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Discrete Movements of Foot Epithelium during Adhesive Locomotion of a Land Snail*

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During the adhesive locomotion of land snails a series of short dark transverse bands, called pedal or foot waves, is visible if a moving snail's ventral surface is observed through a sheet of glass. Moreover, the mucus secreted from the pedal glands and some pedal epithelial cells forms a thin layer which acts as a glue augmenting adherence, while also acting as a lubricant under the moving parts of the snail's foot. The relationships between velocity and the frequency of pedal waves as well as changes in the volume of small air bubbles under foot waves were analyzed by means of digital recordings made through a glass sheet on which the snails were moving. On the ventral surface of a moving snail foot, the adhering parts of the foot constituted about 80% of the total area, while several moving parts only about 20%. The single moving region of the foot (the pedal wave) amounted to about 3% of snail length. The epithelium in the region of the pedal wave was arched above the substrate and was also more wrinkled than the stationary epithelium, which enabled the forward motion of each specific point of epithelium during the passage of a pedal wave above it. The actual area of epithelium engaged by a pedal wave was at least 30% greater than the area of the epithelium as recorded through a glass sheet. In the region of the pedal wave, the tiny subepithelial muscles acting on the epithelium move it up in the front part of the wave, and then down at the end of the wave, operating vertically in relation to the substrate. In the middle part of the wave, the epithelium only moves forward. In summary, during the adhesive locomotion of snails, the horizontal movement of the ventral surface epithelium proceeds as temporally separate phases of upward, forward and downward movement.

Key words: Adhesive locomotion, epithelium, land snail, *Achatina achatina*, pedal wave.

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Strong adherence to the substrate characterizes the behavior of snails in quiescence and during locomotion (snails are also able to move in a "galloping" mode when the foot surface adheres to the substrate only partially). The paradox of adhering firmly and moving at the same time was resolved by observing a series of short waves of muscular contraction running along an adhering snail foot, visible as dark bands if the snail moves on a glass plate and is viewed through it (JONES 1973; LISSMANN 1945a; LUCHTEL & DEYRUP-OLSEN 2001; SIMKISS & WILBUR 1977; LAI *et al*. 2010). Moreover, the mucus secreted by the pedal glands and some pedal epithelial cells forms a thin layer that acts as a glue augmenting adherence, while also acting as a lubricant when the epithelial surface moves above the substrate. It was shown that the viscosity of the mucus under the moving pedal epithelium is much smaller than that under its adhering and immobile parts (DENNY 1980 & 1984; DENNY & GOSLINE 1980). The neuronal mediators influencing this type of locomotive behavior in snails have already been described (PAVLOVA 2001). The problem of how the epithelium functions in the adhesive locomotion of snails and more specifically, in the region of the pedal wave, was addressed only partly in studies devoted to the microscopic analysis of the pedal epithelium structure (MARTIN & DEYRUP-OLSEN 1986; YAMAGUCHI *et al*. 2000). In this study, the problem was examined by means of digital recording of the movements of snails on a glass sheet. The relationship between the velocity of the snail and the frequency of foot waves was quantified, as were changes in the diameter of small air bubbles under the foot during transition between the interwaves and the pedal waves. Based on this last variable, one can infer changes in pressure under the foot of a moving snail. The adhesive locomotion pattern of land snails has been used in the construction of robots (CHAN *et al*. 2005; EWOLDT *et al*. 2007; HOSOKAWA *et al*. 2009).

Material and Methods

The locomotor activity of 142 giant African snails (*Achatina achatina* Lameere) of a body length of 4 to 10 cm and a mass of 26 to 78 g was observed, digitally recorded, and kinematically analyzed. The snails, kindly provided by the Poznań Zoological Garden some years ago, were kept in a moist terrarium on a peat layer at room temperature and fed various kinds of cabbage with the addition of hen egg shells. The experiments consisted of recording a spontaneously moving snail on the upper surface of a horizontal glass sheet. Photographs of the foot surface were taken from under the sheet with a CCD DFK 41 AV02.AS digital camera (The Imagingsource, Germany) equipped with a CCTV 5-50 mm F/1.8 lens (Pentax, Japan), using IC Capture.AS 2.0 software (for AVI and JPG files). Variables were measured or calculated by means of VirtualDub 1.8.8 and/or LabVIEW 2009 software.

The studied snails had their shells marked and were weighed and measured; 45 of them were tattooed by subepithelial injection of carbon particles at least three days before the experiments. Air bubbles were observed between the surface of the snail's foot and the glass substrate in many recordings of spontaneously moving snails.

Recordings of the spontaneous locomotor activity of 97 snails were made for about 10 min each, out of which 20 to 30 s of steady movement was chosen for numerical assessment. Figure 1 shows a schematic drawing of a snail foot with a picture of pedal waves and interwaves as seen through a glass sheet.

The following variables were quantified in spontaneously moving snails: **lg** – body length; **sh** – the length of the forward movement of the snail during time ts; **ts** – duration of the moving episode; **lw** – length of single pedal waves; **nw** – the number of simultaneously occurring pedal waves on the pedal surface; **nwh** – number of pedal waves reaching a specified point during the time ts; **vg** – the speed of the snail (vg = sh/ts); **fw** – frequency of pedal waves (fw=nwh/ts); **cw** – percentage of the pedal surface occupied by all simultaneously occurring pedal waves (cw=lw*nw/lg); **sh/nwh** – length of the forward movement of the snail during one pedal wave reaching the region of the head; **u** – wave effi-

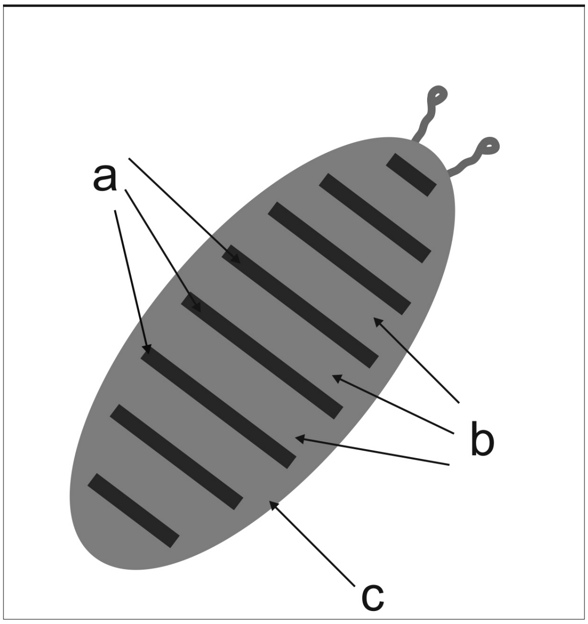

Fig. 1. Schematic drawing of pedal waves on the ventral surface of a crawling snail as seen through a glass sheet; a – pedal waves (moving parts of the ventral surface), b – interwaves (resting segments of the ventral surface), c – rim.

ciency coefficient (the percentage of a pedal wave which is used for forward motion, u=(sh/nwh)/lw).

In order to evaluate the movement of the points tattooed on the snail's foot (as shown in Fig. 3, top scheme) and changes in the air bubbles under the pedal wave (Fig. 4 A1 and A2), some additional variables were needed: **ng** – number of pedal waves which have passed the point marked on the glass during time **tw**; **li** – length of a single interwave; **vw** – velocity of waves calculated for the point marked on the glass (vw=[lw*nw+li*(nw-1)]/tw); **nt** – number of pedal waves that have passed the point tattooed on the snail's pedal surface during time **tt**; **vt** – velocity of waves calculated for the point tattooed on the snail's pedal surface (vt=[lw*nt+li*(nt-1)]/tt); **gt** – intercept between the points marked on the glass and the one tattooed on the snail, after the passage of a single pedal wave; **d** and **h** – the distance between the foot epithelium and the glass plate in the presence of small air bubbles in the wave or interwave region, respectively.

The measured and calculated variables were used to determine how a snail foot epithelium participates in the formation and propagation of foot waves and interwaves.

Results

The measured and calculated variables derived from 45 recordings of spontaneous forward snail locomotion events are presented in Table 1.

It was found that during forward motion only 23±4% of the foot surface (**cw**) moves forward in the form of pedal waves, while some 80% (**1-cw**) firmly and motionlessly adheres to the substrate. The foot waves reaching the anterior part of a snail foot participate in the forward motion of the animal and it was estimated as the foot wave efficiency coefficient **u** (u=(sh/nwh)/lw) on some 28% of each wave. According to this reasoning, the surface of the foot epithelium forming a foot wave consists of the surface related to the forward motion of a snail (**sh/nwh**) and that is equal to the foot wave itself (**lw**), which is shown schematically in Figure 2.

The measurements presented in Table 2 obtained by studying snails with points tattooed on their feet showed that during the passage of a pedal wave, the tattooed point moved past the intercept **gt**.

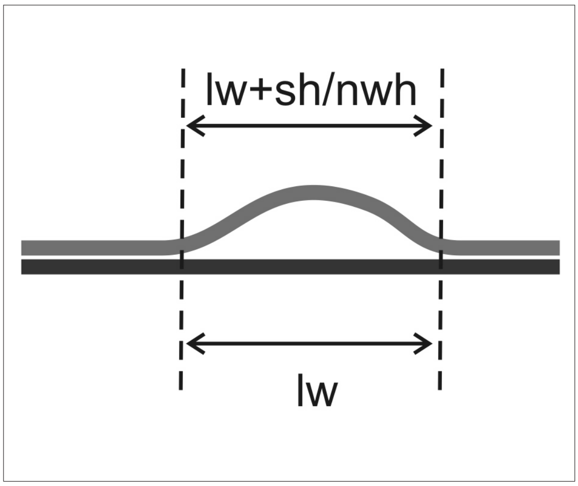

Fig. 2. Schematic drawing of the side view of a snail's integument (the grey line) in the region of the pedal wave during movement along a glass sheet (black line). The meaning of symbols is the same as in Table 1: lw – length of the pedal wave as seen through a glass sheet; sh/nwh – the length of the forward movement of the snail during one pedal wave reaches the region of the head.

Table 1

Relationship between the speed of the forward motion of *A. achatina* snails and the frequency and length of their pedal waves (n=45)

lg [mm]	nw	lw [mm]	cw	sh [mm]	ts [s]	vg [mm/s]	nwh	fw [Hz]	sh/nwh [mm]	u
x ± SD										
50.59 ± 11.81	5.70 ± 1.42	2.00 ± 0.29	0.23 ± 0.04	7.69 ± 2.53	24.48 ± 4.18	0.32 ± 0.11	9.70 ± 1.87	0.40 ± 0.07	0.79 ± 0.23	0.28 ± 0.06

Note: arithmetic means and standard deviations are shown; **n** – number of locomotor events recorded; **lg** – snail foot length; **nw** – number of simultaneous pedal waves on the pedal surface; **lw** – length of single pedal waves; **cw** – the percent of pedal surface taken up by all simultaneous pedal waves (cw=lw*nw/lg); **sh** – the length of the forward movement of the snail head over time ts; **ts** – time of the moving episode; **vg** – snail velocity (sh/ts); **nwh** – number of pedal waves reaching a specified point during the time ts; **fw** – frequency of pedal waves (nwh/ts); **sh/nwh** – the length of the forward movement of the snail during one pedal wave reaches the region of the head; **u** – the wave efficiency coefficient (the percentage of the pedal wave which is used for forward motion, u=(sh/nwh)/lw).

Table 2

Measurements of the forward motion of the pedal wave in relation to two different points - one tattooed on the ventral surface of the foot and the other marked on glass exactly under the first one (n=45)

tw [s]	nw	lw [mm]	li [mm]	vw [mm/s]	tt [s]	nt	vt [mm/s]	gt [mm]
x ± SD								
13.15 ± 2.88	6.16 ± 0.75	2.00 ± 0.29	6.36 ± 1.19	3.52 ± 0.78	15.01 ± 3.06	6.11 ± 0.81	3.12 ± 0.63	0.59 ± 0.22

Note: **tw** – time of passage of pedal waves ng over the point marked on the glass; **ng** – number of pedal waves that passed the point marked on glass during the time tw; **lw** – length of a single wave; **li** – length of a single interwave; **vw** – velocity of waves calculated for the point marked on the glass (vw=[lw*ng+li*(ng-1)]/tw); **tt** – time of passage of pedal waves nt over the point tattooed on the pedal surface of the snail; **nt** – number of pedal waves that passed the point tattooed on the pedal surface during the time tt; **vt** – velocity of waves calculated for the point tattooed on the pedal surface (vt=[lw*nt+li*(nt-1)]/tt; **gt** – the intercept between the points marked on the glass and the one tattooed on the snail after the passage of a single pedal wave.

The quotient **gt/lw** has the same meaning as the coefficient **u** in Table 1 and it amounts to 29.5% of the surface of the foot wave in this series of recordings.

The velocity of the pedal wave relative to the tattooed point (vt) is smaller than that relative to the glass substrate (vw). The reason for this discrepancy is explained schematically in Figure 3.

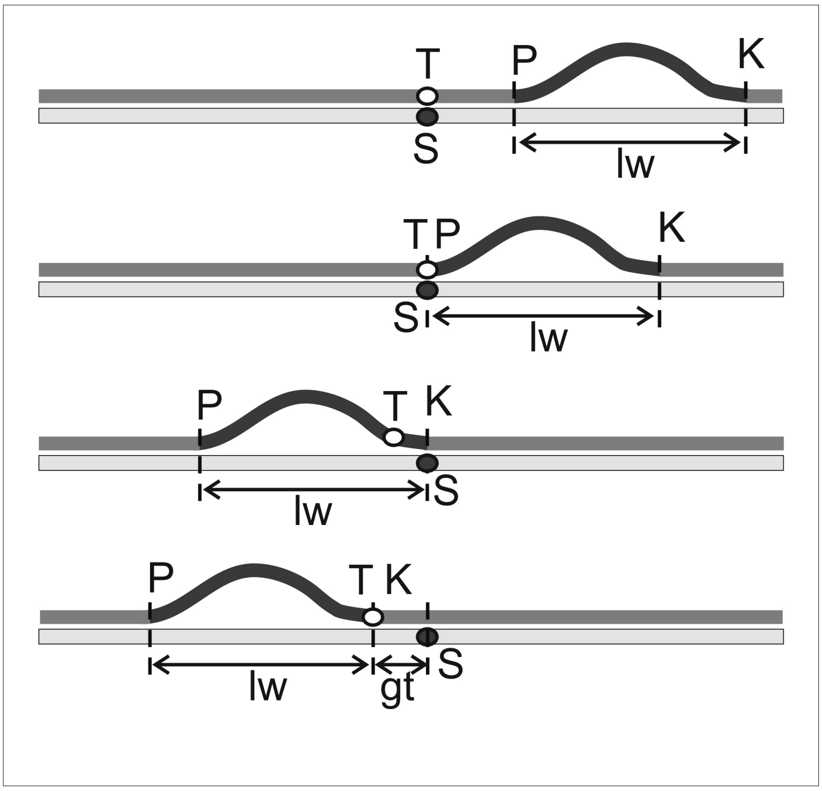

Fig. 3. Schematic explanation of the differences between the assessments of pedal wave (**PK**) velocity in relation to the point tattooed on the ventral surface of the snail foot (**T**) and the point marked on the glass plate (**S**). Although the length of the pedal wave in both methods of calculation is the same (**lw**), it takes more time for it to pass the tattooed point. During the passage of the pedal wave, the tattooed point moved past the intercept **gt**.

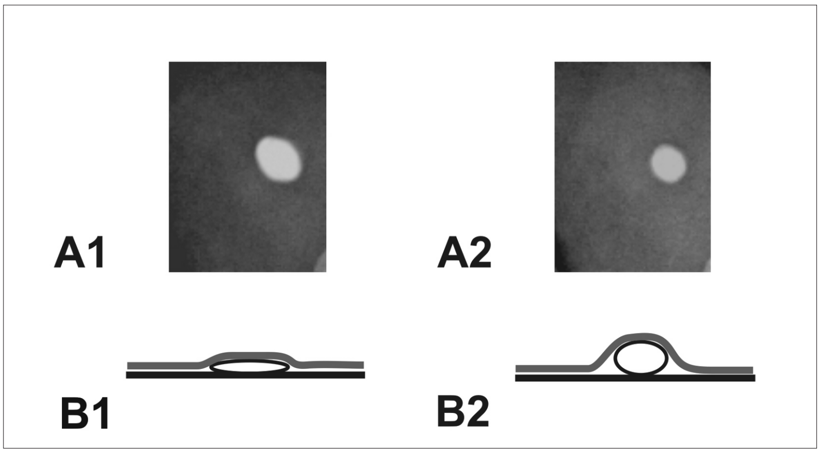

Fig. 4. Changes of the air bubble image surface under a snail foot observed during the passage of an interwave (A1) and a pedal wave (A2). A1 and A2 present images of the same bubble recorded by a digital camera, while B1 and B2 offer a schematic explanation of the surface changes of the bubble in lateral view. Under the interwave the bubble is flattened (B1), while under the pedal wave it is spherical so its diameter is smaller (B2).

The next step in the study was the quantification of changes in small air bubbles under the foot of a moving snail. The surface of every air bubble was greater under the interwave than under the foot wave. An explanation for this phenomenon is presented in Figure 4 and the calculations for 77 recordings are presented in Table 3. The intercept between the foot epithelium and the substrate under the foot wave was almost 2.5 times greater than the intercept under the interwave.

Given that (i) only part of the surface of the foot wave participates in the forward movement of a snail; (ii) during the passage of the foot wave every specific point of the foot is moved forward; (iii) the intercept between the foot epithelium and the substrate is greater under the wave than under the interwave, one can infer that the foot wave consists in arching and folding of the epithelial surface propagating from the posterior to the anterior part of the snail foot. When epithelial folding is transferred to the anterior part of the foot, the snail body extends forward. Based on this data, conclusions on the movement of every specific point on the foot surface and about the forces acting on that point during the passage of the foot wave can also be formulated. In the region of the foot wave, the muscles acting on the epithelium move it upward in the front part of the wave, and then downward at the end of the wave, acting vertically in relation to the substrate. In the middle part of the wave, the epithelium moves only forward. This process is schematically illustrated in Figure 5.

Table 3

Changes in the surface of a small air bubble image under a snail foot observed during passage of a foot wave or an interwave (n=77)

The surface of an air bubble image			
under contraction wave		under interwave	
$x \pm SD$			
[mm^2]	d [mm]	[mm^2]	h [mm]
8.41	3.27	13.24	1.40
±0.76	±0.15	±0.74	±0.16

Note: Distance between the foot epithelium and the glass plate in the region of an air bubble under a contraction wave is **d** and under an interwave is **h**; **d** was measured based on the recordings and **h** was calculated as the height of a cylinder of the volume equal to a sphere of diameter **d**.

Discussion

The current paradigm of adhesive locomotion in gastropods (Luchtel & Deyrup-Olsen 2001; Simkiss & Wilbur 1977) is based on the studies of Lissmann (1945a & 1945b) and Jones (1973 & 1975) on the kinematics and dynamics of mollusk movements as well as those of Denny (1980, 1981,1984; Denny & Gosline 1980) on the function of mucus in this type of movement. According to Lissmann (1945a), the forward motion of snails consists of a series of contraction waves which propel the successive parts of the body starting from the posterior to anterior. The functional units, i.e. the contraction waves consisting of mus-

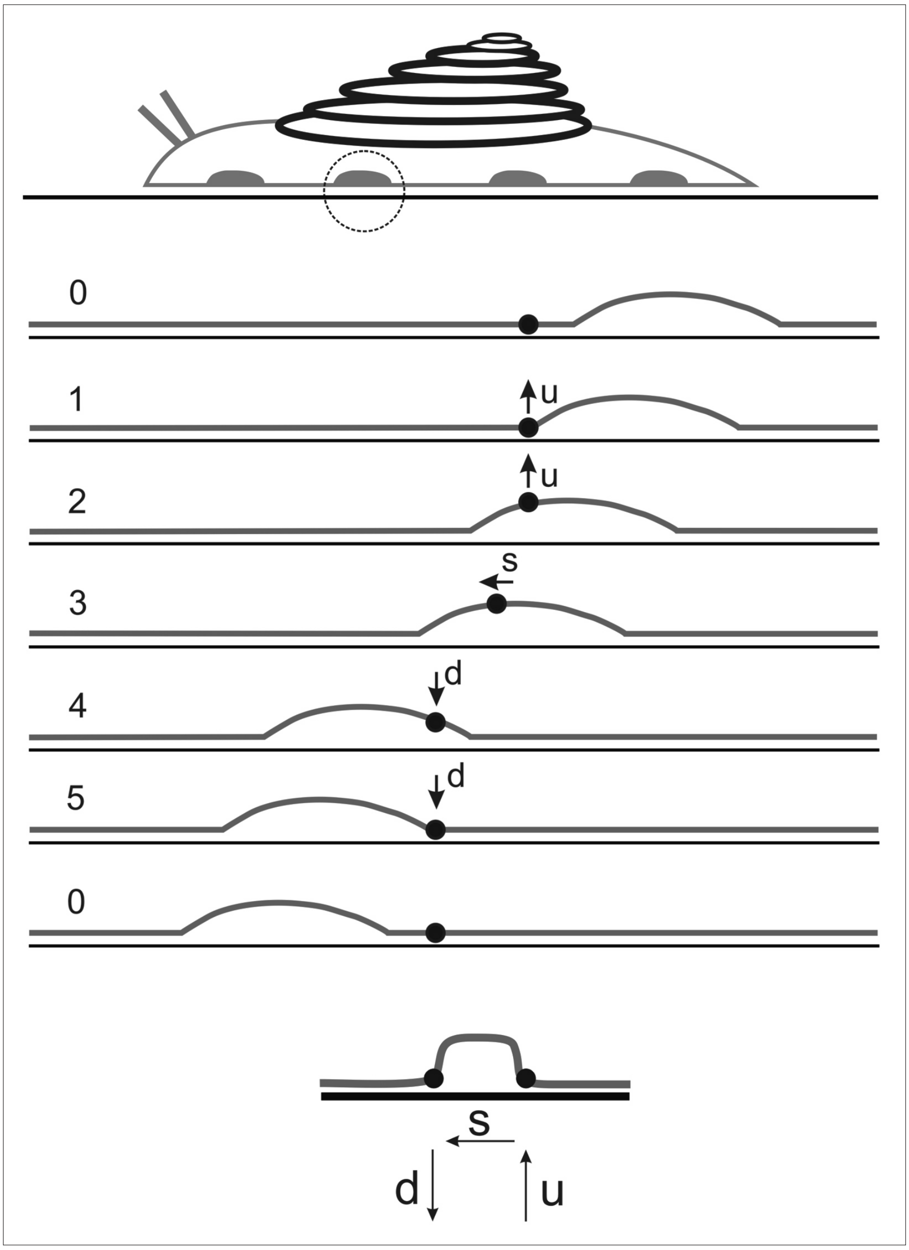

Fig. 5. Diagram of epithelium movements in the region of the foot wave on the surface of a snail foot. A schematic lateral view of four foot waves is shown at the top. The movements of the specified point (●) on the epithelial surface during the passage of the pedal wave are shown in sections 0, 1, 2, 3, 4, 5, and 0. The point ● on the epithelium is moved up (**u**) in the front part of the wave and then down (**d**) in the posterior part of the wave, but it moves forward only in the middle part of the wave (**s**). During the forward movement of a snail, the temporally separated phases of upward, forward and downward movement of discrete areas of its integument occur as shown in lowermost part of the diagram.

cles and neurons, are formed by the contraction of muscle fibers in the front part of wave and their relaxation at the end of the wave, so the different parts of the snail's ventral surface are in two mutually exclusive phases – stationary (relaxation) or mobile (contraction).

The forces of propulsion acting on the moving part of the foot are opposed by the sliding friction of the immobile foot parts and if the second is greater than the first, forward motion is possible.

The careful laboratory analysis of pedal mucus samples carried out by DENNY (1980) shows that the viscosity of the substance is dependent on the rate and magnitude of the applied stress forces. According to these results, the viscosity of mucus in the region of the moving pedal surface is smaller than under the stationary parts, significantly contributing to the efficiency of the movement.

The study of LAI *et al*. (2010) substantially contributed to the modern understanding of snail locomotion. Detailed maps of movements of all points forming the foot surface and also of the forces acting on them were constructed with the application of optical methods. They observed significant differences in the velocity of the foot wave and the rim of the foot and confirmed the motionlessness of the interwaves. The propulsion forces of the snail body act on the interwaves and the regions of the waves and those of the rim oppose these actions.

The analysis of the adhesive locomotion of land gastropods presented in this article focused on the role of the integument in this phenomenon, as its structure, and particularly the pedal epithelium, is the interface between the activity of the muscles and substrate (LISSMANN 1945a & 1945b) and that of mucus (DENNY 1980, 1981 & 1984). Thus, this study aims at a comprehensive explanation of the role of the integument in the formation and propagation of foot waves rather than criticizing the previously presented views.

This study has revealed that the formation and propagation of foot waves is a mechanism of the forward shifting of epithelium (or, broadly speaking, of the integument) that allows the snail to move forward. The epithelial surface of the pedal wave is greater than its image seen through glass (shown schematically in Fig. 2) and both the arching and folding of the epithelium provide the spare surface used for forward movement when pushed toward the anterior part of the snail body.

In accordance with the above reasoning, a snail's velocity (**vg**) is related to the frequency of pedal waves, their length, and also to the wave efficiency coefficient **u** as shown in the formula vg= fw*lw*u.

Additionally, there are differences in pressure between the adhering and moving parts of the pedal epithelium. The distance between the epithelium and the substrate is greater in the region of the wave than under the interwave. This was shown in a kymographic study already in 1939 and has been repeatedly confirmed (as analyzed by LISSMANN 1945a and LAI *et al*. 2010). In this study, calculations based on changes in the dimensions of small air bubbles under the snail foot (Table 3) indirectly indicate that the pressure in the region of the wave is significantly smaller than in the region of adhesion.

Consequently, the propulsion of the wave is not only facilitated by the less viscous mucus as was shown by DENNY (1980), but also by the smaller force pressing this part of the snail foot to the substrate. Both factors diminish the energy cost of locomotion.

The last corollary to the forces acting on the epithelium in the region of the wave is visualized in Figure 5. In the region of the contraction wave, the tiny muscles acting on the epithelium move it up in the front part of the wave and then down towards the end of the wave, acting vertically in relation to the substrate. In the middle part of the wave the epithelium moves only forward.

In summary, in snail adhesive locomotion, the horizontal movement of the ventral surface epithelium proceeds as temporally separate phases of upward, forward, and downward movement.

In conclusions:

1. Forward adhesive locomotion of land snails depends on waves moving along their foot surface, called foot waves.

2. A foot wave consists in the arching and folding of the integument; in this way it forms some spare surface and makes forward movement possible when passing to the front part of the animal.

3. The pressure under the wave is significantly smaller than under the stationary surface.

4. The integument with its epithelium in the region of the pedal wave moves upward in the initial part of the wave, forward in the middle, and then downward towards the end of the wave.

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