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) Males n	$\bar{x}$	s^2	Females n	$\bar{x}$	s^2
Calotropis gigantea[a,e]	0.08	396	42.0	3.202	481	41.1	5.439
Calotropis procera[b,f]	0.03	16	41.4	1.333	13	41.2	2.833
Asclepias curassavica[a]	0.03	-	-	-	30[d]	41.1	4.345
Gomphocarpus fruticosus[b,g]	0.07	11	41.2	2.400	16	40.6	1.600
Tylophora stenoloba[c,e]	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 cardenolides[3]. [b] This species contains cardenolides[3] but strain not tested. [c] Cardenolides absent[10]. [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 ($t_a = 9.6$; $p < 0.001$) 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 suggestion[11] 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 active[13], and *Tylophora* which is negative[10]. 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*) sperm[1]

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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/cm^3) and the rDNA satellite ($\rho = 1.722$ g/cm^3). Satellite DNA (II) has a buoyant density 1.710 g/cm^3, 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/cm^3 in CsCl. Stafford and Guild[2] reported the presence of a heavy satellite DNA in this species. This satellite DNA ($\rho = 1.722$ g/cm^3) was found to contain sequences complementary to 2 subunit ribosomal RNAs[3]. In this paper, the presence of a second satellite DNA, with a buoyant density 1.710 g/cm^3 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-hydroxyquinoline[4] 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 Stafford[3]. Sperm DNA at a concentration of 300 µg/ml in 0.01 M sodium

phosphate (equimolar), pH 6.8, was heated to 79 °±0.2 °C in a flat-bottom flask in a temperature-controlled water bath for 10 min. It was then transferred to an ice-chilled flask embedded in an ice bucket and an appropriate amount of cold polyethylene glycol-dextran was added. After a thorough mixing, 2 phases were separated by a low-speed centrifugation. The top phase (polyethylene glycol) containing mostly the native DNA was pipetted off. The top phase then made up to 23% (w/v) by adding potassium phosphate (equimolar). This brought about separation of polyethylene glycol from DNA. Polyethylene glycol was pipetted off and DNA was dialyzed against 0.01 M sodium phosphate (equimolar), pH 6.8. This DNA was centrifuged in SW 55 rotor (Spino Model L preparative centrifuge) to 55,000 rpm for 20 h at 4 °C. The DNA pellet formed at the bottom of the tube was dissolved in 0.01 M sodium phosphate (equimolar), pH 6.8. To a small amount of this DNA was added solid CsCl to make the initial density of the solution 1.700 g/cm^3. This sample was centrifuged to equilibrium in a Spinco Model E analytical ultracentrifuge at 25 °C. The UV-pictures were taken at 265 nm. The negative was traced by a Joyce-Loebld microdensitometer. Since polyethylene glycol-dextran 2-phase system brings about some impurities[6], the second heavy satellite DNA was isolated by 2 cycles of CsCl-centrifugation of the heavy fraction of sperm DNA. To sperm DNA (100–150 µg/ml) in 0.01 M sodium phosphate-0.001 M EDTA (pH 7.5), solid CsCl was added (1 ml DNA/1.25 g solid CsCl) and 10 ml of this DNA was transferred to polyallomer tubes, and after overlayering them with paraffin oil, they were centrifuged for 4 days at 35,000 rpm, 20 °C. Heavy fractions of the gradient were collected by puncturing a hole in the bottom of the tubes. The pooled fraction was dialyzed against distilled water for 1 h and then centrifuged at 55,000 rpm for 12½ h at 20 °C. The pellets of DNA thus formed were dissolved gently in 0.1 M Tris-0.01 M EDTA (pH 8.5). This DNA was further centrifuged to equilibrium in CsCl and 0.1 ml fractions were collected. By carefully cutting off the lighter side of the gradient, the fractions were pooled for 3rd CsCl-centrifugation which completely separated the second satellite DNA from rDNA satellite.

Results and discussion. The appearance of 2nd satellite DNA (p = 1.710 g/cc) by polyethylene glycol-dextran 2-phase system is shown in figure 1. This DNA was not purified further by CsCl-centrifugation because this method of the isolation of heavy satellite DNA was not considered to be satisfactory[6]. Moreover, the appearance of second satellite DNA band by this method may be a sequel to partial denaturation of some main band DNA, since the method calls for heating of DNA up to 79 °C. But this possibility can be ruled out if one finds the appearance of this satellite DNA (II) using other methods of satellite DNA isolation. When the heavy fractions of sperm DNA is subjected to 2nd CsCl-centrifugation (figure 2), the satellite DNA (II) remains somewhat masked by some main band DNA and the rDNA satellite. When the fraction No. 22 of the 2nd CsCl-gradient was centrifuged in Model E analyti-

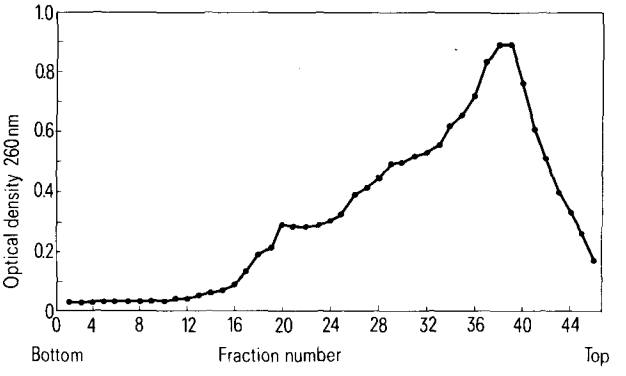

Fig. 2. 2nd preparative CsCl-density gradient profile of the heavy fractions collected after the first preparative CsCl-centrifugation. A 10-ml gradient was run in a polyallomer tube overlayered with paraffin oil. The tubes were centrifuged for 96 h in a 60 Ti rotor at 25 °C. The OD of the fractions was measured at 260 nm in a Gilford 2000 spectrophotometer.

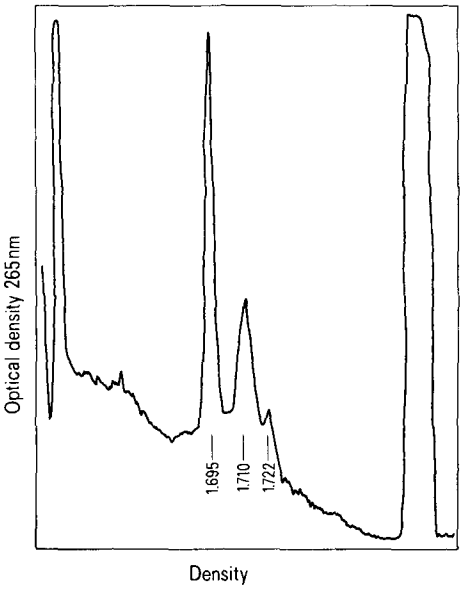

Fig. 1. Microdensitometer tracing of a UV-photograph. DNA from *Lytechinus variegatus* sperm was selectively denatured at 79 °C and the native DNA recovered into the polyethylene glycol-rich top phase, as described in material and methods', was centrifuged at 44,700 rpm, 25 °C, for 24 h in CsCl in a Spinco Model E analytical centrifuge. In between the main band DNA (density 1.695 g/cc) and the rDNA satellite (density 1.722 g/cm^3) is the 2nd satellite DNA (density 1.710 g/cm^3).

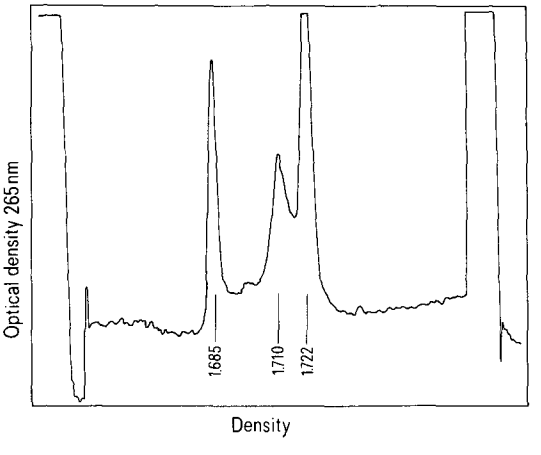

Fig. 3. Microdensitometer tracing of analytical centrifugation of fraction number 22 of figure 2. This sample was centrifuged to equilibrium in CsCl. The banding pattern shows the relative amount of the 2 satellite DNAs, contained in this fraction. In between the marker *Tetrahymena* DNA (density 1.685 g/cm^3) and the rDNA satellite (density 1.722 g/cm^3) is the 2nd satellite DNA (density 1.710 g/cm^3).

cal ultracentrifuge, it contained the 2nd satellite DNA besides the rDNA satellite (figure 3). 3rd CsCl-centrifugation completely separated the second satellite DNA from rDNA satellite[7]. The appearance of satellite DNA (II) by this method establishes its existence. This satellite DNA has a buoyant density 1.710 g/cm^3 in CsCl, corresponding to 50% GC content.

It is difficult to explain why this DNA did not appear in the earlier experiments of Patterson and Stafford[3]. It may, however, be pointed out that these authors isolated DNA from frozen sperms. It is not unlikely that during thawing of frozen sperms, some nuclease may become active and degrade the DNA. The present author has experienced considerable difficulty in isolating a high mol.wt DNA from frozen and preserved sperms.

It would require a large scale purification of the 2nd satellite DNA before its function can be determined. Nevertheless, 2 important characteristic features of the 2nd satellite DNA justify some comments upon its possible function. 1. By looking at the area under the peak (figure 1), it may be suggested that the amount of this DNA may come up to 0.8% of the total amount of DNA of the sea urchin sperm. This amount of DNA is required to encode the histone genes and their associated spacer DNA (Birnstiel et al., 1974; cited by Elgin and Wintraub[8]). 2. The buoyant density of the 2nd satellite DNA suggests that its GC-content is 50%. The GC-content of histone mRNA vary from 51 to 58% (Grunstein et al., 1973; cited by Elgin and Wintraub[8]). Histone genes are repetitious and it is held that they contain AT-rich regions designated as spacer DNA. Assuming that the region of histone DNA, which corresponds to translatable histone mRNA, is conservative, the variation in the AT-rich spacer DNA (Birnstiel et al., 1974; Farquhar and McCarthy, 1973; cited by Elgin and Wintraub[8]) could account for some differences in the overall GC composition of this gene present in different genera of sea urchin.

The data presented in this paper simply point to the existence of a second heavy satellite DNA in *Lytechinus variegatus*. Further characterization of this DNA is required to establish its function.

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Influence of seed size and composition on the dry matter yield of *Cenchrus ciliaris*

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Summary. Higher food reserve in larger seeds of *Cenchrus ciliaris* contributed towards greater forage production, as compared to that of the small seeds, only during the 1st year of establishment.

Large and heavier seeds of a given genotype, having a higher protein content[2,3], has been found to have a higher germination percentage as compared to smaller and lighter seeds[4], and plants obtained from the larger seeds have been reported to have greater seedling vigour[3,5-7] and growth and yield potentialities[8,9] as compared to plants obtained from the small seeds with a lower protein reserve. In *Cenchrus ciliaris*, a prominent fodder grass of the Indian arid zone, seed dimorphism has been found to be associated with significant differences in the growth of the seedlings obtained from them[4]. This report relates the differences in chemical composition of these 2 types of seeds of *C. ciliaris* (CAZRI 358) and describes the results of field performance of plants obtained from them.

Table 1 indicates that the reducing sugar and soluble carbohydrates were relatively more in the small seeds as compared to the large seeds. But the level of starch and extractable protein in the large seeds were very much more than those found in the small seeds. The nitrogen and phosphorus contents of the 2 types of seeds also showed a sharp contrast, but the potassium concentration was slightly more in the small seeds and the difference in the absolute quantities (µg/seed) between the large and small seeds was also small. However, the over-all difference in the nutritional reserve in the 2 types of seeds obviously contributed towards the disparity of early seedling vigour noted earlier[4]. In a perennial grass, like *C. ciliaris*, it was necessary to examine whether this difference in seedling vigour has any relevance with the forage production under field conditions. It is possible that commencement of photosynthesis and equal availability of nutrients may level off the initial differences in growth, as has been observed in relation to

Table 1. Compositional differences in small and large seeds of *C. ciliaris*

Seed size	1000 seed weight (mg)	Reducing sugar (µg/seed)	Soluble carbohydrates (µg/seed)	Starch (µg/seed)	Extractable protein (µg/seed)	Nitrogen (µg/seed)	Phosphorus (µg/seed)	Potassium (µg/seed)
Small	303.00	3.30 (1.09)*	26.00 (8.68)	150.00 (49.60)	1.10 (0.38)	3.99 (1.32)	1.40 (0.47)	0.26 (0.089)
Large	676.00	2.00 (0.30)	22.00 (3.37)	399.00 (58.70)	4.80 (0.71)	20.76 (3.07)	3.90 (0.58)	0.51 (0.075)

* Concentration in mg/100 mg dry seed.