CONTRIBUTIONS TO OUR KNOWLEDGE OF THE BIOLOGY OF MACHILOIDES DELANYI WYGODZINSKY AND CTENOLEPISMA LONGICAUDATA ESCHERICH (HEXAPODA THYSANURA).

by

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"I am a firm believer that, without speculation there is no good or original observation".

Charles Darwin, letter to A.R. Wallace, 22nd December, 1857.

CONTENTS:-

1. RESUME.

Among the South African Thysanura, the order Lepismatoidea has spread over the whole sub-continent, while all except one species of the order Machiloidea are confined to the discontinuous forest belt which lies below the escarpment of the inland plateau. The Machiloidea are not, however, strictly confined to the actual forests within their geographical region, some species invading regions of considerable aridity.

Investigations on the ecology, water relations and orientation behaviour of a representative species of each order have been carried out. These have revealed that:

(i) the physical conditions in the typical niche of the Machiloidea are extremely stable, whereas those in the habitat of the Lepismatoidea are subject to some considerable fluctuation.

(ii) the Lepismatoidea are more resistant to desiccation than the Machiloidea; in both cases this resistance is due in part to physical barriers in the cuticle and partly to an active metabolic process.

(iii) the Machiloidea rely on their eversible vesicles, situated on the abdominal coxosternites, for the uptake of water which cannot be drunk, such as a thin film of water or soil capillary water.

(iv) the Lepismatoidea are able to absorb water from a sub-saturated atmosphere.

(v) the behavioural responses of both in respect of humidity, temperature, light and gravity, are such as to keep them in conditions within the range of their physiological limitations.

From these results it is concluded that the Machiloidea can survive outside the shelter of forests, provided that water is readily available in some form in which it can be absorbed by the animals. The general implications of the results are such as to permit the erection of an hypothesis explaining the distribution of the Thysanura in South Africa in terms of the availability of

water. The results also lead to speculations on the evolution of the Pterygota.

2. INTRODUCTION.

Among zoologists the phrase "well adapted to the land habitat" has almost become synonymous with "locust" or "Cockroach". The Arthropoda are without doubt the most successful of the terrestrial invertebrates and in the myriapod-hexapod line the progressive evolution of adaptive characters to meet the full rigours of the land habitat has culminated in the Pterygota. The Hexapoda are now generally accepted as being descended from a Symphyla-like ancestor (Imms, 1936). The Symphyla, together with many of the primitively apterous hexapods, are animals confined to the equible conditions which obtain amongst the litter and humus of forest floors, and such a habitat must be regarded as the ancestral home of most of the terrestrial arthropods. While much of the success of the Pterygota is generally, and quite justifiably, attributed to the evolution of flight, physiological adaptations must have been of considerable importance in enabling the ancestors of the Pterygota to leave the sheltered habitat of the forest cryptofauna for another, where conditions were such as to permit the evolution of flight, and also in allowing them to survive in the harsh climatic conditions outside the forest.

The Thysanura occupy a unique position among the Hexapoda. They represent the closest living relatives of the Pterygota, but have not evolved the ability to fly. Their relationship to the Pterygota has been established beyond doubt on morphological grounds and, of the two orders comprising the subclass, the Lepismatoidea show the closer affinities to the Pterygota (Snodgrass, 1938; Barnhart, 1961), the Machiloidea having retained many of the characteristics of their Symphyla-like ancestor.

The distribution of the Thysanura in Southern Africa, as shown in Figure 2.01, reveals a marked divergence between the two orders. Southern Africa can be broadly divided into two climatic regions:- a high inland plateau, for the most part treeless and becoming more arid as one moves westward, and a coastal belt with a more even climate. The coastal belt, which

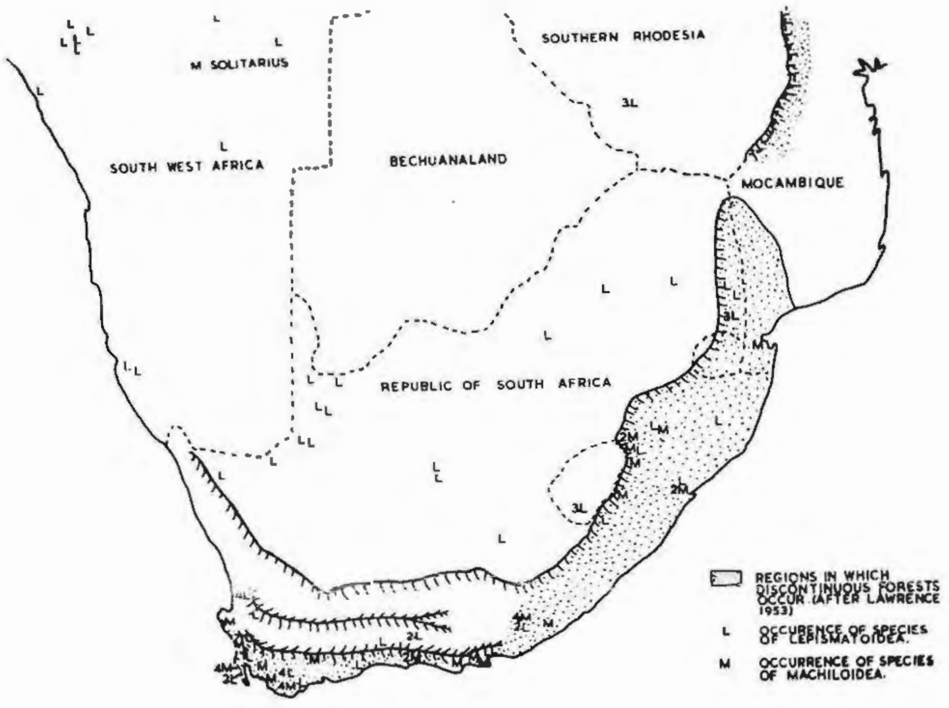


Fig. 2.01: Map showing the distribution of the Machiloidea and Lepismatoidea in Southern Africa (from data in Wygodzinsky, 1955 and personal collections).

lies below the escarpment of the inland plateau supports remnants of once more wide-spread indigenous forest and is referred to by Lawrence (1953) as the discontinuous forest belt. The Lepismatoidea are a wide-ranging group; they have established themselves throughout the subcontinent even to the extremes of the Namib desert (Lawrence, 1959). This implies physiological and behavioural adaptations to the extreme rigours of the terrestrial environment, adaptations not present in the Machiloidea, which are, with one doubtful exception (Machiloides solitarius Silvestri), confined to the discontinuous forest belt. Preliminary investigations on a number of species of Machiloides, the dominant genus of the Machiloidea in Southern Africa, showed that these animals are not only better adapted to terrestrial life than are many of the more typical cryptozoa of the forest floor, but also that within their geographical range they can occur outside the shelter of the forests and even in extremely arid conditions.

A study of the terrestrial adaptations manifested in these two thysanuran orders might well serve to elucidate not only their own geographical distribution but also the evolution of terrestrial adaptations in the Pterygota. The investigation has used three lines of approach:

(i) Ecological, since it is necessary to know the conditions under which the animals live.

(ii) Physiological, since adaptations to the habitat must be ascertained, and

(iii) Behavioural, since the animals need the correct responses to environmental stimuli to keep them within the limits of their physiological capabilities.

The present study of the terrestrial adaptations shown by these two thysanuran orders was undertaken in the belief that it might not only allow us to comprehend the basis of their geographical distribution, but also aid in our understanding of the evolution of terrestrial adaptations in the Pterygota.

An investigation of this nature can initially, of course, only be carried out on one species representing each order, and it must be stressed that the conclusions reached can only be regarded as tentatively valid for the two orders as a whole. Conflicting or contrary evidence may follow when other species are studied in detail.

3. MATERIAL.

Availability was the chief factor governing the choice of experimental material, a species respresenting each of the two orders comprising the sub-class Thysanura being required. Since no other investigations of the character of the present work have been carried out, these animals must initially be regarded, within reasonable limits, as typical of their respective orders.

31. Species Used.

Although at least four species of the genus Machiloides occur in the Grahamstown area, Machiloides delanyi Wygodzinsky (Wygodzinsky 1960) was chosen as representing the Machiloidea, by virtue of its relative abundance. Ctenolepisma longicaudata Escherich (Wygodzinsky 1955) was regarded as representing the Lepismatoidea, though as a widespread household pest, it inhabits a somewhat atypical niche. Although other Lepismatoidea do occur in the Grahamstown area, these are not readily come by in sufficient numbers for an investigation of this nature.

3.2. Habitats of the experimental animals.

Machiloides delanyi has been collected from a number of localities in the vicinity of Grahamstown. It is confined to areas where moisture is fairly readily available, such as the indigenous forests, wooded stream beds, poplar groves, pine forests and even in the so-called "False Karroo" to the North-west of Grahamstown. In the latter region, however, it is only found in the proximity of streams and stock watering dams. Although the animals occur in regions of relative moisture abundance, the actual niche occupied by them is usually dry in comparison with the surroundings. For example, in indigenous forests they are seldom encountered in the moister litter layers but occur in their greatest abundance among the loose rock rubble of a talus slope. In such a habitat there are also found, in the Grahamstown area, three other and undescribed species of Machiloides; it is also the characteristic habitat of Petrobius brevistylus in Great Britain (Delany 1959) and of various species of Machilis, Dilta, Trigoniophthalmus and others in Switzerland (Wygodzinsky 1941). Indeed, the German common name of "Fehlsensprînger" indicates that the animals have long been recognised as petrophils.

Ctenolepisma longicaudata is a household pest, widely distributed throughout both the tropics and the temperate regions of the Old World. Specimens have been collected from old books, cardboard containers and in almost any locality indoors which is relatively undisturbed. These animals are also frequently encountered in baths and hand-basins in bathrooms and in laboratory glassware stored in cardboard boxes, having been trapped by the smooth walls in each instance.

Other Lepismatidae have, during the course of this investigation, been found in a number of localities ranging from open grassland to the semi-desert Karroo. These were always found under stones. The wide range of habitats occupied by the Lepismatidae would seem to find its extreme in the harsh conditions of the Namib desert, where Lawrence (1959) reports a lepismatid species as forming the primary industry in most of the food chains.

3.3. Laboratory Culture Methods.

(i) Machiloides delanyi.

The animals were housed in pairs in plastic dishes measuring 18 x 8 x 4 cm; larger numbers seemed to increase the mortality and made handling more difficult. The shallow dishes greatly facilitated handling which was frequently necessary, both for experimental purposes and for cleaning. Owing to the smooth surface of the dishes, the floor was lined with paper to allow the animals to walk normally and a few stones of suitable size were arranged in the dishes for shelter.

All Machilidae feed on the thallophyte flora encrusting the rocks or growing on the bark of trees in their habitat. They probably also feed on the fungi which abound in forest litter, since they can also be fed on living yeast cells (Wygodzinsky - personal communication). A food supply was provided by stripping bark, supporting a good thallophyte flora, from trees, soaking these pieces of bark in water overnight and allowing the animals to feed on the microflora. The pieces of bark served the dual purpose of a culture medium for the food plants and also the provision of additional shelter for the animals. Under adverse climatic conditions, bark was stripped from trees known normally to support a good growth of algae and lichens; the pieces pieces of bark were soaked overnight in Bristol's Solution and kept under conditions of high humidity for about a fortnight. This treatment produced a good growth of microflora. The animals were found to be very selective in their feeding, rejecting a large number of thallophyte species and acceptable food sources were ascertained only by trial and error. The animals scrape the microflora from the bark together with decaying bark cells and other indigestible material. All this material is indiscriminately ingested, the faeces being composed largely of undigested plant cell remains. The intake of such material is considerable when judged by the rapidity with which the bark is stripped and the quantity of faecal matter produced.

Water was provided by moistening the stones in the culture dishes daily and occasionally placing one or two drops of water on the strips of bark in the dishes. Care was necessary, however, not to allow the humidity in the culture dishes to become too high, since high humidities both render the animals prone to attack by fungi and also apparently affect the facility with which they clear the old integument during ecdysis. The cause of the latter has not been investigated but it has been found that failure to moult effectively was invariably fatal.

In order to keep fatalities to a minimum it was found necessary to clean all faeces from the culture dishes at least every third day and to provide each dish with a fresh paper lining and food supply on these occasions. Once every two weeks the culture dishes and stones were washed in a disinfectant (½% solution of chlorocresol in water) and exposed to the sun for a few hours.

(ii) *Ctenolepisma longicaudata*.

These animals were housed in plastic dishes similar to those already described. Shelter was provided by placing crumpled filter paper in the dishes. The animals were found to show no adverse reaction to fairly high population densities in culture dishes.

Although C. longicaudata was found to subsist quite adequately on a diet of filter paper alone with an occasional watering, they were fed on dried oats with the addition of a pinch of brewers yeast in order to keep them in the best possible condition. Water was provided by means of a cottonwool wick inserted in a small specimen tube filled with water. Whilst no close watch on the humidity was needed for the welfare of the animals themselves, it was found that a Mucor-like fungus established itself on the food supply and the filter paper if the humidity remained high for too long.

These animals suffered neglect without any apparent ill effect and did not require the frequent cleaning of culture dishes which proved essential to the successful maintenance of M. delanyi under laboratory conditions.

4. THE AUTECOLOGY OF MACHILOIDES DELANYI AND CTENOLEPISMA LONGICAUDATA.

From the foregoing it is clear that the Machiloidea are not only restricted in their geographical distribution but are also confined to certain protected localities within their geographical range. The physiological limitations restricting the distribution of the Machiloidea must bear some relation to the conditions obtaining generally in their normal environment and particularly in the actual niche which the animals occupy in such an environment. A comparative study of the habitats of M. delanyi and C. longicaudata was therefore made with a view to establishing what physical or biotic factors limit the distribution of the Machiloidea. Since C. longicaudata inhabits a somewhat atypical niche, the conditions under which other Lepismatoidea are normally found in the field were also investigated; this would probably give a better measure of the field conditions normally experienced by the Lepismatoidea.

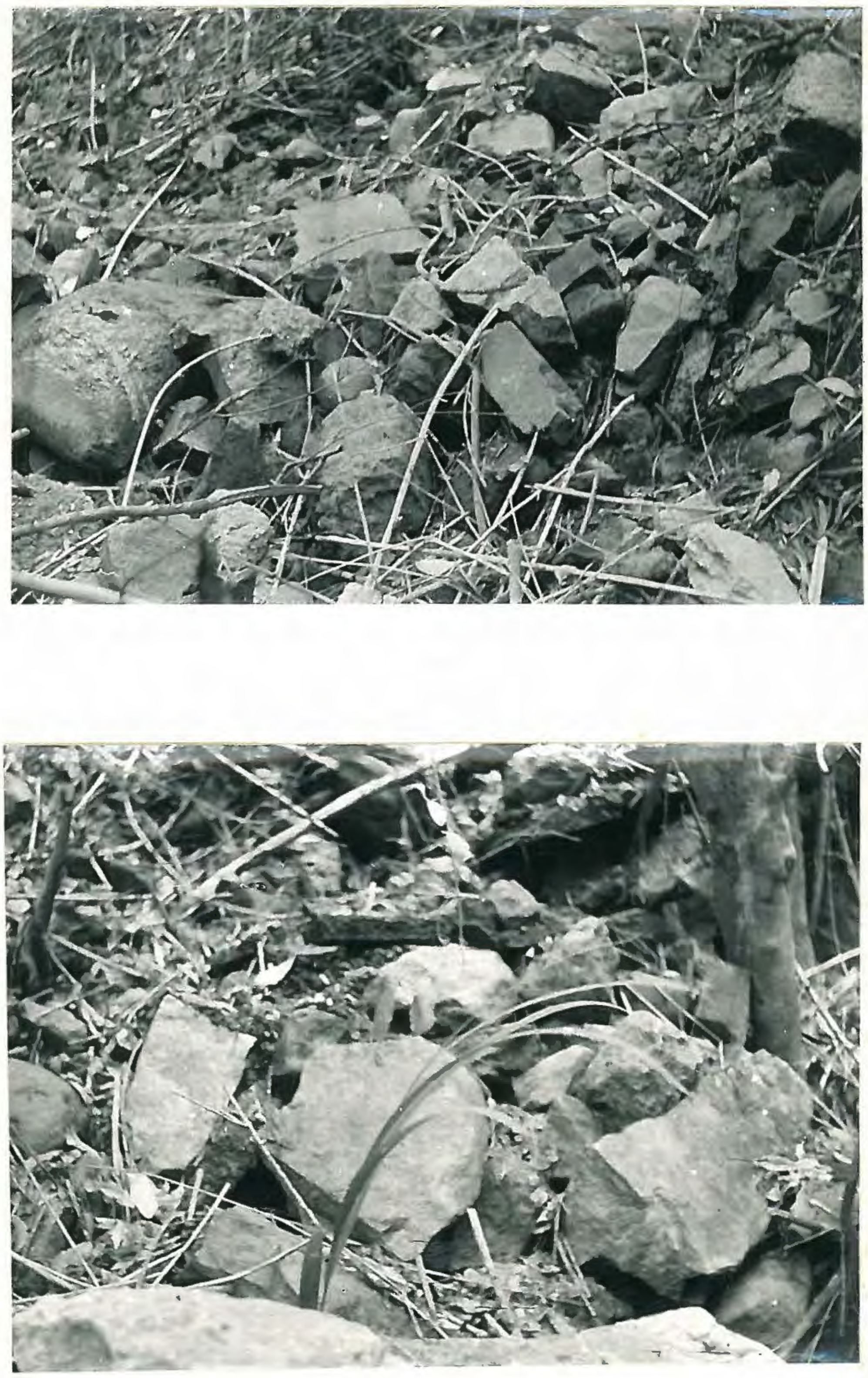
Three factors suggest themselves for investigation;

1. Topographical and edaphic factors which concern mainly the habitat of M. delanyi,
2. Climatic factors, of which both temperature and humidity could be of importance,
3. Biotic factors with particular reference to food, enemies and competition with other organisms.

4.1. Topography and Edaphic Factors of the Habitats.

The habitat taken as typical for M. delanyi was a well-wooded stream valley; one side of the valley comprises a boulder strewn slope, the rocks being loosely packed on one another and housing comparatively large numbers of M. delanyi in the interstices between the rocks. Such a locality can justifiably be regarded as a typical habitat of this species since it corresponds in most details with other regions where the animals have been found in abundance. Plates 4.01 and 4.02 show the general features of the terrain within the valley.

The stratigraphy of the valley floor essentially comprises



Plates 4.01 and 4.02:- A typical habitat of *Machiloides delanyi*.

three layers; the substratum consists of a black, clay-like loam which is covered by a humus layer up to four centimeters deep while a layer of loose leaf litter overlies the whole of the valley floor. The tallus slope, comprising one bank of the valley, consists of boulders of various sizes packed loosely on one another forming shallow pockets on the surface which may be filled with humus and/or litter. Between these boulders there is a labyrinth of interspaces which form the main ecological niche occupied by M. delanyi. The depth of the boulder layer varies from one meter near the bottom of the valley to approximately 30 cm. near the top.

Specimens of M. delanyi have been found in their greatest abundance clinging to the undersurfaces of the rocks and are thus located in the interstices between the boulders of the slope. They also occur amohg the litter overlying the rocks when this is dry and to some extent on the sides of stones embedded in the litter, usually very near to the surface of the litter layer. Their occurrence in the litter in regions where stones are absent is rare and they have never been found in the moister humus layer.

C. longicaudata has been found to occur in a number of localities in the laboratory. Reprint files and stationery boxes which have been left undisturbed for some time may house a number of these animals and the conditions within such files have been regarded as possibly representing those normally encountered by this species. However, since the animals occur quite commonly in many localities within the laboratory, the only apparent essential being that they be left undisturbed, it is difficult to designate them to a definite niche.

A pile of rubble, comprising chiefly large fragments of broken concrete piping and located in open grassland, has been found to house a few specimens of an undescribed species of Ctenolepisma. This was thought to correspond closely to the field conditions generally encountered by the Lepismatoidea and was thus included in the comparative study.

4.2 Climatic Conditions in the Habitats Investigated.

Apparatus and method:

Temperature measurements were, in all instances, made with the aid of mercury thermometers. Ecologists are generally prejudiced against the use of thermometers for microclimatic measurements; this objection is based mainly on the size of the instrument and its tendency to conduct heat away from the site of measurement along the stem. Whilst it is conceded that these considerations may greatly influence the results where the micro habitat is a small confined space, the use of thermometers in the measurement of air temperatures in the comparatively large interstices between the boulders of the talus slope seemed justifiable in the absence of more accurate field measuring equipment. Where used to measure the temperature in the reprint boxes, the whole thermometer was inserted into the box, thus the objection does not apply in this instance. Care was necessary to ensure that the air temperature among the rocks was actually being measured and to this end a rubber collar, two centimeters in diameter was fitted around each thermometer, just above the bulb. This ensured that the bulb was not in contact with the surrounding rocks at any stage. Temperature maxima and minima were recorded using standard maximum/minimum thermometers.

Three means of measuring relative humidity were available for use in this investigation, namely wet/dry bulb thermometers, Edney paper hygrometers and cobalt thiocyanate papers, prepared according to Solomon (1956). Each method was found to have its own merits and demerits for humidity determinations in the various situations required. The determination of the relative humidity in the interstices between the boulders of the talus slope was, after a preliminary investigation, carried out using wet and dry bulb thermometers, which had the advantage of enabling readings to be taken without the lengthy period of equilibration necessary for the other methods. The main objection to the use of wet and dry bulb thermometers in confined spaces is that there may be an increase in the humidity within the microhabitat due to the evaporation of water from the wet muslin enclosing the wet bulb. In order to assess the suitability of wet/dry bulb thermometers for use in the interstices between the rocks, the relative humidity here was measured using all three methods available. The relative humidity was determined as being in the region of 90% on the day preceding the test. Three Edney hygrometers were calibrated for 90% relative humidity over a saturated solution of magnesium sulphate at 20°C and placed at three sites among the rocks. Five cobalt thiocyanate papers, housed in open-ended pieces of glass tubing, the ends of which were closed by means of wide-mesh nylon net, were similarly distributed among the rocks, both at the sites where the paper hygrometers were located and elsewhere. The hygrometers and cobalt papers were now allowed six hours to attain humidity equilibrium with their surroundings, after which the relative humidity at all sites and at five additional sites was measured using wet and dry bulb thermometers. These comprised matched pairs of thermometers, one of which had its bulb enclosed in a piece of wet muslin. The thermometers, which were fitted with rubber collars as previously described, were inserted individually into the region where the measurement was to be made, the dry bulb measurement being made first. It was now possible to effect a comparison between the three methods of humidity determination with the following results:

Wet/dry thermometers:	90% ± 1.5%
Edney paper hygrometers:	89% ± 1.0%

Cobalt thiocyanate papers: between 90 and 93.5%.

Wet/dry bulb thermometers were therefore suitable for use among the rocks. Edney hygrometers calibrated at 90% and 96.5% were used in the determination of the relative humidity in the litter and humus layers respectively; these instruments were also used for the determination of the relative humidity in filing boxes, in which case they were calibrated against a whirling hygrometer in the laboratory.

Results:

Figures 4.01 to 4.04 show the results of the recording of climatic conditions in the various habitats and their immediate

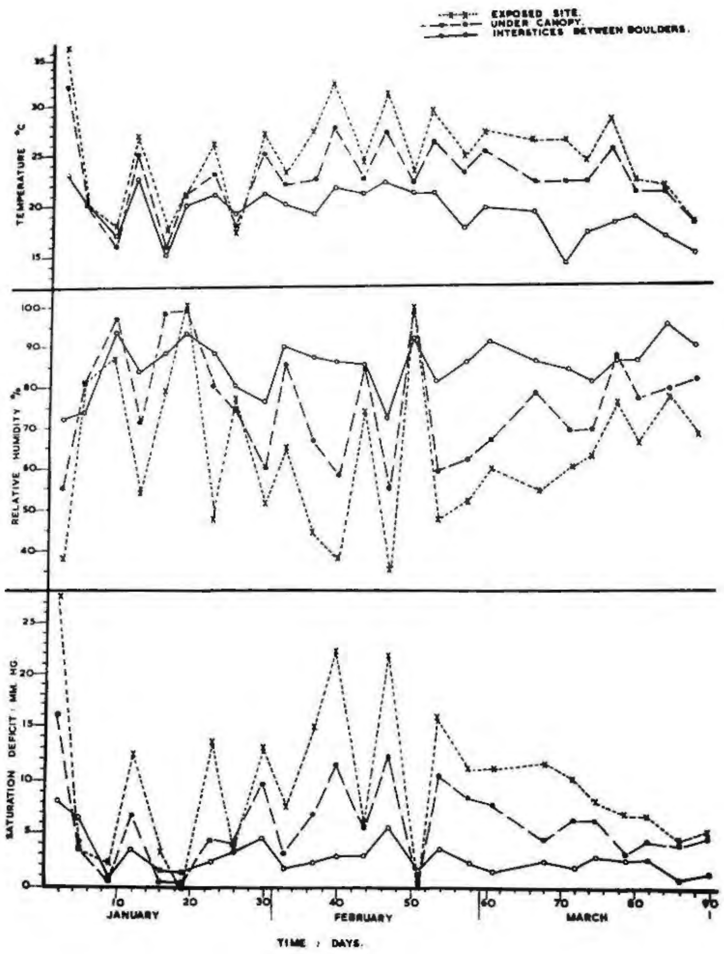


Fig. 4.01 : Conditions of air temperature, relative humidity and saturation deficit obtaining in an exposed site near the wooded valley, under the forest canopy within the valley and in the interstices between the boulders forming the talus slope within the valley.

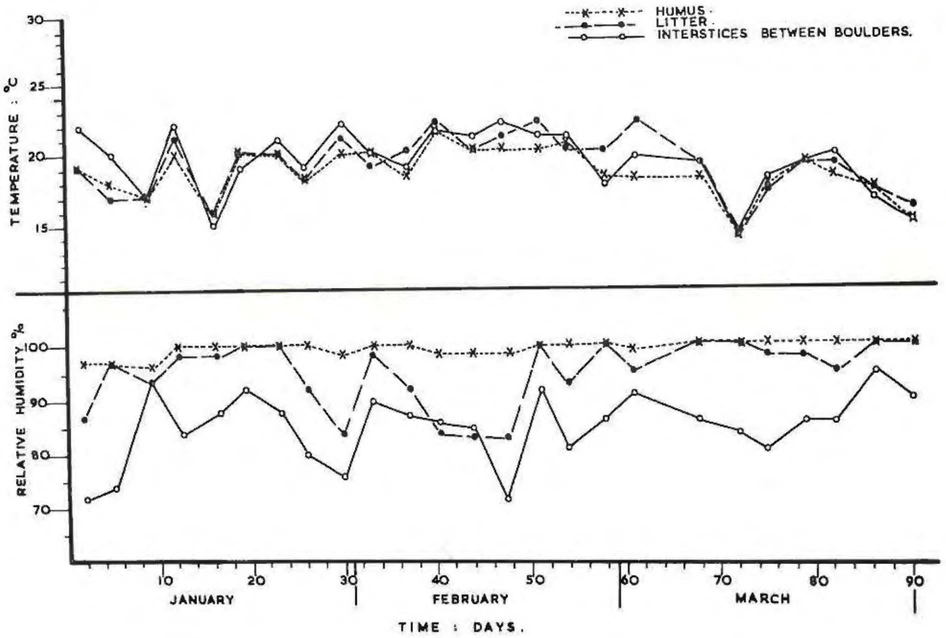
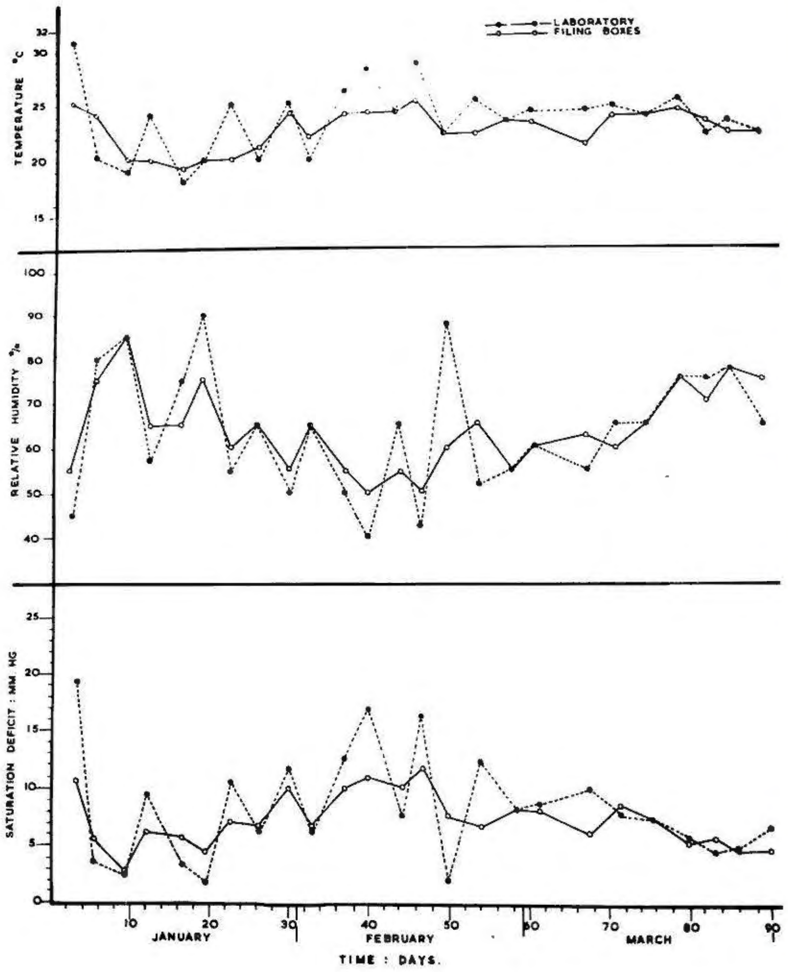


Fig. 4.02 : Conditions of temperature and relative humidity obtaining in the humus and litter layers within the valley compared with the interspaces between the boulders forming the talus slope.



<u>Fig. 4.03</u> : Conditions of air temperature, relative humidity and saturation deficit obtaining in reprint files and boxes compared with the general conditions in the laboratory.

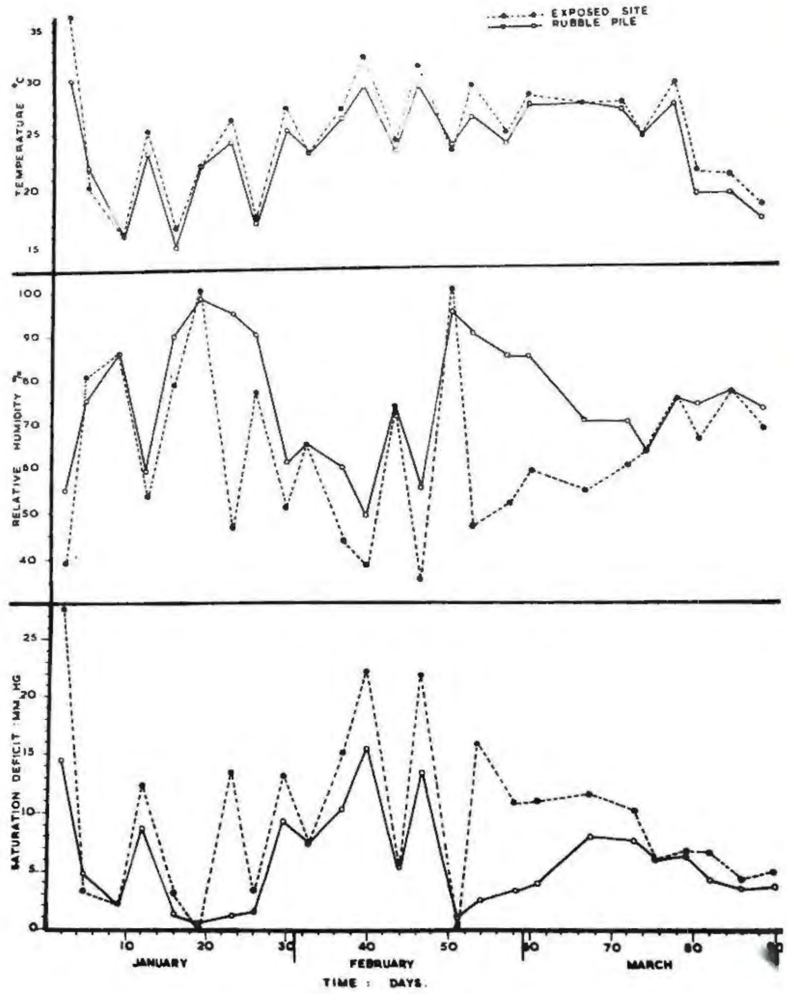


Fig. 4.04 : Conditions of air temperature, relative humidity and saturation deficit obtaining in the interstices between concrete rubble piled in an exposed site compared with the general conditions of the surroundings

surroundings and these are summarised in Table 4.01.

Table 4.01: Summary of the climatic conditions obtaining in the microhabitats and surroundings of M. delanyi, C. longicaudata and an unidentified species of Ctenolepisma, based on recordings made at 2 p.m., twice weekly, over a period of three months.

	Temperature (°C)		Relative Humidity (%)		Saturation Deficit (mm Hg)	
	Mean	Range	Mean	Range	Mean	Range
M.delanyi						
Microhabitat	19.3	14-23	85	72-94	2.7	0.7-8.0
Surroundings	22.5	15-32	75.6	55-98	5.7	0.2-16.0
Litter layer	19.1	14-21	94.8	83-100		
Humus layer	18.5	14-21	99.2	96-100		
C. longicaudata						
"Microhabitat"	22.4	19-25	64.6	50-85	7.4	2.6-11.8
Surroundings	23.4	18-31	64.0	40-90	8.2	1.7-19.4
Ctenolepisma sp.						
Microhabitat	23.3	15-30	74.8	49-98	5.8	0.3-15.4
Surroundings	24.6	18-37	62.5	35-100	10.3	0.0-27.6

Conditions in the humus and litter layers and in the interspaces of the talus slope all show a remarkable degree of constancy when compared with their surroundings. These three habitats owe their stability, at least in part, both to the protection afforded by the vegetation canopy as shown in Figure 4.01 and to their generally protected situation within the valley. This latter consideration is well illustrated by measurements made, using an anomometer, of the wind velocities, at stations outside and within the valley. The maximal wind velocity, recorded at a height of 1.5 meters above ground level in an exposed site adjacent to the valley, was 220 meters per minute while the wind speed at a comparable height within the valley was only 34 meters per minute. Furthermore, the wind velocity within the valley was found to diminish nearer ground level, falling off to three meters per minute at ten centimeters above the ground with no measurable wind at ground level. Changes in the temperature and relative humidity of the surrounding air will, therefore, have only a small effect on conditions in the microhabitats, such effects being dependent largely on conduction, convection and diffusion in the absence of marked air movements.

That the humus-litter complex on a forest floor provides a stable environment has long been recognised (Allee et al, 1949, Lawrence, 1953). The humus has marked water retaining properties, acting as a sponge which soaks up moisture during rain showers and subsequently liberates this water very slowly in the form of water vapour. The litter forms a covering blanket over the humus and, as such, further reduces the rate of evaporation by maintaining a near saturated atmosphere over the humus; it also serves to insulate the underlying layers from the ambient temperature. These factors, together with the protection afforded by the canopy and the physical features of the terrain, give rise to climatic conditions in the humus and litter which are subject to only small fluctuations in response to substantial weather changes in the surroundings. The interstices between the boulders of the talus slope provide an equally stable habitat, as shown in Figure 4.02. The substratum at the bottom of the interstices usually contains a quantity of humus and litter which will retain moisture and the rocks form an overlying cover which serves to insulate the interspaces from changes in the ambient climatic conditions. That the overlying stones provide added protection against extremes and thus contribute to the uniformity of the microhabitat is reflected in the temperature maxima and minima recorded in the valley and in the interspaces of the talus slope; the greatest range recorded among the stones was 6°C. (17 - 23°C), over a period of 24 hours when the range within the valley was 16°C (17 - 33°C).

Owing to the larger air spaces and, presumably, better circulation of air between the rocks, the relative humidity is usually lower than in the humus and litter layers. It may, at times, be even lower than that of the surroundings since very light showers do not penetrate into the interstices and even heavy showers have not been shown to raise the relative humidity here above 93%.

The habitat of M. delanyi is therefore characterised by a degree of climatic stability comparable with that of the humus and litter layers, which together form the habitat of the bulk of the forest cryptofauna; it differs from these habitats, however, in having a consistently lower relative humidity and also affords some protection against actual wetting. Whilst lower than that of the humus and litter, the humidity in the interstices shows but little fluctuation when compared with that of the surroundings and is consistently high enough to keep the saturation deficit of the air at a level where water loss from animals inhabiting the horizon would not be excessive. That M. delanyi avoids the moister regions of its environment is clearly shown by records kept of the apparent moistness or dryness of the locality in which this species has been found in the field; out of 68 animals recorded, only five were found under obviously moist conditions, the remainder having been found to occupy apparently dry situations. Kühnelt (1961) also describes the Machiloidea as "..found regularly in dry forests between leaves and stones". Since the temperature conditions in the litter and humus layers correspond very closely to those of the microhabitat of M. delanyi, the above strongly suggests relative humidity as being a contributing factor in the habitat selection of this species and probably of the order as a whole, since other Machiloidea occupy comparable microhabitats.

Conditions in the microhabitat of C. longicaudata, shown in Fig. 4.03, were found to be extremely variable, particularly in the case of relative humidity. It must be remembered, however, that the habitat was disturbed each time temperature and humidity was recorded, thus the fluctuations may not normally reach the proportions reflected in these results.

The habitat of the field Ctenolepisma also showed considerable fluctuations corresponding to the changes in the surrounding climatic conditions (Fig. 4.04). A heavy downpour of rain would raise the relative humidity within this microhabitat for some days but light showers, which affected conditions in the valley considerably, had no lasting effect here. Temperature conditions, too, are much harsher here than in the habitat occupied by M. delanyi.

In addition to the summer conditions considered above, the temperature maxima and minima were recorded during winter. During a week of heavy frost the minimal temperature recorded in the valley was 4°C, whilst that among the boulders of the talus slope was 7°C.

4.3 Biotic Factors.

M. delanyi shares its microhabitat with relatively few other organisms. Cribellate spiders and less commonly a species of pleoarid bug seem to be the only predators occurring in this horizon; other than these, aggregations of a gregarious species of Psocoptera and a few mosquitoes form the sum total of the associated fauna. The litter layer of the valley floor, on the other hand, houses numerous spiders, chilopods and an occasional onychophoran in addition to large numbers of Collembola and Acarina. The humus layer accommodates an even more diverse fauna, the amphipod Talitrioides eastwoodae and a species of polydesmid diplopod comprising the large phytophagus species, whilst predators include Japygidae and scolopendromorph, geophilomorph and lithobiomorph chilopods.

The niche occupied by M. delanyi, therefore, not only provides conditions of minimal competition with other phytophagus organisms but also minimises the risk of predation. The spiders with which it shares its microhabitat are web-spinners rather than active hunters and it has been found that the scales provide some protection against becoming ensnared in spider webs, the animals simply shedding the scales which adhere to the web. The litter spiders, chilopods and japygids on the other hand, are all active hunters much more likely successfully to capture a machilid, particularly where the overlying litter would impair the animals' normal escape reaction of leaping.

The relatively moist and sheltered conditions in the habitat ensure an abundant food supply for the machilids. Most of the trees and rocks in the valley support a considerable growth of microflora which persists even in conditions of drought.

The biotic factors in the habitat of C. longicaudata are difficult to assess. Spiders seem to be the only obvious predators which occur in any abundance within buildings and competition for food with other organisms seems to be relatively unimportant. Other species of Ctenolepisma have been found under stones together with various other arthropod species, both carnivorous and phytophagus, but too little is known of their biology for any discussion.

5. WATER CONSERVING MECHANISMS.

Land animals, if they are to survive at humidities substantially below saturation, must conserve water. The ability to take up water in order to replace water lost in evaporation is however of primary importance. To establish itself successfully in a dry environment, where a source of replenishment is not available at all times, an animal further needs an impermeable cuticle. Among the terrestrial invertebrata only the Arthropoda have successfully evolved an impermeable integument in response to the need for water conservation; yet even here the degree to which this has been achieved varies from the relatively permeable integument of the Onychophora (Manton and Ramsay, 1937) and terrestrial Crustacea (Williamson, 1951, Edney, 1951, Dandy 1955) to the effectively waterproofed Pterygota. In the various sub-phyla which constitute the Arthropda, there are instances where the water conserving mechanisms have attained different degrees of efficiency in related classes and even orders and this can usually be related to their respective habitats. The work of Perttunen (1953) on the Diplopoda serves to illustrate this very well; Orthomorpha gracilis loses water very rapidly on desiccation and is confined to regions where the water loss incurred would normally be minimal, whilst the wide ranging Schizophyllum sabulosum has attained a high degree of impermeability.

Ecological studies have shown M. delanyi to inhabit a niche where conditions are such that water loss, due to evaporation, apparently never reaches a very high level; Ctenolepisma longicaudata and other Lepismatoidea, on the other hand, have to contend with much higher saturation deficits and marked fluctuations in climatic conditions. Some reflection of the very different conditions, which these two species normally encounter should, therefore, be apparent in their respective abilities to resist desiccation. An understanding of the mechanisms of water conservation of these animals may also contribute to our knowledge of the evolution of an impermeable cuticle by the

Pterygota, since the latter are generally accepted as the closest relatives of the Thysanura.

A study of water conserving mechanisms requires initially that the rate of water loss at a given saturation deficit be established; location and the relative importance of the sites of water loss and the factors affecting the rate of water loss can then be studied in order to learn something of the nature of the mechanisms involved.

5.1 Rate of Water Loss.

A comparative study of the rates of water loss in the two species under consideration was carried out under conditions of extreme desiccation. Preliminary investigations had revealed that M. delanyi was well able to withstand relative humidities of 0% for moderate periods of time and since such desiccation gives rise to easily measurable water losses, this method was again employed.

Apparatus and method:

The experimental animals were subjected to a standard pre-treatment before the commencement of an experiment. They were given free access to abundant food and water over a period of 48 hours, after which they were starved but given water for a further 48 hours. This treatment ensured that the animals were in good condition, being well fed, and also that the alimentary canal was empty, thus precluding the possibility of the animals defeacating during the course of the experiment and upsetting the results by virtue of the water lost with the faeces. A further period of 24 hours in a dry container ensured that most of the moisture adsorbed onto the animal had evaporated; in spite of this precaution the loss in weight during the initial period of desiccation invariably exceeded that for subsequent periods and it is suggested that this is due to evaporation of moisture trapped between the scales and between the overlapping sclerites of the body. The treatment outlined above was followed in both these and subsequent experiments concerned with water loss.

After pretreatment the animals were placed singly in clean, desiccator-dry specimen tubes which had previously been accurately weighed. Included in the weight of each tube were the weight of the material used to close the mouth of the tube and of an elastic band used to hold the latter in place; in the case of M. delanyi the tube mouth was closed by means of a piece of wide mesh nylon net but since C. longicaudata was found to be able to bite through this, plastic window gauze was used for tubes containing this species. The gauze, net and elastic bands were found to be non-hygroscopic and thus suitable for use in these experiments. Each tube was now closed and weighed again; it had been established that the handling involved in closing the tubes had no measurable effect on the weight and the weight of each experimental animal could therefore be accurately determined by subtracting the weight of the tube plus covering material from the weight of the tube plus covering material and experimental animal.

Desiccation of the animals was now effected over anhydrous calcium chloride in a standard desiccator; the desiccator was placed in a constant temperature room at 20°C, which ensured a constant saturation deficit of 17.5 mm. mercury. At intervals of eight and sixteen hours (9a.m. and 5 p.m.) the tubes plus contained animals were reweighed, thus establishing the weight of water lost during the intervening period by each animal and the loss in weight was expressed as a percentage of the body weight of the animal concerned.

Desiccation of M. delanyi was continued until a constant weight had been attained, thus giving a measure of the rate of water loss both before and after death of each animal. C. longicaudata, however, proved much more resistant to desiccation and would die as a result of excessive water loss only after prolonged exposure to a dry atmosphere. To obtain a comparison between the rates of water loss from living and dead specimens it was necessary to kill twelve specimens by means of coal gas and then to desiccate these as previously described.

The use of still air desiccators for experiments of this nature has been subject to some criticism. It has been pointed out that the rate of water loss from an animal in still air is both membrane limited and vapour limited, an humidity gradient being set up between the surface of the animal and the desiccant. The rate of water loss from the animal would therefore reflect not only the degree of impermeability of the cuticle (the membrane limited system) but also the resistance offered to the water molecules by a region of high humidity surrounding the animal due to evaporation (the vapour limited system). Ramsay (1935 a) has, however, pointed out that whilst this criticism is most certainly valid in the case of a large animal which loses water readily, as for example a newt, the vapour limited system may be negligible in the case where the area of the evaporating surface is small relative to the volume of the desiccator and where water is lost very slowly to the surroundings by virtue of cuticle impermeability. In the present experiments from four to six animals, each with a surface area in the region of 150 square millimeters, were housed in a desiccator with a capacity of 1.5 litre and the animals were found to lose weight very slowly; these considerations, together with the fact that the main aim of the experiments was to obtain data for comparative rather than absolute studies, minimises the importance of any errors due to the use of a still air desiccator, provided that temperature conditions were maintained constant for all. A more serious criticism which may be levelled against the use of desiccators as described above, is the disturbance of the humidity within the desiccator when it is opened to remove the tubes for weighing. It was found that the desiccator only re-established its dry atmosphere after about 45 minutes, depending, of course, on the ambient relative humidity. For this reason the animals were only weighed twice daily, thus keeping the number of times the desiccator had to be opened to a minimum. This factor had to be borne in mind in later experiments where the effects may have been significant but in the present comparative study all animals were subjected to the same fluctuations.

Results:

The rate of water loss from M. delanyi was found to be far lower than expected for an animal confined to regions of high relative humidity in a protected microhabitat and also to show a high degree of variability, some specimens losing water rapidly and succumbing after a comparatively short period of desiccation, whilst others displayed remarkable resistance to desiccation. Figure 5.01 shows the mean rate of water loss for a random sample of 12 specimens of M. delanyi compared with those for the longest and shortest lived specimens included in the sample. Since the three rates were to be compared, the best straight line to fit the coordinates was statistically determined in each instance, so as to facilitate subsequent analysis; the regression of the percentage water loss with time, in respect of both the longest lived and the shortest lived animals, differed significantly from the mean for the sample, the probability of the difference being due to chance being less than 0.001 in both cases.

C. longicaudata proved more resistant to desiccation than M. delanyi and also showed less variability in rate of water loss. The longest lived specimen in the sample showed a possibly significant difference from the mean, the probability of the difference being due to chance being less than 0.05.

Both species showed definite evidence for an active control over the rate of water loss in that the rate increased sharply after death. Figure 5.03 shows the rate of water loss from four specimens of M. delanyi which had been desiccated to constant weight. Whilst the rate of water loss from the live animals showed considerable variability, there was, after death, increase to a higher rate which was consistent for all animals tested. Figure 5.04 shows the rate of water loss from 12 dead specimens of C. longicaudata compared with the twelve living specimens: the value for the individual having the highest rate of water loss is also shown. The dead animals again lost weight much more rapidly than did the living specimens; this contradicts the findings of Lindsay (1940) who claims to have

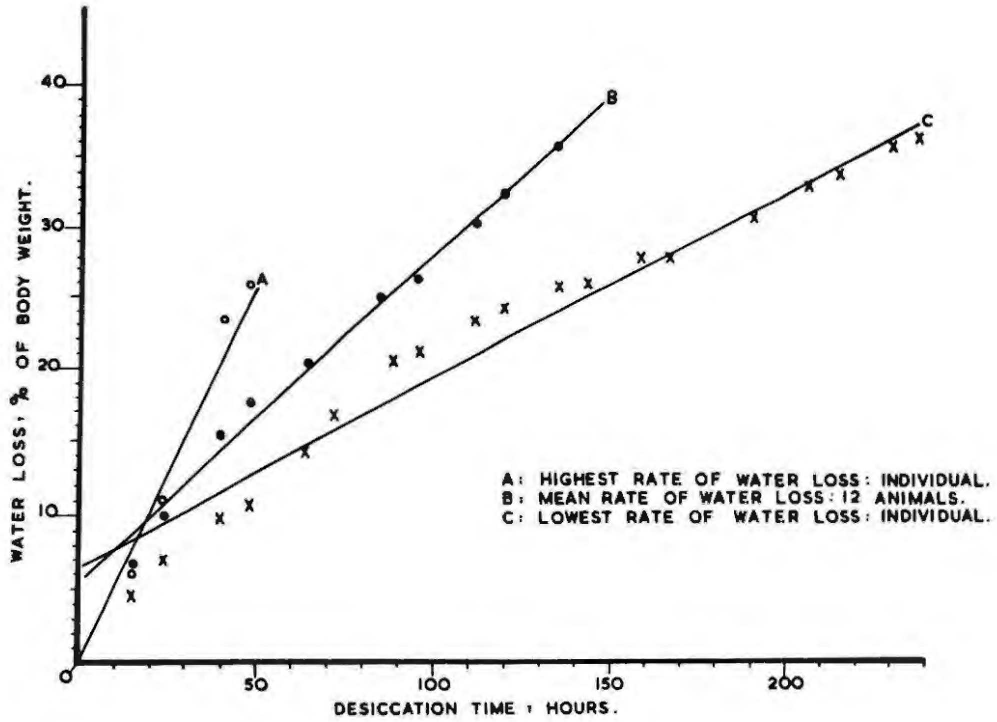


Fig. 5.01 : Rate of water loss from a random sample of living *M. delanyi* when desiccated over calcium chloride at 20°C.

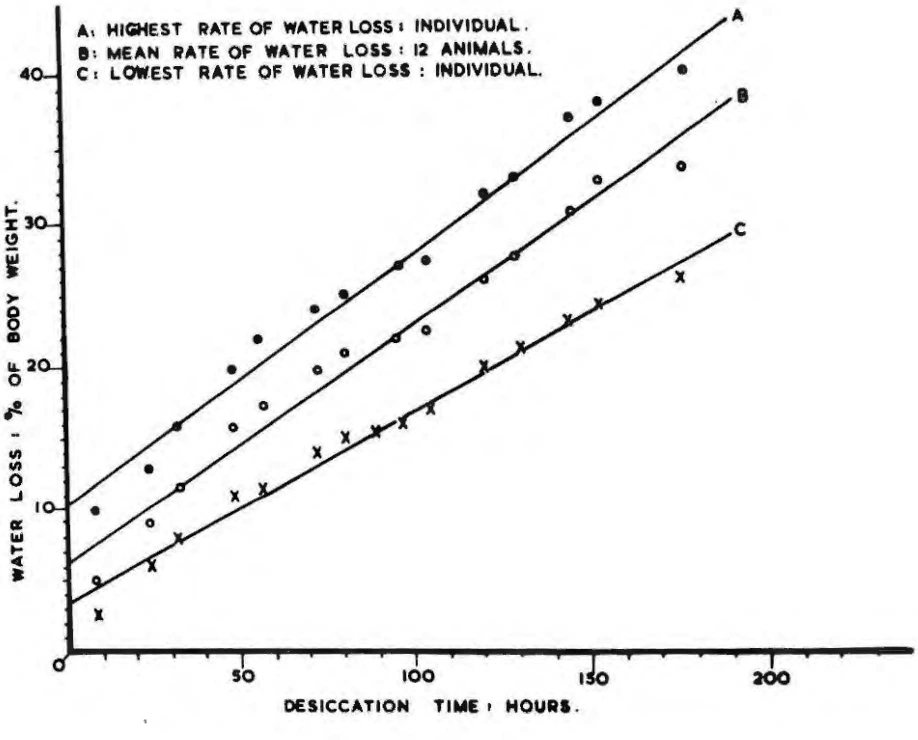


Fig. 5.02 : Rate of water loss from a random sample of living C. longicaudata when desiccated over calcium chloride at 20°C.

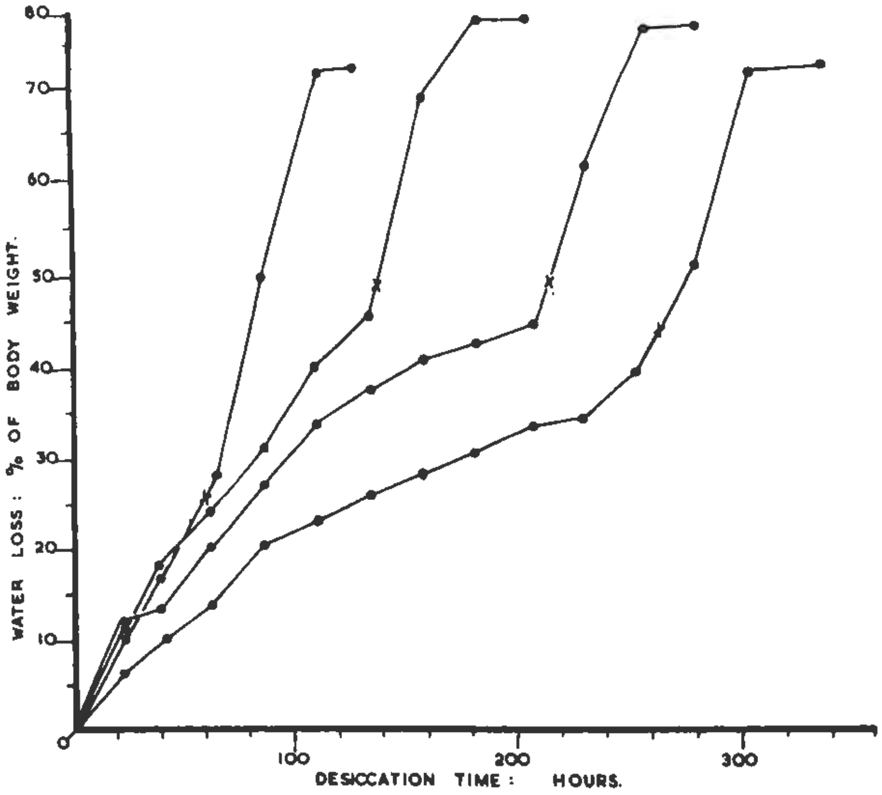


Fig. 5.03 : Rate of water loss from four specimens of *M. delanyi* when desiccated over calcium chloride at 20°C showing the change in the rate after death of the animals. X denotes the point where the animal died.

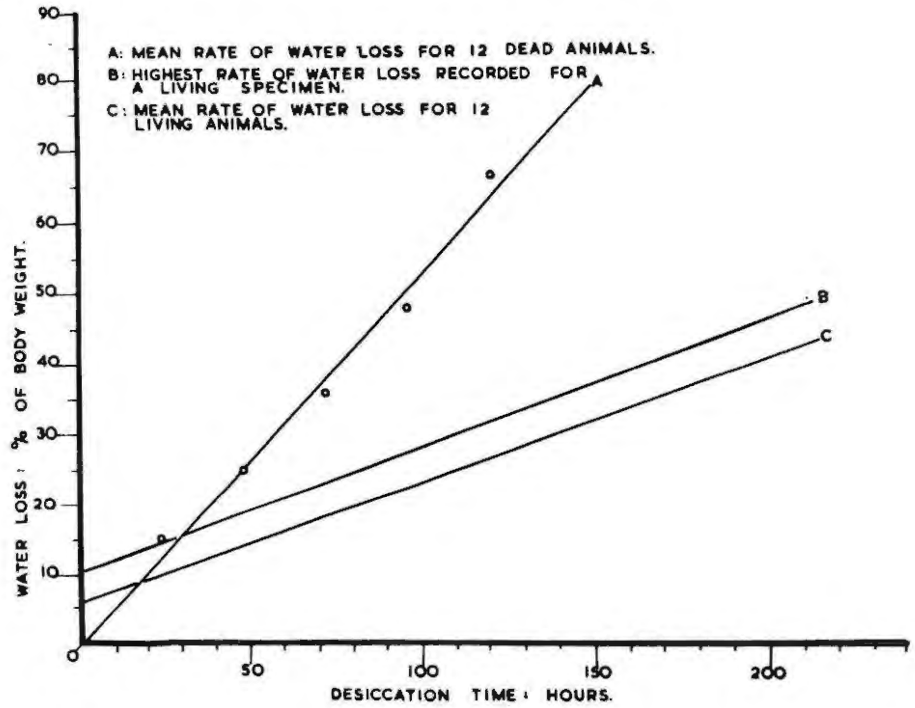


Fig. 5.04 : Comparison between the rates of water loss from living and dead specimens of C. longicaudata when desiccated over calcium chloride at 20°C. Coordinates for B and C are as shown in Fig. 5.02.

found no such difference between the rates for dead and living specimens of C. longicaudata. Lindsay's work cannot be regarded as critical, however, since it was based on the loss in weight of the whole biomass, i.e. the animal plus its food supply.

These experiments showed that whilst M. delanyi loses water at a very variable rate, it is able to withstand considerably more severe conditions of desiccation than those encountered in its typical habitat for considerable periods of time. Its ability to withstand desiccation was compared with that of the amphipod Talitroides eastwoodae, an inhabitant of the humus layer. This animal was found to survive desiccation over calcium chloride for a maximal period of 110 minutes, in which time 73% of its body weight had been lost; the mean survival time for a sample of this species was 70 minutes. The unexpected resistance to desiccation of M. delanyi has not been specifically reported for any other species, although Kühnelt (1961) describes all the machilids as being "....extraordinarily resistant to drying." C. longicaudata shows a greater ability to curb water loss than M. delanyi but is, in this respect, not comparable with such Pterygota as Rhodnius prolixus (Wigglesworth and Gillett, 1936) or Cimex lectularis (Mellanby, 1932) which both lose weight at a rate of approximately 1.5% of body weight per day at 0% relative humidity and 30°C.

The present findings are, to a large extent, in keeping with the habitat studies discussed in Part 4. M. delanyi inhabits a dry niche in an otherwise moist environment and shows a better developed water conserving ability than do typical inhabitants of the moister horizons in the same general environment. C. longicaudata, which in its normal habitat has to contend with higher saturation deficits than those encountered by M. delanyi, has, in turn, developed greater protection against excessive water loss. The ability to control the rate of water loss breaks down on death of the animals in both species, thus suggesting an active rather than a purely structural controlling mechanism.

5.2 Factors Affecting the Rate of Water Loss.

In order to establish more accurately the nature of the water conserving mechanisms, it was necessary to ascertain, as far as possible, the cause of the variability in the rate of water loss within each species. This aspect was best investigated in terms of the factors actually affecting the rate in both living and dead animals. Size and stage in instar both suggest themselves as possibly affecting the rate of transpiration through the cuticle, whilst use of excess carbon dioxide would assist in detecting spiracular control. The presence of a wax layer could also be of extreme importance in water conservation and its presence can be demonstrated by treating the cuticle with abrasives, wax solvents and by subjecting the animals to high temperatures which serves to disrupt the waxy layer in some manner. All these aspects have been investigated in respect of both Machiloides delanyi and Ctenolepisma longicaudata.

(i) Stage in instar.

The stage in instar was considered a possible factor influencing the rate of water loss through the cuticle, since the effective waterproofing of the cuticle may well take a number of days to complete. It had been observed in the preliminary investigation that ecdysis was invariably accompanied by a marked increase in the rate of water loss in both species but it had not been ascertained whether this was in fact due to the loss of moulting fluid when the old cuticle was shed or to an initially higher degree of cuticle permeability.

Apparatus and method:

The experimental animals were marked with a spot of white paint on the thorax, thus enabling any animal which had moulted to be easily recognised; daily examination of the animals in the culture dishes allowed those which had moulted to be isolated and a record of the date of moulting was kept for each individual. A number of animals were starved, with free access to water, for a period of 48 hours, commencing immediately after ecdysis. After a further 24 hours in a dry dish, these animals were desiccated as previously described, desiccation thus commencing three days after moulting. A further batch were allowed seven days feeding under normal culture conditions before being given the usual pretreatment so that, in this instance, desiccation was thus commenced tendays after ecdysis.

It was necessary to desiccate equal numbers from each batch in the same desiccator to ensure that the animals were subjected to the same humidity fluctuations brought about by opening of the desiccator. The experiments were carried out in a constant temperature room at 28°C in the case of <u>M. delanyi</u> and not at the normal 20°C, because of a fault in the thermostatic mechanism of the constant temperature room.

Results:

The results, which are summarised in Table 5.01, show a significant difference between the rates of water loss from three day and ten day animals in the case of <u>M. delanyi</u>. This difference is further reflected in the relative survival times under conditions of extreme desiccation. These animals are, therefore, less able to withstand desiccation shortly after moulting than later in the instar.

Table 5.01: Mean daily water loss, expressed as a percentage of body weight, from specimens of <u>M. delanyi</u> at three and ten days after moulting.

Experiment number.	No. of animals/ category.	3 days		10 days	
		Water loss	Survival time (hours)	Water loss	Survival time (hours)
1	4	14.1	62	11.2	102
2	5	18.6	55	12.0	102
3	1	16.0	32	10.3	96
4	5	20.8	38	12.0	93
Mean for whole sample:		17.4	47	11.5	98

The differences reflected in Table 5.01 are highly significant in respect of both rate of water loss and survival time; the probabilities of the differences being due to chance are less than 0.001 in both instances (F 1/22 = 23 for difference in water loss and 19 for survival time). The analysis further showed no difference between the results of individual experiments for both water loss and survival time (F 3/22 = 1.0 and 0.6 respectively) and it was therefore not necessary to ensure that equal numbers of animals in each category were housed in the same desiccator in future experiments, although this was still done wherever possible.

C. longicaudata showed no difference between the rates of water loss from animals desiccated three days and ten days after moulting. Table 5.02 shows the results obtained for this species; these show no significant difference between the rates (probability greater than 0.1 that the difference is due to chance, F 1/20 = 3.36) and all animals survived 168 hours desiccation

Table 5.02 : Mean daily water loss, expressed as a percentage of body weight, from specimens of C. longicaudata at three days and ten days after moulting. Mean daily water loss based on determinations for first 72 hours of desiccation only.

Experiment number	No. of animals/ category	3 Days Water loss	10 Days Water loss.
1	3	5.0	4.6
2	5	5.1	4.9
3	3	5.9	4.4
4	2	6.1	4.4
5	2	4.9	3.4
Mean for whole sample:		5.4	4.5

From these experiments it was clear that in further experiments on factors influencing the rate of water loss from

M. delanyi it would be necessary to use animals at the same stage in an instar. However, further experiments showed no differences between the rates of water loss from animals at seven, ten and twelve days after moulting and it was therefore made standard practice before using them for experimental purposes to keep animals until seven days after their first observed moult. C. longicaudata was used at any stage between three and ten days after moulting, the latter limit being imposed to preclude the possibility of the animals moulting during any experiments involving prolonged desiccation.

(ii) Size.

The effect of size on the rate of water loss could be significant if there is a major loss of water through the cuticle, since, assuming that the cuticle is uniformly permeable, the rate of transpiration would be higher in smaller specimens where the surface to volume ratio is highest. Since the data obtained in the preceeding experiment eliminates any effect of stage in instar, the results were analysed to determine whether there was in fact any correlation between size and rate of water loss.

The initial weight of each animal was taken as a measure of its size and, using the product-moment method, correlation coefficients for the association between size and mean daily water loss was calculated for each of the three day and ten day samples. The results of this analysis are shown in Table:5.03.

Table 5.03 : Results of a product-moment analysis to determine whether any correlation exists between size and mean daily water loss in M. delanyi and C. longicaudata.

Sample	No. of Animals.	Correlation coefficient (r)	$\frac{1}{2}\log_e\frac{1+r}{1-r}$ (z)	P*
M. delanyi				
3 days after moult	15	0.11	0.115	0.9
10 days after moult	15	0.192	0.197	0.5
C. longicaudata				
3 days after moult	15	0.213	0.214	0.5
10 days after moult	15	0.05	0.05	0.9

* P = probability of the association between size and mean daily water loss varying from random due to chance.

The results reflect no correlation between size and rate of water loss and it was therefore not necessary to match experimental animals for size in subsequent experiments. The absence of such a correlation cannot, however, be regarded as evidence precluding the general body surface as an important site of water loss; not only are animals small but the available size range was itself so small that any difference in the rate of transpiration which might be attributed to size difference would in all probability be masked by the normal variation in the rate of water loss.

This can be seen from the following calculation, based on the data obtained from a batch of nine specimens of M. delanyi, desiccated ten days after moulting:

Mean body weight per individual for sample	27.5 mg
Mean rate of water loss per individual	12% of body wt / 24 hrs.
Surface area calculated from measurement of a specimen weighing 27.2 mg (nearest approximation to mean available)	154.5 sq. mm.

Now, if all water were lost via the general body surface, then the mean rate of transpiration would be 3.3 mg per 154.5 sq. mm. or 0.0213 mg/sq mm.

Calculated surface areas for the largest and smallest animal in the sample were as follows:

Largest specimen weighing 37.2 mg, surface area = 228.1 sq.mm.

Smallest specimen weighing 13.6 mg, surface area = 95.12 sq.mm.

If we assume the rate of water loss per square millimeter to be constant, the largest and smallest animals would be expected to lose water at rates of 13.0% and 14.9% respectively. The observed range of variability in the rate of water loss for four of the animals, whose weights ranged from 34 to 37.2 mg, was found to lie between 7.0 to 19.5% of body weight per 24 hours. From this it is clear that the small difference of 1.9% between the largest and smallest animals which can be attributed to the size difference between the two, would most certainly not be apparent in any of the experiments.

These results were of value in that they obviated the necessity of matching experimental animals for size in further experiments.

(iii) Removal of cuticular scales.

The Thysanura continue to moult at regular intervals throughout their lives. The instars are short in the newly-hatched young of the Lepismatoidea but become progressively longer in older individuals until sexual maturity is reached, when, in the case of Ctenolepisma quadriseriata, the instars range from 18 to 36 days but remain fairly constant for each individual (Sweetman, 1951). M. delanyi adults have been found to moult once every 19 to 27 days whilst the values observed for C. longicaudata proved to be comparable with C. quadriseriata. Each moult provides the animal with a new layer of cuticular scales, which may, depending on the activities of the animal, be partially or almost completely lost during the course of the instar. It was thought possible, therefore, that the scales played some role in the water economy of the Thysanura which necessitates their frequent replacement. To this end a comparison between the rates of water loss of normal and descaled animals was effected for both species under consideration.

Apparatus and method:

Five days after moulting, the experimental animals were subjected to chilling in a refrigerator to induce a state of chill coma, after which the scales were removed from half the animals in each batch. Chilling was found to be the most suitable means for rendering the animals manageable; coal gas and ether both proved unsuitable since these often caused fatalities and on occasions affected the rate of water loss from normal animals which had not had their scales removed. The scales were removed by lightly brushing the animals with adhesive cellophane tape to which the scales would adhere but which did not stick to the cuticle. The remaining half of each batch were not treated in any way and were used as controls to ensure that chilling in no way affected the rate of water loss. Both batches of animals were allowed to recover at room temperature and were returned to their culture dishes where they were allowed food and drink for a period of 48 hours. After this period they were afforded the normal pretreatment prior to desiccation.

Results:

The results for both M. delanyi and C. longicaudata are shown in Table 5.04.

Since the means obviously correspond very closely, the results need not be subjected to statistical analysis, and there seems, therefore, to be no difference between the rates for normal and descaled animals. M. delanyi shows a slightly higher rate in descaled animals and the removal from the data in Table 5.04 of animal marked *. which has a markedly higher rate, reduced the mean for the normal animals to 8.2%, but an analysis of variance revealed that even here there was no significant difference between the two groups (F 1/9 = 0.22).

Table 5.04 : Effect of scale removal on the rates of water loss (% of body weight) from M. delanyi and C. longicaudata.

Batch No.	M. delanyi		C. longicaudata	
	Normal	descaled	Normal	descaled
1	10.9	7.0	4.8	4.7
	6.9	12.5	3.3	4.9
			4.6	5.3
2	8.2	10.3	6.3	5.4
	7.9	9.4	5.3	5.1
	13.9 *	6.4		
	6.8	7.4		
3			4.5	4.3
4	7.0	8.1	4.7	4.9
			4.5	3.8
5	8.8	9.9	3.7	4.4
	9.3	10.1	5.1	4.5
Mean	8.85	9.01	4.7	4.7

The scales of the two species play only a minor role, if they play any role at all, in the prevention of excessive water loss and may, in the absence of contrary evidence, be regarded as unimportant in this respect for the Thysanura as a whole. The function of the scales seems to be largely protective; the escape from spider webs by M. delanyi has already been mentioned and preliminary experiments carried out on normal and descaled animals showed that the normal animals very often, although not invariably, managed to escape from a web, whereas descaled animals were never able to do so. The scales also enhance the escape of the animals from direct capture by predators; shedding of the scales makes the animals difficult to hold in a grip, the shed scales acting as a fine-particle lubricant like graphite and causing the captive to slip from the restraining grasp if not firmly caught between the mouthparts of the predator at first attempt. If this is indeed the function of the scales, regular replacement would be necessary if the selective advantage is to be maintained and this is only possible where moulting persists in the adult.

(iv) Abrasion of the cuticle.

The impermeability of many pterygote cuticles is dependent upon the presence of an epicuticular wax layer. This has been adequately demonstrated by many workers, notably Ramsay, (1935 b) Wigglesworth, (1945) and Beament (1945); further evidence by other workers is adequately reviewed by Richards (1951). Both Wigglesworth (1945) and Beament (1945) have shown that abrasion of the cuticle of various highly impermeable insects greatly increases the rate of water loss from these animals and this increase in the cuticle permeability is attributed to the removal of the waterproofing lipid layer. The presence of such a lipid layer in the epicuticle of the two species of Thysanura under investigation could, if present, be demonstrated by such cuticle abrasion.

Apparatus and method:

The animals were subjected to scale removal and pretreatment as described for the previous experiment. After desiccation over calcium chloride in a constant temperature room at 20°C over a period of 51 hours, the mean daily rate of water loss being calculated for the final 48 hours thus eliminating the problem of adsorbed moisture. After this initial period of desiccation they were subjected to further chilling and each batch was divided into three groups. The first of these was rubbed over the dorsal surface with fine powdered aluminium, which has the property of not only abrading the cuticle but may also cause cuticular lipids to become adsorbed and thus remove the wax over a considerable area. The animals in the second group were gently rubbed over the dorsal surface with find sand paper which would abrade the cuticle severely, whilst the third group were used as controls to ensure that the second chilling in no way affected the normal rate of water loss. The animals were allowed to recover at room temperature, after which they were desiccated for a further 51 hours.

Results:

The results are shown in Table 5.05.

Table 5.05 : The effects of abrasion of the cuticle on the rate of water loss (% of body weight/24 hours) from *M. delanyi* and *C. longicaudata*.

Treatment and experiment no.		*M. delanyi*		*C. longicaudata*		
	No. of animals	Rate before abrasion	Rate after abrasion	No. of animals	Rate before abrasion	Rate after abrasion
Aluminium dust.						
1	3	8.7	8.5*	3+	3.3	3.4
2	2+	5.8	6.2	2	4.2	4.7
3	1	10.3	8.8	2	5.3	5.2
4	1	7.9	7.5	1	4.9	5.0
Sandpaper						
1	2	10.7	11.9*	3+	3.8	3.4
2	2+	5.1	5.4	1	4.3	4.2
3	1	7.9	8.0	2	4.7	4.6
Mean for abraded animals:		8.1	8.0		4.4	4.4
Controls						
1	2	10.3	9.4*	2+	2.3	2.5
2	1+	5.1	5.5			
3				2	2.7	2.7
4	2	7.9	7.3			
Mean for controls:		7.3	7.4		2.5	2.6

+ Desiccation carried out at 15°C owing to incorrect setting of thermostat.

* Wholly or partly based on a single 24 hour period of desiccation due to death of some or all of the experimental animals.

The foregoing results make the presence of a lipid layer in the epicuticle, comparable with that known to exist in the Pterygota, unlikely. Lower (1958) reports the presence of a very resistant lipoid layer as forming the outermost layer of the epicuticle of C. longicaudata but this does not appear to be homologous with the epicuticular wax of the Pterygota for reasons which will be discussed later.

(v) Temperature.

The effect of temperature on the rate of water loss from an arthropod may be threefold. The first and most important of these is an indirect effect; an increase in temperature, in the absence of a comparable increase in the absolute humidity, gives rise to an increase in the saturation deficit and thus to the evaporating power of the atmosphere surrounding the animal. It is not the intention to enter into a discussion on the validity of the "Saturation Deficit Law" as proposed by Buxton (1931) and Mellanby (1935), other than to point out that the temperature/humidity/evaporation relationship is an extremely complex one, particularly in respect of biological systems, as has been shown by Ramsay (1935 a). Although much has been written about the validity of the "Law", there is no a priori reason for rejecting the view that water evaporates from an animal at a rate proportional to the saturation deficit.

Temperature may also affect the rate of evaporation from an arthropod directly by altering the properties of the cuticle, particularly where a thermolabile epicuticular wax layer is present. Ramsay (1935 b) was able to show that water loss from a cockroach into dry air increased steadily with an increase in temperature up to a temperature of 30°C, after which there was a sharp increase in the rate. The initial steady increase could be attributed to an increase in the evaporating power of the air and the transition to the higher rate was suggested as being due to a change of state of the lipoid material covering the cuticle thus changing the permeability of the cuticle as a result. Beament (1945) has put forward the hypothesis that the lipid imparting the greatest degree of impermeability to the cuticle is an "orientated and closely packed monolayer" arranged next to the epicuticle. The physical nature of the transition is envisaged as a thermally induced mobility of these lipid molecules which disrupts their "orientated and closely packed" arrangement, producing a random and normal distribution of the molecules and consequently an increase in the permeability of the cuticle, which is not reversable (Beament, 1954, 1961 a) since the original organised arrangement is not re-established on cooling. Cloudsley-Thompson (1950), Edney (1951) and Mead-Briggs (1956) have, however, all shown that temperature has an effect on the permeability of some Myriapoda which have no demonstrable epicuticular wax layer. It was necessary, therefore, to investigate the effects of temperature on the rate of water loss from M. delanyi and C. longicaudata in spite of the absence of epicuticular wax.

Edney, (1956) and Mead-Briggs (1956) have pointed out that an increase in temperature also increases the diffusion rate in the vapour limited system and Mead-Briggs has in fact corrected his results to compensate for this effect. Beament (1961 a) questions the validity of the correction, derived from an equation developed by Jeffreys (1918 quoted in Ramsay, 1935 a), on the grounds that it applies to an entirely vapour limited system in still air whilst Mead-Briggs' results are for a system both membrane and vapour limited in slowly moving air. Mead-Briggs' results show the correction to be necessary only for temperatures in excess of 35°C, however and since the temperature range covered in the present experiments falls below this, no compensation for increased diffusion was attempted.

In order to establish whether temperature does have any effect on the cuticle permeability of the Thysanura it was necessary to determine whether a constant relationship exists between the rate of water loss and temperature at constant relative humidity and, if so, whether any changes in permeability of the cuticle are apparent when the temperature increases at constant saturation deficit.

Apparatus and method:

The effect of temperature at constant relative humidity was investigated by desiccating the animals over a solution of potassium hydroxide, prepared according to the data provided by

Solomon (1951); the method of preparation will be discussed in Part 7. Experimental animals were pretreated in the usual manner and desiccation was carried out at 7°C, 12°C and 20°C on three consecutive days for one batch of animals; a further batch was desiccated over the same solution at temperatures of 25°C, 30°C and 34°C. The water loss during the 24 hours at each temperature was determined by weighing as before.

In order to determine whether temperature affected the rate of water loss in any way other than increasing the evaporating power of the atmosphere in the desiccator, the experimental animals were also desiccated over solutions giving approximately constant saturation deficit over the temperature range. The relative humidities used at each of the various temperatures were as shown in Table 5.05 and these were achieved by means of potassium hydroxide solutions prepared according to Solomon (1951).

Table 5.05 : Details of solutions used to give a near constant saturation deficit over a wide temperature range.

Temperature	*M. delanyi.*		*C. longicaudata.*	
	Relative humidity (%)	Saturation deficit (mm Hg)	Relative humidity (%)	Saturation deficit (mm Hg)
7°C	0	7.51	-	-
12°C	25	7.89	0	10.52
20°C	55	7.89	40	10.52
25°C	70	7.16	55	10.69
30°C	75	7.96	65	11.12
33°C	80	7.55	70	11.32

Each batch of animals was desiccated for periods of 24 hours each at three different temperatures, desiccation being carried out over the appropriate solution in each instance.

Results:

The results showed that for both species the rate of water loss increases as the saturation deficit increases, as shown in Figure 5.05; there is no sharp increase in the rate indicating

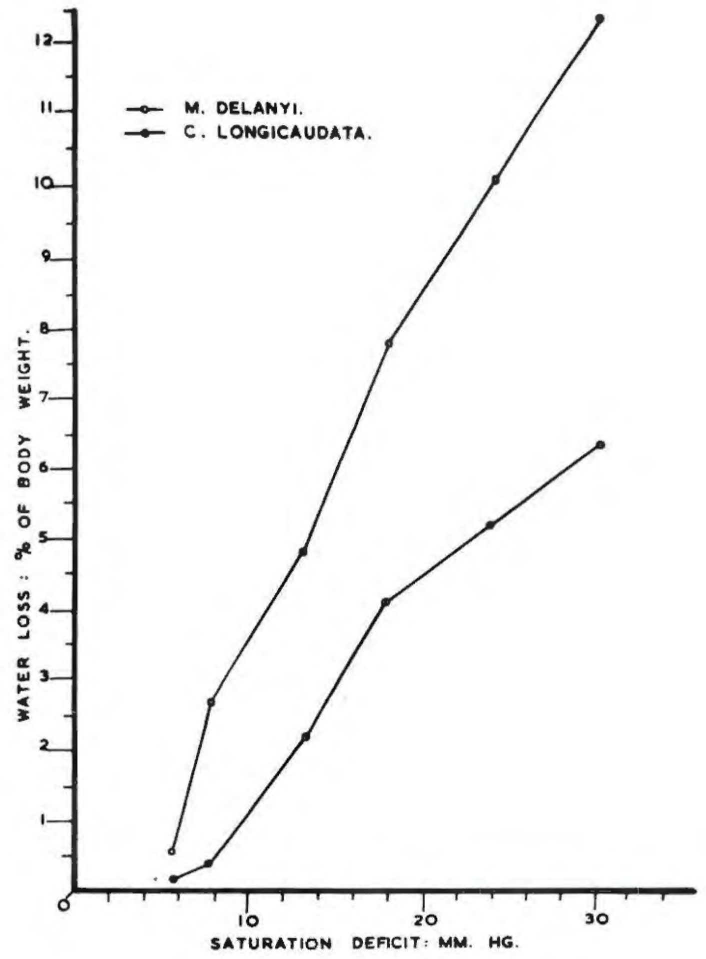


Fig. 5.05 : Effect of increasing saturation deficit, brought about by increasing the temperature at constant relative humidity, on the rates of water loss from living specimens of M. delanyi and C. longicaudata.

a transition within the temperature range tested for M. delanyi, although C. longicaudata shows an apparent transition above 12°C. This latter phenomenon can possibly be explained by the ability of C. longicaudata to absorb moisture from an undersaturated atmosphere, as will be discussed later.

Table 5.06 shows the rate of water loss at different temperatures under conditions of constant saturation deficit.

Table 5.06 : Rate of water loss (% of body weight per 24 hours) from M. delanyi and C. longicaudata at different temperatures and constant saturation deficit.

Temperature.	M. delanyi.		C. longicaudata.	
	No. of animals.	Mean rate	No. of animals.	Mean rate.
7°C	8	2.50	-	-
12°C	8	2.50	10	1.98
20°C	8	2.60	9	1.85
25°C	11	2.67	9	2.17
30°C	11	3.43	9	2.57
33°C	11	3.84	9	2.71

There is a slight increase in the rate of water loss at the higher temperatures in the range as shown in Figure 5.06, and this may be indicative of a change in permeability due to temperature increase. Such a change may be the result of the effect of temperature on lipids impregnating the procuticle as in some Myriapoda (Blower, 1951) although the presence of such impregnating lipid material has not been demonstrated in C. longicaudata by Lower (1958). Perhaps a more plausible explanation lies in the general inaccuracy associated with the use of potassium hydroxide solutions for obtaining a given relative humidity. This is discussed in detail in Part 7; small inaccuracies in the relative humidity will not seriously affect the saturation deficit where the saturation vapour pressure is low but as the temperature increases, so does the saturation vapour pressure and a small inaccuracy in relative humidity may give rise to a

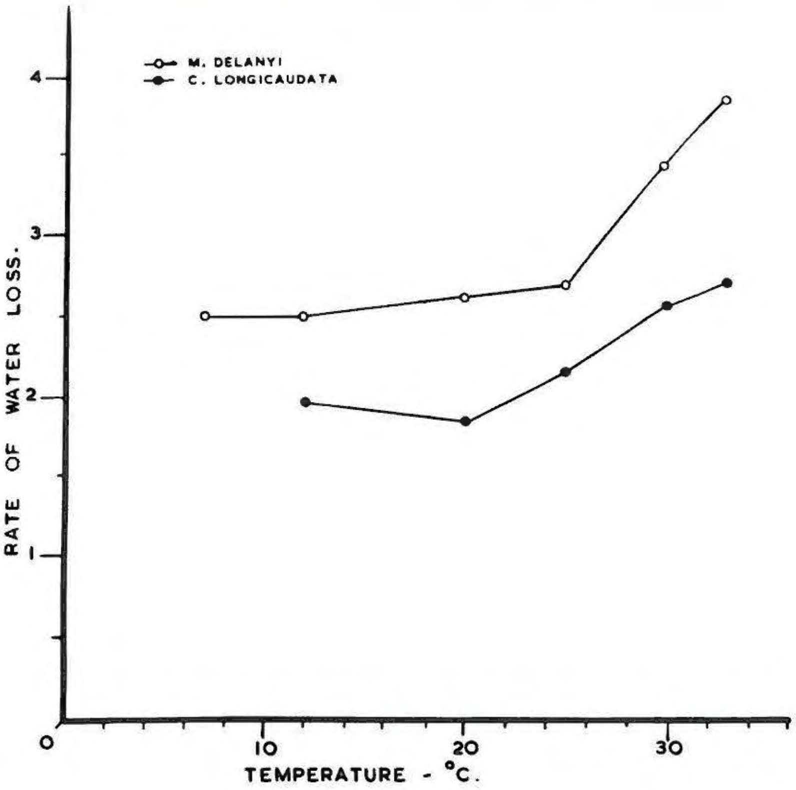


Fig. 5.06 : Effects of temperature on the rates of water loss from living specimens of M. delanyi and C. longicaudata at approximately constant saturation deficit.

substantial difference in saturation deficit, these differences becoming more marked at higher temperatures.

It is of course possible that a transition takes place at a temperature higher than the range covered by the above experiments; the investigation of this would require using dead animals and was not done owing to the scarcity of material.

(vi) Wax solvents.

Experiments carried out in order to establish the relative importance of the cuticle and tracheal system as sites of water loss suggested the possibility of an acetone soluble factor being present in the cuticle of M. delanyi. In view of the results obtained in the previous experiment, it was thought that this factor might possibly be some lipid material which confers a certain degree of impermeability to the cuticle. If a lipid was in fact present in the cuticle, the treatment of dead animals with a wax solvent such as chloroform should increase the rate of water loss.

Apparatus and method:

Experimental animals were pretreated as before, killed, descaled and dipped into chloroform for a period of ten seconds. After treatment the animals were ventilated until the smell of chloroform was no longer detectable and the animals were desiccated for a period of twelve hours over calcium chloride.

Results:

The results are shown in Table 5.07.

Whilst the chloroform had no effect on the rate of water loss from C. longicaudata, there was a marked increase in the rate for M. delanyi, indicating the presence of a chloroform and acetone-soluble waterproofing principle in the cuticle of the latter. Such a principle could conceivably be lipid in nature and by virtue of its solubility in cold acetone would be a simple or a derived lipid (Casselman 1959). The apparent change in the permeability of the cuticle of M. delanyi when subjected to high temperatures may therefore well be due to a change in the state of lipids impregnating the cuticle. The absence of any such effect on the cuticle of C. longicaudata confirms that for this species the epicuticular lipids reported by Lower (1958) are not extractable by wax solvents in the cold.

Table 5.07: The effects of immersion in chloroform on the rate of water loss (% of body weight per 12 hours) from M. delanyi and C. longicaudata.

Species	Specimens treated with chloroform.		Untreated specimens	
	No. of animals	Rate of water loss	No. of animals	Rate of water loss
M. delanyi	9	40.1%	8	16.4%
C. longicaudata	5	7.8%	12	7.5%*

* Calculated from 24 hourly rate.

(vii) Excess carbon dioxide.

The foregoing experiments have all centred on water loss through the cuticle but this does not constitute the only site of water loss from the animals. Mellanby (1934) has stressed the now well-known fact that since the respiratory surfaces must perforce be permeable to water, these constitute the main site of water loss from the Pterygota; the ability to occlude the spiracles is therefore of extreme importance in the water economy of these animals. The duration of the period during which the spiracles are open depends on the partial pressure of carbon dioxide present in the tracheal system and Wigglesworth (1936) has shown that in the case of the flea Xenopsylla the spiracles will remain permenently open in air containing 2% carbon dioxide. Wigglesworth and Gillett (1935) have also found that Rhodnius prolixus, which is normally very resistant to desiccation, will die after about three days in a dry atmosphere if its spiracles are kept open by administering 5% carbon dioxide.

Although no spiracular closing mechanism has been described for the Thysanura, it was considered necessary, in view of the marked difference between the rates of water loss for living and dead animals, to investigate the effect of excess carbon dioxide on the animals under consideration.

Apparatus and method:

It was found to be impossible to administer the excess carbon dioxide in a still air desiccator such as that described for the previous experiments and it was therefore necessary to devise an airflow desiccator which would allow the required admixture of carbon dioxide to the dry air. Such a desiccator had to meet the following requirements:

1. The air flowing through the apparatus must be at the same temperature as that of the constant temperature room in which the experiment is carried out (20°C).
2. The air flowing through the desiccation chambers must be dry, comparable with that in a still air desiccator.
3. The air passing through the desiccation chamber must constitute a regular flow.
4. The velocity of the air stream must be the same for each desiccation chamber.

The apparatus used is shown diagrammatically in Figure 5.07 The main airflow was obtained from the laboratory compressor via an outlet in the constant temperature room. The air stream was passed through a motorcar radiator inside the constant temperature room, thus bringing the air into temperature equilibrium with the interior of the constant temperature room. On leaving the radiator, the airstream was partially dehydrated by the calcium chloride in desiccator D.1 and passed, via a cottonwool dust filter (FI.1) and a flowmeter (F.1) to the main desiccators (D.3 - 5) where dehydration was completed. From the desiccators the air was led through a further dust filter (FI.2) to the distributor, which served to divide the airstream between four desiccation chambers. The carbon dioxide source was a pressure cylinder fitted with a reducing valve to give better control over the rate of flow; the cylinder was housed inside the constant temperature room and the gas was found to be at only a

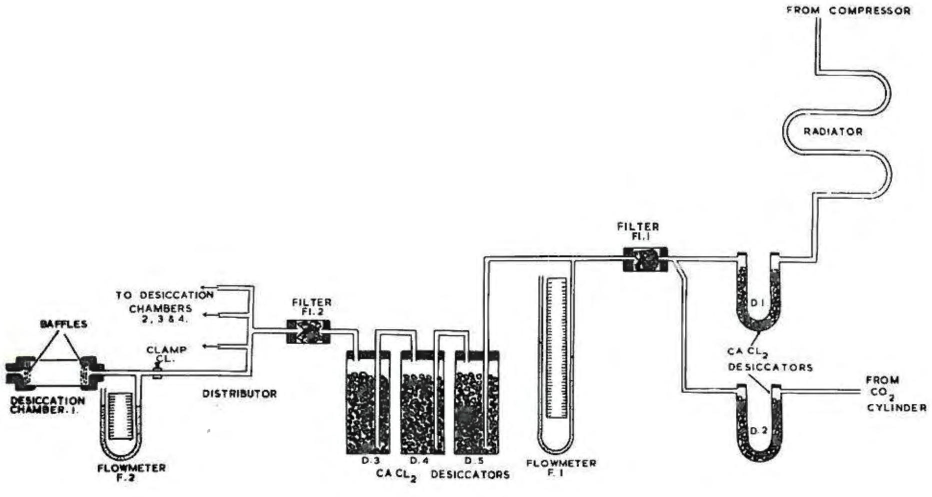


Fig. 5.07 : Airflow desiccator used to administer excess carbon dioxide during desiccation.

slightly lower temperature than that of the room on leaving the cylinder; its passage through the apparatus was sufficient to bring it into temperature equilibrium. After partial dehydration in desiccator D.2, the carbon dioxide joined the main stream at the dust filter FI.1, which served not only to ensure that no calcium chloride particles were carried over into the flowmeter from desiccators D.1 and D.2 but also assisted in mixing the air and carbon dioxide streams.

The desiccation chambers each comprised a length of glass tubing measuring 6 x 1.5 cm. and closed at each end by means of inverted vaccine bottle tops, each of which had a hole bored through its centre to allow a throughflow of air. The air stream entering the chamber was broken up by means of a baffle consisting of a wad of open-mesh nylon gauze and each chamber was equipped with a flowmeter to measure the rate of flow of the airstream through the chamber.

A thermometer inserted into desiccator D.6 confirmed that the temperature of the air in the desiccator was in fact the same as that of the interior of the constant temperature room. The effectiveness of the baffles could be demonstrated in two ways. The cotton wool was removed from filter FI.2 and tobacco smoke was gently blown into it. This smoke was then immediately forced through one of the chambers by a slow flow of air, the remaining three chambers having been isolated from the apparatus by closing the clamps CL. The smoke was observed to pass evenly through the chamber and to fill it completely; it could be assumed therefore that there was a regular flow of air through each chamber. Further, a thin strip of cobalt zinc chloride paper was inserted into each chamber and the apparatus set into operation; these papers took on a blue colour which was even along the length and diameter of the tube, indicating an even distribution of the dry air. By comparing these papers with control papers from the same strip which had been kept in a still air desiccator for a period of four hours, it was ascertained that, as nearly as could be established, the air passing through the desiccation chambers was as dry as that in the still air desiccators used.

In order to ascertain whether each chamber was in fact receiving the same volume of air per unit time and, more important, whether the carbon dioxide was completely mixed in the air stream, the desiccator was set up to give a moderate flow of air (7 cm mercury on flowmeter F.1) containing approximately 10% carbon dioxide. The airflow through each chamber was adjusted by means of the clamps CL to give a reading of two millimeters of its respective flowmeter. A solution of calcium hydroxide was prepared by hydrolysing 5.7 grams of calcium oxide in one litre of distilled water; four volumes of 100 ml. each of this solution were accurately measured into 150 ml beakers. The gas from the outlet of a desiccation chamber was bubbled through one of these solutions for a period of 30 seconds, after which the precipitated calcium carbonate was filtered out of the solution. 50 ml. of each of the filtrates was now titrated against an approximately 0.1 N. solution of hydrochloric acid, using methyl orange as an indicator. Since both the calcium hydroxide and the hydrochloric acid used were identical for all four gas samples, the same volume of gas passing through each of the chambers in 30 seconds should result in equal volumes of hydrochloric acid being required to bring about the colour change in each titration. Titrating the hydrochloric acid against the original calcium hydroxide solution required 14.2 ml. HCl; the results obtained for the filtrates after the calcium carbonate had been removed were 9.7, 9.2, 9.0 and 9.5 ml HCl respectively, showing that each chamber was, in fact, receiving nearly the same volume of carbon dioxide per unit time as the others, although the two outer chambers received slightly less.

In order to minimise the errors due to possible differing conditions between the desiccation chambers each animal was desiccated without and with an admixture of carbon dioxide to the air stream. This obviated the necessity of running a control experiment and thus reduced the variability in the results. The desiccation procedure was as follows:

1. Pretreated animals were weighed and placed singly in the

desiccation chambers

2. The compressed air was turned on and regulated to give a reading of 6 cm mercury on the flowmeter F.1.
3. The rate of flow through each desiccation chamber was regulated by means of the clamps CL to give a reading of 1 mm on each flowmeter F.2.
4. Desiccation was continued for a period of 24 hours, after which the animals were weighed again.
5. The animals were returned to their respective desiccation chambers and the airflow was regulated to give a reading of 5.4 cm mercury on the flowmeter F.1. Carbon dioxide was led into the apparatus, the flow being regulated by means of the reducing valve on the cylinder to bring the total gas pressure at flowmeter F.1. up to 6 cm once again.
6. The rate of flow through the desiccation chambers was checked but these were never found to require readjustment.
7. After a further 24 hour period of desiccation in the air + carbon dioxide mixture, the animals were weighed again and the results for the two periods compared.

Results:

The results, shown in Table 5.07, showed that carbon dioxide had no effect on the rate of water loss from M. delanyi and C. longicaudata.

Table 5.08 : The rates of water loss (% of body weight per 24 hours) from M. delanyi and C. longicaudata in moving air, before and after an admixture of excess carbon dioxide.

Species	No. of animals	Rate of water loss in air stream	
		$- CO_2$	$+ CO_2$
M. delanyi	8	8.56	8.33
C. longicaudata	8	4.30	4.27

The foregoing, whilst showing that carbon dioxide has no effect on the rate of water loss, cannot be taken as proof positive that no mechanism exists for closing the spiracles. Whilst such a mechanism seems unlikely for C. longicaudata, a possible means of closing the spiracles of M. delanyi will be discussed later.

5.3 The Sites of Water Loss.

Water may be lost from the haemocoel and tissues of terrestrial arthropods via the cuticle and via the respiratory surfaces. Where the cuticle has not been effectively waterproofed in some manner, considerable water may be lost over the general body surface; those arthropods which have achieved a measure of waterproofing of the cuticle usually incur the greater proportion of their total water loss via the tracheal system and of the tracheate arthropods most, if not all, fall into the latter category. However, even in poorly waterproofed animals such as the terrestrial crustacea, the integument always forms a barrier against the evaporation of body water, while in the better adapted terrestrial arthropods, it always allows the passage of some water, however small the amount. It is necessary, therefore, to establish the relative importance of the general body surface and the tracheal system as sites of water loss in both species under consideration.

Apparatus and method:

An attempt was made in two different batches of experimental animals of both species, to block the spiracles, and to apply waterproofing agents to the general body surface while leaving the spiracles uncovered. The exact location of the spiracles in each species was ascertained by using the cobalt tracheal injection technique developed by Wigglesworth (1950); the condition of the tracheal system and spiracles in M. delanyi was found to be almost identical to the illustration of the respiratory system of Petrobius maritima (Oudemans, 1888) whilst that of C. longicaudata agreed in all essential details with Lepisma saccharina as described by Sulc (reproduced in Paclt, 1956).

Experimental animals were pretreated in the usual manner and then killed by means of coal gas. Half of each group was painted with a waterproofing agent, applied to the whole of the dorsal surface, the head and the ventral surface other than two strips where the spiracles were known to be located; the other half had only the spiracular region covered by the waterproofing agent. The animals were weighed before and after the application of the waterproofing agents and then desiccated over calcium chloride in the usual manner.

Three waterproofing agents were used and not one of these proved satisfactory. "Samsonite" proved to be both difficult to apply and insufficiently impermeable to water (perhaps due to inadequate bonding with the cuticle), whilst "glyptol", although readily applied, imparted no additional impermeability to the cuticle. Paraffin wax of low melting point (54°C) was found to be suitable in some instances and was in fact the only substance to produce some results. Two quick-drying enamel paints which were tested for suitability proved to lose weight slowly over a period of as long as 12 hours and could therefore not be used.

Results:

The application of "Samsonite" and "Glyptol" dissolved in acetone brought about an increase in the rate of water loss from M. delanyi. This may be attributed to the solvents in these waterproofing agents upsetting the cuticle structure, and gave rise to the investigation on the effects of wax solvents on the cuticle described in Part 5.2(vi). Painting M. delanyi with molten wax also produced no results since, as the animal lost water and its volume decreased, the body surface was pulled away from the wax and an increased rate of water loss followed. The application of the molten wax may also have upset the organisation of lipids in the cuticle and thus have increased the permeability. No results were, therefore, obtained for M. delanyi.

The application of Samsonite and of Glyptol had no significant effect on the rate of water loss from C. longicaudata. The rate for animals which had their general body surface covered by "Glyptol" was slightly lower than for those of the controls and also for animals with the spiracles only covered but this difference was extremely small (2.2%). Since C. longicaudata loses water more slowly than does M. delanyi and therefore decreases in volume more slowly when desiccated, some results were obtained for this species which suggest the general body surface as being the chief site of water loss in the dead animal. All results obtained for C. longicaudata are shown in Table 5.09.

Table 5.09 : Effects of blocking the spiracles and of waterproofing the cuticle on the rate of water loss (% of body weight per 24 hours) from dead specimens of C. longicaudata.

Waterproofing agent.	Spiracles blocked.		Body surface covered.	
	No. of animals.	Rate of water loss.	No. of animals.	Rate of water loss.
Samsonite	5	17.3	6	15.9
Glyptol	4	15.4	5	13.2
Paraffin wax	6	15.5	6	8.9
Controls	12	15.0		

These experiments have, on the whole, proved unsatisfactory and until such time as a waterproofing agent is found which is suited to the cuticle of these animals it can only be tentatively assumed that the chief site of water loss in the dead animal is the body surface. Some indirect evidence in support of this assumption will be discussed when possible mechanisms controlling the rate of water loss are considered.

5.4 Possible Mechanisms Controlling the Rate of Water Loss.

In the Pterygota water loss is entirely controlled by the spiracles, since the integument has, in most instances, achieved a high degree of impermeability. A feature of all the apterygote sub-classes of the Hexapoda is the lack of any mechanism whereby the spiracles may be occluded (Oudemans, 1888, Lawrence, 1953, Paclt, 1956). This is true even in those groups where the tracheal system itself is highly developed as for example in the Japygidae (Diplura) and the Lepismatoidea. The fact that the Thysanura are undoubtedly able to exercise considerable control over the rate of water loss from their bodies made it necessary to ascertain whether spiracular muscles may have been overlooked by previous workers or whether an indirect method of closing the spiracles is possible.

Serial sections and dissection of injected specimens stained with picro-carmine revealed no muscular control mechanism by means of which the spiracles could be closed. A possible indirect mechanisms for controlling the rate of waterloss was apparent in M. delanyi but no means of covering the spiracles could be found for C. longicaudata. The abdominal spiracles of M. delanyi are all situated under the paratergal expansions which overlie the pleura of each segment and it is possible that by pulling the paratergal expansions in closer to the body, the spiracles can be covered more effectively. The large mesothoracic spiracle, situated at the anterior margin of the mesothorax must, by virtue of its size, constitute the main site of water loss from the tracheal system. It can also possibly be closed by movements of the tergites. The posterior attachment of the pronotum and the anterior attachment of the mesonotum lie respectively at the anterior and posterior margins of the mesothoracic spiracle. While the pronotum overlaps the mesonotum in the normal manner above the level of the spiracle, the overlap is reversed below this level so that the mesonotum overlaps the pronotum. This condition is shown in Figure 5.06. The region where the overlap between the two sclerites changes thus forms a pivot or hinge between pro- and mesothorax which could allow for opening (Figure 5.06 A) and closing (Figure 5.06 B) of the spiracle.

However attractive the above hypothesis seems, most of the available evidence is in contradiction to the proposed scheme. When animals were killed by means of coal gas or other poisonous vapours, many died in a position where, according to these ideas, the mesothoracic spiracle, at least, should be closed. These individuals showed no difference in their rate of water loss from those in which the spiracle would be open.

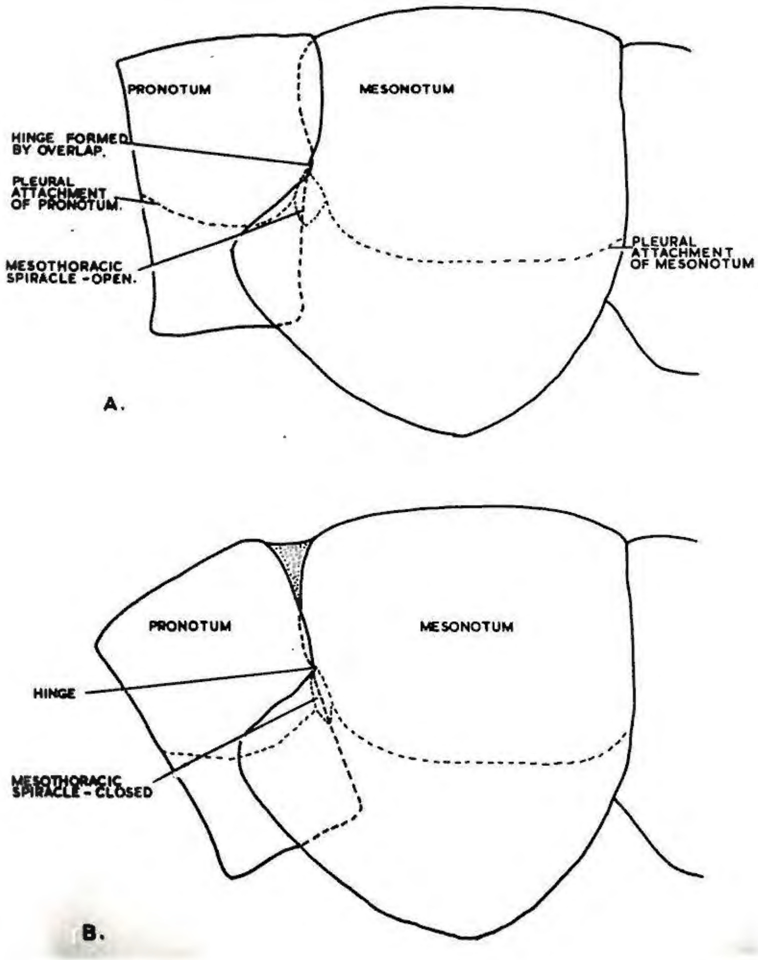


Fig. 5.08 : Positional relationship between pro- and mesonotum and the mesothoracic spiracle of *M. delanyi*, showing the possible means for occluding the spiracle as discussed in text.

Further evidence against spiracular control can be deduced from the fact that carbon dioxide has no effect on the rate of water loss. If a spiracular mechanism exists, the opening and closing of the spiracles must be regulated in response to the partial pressure of gases in the tracheal system. Ridding the tracheal system of carbon dioxide is of primary importance here and it has been found in those Pterygota which have been investigated that the partial pressure of carbon dioxide governs the duration of the period during which the spiracles remain open. The lack of such a mechanism, in a tracheal system which can be completely closed, would prove a danger to the animal unless the spiracles were opened and closed rhythmically under all conditions. This would lead one to expect that in M. delanyi regular movements of the tergites should be apparent. No movements which could be interpreted as opening and closing of the spiracles could, however, be seen during two hours observation through a lens.

The bulk of the evidence, therefore, points to water loss being controlled by means other than spiracular occlusion; this is particularly true for C. longicaudata where, on available evidence, such a method of control is impossible. The case for spiracular control in M. delanyi, however improbable, cannot be be finally rejected, since the control might be governed by the partial pressure of oxygen in the tracheal system, a possibility which has not been investigated.

Since spiracular control of water loss appears to be eliminated, the control of evaporation of body water must be exercised by the integument. Physical barriers against the passage of water molecules are certainly present in both species. Lower (1958) has shown the epicuticle of C. longicaudata to consist of a protein layer covered by a sudanophil layer which is resistant to even the action of boiling wax solvents. This sudanophil layer may be a lipoprotein but Lower claims it to be Millon negative and has no evidence for a protein constituent. Whatever its nature, this epicuticular layer may well impart a considerable degree of impermeability to the cuticle. The action of wax solvents on the cuticle of M. delanyi suggests that here too a substance exists in the cuticle which is responsible for reducing the rate of water loss. This substance may also be a lipid.

However, the physical barriers discussed above cannot account for the differences between the rates of water loss from dead and living animals. It is necessary to look for a mechanism involving active metabolic work on the part of the animal, one which would break down on death, and in this connection the following observations are of interest:

(i) An active inward secretion, as a means of retaining water, is not peculiar to the Thysanura. It has been reported for spiders (Davies and Edney, 1952) and for various species of ticks (Lees, 1947). These animals all have much better developed physical barriers against water loss than the animals considered in this investigation.

(ii) Prolonged exposure of the animals to coal gas, while it may be insufficient to cause their death, nevertheless severely upsets their control of water loss on recovery, although locomotion appears in no way impaired. C. longicaudata, under these conditions, loses weight at a rate almost comparable with that of a dead specimen, while M. delanyi will lose as much as 7% of its body weight in 24 hours at a saturation deficit of 1.4 mm Hg, where the expected water loss would be between 0.5 and 1.0%. These results strongly suggest the upset of a metabolic process being the cause of the breakdown in control of water loss. The possible nature of this process will be discussed after the results of the following section have been considered.

To sum up, both M. delanyi and C. longicaudata have attained a good measure of protection against desiccation in the form of physical barriers in the cuticle and some active water retaining mechanism. C. longicaudata has developed these to a higher degree of perfection than has M. delanyi, as would be expected from the study of the environmental conditions under which the two species live.

6. WATER UPTAKE.

However well the water conserving mechanisms of an animal are developed, transpiration still takes place, by way both of the general body surface and the respiratory surfaces. In order to survive it is therefore necessary from time to time to replenish the water lost in this way. The less effective the water conserving mechanism, the more important becomes the ability to gain water from the surroundings and it is doubtless the ability to utilise available sources of moisture which has allowed the invasion of the land by the Arthropoda, an invasion which probably took place long before an impermeable cuticle had evolved.

The intake of water may be effected in a number of ways. Provided that the necessary secretory mechanisms are present in the alimentary canal to permit absorption, water taken in with food can adequately supply the needs of such arthropods as sap-feeders and blood suckers, while even some carnivorous species may take in considerable quantities of water with the tissue fluids of their prey. Many phytophagus forms such as lepidopterous caterpillars may also take in appreciable amounts of water contained in the plant tissues on which they feed. Animals feeding on comparatively dry foods, must however, obtain additional water to replenish their internal water store. C. longicaudata certainly falls into this category. M. delanyi may gain some moisture from the thallophytes on which it feeds, but it seems unlikely that the amount of water contained in these would be adequate for its needs.

Water, other than that taken in with food, can be aquired in three ways. Oral drinking is said to be of common occurrence among adult insects (Lelercq, 1946, quoted in Edney, 1957), but this requires that the water occurs in droplet form; a surface film of water on a stone or capillary water in soil cannot normally be taken in without special structural modifications, but such uptake has been shown to occur. The spiders Tarantula barbipes and Lycosa radiata are able to extract capillary water from a bed of fine graphite particles (Parry,1954) and various terrestrial Isopoda are able to take in capillary water by both mouth and anus (Spencer and Edney, 1954). Absorption of water through the cuticle is also of common occurrence among the terrestrial arthropods; here the eversible vesicles, located on the abdomen or on the coxae of the abdominal appendages, are important sites of water uptake. The "collophore" or ventral tube of Onychiurus armatus (Collembola) has been shown to be its main water absorbing organ (Nutman, 1941) and the eversible vesicles of Campodea (Diplura) serve a similar function (Drummond, 1956). Eversible vesicles are also known to be concerned with water uptake in other arthropods including Opisthopatus cinctipes (Onychophora) (Alexander and Ewer, 1955), Hanseniella agilis (Symphyla) (Tiegs, 1947) and Callipus longobardius (Diplopoda) (Manton, 1958). The vesicles have been shown to be capable of extracting capillary water (Tiegs, 1947, Alexander and Ewer, 1955) and would thus also be capable of absorbing water from a surface film. Preliminary experiments had shown that the eversible vesicles of M. delanyi are also concerned with water uptake, but no quantitative measure was made of the extent of their function.

Water vapour porvides yet another source for the replenishment of a depleted internal water store. A number of well authenticated cases of arthropods absorbing water from an undersaturated atmosphere can be quoted: notable among these are Tenebrio larvae (Buxton 1930; Mellanby 1932), ticks (Lees, 1946; Browning, 1954) and rat fleas of the genus Xenopsylla (Edney, 1947). Lindsay (1940) reports C. longicaudata as being able to absorb water from an atmosphere at 99% relative humidity, but work carried out so near saturation must be suspect, since even slight temperature changes could bring about precipitation.

Finally, some animals make use of metabolic water to increase the body water content. Mellanby (1932) has shown Tenebrio larvae metabolise considerable quantities of their fat stores and in so doing replace the water lost in evaporation by the water resulting from this fat metabolism. Although the animals lost weight, the proportion of dry to wet weight remained constant, indicating that the water loss was equalled by the replacement due to oxidation of the fats. The above is but one example among many; the subject has been reviewed by Edney (1957).

The present investigation set out to determine which means of gaining water are used by the two species of Thysanura under consideration and under which conditions they are used.

6.1 Uptake of Water in the Liquid State.

Liquid water would normally be encountered by land arthropods in any one of the three conditions mentioned above, i.e. as free water, water forming a thin film over surfaces and capillary water. *C. longicaudata*, by virtue of its atypical habitat, may well encounter none of these over considerable periods, whilst during certain seasons, free water may be rare, even in the habitat of *M. delanyi*. The ability of both species to benefit from all three possible water sources, and the mechanisms used in each instance have been investigated.

Apparatus and method:

The experimental animals were desiccated over calcium chloride in the normal manner; *M. delanyi* was subjected to between 24 and 48 hours desiccation, while *C. longicaudata* was desiccated for as long as 120 hours to give an approximately comparable water loss. The desiccated animals were divided into three batches, each of which was given water in one of the following ways:

(i) Free water; water droplets scattered over stones in a plastic dish.

(ii) Water film; stones which had been wetted by immersion in water and then shaken to remove all but a thin film of water covering each stone.

(iii) Capillary water; an unglazed china plate which had been soaked in water.

After 12 hours access to the respective water sources, the animals were subjected to a further three hours of desiccation over calcium chloride to remove any adsorbed moisture and then weighed again in order to establish whether any water had been gained.

The experiments were repeated using a weak aqueous solution of Light Green instead of water. After each experiment had been completed, the animals were killed by means of ethyl acetate vapour and dissected in order to ascertain the distribution of the dye within each animal; preliminary experiments on M. delanyi had proved the dye to be easily detectable in the animals, particularly in cuticular structures such as the foregut and vesicles.

Results:

Both species were shown to gain weight under all conditions in these experiments. Table 6.01 shows the mean water gain by the two species.

Table 6.01 : Water gain from three different water sources by M. delanyi and C. longicaudata. Water lost during desiccation and gained from water source expressed as percentage of original body weight.

Species and water source	No. of animals.	Mean water loss.	Mean water gain.	Percentage of water lost recovered.
M. delanyi				
Free water	6	9.5	14.8	156%
Water film	7	20.1	15.9	79%
Capillary water	7	17.5	10.9	81%
C. longicaudata				
Free water	6	18.1	18.6	103%
Water film	5	17.6	11.0	63%
Capillary water	6	19.4	16.2	84%

Using water coloured with Light Green showed the modes of uptake to be different in the two species. Whilst M. delanyi used its vesicles to obtain water not readily available for oral drinking, C. longicaudata, which has no such structures, showed no uptake of the dye at all. Table 6.02 shows the distribution of the dye in the two species after each had shown gains comparable with those reflected in Table 6.01.

Table 6.02: Distribution of dye (Light Green) in M. delanyi and C. longicaudata after water uptake from three different sources.

Water Source	M. delanyi			C. longicaudata	
	Total no. of animals	No. with dye in gut	No. with dye in vesicles	Total no. of animals	No. with dye in gut
Free water	8	3	5	7	7
Water film	6	1	6	6	0
Capillary water	8	0	8	7	0

Both species are, therefore, able to drink water, and do so readily when water is available in drinkable form. M. delanyi, however, uses its vesicles for the uptake of water from other sources when drinking is not possible. C. longicaudata shows no visible evidence of water uptake through any part of the cuticle, under conditions in which water could not be drunk, although it must gain water in some way since it shows a comparable increase in weight. The only way in which water could have entered these animals was in the form of water vapour.

6.2 Uptake of Water Vapour.

Lindsay (1940), claimed that C. longicaudata was able to absorb water from an atmosphere at 99% relative humidity. In view of the findings in the preceding experiments this aspect was reinvestigated. The possibility of M. delanyi sharing this ability could not be rejected merely on the grounds of the presence of an alternative water absorbing mechanism, the eversible vesicles, so the ability of this species was also investigated.

Apparatus and method:

The experimental animals were desiccated as previously described, and subjected in a uniform humidity chamber to various relative humidities, at a constant temperature of 20°C. The details of the construction of the uniform humidity chamber used will be found in Part 7 of this thesis. The desired relative humidity was obtained by using saturated solutions of various salts or solutions of potassium hydroxide prepared according to Solomon (1951). In order to preclude the possibility of the animals eating the nylon fabric covering the false floor of the chamber (a distinct possibility in the case of C. longicaudata) this was replaced by a layer of plastic window gauze.

After an initial desiccation the animals were exposed, for a period of 24 hours, to some particular humidity, and then desiccated again for a period of three hours before weighing. The use of dead controls proved this period to be more than adequate for the removal of any water trapped between both the scales and overlapping sclerites. The animals were returned to the uniform humidity chamber for a further 24 hours after weighing, in order to ascertain whether there was any further gain in weight during this period.

Results:

While M. delanyi proved incapable of absorbing water vapour from even a saturated atmosphere, C. longicaudata was found to be able to replenish its water store in this way from an atmosphere with a relative humidity as low as 60%. It is not clear why Lindsay (1940) claims the limit of this ability to be at 99% relative humidity, since nowhere in the text of her paper does she mention having conducted experiments at any lower humidity.

The details of these experiments are tabulated in Table 6.03.

Table 6.05: Water gain from saturated and subsaturated atmospheres by M. delanyi and C. longicaudata. Water loss and water gain are expressed as a percentage of the original body weight.

Species & relative humidity	No. of animals	Mean water loss	Mean water gain 24hrs.	48hrs.	Percentage of water lost recovered.
M.delanyi.					
100%	7	26.4	-0.6	.	-
98%	8	27.2	-1.3	-	-
C.longicaudata					
100%	6	34	+25	-	75%
98%	8	27.3	+20	+21	78%
90%	7	26.0	+18	+21	78%
75%	6	17.8	+16	+17	93%
60%	7	23.1	+ 6	+ 6	27%
60%	3*	21.5	+15	+17	80%

* Includes only those animals in the preceding batch which were able to absorb water at 60% relative humidity.

The results obtained in the preceding experiment on water uptake can therefore be explained by C. longicaudata absorbing moisture from the near saturated atmosphere surrounding the wet stones and the saturated porous plate.

It has been shown that water loss from C. longicaudata is proportional to the saturation deficit of the surrounding atmosphere and it seemed more than likely that the same parameter imposed a limit to the ability to gain water in the form of water vapour. In order to ascertain whether this was in fact true, five specimens were desiccated over calcium chloride and placed in a uniform humidity chamber at 75.5% relative humidity and 35°C. After a period of 24 hours, the animals were desiccated over calcium chloride for three hours and reweighed. All were found to have lost weight, as did five undesiccated specimens, which were included to observe the effect of high humidity on animals whose water balance was normal.

The chamber was now left open in a constant temperature room at 20°C, and allowed to cool slowly so that no condensation of moisture occurred. The animals were reintroduced into the chamber and left for a further 24 hours after which they were again briefly desiccated and then weighed. All were found to have gained weight, but while the desiccated specimens absorbed considerable quantities of water to compensate for water lost, the undesiccated specimens took up only sufficient to regain their original weight. The results are shown in Table 6.04.

Table 6.04: Effect of temperature on the water uptake desiccated and normal specimens of C. longicaudata from an atmosphere at 75.5% relative humidity.

Condition.	No. of animals.	Mean water loss.	Mean water uptake at 35°C	20°C
Desiccated	5	10.0	-1.2	+7.2
Normal	5	-	-3.1	+3.4

These experiments show the ability of C. longicaudata to absorb water vapour from an undersaturated atmosphere to be limited by saturation deficit when the relative humidity is high, a result which is at variance with the findings both of Mellanby (1932) for the larvae of Tenebrio and of Edney (1947) for the prepupal stage of the rat flea Xenopsylla brasiliensis. Unfortunately no experiments were done to ascertain whether relative humidity limits the ability to take up water at low saturation deficits. It will be seen in Figure 5.05 that there is a slight water loss at a relative humidity of 25% and a temperature of 7°C. The saturation deficit here would be only 5.7 mm Hg, only 1.3 mm Hg higher than the saturation deficit at 75% relative humidity and 20°C and 1.3 mm HG less than the saturation deficit at 60% relative humidity at 20°C, yet water uptake has been demonstrated from both the latter two, while none of the animals tested at the low relative humidity showed any gain in weight. Relative humidity cannot, therefore, be eliminated as one of the limiting factors involved in water uptake.

6.3 The Utilisation of Metabolic Water.

This aspect has not been investigated, but one observation is of possible importance here. The abdomen of M. delanyi houses a well developed musculature used in leaping. Such leaps are effected by forcibly beating the abdomen onto the substratum. C. longicaudata, on the other hand has a very poorly developed abdominal musculature, and the abdomen of this species contains an extremely large visceral and parietal fat body. Such large fat stores, which occupy most of the haemocoelic space in the abdomen, would make the utilisation of metabolic water highly probable.

6.4 Extrusion of the Eversible Vesicles by M. delanyi.

The eversible vesicle of M. delanyi and, indeed those of other arthropods which possess these organs, must be extruded by some form of internal hydrostatic pressure. Since each vesicle is in direct contact with the haemocoel, localised increases in blood pressure must be affected by the internal water content of the animal, desiccation should have a marked effect on extrusion; the greater the water loss the less easily the vesicle should be extruded. The limitations of this system whereby the vesicles are extruded were investigated by desiccating specimens of M. delanyi for different times and observing the effect on the ability to extrude the vesicles.

Apparatus and method:

Experimental animals were desiccated in the usual manner for periods varying from 6 - 172 hours, and the total water loss during this period was determined. Each animal was now placed on a sheet of glass raised 15 cm. above a magnifying mirror, so that its ventral surface could be readily observed. Before placing an animal on the glass plate, a thin film of water was allowed to condense on the glass by gently breathing over it. The animal was then placed on the water film and covered with a small petri dish. The time elapsing between placing an animal on the water film and the maintained extrusion of (i) the first extruded vesicle and (ii) more than four pairs of vesicles, was noted in each instance. If the water film had evaporated before any extrusion of the vesicles had taken place, a fresh water film was allowed to condense on the glass next to the petri dish and the animal was gently coaxed onto this.

Results:

Up to a water loss of 15% the animals would extrude all their vesicles almost simultaneously although the more anterior vesicles were usually, although not invariably extruded first. The time to extrusion was very variable, some extruding the vesicles almost immediately on being placed on the water film, while others did so only after five and on one occasion, even ten minutes. Between 16 and 20% water loss, however, the vesicles were extruded almost immediately on being placed on the water film, extrusion still being almost simultaneous.

Above 20% water loss the vesicles were no longer extruded simultaneously. One or two anterior vesicles were extruded first, followed by one or two more after a few minutes. The time lapse between successive extrusions became progressively less and the last four or six vesicles were usually extruded simultaneously. As the water loss increased above 20% so the time to the first extrusion became longer, as did the time between successive extrusions of the more posterior vesicles.

Figure 6.01 summarises the results of this experiment. The numbers of animals from which the results at the higher percentages of water loss are based were small because of a high mortality during desiccation and the inability of many of the survivors to extrude their vesicles at all.

Not only does it take longer to extrude the vesicles after prolonged desiccation, but it also becomes more difficult for the animals to maintain the vesicles in the extruded position. Those animals which, after prolonged desiccation, were able to

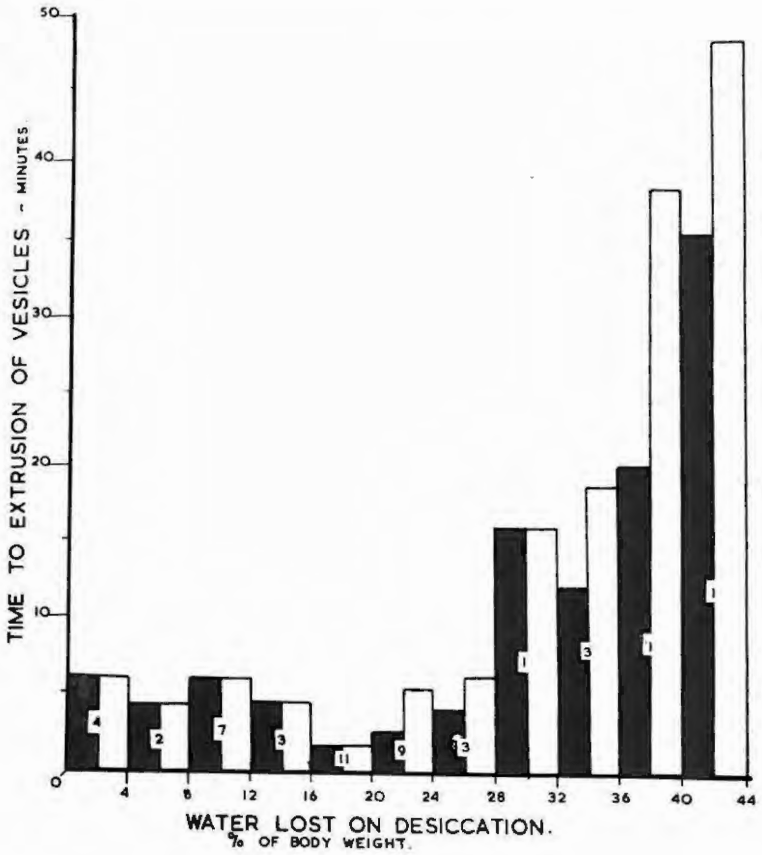


Fig. 6.01: Effect of water loss on the time taken by <u>M. delanyi</u> to extrude the first (shaded) and the first four pairs (unshaded) eversible vesicles. The figures in each histogram denote the number of animals in each category.

extrude their vesicles, would initially extrude only a single anterior vesicle, which they would maintain only momentarily in the extruded position. Only after several attempts, each usually a little more successful than the preceding one, was the vesicle permanently maintained in the extruded position. This also applied to some of the subsequent extrusions.

The difficulty experienced in extruding the vesicles can be attributed to a reduction in the volume of the haemolymph. This is supported by the observation that extrusion of the vesicles by animals which had previously been found unable to do so, was facilitated by giving them water in drinkable form. Once such animals had drunk, they were not only able to extrude the vesicles to assist in further uptake of water, but were able to do so without any apparent difficulty.

The actual mechanism whereby the hydrostatic forces in the haemocoel are distributed to effect extrusion have not been investigated. That the body muscles are involved can be deduced from there being a general shortening of the abdomen, presumably brought about by contraction of the abdominal musculature. This contraction of the abdominal muscles starts at the posterior end, passing forward. The fact that the anterior vesicles are always the first to be extruded suggests that the heart may also be concerned with the distribution of pressures and that the pressure head caused by abdominal contraction meets an opposing pressure head caused by the pumping action of the heart in the region of the most anterior vesicles.

All the phenomena observed in these experiments are explicable in terms of the water content affecting the ability to extrude the vesicles. The desiccated animal experiences considerable difficulty in extruding a single vesicle, but once extruded this vesicle replenishes the depleted water store to some extent, thus facilitating the extrusion of further vesicles, each of which, by virtue of their water absorption renders subsequent extrusions easier.

The vesicles of M. delanyi are, therefore, efficient water absorbing organs but their use is to some extent limited by their being rendered inextrusible under conditions of extreme water loss.

6.5 Absorption of Water Vapour in Relation to Active Water Retention.

We have seen that *C. longicaudata* is capable of active water absorption through the cuticle from an unsaturated atmosphere. This is a property shown by a number of arthropods including such insects as the larvae of *Tenebrio* (Mellanby, 1932), and the prepupae of *Xenopsylla* (Edney, 1947) and also certain ticks (Lees, 1946, 1947; Browning, 1954). No fully satisfactory explanation for this mechanism of active uptake of water vapour has as yet been put forward. It has been suggested that the hypodermis contains an active pumping mechanism which effects an inward secretion of water molecules against a concentration gradient (Lees, 1946, 194). The mode of operation of such a pump has not been determined; a purely osmotic mechanism seems highly unlikely, since this would require the development within the hypodermis with an osmotic pressure equal to 140 atmospheres to prevent water loss at 90% relative humidity and 20°C and, of course, even higher concentrations to absorb water under these conditions. Beament, (1954) has attempted to overcome this difficulty by suggesting that the physical nature of the cuticle lowers the gradient across which the pump has to work. He has studied the properties of a compound membrane formed of tanned gelatin covered with wax. This, like cuticle, is asymmetrical in its behaviour towards water. Water will pass readily through the membrane if applied to the waxed surface but far less readily if applied to the surface of the tanned protein. Using this model, he has determined that it is only necessary to reduce the water content of a saturated tanned gelatin layer by 1% to counteract the suction force exerted at the wax surface by the saturation deficit obtaining at 90% relative humidity and 20°C. The lipid/cuticulin complex of the epicuticle can be regarded as a compound membrane similar to Beament's model, and he suggests that the reduction in water content of the cuticulin layer by the required amount is effected by the processes of the hypodermis which pass through the pore canals of the procuticle and thus lie in contact with the cuticulin layer of the epicuticle.

Edney (1957) has pointed out that the differential permeability suggested by Beament in no way reduces the necessity for a pump with a very high degree of efficiency, since if the suction force exerted by a 99% saturated tanned protein can resist evaporation from its surface into an atmosphere at 90% relative humidity, then the force required to remove the same water from the cuticle and into the tissues must also be in equilibrium with 90% relative humidity. Thus the arrangement proposed by Beament in no way reduces the force which the hypodermal pump must develop. However, forces of this order of magnitude are known to be developed by active transport systems elsewhere in the animal kingdom; the removal of water from the shell of cephalopod molluscs, important to these animals in the attainment of a neutral buoyancy, may require a suction force of as much as 175 atmospheres (Denton, 1961). Thus the large forces which have to be developed are themselves no barrier to the hypothesis that active uptake of water vapour depends upon an active inward secretion by the hypodermis.

It is thus possible to visualise the mechanism of active water uptake from an unsaturated atmosphere as depending upon "water pumps" in the hypodermal syncytium (or hypodermal cells) which can withdraw water from the cuticle. The cuticle itself will probably display hygroscopic properties which will facilitate uptake by creating a readily filled water compartment in the cuticle. Such a postulate is reasonable in the light of our present knowledge of the properties of tanned protein membranes. Lastly, it is desirable that the cuticle should be asymmetric in relation to water movement, allowing a ready inward passage of water molecules and impeding their outward passage. This condition is not only satisfied by Beament's model, but also by natural cuticles.

It is possible to carry the argument one stage further. If active absorption of water from an unsaturated atmosphere depends upon the removal of water molecules from the cuticle by the hypodermal cells, it should also follow that water molecules which escape into the cuticle from the hypodermis may be drawn back into it again. In this way active water transport by the hypodermis will also serve to reduce the rate of water loss and can in itself be a mechanism of water retention.

That indeed both retention of body water and water uptake are active processes is beyond doubt. The effect of coal gas on both M. delanyi and C. longicaudata has already been discussed and to this may be added Browning's (1954) findings that anaesthesia caused by excess carbon dioxide (concentrations of the order of 30-50% and thus far in excess of those used in the present investigation) will not only destroy the ability to take up water vapour but at the same time upsets water retention in the tick Ornithodorus moubata.

It is desirable to investigate these ideas further in the light of results obtained from other animals. In ticks it has been shown that the ability to absorb water vapour is destroyed, not only by carbon dioxide anaesthesia and death, but also by abrasion of the cuticle. These treatments also all result in a general increase in the rate of water loss. In C. longicaudata, however, while death and carbon monoxide poisoning cause an increased rate of water loss and destroy the ability of the animals to take up water vapour, abrasion has no effect on the rate of water loss. (Its action on active water uptake has not been investigated).

It is generally accepted that the action of abrasives is to remove the epicuticular waxes and by this action alone to permit a more rapid rate of water loss. Why abrasives should inhibit active water uptake on such an assumption is not clear. The present results with C. longicaudata throw doubt on the validity of this idea. The hypothesis may be saved if it is assumed that the transpiratory properties of the tanned protein of cuticle differ from those of the cuticular protein of C. longicaudata which is said (Lower, 1958) not to be tanned. But there is another possibility worthy of consideration. C. longicaudata differs from other arthropods in which active water absorption occurs in the absence of pore canals. This suggests that the major action of abrasives is damage to the hypodermal cell processes which pass into the pore canals. This damage may result in structural disorganisation of the plasma membranes leading to a cessation of action of the "water pumps". If the "water pumps" cease to function, not only will active water uptake from the environment cease, but the rate of water loss will increase as resorption of water from the cuticle will stop.

In this connection it is interesting to note that if Ixodes is allowed to recover after abrasion, active water uptake recommences before fresh wax is secreted over the abraded surface has been completed. While it is possible to postulate that the original permeability properties of the cuticle have been restored by the secretion of an invisible lipid monolayer, it seems equally reasonable to suggest that the normal conditions of water balance are restored as soon as the hypodermal cells have recovered from injury and the water pump system is once again at work.

The function of pore canals is one which is obscure. While it is commonly held that they play a role in the transport of material to the epicuticle, this idea meets with the difficulty that insects with thin cuticles lack pore canals. Pore canals are thus not essential for the formation of the epicuticle. If we postulate that a major function of the pore canals is concerned with water balance, their absence from thin cuticles becomes less surprising. The procuticle of C. longicaudata is only 10 mu. thick and the distance separating the pore canals of larval Sarcophaga is of the same order (Richards in Roeder 1953). The absence of pore canals in aquatic forms like mosquito larvae is in keeping with the present hypothesis, but against this must be weighed the presence of such canals in the cuticle of the freshwater crayfish Astacus.

In sum, there is evidence which justifies the further study of the idea that the hypodermal projections into the pore canals are concerned with control of water flow through the cuticle and that a major action of abrasives depends upon physical injury to these structures. This is not to deny that waxy epicuticular layers may contribute greatly to reduction of transpiration through the cuticle; clearly a physical barrier is more economic of energy than a process of active transport, nor is it to deny the hypodermal filaments in the pore canals other roles than water absorption in the economy of the insect.

A second suggested property of the cuticle, namely a hygroscopic character, calls also for consideration. The hygroscopic properties of cuticle will be a function of relative humidity, albeit the relation is not linear (Pielou, 1940). In both Tenebrio and Xenopsylla it has been shown (Mellanby, 1932; Edney, 1947) that the rate of water absorption from the atmosphere is a function of relative humidity, not saturation deficit. In C. longicaudata this is not the case. The animal is unable to take up water from an atmosphere whose relative humidity is within the range where absorption is possible, if the saturation deficit is high. This difference may be quantitative. In Tenebrio and Xenopsylla the physical barriers in the cuticle impart to it a high degree of impermeability so that, even at high saturation deficits, the rate of water loss is low and a nett gain of water may be observed. In C. longicaudata this is not the case and the rate of water loss at high saturation deficits may be sufficient to result in a nett loss of water, thus giving the impression that no active water uptake is occurring.

Finally, and speculatively, we may examine the consequences of the suggestion that active water absorption and water retention by active absorption are related. The vast importance of "water getting" for terrestrial animals had already been stressed. It seems possible that the ancestral forms in the myriapod insect line may early have evolved the ability to absorb water droplets through the cuticle, an ability retained today by the cockroach (Beament 1961). Such water absorption depended upon the development of hypodermal "water pumps". By their very development the rate of water loss through the cuticle was lowered and the invasion of drier areas became possible. A new selective force, the need to restrict water loss by transpiration, now came into operation and physical barriers to the passage of water through the cuticle developed, but the old ability to absorb water droplets was thus reduced. Two pathways were open. The one was to limit the sites of water absorption to certain regions of the body, regions which, because of their high permeability, must not normally be exposed. This led to the development of eversible vesicles and to the condition which we find in the Machiloidea. The other was to increase the efficiency of the water pumps so that water could be absorbed from a sub-saturated atmosphere. This is the solution found in the Lepismatoidea.

But the invasion of still drier areas required the development of greater mechanical protection and strength: the cuticle was thickened further and as it thickened the hypodermis invaded it establishing the pore canals to play a part both in water retention and water absorption.

7. BEHAVIOUR AND THE ECOLOGICAL NICHE.

The importance of behaviour in the invasion of a new environment is now well established. The work of Edney (1954) on the terrestrial Isopoda is but one striking example of how innate behaviour in the form of taxes and kineses orient animals in such a way as to avoid the harsher conditions of the enviornment, restricting them to physical conditions within the range of their physiological capabilities. Such orientation behaviour contributes in no small measure to the success of a species in an otherwise hostile environment.

Any investigation which seeks to elucidate the divergent distribution of two related groups such as Machiloidea and Lepismatoidea must include a consideration of their responses to physical conditions obtaining in the environment: this involves not only the ascertaining of the direction and intensity of the responses involved but also their nature. Where possible the sense organs should be located and possibly the underlying neuro-physiological mechanisms determined.

Of primary importance to any terrestrial animal are both the maintenance of an adequate internal water balance for the proper functioning of its cellular mechanisms and the keeping of the body temperature within working limits. In considering these aspects it was therefore necessary to establish the behavioural responses of the experimental animals to the following factors:

1. Temperature; for it affects both cellular function and the rate of water loss from the body.
2. Light, which can be equated with solar radiation in nature and thus involves both temperature and water loss.
3. Humidity, for it affects both water loss and water uptake, and

4. Gravity, which may indirectly assist in leading the animal to optimal conditions.

Whilst the behavioural responses to each of the aforementioned stimuli are in themselves of primary importance, it is of course necessary to establish the interaction of the responses since all the stimuli will impinge upon the animal's sensory apparatus at any one time. Equating these findings with physiological and ecological studies may thus contribute to the solution of the problem under consideration.

7.1. Behavioural Responses to Temperature.

The effects of temperature on a terrestrial animal are twofold. Its role in determining the saturation vapour pressure and consequently the saturation deficit of the atmosphere in a particular environment has a direct bearing on the rate of water loss from the body of an animal in the environment. This has been adequately shown by many authors, notably Johnston (1942) and Edney (1945, 1947, 1956, 1957). In addition temperature affects the cellular mechanisms of an organism, lethal temperatures being encountered both above and below the physiological range (Buxton 1931, Mellanby 1932). High temperatures upset metabolic processes, particularly with respect to phosphate metabolism (Fraenkel and Hopf 1940, Hopf 1940). Low temperatures slow down the rate of metabolic processes, initially causing chill coma in which condition the animals are moribund; this may be followed by death if the tissue fluids freeze, such death being attributed to both dehydration of the tissues and rupturing of the cells by the ice crystals formed.

An investigation of the temperature relations of an animal therefore involves two considerations in that it is necessary to not only establish what is the preferred temperature of the animal but also what temperatures outside the preferendum constitute a barrier to it. Clearly animals cannot be strictly confined to their preferred temperature range at all times, since movement into less favourable temperature zones is bound to become necessary on numerous occasions in response to other drives. Such an excursion may, however, lead the animal into a region where the temperature is lethal unless some mechanism exists whereby this is avoided. More important still, temperature conditions in the habitat may become near lethal due to extraneous factors and the animal must have the necessary behavioural mechanisms to take it out of such a habitat if it is to survive. The preferred temperature is therefore the temperature at which the animal usually comes to rest with some degree of latitude on either side before lethal temperatures are encountered which must be avoided. Both aspects have been investigated for both <u>Machiloides delanyi</u> and <u>Ctenolepisma longicaudata</u>.

(i) <u>Preferred and critical temperatures</u>.

Apparatus and method:

The apparatus used for the establishment of temperature gradients and barriers was a "Temperature Organ" shown in Fig. 7.01. It comprised two brass troughs measuring 61 x 5.5 x 5.0 cms and 15.0 x 5.5 x 5.0 cm respectively, welded to a stout brass bar measuring 91.0 x 6.5 x 3.0 cm, which thus formed the floor of both troughs. Drilled into the region forming the floor of the larger of the two troughs were eleven holes, evenly spaced at 5.0 cm apart; into these matched thermometers were inserted, enabling the temperatures at these points to be recorded. The smaller trough was fitted with brass tubing so as to allow a continuous flow of water through it by connecting it to a water tap, thus providing a means of cooling one end of the larger trough, which contained the experimental animals.

Since both <u>M. delanyi</u> and <u>C. longicaudata</u> showed a marked negative geotaxis it was found necessary to insert a piece of glass at each end of the larger trough as the welding in the corners provided footholds enabling the animals to climb upwards and thus out of the effective area.

A temperature gradient could be established along the length of trough A by heating the free end of the brass floor by means of a bunsen burner and cooling the other end by circulating water through trough B. The effect of the cooling was extremely localised, probably due mainly to the large size of the brass bar forming the floor; this resulted in very steep gradients being set up in the apparatus. If heat was applied slowly a large neutral zone, in which there was no gradient of temperature, was established in the middle of the trough; this condition was unsatisfactory in that it was impossible to tell whether the presence of animals in this zone was due to temperature or chance and it was therefore necessary to apply sufficient heat to allow the influences of heating and cooling to meet. Whilst this did not affect the experiments concerned with establishing the upper critical temperature, where the retreat of the animals before an advancing temperature barrier was observed, it was important in the determination of the temperature preferendum where a given gradient had to be maintained for some considerable time. Ideally the gradient in the latter instance should have covered a range from the lowest possible at the cold end to the upper critical temperature at the hot end but this was found impossible to achieve and thus the region in which the animals were free to move covered only about half the apparatus.

A plan view of the apparatus is shown in Fig. 7.01 showing the numbering of the thermometers later to be referred to in considering the results. Points 0 and 12 refer to temperatures recorded from thermometers

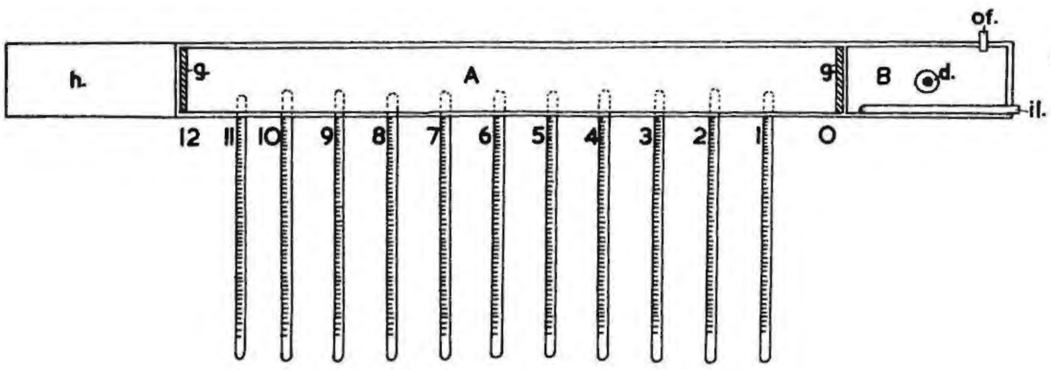


Fig. 7.01 : Plan of the Temperature Organ. A - trough containing experimental animals; B - cooling trough; d - draining hole of cooling chamber; g - glass plate; h - point of applying heat; il - water inlet pipe of cooling trough; of - overflow of cooling trough; 0 - 12 - points at which temperature was recorded.

placed with their bulbs resting on the floor of the trough at the two extreme ends. These thermometers had their bulbs insulated from the ambient temperature by means of a layer of cotton wool, only the base of the bulb in contact with the floor of the trough being exposed.

The apparatus was allowed to come into equilibrium with the temperature in the laboratory and all the thermometers (0 - 12) were read to ensure a uniform temperature throughout. A batch of from ten to twenty experimental animals was then liberated in trough A, the trough covered by a sheet of glass wrapped in black paper and the animals were left to distribute themselves in the apparatus. After an hour the animals had normally come to rest and rarely moved even when the lid was raised in weak diffuse light. The numbers of animals between each thermometer and the next was recorded, the lid closed and the experiment commenced.

In order to investigate the upper critical temperature into which the animals would venture, water was circulated through trough B in order to keep this end from attaining a lethal temperature level and thus providing a retreat. The other end of the brass bar was heated by means of the bunsen burner and a close watch was kept on the temperatures along the length of the floor. At frequent intervals the numbers of animals in the various temperature ranges were observed in a weak diffuse light. In addition, a number of experiments were carried out with but a single animal in the apparatus in order to establish the nature of the response. These experiments were carried out under bright light and the animal was thus normally active.

The determination of the preferred temperature involved a modification in method. The temperature of the cold end had to be reduced well below room temperature and in order to facilitate this, the "Temperature

Organ" was equilibrated in a constant temperature room at a temperature of 14°C. Since the experiment could not be carried out in the constant temperature room, owing to the absence of gas outlets for the operation of the bunsen burner, the apparatus had to be moved into a laboratory where the temperature was usually in the region of 20°. In order to minimise the increase in the temperature of the apparatus concomitant with this transfer, the animals were allowed only fifteen minutes in which to distribute themselves in the trough before the temperature gradient was set up, this period having been found to be the minimum required for settling. After the positions of the experimental animals had been recorded trough B was filled with ice to cool the cold end of the gradient to as low a temperature as possible, this temperature varying between 5 and 8°C, depending on the ambient temperature. Once this had been achieved, heating of the hot end was commenced until a suitable gradient had been established, after which heating was carefully controlled in order to maintain this gradient for a period of one hour. The numbers of animals in each temperature range were now recorded, the area of greatest aggregation representing the preferendum.

Results:

Both M. delanyi and C. longicaudata showed a marked avoidance of high temperatures. Figures 7.02 and 7.03 show the distribution in the apparatus at ten minute intervals for the two species respectively. These results suggest that C. longicaudata avoids temperatures above 40°C. whereas the avoidance reaction of M.delanyi is evoked at a somewhat lower temperature. This was borne out by the experiments carried out on individual animals, where, as nearly as could be established, the upper critical temperatures appeared to be between 40°C and 43°C for C. longicaudata and between 35°C and 36°C for M. delanyi.

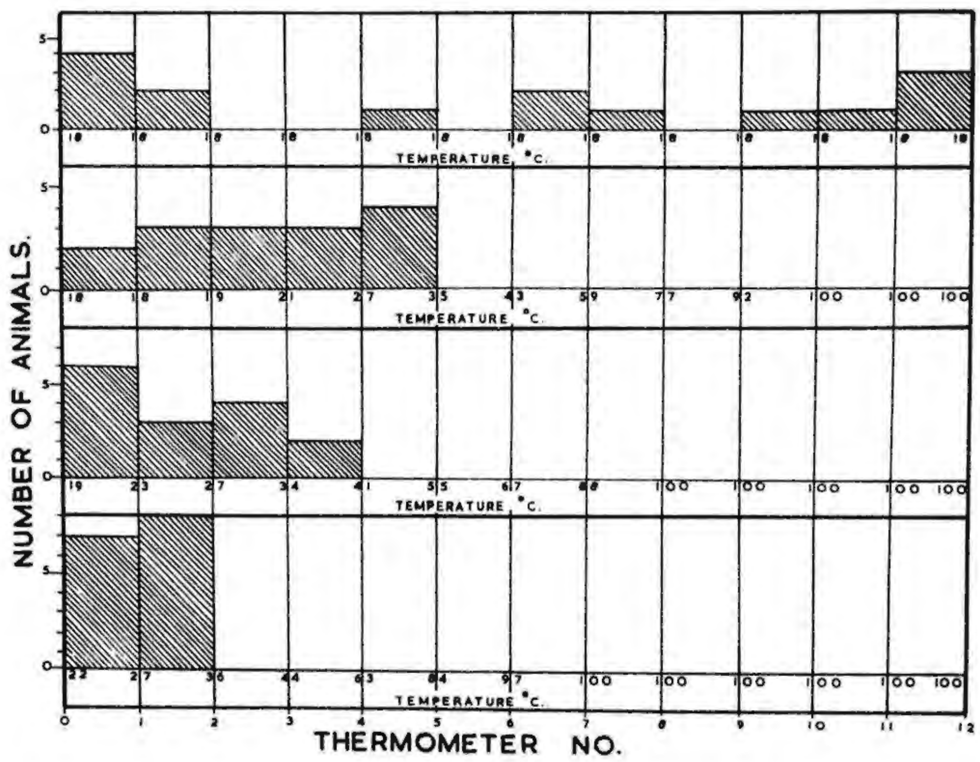


Fig. 7.02 : The distribution of M. delanyi in a temperature gradient of increasing steepness, recorded at ten minute intervals.

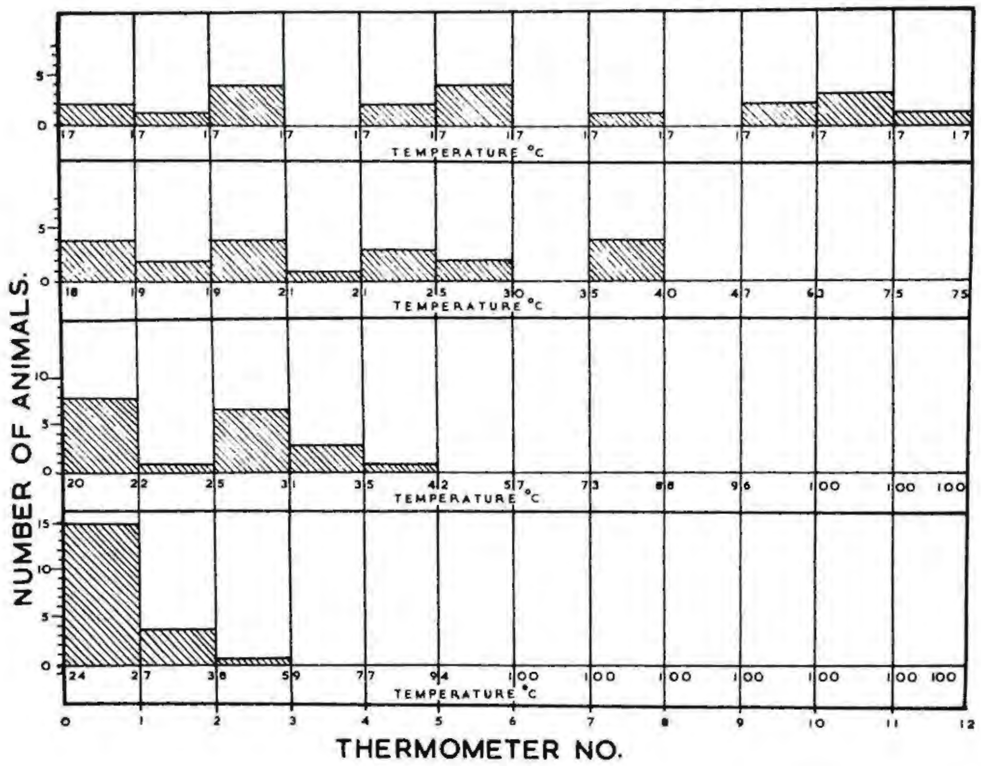


Fig. 7.03 : The distribution of C. longicaudata in a temperature gradient of increasing steepness, recorded at ten minute intervals.

The nature of the response appeared to be a klinokinesis and this was subsequently shown to be so (see following section 7.1 (ii)). The animals turned sharply upon reaching the critical temperature and then retreated but owing to the narrowness of the chamber restricting the movements of the animals to some extent, it was necessary to conduct separate experiments in a chamber which allowed greater freedom of movement.

C. longicaudata showed a well-defined optimal temperature zone in the range 12°C to 25°C, the majority of the animals having aggregated here in the gradient as shown in Figure 7.04. M. delanyi, on the other hand, aggregated in the cold end of the gradient without showing any cold avoidance as evident in C. longicaudata, as shown in Figure 7.05. It was therefore necessary to establish whether these animals responded to the lowest temperature available, or whether the temperature preferendum was lower than the minimum provided in the gradient. To this end a further experiment was conducted in a gradient with a maximum of 14°C and a minimum of 5° (the lowest obtainable in the apparatus). Figure 7.06 reflects the results of this experiment, indicating an avoidance of extremely low temperatures. The solitary animal in the coldest zone was found to be in a state of chill coma. When allowed to recover and re-introduced to its original position in the gradient, it moved out of the low temperature zone; repeated re-introductions into the cold zone had the same result, thus the animal's presence originally in this portion of the gradient may be attributed to trapping.

(ii) Nature of the temperature responses.

Apparatus and method:

The difficulties experienced in establishing the nature of the responses to temperature using the "Temperature Organ" have already been alluded to. A new apparatus allowing for greater freedom of movement

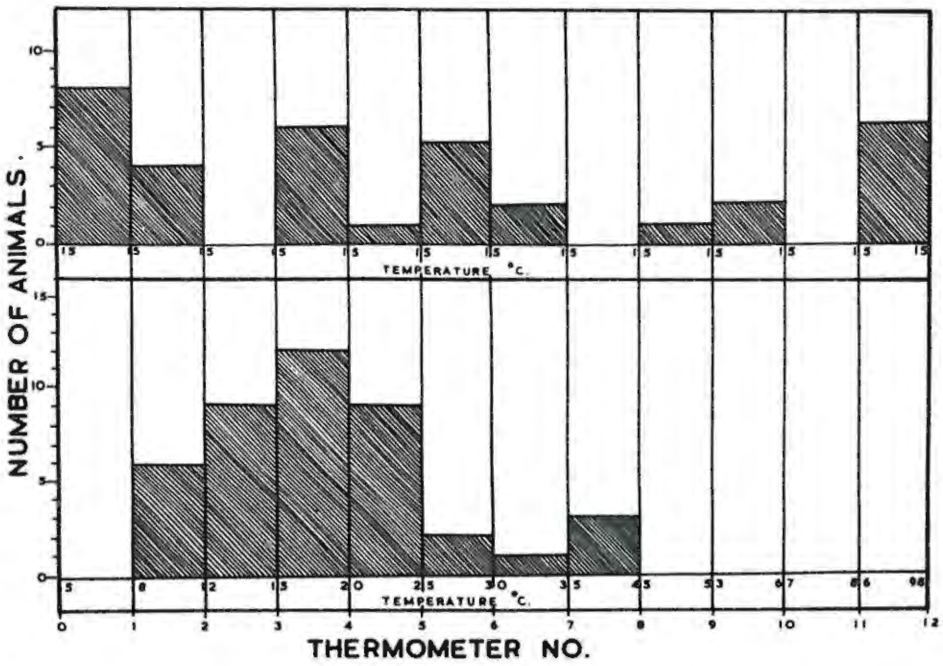
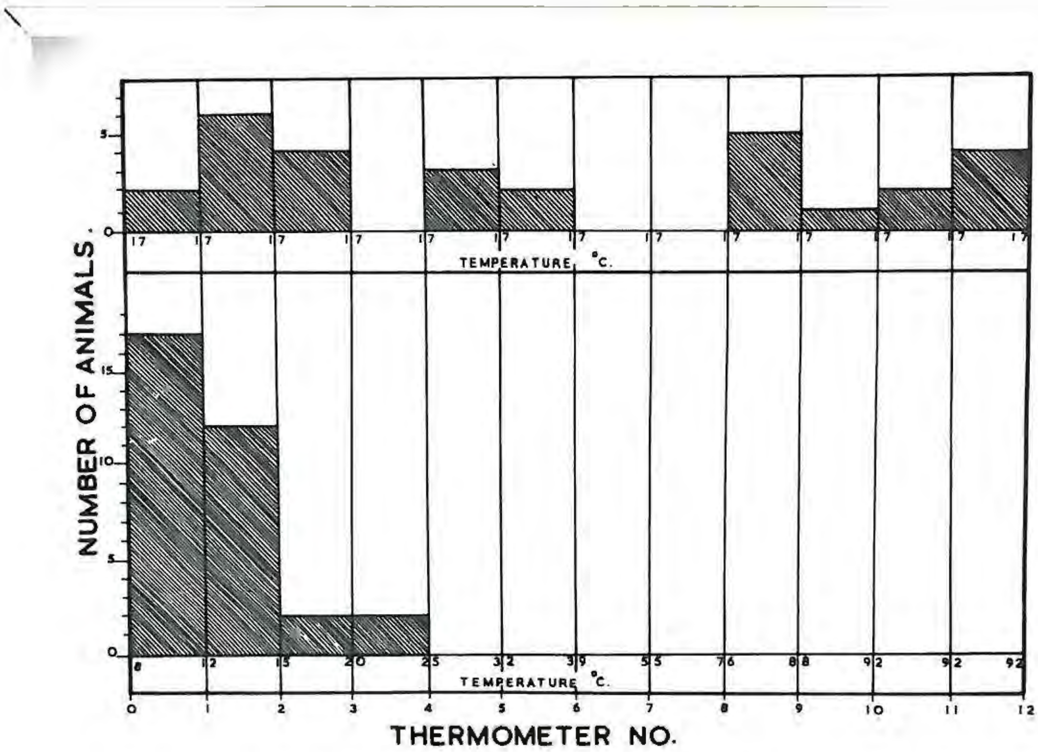
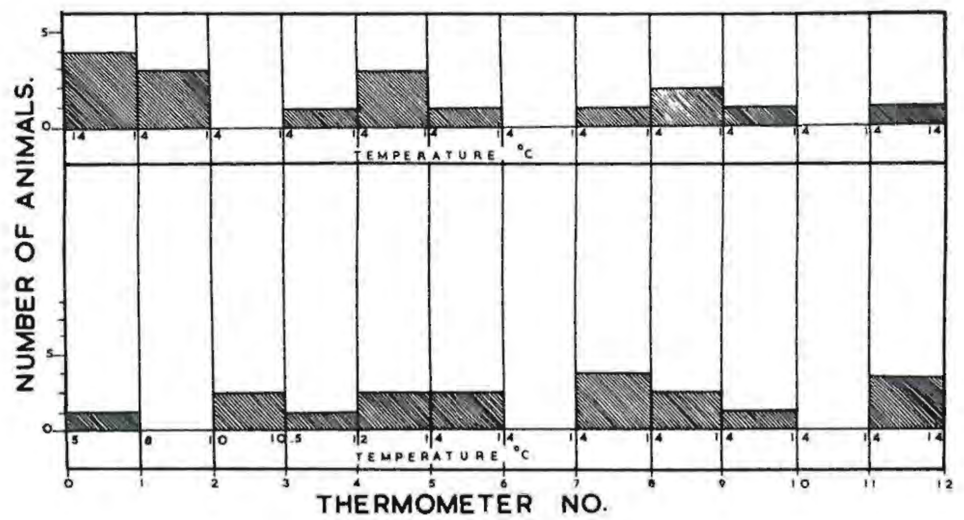


Fig. 7.04 : Distribution of C. longicaudata in a temperature gradient showing the preferred temperature range to lie between 12°C and 25°C.



<u>Fig. 7.05</u> : Distribution of <u>M. delanyi</u> in a temperature gradient showing aggregation in the lowest temperature zone.



<u>Fig. 7.06</u> : Distribution of <u>M. delanyi</u> in a temperature gradient showing some avoidance of extreme cold.

had to be employed to confirm the observations made and a circular gradient, which had been used by other workers for this type of investigation, seemed to meet the requirements here. The chamber used was made of tin, and measured 23 cm. in diameter with four concentric circles inscribed in the floor, thus dividing it into five zones. The experimental animals were introduced into the chamber singly and their movements recorded by tracking for five minutes on a scaled-down plan of the chamber under a dim red light which had been shown to elicit no photoresponses. The animal was then removed and a temperature gradient was set up by heating the centre of the chamber from underneath by means of a 100 watt electric lamp in a constant temperature room at 8°C. The gradient thus set up ranged from 59°C in the centre to 14°C at the periphery, the temperatures having been measured using a thermometer insulated from the ambient temperature by means of a layer of cotton wool so that only the base of the bulb was in contact with the floor of the chamber. Using the "Temperature Organ" to test the efficacy of this method of measuring temperature it was found that a thermometer thus placed, although slower to come into equilibrium, never read more than 1°C lower than the thermometer with its bulb actually in the floor. Over the range tested, the usual difference was less than 0.5°C.

Once this gradient had been established the animal was re-introduced into the coldest zone of the chamber and its movements tracked for a further five minutes as previously described. If the animal remained stationary for more than ten seconds, it was stimulated to resume activity by tapping the chamber underneath the spot where it had come to rest. Four specimens of <u>C. longicaudata</u> were tested in this way.

A more satisfactory method of observing the response to high temperature was evolved from the foregoing quite by accident. It was found that when animals in the chamber were subjected to overhead lighting from a 100 watt electric lamp situated 150 cm above the chamber, they would confine their movements to the periphery of the chamber and remain active for long periods of time. By placing a temperature barrier in the path of the animal in the chamber, it was now possible to track it and observe its reactions to the barrier in normal light. In addition, this method ensured numerous encounters with the barrier. Both high and low temperature barriers were used in the apparatus. These were obtained by placing either a 100 watt electric lamp or a block of ice under the floor of the chamber at its periphery as required.

M. delanyi showed the same response to the periphery of the chamber under conditions of overhead lighting. Tracking of these animals was complicated by their ability to leap, particularly when stimulated to movement after having come to rest for longer than ten seconds.

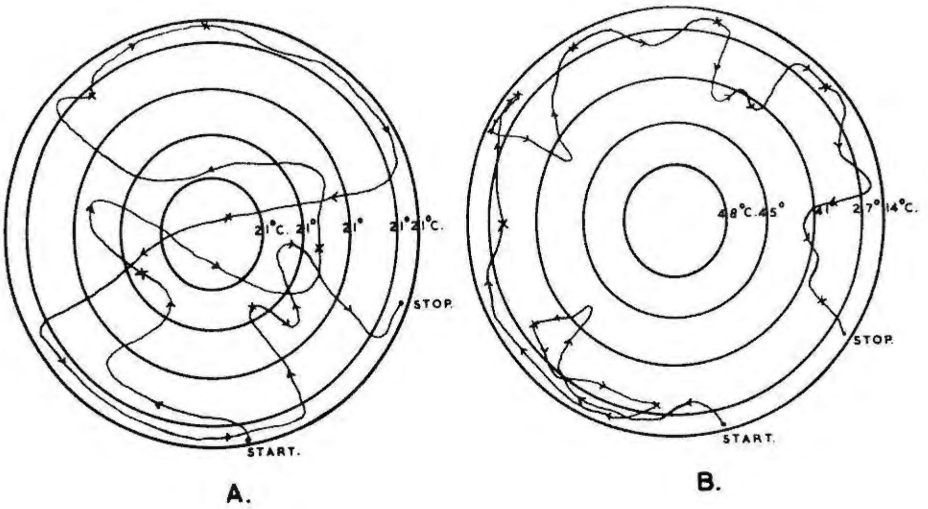
Whilst the foregoing would demonstrate the reactions of the animals to encountered unfavourable temperature conditions, there is clearly also a need for a response to environmental temperatures changing to a dangerous level. The animal would, in such circumstances, find itself already within the zone of unfavourable stimulus and would, therefore, not be able to take avoiding action as such.

In order to investigate this aspect of the temperature responses, the animals were placed singly in glass-topped metal containers 12 cm in diameter, the floor of which had been inscribed with two concentric circles and divided into four quadrants in order to facilitate localisation of the animals on a plan. The container was allowed to come into equilibrium at a given temperature in a constant temperature room in the dark, and the position of each animal recorded at half-hourly intervals using a dim red light. This would reflect whether the animal had moved during the preceding half hour or not and thus give a measure of activity. This experiment was carried out at 10°C, 15°C, 21°C, 30°C, 35°C and 40°C for C. longicaudata whilst for M. delanyi 40°C was omitted and 7°C included in the range instead. Temperatures of 35°C and 40°C were attained by localised heating from a thermostatically controlled hotplate, the containers being suspended approximately one cm above the surface of the hotplate. Temperature was, in all instances, measured against the sides of the containers. Eight readings were taken for each of twelve animals at each temperature.

Results:

The experiments carried out in the circular chamber served to confirm the observations made in the "Temperature Organ", the avoiding reaction to high temperatures being indeed a klinokinesis in the sense defined by Fraenkel and Gunn (1940). Figure 7.07 shows the movements of C. longicaudata in the chamber before and after setting up of the gradient; abrupt turning when an unfavourable stimulus is encountered is clearly evident. The results from the other two animals tested in this way was essentially the same. The klinokinesis was even more pronounced in the results obtained by placing a temperature barrier in the path of the animals as shown Figures 7.08 and 7.09 for C. longicaudata and M. delanyi respectively. This avoiding reaction was, however, only apparent when a high temperature barrier was encountered, low temperatures evoking no such reaction from either species.

Both species showed increased activity under adverse temperature conditions; such an orthokinesis could serve to take the animals out of unfavourable temperature conditions in which they may find themselves. Figures 7.10 and 7.11 reflect the numbers of animals active and inactive during each of the half-hour intervals between readings, for the various temperatures tested. Figure 7.12 summarises comparatively, the



<u>Fig. 7.07</u> : Path followed by <u>C. longicaudata</u> in a circular metal chamber before (A) and after (B) the setting up of a temperature gradient, showing a typical klinokinesis in order to avoid unfavourable temperature stimulus.

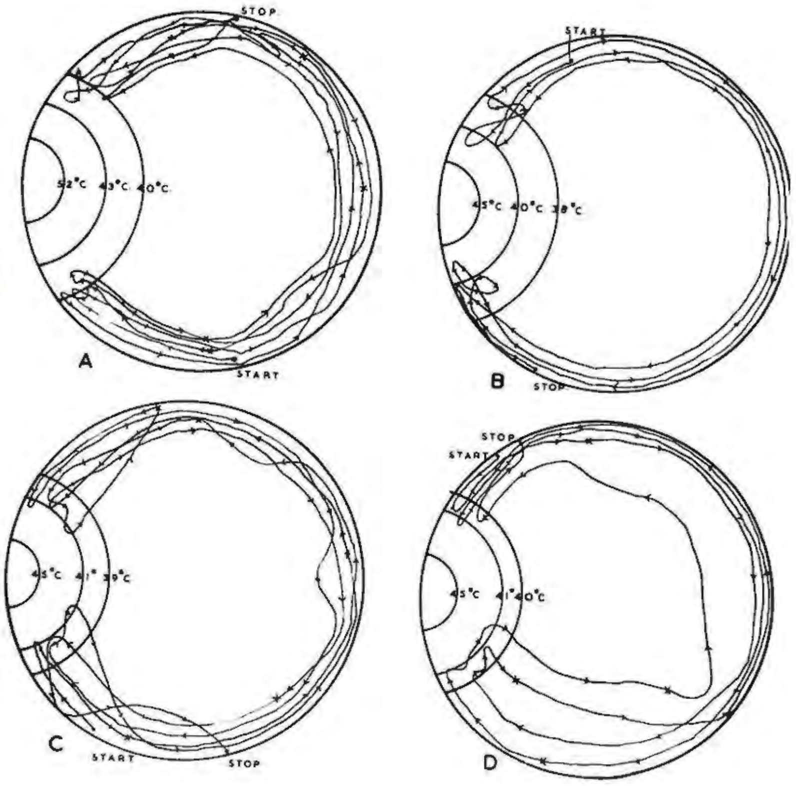


Fig. 7.08 : Reactions of C. longicaudata to a temperature barrier across its path showing the klinokinesis even more markedly.

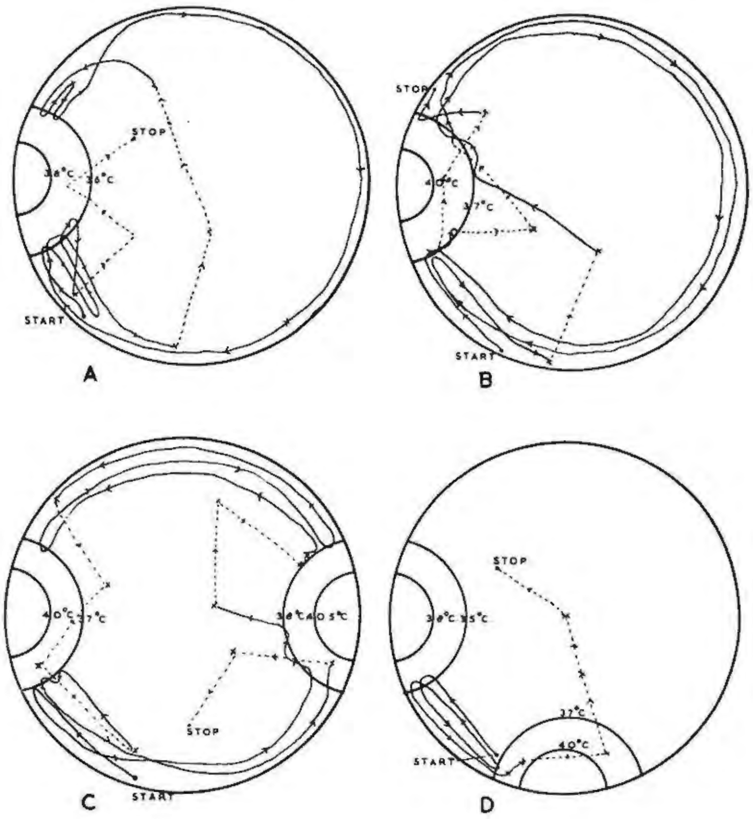


Fig. 7.09 : Reactions of M. delanyi to a temperature barrier across its path. Dotted lines indicate magnitude and direction of leaps.

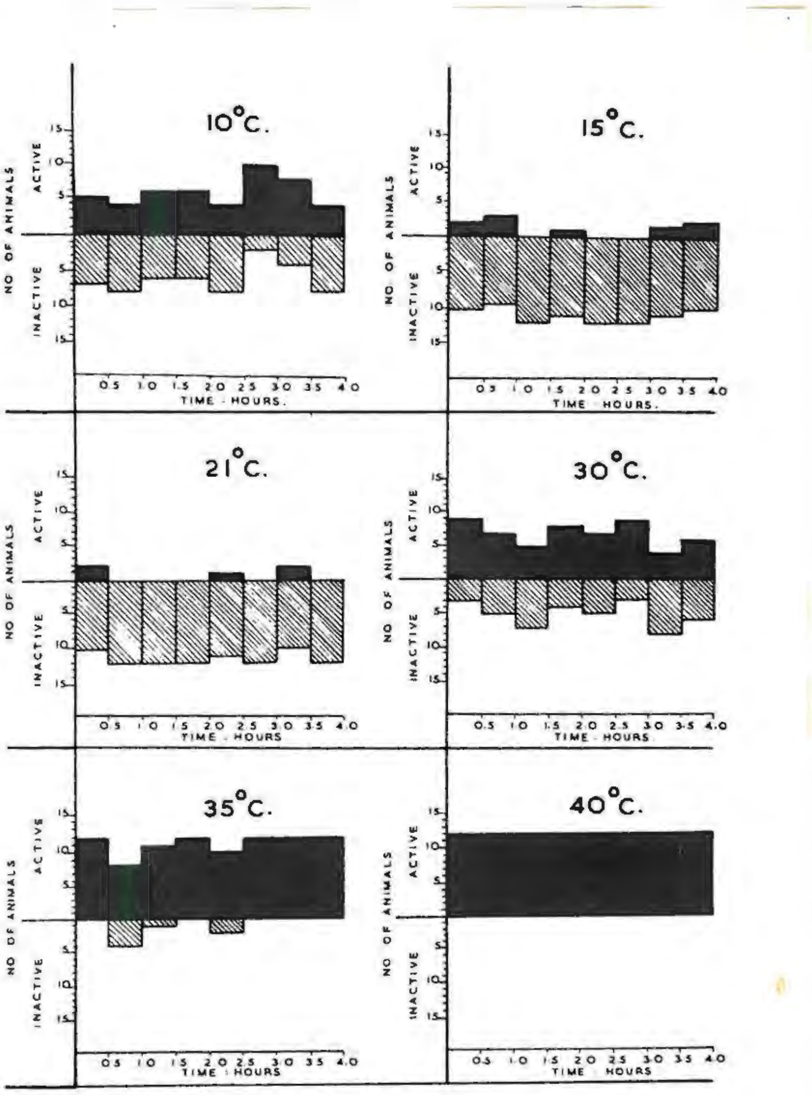
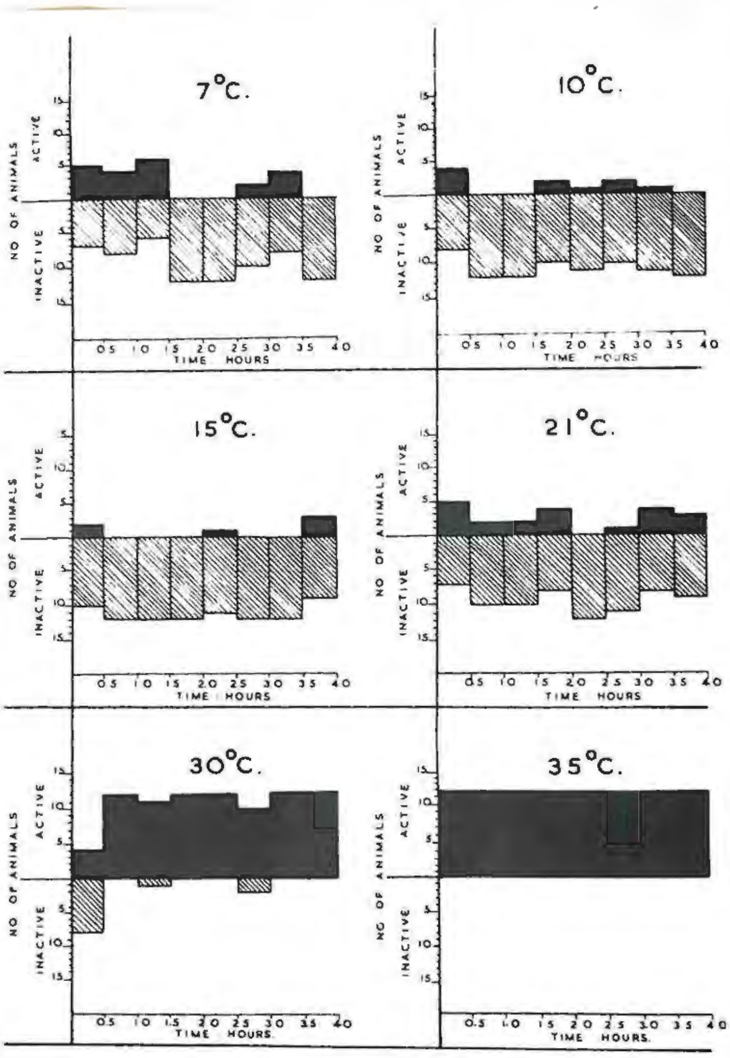


Fig. 7.10 : Activity of C. longicaudata at different temperatures, based on half-hourly readings, suggesting an orthokinesis under conditions of unfavourable temperature stimulus.



<u>Fig. 7.11</u> : Activity of <u>M. delanyi</u> at different temperatures, based on half-hourly readings, also suggesting an orthokinetic response to unfavourable temperature conditions.

results obtained from these experiments and further serves to confirm the temperature preferenda of the two species as established using the temperature organ, the animals becoming progressively more active as the temperature deviates further from the eccritic.

An activity orthokinesis is, therefore, manifest in both species, providing an escape mechanism which guards against their becoming trapped in regions where adverse temperatures, both high and low, may be brought about by gradual changes and where no barriers eliciting an avoiding reaction will, therefore, be encountered. This, together with the avoiding reaction, would serve to minimise the risk of the animals ever finding themselves in lethal temperatures.

(iii) The site of the thermoreceptors.

Apparatus and method:

The only means whereby the thermoreceptors could be localised was to ablate various appendages or parts of appendages which could possibly carry these sense organs and then to note the effect on the temperature responses of the animals. The appendages which were considered most likely to be concerned with temperature perception were the antennae, the maxillary palps and the tarsi of the legs. Thermoreceptors on the abdominal styles, cerci or median caudal filament would be of little use since the animal would find itself already well within the zone of unfavourable temperature before such receptors are stimulated.

Experimental animals were tested for avoiding behaviour when faced with a temperature barrier as described under 7.1 (ii) in order to ensure that they were behaving normally. 24 specimens of each species were then cooled in a refrigerator to induce a chill coma which allowed the various operations to be carried out with little difficulty. These groups were then divided into four batches of six animals each and

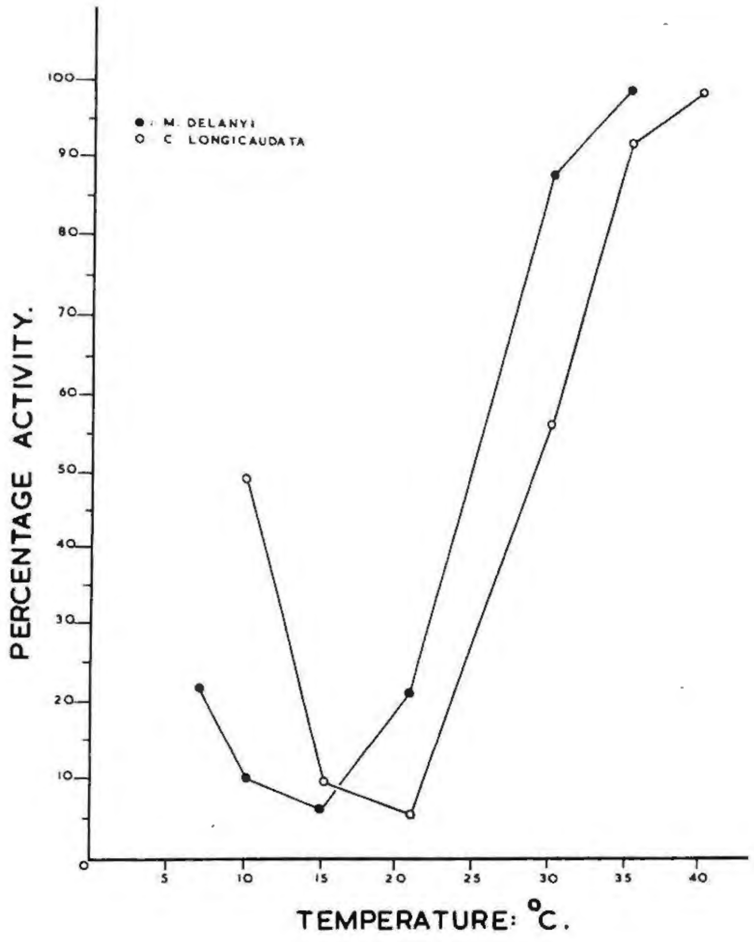


Fig. 7.12 : Percentage activity of both C. longicaudata and M. delanyi at different temperatures based on all the readings shown in Figures 7.10 and 7.11.

treated as follows:

Batch 1 - flagellae of antennae removed as close to the pedicel as possible: Batch 2 - maxillary palps removed: Batch 3 - antennal flagellae and maxillary palps removed and Batch 4 - controls, in order to ensure that chilling had in no way affected the normal temperature responses of the animals. All the animals were kept in a constant temperature room at 21°C with access to food and water for a period of 48 hours before commencement of the experiment, in order to allow them to recover from any possible effects of the operations.

After the recovery period, the animals' responses to a temperature barrier were again tested as previously described, their movements being recorded on a scaled down plan of the chamber.

Results:

These experiments showed the presence of at least two thermoreceptors in both M. delanyi and C. longicaudata with a strong possibility of a third being present in both.

The path followed by three of the six specimens in each batch of M. delanyi are shown in Figure 7.13, the remaining animals differing but little from these. It will be seen here that ablation of either antennae or maxillary palps by themselves had no apparent affect on the temperature responses of the animals since the klinokinesis was still apparent at the same temperatures at which the controls showed this response. Removal of both antennae and maxillary palps, however, resulted in the avoiding behaviour breaking down, the animals would now move into the zone of highest temperature. Once here they showed obvious signs of distress, leaping out of the unfavourable stimulus zone only to re-enter it again on the next circuit of the chamber.

C. longicaudata as Figure 7.14 shows, behaved normally towards the temperature barrier only so long as the antennae were intact, there being no difference between animals without maxillary palps only and the controls. When the antennae were removed but the palps left intact the animals still showed an avoiding reaction but instead of this being at the normal temperature of 40 - 43°C the response was now evoked only at 48 - 50°C. It was thought that the difference might be due to the long antennae stretching out in front of the animal but the length of a 5°C difference in the temperature gradient was found to be at least twice the antennal length. Furthermore, *M. delanyi* has relatively longer antennae than *C. longicaudata* but showed no difference in the temperature which it would avoid with or without antennae, so it would appear that the two receptors of *C. longicaudata* have different thresholds. Ablation of both the antennae and the maxillary palps caused a complete breakdown of the avoiding reaction in *C. longicaudata*, the animals passing through the barrier without deviation. There was, however, a marked increase in the speed of locomotion when the animals passed through the high temperature zone, which may indicate the presence of a third receptor.

It seemed possible that this third receptor, if present, might be located on either the tarsi of legs or on the labial palps. Amputation of the tarsi or covering them with "Samsonite" so impaired locomotion as to render the animals useless for experimental purposes, whilst amputation of the labial palps had no apparent effect. In these instances the animals were confronted with a barrier of as much as 70°C and although this seemed to elicit leaping somewhat further from the centre of the barrier in the case of *M. delanyi*, *C. longicaudata* would still pass through it without deviation, often succumbing to heat shock within the range of the adverse temperature.

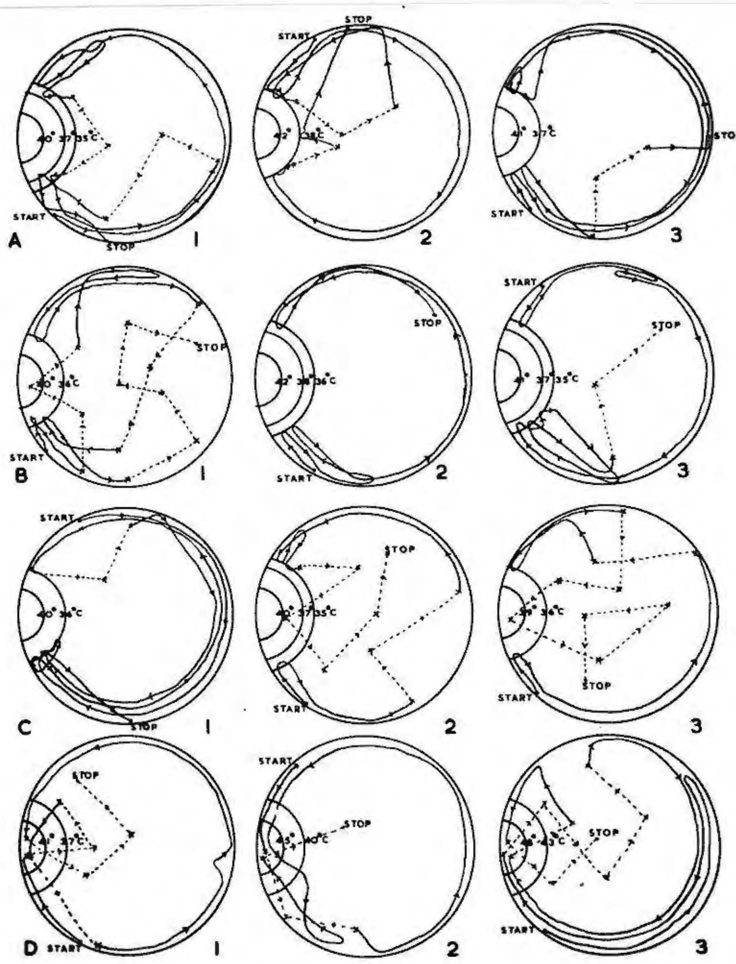


Fig. 7.13 : Effects of Ablation of Antennae (B), Maxillary Palps (C) and both antennae and maxillary palps (D) on the avoidance reaction to high temperatures of M. delanyi. (A) shows reactions of controls.

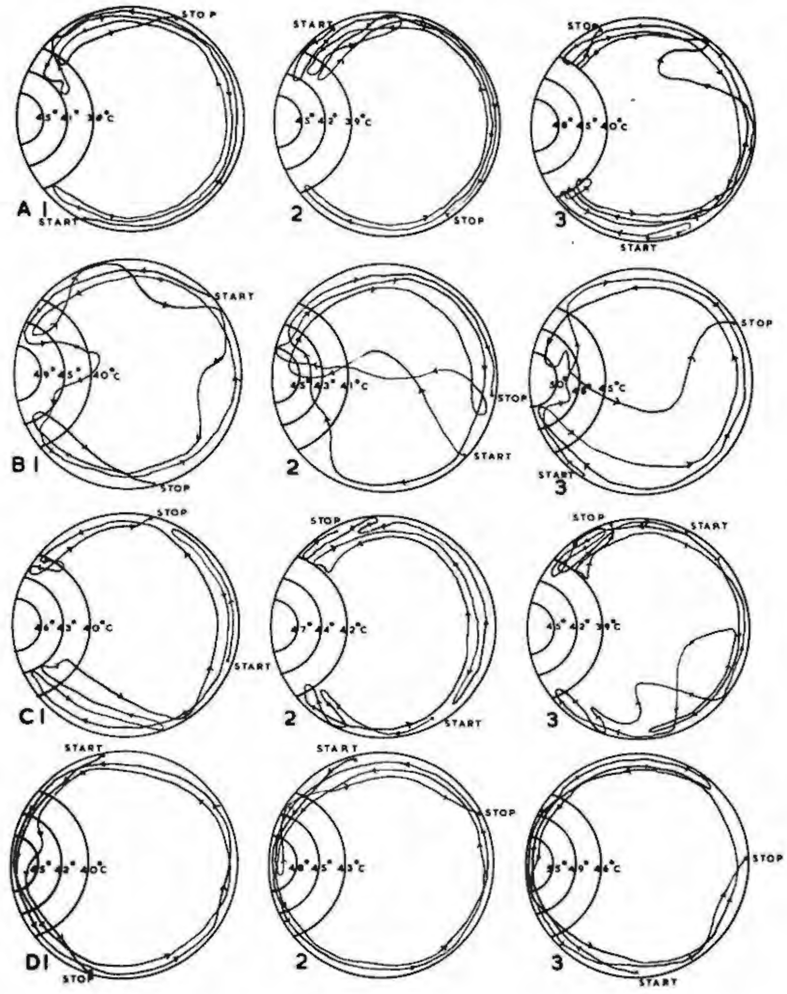


Fig. 7.14 : Effects of ablation of antennae (B), maxillary palps (C) and both antennae and maxillary palps (D) on the avoidance reaction of C. longicaudata to high temperatures. (A) shows reactions of controls.

7.2 Behavioural Responses to Light.

The absorption of solar heat will also rapidly raise the temperature of many animals above the critical level; this is certainly true for *Machiloides delanyi* which will succumb to what can only be assumed to be hyperthermia after but 30 minutes exposure to the full summer sun. *Ctenolepisma longicaudata* will survive for somewhat longer, two to four hours having been recorded although specimens of an hitherto unidentified lepismatid species from the semi-desert Karroo region near De Aar survived only 90 minutes exposure. The latter were housed in a plastic dish after collection and inadvertantly left in the sun. Since the dish was made from a transparent plastic material and had its lid in position, the temperature within must have been raised considerably in much the same way as in a greenhouse where there is no dissipation of heat by convection and general circulation of air. The instances recorded for *M. delanyi* and *C. longicaudata* were of animals housed in open translucent plastic containers placed in a window-sill exposed to the full sun and therefore, not comparable with the case of the Karroo lepismatids.

In colder climates the ability to absorb radiant heat from the sun is often capitalised by poikilotherms inhabiting these regions, dark body pigmentation enhancing the absorption of infra red rays. One record of a thysanuran thus absorbing solar heat comes from the region immediately below the snowline in the Himalayan Mountains, where a machilid, *Machilanus sp.*, as well as other arthropods, is dependent upon the sun to raise its body temperature to a level where activity becomes possible under conditions where the air temperature is but little above freezing point (Swan, 1961). In warmer climates, however, the continuous absorption of solar heat is, as we have seen, likely to result in death, particularly in small animals where the surface to volume ratio is high and, unless a mechanism whereby such heat may be dissipated is present, the animals need a behavioural response to keep them from exposure to direct sunlight.

The nocturnal habits of both *M. delanyi* and *C. longicaudata* have already been referred to, suggesting that both possess a behavioural mechanism which orients them away from light, thus avoiding the problem of having to lose excess heat to their surroundings. Such a photoresponse has, however, still to be demonstrated experimentally.

(i) Behaviour in a light/dark choice chamber.

Apparatus and method:

Both species were given a choice between light and dark in a choice chamber measuring 12 x 12 x 4 cm. The chamber consisted of a plastic dish the walls and floor of which were lined with black paper while one half was effectively darkened by means of a double masking layer of black paper. Communication between the two halves of the chamber was confined to a gap, 0.5 cm high, between the floor and a black paper screen running the full width of the chamber. A section through the length of the apparatus is shown in Figure 7.15.

Batches of from four to twelve dark-adapted animals were placed in the "light" side of the chamber under a dim red light and confined to this side by a strip of celluloid placed in front of the interleading gap. The animals were allowed to come to rest in the chamber in darkness over a period of one hour after which the celluloid strip was removed, giving free access to the "dark" side of the chamber and a 60 watt electric lamp placed 60 cm. above the choice chamber was switched on. The numbers of animals in the light side of the apparatus were determined at short intervals ranging from one to four minutes. 82 specimens of M. delanyi and 35 specimens of C. longicaudata were thus tested.

A control experiment was done with 20 animals of each species. These were treated exactly as described above with the exception that no light stimulus was given until the animals had had full access to the whole of the choice chamber for a period of three hours.

The experiments were carried out in a constant temperature room at a temperature of 20°C.

Results:

Both M. delanyi and C. longicaudata showed a strong negative photoresponse, rapidly leaving the light side of the choice

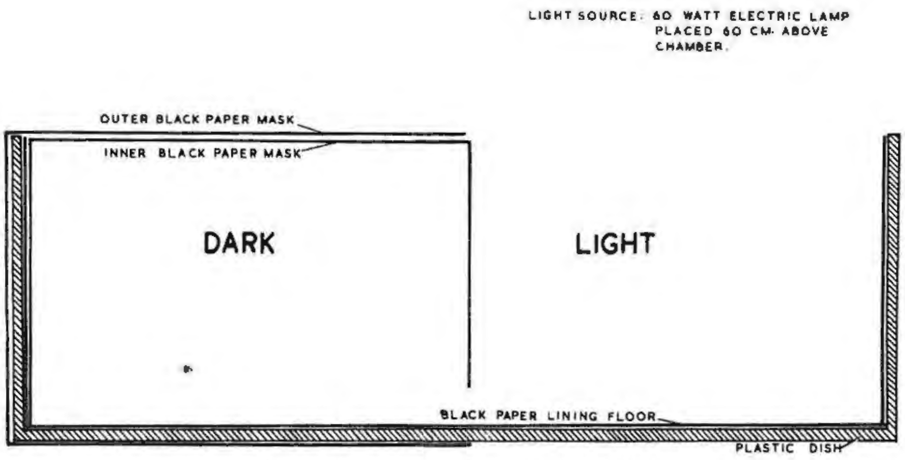


Fig. 7.15 : Section through the length of the Light/Dark choice chamber.

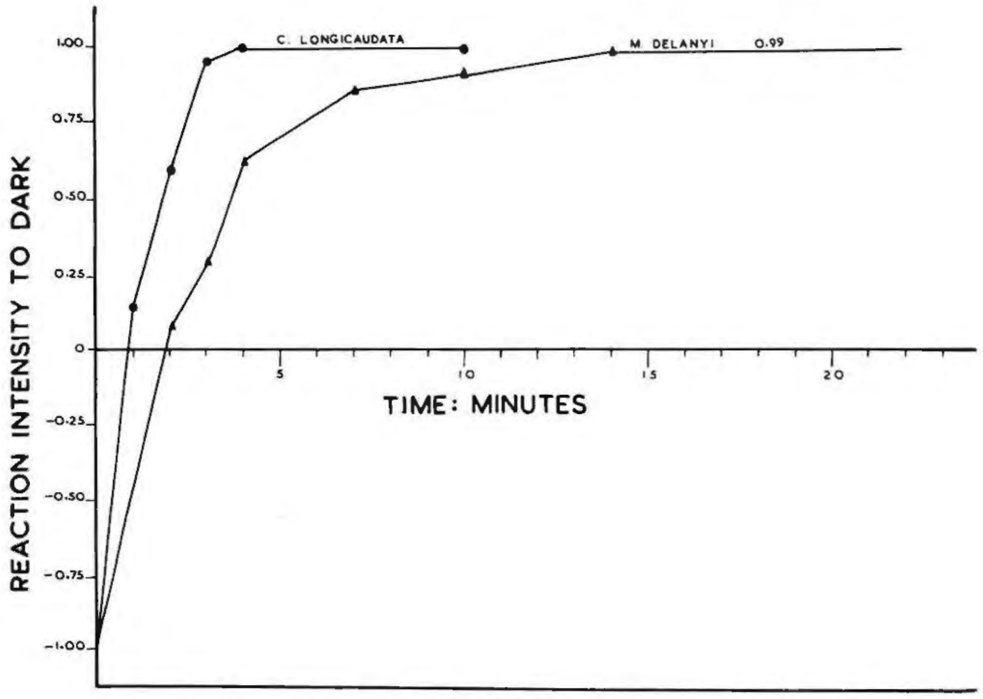


Fig. 7.16 : Reaction intensity of C. longicaudata and M. delanyi to the dark when in a Light/Dark choice chamber at 20°C.

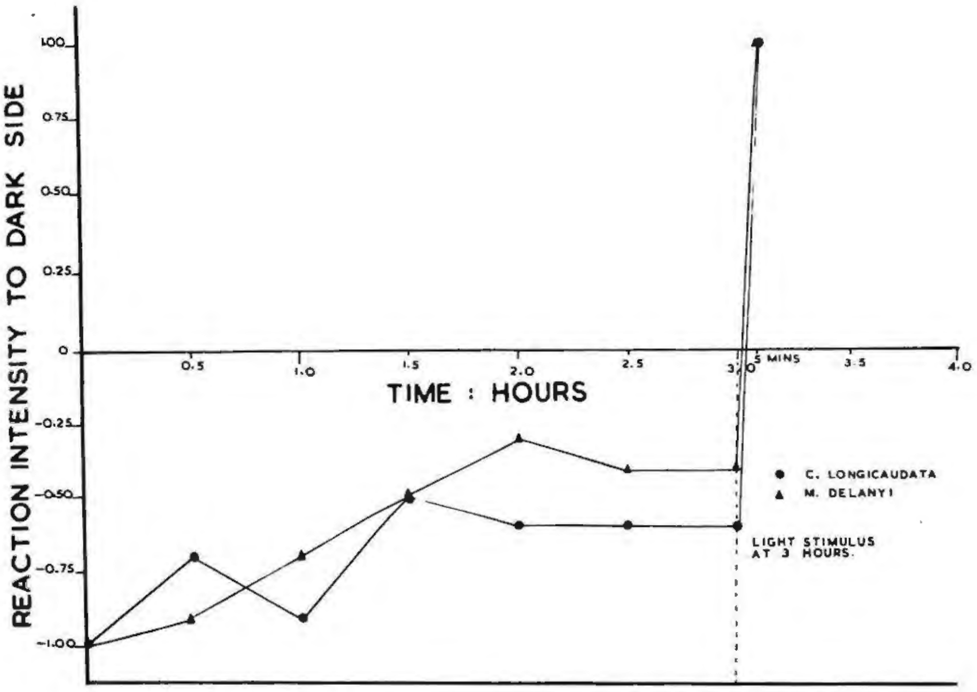


Fig. 7.17a : Reaction intensity of *C. longicaudata* and *M. delanyi* to the dark side of Light/Dark choice chamber under conditions of total darkness with light stimulus given after three hours.

chamber. Figure 7.16 shows the changes with time of the reaction intensity towards the dark, the reaction intensity being expressed as number of animals in dark minus number of animals in light over total number of animals. This method of expressing the reaction intensity thus gives a range from 1 (all in dark) through 0 (completely random) to -1 (all in light). Figure 7.17a shows the results obtained in the control experiment.

It appears from the results that the negative light response is much more rapidly evoked in C. longicaudata than in M. dalanyi and this is further borne out by observations on individual animals subjected to a light stimulus. C. longicaudata will almost immediately become active upon stimulation whilst there is a short latent period in M. delanyi. This phenomenon was subjected to further investigation, the details of which appear in Part 7.2 (ii). Further evidence for a lesser sensitivity to light in M. delanyi can be inferred from the readiness with which it becomes light adapted: specimens of this species which have been kept in culture dishes with no shelter for a few days show no response to such shelter when provided, as lohg as the light intensity is not too high but C. longicaudata has never been observed to behave in such a manner. The possibility of a thigmotaxis being responsible for the condition in C. longicaudata cannot, however, be excluded and the whole question of light and dark adaptation in both species has not been critically investigated.

(ii) Nature of the Photoresponse.

In determining the nature of the photoresponse the following questions need to be answered:

1. Does light stimulate the animals to activity even in the absence of darkness affording a refuge, as was the case in the light/dark choice chamber?
2. Do the animals reach the dark side of the choice chamber by virtue of a negative response to light (negative phototaxis) or a positive reaction to darkness (skototaxis) or perhaps both?
3. Is the directional response a klino, tropo, or telotaxis?

It is therefore necessary to establish the animals' reactions to uniform light, directional light and to more than one source of stimulus. Unilateral blinding would also assist in determining the nature of any directional response.

Apparatus and method:

The effect of uniform light on the animals was observed using the circular metal chamber in which the temperature responses were investigated. The floor of the chamber was lined with white paper to provide a suitable footing and the chamber was set up 150 cm. below a 100 watt lamp. The inner walls of the chamber were reflective thus light conditions within were uniform and non-directional. The experimental animals were kept in the dark for a period of 48 hours in order to ensure that they were dark adapted, after which they were placed in the chamber without exposing them to light at any stage and left to distribute themselves in the dark. Observation using a faint red light ensured that the animals were all at rest after a period of one hour and the lamp overhead was now switched on. The numbers of animals actively moving (active), moving without changing position, e.g. waving antennae etc. (partially active) and completely inactive were periodically recorded.

Responses to directional light were observed in an arena measuring 50 x 22 cm., and constructed from black cartridge paper. The lower parts of the walls of the arena were lined with strips of glass to prevent the animals from climbing out. An 11 cm. gap was cut out of the centre of one of the shorter walls into which was fitted a museum jar filled with water to act as a heat filter to eliminate radiant heat from a 60 watt electric lamp situated 10 cm. behind it. The efficacy of this filter was tested using a black-bulb thermometer contained in an evacuated glass tube and placed at a spot 5 cm. from the filter; this showed an increase of only 1.7°C (3°F) over a period of ten minutes. Since the animals were never in this position for more than a matter of second, the filter was thought to be adequate.

Dark adapted animals were used as in the previous experiment. The animals were individually placed at a spot 5 cm. from the centre of the heat filter and the light immediately switched on. The animals were tracked on a scaled-down plan of the arena, the experiment being regarded as complete for any particular individual when it reached the wall opposite the light source.

It must be stressed here that the light beam used was not perfect in that although a large sheet of black paper was placed against the wall of the constant temperature room immediately behind the apparatus, the roof and walls further away were not blacked out, thus these would reflect a certain amount of light. Further, the strips of glass lining the walls to prevent escapes would also give rise to some reflection.

In order to determine whether a skototaxis was at least part of the directing mechanism the animals were given a choice between alternate patches of light and dark under uniform light conditions. Here again the circular metal chamber was used, but four pieces of black cartridge paper 9 cm. square were placed around the periphery of the chamber at 90°intervals, thus effectively dividing the circular wall into eight equal alternating areas of light and darkness when lit from overhead. Thin lines drawn with a hard pencil were extended from the edges of each sheet of black paper to the centre of the chamber. A circle, two centimeters in diameter was drawn on the floor in the centre of the chamber from which the animals were started in each instance. Individual dark-adapted animals were placed in the centre circle from a specimen tube enclosed in black paper and tracked on a scaled-down plan of the apparatus, care having been taken to change the direction of liberation by 45° for each successive animal tested. The experiment was regarded as having been concluded for any individual animal when it reached the peripheral wall of the chamber. The pencil lines on the floor enable a distinction to be drawn between animals orientated directly towards a black screen and those orientated between screens on leaving the central circle.

Further experiments of this nature were conducted in an arena similar to that described for the investigation of responses to directional light but with white walls and floor instead of black. Sheets of black paper were placed at various positions in the arena, at right angles and parallel to the direction of the light rays. The paths of the animals here too were tracked on a scaled down plan of the apparatus.

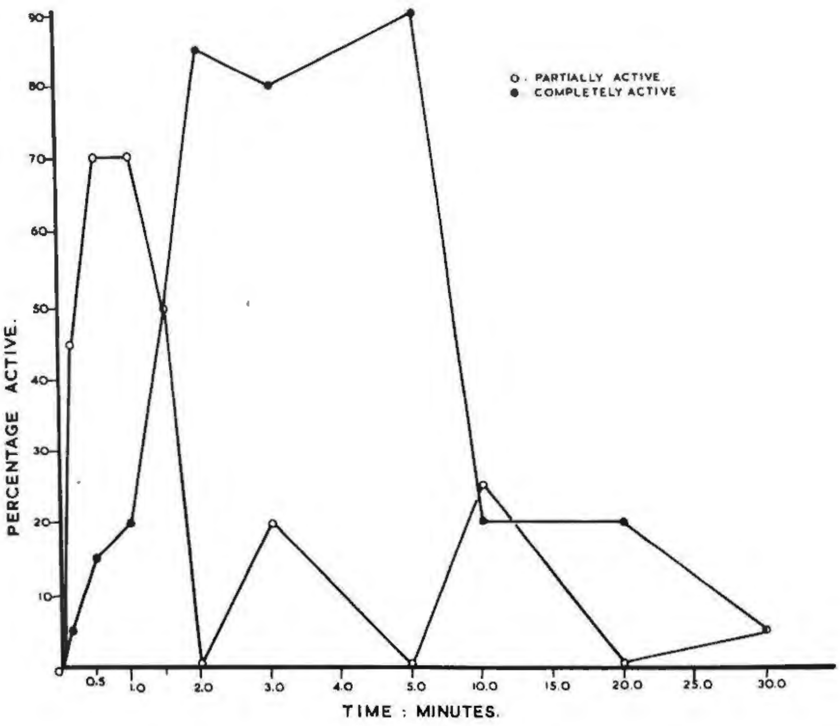
Unilateral blinding was effected by the application of a quick-drying black enamel paint to one compound eye. It was necessary to bring the animals into a state of chill coma before the paint could be applied and this required a control experiment to ascertain whether chilling and paint application had any effect on the light responses. To this end seven animals of each species were chilled together with the experimental animals but instead of blacking out the compound eye, paint was, in this case, applied to the prothorax. These animals were then tested in a light/dark choice chamber where their light responses were found to be normal. The reactions of the unilaterally blinded experimental animals were then observed under conditions of uniform and directional illumination.

Owing to the lateral ocelli of <u>M. delanyi</u> lying in very close proximity to the compound eyes, the application of paint to the latter invariably resulted in the ocelli being partially covered as well. In order to ensure uniformity, care was taken to cover the ocellus on the blinded side completely in every instance. How this affected the results of these experiments is not known.

Results:

Both <u>M. delanyi</u> and <u>C. longicaudata</u> were almost immediately stimulated to activity by light, there being a short latent period between the application of the light stimulus and time when the majority of the animals were active. Figures 7.17 and 7.18 show the percentage of the total numbers of animals fully and partially active as a function of the time of stimulation. Both species showed the same response to the periphery of the chamber already described for the circular temperature gradient but whether this was purely a photoresponse or a photoresponse together with a touch response is not known.

Both species show a negative response to directional light, their paths in a light beam being roughly the same as the direction of the light rays as shown in Figure 7.19. This was further



<u>Fig. 7.17</u> : Effect of uniform light stimulus on the activity of *M. delanyi*.

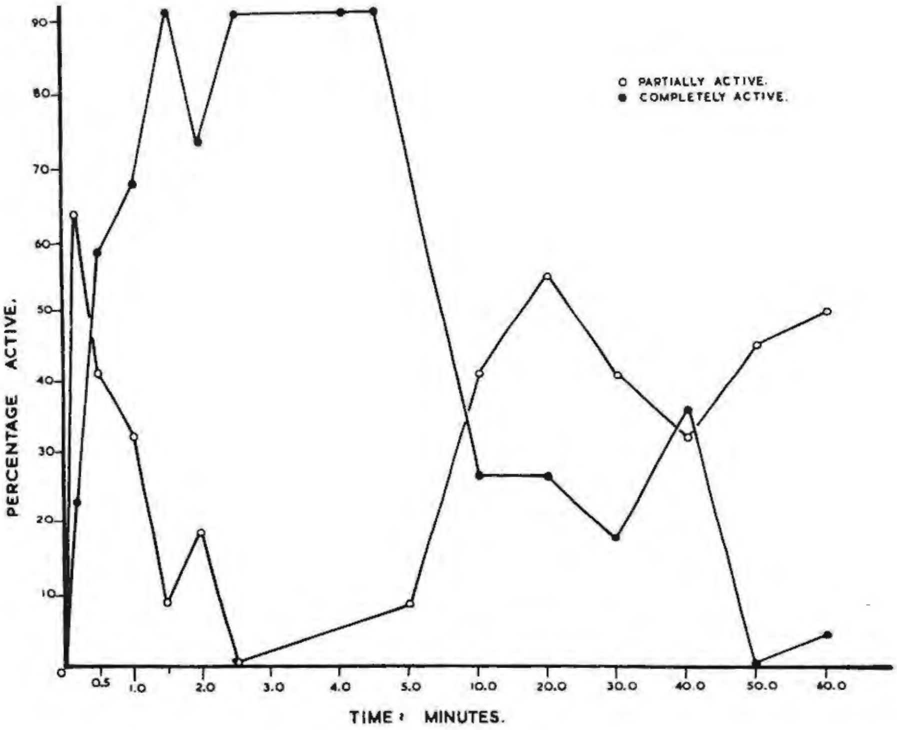


Fig. 7.18 : Effect of uniform light stimulus on the activity of *C. longicaudata*.

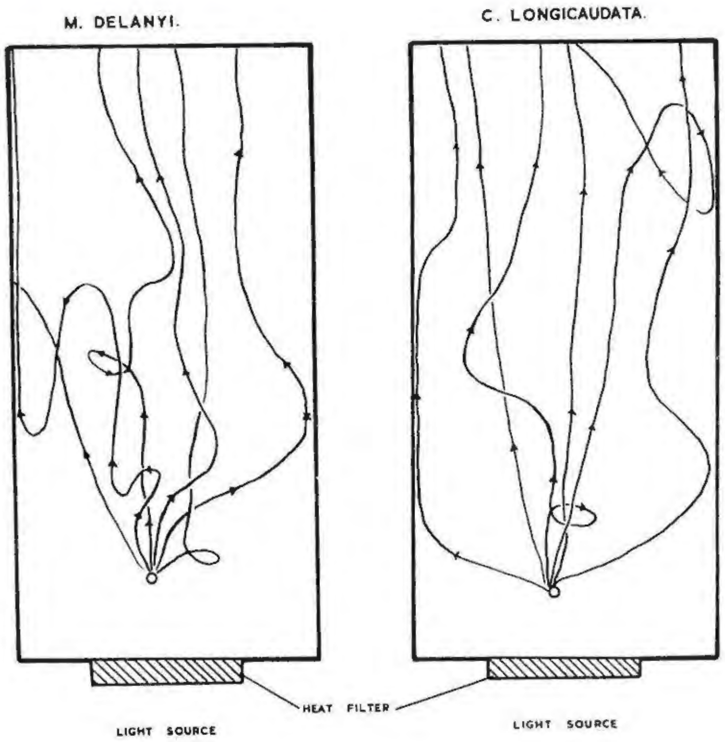


Fig. 7.19 : Paths followed by dark adapted specimens of M. delanyi and C. longicaudata under the influence of directed light in a black-walled arena.

borne out by observation of animals in transparent plastic humidity choice chambers, where they would almost invariably move to the side away from the light, regardless of humidity. Both species therefore show, in addition to an activity orthokinesis, a negative phototaxis, the exact nature of which was not determined.

When given a choice between alternate areas of light and darkness the animals showed a positive response to the dark patches in the chamber, as shown in Figure 7.20. The lines drawn on the floor of the chamber allowed a distinction to be drawn between animals which moved directly towards one of the black screenes, those which started off on a line between the black screens but which deviated to a black screen during the period of locomotion to the periphery and those which started off on a line between the screens and kept on this line of movement. For the eighteen animals tested thus, the results were as reflected in Table 7.01.

Table 7.01. Orientation of M. delanyi and C. longicaudata to screens of black paper in uniform light.

Course:	M. delanyi.	C. longicaudata.
Directly to screen	13	9
Start between screens but deviate towards a screen.	4	5
Start and finish between screens	1	4

In each instance a significantly higher proportion of the animals reached the black screens, the probability of this being due to chance being less than 0.001 (Chi-squared = 16.0) in the case of M. delanyi and less than 0.01 (Chi-squared = 6.8) in the case of C. longicaudata. However, whilst a significantly higher proportion of the specimens of M. delanyi actually started directly towards one of the black screens, (Chi-squared = 4.5, P = less than 0.05), the initial orientation of C. longicaudata was apparently completely random.

Since the animals would normally head for the periphery of

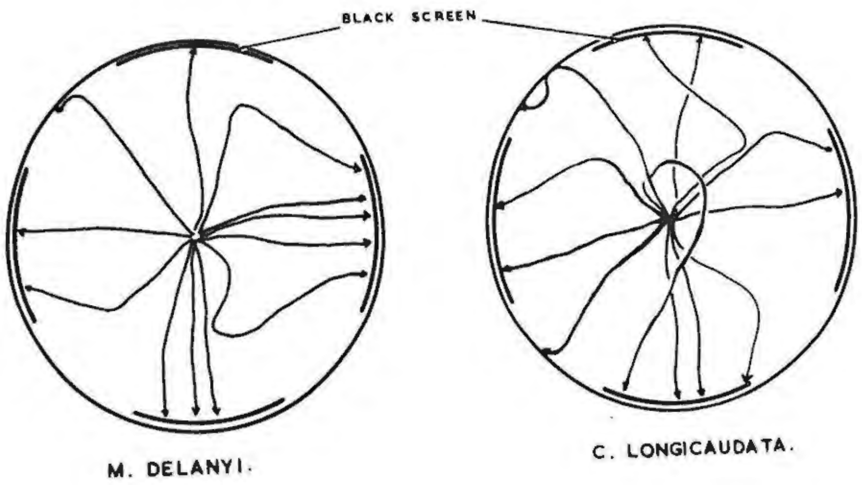


Fig. 7.20 : Responses of M. delanyi and C. longicaudata to alternate areas of light and darkness under conditions of uniform light stimulus.

the circular chamber, these experiments could not be regarded as absolutely critical and the remaining results were obtained in the white-walled arena already described. When the animals were placed in the arena with the wall opposite the light source completely blacked out by means of a black screen, the movement away from the light was much more striking as shown in Figure 7.21 the negative phototaxis here probably being re-inforced by the skototaxis. When the single black screen was replaced by two smaller screens, each 7.5 cm. wide, with a space of 8 cm. between them, most of the specimens of M. delanyi tested, moved to one or other of these but two of the animals (numbers 1 and 4 in Figure 7.22) showed a response not hitherto observed in that they moved out of the beam and into an area of less intense illumination immediately adjacent to the heat filter. C. longicaudata again showed much the same behaviour, but here three of the nine animals tested orientated between the two screens, eventually coming to rest behind them. The paths followed by the animals in these experiments is shown in Figure 7.22.

The two screens were placed so that one was parallel to the path of the light rays from the main light source 7.5 cm. from the corner, whilst the other was placed at an equal distance from the corner at right angles to the path of the rays. All specimens of M. delanyi tested with the exception of one moved directly to the screen at right angles to the light rays, the remaining one again finding its way to the region of lower illumination intensity next to the heat filter. Of ten specimens of C. longicaudata two moved to the screen parallel to the light rays, whilst the remainder, although often starting off directly towards the screen at right angles to the path of the main light source rays, became directed between the two screens as shown in Figure 7.23. When these screens were replaced with larger screens 15 cm. in width and separated by greater distances, the parallel and transverse screens now having their edges placed 13 cm. and 3 cm. from the corner respectively, M. delanyi showed a far greater response to the parallel screen, six animals moving directly towards it, although their initial path was towards the transverse screen.

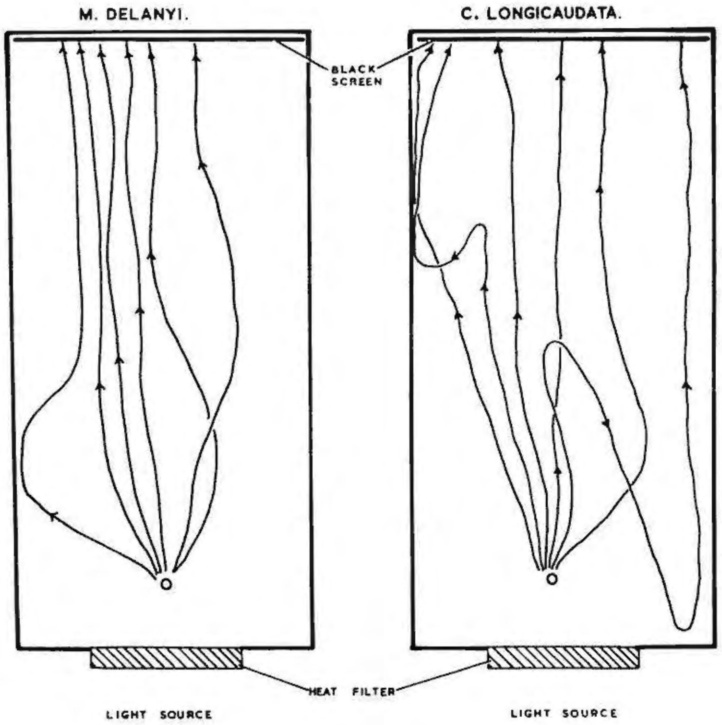


Fig. 7.21 : Paths followed by dark adapted specimens of M. delanyi and C. longicaudata under the influence of directed light in a white-walled arena with a black screen placed at the end opposite the light source.

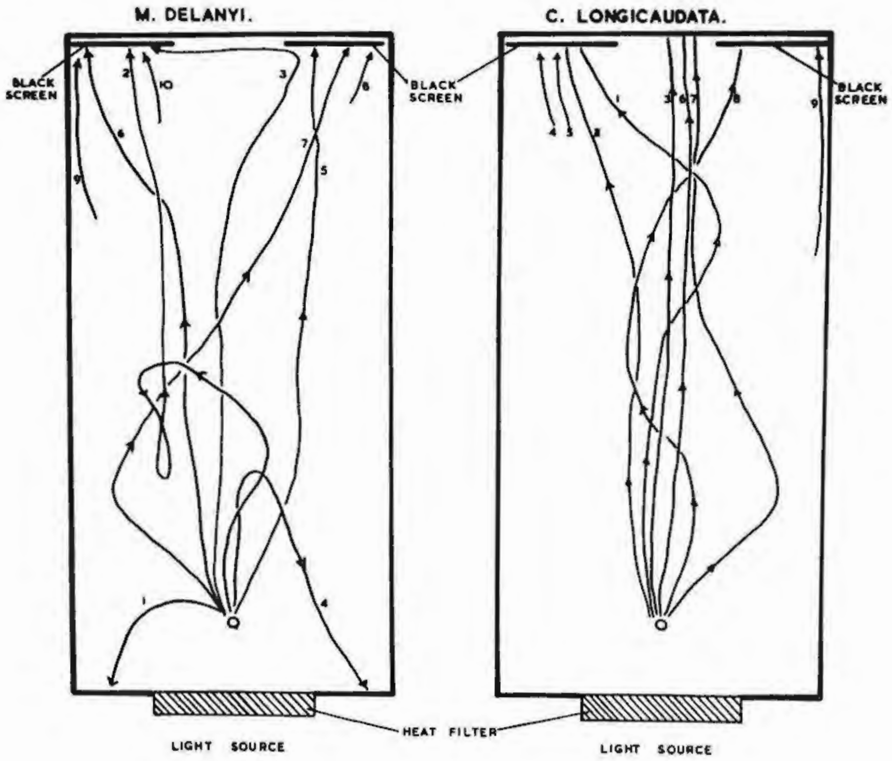


Fig. 7.22 : Orientation of dark adapted specimens of *M. delanyi* and *C. longicaudata* when faced with two screens placed at right angles to the rays from the main light source in a white-walled arena.

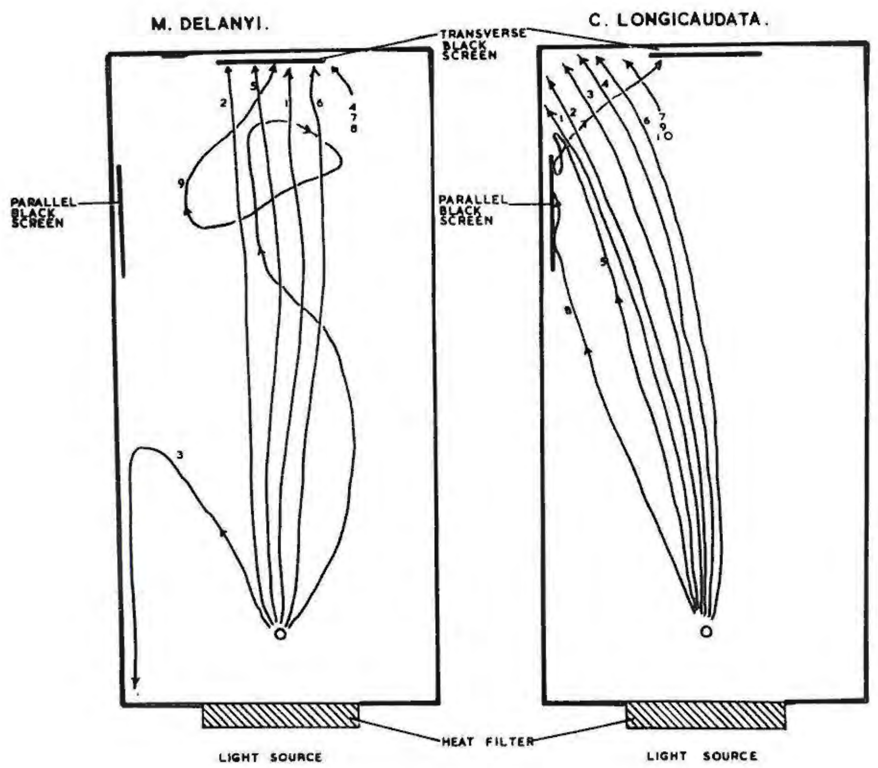


Fig. 7.23 : Orientation of dark adapted specimens of *M. delanyi* and *C. longicaudata* to two black screens in a white-walled arena; one screen is parallel to the direction of the light rays from the main light source, the other is at right angles to these.

The responses of C. longicaudata under these conditions were extremely erratic, some moving to one or the other of the screens, whilst others moved into the region of reduced illumination next to the filter. The paths followed by the experimental animals under the conditions of stimulus described are shown in Figure 7.24. A glass-walled arena allowed these latter two experiments to be repeated with considerably less contrast between the screens and the background. In this instance the parallel screen was placed on the opposite side of the arena, i.e. on the right of any animal moving directly away from the light source, in order to ensure that the animals do not have an inherent tendency to turn towards the left under the conditions obtaining in the arena. The results of these experiments were essentially the same as those described above.

The results obtained up to this stage strongly suggest a skoto-telotaxis in the case of M. delanyi and a skoto-tropotaxis in in the case of C. longicaudata. Unilateral blinding did little to clarify the position any further since no clear-cut circus movements were discernible in either case.

The unilaterally blinded animals were placed in the uniform light chamber where only one specimen of C. longicaudata showed what may have been a single circus movement. Upon reaching the periphery four of the six animals of this species turned towards the blinded side the remainder towards the seeing side. M. delanyi showed no signs of circus movements or more frequent turning towards the blinded side.

When placed in the white-walled arena, facing the light source, the animals would turn and move away from the light. Here, too, no recognisable pattern emerged in the case of M. delanyi but two specimens of C. longicaudata showed what may be interpreted as circus movements. There was a tendency here for the animals to orientate themselves with the blinded eye towards the main source of stimulus, which is interpreted by Fraenkel and Gunn (1940) as being equivalent to a circus movement, in which case these experiments would lend some weight to the suggestion of a tropotactic component in the light responses of

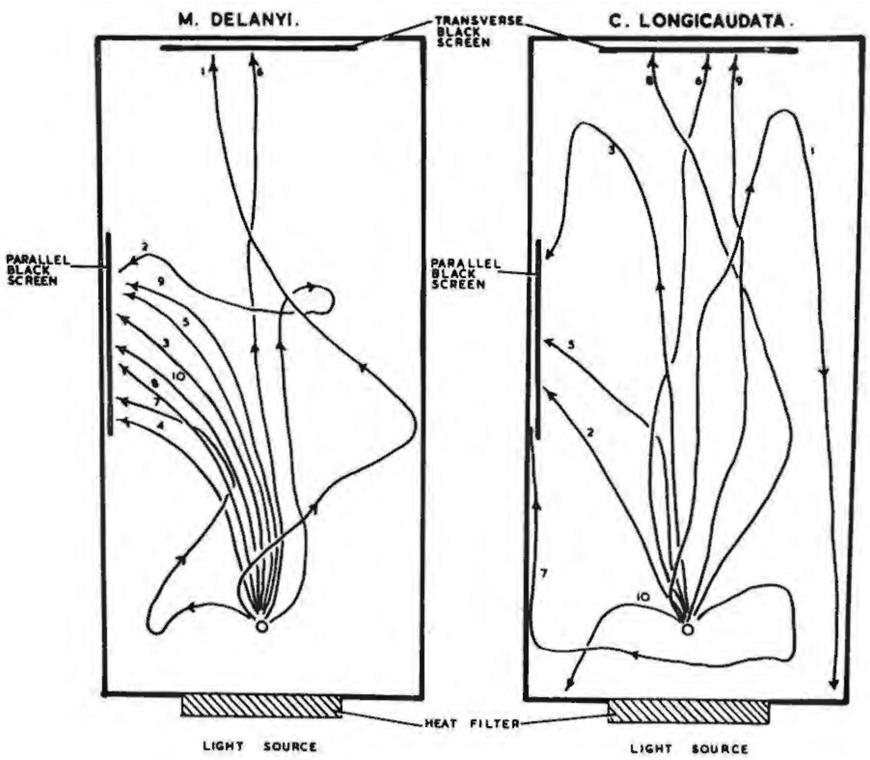


Fig. 7.24 : Orientation of dark adapted specimens of *M. delanyi* and *C. longicaudata* to two black screens placed as in Fig. 7.23 but placed further apart.

C. longicaudata.

The interpretation of the light responses put forward here may find some confirmation in the structure of the compound eyes of the two species under consideration. The large compound eyes of the Machiloidea are so conspicuous as to be regarded as a diagnostic character of the order as a whole. They are contiguous on the vertex and composed of a large number of ommatidia pointing in different directions, the latter feature being a requirement for a telotactic light response; the compound eyes of the Lepismatoidea are, on the other hand, reduced comprising only a few ommatidia and situated laterally on the head. The angle of vision in C. longicaudata was determined by making a camera lucida drawing of the head of a freshly killed specimen. The drawing gave a 100x enlarged representation of the head and particular attention was paid to the orientation of the ommatidia. This showed that an area falling within an angle of between 38° and 45° to either side of the long axis of the body, directly in front of the animal would be outside its field of view. Nothing is known of the angular limits of the field of view for a single ommatidium in the case of C. longicaudata but assuming this to be of the same order of magnitude as that of the locust (Burtt and Catton, 1954) the figure may be reduced to between 28° and 35°, which would perforce necessitate orientation by comparison of the stimuli received by each of the compound eyes.

Consider Figures 7.23 and 7.24 in the light of the above. In Figure 7.23 M. delanyi can be seen to respond telotactically to the transverse screen since its full frontal vision places this source of stimulus within its field of view. The parallel screen only comes into the field of vision when the animal comes almost abreast with it, but the stimulus from this source is ignored, since the original telos, the transverse screen, has now almost been reached. C. longicaudata on the other hand has neither screen in view at the start of a similar experiment and probably starts moving towards the transverse screen by virtue of its negative phototaxis. The transverse screen will come into the field of vision when the animal is at a distance of between

3.5 and 7.5 cm. away from it, by which time the left compound eye has already received the full stimulus from the parallel screen, which will have deflected the line of movement towards the left but in doing so the transverse screen now comes into the field of the right eye and consequently the animal becomes oriented between the screens. When the screens are larger and further apart, as in Figure 7.24, M. delanyi still starts off towards the transverse screen, but on encountering the nearer parallel screen, may deviate towards the latter, which behaviour is not inconsistent with a telotactic response. C. longicaudata again has neither screen in view on starting, its direction being determined by the negative phototaxis but whether it deviates towards the parallel screen on passing it depends on the intensity with which the phototaxis is still exerting its influence, since the animal is still close to the source of light stimulus. Thus some of the animals may continue directly towards the transverse screen. If the animal does respond to the stimulus from the parallel screen, the source of stimulus is now sufficiently wide to allow for stimulation of both compound eyes and the animal will move directly towards it. If the skototaxis manifests itself only at a stage where the animal has almost passed the parallel screen, orientation between the screens, as in the case of animal number 3 in Figure 7.24, may occur. No explanation is offered for numbers 1, 7 and 10 in Figure 7.24 which may illustrate a slight telotactic response towards regions of lower illumination intensity or a reaction towards the junction between wall and floor. However, one cannot expect animals to show the correct response at all times as one would a machine.

In view of the evidence for a skoto-tropotaxis in C. longicaudata one would expect marked circus movements from unilaterally blinded animals of this species. The absence of these movements may be due to a very rapid adaptation, no other explanation being readily apparent.

Clearly, the experiments done here are insufficient to be regarded as anything more than preliminary, giving an indication of the possible mechanisms involved. More detailed studies, using larger numbers of animals and taking into account variations of reaction intensities with variations in stimulus intensities are required to explain the conditions in the two animals fully. However, both groups have been shown to shun light and the mechanism by means of which this is achieved seems undoubtedly to be the combined effect of a negative phototaxis and a skototaxis in both cases, which will enable the animals to shelter from exposure to the sun.

7.3 Behavioural Responses to Atmospheric Humidity.

Of primary importance in the maintenance of an adequate internal water balance is the ability to take up water in order to replenish water lost by evaporation. The frequency with which such replenishment must take place is largely dependent upon the permeability of the cuticle, by far the most important factor in water conservation; once a completely impermeable cuticle has been evolved the only essential behavioural response to humidity which the animal requires is a water drive, i.e. a response which will lead it to a source of water when the internal water content falls to a level where replenishment becomes necessary. Where an effective waterproofing mechanism has not been evolved, however, the animal must, in addition to a water drive, possess the necessary innate behaviour which will cause it to avoid regions of low humidity, thus reducing water loss to a minimum.

It has been established in Part 5 that, whilst C. longicaudata is able to withstand considerable desiccation at the expense of a relatively small water loss, M. delanyi has achieved only moderate protection against the evaporation of its body water. Humidity would therefore be expected to play an important part in the choice of niche of the latter. Furthermore, the internal water balance of either species at any given time would to a large extent govern the humidity response, initiating a search for a source of replenishment when necessary or inhibiting such a hygroresponse when replete.

The humidity responses of both M. delanyi and C. longicaudata were investigated under conditions of normal and low water content.

(i) Responses to saturated and dry atmospheres.

Apparatus and method:

Experiments were conducted which gave the animals a choice between conditions of saturated vapour pressure and complete desiccation. The apparatus used was a simple choice chamber comprising a plastic dish measuring 12 x 12 x 4 cm., on the floor of which two plastic trays each measuring 11.5 x 6 x 1.5 cm. were placed, each tray thus occupying half the chamber floor. One of these trays contained distilled water, the other anhydrous calcium chloride, which would subtend relative humidities of 100% and 0% respectively. However, when the reagents are in juxtaposition, as was the case in the choice chamber, there is a diffusion of water vapour from the wet to the dry side resulting in values of between 92 and 96% relative humidity in the wet side and between 20% and 30% in the dry. These humidities were determined using cobalt thiocyanate papers prepared as described by Solomon (1957), the standards being prepared by suspending papers over saturated solutions of salts known to subtend a given relative humidity. A false floor of perforated zinc was laid over the trays and this was covered by a sheet of "Paper Nylon", a fine-mesh paper-like fabric which is non-hygroscopic. This served both to prevent smaller animals from climbing through the perforations in the zinc floor and to provide a suitable surface for the animals to walk on. The whole of the false floor was kept in place by means of a length of plastic covered expanding curtain rod, fashioned into a circular collar of circumference equal to the internal circumference of the choice chamber. The lid of the chamber had a hole 2.5 cm. in diameter drilled through its centre in order to permit introduction of the experimental animals without the necessity for raising the lid. This hole was closed by means of a tight fitting rubber bung. A transverse section through the apparatus is shown in Figure 7.25.

Before the animals were introduced into the choice chamber the atmosphere within had to be allowed to come into equilibrium with the subtending distilled water and calcium chloride. The time taken for this to come about was determined by placing a

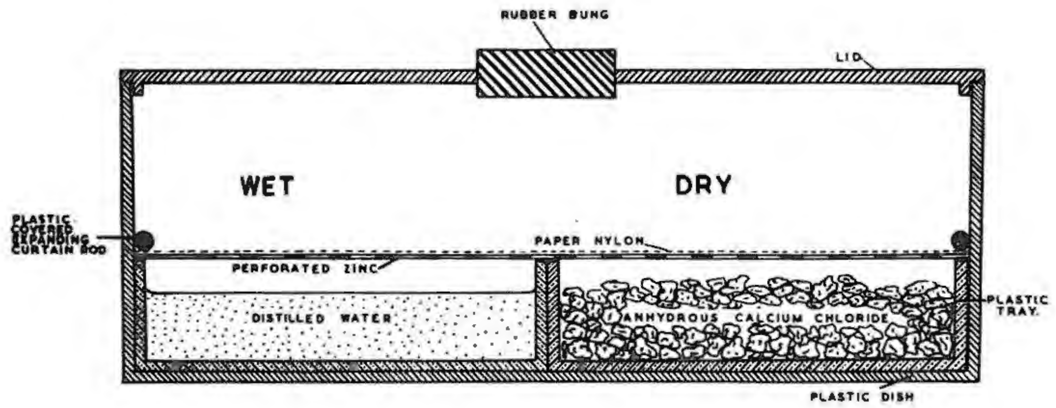


Fig. 7.25 : Section through the Wet/Dry choice chamber used.

strip of filter paper, impregnated with cobalt chloride as described by Solomon (1955), on the floor of the choice chamber positioned so that it would come under the influence of both the reagents. The lid was then closed and the edges were sealed with vaseline. The time taken for the cobalt paper to assume a bright pink colour in the wet side of the choice chamber and a bright blue colour in the dry side, was regarded as the minimal time required for the chamber to come into equilibrium. For all experiments using the choice chamber described above, twice the time taken for the control chmaber to come into equilibrium was allowed to elapse between the setting up of the chamber and the commencement of the experiment. Twenty minutes was the usual time taken for the colour change to come about and thus forty minutes equilibrating time was allowed. The cobalt paper strip also served to show how effective such a chamber was, since there was a very sharp line of demarcation between the pink and the blue with no gradual merging from one colour into the other. This ensured that the choice offered the animals was between the extremes only with no intermediate gradient.

The choice chamber was set up and allowed to come into equilibrium in a constant temperature room at 20°C. The experimental animals which had had access to food and water overnight were then introduced in batches of eight or fewer animals per chamber and the constant temperature room was darkened. Previous experiments using at least three species of Machiloides and C. longicaudata had shown that the animals took from 15 to 20 minutes to come to rest after having been disturbed and in these experiments one hour was allowed to elapse from the time of introduction of the animals until a count of the numbers in each half of the choice chamber was made. The counts were made under under faint red light to which the animals were known not to respond. After each counting the animals were stimulated to activity in the chamber by tapping the sides and lid with a pencil. This resulted in random movements within the chamber and a further count was taken one hour later when the animals had once more come to rest. In this way seven countings were recorded for each batch of animals and the mean of these countings were regarded as the actual distribution of a particular batch of animals in the chamber.

As a control experiment each batch of animals was again given access to food and water overnight and then introduced into a choice chamber similar to that described above but with clean, dry, empty trays under the false floor, thus having a uniform relative humidity approximating to that in the constant temperature room throughout. Seven hourly readings were taken as in the wet/dry choice chamber.

Results:

The results of the foregoing experiments are summarised in Tables 7.02 and 7.03.

Table 7.02.

Distribution of C. longicaudata in a Wet/Dry choice chamber. P = probability of distribution varying from random due to chance.

Experiment No.	Choice		Control	
	Number of Animals.		Number of Animals	
	Wet	Dry	Left	Right
1.	11	9	10	10
2.	8	10	11	7
3.	5	6	7	4
4.	3	6	5	4
Total:	27	31	33	25
P	Greater than 0.5		Greater than 0.5	

Table 7.03.

Distribution of M. delanyi in a Wet/Dry choice chamber. P = probability of distribution varying from random due to chance.

	Choice		Control	
Experiment No.	Number of Animals.		Number of Animals.	
	Wet	Dry	Left	Right
1.	1	3	3	1
2.	2	6	3	5
3.	1	13	9	5
4.	7	11	8	10
5.	5	16	9	12
6.	3	14	10	7
Total	19	63	42	40

P Much less than 0.001 0.95

The results clearly show that C. longicaudata is normally indifferent to atmospheric humidity, which is what its ability to absorb water vapour might lead one to expect. M. delanyi, on the other hand showed a definite preference for the dry side of the chamber, which is not compatible with either its relative inability to withstand desiccation or with the conditions prevailing in its ecological niche. This finding was foreshadowed by earlier work already referred to, where the same result was obtained using mixed batches of 2 or 3 species of Machiloides, including M. delanyi. It seemed therefore that the eccritic humidity must lie somewhere below saturation.

(ii) Effects of desiccationn on the responses to saturated and dry atmospheres.

Apparatus and method:

The choice chamber and method used were the same as that described in the foregoing section but the animals used were subjected to desiccation over calcium chloride for 48 hours in the case of *M. delanyi* and 120 hours in the case of *C. longicaudata*. The latter desiccation time would subject *C. longicaudata* to approximately the same percentage water loss as *M. delanyi* during 48 hours desiccation.

Only three readings were taken for each batch of *C. longicaudata*; having the ability to absorb moisture from an atmosphere near saturation it could restore its normal water balance if the experiments were prolonged and thus affect the result. *M. delanyi* was left in the choice chamber for the full seven hours.

Results:

Both species showed a distinct preference for the near saturated atmosphere of the wet side of the chamber. This is reflected in Tables 7.04 and 7.05. The response was far more marked in *C. longicaudata* than in *M. delanyi* probably due to the extreme variability in the rate of water loss shown by the latter (Part 5).

Table 7.04.

Distribution of *C. longicaudata* in a Wet/Dry choice chamber after 120 hours desiccation over Calcium Chloride.

Experiment No.	Number of Animals.	
	Wet	Dry
1.	8	0
2.	10	2
3.	11	0
4.	14	1
Total:	43	3

Table 7.05.

Distribution of M. delanyi in a Wet/Dry choice chamber after 48 hours desiccation over Calcium Chloride. P = probability of distribution varying from random due to chance.

Experiment No.	Number of Animals.	
	Wet	Dry
1.	8	4
2.	12	4
3.	15	5
4.	14	7
5.	11	7
Total:	60	27
P	Less than 0.001	

This response in both animals would ensure that they go in search of water in order to replenish a depleted internal water content once a critical level is reached. That this condition does arise even in an animal able to absorb water vapour from an undersaturated atmosphere is borne out by some results obtained during the preliminary investigation, which were thought puzzling at the time and for a while could not be repeated. The animals in question, ten specimens of C. longicaudata, had been freshly caught from a map store. They were immediately placed in a wet/dry choice chamber, without any pre-treatment and gave the results shown in Table 7.06 for the first three two-hourly readings.

Table 7.06.

Distribution of Ten Specimens of C. longicaudata in a Wet/Dry choice chamber immediately after capture.

Count No.	Number of Animals	
	Wet	Dry
1.	8	2
2.	6	4
3*	4	5

* One animal managed to find its way below the false floor and was not counted here.

The above result was not statistically significant, largely due to the small number of animals. For the first count Chi-squared was equal to 1.272 and a probability of 0.1 that the distribution differed from random due to chance. These results can now be explained. The animals had probably been subjected to considerable water loss in the dry map store, accounting for the initial reaction towards the wet. The fall in reaction intensity towards the wet would then follow as the animals restored their internal water balance to normal.

(iii) Distribution during a prolonged stay in a wet/dry choice chamber.

The effects of desiccation on the behaviour of the two species under consideration could further serve to confirm the ability of C. longicaudata to absorb water vapour and the inability of M. delanyi to do so, by the behaviour of both desiccated and normal animals during a prolonged stay in a wet/dry choice chamber. This would also serve to confirm the reversible hygroresponse of M. delanyi.

Apparatus and method:

Experimental animals of both species were divided into two groups each, one of which was subjected to desiccation over calcium chloride for 176 hours in the case of C. longicaudata and 48 hours in the case of M. delanyi, the remaining group being normally watered but not fed. Each group was then subdivided into batches of ten or fewer and each batch placed in a wet/dry choice chamber as before. Counts were made at varying times over a period of 100 hours. The group of M. delanyi which had been subjected to desiccation was given water in the form of a piece of moistened filter paper placed in the wet side of the choice chamber after 48 hours as it was feared that the animals would die as a result of prolonged water deficiency.

Results:

Figures 7.26 and 7.27 show the reaction intensity to the dry side of the choice chamber as it varied with time spent in the choice chamber. The reaction intensity was, as in the case of the light responses, calculated as being the number of animals in dry less the number in wet divided by the total number of animals used.

These results bear out findings already described, namely (i) that M. delanyi normally avoids conditions of saturated vapour pressure and that this response is reversed upon desiccation but re-established when the water balance is restored to normal; (ii) that M. delanyi is unable to absorb water vapour from the atmosphere; and (iii) that C. longicaudata is normally indifferent to atmospheric humidity but reacts positively to conditions of high relative humidity when its internal water content falls below a critical level, restoring this to normal by absorbing water vapour from the atmosphere.

(iv) The eccritic humidity of M. delanyi.

It was obvious from all the foregoing results that the response to the dry shown by M. delanyi in the wet/dry choice chamber could not be the only humidity response manifest in this species. Clearly, there had to be a preferendum below saturation, which the following experiments were designed to determine.

Apparatus and method:

A choice chamber similar in construction to the wet/dry choice chamber previously described was used, but solutions known to subtend specific relative humidities were contained in the trays below the false floor instead of distilled water and

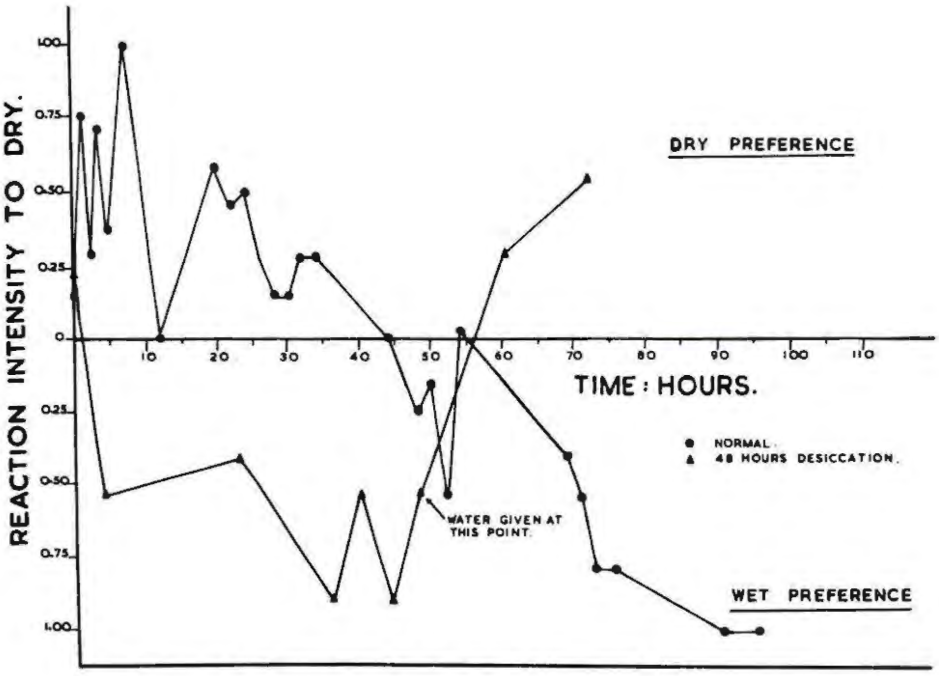


Fig. 7.26 : Reaction intensity of normal and desiccated *M. delanyi* to the dry side as it varied with time during a prolonged stay in the wet/dry choice chamber.

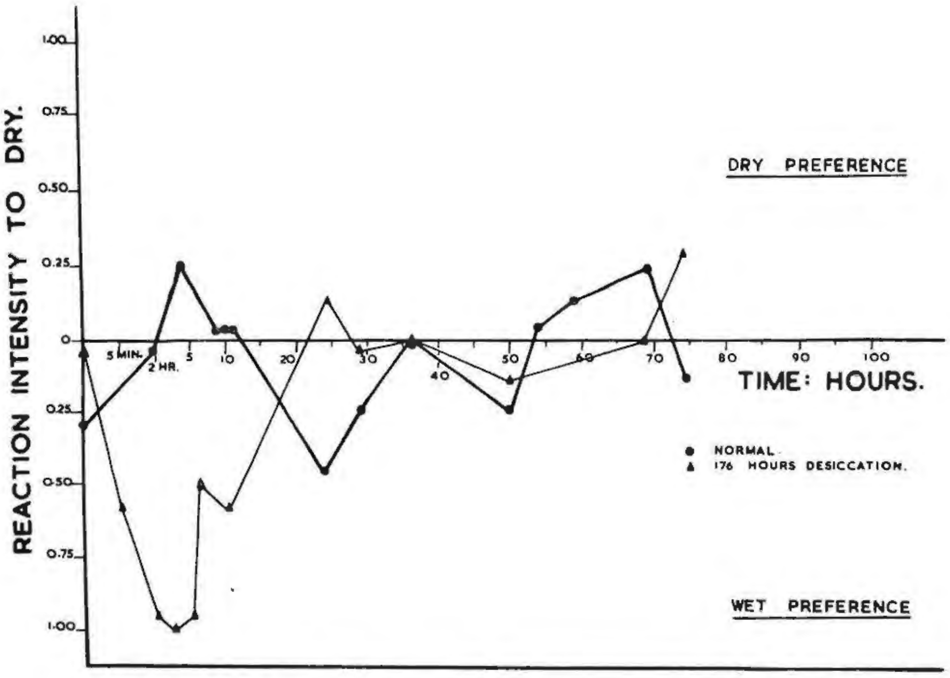


Fig. 7.27 : Reaction intensity of normal and desiccated *C. longicaudata* to the dry side as it varied with time during a prolonged stay in the wet/dry choice chamber.

anhydrous calcium chloride. In the initial experiments various concentrations of potassium hydroxide were used and the results thus obtained were confirmed in the critical range using saturated solutions of various salts.

The potassium hydroxide solutions were made up to the concentrations determined by Solomon (1951) in two ways. Initially the required amount of potassium hydroxide required per 100 g. of solution was weighed out as accurately as possible and distilled water was added to make up the 100 g. of solution. This was found to be somewhat inconsistent in that the densities of two solutions made up independently to the same concentration were usually significantly different, not only from one another but also from the density required. More accurate results were obtained using the gravimetric method described by Solomon. Here approximately 100 g. of KOH pellets were dissolved in 100 ml. of distilled water. The density of this solution was now determined by accurately pipetting three volumes of 10 ml. each of this solution, cooled to 15°C, into clean, dry, specific gravity flasks which had been previously weighed and then weighing again. The sum of the weights of these three volumes divided by thirty was taken to be the density of the solution. A graph showing the relationship between weight % KOH and density of KOH solutions was prepared from the data given by Solomon and from this the weight % of each batch of solution prepared could be determined. This stock solution could now be diluted to the required concentration by adding distilled water, the weight of which was determined using the formula:

$$W = \frac{S}{A} - 1$$

where W = weight of distilled water to be added to 1 g. of stock solution; S = weight % KOH in stock solution and A = weight KOH % in required solution. The required volume of water was added from a burette, taking 1 ml. of distilled water to weigh 1 g.

Three criticisms may be levelled against the above technique:

1) The gravimetric method of density determination is not absolutely accurate.

2) No account is taken of the presence of potassium carbonate in the solutions.

3) The solutions are not very stable, the more concentrated solution becoming progressively more diluted by the absorption of water from the solution of lower concentration in a choice chamber.

The chief source of error in the determination of density by gravimetric and volumetric methods is the accurate measurement of the volume of solution pipetted into the specific gravity flasks. A strong alkali such as potassium hydroxide precludes the use of a burette and a graduated 10 ml. pipette had to be used. More accurate control over the pipette was obtained by connecting a short length of rubber tubing over the mouthpiece of the pipette, wiring it on as tightly as possible to make the joint airtight. This rubber tubing was then occluded by means of a strong spring clip, the manipulation of which allowed accurate control over the outflow of liquid from the pipette which was held in a burette stand. A double check was thus possible, using both the calibrations of the pipette and the calibration mark on the specific gravity flask. In order to check the accuracy of this method it was used to determine the density of laboratory distilled water, which was found to be 1.0022. Checking the densities of some of the finally prepared solutions against the densities given by Solomon showed a greater discrepancy the differences ranging from +0.007 to -0.004.

The amount of potassium carbonate present in the solutions was not known and no attempt was made to ascertain this. It could have been determined by titration against a standardised concentration of hydrochloric acid, using both phenolphthalene and methyl orange as indicators but it was felt that the general inaccuracy of the method as practised did not justify this.

Deterioration of the solutions was minimised by using each batch only once, even if the same concentration was required in the choice chamber immediately after one experiment was completed.

The choice offered the animals never involved a difference of less than 10% relative humidity and where measured using an

Edney Paper Hygrometer calibrated either over saturated salt solutions or against wet and dry bulb thermometers, depending on the range required, the error involved in the preparation of the solutions was never more than 2% relative humidity. It seemed, therefore, that for a preliminary determination of the eccritic humidity these solutions would suffice.

The animals were tested for humidity preference in the choice chamber as previously described, seven readings being taken for each batch. Using an alternate choice chamber rather than a humidity gradient had the advantage of obviating the necessity for measuring the relative humidity along the gradient, which would require hygrometers actually in the apparatus at all times.

Once the region of the eccritic humidity had been established using potassium hydroxide solutions, confirmatory experiments were done using the following saturated solutions:

Potassium dihydrogen phosphate	RH	96.5%
Potassium chloride	RH	85.0%
Ammonium sulphate	RH	80.5%
Sodium chloride	RH	76.0%
Glucose	RH	55.0%

all at 20°C. These solutions were made up as described by O'Brien (1948) and by Winston and Bates (1960).

No measurements of the actual humidities in each side of the choice chambers were made in the case of preliminary determinations using KOH solutions for humidity control. In the case of the confirmatory experiments using saturated solutions, however, control chambers were set up with two cobalt thiocyanate papers kept from contact with the false floor by plastic gauze placed in each side of the chamber. One of these papers was removed after one hour, the other after seven hours (the normal duration of an experiment) and mounted under liquid paraffin. These papers were compared with papers from the <u>same strip</u> which had been kept in a uniform humidity chamber over the controlling solutions used. No observable difference in colour between the papers from the choice chambers and the uniform humidity chambers was apparent where the difference in relative humidity between the two sides of the choice chamber was 16.0% or less. Where this difference exceeded 16.0% the papers from the side of the chamber having the higher relative humidity appeared to be a slightly darker pink colour than the controls suggesting a small loss of water vapour to the drier side. The papers from the drier sides of these choice chambers, however, showed no comparable difference. It would seem, therefore, that the relative humidity difference in these chambers was slightly less than that indicated in the results.

All the experiments in this investigation were carried out in a constant temperature room at 20°C, which was, unfortunately, somewhat higher than the temperature optimum as the investigation on the temperature relations of the animals was actually carried out after these experiments had been largely completed.

Results:

The results of these experiments are summarised in Tables 7.07 and 7.08, showing the humidity preferendum to be a fairly wide range between 85% RH and 70% RH.

<u>Table 7.07</u>.

Reaction of <u>M. delanyi</u> to different relative humidities, using potassium hydroxide solution for humidity control. "P" = probability of distribution differing from random due to chance.

Humidity choice.	No. of Animals.		Reaction intensity to dry	Chi-squared	"P"
	Wet	Dry			
100 - 90	2	22	0.83	16.7	<0.001
90 - 75	8	34	0.62	16.1	<0.001
90 - 80	5	19	0.58	4.0	<0.05
85 - 75	22	16	-0.16	0.94	<0.5
80 - 70	18	16	-0.06	0.12	<0.9
80 - 60	25	7	-0.56	10.0	<0.01
75 - 60	25	5	-0.67	16.7	<0.001
60 - 50	24	9	-0.39	6.8	<0.01
50 - 25	26	29	0.05	0.16	<0.5
25 - 15	27	22	-0.10	0.51	<0.5
15 - 0	21	3	-0.75	13.5	<0.001

Table 7.08.

Reaction of M. delanyi to different relative humidities, using saturated solutions for humidity control.

Humidity choice.	No. of Animals.		Reaction intensity to dry.	Chi-squared	"P"
	Wet	Dry			
96.5 - 80	2	18	0.80	12.8	<0.001
96.5 - 75	3	15	0.67	8.0	<0.01
85 - 75	14	17	-0.13	0.3	<0.5
80 - 75	16	13	0.10	0.32	<0.5
80 - 55	16	2	-0.78	11.6	<0.001
75 - 55	19	3	-0.73	9.8	<0.01

The lower relative humidities were not retested using saturated solutions instead of potassium hydroxide as there seemed to be little of consequence to the investigation to be gained from this. The inability of the animals to distinguish between two humidities well outside their normal range is not peculiar to M. delanyi but has also been shown for the crab Potamon depressus by Dandy (1955).

The eccritic humidity thus determined corresponds very well with the relative humidities measured in the actual hiche which M. delanyi inhabits and it seemed unnecessary, therefore, to attempt to narrow the range down any further.

(v) The Site of the Humidity Receptors.

As in the case of the thermoreceptors, it was thought that the most probable site of the hygroreceptors would be the antennae. Amputation experiments were carried out on both M. delanyi and C. longicaudata in order to either confirm or disprove this.

Apparatus and method:

The antennae of a number of experimental animals were removed in the manner previously described and a twenty four hour period was allowed for them to recover from the effects before commencement of the experiment. During this period the animals were given free access to food and water.

After the recovery period the humidity responses of the animals were tested in the wet/dry choice chamber in the manner previously described. The animals were then desiccated over calcium chloride for periods of 48 hours and 176 hours in the cases of M. delanyi and C. longicaudata respectively after which their responses to humidity were retested.

A control experiment was conducted in each case, in which the animals were subjected to chilling but the antennae were not amputated. Subsequent treatment of these animals was as described above.

Results:

The results of these experiments are shown in Tables 7.09 and 7.10. In neither case is there any indication of a hygro-response, whether positive or negative, thus indicating that the hygroreceptors are situated on the antennae.

Table 7.09.

Distribution of M. delanyi in a wet/dry choice chamber before and after 48 hours desiccation.

Condition	Before Desiccation.		After Desiccation.	
	Wet	Dry	Wet	Dry
Normal	5	17	16	6
Antennae removed.	15	15	13	17

Table 7.10.

Distribution of C. longicaudata in a wet/dry choice chamber before and after 176 hours desiccation.

Condition	Before Desiccation.		After Desiccation.	
	Wet	Dry	Wet	Dry
Normal	10	7	15	2
Antennae removed.	14	9	10	13

Examination of the antennae has failed to reveal any recognisable sensillae which may serve as hygroreceptors. There are certainly no structures resembling the tuft organs of Pediculus humanis (Wigglesworth 1941) or the pit-peg organs of Tenebrio molitor (Pielou 1940) recognisable on the entire antenna, where only trichoid sensillae of two different sizes could be seen. No serial sections of the antennae have been cut, and this may well reveal the presence of sensillae other than these. The trichoid sensillae cannot, of course, be excluded as possible hygroreceptors, but this would be difficult to prove by means of behaviour experiments, since they cover the antennae from the tip of the pedicel.

(vi) The Nature of the Hygroreceptors of M. delanyi.

Two types of hygroreceptor are known to occur in arthropods, namely "Hygrometers" which respond to relative humidity, being dependent upon the uptake of moisture from the surrounding atmosphere and "Evaporimeters" which depend on the rate of water loss from the animal and thus respond to the saturation deficit of the atmosphere. Whilst the former type would be effective in keeping the animal out of both too high and too low an humidity, it would appear from the nature of the latter that it would serve only to keep the animal out of conditions where it encounters too high a rate of water loss.

An attempt was made to ascertain whether M. delanyi was in fact responding to relative humidity or to saturation deficit; C. longicaudata was excluded from this investigation since it is normally indifferent to humidity.

Apparatus and method:

It is possible to change the saturation deficit within an enclosed chamber quite considerably by merely altering the temperature. The relative humidities over the solutions used in this investigation vary but little with the temperature, whilst the saturation vapour pressure and therefore the saturation deficit are very much affected. The temperature/relative humidity/saturation deficit relationships of the solutions used in this investigation are shown in Table 7.11.

Table 7.11.

Temperature/relative humidity/saturation deficit relationships over saturated solutions used (from Winston and Bates, 1960).

Temperature	$10^{\circ}C$		$20^{\circ}C$		$25^{\circ}C$	
Saturated solution	R.H. %	S.D. mm.Hg	R.H. %	S.D. mm.Hg	R.H. %	S.D. mm.Hg
KNO_3	96	0.37	95.5	1.14	92.5	1.78
NaCl	76.5	2.16	76	4.21	75.5	5.8
Glucose	56	4.05	55	7.81	55	10.7

The choice chamber used was similar in construction to that previously described with the exception that a hole was bored through the rubber bung allowing a thermometer to be inserted into the chamber and the temperature within to be measured. Initially, the chamber was set up with saturated solutions of KNO_3 and NaCl as alternatives and allowed to come into equilibrium overnight in a constant temperature room at 10°C. Experimental animals which had been pretreated in the usual manner were then introduced into the chamber and their distribution recorded over seven hourly intervals after which they were returned to their culture dishes and given food and water. The choice chamber was now allowed to come into equilibrium at 20°C overnight and the experimental animals were re-introduced for a further seven readings. The experiment was repeated on the following day at 25°C.

After a resting period of three days the experiment was repeated using the same animals but in this instance, the choice provided in the alternative chamber was between NaCl and glucose. Owing to what proved to be abnormal behaviour in one particular batch it was necessary to use a larger number of animals in this latter experiment.

Results:

These experiments showed the hygroresponses of M. delanyi to be reactions to relative humidity rather than to saturation deficit. Since the animals are euryhygrous, the eccritic humidity being a range between 70 and 85% relative humidity at 20°C, they should, if the receptors involved are evaporimeters, have a preferred range of saturation deficits falling within the limits determined by the relative humidity preferendum. Such a saturation deficit preferendum would therefore be the range between 2.63 and 5.25 mm. Hg and one would expect avoidance of saturation deficits below and above this range. Table 7.12 shows the results obtained, which are not in keeping with a response to saturation deficit.

Table 7.12.

The effects of increasing saturation deficits on the humidity response to M. delanyi at constant relative humidity.

Temperature	10°C		20°C		25°C	
Relative Humidity %	96	76.5	95.5	76	92.5	75
Saturation Deficit (mm Hg)	0.37	2.16	1.14	4.21	1.78	5.82
No. of Animals	4	16	3	17	7	13
Relative Humidity %	76.5	56	76	55	75	55
Saturation Deficit (mm Hg)	2.16	4.05	4.21	7.81	5.82	10.7
No. of Animals	46	26	54	18	50	21

There is a consistent response to 75% relative humidity throughout, even where its associated saturation deficit does not fall within the preferred range. More critical analysis is possible in the case of the 76.5 - 56% relative humidity choice at 10°C, where the associated saturation deficits fall below and within the preferendum respectively. The expected distributions from possible combinations of evaporimeter and hygrometer type hygroreceptors would, in this instance, be as follows:

1. Wet and dry mediators are evaporimeters: Reaction to dry, since 2.16 mm. Hg is below preferendum.

2. Dry mediator an evaporimeter, wet mediator an hygrometer: Reaction to dry since 2.16 mm. Hg below preferendum and dry response is always dominant.
3. Dry mediator an hygrometer, wet mediator an evaporimeter: Random distribution since each would be within its respective preferendum.
4. Both wet and dry mediators are hygrometers: A wet response.

The evidence therefore strongly favours both hygroreceptors being of the hygrometer type. The reaction intensity towards the 76.5% relative humidity in the 76.5 - 56% relative humidity choice at 10°C is somewhat lower than that at 20°C but statistical analysis has shown this difference to be not significant, the probability of it being due to chance being between 0.1 and 0.5 (Chi-squared = 2.1).

(vii) Nature of the Humidity Responses.

In the case of M. delanyi we are faced with the elucidation of an avoidance of both high and low humidities in normal animals and a water drive to the highest humidity available in desiccated specimens, whilst in C. longicaudata only the latter response is evident. Five possible means of investigation suggest themselves in order to determine the nature of each hygroresponse:

1. Observation of normal and desiccated animals in an alternative wet/dry choice chamber, providing a choice between the extremes.
2. Observations of normal animals in an alternative choice chamber providing a choice between the eccritic humdity and calcium chloride (M. delanyi only).
3. Observations on normal and desiccated animals in uniform humidity chambers at different relative humidities.
4. Observations on desiccated animals in an humidity gradient to detect any directive response which may be present.
5. The effects of unilateral removal of the hygroreceptors on the behaviour of desiccated animals in an humidity gradient and in a uniform humidity chamber.

Preliminary experiments of this nature were carried out for both species under consideration.

Apparatus and method:

The alternative choice chamber used was the same as that previously described. Initially, specimens of M. delanyi which had had free access to food and water were introduced, one at a time, into the choice chamber which contained distilled water and anhydrous calcium chloride as alternatives. Each individual was tracked on a plan of the chamber for a period of ten minutes and a record was kept of the number and duration of all periods during which each animal was stationary. The experiment was then repeated, using specimens of M. delanyi which had been desiccated over anhydrous calcium chloride for a period of 48 hours and of C. longicaudata which had been subjected to 178 hours desiccation.

Normal specimens of M. delanyi were also tracked for periods of ten minutes in a choice chamber in which the two halves contained a saturated solution of sodium chloride and anhydrous calcium chloride respectively, thus providing a humidity choice between approximately 75% and a very low relative humidity.

The uniform humidity chambers used were merely alternative choice chambers in which both sides contained the same reagent, thus providing a uniform humidity throughout. The paper nylon on the false floor of these chambers was divided into sixteen squares of equal area by means of faint pencil lines to which the animals were known not to react. Chambers of this type were set up using anhydrous calcium chloride, saturated solutions of glucose, sodium chloride and potassium nitrate, and distilled water, providing relative humidities of 0%, 55%, 75%, 85% and 100% respectively. Six animals were placed in a chamber at any one time and these were allowed to distribute themselves in the dark for a period of 30 minutes, after which the position of each animal in the chamber was noted under a diffuse red light and plotted. Position plotting was done on a scaled down plan of the chamber, the squares on the false floor facilitating accurate location. The positions of the animals were now recorded at fifteen-minute intervals over a period of 3½ hours, enabling an estimate of the degree of activity to be made. Twelve animals were used at each relative humidity, thus giving a total of 168 readings and, in addition, desiccated specimens of both species were tested at 0% relative humidity.

A humidity gradient was obtained in an alternative choice chamber by placing distilled water and calcium chloride in compartments in the trays under the false floor so as to be located at diagonally opposite corners of the choice chamber respectively. The area thus falling under the direct influence of each reagent was in this way limited to four square centimeters with a long gradient between the two extremes. Desiccated animals of both species were introduced into the middle of this gradient and tracked on a plan of the chamber for a period of ten minutes.

Amputation of a single antenna was effected in the usual manner, by cooling animals into a state of chill coma and then removing the flagellum of one antenna under a binocular microscope, using scissor forceps for the operation. These animals were, after a recovery period of 48 hours, subjected to desiccation for periods appropriate to each species after which their behaviour in uniform humidity chambers at 0% and 100% relative humidity and in a humidity gradient were observed.

Results:

Normal specimens of M. delanyi showed no avoidance of the wet side of the wet/dry alternative choice chamber, wandering freely into and out of the region of near saturation. There was, however, a tendency to come to rest for longer periods in the dry side of the chamber,as reflected in Table 7.13.

Table 7.13.

Numbers and durations of stops by six specimens of *M. delanyi* in a wet/dry alternative choice chamber during a period of ten minutes.

Animal No.	Wet.		Dry	
	No. of stops.	Total duration	No. of stops	Total duration
1	5	1.25 min	7	6.5 min
2	4	2.00	4	7.0
3	3	0.75	4	6.2
4	5	3.60	3	6.0
5	2	4.25	3	3.9
6	4	0.40	5	6.2
Total:	23	12.25	26	35.8
Mean per individual	4	2.04	4.5	6.0

The difference between the total times spent at rest in the wet and dry sides of the chamber is statistically highly significant, the probability of the difference being due to chance being less than 0.001 (chi-squared = 12.7; $F_{1/10}$ = 25.1).

These figures strongly suggest an activity orthokinesis, instrumental in keeping the animals out of conditions of too high a humidity. Confirmation of this was obtained from the experiments carried out in uniform humidity chambers, where the animals showed a far greater activity in a saturated atmosphere than at lower relative humidities, as shown in Table 7.14. The activity orthokinesis seems to function only at humidities above the preferendum, there being no significant difference between the numbers active at the lower relative humidities. There is, however, a significant difference between the numbers active at 85% relative humidity and the three lower humidities, suggesting that the former value is very close to the upper limit of the preferendum. Table 7.15 shows the probabilities of the differences between all values reflected in Table 7.14 being due to chance.

Table 7.14. Numbers of changes in position recorded for twelve specimens of M. delanyi at each of five different relative humidities.

Relative humidity.	No. of animals moved (possible 168)	Percentage activity.
0%	27	16.1%
55%	23	13.6%
75%	31	18.4%
85%	45	26.7%
100%	81	48.2%

Table 7.15. Results of a statistical analysis of the data reflected in Table 7.14 (P = probability of difference being due to chance).

	55%		75%		85%		100%	
	P	Chi^2	P	Chi^2	P	Chi^2	P	Chi^2
0%	0.9	0.37	0.9	0.37	0.05	5.7	0.001	39.7
55%	-	-	0.5	1.4	0.10	8.9	0.001	46.8
75%	-	-	-	-	0.10	3.3	0.001	33.4
85%	-	-	-	-	-	-	0.001	16.4

A measure of the extent to which the activity orthokinesis accounts for the whole dry response may be reflected in a comparison between the percentage activity at two different humidities as per Table 7.14 and the distribution in a choice chamber offering these same humidities as alternatives. Table 7.16 shows the results of such an analysis.

Table 7.16. Comparison between expected and observed reaction intensities of the dry response of M. delanyi.

Humidity Range	Reaction Intensity to dry.		Extent to which orthokinesis accounts for reaction.
	Expected (vide Table 7.13)	Observed	
100 - 90/85%	0.28	0.83	34%
100 - 75%	0.45	0.46	97%
100 - 55/50%	0.55	0.68	81%
100 - $CaCl_2$	0.50	0.54	93%
85 - 75%	0.18	-0.037	100% +

It appears that the activity orthokinesis could account for the whole of the dry response where the alternatives are saturated air and humidities below the eccritic range. There may, however, be another response involved at high humidities, since the observed reaction intensity in the 100 - 90% choice is very much higher than the expected. Whilst it is felt that the possibility of such a response cannot be rejected, the results can not be regarded as proof of its existence. The activity at 100% relative humidity may well be considerably higher than indicated, particularly when considered in the light of the results shown in Table 7.13. All that the results of the experiment now under consideration indicate is whether an animal has moved during the 15 minutes immediately preceding a particular reading and not the actual number of moves which the animal has made during this period. Furthermore, the intensity of the orthokinesis was found to fall off with time, which would also affect the results, since these represent the total of 14 readings.

The mechanism keeping M. delanyi out of too low a humidity was found to be less clearly defined. The animals showed very little movement in the choice chamber, most often going directly to the half of the chamber in which the relative humidity was 75%. Prolonged observation showed that the animals would usually stop upon reaching the humidity barrier, often remaining stationary at this point for as long as ten minutes, after which they would turn back into more favourable humidity conditions. In a few instances the animals moved sufficiently often for tracking experiments, the results of which are shown in Figure 7.28. These tracks do show a tendency for the animals, once in the dry side of the chamber, to move back to more equible conditions but no clear directive response was evident.

Desiccated individuals of both species showed a positive response to the wet side of the wet/dry alternative chamber and a clear avoidance of the dry. Both species would come to rest in the wet side of the chamber but whilst M. delanyi would still frequently change their position within the wet side of the chamber, C. longicaudata would settle down very quickly, after which movement was rare. Figure 7.29 shows the results of tracking experiments carried out on both species.

It was found that some desiccated specimens showed very little tendency to move at all, regardless of whether they were in the wet or dry side of the chamber. These were found to have suffered too great a water loss during desiccation which had inactivated them. A number of these animals actually died during the course of the experiment.

Experiments carried out in a humidity gradient indicated the possible presence of a directing mechanism which would lead the animals to a region of high humidity when suffering extreme water loss in desiccated specimens of both species. The animals move more or less directly towards the region of higher humidity as shown in Figure 7.30, M. delanyi again showing some activity even after the region of higher humidity had been reached, whilst C. longicaudata would settle quite rapidly and not necessarily in the region of higher humidity but in fairly close proximity to it. It was hoped that amputation of the hygroreceptors on one side would give some indication of the nature of this response but this had no effect. Desiccated animals with only one antenna could still locate the region of high humidity in a gradient and showed no circus movements in conditions of uniform high humidity. This is hardly surprising, since the mobility and thus the scanning ability of the antenna would make a tropo-

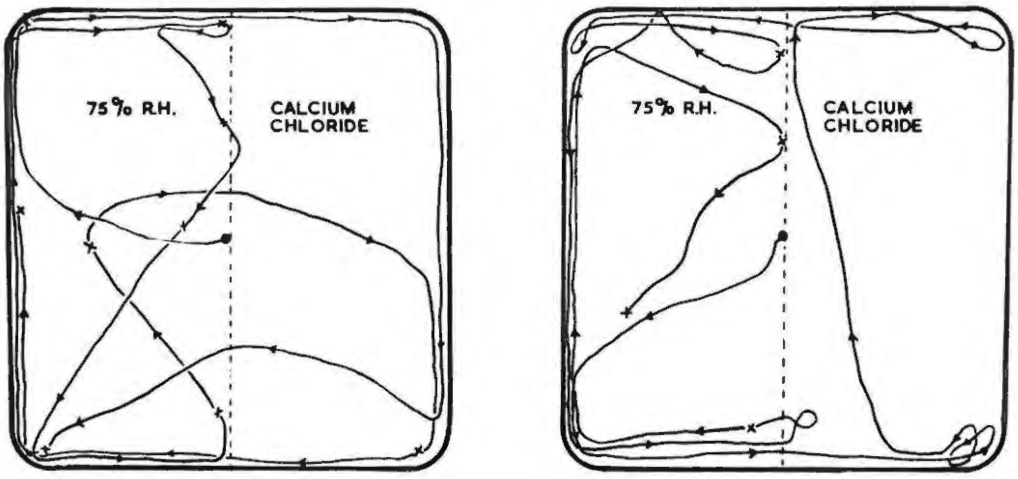


Fig. 7.28 : Path followed by M. delanyi in an alternative choice chamber showing avoidance of humidities below the preferendum.

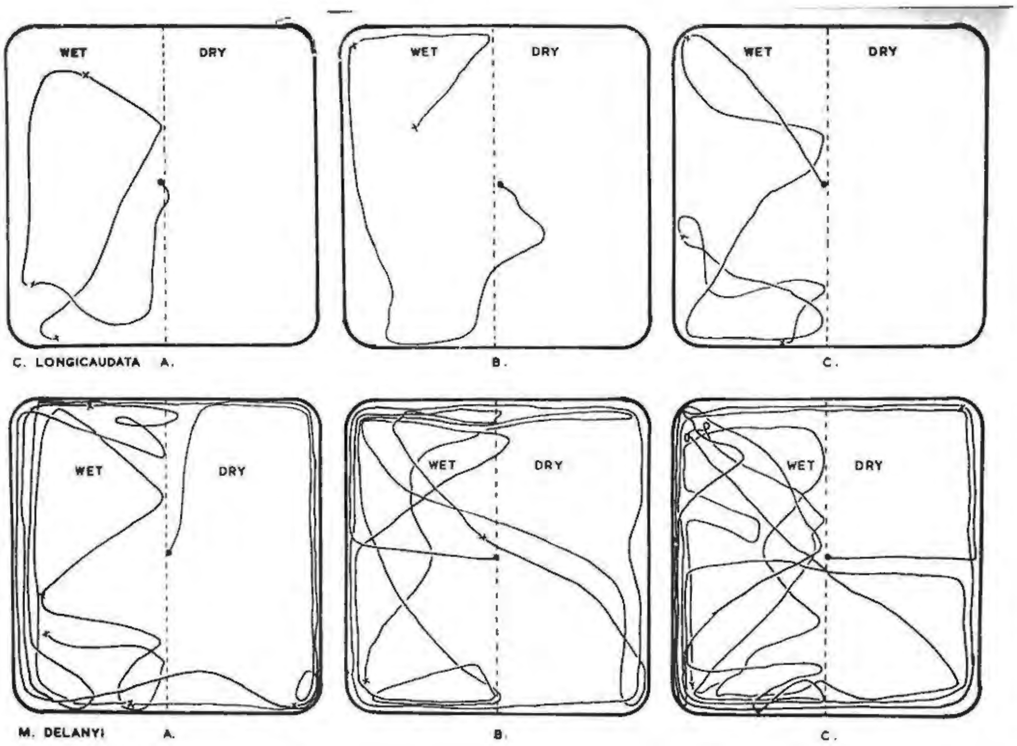


Fig. 7.29 : Paths followed by desiccated specimens of M. delanyi and C. longicaudata in an alternative choice chamber showing marked klinokinesis away from the dry.

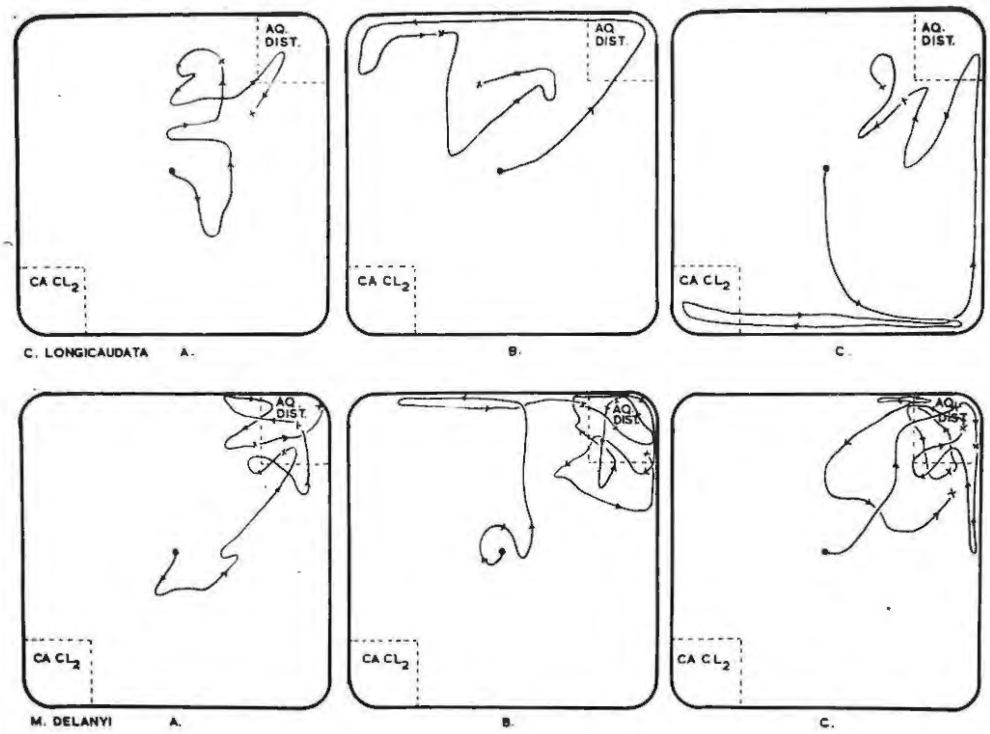


Fig. 7.30 : Paths followed by desiccated specimens of M. delanyi and C. longicaudata in a humidity gradient.

taxis unlikely. A klinokinesis avoiding dry conditions could easily account for the animals being directed towards the wet since it will always tend to prevent them from entering conditions which are drier than their present surroundings. The mechanism would thus probably be a klinotaxis but since the antennae are normally moving when the animal is active, this is not easily confirmed.

Desiccation had some effect on the activity of both species under uniform dry conditions when compared with normal animals but this effect was not as marked as one would expect, perhaps due to adaptation of or damage to the sensory apparatus. Table 7.17 shows a comparative analysis of the degree of activity between normal and desiccated animals in a uniform humidity chamber at 0% relative humidity.

Table 7.17. A comparison between the activity of normal and desiccated specimens of M. delanyi and C. longicaudata in a uniform humidity chamber at 0% relative humidity.

	M. delanyi.		C. longicaudata.	
Experiment No.	Normal	Desiccated	Normal	Desiccated
1	17	18	7	16
2	10	25	10	13
Total	27	43	17	29
Chi-squared:		4.6		3.62
Probability		0.05		0.05

7.4 Behavioural Responses to Gravity.

The negative geotaxis shown by both Machiloides delanyi and Ctenolepisma longicaudata has already been alluded to. Because of the observations made in some of the experiments previously described, a close watch was kept on the positions in which the animals were normally found, both in the culture dishes and in the field. M. delanyi was most often found hanging, ventral surface uppermost, from the lower surface of stones or pieces of bark, both in cultures and in the natural habitat, whilst C. longicaudata was found normally to take up a similar position on the filter paper in the culture dishes. These observations had to be confirmed by means of experiments, where the animals were not influenced by stimuli other than gravity.

Apparatus and method:

A simple apparatus was employed to confirm the negative response to gravity shown by both species. Three strips of boxwood were placed in a deep plastic dish, each with one end resting on the floor of the dish and the other resting against one of the walls. Ten or fewer animals, which had been fed and watered before commencement of the experiment, were placed on the floor of the dish which was then left in a darkened constant temperature room at 20°C overnight, after which period the positions of the animals in the apparatus were recorded.

The dishes were covered by means of perforated lids to admit free access of air and each chamber was tested for uniformity of the humidity within by means of two cobalt thiocyanate papers, one of which was suspended from the lid, the other placed on the floor under a screen of plastic gauze. When these were compared, no difference in colour could be detected, therefore the humidity was assumed to be nearly uniform throughout the apparatus. In order to ensure that the animals were not attracted to some substance present at one or other site on the boxwood strips, the positions of these were changed so that the lower end, resting on the floor of the dish, in one experiment, became the upper end, resting against the wall, in the next.

Results:

The results were expressed simply as the numbers of animals found at rest on the floor of the dish and on the upper, lower and lateral surfaces of the strips respectively as reflected in Table 7.18 and serve to confirm the earlier observations.

Table 7.18. Sites of settling of M. delanyi and C. longicaudata in the apparatus described in text, showing a negative response to gravity.

Site of settling	No. of Animals	
	M. delanyi	C. longicaudata
Floor of apparatus	4	7
Upper surface of strip	1	3
Lower surface of strip	17	14
Lateral surface of strip	4	0

Both species show a negative geotaxis and possibly a dorsal gravity response. No attempt has been made to locate the sense organs mediating the gravity responses of the animals or to determine the exact nature of the response.

5. Interaction of the Responses to Temperature, Light and Humidity.

Animals are never subjected to only one category of stimulus, to the exclusion of all others, at any given time, thus whilst one physical factor in a particular part of the environment may be within the preferred range, another may be unfavourable. Clearly, there must be some interaction between the responses to all the stimuli impinging on the sense organs, which will decide whether the animal will avoid a particular niche or occupy it. Whilst it would be impossible to investigate combinations of all the possible factors which may be encountered, the more important of these, temperature, light and humidity were considered for both Machiloides delanyi and Ctenolepisma longicaudata. For obvious technical reasons only two factors were considered at any one time.

Apparatus and method:

The interaction between temperature and light were investigated using the "Temperature Organ" already described. A mask of black cartridge paper was constructed which could be used to darken 10 cm. of the length of the trough holding the experimental animals, leaving only a space of 0.5 cm. between the floor of the trough and the mask to allow the animals free entry to and exit from the darkened area. The mask was placed so as to darken en the region between thermometers 5 and 7 and two 60 watt electric lamps were placed approximately 35 cm. above the apparatus, adjusted to light the interior of the trough as evely as possible.

The experimental animals were placed in the apparatus and allowed to distribute themselves in the dark over a period of one hour and their positions were recorded. The trough was now lit for a period of 30 minutes by means of the overhead lamps, after which a further record of the distribution was made. Heat applied to the apparatus with the overhead lights remaining on, set up a temperature gradient in the apparatus and the distribution of the animals was noted as the gradient steepened.

A similar experiment was done using a directive light beam, achieved by fixing a mirror at the cold end of the apparatus and a black paper mask, similar in construction to the one described for the previous experiment, but allowing entry and exit only from the side facing the mirror, was positioned so as to darken the area between thermometers 3 and 5. The light beam from a microscope lamp was directed onto the mirror to give a beam of directive light along the length of the trough. Dark adapted animals were placed in the light beam to ensure normal responses to directive light and once the animals had made their way into the dark a temperature gradient was set up. The reactions of the animals to the conflicting stimuli was noted as the gradient steepened.It was found necessary to fix a strip of glass to the far wall of the mask in order to prevent the animal from climbing up the paper and thus out of the region of the temperature stimulus.

The interaction between light and humidity was investigated in an alternative wet/dry choice chamber, half of which could be darkened by means of a black paper mask. The experimental animals, which had been dark adapted and had had free access to food food and water before the experiment, were given a choice between near-saturated, dark conditions and dry, light conditions, which would evoke conflicting responses, in one experiment and between near-saturated light and dry dark conditions in a control experiment. These experiments were repeated using animals which had been desiccated for 48 and 176 hours in the cases of M. delanyi and C. longicaudata respectively. In the latter case, however, a further control experiment was necessary in order to determine whether desiccation had any effect on the normal light responses of the animals. Here desiccated animals were placed in a light/dark choice chamber with a uniform humidity throughout and their light responses were noted.

Temperature-humidity interaction was investigated only in so far as it affected the activity orthokinesis of M. delanyi at the eccritic humidity. The animals were placed in a uniform humidity chamber over a saturated solution of sodium chloride; the relative humidity within the chamber was approximately 76% at 15°C. The chamber was placed in a darkened constant temperature room at 15°C and the animals introduced. The positions of the animals were plotted at 30 minute intervals over a period of four hours, after which the temperature of the constant temperature room was increased to 30°C and a further ten half-hourly plots were made. The effects of temperature on the actual choice of the animals in an alternative chamber was not determined since it was found impossible to set up suitable temperature alternatives in such a chamber.

Results:

Temperatures above 30°C would cause M. delanyi to leave the shelter of the black paper mask in the temperature organ, whilst C. longicaudata would remain in the dark until a temperature of about 35°C was reached. These temperatures are somewhat lower than the temperatures normally avoided by the animals, but approximately correspond to the temperatures at which the temperature activity orthokinesis elicits a high level of activity in each case. The results are shown in Figures 7.31 and 7.32. Where the light beam was used, the temperatures at which the animals emerged from the shelter of the mask were somewhat higher, namely 33°C and 37°C for M. delanyi and C. longicaudata

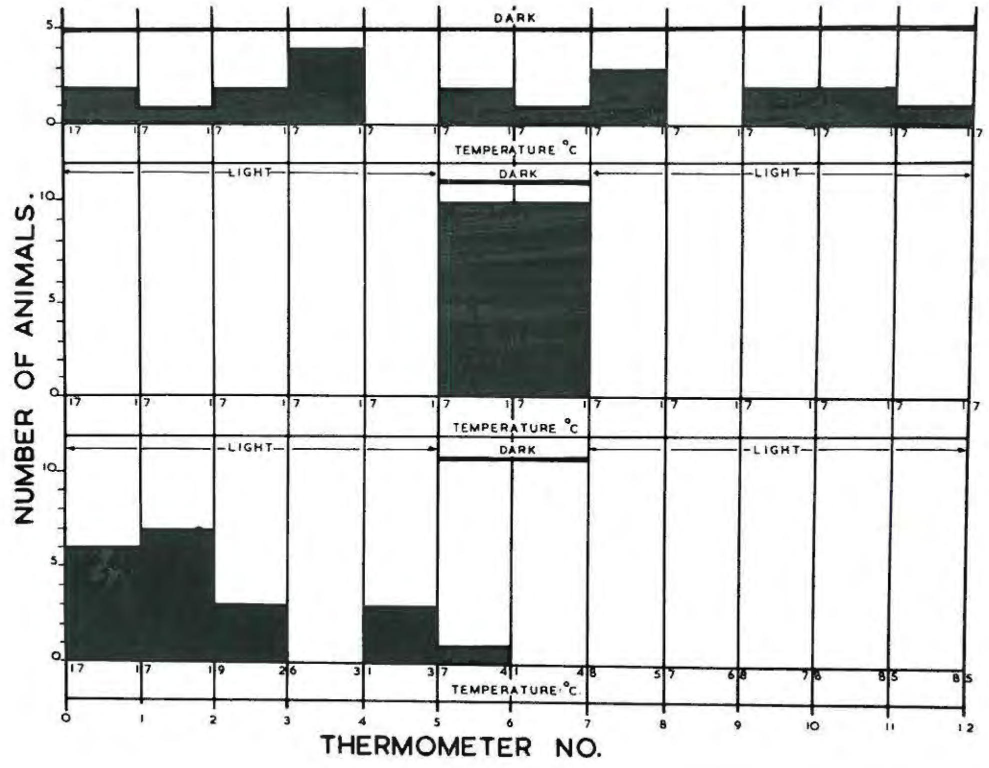


Fig. 7.32 : Distribution of C. longicaudata in a temperature organ, provided with shelter from light, before and after the establishment of a temperature gradient.

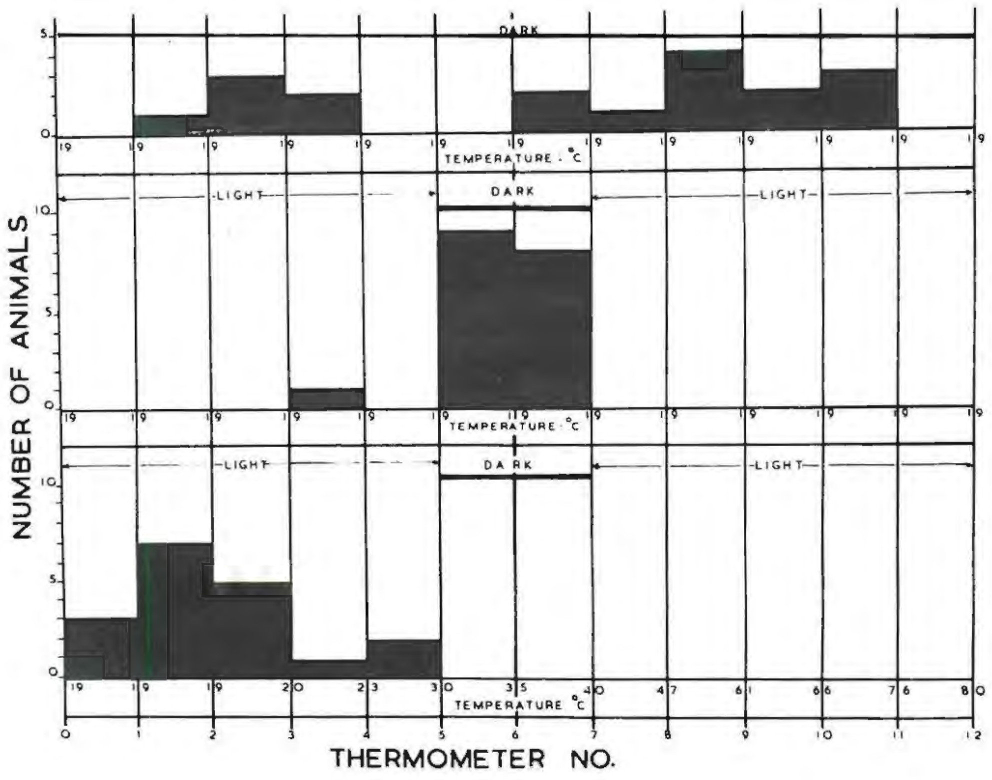


Fig. 7.31 : Distribution of M. delanyi in a temperature organ provided, with shelter from light, before and after the establishment of a temperature gradient.

respectively. This latter result is probably due to the intensity of the light stimulus being considerably higher in the case of the directive light beam.

In normal animals the light response takes precedence over the humidity responses as determined in previous experiments, the animals showing a 100% reaction to the dark regardless of the associated relative humidity. However, desiccation of the animals reverses the order of precedence of these two responses and the animals will congregate in the region of highest humidity irrespective of whether this region is dark or not. These results are summarised in Table 7.19.

Table 7.19. Distribution of M. delanyi and C. longicaudata in an alternative humidity choice chamber with one half darkened so as to provide conflicting and reinforcing light stimuli to the humidity responses.

	No. of Animals:			
Species and Condition	Wet Dark	Dry Light	Wet Light	Dry Dark
M. delanyi				
Normal	16	0	0	16
Desiccated	18	0	15	3
C. longicaudata				
Normal	15	0	0	15
Desiccated	18	0	17	1

Desiccation was found to have no effect on the normal light responses of the animals.

In the case of M. delanyi the temperature activity orthokinesis was found to come into effect even when conditions of relative humidity were in the preferred range. The percentage activity increased from 7% at 15°C to 90% at 30°C, based on the results obtained from twelve animals.

Temperature therefore appears to have an overriding influence over the humidity and light responses, whilst the light responses will suppress humidity responses in normal animals but not in desiccated animals, where the water drive is dominant.

7.6 The Adaptive Nature of the Behavioural Responses.

The adaptive nature of the behavioural responses considered in this investigation is self evident. The temperature responses ensure that the animals are kept out of conditions likely to be lethal, while the light responses ensure that they are not exposed to the full rigours of the environment. The humidity reactions of M. delanyi confine it to its typical niche where it enjoys some measure of freedom from predators, parasites and disease whilst suffering minimal water loss. Escape from competition, predators and parasites probably also accounts for the negative geotaxis shown by M. delanyi. This response would further assist in leading the animals to a position where the humidity is usually within the preferred range. In the habitat where the bulk of the material used in this investigation was collected, and where the study of physical conditions in the environment was made, the niche on the lower surface of the stones on the talus slope was shared only by a relatively small number of spiders and ploearid bugs and occasionally a few other hexapods such as Psocoptera, whilst the litter, humus layers and the bases and undersides of stones lying in the humus supported a considerable fauna. The significance of the negative response to gravity in the case of C. longicaudata is not at all clear, since other Lepismatoidea encountered in the field do not apparently respond to gravity in their settling behaviour, always being encountered on the ground under the stones forming the habitat. Thigmotaxis suggests itself as being important here, and possibly also in the case of C. longicaudata, but this aspect has not been investigated.

Experiments have shown that the avoidance of high temperatures takes precedence over all other behavioural responses investigated, since it constitutes the factor most likely to be lethal. The reaction to darkness normally dominates the humidity responses since leaving shelter not only exposes the animals to the risk of hyperthermia due to solar heat, but also to attack by diurnal predators. The dominance of the water drive over the positive response to darkness is perhaps explicable in terms of the lethality of excessive water loss but it is doubtful whether the need for replenishment is ever so urgent under anything like normal conditions as to necessitate a search for water by day, since the rate of water loss from both M. delanyi and C. longicaudata is sufficiently low to allow for a water search to be deferred until after nightfall. This increase in impermeability probably accounts for the fact that the desiccation has no effect on the light responses of the Thysanura, as is the case in Porcellio scaber (Latrielle) where exposure to dry air reverses the normal negative light response, causing the animals to leave a dry shelter in search of moisture (Abbott, 1918, quoted in Edney, 1954). A similar reversal of the light response has been shown for Tenebrio molitor, an animal extremely resistant to desiccation (Perttunen and Lahermaa, 1958) but here the response may well be necessary since the animal both inhabits a very dry environment, where free water is not always readily available and has not been shown to have the ability to absorb water from the atmosphere.

8. DISCUSSION

It is now necessary to view, as a whole, the results obtained from this investigation. These permit us to put forward a tentative explanation for the distribution of the two orders comprising the Thysanura and also to draw at least some conclusions pertaining to escape from the shelter of the forest floor, a step which must have been of the utmost importance in the evolution of the Pterygota. Associated with changes in habitat are changes in orientation behaviour, ensuring that this meets the requirements of the new environment; physiological adaptation must go hand in hand with behavioural adaptation to ensure the success of a species in the invasion of a new habitat. Such changes in ehaviour, in turn, may involve changes in the sensory apparatus of the animals concerned and since this aspect, together with the evolution of some behavioural responses, would at times involve a digression from the main theme outlined above, these will be dealt with separately.

8.1 Factors Affecting the Distribution of the Thysanura.

From studies on the habitats of the two species under consideration, it is apparent that the only factor common to both is darkness. M. delanyi typically inhabits a niche characterised by a stable microclimate; the humidity in this habitat seldom, if ever, reaches saturation, nor does it fall to a level where evaporation would be excessive. The animals have developed a moderate resistance to water loss but the degree of resistance is very variable, being dependent on the stage within an instar and probably on a number of other hitherto underdetermined factors. By limiting itself to the protection of its typical habitat M. delanyi is, therefore, subject to the minimal risk of suffering excessive water loss. Temperature extremes also never occur in the typical habitat, although specimens have been encountered where the temperature is higher than the determined preferendum. The animal's orientation behaviour is such as to ensure that it attains the conditions which prevail in its typical habitat.

The typical habitat of C. longicaudata shows considerable fluctuations in its climatic conditions. The cover of the stones provides shelter from temperature extremes, but the humidity is very variable. Greater resistance to water loss probably accounts for the humidity indifference of this species and, while temperature will limit its choice of niche, it attains the shelter of its typical habitat largely by means of its light responses.

Three factors must be considered as of some importance in governing distribution. These are the availability of food, the possible restrictions imposed by temperature and finally humidity. Food can be dismissed as a factor limiting the distribution of the Machiloidea since they are known to feed on the leaves of many plants in the absence of their usual food plants (Kühnelt, 1961) and there seems to be no reason why they should not adapt wholly to this diet. Temperature as a limiting factor, however, requires a more thorough consideration.

C. longicaudata has a higher temperature range than M. delanyi, both in its preferendum and its upper critical temperature, but it seems hardly likely that a lower temperature range has been the limiting factor in restricting the distribution of the Machiloidea. Delany (1954) reports a higher temperature tolerance than that shown by M. delanyi in the shore living machilid Petrobius marititimus (Leach) on Lundy. Field measurements of the temperatures under stones inhabited by this species showed it to occupy niches where the temperature was as high as 28.6°C, although the relative numbers of animals found at this extreme and at lower temperatures are not given. If indeed the animals do consistently occupy niches where these high temperatures obtain, this can be seen as an example of a machilid species which, like the Lepismatoidea, has abandoned the more sheltered, environment common to most Machiloidea, for the harsher conditions of the unprotected sea shore and which has had to adapt both its temperature tolerance and temperature responses to suit the new environment.

This is not, however, to minimise the importance of *accepting* higher temperatures on leaving the shelter of the forest. If other factors permit the invasion of harsher conditions and the animal is unable to adapt to the temperature conditions obtaining in the new surroundings, the invasion of the new environment is doomed to failure. However, the experiments carried out in the present investigation have shown that, while temperatures above the eccritic do result in avoiding or escape behaviour, these conditions are not necessarily lethal. *M. delanyi* survives prolonged exposure to a temperature of 30°C and clearly if the population pressure were sufficiently strong to force the invasion of an area where temperatures of this order prevailed in the typical niche, the animals should, like *Petrobius maritimus*, be able to adapt to the new temperature conditions.

The higher temperature tolerance of *C. longicaudata* is a necessary adaptation to the environment in which it lives but cannot be regarded as the factor which has allowed it to invade these conditions. Such tolerance must have evolved under the new conditions.

Thus it would appear that humidity is the factor most likely to be limiting the distribution of the Machiloidea. It seems that the greater water loss suffered by *M. delanyi* limits it to regions where this would not be excessive. Both *M. delanyi* and *C. longicaudata*, however, lose water faster than do the Pterygota and other arthropods which have invaded the more arid regions. We have, therefore, to elucidate not only why, despite this physiological shortcoming, the Lepismatoidea are so widely spread but also why the Machiloidea occur in areas which might be regarded as physiologically unsuited to them.

Now if an animal is to invade a dry area, the risk of a high rate of water loss being fatal is not great if the loss can be made good before the internal water store has been depleted to a degree where cellular function is impaired. But the invasion of a dry region usually requires the organism to have an eccritic humidity that is below saturation. Consequently, when its water content falls, a water drive must be displayed, i.e.

the humidity response must reverse.

Such a reversal of a dry response after desiccation has been shown, among other examples, for Blatta orientalis (Gunn and Cosway, 1938), Ptinus tectus (Bentley, 1944), Tribolium castaneum (Willis and Roth, 1950), Tenebrio molitor (Dodds and Ewer, 1952), the tick Ixodes ricinus (Lees, 1948) and for the diplopods Schizophyllum sabulosum and Iulus terrestris (Perttunen, 1953). Indeed it seems certain that such a reversal must occur in any species whose eccritic humidity is below saturation and it certainly does in both species of Thysanura under investigation.

By virtue of its moderate impermeability and its water drive M. delanyi should be able to survive outside the shelter of the forest canopy and to invade the habitat of the Lepismatoidea. This indeed it does, but only in certain localities within its geographical range. Such an invasion would require only two things; that the animal accepts a somewhat higher temperature range and that it can replenish its internal water store more frequently than a typical lepismid. In fact M. delanyi and at least one other undescribed species of Machiloides have penetrated the arid region known as the False Karroo to the north-west of Grahamstown. In this area the animals are found next to streams and stock water dams. Wygodzinsky (1955) reports that species of Machilinus are found in comparable niches in extremely arid regions in Argentina. These animals thus seem to be able to invade these more arid areas, provided a supply of water is continually available. Machilinus may in all probability have invaded these arid regions by following the courses of rivers down from the more equible conditions of the Andes. M. delanyi may have invaded the more arid regions of the False Karroo in the same manner, since the watershed of the streams flowing through this region lies in a mist belt and supports many remnants of indigenous forest.

Thus their general inability to curb water loss would seem to limit the distribution of the Machiloidea only insofar as it necessitates frequent replenishment of theinternal water store and that the chief factor limiting their distribution is the availability of water. Wygodzinsky (1955) points out that the South African Machiloidea, of which Machiloides is the dominant genus, are confined to regions where the assured rainfall is between 20 and 30 inches. One can take this a step further and say that they are confined to a region where, on the average for the thirty year period 1921 to 1950, seldom a month passes without a rainfall of at least 10 mm. (Weather Bureau, 1957). This region corresponds to the discontinuous forest belt which lies below the Great Escarpment, as shown in Figure 2.01. During the summer months a low pressure region persists over the inland plateau of the subcontinent, i.e. over the region above the escarpment. This low pressure region allows an influx of moist air from the coast into the interior and results in summer rains, not only in the interior but also in the region below the escarpment. The western tip of the forest belt receives much less rain during these summer months but is never subject to long periods without any form of precipitation. During the winter months, however, the low pressure system over the interior is very weak, if it exists at all, and the weather of the subcontinent is greatly influenced by deep cyclonic depressions moving from west to east past the southern tip of Africa. These give rise to winter rains along the western and southern coastal regions, but no rain falls over the interior during this time of the year. The eastern aspects of the forest belt will receive less rain in winter than the southern and western parts, but they are subject to periodic influxes of moist air from the coastal regions, resulting in mist and light rain. This moist air does not penetrate the interior, being stopped by the escarpment. Thus while the discontinuous forest belt receives a measure of precipitation throughout the year, the inland plateau may have no rainfall at all during the period from May to September. Now even at moderate saturation deficits, M. delanyi will lose water at a rate which will necessitate regular uptake of water. This requirement cannot be satisfied on the inland plateau during its prolonged dry period.

C. longicaudata shows an advance over M. delanyi in at least two aspects of its water economy. It has acquired both a higher resistance to water loss and the ability to absorb water from a subsaturated atmosphere. The former reduces the need for frequent replenishment of the water store, while the latter enables the animals to effect such replenishment under conditions in which M. delanyi loses water. Further, there is the possibility that C. longicaudata can retain metabolic water, which may help it tide over periods of drought; certainly the large fat stores make this a likelihood.

Even in the most arid regions, humidities from which the lepismids can absorb water vapour must occur far more frequently than actual precipitation and the widespread distribution of the Lepismatoidea can therefore be attributed to the water gaining mechanisms which has made them independent of the presence of liquid water.

In summary then, the Machiloidea are restricted in their distribution by the inadequacy of their water retaining and water uptake mechanisms. These limit them to regions where water in liquid form is always readily available; an increase in the efficiency of either or both of these would permit them to extend their range considerably. Of these two, the water uptake mechanism seems to be the more limiting, the vesicles of the Machiloidea not only being restricted in their use to the uptake of liquid water but also rendered unusable by excessive water loss.

It is now possible to consider, in the light of these data, the evolution of the two Thysanuran orders and of the Pterygota. The ancestral habitat of the terrestrial Arthropoda was almost certainly the moister litter layers of the forest floor, for here the ill-adapted animals would have found sufficient moisture and shelter. To maintain themselves in such a habitat one would expect the eccritic humidity of the early terrestrial arthropods to have been at or near saturation. However, this habitat must have housed not only a considerable population of animals in competition for food and space but conditions would have been such as to favour the spread of various diseases.

One may thus visualise that any animal which could survive in slightly less humid conditions would be at an advantage; that there would be, in other words, selection for an eccritic humidity slightly below saturation. This indeed seems to be the case in *Peripatopsis moseleyi* and Bursell and Ewer (1950) in discussig the dry response of this animal suggest that fungal diseases may provide an important selection pressure for a reaction away from saturation conditions. Such considerations could well apply to the early Thysanura for, in laboratory cultures, *M. delanyi* has been found to be prone to attack by fungi under conditions of high humidity. Wygodzinsky (1941) similarly reports mortality in his cultures due to the fungus *Verticillium terrestre* or a closely related species. He also records infestation both by gregarine protozoa, the infective sporozoites of which are probably more readily ingested where high humidity favours their survival, and by the nymphs of trombidiform mites, also usually associated with the moister humus layer. Thus it seems highly probable that the danger of such parasites, together with predators such as Onychophora and Chilopoda, and also competition with a myriad of other organisms inhabiting the moister layers, would all confer considerable selective advantage on a dry response.

M. delanyi does not constitute the only case where the eccritic humidity does not correspond with the optimum for survival in terms of physiological limitations. The case of *Peripatopsis moseleyi* has already been mentioned. Similarly *Ptinus tectus* Boie has been shown by Bentley (1944) to have a distinct preference for dry air, although Ewer and Ewer (1942) found an optimal humidity of about 70% relative humidity for all stages in its development. In this case, the selective advantage of the dry reaction is obscure.

Thus it appears that the constant pressure for abandoning the moister humus layers resulted in the acquisition of a dry response which was followed by the evolution of better water retention as discussed in Part 6.5. This would result in the physiological condition and humidity responses encountered in

M. delanyi. In these terms the Machiloidea can be regarded as a relict of the early Thysanura which became established in the drier parts of the forests, where they enjoyed some freedom from competition, predators and disease.

Other niches were, however, open which would, in all probability, have been free from competition at the time when the terrestrial cryptofauna was evolving. These included both the forest canopy and also the more open country, where stones and smaller plants would provide shelter to a nocturnal animal, thus ensuring that the full rigours of the environment were cushioned by a more equible microclimate. Both these niches could be invaded by animals which had attained a reasonably efficient water retaining mechanism and the ancestral Lepismatoidea probably first pioneered the more open country. Initially these animals would have been dependent upon eversible vesicles for water uptake; the presence of these organs in the Nicoletidae suggests that they were also present in the ancestral forms. This would have limited them in much the same way as it limits the distribution of the Machiloidea today, but an elaboration of their water retaining mechanism into one of water uptake made the animals independent of the presence of liquid water. Such a step, together with constant selection pressures both for improvement of cuticular impermeability, and for the choice of a protected niche, now enabled the animals to survive under any conditions where the relative humidity periodically exceeded the lower limit of their water uptake ability and where sufficient food material was available.

The Pterygota probably evolved from an arboreal line. Flight could not have evolved in any other than a climbing form. Here too, in all probability, the ability to absorb water from a subsaturated atmosphere would have been of importance and due to transpiration by the plants conditions among the foliage would provide the high humidity to make the uptake of water vapour possible. The ability to absorb water vapour shown by a number of extant insects may well represent the retention of this ancestral character. With the advent of flight came the need for a strengthening of the cuticle and, since the animals now ranged further, the development in the cuticle of physical barriers to water loss, both of these tendencies would culminate in the evolution of the tough impermeable cuticle common to most Pterygota.

Finally, reverting to the distribution of the Machiloidea in Southern Africa, mention must be made of Machiloides solitarius Silvestri. The presence of this species at Okahandja in South West Africa upsets the view expounded here. While there can be no doubt about the correct identification of this species as a Machilid, it must be stressed that to date only the type specimen, a solitary female, has been collected. This casts some doubt on whether the species was in fact collected at Okahandja and it is possible that an error in labelling of specimens may have occurred. If further specimens confirm its distribution, not only will it prove to be an animal of the greatest physiological interest, but the present hypothesis may require significant modification. To develop a more complex picture of Thysanuran evolution upon a unique specimen is not now, however, justified.

8.2 Some Speculations on the Evolution of Behavioural Responses.

Having considered the behavioural and physiological changes which have been instrumental in the emancipation of the Arthropoda from the sheltered environment of the forest floor, there remains for discussion the possible effects of these changes of habitat on the sense organs and the mechanisms of the behavioural responses of the animals. For this it is necessary to include results obtained with other arthropods. While much work has been done on humidity responses of various arthropods, there are nevertheless considerable gaps in our present knowledge of this aspect alone, and the evidence on which the discussion of the temperature and light responses is based is, to say the least, scanty. An attempt at synthesis must, therefore, by virtue of the incompleteness of our present knowledge, be speculative, but as such, it may serve to draw attention to the need for and direction of further investigations.

(i) Temperature receptors and responses.

Before embarking on a discussion of the evolution of thermoreceptors and temperature responses, it is necessary to review our present knowledge of these organs in the antennulate arthropods. The localisation of the main temperature receptors on the antennae, particularly where these are relatively long as in most primitive hexapod groups, allows for due warning of adverse temperature conditions. These appendages are usually stretched out ahead of the animal, and their mobility permits efficient scanning and exploration of the surroundings. Wherever the temperature senses of antennulate arthropods have been investigated these have been found to be centred, at least in part, on the antennae.

Herter (quoted in Wigglesworth, 1953) has shown that removal of the terminal segment of the antennae of Pyrrhocoris sp and of Lygaeus sp (Hemiptera, Heteroptera) causes a considerable upset in the animals' ability to avoid high temperatures; these observations have been subsequently confirmed and extended by Gebhardt (1951, 1953). Pyrrhocoris apterus L., for example, shows an increase in its temperature avoidance threshold of from 45.22 ± 0.15°C in the intact animal to between 50 and 53°C when the antennae are removed; a similar increase upon antennectomy is shown by Lygaeus equestris L. In neither case was an alternative concentration of thermoreceptors,responsible for the avoiding reaction at the new thresholds, apparent. This had led to the suggestion that these are scattered over the whole body surface.

Antennal thermoreceptors have been shown to occur in other arthropods, and, in some of these cases, the presence of additional thermoreceptors can be inferred but they have not been localised; these include some Phasmida (Cappe de Baillon, 1932, quoted in Buddenbrock, 1952, Gryllus bimaculatus L. (Amouriq, 1955), and Lithobius forficatus L. (Bauer, 1955). In the cases listed below, however, alternative receptors have been localised

Collembola - Antennae and end of abdomen (quoted in Buddenbrock, 1952 - no authority given)

Acheta and Liogryllus (Orthoptera - Grylloidea) - Antennae, maxillary palps, tarsi and cerci (Herter, 1924 quoted in Buddenbrock, 1952)

Otiorhynchus ligustici L. (Coleoptera - Curculionidae) Antennae and tarsi (Gebhardt, 1953)

Dorcus parallelepipedus L. (Coleoptera - Lucanidae) - Antennae and maxillary palps. (Gebhardt, 1953)

The problem now poses itself as to why an insect should be equipped with more than one concentration of thermoreceptors and why these should have different avoidance thresholds, the antennal threshold commonly being the lower. It is tempting to interpret this as meaning that the antennal receptors are sensing air temperature while the other receptor groups are concerned with substrate temperature.

Exposed environments, where the substratum is subjected to the full effect of the sun, may attain surface temperatures of as much as 70°C (Alexander and Ewer, 1958) whereas the associated air temperature would seldom exceed a little over half this figure. By virtue of the small area of contact, limited to the tarsi of the legs and, in the case of the Thysanura, the abdominal styles, arthropods may well be able to tolerate higher surface temperatures than air temperatures, provided that there is some air movement between the animal and the surface of the substratum. The work of Alexander and Ewer (1958) on the scorpion Opisthophthalmus latimanus suggests this as a strong possibility which would account for a higher threshold in contact receptors than in air temperature receptors. However, the experiments carried out in this and numerous other investigations on temperature responses of arthropods, are of a nature which precludes any distinction being drawn between responses to air and surface temperatures. Although direct evidence is lacking, it is thus possible that the antennal and palpal thermoreceptors of the Thysanura are concerned with the estimation of air and surface temperatures respectively.

The maxillary palps of the Thysanura are extremely well developed relative to those in the Pterygota, a trend which is evident even in the earliest fossil forms as exemplified by *Dasyleptus brongniarti* Sharov from Permian deposits in Russia (illustrated in Tiegs and Manton, 1958). In this animal the palps appear to be longer than the antennae. This condition would seem to indicate that the maxillary palps are important carriers of sense organs and since they are always in contact with the substratum and situated anteriorly they represent the most likely site for contact thermoreceptors.

The condition where both receptors have approximately the same avoidance threshold, as in the case of *M. delanyi,* may well be primitive and found in animals which have to shun the full rigours of the terrestrial environment. Their need is for behaviour which will serve to confine them to a comparatively sheltered habitat; one where, owing to the infrequency of exposure to the full sun, the substratum is seldom, if ever, likely to attain a temperature as high as that of the surrounding air. High temperatures of both air and substrate must repel and this will be achieved if the avoidance threshold is the same for both sets of organs. This is the condition in *M. delanyi* and also seems to obtain in the case of the diplopods *Paradesmus gracilis* and *Blaniulus guttulatus*, where the antennae have been shown to be sensitive to temperature but antennectomy has no effect on the temperature avoidance reaction (Cloudsley-Thompson, 1951). These animals too inhabit sheltered habitats where the substratum is covered by a layer of humus and where the air/surface temperature relationship would be the same as that described for *M. delanyi*. The nocturnal habit of the Lepismatoidea need not invalidate this argument, since high surface temperatures may be maintained until well after sunset and further the animals may be forced to leave shelter by day for other reasons such as escape from predators.

If indeed the palpi were the primitive contact thermoreceptor, this condition did not persist, for all other hexapods investigated have maxillary palps not normally in contact with the substratum (Liogryllus and Dorcus parallelepipedus) or have highly modified mouthparts, where maxillary palps, if present, are vestigial (Collembola, Curculionidae, Hemiptera). It is possible that with the increase in leg length, in response to locomotory requirements, the palpal receptors, through no longer being in contact with the substratum, may come to respond to air temperature. This certainly seems to be the case in Liogryllus, where Herter has demonstrated a lower threshold to air temperature for the receptors located on the maxillary palps than the antennae. It is significant that this animal has further temperature receptors on the tarsi and the cerci, of which the former at least must be contact receptors. Both these latter sense organs have a higher threshold than those on the antennae and on the palps. Tarsal contact receptors may well be common among the higher insect; they have been shown to occur in the cockroach (Kerkut and Taylor, 1957). Dorcus parallelepipedus also has temperature receptors on the maxillary palps which have a lower threshold than those on the antennae. Examination of the maxillary palps of a number of genera of the family Lucanidae has shown that in every instance these are inserted in such a manner that contact with the substratum is unlikely and in some cases, impossible, owing to the size of the mandibles. The palpal receptors in Dorcus must, therefore, of necessity, be responsible for the determination of air temperature and perhaps are used when the flabellate antennae are withdrawn.

In all the examples investigated, with the exception of the Thysanura, removal of the identifiable thermoreceptors does not abolish the avoiding reaction but merely raises the threshold at which the response is evoked. This suggests the presence of further temperature receptors. Such indeed must also exist in the Thysanura, for neither the speed orthokinesis of C. longicaudata nor the leaping response of M. delanyi depend upon antennal or palpal receptors. The possibility must be remembered that thermal receptors influencing general, as opposed to specific avoiding, behaviour may not be peripheral. Thus Alexander and Ewer (1958) have shown that it is likely that the stilting response of Opisthophthalmus latimanus depends upon central receptors reacting, not to environmental but to body temperature, while Kerkut and Taylor (1958) have recorded a general increase in spontaneous activity from isolated nerve ganglia of the cockroach and other invertebrates when subjected to both high and low temperatures.

(ii) Photoreceptors and Light responses.

The light responses of the two species of Thysanura under consideration are difficult to relate to their respective modes of life. Light, when equated with solar radiation and thus heat absorption, must play a far more important role in the life of an animal which inhabits an exposed environment than one living in the comparative shelter of a forest, and we do indeed find that the activity orthokinesis in response to light is more rapidly developed in C. longicaudata than in M. delanyi. In contrast, however, the photosensory organs of M. delanyi are much better developed than those of C. longicaudata, the former possessing both conspicuous lateral ocelli, relatively much larger than ~~those~~ those of most Pterygota, and well developed compound eyes, whilst in the latter the compound eyes are reduced to aggregations of about 12 ommatidia each and ocelli are entirely lacking. The anomaly whereby the more highly evolved photosensory mechanism occurs in the animal which apparently least needs it may be explained when it is remembered that the more complex compound eyes of the Machiloidea make possible a telotactic response and this is a more effective mechanism for attaining a sheltered position.

The well developed compound eyes of the Machiloidea may also be instrumental in the detection of the approach of a predator. The nocturnal habits of these animals are, however, against this. Much hinges on the illumination threshold for discrimination by the eye and also on whether the animals are fully nocturnal or merely crepuscular. Wygodzinsky (1941) stresses that, though various machilid species in Switzerland are most usually active at night, they cannot be regarded as being wholly nocturnal.

He bases this on observations made of the animals by day during spring or autumn. Diurnal feeding was observed on days when the sky was overcast. He further adds that so long as the beam was not directed onto them the behaviour of the animals could be observed at night by the marginal light of an electric torch. It is possible, therefore, that the animals normally leave shelter before nightfall, as soon as the illumination intensity fall below a given level. The readiness with which M. delanyi becomes adapted to moderate light intensities may lend some support to this suggestion. Extensive field observations alone will provide the answer. My own are as yet too scanty to offer decisive evidence. Finally the ocelli of the Machiloidea may also be associated with a partly diurnal or crepuscular habit. In at least one species, Machilis albiocellata Stach, these organs show a migration of pigment, dependent upon light conditions. The pigment is retracted by night or in the dark and is dispersed by day or in the light (Wygodzinsky 1941). However, until the function of these structures has been determined it will not be possible to consider why they have been developed or retained in the Machiloidea and not in the Lepismatoidea.

(iii) Hygroreceptors and humidity responses.

Before embarking on a discussion of the actual mechanisms involved in the humidity behaviour of the Thysanura, a brief consideration of hygroreceptors is desirable. As previously mentioned, two types of hygroreceptors have been identified in the Arthropoda, namely "Evaporimeters" and "Hygrometers". Evaporimeter type hygroreceptors are invariably associated with a wet response, being dependent upon the rate of evaporation from an animal and therefore most probably situated at a site of maximal water loss. Lees (1943) has suggested that the evaporimeters occurring in the larvae of Agriotes may be located in the thin intersegmental membranes of the head appendages and Bursell and Ewer (1950) have found the wet response of Peripatopsis moseleyi to be mediated by non-antennal receptors apparently scattered over the general body surface. Evidence for the receptors in the latter case being evaporimeters is put forward by the authors and has been confirmed by Dodds (1952) who has also found evidence for non-antennal evaporimeters which mediate in part the wet response of desiccated Tenebrio molitor. A similar condition has been described for Schizophyllum sabulosum (Perttunen, 1955) and for Drosophila melanogaster (Perttunen and Syrjamaki, 1958) but in these latter cases it has not been determined whether the non-antennal wet receptors are hygrometers or evaporimeters. In contrast to the foregoing dry responses are mediated only by hygrometers, evaporimeters being by their mode of operation unsuited to such a function. Dry responses are, as a rule, mediated by receptors located on the antennae; this has been shown to be true for Blatta orientalis (Gunn and Cosway, 1938), Pediculus humanus corporis (Wigglesworth, 1941), Ptinus tectus (Bentley, 1944) Tribolium castaneum (Willis and Roth, 1950) and Blatella germanica (Roth and Willis, 1952) among the Hexapoda and for the diplopod Schizophyllum sabulosum (Perttunen, 1955). Only in the case of Tenebrio molitor have the sense organs involved been definitely identified as hygrometers but it seems likely that the other examples quoted also operate on this principle.

It is, however, not necessary to suppose that all wet responses require an evaporimeter type receptor for there is no reason why an hygrometer should not mediate a wet reaction; its principle of operation is fully compatible with the requirements for mediating a response in either direction, depending on the mechanics of the sensillum. Culex fatigans has been shown to avoid low humidities (Thomson, 1938); the sensory receptors concerned are claimed to be antennal hygrometers by Necheles (quoted in Thomson, 1938) and the presence of antennal wet mediators, which probably also operate on the hygrometer principle, has been showh in Drosophila melanogaster (Begg and Hogben, 1946, Perttunen and Syrjamaki, 1958) as well as in Aedes aegypti (Roth and Willis, 1952). M. delanyi and in all probability C. longicaudata as well, would fall into this latter category, having antennal hygrometers mediating the wet response.

The question arises why the two types of humidity receptors have evolved. Hygrometers, since they depend for their action on the distortion of hygroscopic parts of the cuticle, could meet the requirements of both a wet and a dry response, the direction being dependent upon the structure of the sensillum. Since they are not confined to a region of maximal water loss, they can be located on the antennae, where, by virtue of the scanning ability of these appendages, they would be much more effective in mediating directed hygroresponses. The functional superiority of hygrometers leads us to the conclusion that evaporimeters are probably the primitive type; it may be that the hygrometer type of receptor could only be evolved once the terrestrial arthropods had elaborated a hardened exoskeleton.

In both cases where evaporimeter type hygroreceptors have been identified as the main mediators of the wet response, the eccritic humidity of the animal concerned has been near saturation, viz. 100% relative humidity in the case of <u>Agriotes</u> larvae and 98% in the case of <u>Peripatopsis moseleyi</u>. On the evaporimeter principle, if we take an activity orthokinesis leading to aggregation in the wet as an example, the sensory impulses from the hygroreceptor will continue to be transmitted to the "locomotory centre" as long as water is being lost, the level of excitation, i.e. the frequency of the sensory impulses impinging of the locomotory centre, being proportional to the intensity of stimulation, in this case the rate of evaporation. It can therefore be expected that the intensity of the reaction will be proportional to the saturation deficit up to its maximal intensity, where it would level off.

Now the early land arthropods would have incurred their maximal water loss through the cuticle and their eccritic humidity would have been at saturation, where evaporation would be minimal. In order to attain the eccritic humidity conditions a scattering of evaporimeters over the body surface would be extremely effective. The expected relationship between activity and humidity for such an animal would be as shown in Figure 8.01 A. No example of an animal which has this type of humidity response alone has been discovered. The closest

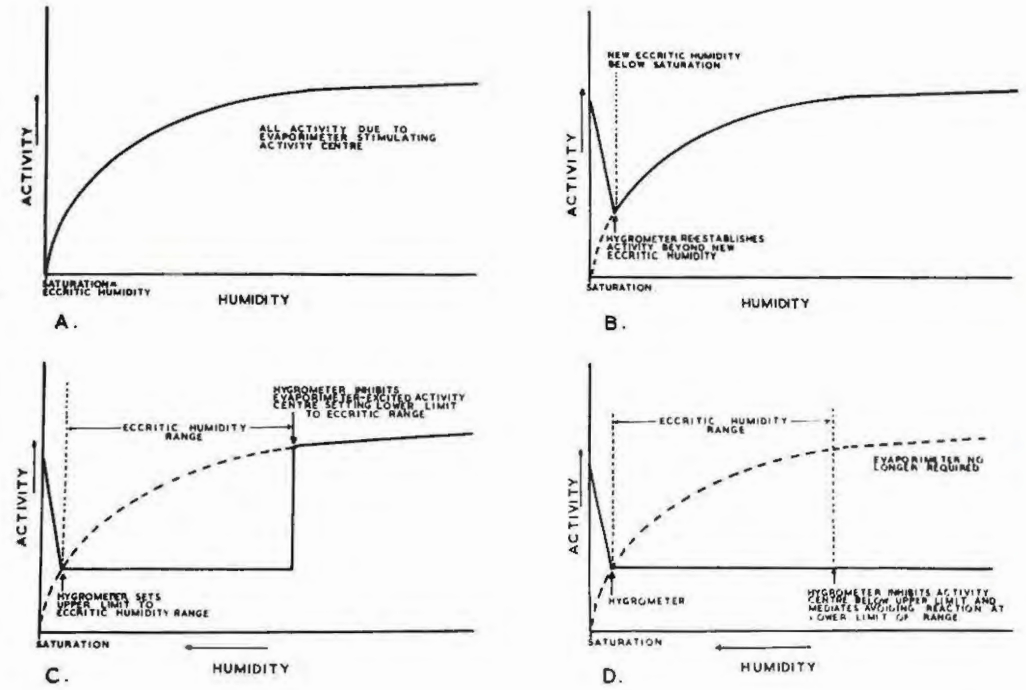


Fig. 8.01:- Suggested steps in the evolution of humidity responses. For explanation see text.

parallel is to be found in the shore-living amphipods Talitrus saltator and Orchestia gammarella (Williamson, 1951) and the diplopod Orthomorpha gracilis, an inhabitant of moist humus (Perttunen, 1953). All three have an eccritic humidity of 100% relative humidity. The nature of the hygroreceptors of the amphipods is inferred as being an evaporimeter, but the point is not proven; those of Orthomorpha have not been studied. In all three examples there is an activity orthokinesis of the type postulated as well as a speed orthokinesis acting to reinforce it. But there is in addition an avoiding reaction to low humidities, whose sensory basis is unknown.

Lowering the eccritic humidity may confer considerable advantage on an animal as previously discussed. To effect this in a stenohygrous animal, there must be involved a second hygroreceptor, one which will increase activity above the new preferendum. Such a receptor can best be an hygrometer operating over a very short range. An hygrometer need, of course, not be confined to a region of maximal water loss and may be located on the antennae. The activity/humidity relationship under these conditions would be as shown in Figure 8.01 B and reflects the condition is Peripatopsis moseleyi (Bursell and Ewer, 1950), an animal which is stenohygrous and ill adapted to terrestrial life, its rate of water loss being about twice that of an earthworm (Manton and Ramsay, 1937). Here, as in the previous example, the activity orthokinesis is reinforced by a speed orthokinesis and an avoiding action of a specialised nature has been shown.

With animals whose eccritic humidity lies below saturation a new problem arises. Once the animal is desiccated it must be able to make good its water loss by drinking. This involves two factors, the suppression of the reaction leading to avoidance of saturation and some type of enteroreceptor to register the state of depleted water load. It is reasonable to assume that the stimulation of the enteroreceptor is itself a source of impulses which inhibit the central responses from the exteroreceptors which normally lead the animal away from conditions of total saturation.

Even a niche below saturation is subject to population pressure, and animals will tend to spread as far as their physiological adaptations will allow. If such physiological mechanisms, be they water conservation or water uptake, allow an animal to become eury-hygrous, even in a limited sense, the eccritic humidity range will become wider. It now becomes necessary to inhibit the effector centre, in this case the locomotory centre, over the preferred range, in order to counter the stimulus of the evaporimeter mediating the wet reaction. Since the inhibition is to be maintained over a humidity range, an evaporimeter would be unsuited to this purpose, but an hygrometer of appropriate mechanical structure would fulfil the requirements. This condition is represented in Figure 8.01 C. The wide eccritic range is ensured by the inhibition of the excitation from the evaporimeter by the newly acquired hygrometer, while the drier conditions are avoided by the high level of activity which follows stimulation of the evaporimeter in these conditions. Possibly this may reflect the condition in the isopod Porcellio scab~er (Gunn, 1937), although the conditions governing the dry response in this animal are not clear. Waloff (1941) has suggested that the sense organs responsible for the wet response shown by Porcellio below its eccritic range, are situated over the general body surface and these are probably evaporimeters.

A widening of the humidity range will be followed by a decrease in cuticle permeability and this will restrict the location of the evaporimeters. Since the region of maximal water loss would now be the respiratory surfaces, the evaporimeters would become confined to the tracheal system. We have seen that, even in forms where there is a very high eccritic humidity, an avoiding action to dry conditions may occur. This may well depend upon generally distributed evaporimeter-type receptors, as appears to be the case in Peripatopsis moseleyi. With the increasingly sheltered localisation of the evaporimeters, they become unsuitable for this task and we may postulate that the second hygrometer, as well as inhibiting the action of the evaporimeters, now acquires the necessary central connections to mediate an avoiding reaction. In this way it can become the dominant controller of the wet reaction.

Further elaboration of the water conserving and/or water uptake mechanisms could give rise to the condition where the wet response is completely suppressed when the animals are in a state of normal water balance. This is the condition in Ptinus tectus (Bentley, 1944), Tenebrio molitor (Gunn and Pielou, 1940 b) and Schizophyllum sabulosum. It may also give rise to complete indifference to humidity as in the case of Iulus terrestris (Perttunen, 1953) and C. longicaudata. With the increasing perfection of physiological adaptations to dry condtions, the preferred range gradually extended until it incorporated all humidities below the upper limit. This is not hard to conceive, particularly where there is a region of humidity indifference well below the lower limit of the eccritic range as in M. delanyi and in Potamon depressus (Dandy, 1955). The effective range of the wet response thus became increasingly constricted until the response was finally suppressed.

However, the need for a change in behaviour upon desiccation remained. It has already been suggested that in forms with an eccritic humidity just below saturation this is achieved by the action of enteroreceptors inhibiting the dry response at high humidities. This mechanisms must be retained but will not be sufficient. In forms such as Tenebrio, where the evaporimeters persist, their inhibition may be raised and this will lead the animal to water. Where the avaporimeters have been lost, it would appear that the responses to impulses from the hygrometers affecting a wet response are modified so as to change the threshold of the avoiding reaction to a far higher humidity. This, coupled with the inhibition of the first hygrometer, will ensure that the animal will now be restricted to an area of high humidity, where its activity will be low. Such would appear to be the condition encountered in M. delanyi following desiccation.

This speculative history of the evolution of humidity reactions is based upon a wide taxonomic assortment of animals and it seems likely that in any particular genus specialisations correlated both with form and habitat have arisen. This is most clearly seen in the burrowing forms like Peripatopsis and Agriotes larvae which have evolved a recoil type of shock reaction yet it seems reasonable to suppose that the conditions in Agriotes larvae are secondary. The history does, however, serve to underline the insufficiencies of our knowledge of the humidity responses within any one group of arthropods if our outlook towards these responses is to be more than to show that habitat and behaviour are correlated. There is clearly need for closer study of some group, such as the Isopoda, in which terrestrial adaptation is yet incomplete or limited, if we are to comprehend the physiological history of this aspect of terrestrial emergence in arthropods.

9. SUMMARY

(1) Ecological, physiological and behavioural studies have been carried out on Machiloides delanyi (Machiloidea) and Ctenolepisma longicaudata (Lepismatoidea) in an attempt to explain the restricted distribution of the former.

(2) An investigation of the physical conditions in the niche occupied by M. delanyi shows it to have a stable micro-climate where temperature and humidity fluctuations are small. The humidity never reaches saturation nor has it ever been found to fall below 70% relative humidity.

(3) The habitat of C. longicaudata, although sheltered from temperature extremes, shows appreciable fluctuations in humidity.

(4) M. delanyi loses water at a slower rate than species of the typical forest cryptofauna. Its rate of water loss is, however, very variable and is higher than that of C. longicaudata.

(5) The rate of water loss from both species increases markedly on death, suggesting an active water retaining mechanism.

(6) The stage within an instar affects the rate of water loss from M. delanyi, the animals losing water more rapidly shortly after moulting.

(7) Size has no demonstrable effect on the rate of water loss from either species.

(8) Removal of body scales and abrasion with aluminium dust or fine sandpaper has no effect on the rate of water loss from living specimens of both species.

(9) Wax solvents increase the rate of water loss from dead specimens of M. delanyi. They have no apparent effect on C. longicaudata.

(10) Carbon dioxide, in concentrations of up to 10%, has no effect on the rate of water loss from either species, suggesting the absence of a spiracular control mechanism. Carbon monoxide poisoning does, however, upset the water retaining ability of both species.

(11) There are some indications that the major site of water loss is the general body surface.

(12) Both species are able to drink water when presented in suitable form.

(13) M. delanyi uses the eversible vesicles located on the ventral surface of its abdomen to extract capillary water from the soil and to absorb water from a thin film of moisture covering stones. Excessive water loss upsets this ability by rendering the vesicles inextrusivle.

(14) C. longicaudata is able to absorb water vapour from a subsaturated atmosphere down to a relative humidity of 60%. This ability is limited by saturation deficit and possibly also by relative humidity.

(15) Retention of metabolic water by C. longicaudata is suggested.

(16) A possible mechanism for the absorption of water vapour from a subsaturated atmosphere, and its relationship to active water retention is discussed.

(17) Both species are eurythermous, the temperature preferenda being between 10° and 20°C for M. delanyi and between 12° and 25°C for C. longicaudata. Their upper critical temperatures are 37°C and between 40° and 43°C respectively.

(18) Aggregation by both species in their respective preferenda is effected by an activity orthokinesis, while a klinokinesis is instrumental in avoiding lethal temperatures. A speed orthokinesis is apparent in C. longicaudata at very high temperatures, under which conditions M. delanyi repeatedly leaps.

(19) Temperature receptors have been localised on the antennae and maxillary palps of both species. The thresholds of these are the same in M. delanyi but in C. longicaudata the antennal receptors have a lower threshold than the palpal.

(20) Both species show a preference for darkness. M. delanyi is directed to a dark niche by a skoto-telo-taxis while C. longicaudata relies on a skoto-tropo-taxis. In

addition, both species exhibit a negative phototaxis.

(21) While C. longicaudata is indifferent to humidity, M. delanyi has an eccritic range between 70 and 85% relative humidity. It always avoids humidities above the upper limit of its preferendum regardless of the alternative humidity offered.

(22) On desiccation, both species react positively to high humidities.

(23) The humidity receptors are located on the antennae and are of the hygrometer type in M. delanyi.

(24) A marked activity orthokinesis directs M. delanyi away from humidity conditions above its preferendum while a klinokinesis causes it to avoid low humidities.

(25) Upon desiccation, the klinokinesis avoiding low humidities becomes intensified in M. delanyi and a similar response is unmasked in C. longicaudata.

(26) Both species show a negative geotaxis and a possible dorsal gravity response.

(27) Temperature avoidance overrides all other responses in both species. The light response is normally dominant over humidity reactions in M. delanyi but on desiccation the positive reaction to high humidity takes precedence over the positive response to darkness. A similar condition obtains in desiccated C. longicaudata.

(28) The distribution of M. delanyi appears to be restricted only by the availability of water and it is suggested that this factor influences the distribution of the Machiloidea as a whole. The ability to absorb water vapour from a subsaturated atmosphere appears to be responsible for the success of the Lepismatoidea.

(29) The implications of these results in relation to the evolution of both the Thysanura and the Pterygota are discussed.

(30) The possible evolution of behavioural responses to temperature, light and humidity, and their respective sensory mediators, are considered.

10. BIBLIOGRAPHY

☆ denotes seen in abstract only.

Alexander, A.J. and Ewer, D.W. (1955). A note on the function of the eversible sacs of the onychophoran Opisthopatus cinctipes Purcell. Ann. Natal Mus. 13, 217-222.

Alexander, A.J. and Ewer, D.W. (1957). Temperature adaptive behaviour in the scorpion Opisthorphthalmus latimanus Koch. J. exp. Biol. 35, 349-359.

Allee, W.O., Emerson, A.E., Park, O., Park, T., and Schmidt, K.P. (1949). Principles of Animal Ecology. Saunders, Philadelphia.

Amouriq, J. (1955). Role de l'antenne du grillon (Gryllus bimaculatus L.) dans la thermopathie. Bull. Soc. Hist. nat. Afrique du Nord, 46, 100-105 ☆

Barnhart, C.S. (1961). The internal anatomy of the Silverfish Ctenolepisma campbelli and Lepisma saccharinum (Thysanura, Lepismatidae) Ann. ent. Soc. Amer. 54, 177-196.

Bauer, K. (1955). Sinnesökologische Untersuchungen an Lithobius forficatus Zool. Jber. 65, 267-300 ☆

Beament, J.W.L. (1945). The cuticular lipoids of insects. J. exp. Biol. 21, 115-131.

Beament, J.W.L. (1954). Water transport in insects. Symp. Soc. exp. Biol. 8, 94-117.

Beament, J.W.L. (1961). The water relations of insect cuticle. Biol. Rev. 36, 281-320.

Begg, M. and Hogben, L. (1946). Chemoreceptivity of Drosophila melanogaster. Proc. roy. Soc. (B), 133, 1-19.

Bentley, E.W. (1944). The biology and behaviour of Ptinus tectus Boie, a pest of stored products. V - Humidity reactions. J. exp. Biol. 20, 152-158.

Blower, G. (1951). A comparative study of the chilopod and diplopod cuticle. Quart. J. micr. Sci. 92, 141-162.

Browning, T.O. (1954). Water balance in the tick Ornithodorus moubata Murray, with particular reference to the influence of carbon dioxide on the uptake and loss of water. J. exp. Biol. 31, 331-340.

Buddenbrock, W. von (1952). Vergleichende Physiologie 1 : Sinnesphysiologie. Birkhauser, Basel.

Bursell, E. and Ewer, D.W. (1950). On the reactions to humidity of Peripatopsis moseleyi (Wood-Mason). J. exp. Biol. 26, 429-439.

/Burtt, E.T. .... p.199.

Burtt, E.T. and Catton, W.T. (1954). Visual perception of movement in the locust.
J. Physiol. 125, 566-580.

Buxton, P.A. (1930). Evaporation from the mealworm and atmospheric humidity.
Proc. roy. Soc. (B), 106, 560-577.

Buxton, P.A. (1931). The law governing the loss of water from an insect.
Proc. R. ent. Soc. Lond. 6, 27-31.

Buxton, P.A. (1932). Terrestrial insects and the humidity of the environment.
Biol. Rev. 7, 275-320.

Casselman, W.G.B. (1959). Histochemical Technique.
Methuen, London.

Cloudsley-Thompson, J.L. (1950). The water relations and cuticle of Paradesmus gracilis (Diplopoda, Strongylosomidae).
Quart, J. micr. Sci. 91, 453-469.

Cloudsley-Thompson, J.L. (1951). On the responses to environmental stimuli and sensory physiology of millipedes (Diplopoda).
Proc. zool. Soc. Lond. 121, 253-277.

Dandy, J.W.T. (1955). Some aspects of the water economy of fresh water crabs of the genus Potamon.
Unpublished - M. Sc. thesis, University of Natal.

Davies, M.E. and Edney, E.B. (1952). The evaporation of water from spiders.
J. exp. Biol. 29, 571-582.

Delany, M.J. (1954). The habitats of the bristletail Petrobius maritimus (Insecta, Thysanura) on Lundy.
Rep. Lundy Fld. Soc. 7, 36-44.

Delany, M.J. (1959). The life history and ecology of two species of Petrobius, Petrobius brevistylus and Petrobius maritimus.
Trans. roy. Soc. Edinb. 63, 501-533.

Denton, E.J. (1961). Buoyancy in cephalopods and fishes.
Progr. Biophys. 11, 179-236.

Dodds, S.E. (1952). Contributions to the study of the humidity reactions of Peripatopsis moseleyi (Wood-Mason) and of Tenebrio molitor L.
Unpublished - M. Sc. thesis, University of Natal.

Dodds, S.E. and Ewer, D.W. (1952). Effect of desiccation on the humidity response of Tenebrio.
Nature, Lond. 170, 578-579.

Edney, E.B. (1945). Laboratory studies on the bionomics of the rat fleas Xenopsylla brasiliensis Baker and X. cheopis Roths. I - Certain effects of light, temperature and humidity on the rate of development and on adult longevity.
Bull. ent. Res. 35, 399-416.

/Edney, E.B. .... p. 200.

Edney, E.B. (1947). Laboratory studies on the bionomics of the rat fleas *Xenopsylla brasiliensis* Baker and *X. cheopis* Roths. II - Water relations during the cocoon period. Bull. ent. Res. 38, 263-280.

Edney, E.B. (1951). The evaporation of water from woodlice and the millipede *Glomeris*. J. exp. Biol. 28, 91-115.

Edney, E.B. (1954). Woodlice and the land habitat. Biol. Rev. 29, 185-219.

Edney, E.B. (1956). A new interpretation of the relation between temperature and transpiration in arthropods. Proc. tenth Int.Congr. Ent. 2, 329-332.

Edney, E.B. (1957). The Water Relations of Terrestrial Arthropods. Cambridge. Cambridge.

Ewer, D.W. and Ewer, R.F. (1942). The biology and behaviour of *Ptinus tectus* Boie, a pest of stored products. III - The effect of temperature and humidity on oviposition, feeding and duration of life cycle. J. exp. Biol. 18, 290-305.

Ewing, H.E. (1942). The origin and classification of the Apterygota. Proc. ent. Soc. Wash. 44, 75-99.

Fraenkel, G. and Hopf, H.S. (1940). The physiological action of abnormally high temperatures on poikilothermic animals. I - Temperature adaptation and the degree of saturation of the phophatides. Biochem. J. 34, 1085-1092 *

Fraenkel, G.S. and Gunn, D.L. (1940). The Orientation of Animals. Oxford. Oxford.

Gebhardt, H. (1951). Lokalisatorischer Nachweis von Thermorezeptoren bei *Dorcus parallelepipedus* L. und *Pyrrhocoris apterus* L. Experientia, 7, 302-303.

Gebhardt, H. (1953). Die Lage der Wichtigsten Thermorezeptoren bei einiger Insekten. Zool. Jber. 63, 558-582 *

Gunn, D.L. (1937). The humidity reactions of the woodlouse *Porcellio scaber* (Latreille). J. exp. Biol. 14, 274-285.

Gunn, D.L. and Cosway, C.A. (1938). The humidity preference of the cockroach. J. exp. Biol. 15, 450-459.

Gunn, D.L. and Pielou, D.P. (1940). The humidity behaviour of the mealworm beetle *Tenebrio molitor* L. III - The mechanism of the reaction. J. exp. Biol. 17, 307-316.

Hale, L.J. (1958). Biological Laboratory Data. Methuen, London.

/Hopf, H.S. ....p. 3.

Hopf, H.S. (1940). The physiological action of abnormally high temperatures on poikilothermic animals. III - Some changes occurring in the phosphorus distribution of the haemolymph of insects under the influence of high temperatures. Biochem. J. 34, 1396-1403. *

Imms, A.D. (1936). The ancestry of insects. Trans. Soc. Brit. Ent. 3, 1-32.

Johnson, C.G. (1942). Insect survival in relation to water loss. Biol. Rev. 17, 151-177.

Kerkut, G.A. and Taylor, B.J.R. (1957). A temperature receptor in the tarsus of the cockroach *Periplaneta americana* J. exp. Biol. 34, 486-493.

Kerkut, G.A. and Taylor, B.J.R. (1958). The effect of temperature changes on the activity of poikilotherms. Behaviour, 13, 259-279.

Kühnelt, W. (1961). Soil Biology. Faber, London.

Lawrence, R.F. (1953). The Biology of the Cryptic Fauna of Forests. Balkema, Cape Town.

Lawrence, R.F. (1959). The sand-dune fauna of the Namib desert. S. Afr. J. Sci. 55, 233-239.

Lees, A.D. (1943). On the behaviour of wireworms of the genus *Agriotes* Esch. (Coleoptera, Elateridae). I - Reactions to humidity. J. exp. Biol. 20, 43-53.

Lees, A.D. (1946). The water balance in *Ixodes ricinus* L. and certain other species of ticks. Parasitology 37, 1-20.

Lees, A.D. (1947). Transpiration and the structure of the epicuticle in ticks. J. exp. Biol. 23, 379-410.

Lees, A.D. (1948). The sensory physiology of the sheep tick, *Ixodes ricinus* L. J. exp. Biol. 25, 145-207.

Lindsay, E. (1940). The biology of the Silverfish, *Ctenolepisma longicaudata*, with particular reference to its feeding habits. Proc. roy. Soc. Victoria 52, 35-83.

Lower, H.F. (1958). The integument of two Apterygota - *Ctenolepisma longicaudata* Esch. (Thysanura, Lepismatidae) and *Smynthurus viridis* Lubbock (Collembola, Smynthuridae). J. Morph. 103, 331-352.

Manton, S.M. (1958). The evolution of arthropodan locomotory patterns. Part 6 - Habits and evolution of the Lysiopetaloidea (Diplopoda), some principles of leg design in the Diplopoda and Chilopoda, and limb structure of the Diplopoda. J. Linn. Soc. (Zool.) 43, 487-556.

/Manton, S.M. ....p. 202.

Manton, S.M. and Ramsay, J.A. (1937). Studies on the Onychophora. III - The control of water loss in *Peripatopsis.* J. exp. Biol. *14*, 470-472.

Mead-Briggs, A.R. (1956). The effect of temperature upon the permeability to water of arthropod cuticles. J. exp. Biol. *33*, 737-749.

Mellanby, K. (1932). The effects of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor* L. Coleoptera.) Proc. roy. Soc. (B) *111*, 376-390.

Mellanby, K. (1932 a). Effects of temperature and humidity on the metabolism of the fasting bed-bug (*Cimex lectularius*) Parasitology *24*, 419-428.

Mellanby, K. (1934). The site of water loss from insects. Proc. roy. Soc. (B) *116*, 139-149.

Mellanby, K. (1935). The evaporation of water from insects. Biol. Rev. *10*, 317-333.

Nutman, S.R. (1941). Function of the ventral tube in *Onychiurus armata* (Collembola). Nature, Lond. *148*, 168-169.

O'Brien, F.E.M. (1948). The control of humidity by saturated salt solutions. J. sci. Instr. *25*, 73-76.

Oudemans, J.T. (1888). Beitrage zur Kenntnis der Thysanura und Collembola. Bijdr. Dierk. 1888, 147-226.

Paclt, J. (1956). Biologie der Primär flügellosen Insekten. Gustav Fischer, Jena.

Parry, D.A. (1953). On the drinking of soil capillary water by spiders. J. exp. Biol. *31*, 218-227.

Perttunen, V. (1953). Reactions of diplopods to the relative humidity of the air. Investigations on *Orthomorpha gracilis*, *Iulus terrestris* and *Schizophyllum sabulosum.* Ann. Zool. Soc. "Vanamo" *16*, 1-69.

Perttunen, V. (1955). The effect of antennectomy on the humidity reactions of normal and desiccated specimens of *Schizophyllum sabulosum* L. (Diplopoda, Iulidae) Ann. Ent. Fenn. *21*, 157-162.

Perttunen, V. and Lahermaa, M. (1958). Reversal of the negative phototaxis by desiccation in *Tenebrio molitor* L. (Col. Tenebrionidae) Ann. Ent. Fenn. *24*, 69-73.

Perttunen, V. and Syrjamaki, J. (1958). The effect of antennectomy on the humidity reaction of *Drosophila melanogaster* (Dipt. Drosophilidae). Ann. Ent. Fenn. *24*, 78-83.

Pielou, D.P. (1940). The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. II - The humidity receptors. J. exp. Biol. *17*, 295-306.

Pielou, D.P. and Gunn, D.L. (1940). The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. I - The reaction to differences of humidity.
J. exp. Biol. 17, 286-291.

Ramsay, J.A. (1935 a). Methods of measuring the evaporation of water from animals.
J. exp. Biol. 12, 355-372.

Ramsay, J.A. (1935 b). The evaporation of water from the cockroach.
J. exp. Biol. 12, 373-383.

Richards, A.G. (1951). The Integument of Arthropods.
University of Minnesota Press, Minneapolis.

Roeder, K.D. (editor), (1956). Insect Physiology.
Wiley, New York.

Roth, L.M. and Willis, E.R. (1952). Possible hygroreceptors in *Aedes aegypti* and *Blatella germanica*.
J. Morph. 91, 1-14 *

Snodgrass, R.E. (1938). The evolution of the Annelida Onychophora and Arthropods.
Smithson. Misc. coll. 97, (6) 1-159.

Solomon, M.E. (1945). The use of cobalt salts as indicators of humidity.
Ann. appl. Biol. 32, 75-85.

Solomon, M.E. (1951). Control of humidity with potassium hydroxide, sulphuric acid and other solutions.
Bull. ent. Res. 42, 543-554.

Solomon, M.E. (1957). Estimation of humidity with cobalt thiocyanate papers and permanent standards.
Bull. ent. Res. 48, 489-506.

Spencer, J.O. and Edney, E.B. (1954). The absorption of water by woodlice.
J. exp. Biol. 31, 491-496.

Swan, L.W. (1961). The ecology of the high Himalayas.
Sci. Amer. 205, 68-78.

Sweetman, H.L. (1953). The number of instars among the Thysanura as influenced by the environment.
Trans. ninth Int. Congr. Ent. 1, 411-415.

Thomson, R.C.M. (1938). The reactions of mosquitoes to temperature and humidity.
Bull. ent. Res. 29, 125-139.

Tiegs, O.W. (1947). The development and affinities of the Pauropoda based on a study of *Pauropus silvaticus*
Quart. J. micr. Sci. 88, 275-336.

Tiegs, O.W. and Manton, S.M. (1958). The evolution of the Arthropoda.
Biol. Rev. 33, 255-337.

Waloff, N. (1941). The mechanisms of humidity reactions of terrestrial isopods.
J. exp. Biol. 18, 115-135.

/Weather Bureau, ...p.204.

Weather Bureau, Union of South Africa (1957). Climate of South Africa. Government Printer, Pretoria.

Wigglesworth, V.B. (1935). Regulation of respiration in the flea *Xenopsylla cheopis* Roths. Proc. roy. Soc. (B) *118*, 397-419.

Wigglesworth, V.B. (1941). The sensory physiology of the human louse, *Pediculus humanus corporis* de Geur. Parasitology, *33*, 67-109.

Wigglesworth, V.B. (1945). Transpiration through the cuticle of insects. J. exp. Biol. *21*, 97-114.

Wigglesworth, V.B. (1950). A new method for injection of the tracheae and tracheoles of insects. Quart. J. micr. Sci. *91*, 217-224.

Wigglesworth, V.B. (1953). The Principles of Insect Physiology (5th edition). Methuen, London.

Wigglesworth, V.B. and Gillett, J.D. (1936). The loss of water during ecdysis in *Rhodnius prolixus* Stal. Proc. R. ent. Soc. Lond. (A) *11*, 104-107.

Williamson, D.I. (1951). Studies on the biology of Talitridae (Crustacea, Amphipoda): Effects of atmospheric humidity. J. mar. biol. Ass. U.K. *30*, 73-90.

Willis, E.R. and Roth, L.M. (1950). The humidity reactions of *Tribolium castaneum* (Herbst). J. exp. Zool. *115*, 561-857. *

Winston, P.W. and Bates, D.H. (1960). Saturated solutions for the control of humidity in biological research. Ecology *41*, 232- 237.

Wygodzinsky, P. (1941). Beitrage zur Kenntnis der Dipluren und Thysanuren der Schweiz. Denkschr. Schweiz. Naturf. Ges. *74*, 112-227.

Wygodzinsky, P. (1955). Thysanura. South African Animal Life *2*, 83-190.

Wygodzinsky, P. (1960). On a new species of *Machiloides* Silvestri from Grahamstown, South Africa. Ent. mon. Mag. *96*, 219-220.