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32. We thank B. M. R. Harvey for helpful advice and the European Commission for a Marie Curie–Research Training Grant (MCFI-2002-00022) awarded to F.K.

Supporting Online Material
www.sciencemag.org/cgi/content/full/301/5630/206/DC1
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28 March 2003; accepted 17 June 2003

Switching Skeletons: Hydrostatic Support in Molting Crabs

Jennifer R. A. Taylor and William M. Kier

Skeletal support systems are essential for support, movement, muscular antagonism, and locomotion. Crustaceans shed their rigid exoskeleton at each molt yet are still capable of forceful movement. We hypothesize that the soft water-inflated body of newly molted crabs may rely on a hydrostatic skeleton, similar to that of worms and polyps. We measured internal hydrostatic pressure and the force exerted during claw adduction and observed a strong correlation between force and hydrostatic pressure, consistent with hydrostatic skeletal support. This alternation between the two basic skeletal types may be widespread among arthropods.

Animals typically have one of two general categories of skeletal support throughout their lives: rigid skeletons (vertebrates, echinoderms, arthropods) or soft hydrostatic skeletons (polyps and vermiform animals) (*1–4*). Those with rigid skeletons are arranged so that muscles attach to and pull on stiff skeletal elements. The forces of muscle contraction are therefore transmitted by the rigid elements. In contrast, animals with hydrostatic systems are arranged so that the force of muscle contraction is transmitted by an incompressible fluid (*5–8*). The fluid typically fills an internal cavity and is surrounded by a flexible container, usually the body wall, which is equipped with muscles and reinforced with connective tissue fibers (*2*, *9*, *10*). Muscle contraction increases the pressure in the fluid, causing the deformations or stiffening required for movement and muscular antagonism.

During molting, crustaceans become soft and inflated with water, thus resembling an animal with a hydrostatic skeleton. Molting involves secretion of a new cuticle beneath the old one, shedding of the old skeleton, and expansion to a larger size. The stomach expands (because of ingestion of water), which causes an increase in hydrostatic pressure (*11*, *12*) that cracks the carapace, which, in turn, facilitates withdrawal (ecdysis). Through a series of coordinated movements, the crustacean extricates itself from the old skeleton (exuviation) (*13*, *14*). They remain in this soft condition for several days before the new cuticle hardens (*11*, *15*, *16*). During this time, the new cuticle is too soft and flexible to resist the compressive forces of muscle contraction. This is often considered to be a period of inactivity (*17*), but crustaceans are in fact capable of vigorous, rapid, and forceful movement and locomotion immediately after the molt (*13*, *14*, *18–20*).

We performed a preliminary experiment to determine whether internal hydrostatic pressure is necessary to maintain shape and support. First, we cut an opening in the cuticle of a newly molted blue crab, *Callinectes sapidus*, to drain the fluid within. We predicted that once the internal fluid was drained, the hydrostatic pressure would be relieved, and the animal would lose mobility. Because of a rapid wound repair mechanism, however, the loss of fluid was minimal. A cheliped of a newly molted crab was then removed at the coxa-basis joint, and the internal fluid was allowed to drain. The cheliped collapsed once the fluid had been drained, indicating that without hydrostatic pressure, the cuticle of a newly molted crab cannot maintain shape or support.

For a more definitive test of the hypothesis, we simultaneously measured the internal hydrostatic pressure and force of muscle contraction in newly molted blue crabs (*21*). Crabs were restrained in an aquarium with the cheliped extended laterally. A force transducer was connected to the carpus of the extended cheliped and a pressure transducer catheter was inserted into a hemocoelic space immediately beneath the arthrodial membrane of the merus, near the merus-carpus joint of the cheliped. Simultaneous recordings of pressure and force were made as the animal attempted to adduct the cheliped. Recordings from a "soft-shell" crab 1 hour after exuviation revealed a strong correlation between the force exerted by the carpus and internal pressure (Fig. 1A). These data are consistent with the use of hydrostatic skeletal support. In order to generate a moment about the merus-carpus joint, the compressional force on the merus from muscle contraction (which would otherwise shorten this segment) must be resisted. Because the internal fluid is incompressible, these forces can be

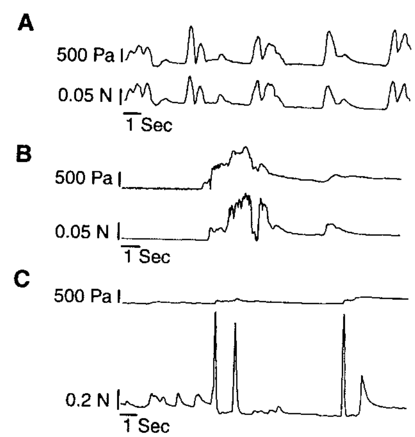

Fig. 1. Pressure and force recordings from post-molt crabs. Upper trace is pressure, lower trace is force. (**A**) Recording from a soft-shell crab 1 hour after exuviation. Peaks of increased pressure correlate with peaks of force. (**B**) Recording from a paper-shell crab 12 hours after exuviation. Again, pressure correlates with force. (**C**) Recording from a hard-shell crab 7 days after exuviation. Large forces are present but there are no corresponding increases in pressure. Note that the scale for force in the hard-shell crab is different. All traces represent about 19.5 s of recording.

Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA.

resisted if the new cuticle resists tension and thereby resists the increase in circumference that would accompany shortening of the segment (*22*). Thus, increase in internal pressure is predicted to accompany muscle contraction if hydrostatic skeletal support is being used.

About 12 hours after exuviation, crabs enter the "paper-shell" stage during which formation of the cuticle is completed, and hardening begins (*23*). Although the cuticle is still soft, it is noticeably more rigid than during the soft-shell stage. Measurements on crabs 12 hours after exuviation also reveal a strong correlation between force and internal pressure (Fig. 1B). This suggests that at least 12 hours after exuviation, hydrostatic skeletal support is still important for movement and locomotion.

Two to 3 days are required for the new shell to harden substantially. In the hard-shell state, the cuticle is sufficiently rigid to resist the compressive and bending forces of muscle contraction. We therefore predicted that in the hard-shell state, no increase in hydrostatic pressure would be observed during muscle contraction. Recordings from a "hard-shell" crab, 7 days after exuviation, are consistent with this prediction. We did not observe increases in pressure as the carpus exerted large forces (Fig. 1C). These data imply that hydrostatic support is no longer used once crabs have returned to the hard-shell condition.

The gradual shift from hydrostatic to rigid skeletal support means that animals experience significantly different stresses in the various stages after molting. The mean maximum pressure increase and force measured change significantly as the animal progresses from the soft-shell stage to the hard-shell stage (Fig. 2). The mean maximum pressure increase among animals in the soft-shell stage was 1383 Pa (SD = 468) and dropped to 812 Pa (SD = 251) in the paper-shell stage and 150 Pa (SD = 116) in the hard-shell stage.

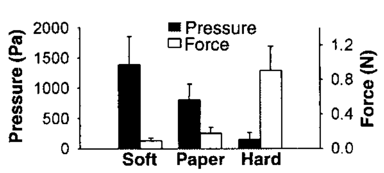

Fig. 2. Average maximum pressure and force recorded from postmolt crabs. Maximum pressure and force peaks from individual crabs were averaged. Bars represent overall average for each treatment (soft-shell, paper-shell, hard-shell). There was a significant difference between the pressures measured in all three treatments (ANOVA, $P < 0.001$; Tukey, $P < 0.01$; $n = 15$, 11, and 5, respectively). Force measurements were significantly different between hard-shell crabs and both soft- and paper-shell crabs (ANOVA, $P < 0.001$; Tukey, $P < 0.01$; $n = 15$, 14, and 5, respectively) but not between soft-shell and paper-shell crabs. Error bars represent standard deviation.

Thus, an animal in the stages soon after molting must withstand significantly larger pressure-induced stresses throughout its body, with potential implications for both morphology and physiology. Furthermore, the soft, flexible cuticle must have sufficient tensile stiffness to resist deformation by these pressures. Whereas, soon after molting, animals experience large hydrostatic pressures, the forces they exert are lower than those of hard-shell animals. The mean maximum force exerted during flexure of the cheliped was 0.09 N (SD = 0.03) in the soft-shell stage, but increased by over an order of magnitude in the hard-shell stage to 0.9 N (SD = 0.3). These differences may be a result of muscle atrophy during premolt and regeneration during postmolt, but atrophy is only known to occur in the propodus (*24*, *25*) and muscle regeneration occurs slowly, taking up to 3 weeks after ecdysis (*25*). Thus, the use of a hydrostatic skeleton may limit the force that can be exerted by the animal and may be responsible for the reduced speed and agility of movement observed in postmolt crabs.

Soft-shell crabs are often considered to be immobile, so that little attention has been given to how movement is accomplished. When crabs are soft, the claws are less effective for defense, making the animals especially vulnerable to predators, including conspecifics (*17*, *26*). Crustaceans typically spend this vulnerable time in seclusion (*26*, *27*), but are still quite mobile and active. For instance, the lobster *Homarus americanus* readily performs an escape response while in the soft-shell stage (*19*). Some lobsters do not seek shelter, but rather walk about and are capable of intense and coordinated activities (*13*). Stomatopods either present a meral spread threat display or flee when their cavities are invaded by conspecifics (*28*). Soft-shell grapsid crabs move just as actively and rapidly as hard-shell crabs (*20*). Newly molted blue crabs readily swim or crawl away from approaching objects (*29*). Thus, it is clear that the shedding of the exoskeleton does not incapacitate a crustacean.

Although there are examples of individual animals with distinct organs that function with a different skeletal support mechanism, crustaceans represent the only example of an animal that changes the fundamental skeletal support of its motor system from one form to the other repeatedly during life. For example, bivalve mollusks have a hard shell that protects their internal organs and gives the animal shape, but the burrowing foot and siphons rely on a hydrostatic mechanism (*10*). Elephants have a rigid internal skeleton used for posture and locomotion but their trunks depend on a muscular-hydrostatic mechanism (*30*). Likewise, erection in the mammalian penis is maintained by a hydrostatic mechanism (*31*). Crustaceans differ from these examples because the mechanism of skeletal support for the entire body alternates between the two general categories of skeletal support.

Most arthropods grow by molting, and thus, many may undergo this change in skeletal support. For instance, molting dragonfly nymphs swallow air to distend the gut (*32*). Changing from a rigid to a hydrostatic skeleton suggests that, in arthropods, the skeletal support system is not as static as typically thought. It also implies that the arthropod body plan, with numerous jointed appendages arranged in a point-loaded system, can function with hydrostatic skeletal support.

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33. We thank O'Neals Sea Harvest for supplying peeler crabs, J. Thompson for discussions, recording equipment, and comments on the manuscript, and S. Patek for comments on the manuscript. Supported by grants from NSF and NASA.

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22 April 2003; accepted 4 June 2003