Shell Use: An Adaptation for Emigration from the Sea by the Coconut Crab

Abstract. *The coconut crab* Birgus latro *(L.) emigrates from the sea during the postlarval glaucothoe stage. Glaucothoes show ancestral hermit crab behavior of living in empty gastropod shells which protect them during this vulnerable time.*

The use of empty gastropod shells is a behavioral adaptation related to the successful emigration of the coconut crab *Birgus latro* (L.) from the marine to the terrestrial environment. The unique and characteristic behavior patterns of hermit crabs of entering and living in empty gastropod shells are shown by the glaucothoes and persist in young crabs, but are lost in the larger coconut crabs. Since the coconut crab is related to and has evolved from the shell-inhabiting hermit crabs, behavior typical of the hermit crabs which is shown by the glaucothoes can be considered as a retention of ancestral behavior. The behavior is adaptive and presumably has been retained in the glaucothoes and young crabs because it protects them from desiccation and probably other exigencies during this vulnerable stage in their life history.

The complete larval development of *Birgus* including details of the glaucothoe was described recently (*1*). This glaucothoe is similar in appearance to that of other hermit crabs (*2*). Their behavior is typical of glaucothoes of other species of hermit crabs (*3*), except that the glaucothoe of *Birgus* is amphibious and eventually crawls out onto the land, a behavior pattern shared only with the other species of terrestrial hermit crabs.

The glaucothoes may not immediately enter shells, and under laboratory conditions some only explored and sat on small pebbles and shells. However, most of the glaucothoes settled to the bottom of the rearing chambers, and if a suitable substratum was supplied they crawled about exploring small shells, which they eventually entered, and pebbles. Even if a rugose substratum was not supplied, they tended to swim relatively little but lay on the floor of their chambers usually on their backs. With age the tendency to swim decreased. The behavior shown toward shells was similar to that used by other hermit crabs (*4*). These observations and my data on shell utilization by the glaucothoes of *Birgus* confirmed previous observations (*5*), but only in the present work was the parentage definitely known because the glaucothoes were reared from eggs in the laboratory. Like Harms (*5*) at Christmas Island in the Indian Ocean, I have observed glaucothoes in shells among the damp litter on the high beach at Eniwetok Atoll in the Marshall Islands, but until the glaucothoe of *Birgus* had been reared and described it was not possible to distinguish with certainty these glaucothoes from those of the three species of *Coenobita*, the other terrestrial hermit crabs, which also were abundant on the island.

The behavior of the glaucothoes was studied by introducing them into experimental chambers with various combinations of environmental factors (Table 1). The glaucothoes entered shells both in water and on land and emigrated to the various simulated "land" environments regardless of whether or not they had entered a shell. Usually emigration occurred within a few hours of being exposed to the experimental conditions. None however survived on land unless they had entered shells. It appeared that the deaths of the glaucothoes on land were due to desiccation. It is not known why some animals failed to enter shells. Presumably shells also would provide some protection from predation both on land and in water, as well as protection from damage due to waves, sand, and small stones during the emigration across the intertidal region which at Eniwetok consists of rocky areas interspersed with sand beaches. Protection against desiccation is probably the most important factor.

If shells and land were not present, the glaucothoes simply lived in the water, spending most of the time sitting or walking about on the bottoms of their chambers. Eventually they either died or were used in other experiments, but they did not metamorphose to the first crab stage even after as long as 30 days. More work however is needed to determine whether submergence results in delayed metamorphosis. On land, glaucothoes metamorphosed after 21 to 28 days (*1*). Although more data are necessary, it appears that salinity stress did not act as a trigger to either taking shells or emigration. However, it seems that the richer or more complex the simulated terrestrial environmental conditions the more successful was the emigration behavior of the glaucothoes. Again more work is necessary because the test chambers differed in a number of ways not related to the experimental conditions. For example, the water volume, land surface area, slope of the simulated beach, and the chamber size were not identical in each condition. Nevertheless, the results demonstrate that glaucothoes enter shells both in water and on land, that they crawl out of the water with or without shells under a variety of simulated intertidal conditions, and that the shells protect the glaucothoes from desiccation and probably other factors too.

Except for a few cases in the development of locomotion, feeding, and call notes in different groups of birds (6), examples of the retention of ancestral behavior are not well known. In

Table 1. Emigration behavior and survival of the glaucothoes of *Birgus latro* under experimental conditions. Numbers are of glaucothoes (*7*). S, shells present; L, land present. In the first condition, no glaucothoes had metamorphosed by 20 to 30 days.

Conditions	Sample	Emigrated				Remained in water			
		In shell		No shell		In shell		No shell	
		Lived	Died	Lived	Died	Lived	Died	Lived	Died
No S or L	51								
S, no L	69					37	0	7	25
S, L (plastic)	36	0	0	9*	2	0	0	11	14
S, L (sand)	25	2	0	3†	8	1	0	0	11
S, L (sand, coconut husk)	13	0	0	1†	5	0	0	0	7
S, L (sand, soil, plants, coconut husk and meat, coral pebbles)	52	31	0	4†	3	2	0	0	12
		Salinity stress (percentage of seawater)							
S, no L, 60–75	7					4	0	0	3
S, L (sand), 60–75	11	1	0	1†	1	0	0	0	8
S, L (sand), 150	6	0	0	1†	0	0	0	0	5

* Glaucothoes returned to water, survived to end of experiment. † Glaucothoes entered shells on land, survived to end of experiment.

this regard, the patterns of behavior characteristic of hermit crabs which the glaucothoes and young crabs of *Birgus latro* show toward empty gastropod shells are of great interest and represent an excellent example of the retention of ancestral behavior during development.

ERNST S. REESE
Department of Zoology and *Hawaii Institute of Marine Biology, University of Hawaii, Honolulu*

References and Notes

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2. A. J. Provenzano, Jr., *Crustaceana* **4**, 207 (1962); *Pac. Sci.* **21**, 457 (1967).
3. E. S. Reese, *Anim. Behav.* **10**, 347 (1962); B. A. Hazlett and A. J. Provenzano, Jr., *Bull. Mar. Sci.* **15**, 616 (1965).
4. E. S. Reese, *Behaviour* **21**, 78 (1963).
5. J. W. Harms, *Z. Naturwiss. Jena* **71**, 1 (1937).
6. R. J. Andrew, in *A New Dictionary of Birds*, A. L. Thomson, Ed. (Nelson, London, 1964), pp. 86–90.
7. To keep the experimental age of the glaucothoes as constant as possible, it was necessary to work with them as they appeared, usually within 24 to 48 hours after the terminal molt. They were tested with whatever shell sets and experimental chambers were available at that time. The opportunistic nature of the program is reflected in the different sample sizes. The duration of the experiments was variable, usually about a week, but occasionally a few days to 2 weeks. The responses shown in Table 1 represent the situations at the conclusion of the experiments at which time none of the glaucothoes had metamorphosed to the first crab stage.
8. I thank Drs. D. F. Dorward and J. E. Nelson, both of Monash University, Australia, for commenting on this paper. Supported in part by NSF grants GB-1003 and GB-3651 and by the Eniwetok Marine Biological Laboratory. Contribution No. 297 from the Hawaii Institute of Marine Biology, University of Hawaii, Honolulu 96822.

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Density Gradient Centrifugation: Fixation of Bands by Photopolymerization of Acrylamide

Abstract. *A method for immobilization of proteins in a density gradient is described. This method eliminates the collection of drops or the use of a schlieren optical system, but visualizes the results of the Amido Schwarz stain. Although only sucrose gradients have been examined, the method possibly may be extended to other gradients.*

The widespread use of density gradient centrifugation has led us to develop a technique for immobilization of the separated materials *in situ*. The gel which results from the polymerization of the tube contents may then be treated in the same manner that gels of disc electrophoresis are treated. Banding, immobilization, and localization of some proteins are reported here.

An all-glass gradient-maker was used in pouring the gradients (*1*). The solutions used for preparation of the light and heavy components of the gradient are made by mixing aliquots of stock solutions B, D, and E, and water and sucrose. Stock solution B is 11.4 g tris buffer, 1.2 ml *N,N,N′,N′*-tetramethylethylenediamine (TEMED, practical grade, Matheson Coleman and Bell 8563), and 98 ml of distilled water; the *p*H is adjusted to 6.9 with 85 percent phosphoric acid. Solution D is 24 g of acrylamide, 5 g *N,N′*-methylenebisacrylamide (Bis, Eastman 8383), and distilled water to give final volume of 100 ml. Solution E is 8 mg of riboflavin in 100 ml of distilled water (*2*). Solutions B and D as well as the sucrose solutions are stored in the refrigerator; E is prepared fresh daily. The polymerizable solution consists of 1 part B, 2 parts D, 1 part E, and 4 parts water. The sucrose solutions are prepared in double strength; for example, 10 and 50 percent sucrose solutions are prepared for a final 5 to 25 percent sucrose gradient. Each double-strength sucrose solution is diluted with an equal volume of polymerizable solution. Three milliliters of each of these two resulting solutions are used for pouring the gradient into 5 by 1.5-cm cellulose nitrate tubes.

The sample in a volume of 0.05 ml is layered onto the surface of the poured gradient mixture with a microliter pipette. In this work a 50SW swinging bucket rotor was used in a Spinco model L preparative ultracentrifuge. The tubes were spun at 50,000 rev/min for the appropriate time.

When the run is completed, the tubes are removed from the buckets and overlayered with about 2 mm of water with a fine capillary pipette. The overlayering helps produce a sharp edge at the top of the tube contents after polymerization is completed. The tubes are photopolymerized by a fluorescent light in about 30 minutes. The gels are removed from the tube by shaking or by puncturing the bottom of the tube, whereby they slide out easily.

For total protein, the gels are stained in a 1 percent solution of Amido Schwartz dye (Naphthol Blue Black C, 1; No. 20470, Allied Chemical Corp.) in 7 percent acetic acid. This procedure stains the proteins and binds them to the gel. Excess dye may be removed by washing repeatedly in 15 percent acetic acid or by electrophoretic destaining in 15 percent acetic acid. The destained gels may be viewed and stored in 15 percent acetic acid. Figure 1 shows the results of a typical run. The conditions are noted in the legend.

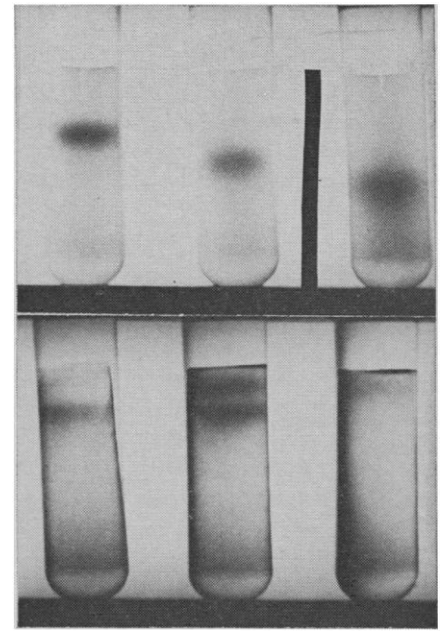

Fig. 1. (Top) Left to right, 40 μg of bovine serum albumin centrifuged for 19, 24, 30 hours in a 5 to 25 percent sucrose gradient at 50,000 rev/min. (Bottom) Left to right, 40 μg of lysozyme, 40 μg of lysozyme and 40 μg of alcohol dehydrogenase, and 40 μg of alcohol dehydrogenase centrifuged for 6 hours in a 5 to 10 percent sucrose gradient at 50,000 rev/min. The black bar indicates a length of 46 mm.

The results of density gradient centrifugation are usually analyzed by schlieren optics or by fractionation of the run by drop collecting. For work involving only localization of the sample, this immobilization technique may be advantageous. The technique described here may be of use in enzyme studies; the techniques for visualization of many enzymes by specific reagents on polyacrylamide gels are available (*3*).

THOMAS A. COLE
THOMAS W. BROOKS, JR.
Department of Biology, Wabash College, Crawfordsville, Indiana 47933

References and Notes

1. The gradient-maker was a gift from Dr. George D. Guthrie (Indiana University, Bloomington).
2. These solutions are double-strength preparations for those used in disc electrophoresis: J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
3. Disc Electrophoresis Information Center, 5635 Fisher Lane, Rockville, Md. 20852.
4. Supported by PHS research grant GM11860. We thank Dr. A. E. Brooks for his help during this work.
5. After this manuscript was accepted for publication, a similar method was brought to our attention [W. B. Jolley, H. W. Allen, O. M. Griffith, *Anal. Biochem.* **21**, 454 (1967)].

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