Ionic identity of pore water influences pH preference in Collembola

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Title: IONIC IDENTITY OF PORE WATER INFLUENCES PH PREFERENCE IN COLLEMBOLA

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Abstract

A test system described by Van Straalen and Verhoef (1997) was used in order to check whether the endogeic Collembolan *Heteromurus nitidus* was repelled by acid pH. In each of eight experimental runs sixteen naive animals were allowed to select sectors in a circular pH gradient made of pure quartz sand impregnated with McIlvaine's buffer solutions at constant osmolarity. Disodium or dipotassium hydrogen phosphate was mixed with citric acid in varying proportions, giving rise to acidity levels ranging from pH 2 to pH 9. The animals reacted quite differently according to whether Na or K was used as the metallic cation. With potassium, a strong variation was observed from one experimental box to another, *H. nitidus* aggregating at pH levels varying from 4 to 8, the most frequent aggregation being observed at pH 6. With sodium, aggregation occurred over a more restricted range, from pH 7 to pH 9, most frequent aggregation being at pH 8. It was concluded that the most acidic pH range (2-3) was avoided by the animals and that the chemical composition of buffer solutions strongly influenced results of pH-preference tests. If we consider that i) the absence of *H. nitidus* from acid soils (pH 5 to 3.5) can be explained by biological rather than by chemical effects, ii) this species can be cultured in acid as well as alkaline soils in the absence of predators, it ensures that potassium gives results that are more representative of the distribution of *H. nitidus* according to soil acidity than sodium.

Keywords : Aggregation, Arthropod, Base metal, pH, Preference test.

1. Introduction

The preference for acid, neutral or alkaline pHs by soil Collembola might explain the distribution of species over gradients of soil acidity (Hågvar and Abrahamsen, 1984; Chagnon et al., 2000; Loranger et al., 2001) and changes in the species composition of communities when subjected to anthropogenic acid or alkaline deposition (Hågvar and Kjøndal, 1981; Heungens and Van Daele, 1984; Kopeszki, 1992). It has been demonstrated that some Collembola, when allowed to chose between different chemical solutions, exhibited strong preferences according to pH, osmolarity and electrical conductance, and it was suggested that these preferences were indicative of ecological requirements of species or groups of species (Mertens, 1975; Hutson, 1978; Jaeger and Eisenbeis, 1984). This encouraged the development of a standardized bioindicator system for soil acidity (Van Straalen et al., 1987; Soejono Sastrodihardjo and Van Straalen, 1993; Van Straalen and Verhoef, 1997), presenting the opportunity to test a wide range of animal groups in the same, controlled, reproducible, experimental conditions. Nevertheless Hågvar (1990) demonstrated that the issue of culture experiments at different pH levels was strongly influenced by the fact that species were cultured alone or as part of a community. Recent studies have shown that the absence of the endogeic entombryid *Heteromurus nitidus* (Templeton 1835) from soils at pH less than 5 could be due to biological effects rather than to chemical sensitivity (Salmon and Ponge, 1999; Salmon, 2001; Salmon and Ponge, 2001). Thus it was judged interesting to study the behaviour of this animal when placed in the standardized system devised by Van Straalen and Verhoef (1997). Given that the chemical composition of buffer solutions was not definitively fixed by these authors, potassium or sodium being considered as playing the same role in pH preference, we decided to verify whether base metals of similar ionic strength but of different nature could be actually interchanged in standardized tests.

2. Material and methods

Circular preference boxes offering a double gradient of pH (Van Straalen and Verhoef, 1997) were used in order to test a wide range of acidity spanning from pH 2 to pH 9, thus embracing a much broader range than the natural range of *H. nitidus* (Ponge, 1983; Ponge, 1993; Salmon and Ponge, 1999). A detailed description of the boxes is given in Van Straalen and Verhoef (1997). The bottom of each box was made of 16 compartments filled flush with pure quartz sand impregnated with a buffer solution, without any possible diffusion from one compartment to the other. Animals were allowed to move freely over the sandy bottom but compaction of the sand prevented them from burrowing in the substrate. The different pH values were regularly ordered in a continuously increasing then decreasing order such that two adjoining compartments never differed by more than one pH unit, and all pHs were presented twice.

McIlvaine buffer solutions were prepared by mixing citric acid ($C_6H_8O_7,H_20$) with di-sodium or di-potassium hydrogen phosphate (Na_2HPO_4 or K_2HPO_4, $2H_20$) in varying proportions. Ratios were calculated according to Elving (1963) in order to achieve the desired pH values. Correction for osmolarity was made by diluting the concentrated solutions to a ca. 135 mM concentration. Osmolarity (Wescor osmometer 5100B) and pH (Orion 701A) were checked before each experimental run.

Eight boxes (replicates) were simultaneously used for each of two experimental runs, one with potassium, the other with sodium as the metallic cation. Sixteen adult or subadult *H. nitidus* were introduced into each box, one onto each compartment. Animals were experimentally naive, having never been in contact with buffer solutions before the experiment. All were derived from a batch culture started six weeks before with individuals extracted from a calcic mull at pH 7.5 (black rendzina under hornbeam) described by Bouché (1975). After introducing the animals, boxes were placed in a climate chamber at constant temperature and relative humidity (20°C, 50% R.H.), under a 14h:10h night:day photoperiod. Counting was carried out after 19h under fluorescent light, care being taken that no gradient of light could force the animals to move towards darker parts of experimental boxes (Salmon and Ponge, 1998).

Data for each experimental run were numbers of animals found at each pH value at the end of the experiment. Comparisons were done between pHs using a two-way ANOVA without replication with boxes as a trivial factor (used for the calculation of the error term) and pHs as the treatment factor (Sokal and Rohlf, 1995). A posteriori comparisons between means were achieved by the SNK procedure (Glantz, 1997). Homogeneity of boxes was checked by chi-square test after computing a G-statistic (Sokal and Rohlf, 1995). Standard deviation of animal numbers found at the different pH values was calculated for each experimental box and compared to that of mean values (after pooling the eight boxes) by help of a t-statistic (Sokal and Rohlf, 1995), in order to check for aggregational patterns.

3. Results

3.1. Potassium experiment

The distribution of pooled numbers of animals at the different pH values exhibited a bell-shaped curve, with a mode at pH 6 (Fig. 1). Variation among pHs was highly significant (F = 4.2, P = 0.001). Significantly more animals were found at pH 6 than at pH ≤ 4 or at pH 9 but, despite a preference for pH 6, there was a wide array of pH values (5-8) which did not differ significantly from the average preferred pH.

Heterogeneity among boxes was highly significant (G = 194, P = 3.10^{-19}). Examination of individual boxes showed that in each box animals preferred a given pH where they aggregated, but that this pH value differed from one box to another, varying from pH 4 (one box) to pH 8 (one box). The degree of aggregation of *H. nitidus* in individual boxes can be estimated by computing in each box the between-pH standard deviation and comparing the mean value of this statistic (3.6) to the standard deviation of mean values, calculated by averaging the 8 boxes (1.7). The mean standard deviation of individual boxes was significantly higher than the standard deviation of a mean box (t = 6.4, P = 0.0004).

3.2. Sodium experiment

The mean distribution of animal counts was much more restricted than with potassium (Fig. 2), tightly centring around pH 8. The number of animals found at pH 8 was significantly higher than at all other pH values. Variation among pHs was highly significant (F = 11.6, P < 0.00001).

Heterogeneity among boxes was highly significant (G = 114, P = 4.10^{-7}). Aggregation occurred at pH 8 in six boxes out of eight, the remaining two boxes showing aggregation at pH 7 and pH 9, respectively. The mean standard deviation of compartments within individual boxes (4.1) was slightly but significantly higher than the standard deviation of compartments within a mean box (3.2, t = 2.8, P = 0.03), and did not differ from the value observed with potassium (3.6, t = 0.95, P = 0.18).

4. Discussion

In each experimental box, aggregation was found to occur at a given pH value, which may nevertheless differ from one box to another, except at extreme acidic pH values. As a result, count numbers exhibited a high degree of variation between compartments (pHs). Their standard deviation can be used as an aggregation index, since the mean number of animals per compartment (= 1) was constant. This index did not differ between the two experiments, indicating that the choice of sodium or potassium as the metallic cation did not affect the aggregation process. This aggregation phenomenon is consistent with what we know of the deposition of attraction pheromones by adult *H. nitidus* (Krool and Bauer, 1987) as well as by other entomobryid species (Verhoef et al., 1977a; Verhoef et al., 1977b). Even though aggregation has been demonstrated to have a high survival value in the field (Joosse, 1970; Verhoef and Nagelkerke, 1977), the accumulation of a molecular signal occurs just because an animal remains for enough time at the same place (Usher and Hider, 1975; Verhoef et al., 1977b; Leonard and Bradbury, 1984), which does not occur when this place is unfavourable (pH < 4).

Given that aggregation occurred over a wide range of pH values when potassium was used as a metallic cation, i.e. from pH 4 to pH 8, we cannot conclude that pH 6 was the preferred value, despite its modal position on the histogram depicted on Figure 1. Rather we conclude that the animals were repelled by strong acidity (pH < 4) and alkalinity (pH > 8), but did not express clear preferences within the range 4-8. This is consistent with results of culture experiments, which demonstrated that *H. nitidus* tolerates acidic soil (pH 4) (Salmon and Ponge, 1999).

Results of the sodium experiment differed to a great extent from those of the potassium experiment. Aggregation occurred at neutral to alkaline pH values, with a clear preference for pH 8. Thus potassium and sodium do not play the same role in the preference behaviour of *H. nitidus*. Since pH itself, at least above pH 3, was not responsible for the observed aggregational pattern (this would occur with K as well), then attraction for sodium (and not for potassium) can explain that animals aggregated in compartments richer in sodium, with the exception of the most alkaline compartment (pH 9) where aggregation occurred only once. An attraction to solutions rich in Na is difficult to explain since increased external salinity (NaCl) affects negatively osmotic and ionic concentration of haemolymph (Witteveen et al., 1987), absorption of water by ventral tube vesicles (Eisenbeis, 1982), and fecundity (Barry and Hutson, 1978) of Collembola. However neither the effects of K, which is more abundant than Na far from the sea shore (Seasted and Crossley, 1981), nor the physiological use of K and Na have been studied in Collembola. Nevertheless, Na accounts for a great part of the total osmolar concentration of the haemolymph of Collembola (Eisenbeis, personnal communication) as it is the case for most primitive insects (Wigglesworth, 1965), which could explain the attraction of *H. nitidus* to solutions at increased Na levels. Such an attraction was not observed for potassium despite the fact that Collembola in general have a relatively high K content, compared to other soil invertebrates (Reichle et al., 1969 ; Pokarzhevskij et al., 1989 ; Teuben and Verhoef, 1992). This behaviour may reflects field conditions where K levels in soils exceed K levels required by Collembola since Collembolan faeces exhibit a higher relative availability in K than ingested litter (Teuben and Verhoef, 1992).

The importance of the chemical composition of buffer solutions used for the assessment of pH preferences must be highlighted. Repellent as well as attractive ions should be avoided, since they superimpose their effect to that of pH itself. Despite the absence of discrimination between Na and K evoked by Van Straalen and Verhoef (1997) for two other microarthropod species, the case of *Heteromurus nitidus* illustrates the need for using potassium rather than sodium in standardized tests for animals.

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Figure Captions

Fig. 1. Mean distribution of *Heteromurus nitidus* in the pH gradient, with potassium as the metallic cation in buffer solutions. Values are means of eight replicate boxes, with their standard errors. Significant differences are indicated by letters (same letter = not significant, different letter = significant).

Fig. 2. Mean distribution of *Heteromurus nitidus* in the pH gradient, with sodium as the metallic cation in buffer solutions. Values are means of eight replicate boxes, with their standard errors. Significant differences are indicated by letters (same letter = not significant, different letter = significant).

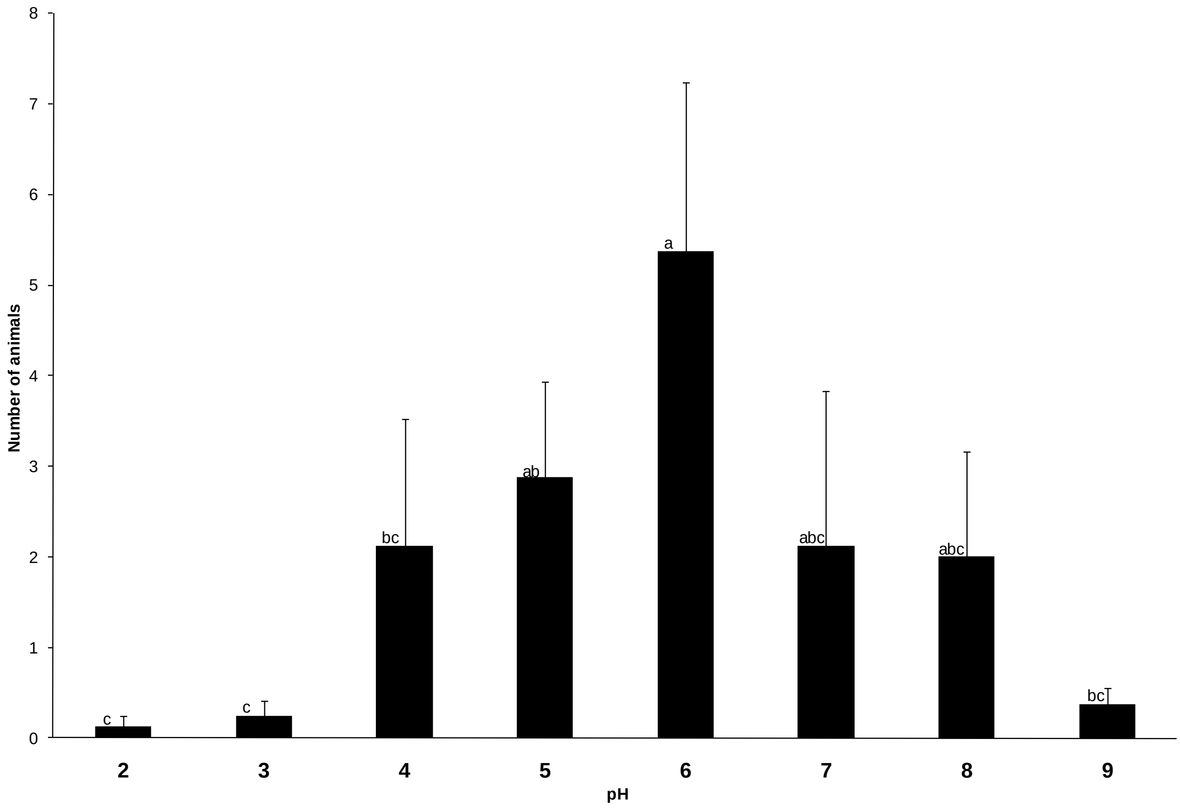

Fig. 1

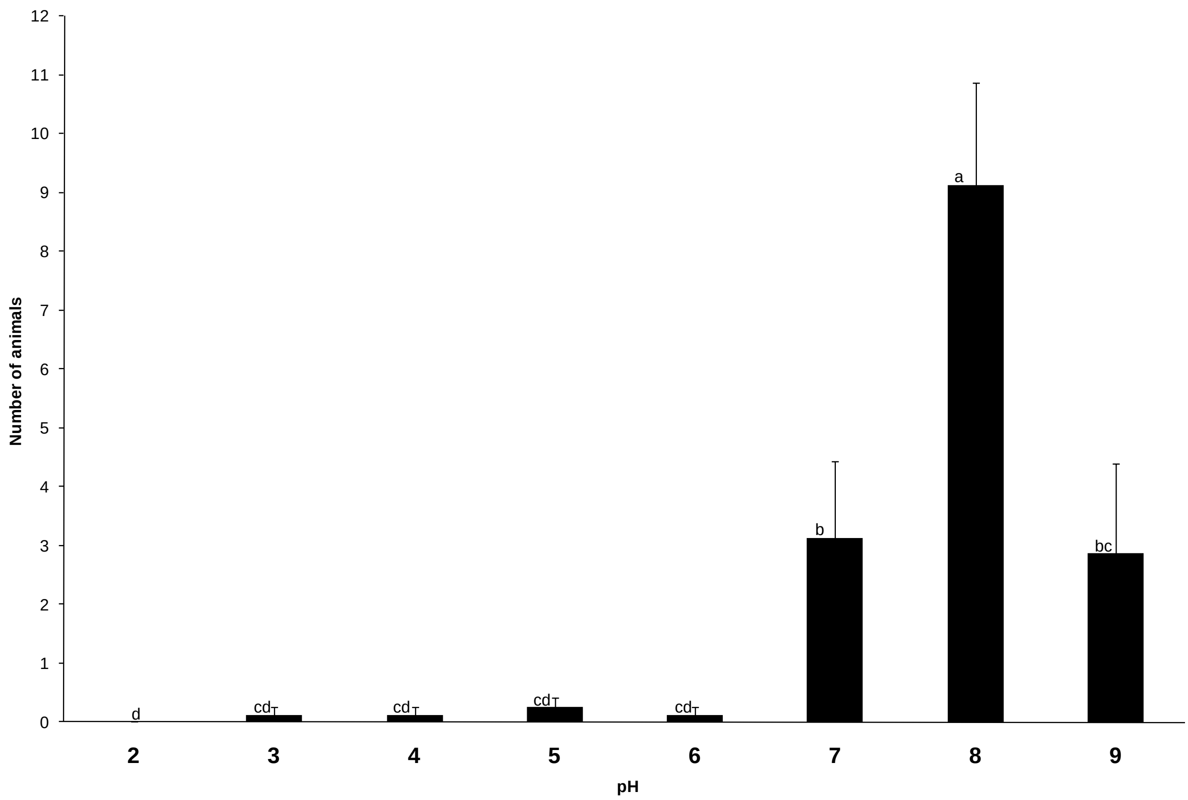
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Fig. 2