that had the highest courtship propensities, in order to maximize the probability of obtaining animals willing to court under experimental conditions. We are aware that such prescreening may bias the results by increasing overall courtship rates. Any bias, however, would tend to minimize the probability of demonstrating courtship pheromone effects and thus render significant results more reliable. The experiment comprised eight trial nights, with three "days off" between each trial night. Courtship encounters were staged by placing male-female pairs into individual courtship boxes at dusk. Delivery of male courtship pheromones was controlled experimentally because each male lacked a mental gland. The time a pair spent in tail-straddling walk was recorded. An increase in female receptivity was operationally defined as a decrease in the time a pair spent in tail-straddling walk. A female that engaged in tail-straddling walk became a candidate for treatment delivery when the courting male turned back and physically contacted the female's snout with his chin. At this time, the first of three 5-μl drops of the appropriate treatment solution was delivered to the nares of the female. Typically, the male delivers courtship pheromones multiple times during tail-straddling walk. The experimental delivery of three drops (10 min apart) mimics natural male delivery. Females received either a pheromone (purified PRF pool from the G-75 column) solution or saline (control) solution. If a female received a treatment solution on the first trial night, the female received the opposite treatment on the following trial night. The saline (control) solution was the same vehicle used in the purification of PRF.

11. A third of the amino acid sequence of the 22-kD PRF protein was determined by direct NH_2-terminal sequencing of PRF or tryptic peptides on an Applied Biosystems 470A Gas Phase Peptide/Protein Sequencer coupled to a 120A HPLC [R. C. Feldhoff unpublished material]. We isolated mRNA from frozen mental glands, then used reverse transcriptase polymerase chain reaction (RT-PCR) for first-strand cDNA synthesis (Invitrogen, Carlsbad, CA). The 5′ and 3′ ends of the PRF transcript were isolated with degenerate oligonucleotides (designed from NH_2-terminal amino acid sequence) and RACE-PCR techniques [M. A. Frohman, *PCR Protocols: A Guide to Methods and Applications*, M. A. Innis Ed. (Academic Press, San Diego, 1990), chap. 4]. PRF cDNA–specific primers were then constructed to amplify a 648–base pair coding region from single-stranded cDNA. PCR products were ligated into a derivative of pZerO-2.1 (Invitrogen, Carlsbad, CA) and chemically transformed. Sequencing templates were prepared by PCR directly from five colonies with standard primers. Templates were sequenced with a dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and run on an ABI377XL Automated Sequencer. The translated cDNA sequences showed homology (see GenBank accession numbers AF181480–181483) with independent amino acid sequencing.

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17. We thank S. J. Arnold, E. Adams, P. Andolfatto, P. Feldhoff, M. Kreitman, T. Morton, C. Palmer, E. Stahl, R. Steinberg, and J.-B. Warne-Reese for technical advice, help in the field, or manuscript review. We also wish to thank the Highlands Biological Station (HBS) and R. Bruce for the use of their facilities. Supported by NSF grants IBN-9709355 (R.C.F.) and IBN-9615473 (S.J.A. and L.D.H.), NSF dissertation improvement grant 9801210 (S.M.R.), Hinds Fund University of Chicago (S.M.R.), and HBS Grants in Aid (L.D.H. and S.M.R.).

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Water Vapor Absorption in Arthropods by Accumulation of Myoinositol and Glucose

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Hydrophilic soil arthropods have been thought to respond to soil desiccation exclusively by migrating to deeper soil layers. Numerous studies have shown that their survival below 90 percent relative humidity dry weight, is limited to hours. However, little attention has been paid to physiological adaptations to more realistic desiccation regimes, such as at the permanent wilting point of plants (98.9 percent relative humidity). A water vapor absorption mechanism is described that allows a common soil collembolan, *Folsomia candida*, to remain active down to below the permanent wilting point. A reevaluation of the water physiology of this widespread and diverse animal group is required.

Soil-dwelling arthropods have several characteristics that distinguish them from surface-living forms, in particular with respect to water balance. These characteristics include small size, epidermal respiration, and high integumental permeability to water (*1*). In fact, soil-dwelling Collembola are reported as having no physiological or metabolic means of regulating water loss and generally have high integumental permeability compared with other terrestrial arthropods (*2*, *3*). These characters seem to match the soil environment, where the pore humidity is normally very close to 100% relative humidity (RH). However, during dry periods, evapotranspirative removal of water throughout the root zone may reduce pore humidity. At about 98.9% RH, at which the water potential is at −15 bars, plants normally wilt (*4*). The hemolymph osmolality of soil arthropods, such as Collembola, is usually about 300 mosm/kg (*5*), roughly corresponding to an osmotic pressure of −8 bars and 99.4% RH. Thus, at soil pore relative humidities below 99.4%, which occur frequently, Collembola and other soil arthropods must tolerate, or in some way avoid, a net efflux of water.

Folsomia candida (Willem) is able to survive more than a week at 98.2% RH (*6*). This RH corresponds to a water potential deficit of about 17 bars between the environment and the normal body fluid osmotic pressure of these animals. We investigated this phenomenon by monitoring the water content, the hemolymph osmotic pressure, and the sugar and polyol (SP) contents of *F. candida* over a 7-day period of dehydration in an atmosphere maintained at a constant 98.2% RH. The response of these animals was compared with that of animals kept in an atmosphere at 99.6% RH without free water and with animals kept at 100% RH with access to free water.

As was expected, animals held in an atmosphere at 98.2% RH dehydrated rapidly during the initial 24 hours. They lost a third of their initial total water content, equivalent to a loss of half of their osmotically active body water (OAW) (Fig. 1), and became wrinkled in appearance and inactive, responding only to strong tactile stimulation. Controls in 99.6 and 100% RH remained unchanged in the same period. This pattern was repeated in the osmotic pressure of the body fluids, where the dehydrated animals approached water potential equilibrium with their environment at −24 bars (Fig. 2). Net water efflux was therefore close to zero at this time. The initial osmotic pressure of the animals was −7.5 bars. A loss of half of their OAW should result in an osmotic pressure of about −15 bars, so these animals should still be suffering from a water potential deficit with their environment. However, the animals actively increased their osmotic pressure to halt water efflux. Part of the explanation for this active increase in osmolality can be found in the synthesis of myoinositol (Fig. 3). This polyol, undetectable in control animals, constituted 1.4% of the dry weight of animals after the first 24 hours. Under the assumption that all myoinositol was dissolved in OAW, the contribution to the osmotic pressure would be about 5 bars, leaving less than 4 bars to be explained by other osmolytes.

The animals did not become iso-osmotic but within 48 hours of desiccation reestablished their hyperosmoticity to their sur-

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roundings at about the same level as under normal conditions (Fig. 2). This resulted in a net influx of water by passive absorption from the atmosphere. Accordingly, between the point of maximum dehydration at 24 hours and the end of the dehydration period at 169 hours, the animals increased their OAW content from 0.51 to 0.91 g of H_2O g^{-1} of dry weight (Fig. 1). At the outset of the experiment, the animals contained 0.99 g of OAW g^{-1} of dry weight, meaning that they had, in the following 6 days, recovered almost all of the water lost during the first 24 hours. This rehydration was accompanied by a clear return to the animals' normal appearance, which again was fully active as soon as their containers were opened instead of requiring strong tactile stimulation to induce movement. The increase in osmotic pressure was associated with sharp increases in myoinositol and glucose (Fig. 3), which had stabilized after 5 days at a combined concentration of 8% of the animals' dry weight. Under the assumption that all SP molecules made a colligative donation, these chemicals contributed 15 bars to the increased osmotic pressure after 48 hours (Fig. 4). At this time, the combined effects of water loss and SP production left only 11% of the increase in body fluid osmotic pressure unexplained. SP synthesis continued and reached a plateau at 120 hours; however, at this time, animals had rehydrated more than could be explained by SP production alone. A comparison of the animals' water content, SP content, and body fluid osmotic pressure leaves about 9 bars to be explained by unidentified osmolytes. At day 7, the dehydrated animals were returned to an environment at 100% RH with free liquid water. This abrupt shift to a rehydrating environment forces the animals to remove osmolytes from their body fluids to avoid a lethal influx of water. Indeed, about 50% mortality was observed during the following 48 hours. However, surviving animals absorbed water rapidly (Fig. 1), and the SPs disappeared completely in that time (Fig. 3).

Synthesis of SPs with colligative properties causing a lowering of the body fluid melting point, synonymous with depression of vapor pressure, has been reported in cold-hardy arthropods (*7*). It has previously been suggested that SP accumulation may therefore also reduce water loss under desiccating conditions (*8*, *9*). Accumulation of SPs is also associated with anhydrobiosis, well known in soil invertebrates including tardigrades (*10*), nematodes (*11*), and Collembola (*12*). However, in anhydrobiosis, the adaptive value of SP accumulation has been primarily ascribed to noncolligative properties involved in the protection of membranes and proteins (*13*). The role of SP accumulation in *F. candida* is apparently not associated with anhydrobiotic properties because the lethal limit for drought tolerance is above 95% RH (*6*). Instead, it appears that the SP accumulation in the present study is linked to water vapor absorption at low soil humidities.

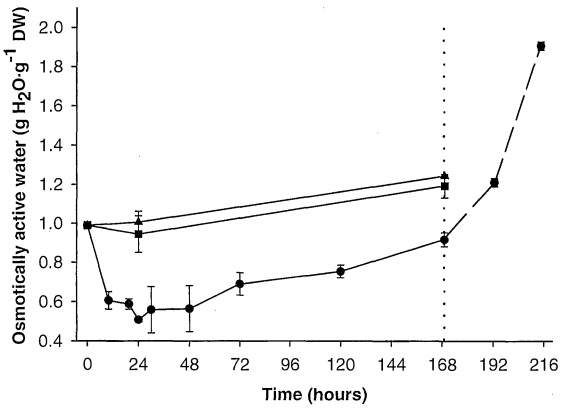

Fig. 1 (left). Delayed passive water vapor absorption in *F. candida* exposed to severe drought stress (*18*). Osmotically active water (OAW; mean ± SE; $n = 5$) in animals exposed to 98.2% RH (circles), 99.6% RH (squares), or 100% RH with access to free water (triangles). The vertical dotted line indicates the time at which animals were returned to Petri dishes with access to free water.

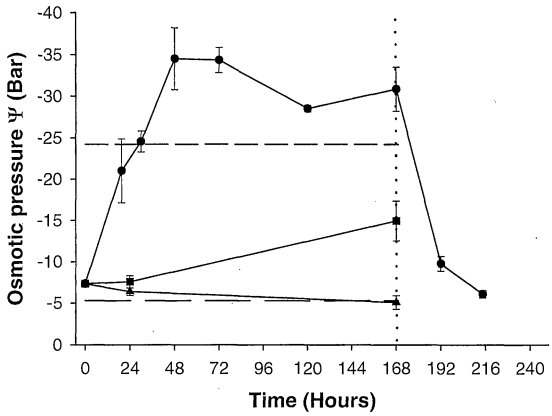

Fig. 2 (right). Drought-stressed animals reestablished hyperosmoticity to their environment within the first 48 hours and were thus able to absorb water vapor. Body fluid osmotic pressure (mean ± SE; $n = 3$) in *F. candida* exposed to 98.2% RH (circles), 99.6% RH (squares), or 100% RH with access to freewater (triangles), measuredas described (9). The horizontal lines indicate the corresponding water potential at 98.2% RH (short-dashed line) and 99.6% RH (long-dashed line). The vertical dotted line indicates the time at which animals were returned to Petri dishes with access to free water.

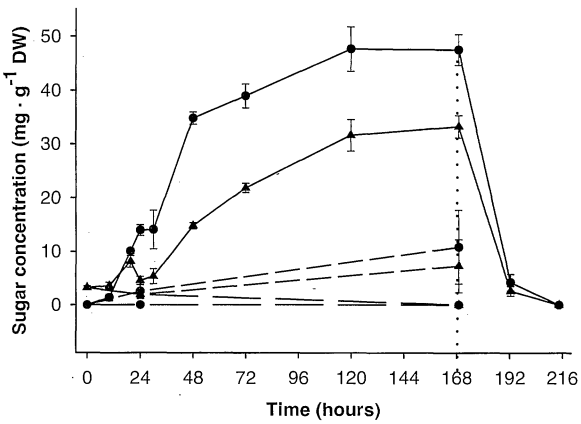

Fig. 3 (left). Sugar and polyol production is rapidly induced in drought-stressed animals (*19*). Contents (mean ± SE; $n = 5$) of myoinositol (circles) and glucose (triangles) in *F. candida* exposed to 98.2% RH (solid line), 99.6% RH (short-dashed line), or 100% RH with access to free water (long-dashed line). The vertical dotted line indicates the time at which animals were returned to Petri dishes with access to free water.

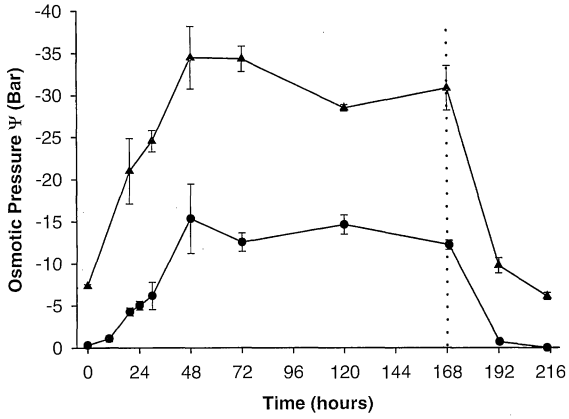

Fig. 4 (right). Sugars and polyols make a major contribution to the osmotic pressure in drought-stressed animals. Combined osmotic contribution (mean ± SE; $n = 5$) of myoinositol and glucose in body fluids of *F. candida* exposed to 98.2% RH (circles). For comparison, the total body fluid osmotic pressure is also shown (triangles). The vertical dotted line indicates the time at which animals were returned to Petri dishes with access to free water.

A number of terrestrial arthropods have the capability to balance their water budget by actively absorbing water from highly unsaturated atmospheres. These truly terrestrial arthropods achieve this balance by a combination of a cuticle with very low permeability and locally creating extremely low water activity in specialized tissues (*3*). In common with most soil arthropods, *F. candida* has a highly permeable integument, making localized active water absorption inappropriate. This animal is therefore forced to maintain all its body fluids hyperosmotic to its surroundings to allow net water uptake from the atmosphere by passive diffusion along the gradient in water potential. We have shown that glucose and myoinositol account for a large portion of the measured increase in osmotic pressure. This type of water vapor absorption confers the capability of meeting the water requirements of a terrestrial arthropod under prolonged drought stress (*14*). The adaptive importance of this mechanism is obvious, allowing *F. candida* to remain active in the same range of drought intensities that plants are capable of surviving and that must therefore occur throughout the root zone (*15*). Hitherto, experimental designs in studies of hygrophilic soil arthropods have masked the discovery of such water-regulating abilities. The physiological adaptations of these animals to desiccation require reevaluation.

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14. *Folsomia candida* is adapted to the range of humidities it will encounter in the soil. The water vapor absorption by *F. candida* at 98% RH is therefore of equal ecological importance as the water uptake of a desert insect at 50% RH.
15. We did not investigate the process at lower RH, but we expect it to work at even lower humidities because increasing mortality only occurs at RH below 97%. The soil is a very buffered environment, and drying of the soil is a rather slow process. This implies that SP accumulation in *F. candida* will be induced well before the lethal limit is reached.
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18. One- to two-month-old adult *F. candida* were placed in food-free Petri dishes containing a 5-mm-thick layer of water-saturated active charcoal and plaster of Paris for 48 hours to void their gut before the experiment. In the two groups of animals without access to free water, animals were randomly removed from the Petri dish at time zero and placed in small plastic containers (replicate), where the relative humidity of the air was precisely controlled at 99.6 or 98.2% RH with 7 and 31.6 g of NaCl liter^{-1} of water, respectively, as described (*6*). Each of these replicates contained 10 animals for measurement of water content and SPs and 20 for the measurement of body fluid osmotic pressure. The animals with access to free water were kept on the Petri dishes until sampling, when the appropriate numbers were randomly chosen for analyses. During the following 7 days, water content, osmotic pressure of body fluids, and the concentration of SPs were measured at the intervals shown in figures. At each sampling time, three replicates were removed for measurement of osmotic pressure and five for measurement of water content and SP concentration. Total water content was determined gravimetrically (grams per gram of dry weight) and converted to OAW by use of the relation given by (*16*).
19. After measurement of water content, animals were placed in 600-μl Eppendorf tubes. One hundred microliters of 40% ethanol containing the sorbitol internal standard was added (undetectable in crude extracts of both drought-stressed and control animals), and the animals were homogenized with a rotating glass rod. The rod was rinsed into the Eppendorf tubes with 2× 100 μl of 40% ethanol and the homogenate placed in an ultrasonic bath (Branson 5200; Branson Cleaning Equipment Company, Shelton, CT) for 30 min. The tubes were warmed to 80°C for 5 min and subsequently centrifuged at 20,000*g* for 10 min. The supernatant was removed to a 1.5-ml Eppendorf tube, and the pellet was rinsed twice with 100 μl of warm 40% ethanol and once with 100 μl of warm 20% ethanol. These extracts were left in a heat block at 60°C for about 15 hours until dry. SPs were redissolved in 500 μl of H_2O and filtered through a 0.2-μl filter (Nylon acrodisc; Gelman Sciences, Ann Arbor, MI) ready for high-performance liquid chromatography (HPLC) analysis. Samples of 25 μl were run in triplicate on a Shimadzu (Tokyo, Japan) HPLC system with a LC-6A pump, a SIL-6B auto-injector, and a C-R4AX integrator. SPs were separated by ion exchange chromatography with a Supelcogel-Ca column (30 mm by 7.8 mm) and a Supelcogel-Ca guard column maintained at 55°C with pure water as the mobile phase. SPs were detected with an evaporative light scattering detector (Sedex 55). Concentrations were calculated from a standard curve with SP standards including trehalose, myoinositol, glucose, mannitol, and sorbitol. Recovery of internal standard averaged 75% (SD 5%). The colligative contribution of SPs to the animals' osmotic pressure was calculated with the conversion table in (*17*). A supplementary gas chromatography analysis showed that glycerol content was not elevated in drought-stressed animals and that no traces of erythritol, ribitol, fructose, or sucrose were found.

20. This study received financial support from the Danish Natural Science Research Council. We are indebted to M. H. Petersen for technical assistance in chemical analyses and to H. Ravn for advice concerning the HPLC analysis.

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Coincident Induction of Long-Term Facilitation in *Aplysia*: Cooperativity Between Cell Bodies and Remote Synapses

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Induction of long-term synaptic changes at one synapse can facilitate the induction of long-term plasticity at another synapse. Evidence is presented here that if *Aplysia* sensory neuron somata and their remote motor neuron synapses are simultaneously exposed to serotonin pulses insufficient to induce long-term facilitation (LTF) at either site alone, processes activated at these sites interact to induce LTF. This coincident induction of LTF requires that (i) the synaptic pulse occur within a brief temporal window of the somatic pulse, and (ii) local protein synthesis occur immediately at the synapse, followed by delayed protein synthesis at the soma.

Synaptic plasticity, commonly thought to be a neuronal substrate for learning and memory, can exist in several temporal phases. In both hippocampus (*1*) and *Aplysia* (*2–6*), temporal phases of plasticity range from short term (minutes) to long term (hours to days). Recent evidence from both systems has shown that long-term changes in synaptic strength induced at one synaptic site can facilitate the induction of long-term changes at another site (*7, 8*). We examined induction of long-term synaptic plasticity in the intact central nervous system of *Aplysia* by exploring the temporal and spatial constraints on interactions between two structurally remote cellular compartments: (i) the cell bodies of tail sensory neurons (SNs) and their proximal synapses onto interneurons (*9*), located in the pleural ganglion, and (ii) their distal synapses onto tail motor neurons (MNs) (2 to 3 mm

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