl, 2 heavy satellite bands appear beside the main band DNA. Satellite DNA (II) appears in between the main band DNA ($\rho = 1.695$ g/cm³) and the rDNA satellite ($\rho = 1.722$ g/cm³). Satellite DNA (II) has a buoyant density 1.710 g/cm³, corresponding to 50% GC content. It is speculated that the satellite DNA (II), which appears to be of high mol.wt, might contain the sequences complementary to histone mRNA.

The main band DNA from the sperm of sea urchin (*Lytechinus variegatus*) has a density 1.695 g/cm³ in CsCl. Stafford and Guild² reported the presence of a heavy satellite DNA in this species. This satellite DNA ($\rho = 1.722$ g/cm³) was found to contain sequences complementary to 2 subunit ribosomal RNAs³. In this paper, the presence of a second satellite DNA, with a buoyant density 1.710 g/cm³ in CsCl, is reported.

Material and methods. Isolation of sperm DNA. Freshly collected sperm of *Lytechinus variegatus* was used for DNA isolation. 1 ml of sperm was suspended in 20 ml 0.05 M EDTA-1% sodium lauryl sulfate mixture. The suspension was shaken gently for a few minutes until the sperms appeared lysed. To the sperm lysate was added an equal volume of phenol-m-cresol-8-hydroxyquinoline⁴ and the sperm lysate was shaken for 1 h. The protein precipitate

was removed and 2 more phenol extractions were carried out. Remaining phenol was dialyzed out and DNA was treated with nuclease-free pronase. This was followed by 3 extractions with chloroform-isoamyl alcohol. DNA was then exhaustively dialyzed against 0.01 M sodium phosphate (equimolar)-0.001 M EDTA (pH 8.5) and stored at 4 °C in chloroform.

Isolation of second satellite DNA. Main band DNA (100-300 µg/ml) of high mol.wt was used for the isolation of second satellite DNA. 2 different methods were employed for this purpose. The first method involves the selective denaturation of main band DNA followed by separation of the native DNA from the denatured DNA in a mixture of polyethylene glycol-dextran^{3,5}. The phase components were prepared as described by Patterson and Stafford³. Sperm DNA at a concentration of 300 µg/ml in 0.01 M sodium