

THE LOCOMOTION OF LIMAX.

I. TEMPERATURE COEFFICIENT OF PEDAL ACTIVITY.

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The creeping of such a gasteropod as *Limax maximus* results from (Fig. 1) a rhythmic succession of evenly spaced, progressive wave-like deformations of the pedal surface. It should be possible to utilize these waves for the study of neuromuscular physiology in the intact animal, avoiding in this way effects due to lack of proper circulation and the like, as when isolated organs are employed (Vlès, 1908; Crozier, 1922-23).

The pedal activities of a number of chitons and gasteropods have been described and classified in a comparative way (Vlès, 1907, 1913; Parker, 1911, 1914; Olmsted, 1917-18; Crozier, 1918-19). Experiments by Biedermann (1905), Künkel (1903), Bethe (1903), Arey and Crozier (1919), and ten Cate (1923) agree in pointing to the fact that the formation of the pedal waves and their procession over the foot depend essentially upon the intrinsic nervous mechanism of this organ, and presumably upon its nerve net. The mechanism of creeping is normally released, reflexly, by impulses traveling over the central ganglia and pedal nerves.

The neuromuscular creeping organ of *Limax* is definitely polarized, as is true of the majority of gasteropods; the pedal waves are *direct*, coursing from posterior to anterior end of the foot. The waves occupy the median third of the foot. About eleven to nineteen waves are present at one time, depending chiefly upon the size of the animal, although in very rapid creeping the number may be increased. Each wave is a region of the creeping surface lifted free from the substratum; within this temporarily lifted zone the contractile elements of

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the foot serve to move the pedal substance forward. This conception of the pedal wave (Parker, 1911; Olmsted, 1917-18) has been questioned by certain observers (van Rijnberk, 1918-19; ten Cate, 1923). Our observations on *Limax* agree with those of Parker (1911). The correctness of this general interpretation is supported by the results of inspecting cases where the pedal wave is of gross dimensions, not only in mollusks (Parker, 1914, 1917-18, 1921) but also in representatives of other phyla practicing essentially this method of locomotion (*Metridium*, Parker, 1917; larvæ of slug-moths, Crozier, 1923-24).



FIG. 1. *Limax maximus*, ventral view showing character of pedal waves.

The precise negative geotropism of *Limax* (Davenport and Perkins, 1897) makes it easy to study the creeping of the slug under conditions such that the rate of doing work (lifting the body vertically) varies directly with the animal's weight and with the velocity of upward movement. A slug is placed at the bottom of a large cylindrical glass jar about 3 feet high, the inside of the jar being previously moistened by rinsing with tap water. On beginning movement, the wall of the jar is quickly encountered and the animal in practically every instance orients in such fashion as to move upward in a straight line on the wall

of the cylinder. The jar is covered to exclude air currents, which may otherwise result in irregularities of creeping. A thermometer in the jar, suspended from the cover, gives the temperature.

In our measurements we have taken account of the following: (1) the weight of the individual, (2) the dimensions of the pedal surface, (3) the speed of creeping (V), (4) the frequency of pedal waves (F), (5) the upward movement due to a single wave (A), (6) the velocity with which a single pedal wave traverses the foot (v), and (7) the

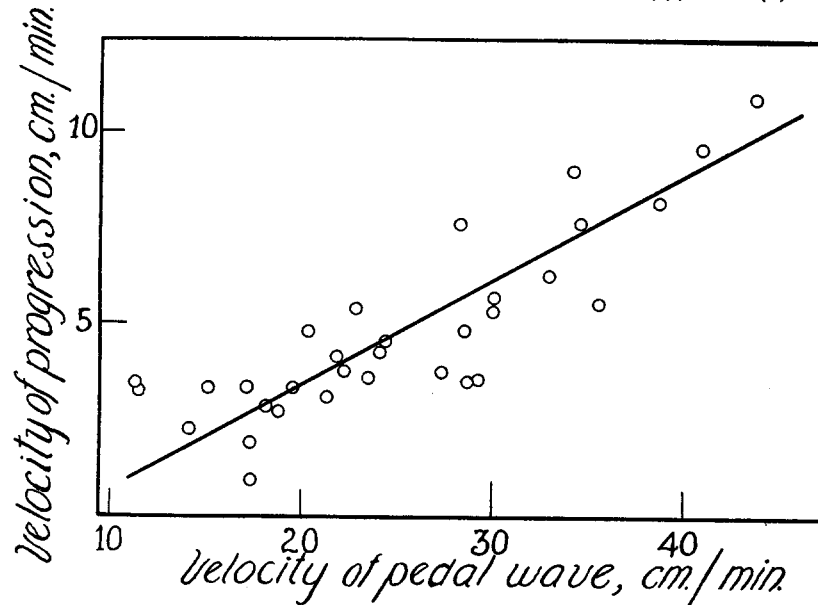


FIG. 2. The relation between velocity of a single pedal wave and the simultaneous velocity of upward creeping.

temperature. Since (3), (4), and (6) are not independent quantities, and since the speed of upward movement varies from moment to moment, the observations (particularly as to (3), (4), and (5)) must be made concurrently during brief intervals. This is accomplished by noting the time and the number of pedal waves required to move the animal upward by a known distance (usually 1 to 2 cm.) marked upon the surface of the glass jar. The temperature was varied between 11° and 28°C.; these represent about the extreme limits for active creeping. The adhesive power of the foot depends largely upon its

extruded mucus, and this may constitute a slight error in comparing the effectiveness of the pedal mechanism at different temperatures, since at lower temperatures the animal is doing work against the fixation due to a less fluid slime. Other sources of variation greatly outweigh this one, however. The results described are based upon series of readings with each of thirty-four *Limax maximus* varying in weight from 0.18 to 15.2 gm.

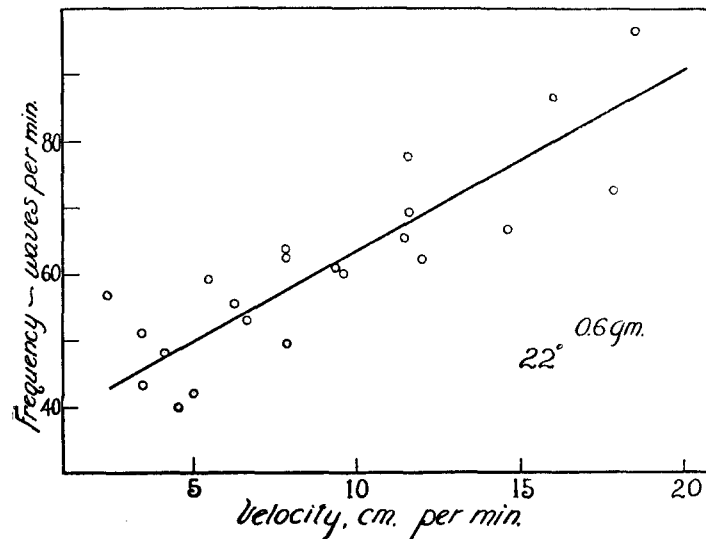


FIG. 3. The relation between frequency of pedal waves arriving at the anterior end of the foot, and the simultaneous velocity of creeping, for four individuals of the same weight (0.6 gm.) and at the same temperature.

The velocity of a single pedal wave is directly proportional to the speed of upward creeping (Fig. 2). Variations from strictest proportion unquestionably arise in large part from the complex character of the pedal musculature; a deficiency in wave frequency may thus be compensated by greater dimensions of the single waves. But in general trend the proportionality of v to V is unmistakable, and in spite of the complex organization of the foot, and of the considerable likelihood of slight observational error, seems to hold regardless of the weight of the animal, or of the temperature. The actual magnitudes of v are of the order associated with other measurements of

(presumably) nerve net-conducted processes (Parker, 1918-19), but this correspondence is not in itself conclusive evidence of nerve net transmission (Crozier, 1923-24).

The velocity of progression is also directly proportional to the number of pedal waves per minute passing a given point on the foot (F). Data in Fig. 3 were obtained from tests with four animals

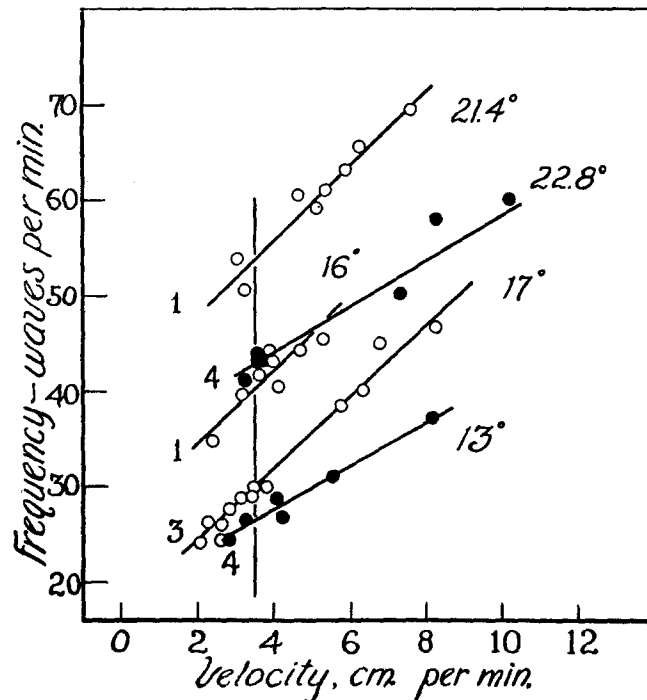


FIG. 4. The relation between wave frequency and velocity of progression is illustrated for different individuals (numbered 1, 3, 4) and at different temperatures. The slope of the lines for *Limax* 4 is unusual, but consistent at different temperatures.

of identical weights (0.6 gm.). In general, this type of relation is found, and with the same factor or proportionality (slope), when observations pertaining to a single individual are considered. The position of the line (Figs. 3, 4) is shifted toward the V axis at lower temperatures, but the slope of the line remains constant. Occasionally an exceptional individual is found for which the slope of the line con-

necting V with F is different; the same slope is retained in the observations from such an individual at a different temperature (Fig. 4).

Vlès and his collaborators (Vlès, 1908; Peyrega and Vlès, 1913; Vlès and Bathellier, 1920) have studied the relations between pedal waves and locomotor efficiency in gasteropods. Vlès and Bathellier (1920) propose an equation of the form

$$V = Ae^{BF}$$

to express the connection between velocity (V) and wave frequency (F) in vertical ascension by various snails; in this formula A and B are constants. The actual data are quite scattered, and nothing more than a crude fit could be expected. From this equation, Vlès proposes to deduce certain properties of the contractile elements in the foot. By plotting all our observations with *Limax* (at room temperature) upon one frame, it is possible to observe such a trend as Vlès has endeavored to characterize. But if the data are considered in terms of the single individuals involved, it is scarcely possible to regard Vlès' equation as having physical significance. Very large *Limax*, and an occasional smaller one, may tend to give a proportionately greater increase in velocity for a given increase in wave frequency; and for such large individuals the line of observations is shifted away from the F axis, so that when readings from numbers of specimens are lumped together the curve passing through their midst seems an exponential one. It is also true that the relation between V and F is responsive to slight differences of temperature, which assists in giving a vague scatter to the results.

The velocity of creeping at any moment is determined by the frequency of the waves and by the advance due to a single wave; the latter is dependent upon the interplay of two general sets of muscle fibers in the foot, respectively oriented dorsoventrally and longitudinally (Car, 1897). The advance due to a single wave is measured as an average from the number of waves disappearing at the anterior end of the foot while the anterior border of the foot is advancing over a measured distance. This quantity, A , is of course directly proportional to the velocity of creeping, but the slope of the line of proportionality becomes greater at lower temperature (Fig. 5). At constant temperature the slope of the line for different individuals is the same

(Figs. 6, 7). In general, with animals of increasing weight the line is displaced from the axis of velocities (Fig. 7); individuals of the same weight yield points falling upon the same line (Fig. 6).

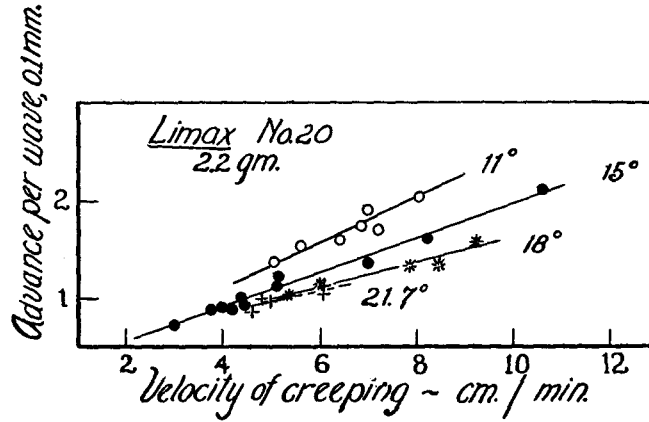


FIG. 5. The advance due to one wave is directly proportional to the velocity of creeping, but the slope of the line varies inversely with the temperature.

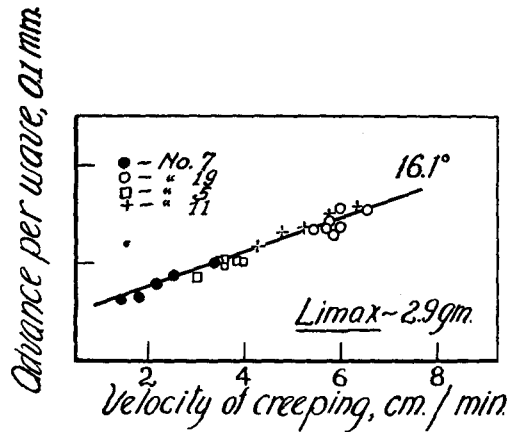


FIG. 6. The advance per wave is proportional to the velocity of creeping; the relationship is identical for animals of the same weight.

From these findings it is possible to estimate the effects of temperature upon pedal activity.

The frequencies of waves required at different temperatures to produce a given velocity of upward creeping were measured for twenty-

four animals. By interpolation upon such curves as are shown in Fig. 4, the frequency at each experimental temperature was obtained which corresponded to a velocity of 3.5 cm. per minute. Curves were drawn relating these frequencies to temperature. The separate curves overlapped in the region of 17°C. The frequency corresponding to this temperature was therefore taken (on the curve) as 100 per cent for each individual and the observations at the other temperatures were reduced to this scale. Variations due to differing weights of individuals and other specific causes were thus largely eliminated. The corrected frequencies emerging from this process are plotted logarithmically in Fig. 8, against the reciprocal of the absolute tem-

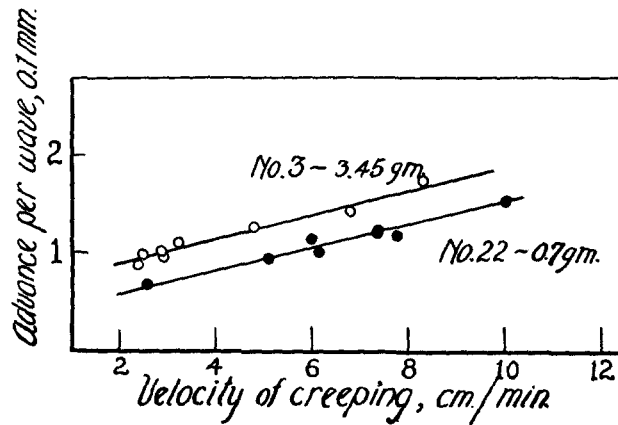


FIG. 7. For *Limax* of differing weights, the lines expressing the relation of advance per wave to velocity are parallel.

perature. Although subject to considerable variation, the trend of the observations is unmistakably clear. The frequency of pedal waves required to accomplish a given amount of work in a given time is affected by the temperature in such a way that the logarithm of the frequency is inversely proportional to the absolute temperature, as required by Arrhenius' (1889) formula. For the present case the value of μ in this formula is about 10,700 (Q_{10} for 11° to 21° = 2.1).

The velocity of the individual pedal wave (v) is the same for a given velocity of creeping (V), regardless of the temperature. Hence it is impossible to obtain a direct measure of the temperature coefficient

for the "velocity of a single wave." The constancy of v for fixed V comes about from the fact that with lower temperatures the advance due to any single wave is enhanced. This may be measured by reading from curves such as shown in Fig. 7, the increase in average "advance due to one wave" which is requisite for a given increment in velocity. This amount varies inversely with the temperature. The

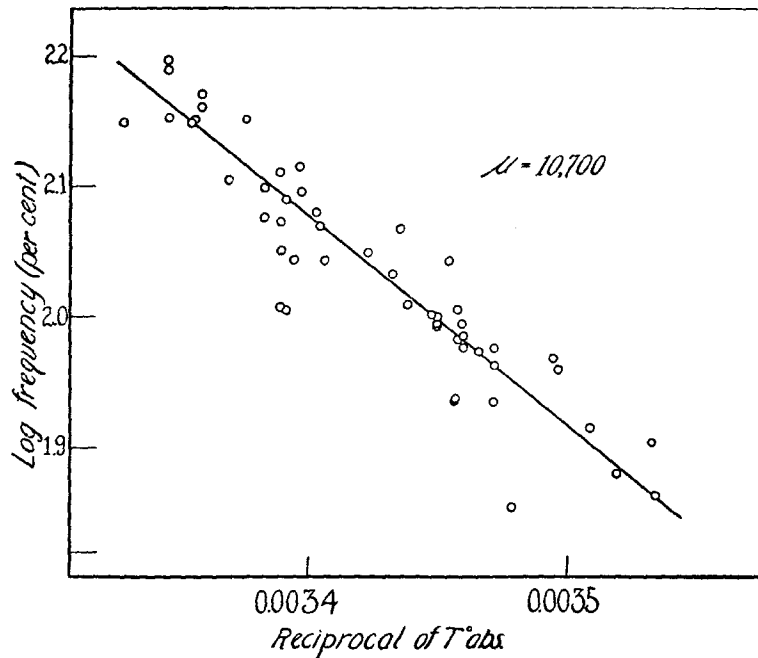


FIG. 8. The logarithm of the frequency of pedal waves required to lift the body of the animal vertically at a fixed rate is inversely proportional to the absolute temperature. The equation of the line drawn is $\log F = \frac{10,700}{2T} + C$, where T is the absolute temperature.

results (Fig. 9) permit only a rough estimate of the temperature coefficient, which is about $Q_{10} = -2.2$. At lower temperatures the individual wave is more effective, under conditions permitting comparison, probably due to the heightened tonus of its contractile fibers; that is, the forward movement of the temporarily lifted portion of the foot is more extensive.

Indirectly, the temperature coefficient for speed of a pedal wave (v) may be deduced from the fact that F and V are directly proportional, as also v and V , while the temperature coefficient of F is known. The coefficient for v should therefore be the same as that for F .

It is not without significance that the temperature coefficient for pedal activity in *Limax*, which may be regarded as the coefficient of

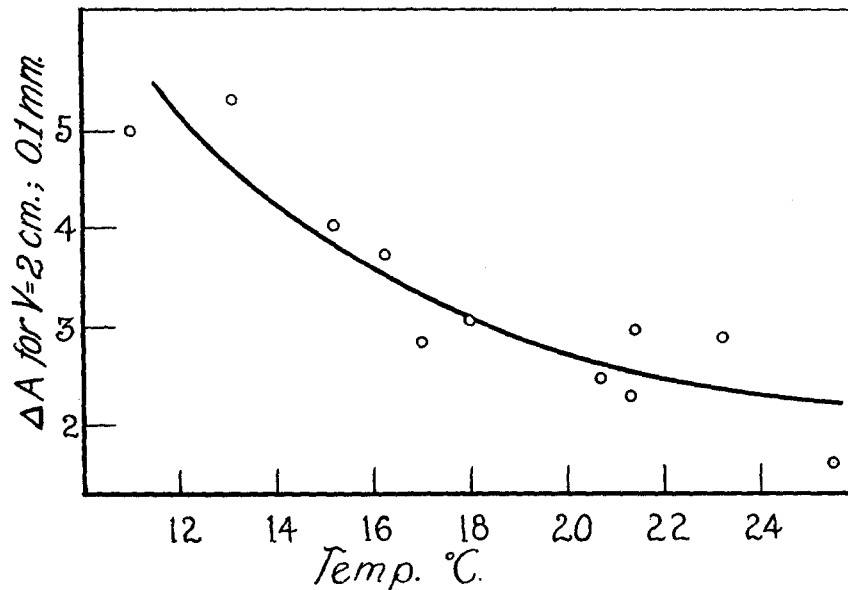


FIG. 9. The relation of temperature to the vertical advance per single wave (ΔA) required to effect a given increment in velocity of progression (2 cm. per min.). The points are each the average to two or three determinations; Q_{10} = approximately - 2.3.

nervous initiation of waves and also of their conduction, is similar to that obtained in another instance of nerve net transmission. From Parker's (1920) data giving the rate of transmission in the colonial body of *Renilla* at several temperatures I calculate a value of μ of about 11,200,¹ which is probably not significantly different from that found with *Limax*. Comparison on this basis is of interest not only

¹This applies to the temperature interval 11° to 22.8°; from 22.8° to 31°, the value of μ is higher (16,100). The occurrence of a "break" in the temperature relation at about the temperature of the normal environment is not at all unusual in such data; it is planned in another connection to consider its significance.

because μ gives a constant covering a wide range of temperatures, which the commonly employed " Q_{10} " does not, but also because the physical meaning of μ as a "heat of activation" holds out the prospect of classifying protoplasmic processes when investigated from this standpoint.

SUMMARY.

Pedal progression of the slug *Limax maximus* was studied to obtain relations between wave velocity on the sole of the foot, wave frequency, the advance due to a single wave, and the velocity of vertically upward creeping. Each of the first three quantities is directly proportional to the simultaneous velocity of progression. Under comparable conditions, that is when work is done at a constant rate, the frequency of pedal waves is influenced by the temperature according to the equation of Arrhenius, with $\mu = 10,700$ (Q_{10} for 11° to $21^{\circ} = 2.1$). The velocity of a single wave must have very nearly the same "temperature characteristic," which is found also in another case of nerve net transmission (in *Renilla*).

BIBLIOGRAPHY.

- Arey, L. B., and Crozier, W. J., 1919, *J. Exp. Zool.*, xxix, 187.
 Arrhenius, S., 1889, *Z. physik. Chem.*, iv, 226.
 Bethe, A., 1903, *Allgemeine Anatomie und Physiologie des Nervensystems*,
 Leipsic.
 Biedermann, W., 1905, *Arch. ges. Physiol.*, cvii, 1.
 Car, L., 1897, *Biol. Centr.*, xvii, 426.
 ten Cate, J., 1923, *Arch. n  erl. physiol. homme et animaux*, viii, 377.
 Crozier, W. J., 1918-19, *J. Exp. Zool.*, xxvii, 359; 1923-24, xxxviii, 323; 1922-
 23, *Anat. Rec.*, xxiv, 398.
 Davenport, C. B., and Perkins, H., 1897, *J. Physiol.*, xxii, 99.
 K  nkel, K., 1903, *Zool. Anz.*, xxvi, 560.
 Olmsted, J. M. D., 1917-18, *J. Exp. Zool.*, xxiv, 223.
 Parker, G. H., 1911, *J. Morphol.*, xxii, 155; 1914, *Contributions from the Bermuda
 Biological Station for Research, No. 31*, iii; 1917, *J. Exp. Zool.*, xxii, 111;
 1917-18, *J. Exp. Zool.*, xxiv, 139; 1918-19, *J. Gen. Physiol.*, i, 231; 1920,
Proc. Am. Phil. Soc., lix, 171; 1921, *J. Exp. Zool.*, xxxiii, 205.
 Peyrega, E., and Vl  s, F., 1913, *Bull. Soc. zool. France*, xxxviii, 251.
 van Rijnberk, G., 1918-19, *Arch. n  erl. physiol. homme et animaux*, iii, 539.
 Vl  s, F., 1907, *Compt. rend. Acad.*, cxlv, 276; 1908, *Bull. Soc. zool. France*, xxxiii,
 175; 1913, *Bull. Soc. zool. France*, xxxviii, 242.
 Vl  s, F., and Bathellier, J., 1920, *Compt. rend. Acad.*, clxxi, 1083.