



Soft-cuticle biomechanics: A constitutive model of anisotropy for caterpillar integument

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ABSTRACT

The mechanical properties of soft tissues are important for the control of motion in many invertebrates. Pressurized cylindrical animals such as worms have circumferential reinforcement of the body wall; however, no experimental characterization of comparable anisotropy has been reported for climbing larvae such as caterpillars. Using uniaxial, real-time fluorescence extensometry on millimeter scale cuticle specimens we have quantified differences in the mechanical properties of cuticle to circumferentially and longitudinally applied forces. Based on these results and the composite matrix–fiber structure of cuticle, a pseudo-elastic transversely isotropic constitutive material model was constructed with circumferential reinforcement realized as a Horgan–Saccomandi strain energy function. This model was then used numerically to describe the anisotropic material properties of *Manduca* cuticle. The constitutive material model will be used in a detailed finite-element analysis to improve our understanding of the mechanics of caterpillar crawling.

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1. Introduction

Soft-bodied invertebrates such as caterpillars do not have a rigid skeleton and must use soft tissues and muscles to transmit forces during movements and to hold the body contents in place. If the body morphology is maintained by hydrostatic pressure then the body wall must be under constant stress (Niebur and Erdos, 1991). Under these circumstances the passive properties of the soft body wall could exert a major influence on the morphology and deformation of the animal.

Many soft-bodied animals can be described mechanically as constant volume hydrostats with a tendency to form spherical structures (Wainwright, 1988). However, in the periods between each molt, cylindrical caterpillars dramatically increase in size while their proportions (ratio of diameter to length) do not change significantly. Early work by Carter and Locke directly addressed this paradox. Using microscopic and histological techniques, they showed how during growth soft cuticle expands

more in the axial than the circumferential direction through the unfolding of microscopic pleats in the endocuticle (Carter and Locke, 1993). While stretching causes reinforcing fiber realignment in the circumferential direction, the axial pleats reduce this realignment along the animal's axis. The unfolding of pleats is essentially a structural compensation to maintain proportions during growth. However, aside from growth considerations caterpillars are still pressurized cylinders in which hoop stresses are twice the axial stresses and it is unknown how the material properties of the body wall are adapted to this difference.

Although internal muscles attached to the body wall have been previously modeled and shown to be nonlinearly pseudo-elastic (Dorfmann et al., 2007, 2008), no comparable work has been done on the integument. According to the limited data on *Manduca* body pressure, the cuticle has to withstand a baseline pressure of several kPa (Mezoff et al., 2004). During locomotion and vigorous body swings (such as the strike reflex to noxious stimuli), local cuticle stretch may increase significantly via muscle action as well as pressure fluctuation. There are three aims in this project. First, we experimentally characterize the soft-cuticle properties in comparison to known tissue mechanics. Secondly, we formulate a phenomenological constitutive model to capture the mechanical response and finally, we explore the mechanics in the functional context of locomotion.

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Biological materials have stimulated a renewed interest in understanding the mechanical behavior of complex composites (Freier et al., 2005; Chen et al., 2006b; Vincent and Wegst, 2004). Insect cuticle is particularly attractive due to its light weight, adjustable strength, and bio-compatibility (Vincent, 2002). Insect integument consists of many components including stiff chitin microfibrils, elastomeric proteins such as resilin, lipids and heavy metals: their mechanical properties can change dramatically with small changes in composition such as the water content (Hepburn, 1972). In fact, insect cuticle stiffness can vary by eight orders of magnitude from 100 kPa to tens of GPa while appearing to have the same basic structure (Vincent and Wegst, 2004). More specifically, endocuticle seems to be responsible for most of the mechanics in soft cuticle. It often develops anisotropy, depending on the pre-stress during morphogenesis (Neville, 1967a).

Endocuticle is a continuously growing structure consisting of a silk-like protein matrix embedded with hydrophobic chitin nanofibrils (Wolfgang and Riddiford, 1986). Within this complex, layers of parallel fibers shift in orientation over successive lamina (Hepburn and Ball, 1973; Dennell, 1976; Hepburn, 1976), causing the well-known helicoidal appearance as described by Neville (1975) and Neville and Levy (1984). This cross fiber reinforcing strategy is biologically common (Locke, 1958) and has been intensely studied for its biomedical applications (Sanchez et al., 2005; Giraud-Guille, 1992; Belamie et al., 2006). In addition, cuticle chitin orientation changes by daily growth layers and has been documented in many insects (Neville, 1967b) and shown by scanning electron microscopy (Hughes, 1987).

The predominant molecular conformation of soft insect cuticle protein is antiparallel beta-pleated sheet (Iconomidou et al., 2001), and alpha chitin crystals are likely to bind to these proteins via H-bonds (Atkins, 1985). It has been proposed that protein hydrophobicity could take part in the control of cuticle hydration (Vincent, 2002). Further analysis on *Manduca* cuticular protein reveals the dynamic trafficking of proteins between endocuticular laminae and the hemolymph (Csikos et al., 1999). Like mammalian skeletons, caterpillar cuticle is more than just a passive structure; it is subject to constant chemical modulation by the body and acts as a protein storage and buffer, even though the chitin fibers do not change once laid down (Condoulis and Locke, 1966). Such dynamics can be demonstrated in the reversible 5-HT mediated plasticization of *Rhodnius* larvae cuticle (Reynolds, 1975).

Anatomically, *Manduca* only has a few oblique muscles and no circumferential muscles. The internal hemocoel is not physically compartmentalized so hydraulic pressure must be distributed over the entire body to minimize the energy. To accommodate about 10-fold change in volume within each intermolt period, the cuticle stretches with intussusceptions and unfolding. It has been shown that new 5th instars can be inflated to roughly twice their size, whereas the late 5th instar animals typically quadruple in volume (Wolfgang and Riddiford, 1981). This implies that *Manduca* caterpillars do not wrinkle less as they get bigger. In fact, their growth is close to linear scaling until the cessation of cuticle deposition. In 5th instar *Manduca*, cuticle thickness increases linearly with the body weight, but its stiffness declines due to growing hydration (Wolfgang and Riddiford, 1987). If the cuticle must maintain caterpillar's cylindrical morphology, anisotropy is expected to reinforce the circumferential direction as previously illustrated (Wainwright, 1982). While no such anisotropy has been reported, we hereby examine the mechanical properties of caterpillar cuticle in more detail using high resolution material testing equipment. For analytical purposes, we divide the endocuticle into three components: a homogeneous three-dimensional protein gel, an amorphous fibril two-dimensional reinforcement, and some fiber network with a preferred direction.

Following the tradition of nonlinear solid mechanics, we can characterize soft fiber-reinforced materials by deriving the constitutive relationship from an objective strain energy function (Holzapfel et al., 2000; Holzapfel, 2006). The proposed constitutive model accounts for large deformation, for the nonlinear pseudo-elastic material response and is suitable for implementation into a finite-element code. The long-term goal of this work is to use such material models in a detailed finite-element analysis of the caterpillar to improve our understanding on how the mechanical properties of soft tissues are exploited to control supply movements.

2. Experimental methods

Cuticle morphogenesis is rapid during the fifth instar so *Manduca* caterpillars were developmentally synchronized (Copenhaver and Truman, 1982) and used at 12 h post-ecdysis. Caterpillars were dissected, and two cuticle strips (5×1 mm) were harvested near the 3th abdominal segment (A3) dorsal midline from each animal. The circumferential strip was taken from the second annulus (there are eight annulus per segment) of A3, while the longitudinal strip was taken along the rest of the dorsal midline as shown in Fig. 1. Pilot tests showed that other abdominal segments are mechanically similar. The cuticle thickness (0.05 mm) was determined by identifying the endocuticle span in tissue cross section scanning electron micrograph. Great care was taken to avoid the inter-segmental folds for the longitudinal specimen and the inter-annulus crests for the circumferential specimen. Dissected cuticle strips were fragile, so the bonding margins were first treated with biological glue and then encapsulated by UV cured epoxy (Loctite, modified acrylamide) to produce specimen handles.

Uniaxial tests—The mechanical tests were performed in *Manduca* saline at room temperature (27°C). One end of the specimen was fixed on an epoxy platform submerged in saline via the specimen handle. The other handle was attached to a Dual-Mode Lever System (Aurora Scientific Inc., Ontario, Canada) via a steel pin. The servo motor could be controlled by an analog voltage signal and the coupled load cell measured the instantaneous torque. Data were digitized at 100 Hz (NI PCI-2669 data acquisition board, National Instrument, Austin, TX). See the schematic in Fig. 2 for details.

Real-time extensometry—Green fluorescent microspheres (169 μm/468 nm peak excitation/508 nm, peak emission; Duke Scientific Co., Fremont, CA) were fixed about 2 mm apart along the stretching axis on the epicuticle side using lanolin. Four blue LEDs (465 nm; LEDtronics Inc., Torrance, CA) provided the optical excitation under a custom made shade. A black and white CCD camera Basler A602 (Basler Vision Tech., Ahrensburg, Germany) captured the specimen image via a compound dissection scope at $\times 12$ magnification as shown in Fig. 3. A custom LabView program (National Instrument, Austin, TX) acquired and analyzed the

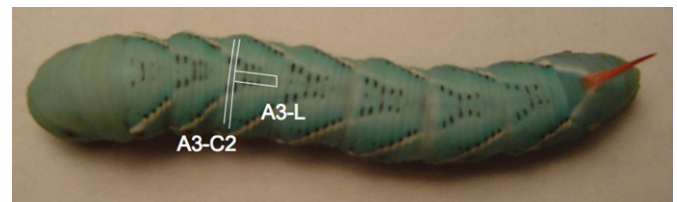


Fig. 1. Cuticle specimens were harvested from the dorsal abdominal segment three of a 12 h post-ecdysis *Manduca sexta* caterpillar. This figure shows the exact locations of the circumferential strip (A3-C2) and the longitudinal strip (A3-L) as labeled.

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