Behavior of *Epiperipatus biolleyi* (Onychophora: Peripatidae) under laboratory conditions

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Abstract: The behavior of *Epiperipatus biolleyi* Bouvier was studied in the laboratory. In choice tests, bryophyte vegetation and its associated soil were preferred to grass and its soil. In 87 hr the animals changed artificial burrows 2.89 times. They enter burrows mostly by walking forward and show a tendency to rest facing the entrance. No agressive competition for burrows was observed. Pairs rest with some body contact about half the time. Seven resting body postures were identified. They hide from direct sunlight in 189 s (mean) when placed over moss and appear to avoid light around 470-600 nm. Walking speed was near 1 cm/s. They float and become tergid in freshwater but drown in sea water. Their adhesive secretion has a bitter taste and dissolves in less than 3 s in sea water but remains adhesive under freshwater for at least 20 hr. In nature, animals bear scars and mutilated oncopods. Death is often preceded by a retraction of the antennae and expulsion of saliva, adhesive substance, faeces and sometimes embryos. Ecdysis occurs aproximately every 15 days. At least one bird *(Turdus grayii)* and one snake *(Micrurus hemprichii)* are known to prey on other onychophoran species in the wild.

Key words: Onychophora, velvet worm, peripatus, ethology, predation.

New findings about Cambrian invertebrates (Hou *et al.* 1991) have revived interest in their presumed living descendants, the Onychophora. Unfortunately, almost all knowledge on the behavior of living onychophorans is based on anecdotal observations (see Read 1985 and Ruhberg 1985 for reviews). The only quantitative experimental data are from a study of the feeding behavior in *Macroperipatus torquatus* from Trinidad (Read and Hughes 1987).

This paper presents observations on the behavior of *Epiperipatus biolleyi* Bouvier, 1902, under laboratory conditions in Costa Rica. The topics chosen cover aspects of the little known behavioral ecology of onychophorans: selection of vegetation, soil types and humidity levels; burrowing behavior; interactions among individuals and body posture.

MATERIAL AND METHODS

Twenty specimens of *E. biolleyi* were collected from the Las Nubes-Cascajal area, Coronado, San José, Costa Rica (83° 57'37" W, 10° 00' 18" N, 1750-1800 m elevation) in September and October of 1992. The habitat is classified as Low Montane Very Moist Forest (Holdridge 1967) but is currently cattle grassland with patches of secondary (basically riparian) forest. The animals were transported in closed styrofoam containers within 1-3 hr to a laboratory at the Universidad de Costa Rica. Soil and living vegetation used for all tests were collected in their natural microhabitat and transported in closed plastic bags. Experiments were done in darkness under laboratory conditions (natural light cycle, around 18-23° C). The onychophorans were adults (nine females, 11 males) weighing
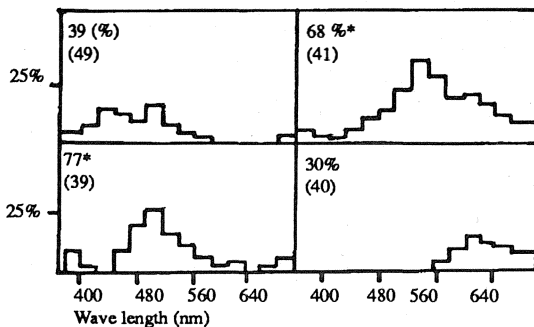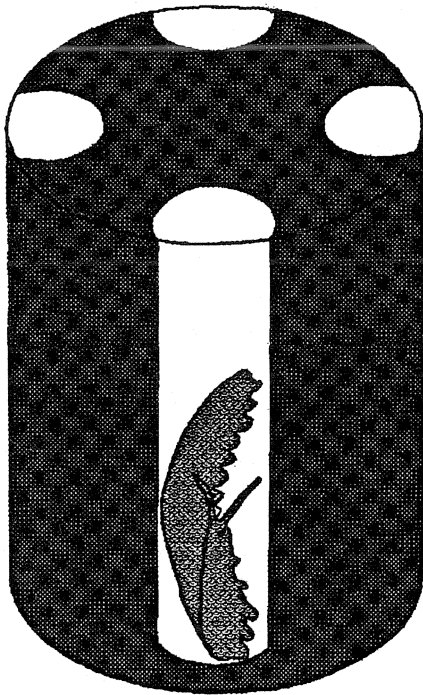
Fig. 1. Experimental burrows design (not to scale) and % of cases in which the animals moved to darkness when exposed to light of the illustrated wavelength composition. In parenthesis: number of replications. Cases with asterisk were significant (Binomial, p<0.05).

x=0.288 g (SD 0.207, min. 0.0582, max. 0.731) at the time of arrival to the laboratory. To prevent conditioning, choice tests were not repeated more than three times with each individual. The possible effects of sex and size were not analysed. Voucher specimens will be deposited in the Museo de Zoología, Universidad de Costa Rica (UCR) and American Museum of Natural History, New York.

Choice tests: Lidded petri dishes were used as terraria and kept saturated with moisture.

The largest animals measured 5 cm in length and had enough space to select between the halves of the 50 cm² petri dishes (8 cm diameter, 2 cm depth). For the vegetation test, the bottom was covered with a 0.4 cm layer of loose soil and above it, one half of the dish was covered with bryophytes (chiefly *Marchantia polymorpha*) and the other with complete grass blades. For the soil selection tests, vegetation was absent and each half of the dish had, respectively, soil collected from under bryophites and from under grass. To test reaction to humidity, petri dishes with four compartments were used. The soil was previously dried for 96 hours at 80-90° C and seived with a 0.96 mm mesh to eliminate large particles. Each compartment was provided with loose soil in one of the following treatments: 0, 33, 55 and 78 % humidity. The tests ended when evaporation began to obliterate moisture differences.

Experiments with burrows: Specimens were placed on loose commercial garden soil (treatment) or moss from their natural habitat (control) in glass jars (10 cm high, 5 cm diameter). These were exposed to sunlight for 5 min to elicit the burrowing response. Similar jar terraria were filled with compacted commercial garden soil and four burrows (approximately 5 cm deep and 7 mm diameter) were made at the perimeter (burrow interior was visible through the glass, Fig.1). One animal was placed in each terrarium and the occupation of the individually marked burrows recorded every 12 hr during daytime. For this test, the terraria were exposed to the natural daylight cycle but light was blocked from burrows with aluminum foil. This design was also modified by placing two individuals in single-burrow terraria. The location and any interactions of the animals were recorded every 2 hr.

Light avoidance: Observations on the reaction to light were done by placing the animals in the middle of lidded petri dishes with one half covered with light-proof tape and recording after two minutes if they had moved to the lighted (left side) or the dark (right) half. Cases in which the animal did not move were excluded. The light source was a standard 25 w Sylvania cool white F40T12 daylight lamp placed 18 cm above the petri dish and covered

with colored translucent plastic whose transmittance curves apperar in Fig. 1. Transmittance was measured with a light scanning spectrometer (30 w Deuterium light source) and corrected according to lamp spectral energy data provided by the manufacturer. Totally darkened petris were used as controls.

Other observations: A sketch of the body posture of each individual was made while recording results of the other experiments. The lengh of intermoult periods was estimated by noting the time to disappearance of a drop of white water-soluble typist corrector on the back of some of the animals. Marking did not noticeably affect behaviour when compared with unmarked specimens. Locomotory speed was measured for individuals moving away from the

light and heat of a 60 w incandescent light bulb (initially located at a horizontal distance of 30 cm) over standard white bond paper. The distance travelled was marked every 5 s.

To test reactions to fresh and sea water, specimens were floated in petri dishes containing tap water and sea water (salinity 31 parts per thousand, around 21 °C). Some specimens were forced to expel adhesive under water by gentle squeezing between fingers. The same procedure was used to squirt adhesive on the tongues for taste assessment. Faeces were observed in fresh samples smeared on a microscope slide, without staining. All statistical values are in the following format: mean ± standard deviation, minimum, maximum, N= number of cases.

RESULTS AND DISCUSSION

Choice tests: In most cases, the bryophyte-containing half of the terrarium was preferred to the grass section (Fig.2, Binomial test, p<0.05). At the conclusion of the experiments, humidity was measured by drying to constant weight. It was higher in the bryophyte half (84.2±3.4, 76.5-87.9 %, N=10 for bryophite part; 71.1±4.4, 66.8-80.0 %, N=10 for grass part, Mann-Whitney U, p<0.001).

Bryophyte soil was preferred over grass soil, although in a few cases the animal stayed at their junction (Fig.2, Chi-square, p<0.01). The bryophyte soil used in this test had a higher water content (24.9±2.3, 21.6-28.9 %, N=9, against 19.4±1.9, 17.2-23.7 %, N=9, of grass soil, Mann-Whitney U, p<0.001). Nevertheless, the experiment described below suggests that humidity was not the reason for these results. Perhaps they preferred options with the smell of bryophytes.

The number of onychophorans selecting each humidity level were: dry 5, slightly moist 1, moist 2 and wet 7 (N=15). This experiment was discontinued because the animals which moved to the dry section died of dehydration. This result was unexpected and sheds doubts on the animal's capacity to estimate moisture in the dark.

In the field, *E. biolleyi* is usually found at the moss-substrate interface and in burrows in the soil (in prep.). Under experimental conditions, the animals stayed chiefly within the
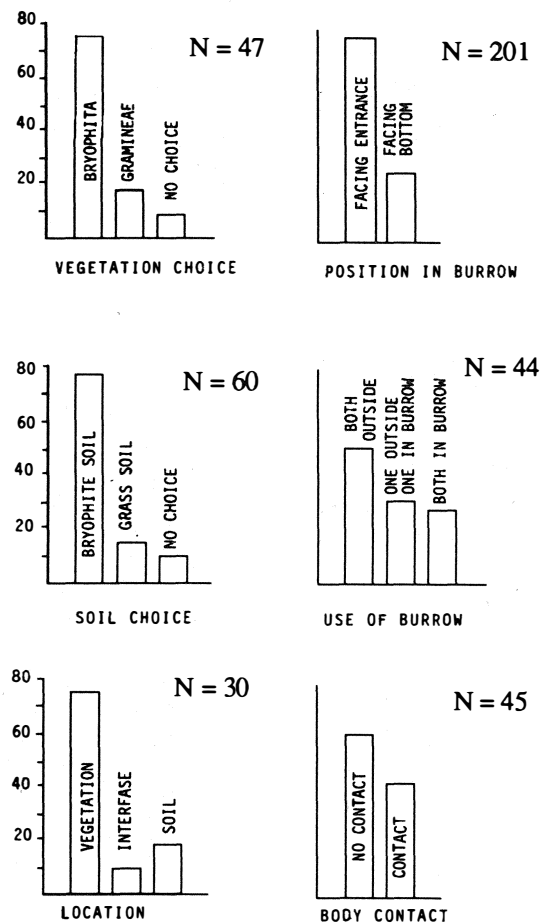

Fig. 2. Responses of *Epiperipatus biolleyi (%)* in choice experiments.

vegetation and rarely at the interface or within the loose soil (Fig.2).

Experiments with burrows: It is generally stated that onychophorans cannot make burrows (see Read 1985, Ruhberg 1985), but to our knowledge this had not been tested before. In moss, individuals escaped from the light by entering it; in soil none did (N=10). The hiding time, measured from begining of test to when the animal reached full cover under the moss, was: 189±75s, range 60-300 s, N=9 (one did not hide).

The animals on soil searched actively on the ground, by inserting antennae and head among soil particles, apparently looking for crevices or burrows, but failed to construct their own. In the field their feeding and mating grounds may be limited to areas offering appropriate burrows. Read (1985) reached the same conclusion for *M. torquatus*.

No onychophoran showed fidelity to any of the four burrows available per individual. Throughout the study (87 hr), they switched burrows a mean of 2.89 times±1.33, 1-5. Ruhberg (1985) applied Lawrence's generalization that cryptozoa show site fidelity to microhabitats. Under experimental conditions, the generalization does not apply to the burrow itself. If the experiment reflects natural conditions, it means that *E. biolleyi* shows an opportunistic behavior, entering any burrow or similar space found nearby when "resting" time approaches. *M. torquatus* can use several burrows but tends to prefer one (Read 1985).

The animals mostly walked forward into the burrow (N=31) but could also enter backwards if their rear oncopods happened to pass the entrance while they were moving on the surface (N=2). In the first case, they often continued walking 1-2 cm after reaching the end of the burrow: as a result, the head tended to face the entrance (Figs. 1 and 2, p<0.05, Binomial Test), which may allow a rapid reaction to enemies entering the burrow and to changes in humidity, temperature or light. A similar behavior in *M. torquatus* is believed to increase the probability of detecting prey and enemies (Read 1985).

When two individuals were put in a terrarium with only one burrow, they randomly stayed: (1) both outside the burrow, (2) only one in the burrow and (3) both in the burrow (Fig.2, Chi-square test, p>0.05). No agressive competition for the only available burrow was observed. Nevertheless, two *E. biolleyi* placed in pairs in small terraria (5 cm high, 3 cm diameter) for transportation from the field to the laboratory (a few hours) died of injuries apparently produced by their companion, possibly because of the capture stress. *M. torquatus* may share burrows independently of size and sex, and rarely bite each other for burrows or food (Read 1985).

Pairs of individuals sharing a terrarium were seen resting with some form of body contact among themselves almost as often as not (Fig.2, Binomial Test p>0.05). Thigmotaxis is widespread in the phylum (review in Ruhberg 1985). By increasing body contact, they may reduce desiccation and thermal stress. Future research could test these hypotheses by measuring body contact under different humidity and temperature conditions.

Reactions to light: The animals moved more frequently to the dark part of the petri dish when exposed to light 470-600 nm in wave length. In the two sets of experiments with the higher wave lengths the controls tended to select the right half of the petri dish (Binomial test, p<0.01). That half was closer to the lamp base and they might have responded to vibrations of the lamp transformer.

We are aware of no previous information about reaction to light in the phylum other than the traditional use of red light to avoid much disturbance during observation (review in Ruhberg 1985). These data are presented as a baseline for future research because (1) the observation that the animals do not avoid some light frequencies does not mean that they are blind to those frequencies and (2) a more proper test requires use of special light sources and measurements in the nerve cells (both are beyond our possibilities).

Seven resting body postures were noted (Fig.3), here called *Line, U, S, J(head), J(tail), Roll* and *Ring*. A slight tendency to rest more often in *S* while on the surface was found (Fig.3, Kendall Tau significant but just below p<0.05).

Onychophoran body posture has not been studied apart from reports about coiling or shortening in response to desiccation or to

LINE   U   S   J - TAIL

29/37   21/25   23/13   8/10

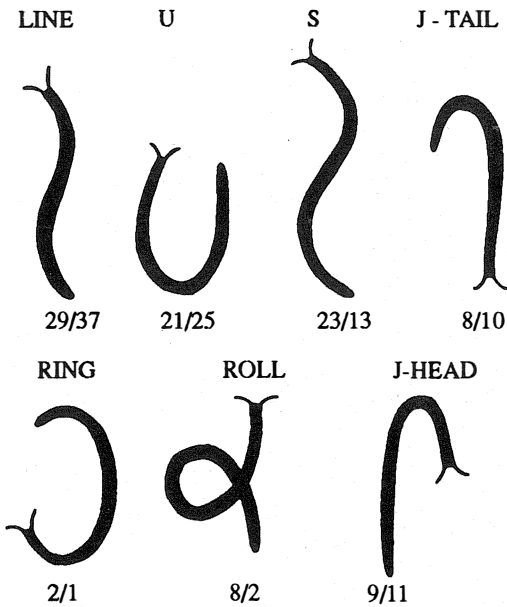RING   ROLL   J-HEAD

2/1   8/2   9/11

Fig. 3. Body posture repertoire of *E. biolleyi*. Numbers: frequency of posture displayed on surface (left) and in burrow.

manipulation (review in Ruhberg 1985). Here, the number of identified postures is increased from three (see Ruhberg 1985) to seven. One important difference is that these seven postures are assumed normally, without disturbing the animals. They may reflect a variety of factors, such as substrate and vegetation contour and mechanical body limitations. Other observations: Walking speed was measured in two individuals. One weighing 0.0597 g moved at 1.1 ± 0.3 (0.7-1.5) cm/s, N=19 measurements. The other weighed 0.0423 g and moved at 1.3±0.4(0.6-2.0) cm/s, N=17. These values are about 18 times higher than those reached by undisturbed *M. torquatus* in nature, but within the range for onychophorans in laboratory (review in Read 1985).

On freshwater, the animals floated and became slightly turgid after 25 min (test duration; N=5). They remained in good health after the test, unlike those floated on sea water (N=4), which died 14-18 min after the onset of the experiment (it was therefore suspended). One individual repeatedly tried to penetrate the sea water, inserting its head to a depth of 1 cm. After 1 min, their skin began to get moist. This suggests that onychophorans could survive contact with water while being transported (*e.g.* during floods) over freshwater, but that dry parts are required in natural rafts during dispersal across sea.

E. biolleyi produces an adhesive lacking smell or color and which does not seem to be poisonous. The animal uses the mouth to remove any adhesive sticking on its own skin. The fresh adhesive tastes bitter (N=9, four tasters), but loses its taste as it hardens. Perhaps the original function of the secresion was defense (as emetic and adhesive in the mouth of predators) and secondarily it was used to capture prey. Read (1985) reported a case of adhesive successfully used in this fashion against a snake.

In the laboratory, the adhesive substance is expelled as a defense when the animal is manipulated or finds itself in a hostile environment (one expelled adhesive when exposed to a temperature of about 5 °C, another when floated on sea water, and all when anesthetized). In air, the adhesive dries in less than 3 s, while in sea water it becomes a mucilage and finally dissolves (N=5). A spherule of adhesive 0.5 mm in diameter dissolves completely in 1 s; one with a diameter of 2 mm in 2 min. Under freshwater, the adhesive (N=4) sticks to the fingers, and one sample remained operative for 20 hr but hardened normally as soon as exposed to air. These observations suggest a chemical hardening mechanism, as supposed by other authors (Manton and Heatley 1937, Röper 1977) rather than the mechanical principle hypothetized by Read (1985).

When specimens are kept in terraria with accompanying fauna suspected to be their predators (*Bolitoglosa subpalmata* salamanders 15 cm in total length, Opiliones 2.5 cm leg length) or prey (*e.g.* Isopoda 1 cm length), there is no evidence of predation, with the exception of an opilion that was eaten by an onychophoran. Read and Hughes (1987) published the first non-anecdotal list of prey consumed by onychophorans in the field, based on *M. torquatus*. The list includes crickets, cockroaches, earwigs, centipeds, millipeds, spiders and woodlice. All are commonly associated with onychophorans (Ruhberg 1985) and are found in the natural microhabitat of *E. biolleyi* (Monge-Nájera, in prep.).

Fig. 4. In nature, *E. biolleyi* individuals sometimes show healed injuries marked by pink scars (photograph: H. Ruhberg).

Isopods, which have been found eating the corpses of *E. biolleyi*, even crawl on living individuals without being attacked. This confirms a doubious previous report for another species (see Ruhberg 1985).

Some *E. biolleyi*, examined shortly after collecting, show healed injuries (Fig.4) and even missing portions of legs (Monge-Nájera, pers. obs.). Similar observations were reported by Lavallard *et al.* (1975) for Brazilian onychophorans. Reliable information about their predators in the field was not found by Ruhberg in her review (Ruhberg 1985). Read (1985) mentioned a chilopod and a spider. Here we bring attention to several overlooked reports of field predation on onychophorans. Dyrcz (1982) observed the bird *Turdus grayi* feeding onychophorans to their nestlings in Panama. The Neotropical ground snake *Micrurus hemprichii* almost exclusively feeds on onychophorans in nature (Greene 1973, Cunha *et al.* 1978, Roze 1982). While collecting in the habitat of this snake in Loreto (Peru: Tahuayo river) and Tigüino (Pastaza, Ecuador), W.

Lamar (1993 pers. comm.) found inactive onychophorans on the upper surface of leaves (about 0.5 m above ground level), with a typical posture: body elongated and antennae extended together to the front. Once he saw a pair on a single leaf (they were greyish sprinkled with brown). Perhaps these onychophorans escape ground snake predation by resting above in the foliage (Lamar 1993 pers. comm.).

No clear evidence exists for diurnal activity in onychophorans (Read 1985). V. van der Lande has confirmed (1992 pers. comm.) that there are no field observations of daytime activity in *Peripatoides gilesii*. Brink (1957) mentions onychophorans wandering during twilight after late rains. One *E. biolleyi* was seen (E. Domínguez, pers. comm. 1992) wandering in plain daylight (6 am) on the surface of moist soil in the yard of a semirural home in San Pedro de Coronado, San José, Costa Rica (83° 59' 30" W, 09° 50' 28" N, 1500 m elevation, formerly Low Montane Very Moist Forest). Daytime activity is probably

atypical and in any case relates to periods of high atmospheric humidity. Similarly, the introduced snail *Helix aspersa*, which is normally nocturnal, becomes active early or late in the day, following heavy rains in Costa Rica.

One female kept in a 17 X 19 cm terrarium (with natural soil, vegetation and fauna) in San Pedro de Coronado, a few km from the site where it was collected, lived 150 days. Those kept in our laboratory lived 11-37 days, slightly less than a troglophilic *Peripatoides indigo* transported by Ruhberg (1985) from New Zealand to Germany. The short captive longevity may reflect the stress caused by the experiments and *E. biolleyi* seems to be a fragile species.

A few hours before death, these animals show a characteristic retraction of the antennae, which become flaccid and curved downward (sometimes crossed in *X*). Distress is often marked by elimination of some body contents (saliva, adhesive and faeces) and may cause females to deliver premature embryos (Ruhberg 1993, pers. comm.). Fresh corpses assume the shape given experimentally. The deep red skin pigment fades rapidly and a few hours after death, the corpse turns light pink.

Like other onychophorans, this species avoids air currents (we tested by blowing at them), a reaction interpreted as defense against desiccation (see Ruhberg 1985).

Faeces extracted from the intestine of two females collected two days earlier had abundant corpuscles that may be excretory granules (see Manton and Heatley 1937) or coccus type bacteria. It is interesting that after 48 hours without feeding, both animals had filled digestive tracts. In contrast, other species eliminate food remains in about 18 hr (Manton and Heatly 1937, Ruhberg 1985).

Moulting time under laboratory conditions was 14.71 ± 2.69 (12-19) days (N=7), very similar to that of other species (Manton 1938, Read 1985).

ACKNOWLEDGEMENTS

RESUMEN

El comportamiento básico de *Epiperipatus biolleyi* Bouvier, 1902 se estudió mediante experimentos de laboratorio usando 20 individuos. En pruebas de escogencia, prefirieron briófitos y su suelo, a gramíneas y su suelo. Se ocultaron principalmente entre la vegetación y raramente en la interfase o en el suelo. No parecen ser capaces de excavar sus propias madrigueras. Entran en ellas principalmente caminando hacia adelante y muestran tendencia a descansar enfrentando la entrada, tal vez para responder con rapidez ante enemigos, presas y cambios climáticos. En 87 hr cambiaron de madriguera 2.89 veces. No se observó agresividad intraespecífica cuando había solo una madriguera. Cuando están en pares, la mitad de las veces descansan con algún tipo de contacto corporal. Se identificaron e ilustran siete posturas corporales básicas. Si se les coloca sobre musgo y se les expone a la luz solar, se ocultan en una media de 189 s. La velocidad de locomoción mostró medias individuales de 1.1-1.3 cm/s. Flotan y se vuelven turgentes en agua dulce, pero perecen en agua de mar. Su sustancia adhesiva es amarga, se disuelve en menos de 3 s en agua marina y permanece líquida en agua dulce, lo cual apoya la hipótesis del secado químico y no la del secado mecánico. En la naturaleza, los animales pueden tener cicatrices y oncopodios mutilados. Un ave (*Turdus grayii*) y una serpiente (*Micrurus hemprichii*) son depredadores de otras especies de onicóforos en el campo. En cautiverio y con alimentación constante, *E. biolleyi* sobrevivió hasta 150 días. La agonía incluye retracción de las antenas y eliminación

de saliva, sustancia adhesiva, heces y oca-
sionalmente embriones prematuros. Las heces
tienen gran cantidad de esférulas que podrían
ser desechos metabólicos o bacterias tipo coco.
Estos animales evitan las corrientes de aire y
mudan cada 15 días, aproximadamente.

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