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Short communication

Enhanced water conservation in clusters of convergent lady beetles, *Hippodamia convergens*

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Introduction

Large dense aggregation is one of the most well-known behavioral features by adults of the convergent lady beetle, *Hippodamia convergens* (Guerin-Meneville) (Coleoptera: Coccinellidae), where several thousand individuals clump together during diapause under leaves or debris or while feeding on aphids (Hodek, 1973). Living in an aggregation offers such benefits as defense and access to mates (Hodek, 1973), but are there other attributes that could be garnered as a result of group living? One possibility could involve water balance. The maintenance of adequate levels of body water (water balance) is a problem for all arthropods (Hadley, 1994), particularly for small-bodied insects such as lady beetles whose surface area is great in relation to their volume, which favors water loss (Schmidt-Nielsen, 1984). In adults of the tropical fungus beetle, *Stenotarsus rotundus* (Coleoptera: Endomychidae) the formation of large clusters during diapause (Denlinger, 1994) has a profound impact on water conservation by lowering water loss rates: as group size increases, water loss decreases (Yoder et al., 1992). It may be that lady beetles in clusters retain water more effectively than isolated specimens. Accordingly, we derived rates of net water loss for adult female *H. convergens* beetles in groups of different sizes. In addition, water content and dehydration tolerance limit were determined.

Materials and methods

Beetles and test conditions. *H. convergens* were collected from Baltimore, MD and were reared on aphids and tap water at L15:D9, 22–24 °C. Adult females, about 3 weeks of age, were used in the experiment. Beetles were held without food and water at 22–24 °C, 33% r.h. ($MgCl_2$; Winston & Bates, 1960), L15:D9, for at least 24 h and until 4–6% body mass had been lost, minimizing the effects of ingestion, defecation, excretion and reproduction on mass changes. Changes in mass thus reflect changes in the mass of water (Wharton, 1985).

Beetles were weighed and monitored singly using an electrobalance (Perkin-Elmer), with precision of ± 0.2 μg SD and accuracy of ± 6.0 μg at 1 mg. Calcium sulfate provided a dry atmosphere of 0% r.h. All experiments were conducted in an environmental room (22–24 °C, L15:D9). Beetles were removed from test conditions, weighed and returned within 1 min, and no anesthesia was used. Individuals monitored in groups were marked on the elytra with paint (Pactra, Van Nuys, CA); paint had no effect on mass changes (data not shown).

Determination of water balance characteristics. To determine water content, beetles were weighted (initial mass) and dried (0% r.h., 90 °C) to constant mass (dry mass); the amount of water lost (water mass) was expressed as a percentage of initial mass (% body water). Wharton's (1985) method was used to derive net water loss rates (integumental plus respiratory water loss). Isolated beetles (or paint-marked individuals from groups) were weighed, returned to the group and then transferred to 0% r.h., 22–24 °C, L15:D9. Beetles were reweighed every 12 h for a total of five readings of mass. Under 0% r.h., mass changes are due solely to loss; the slope of a regression on a plot of ln m_t/m_0 (m_t = water mass at time t; m_0 = initial water mass) *versus* time is the rate of net water loss and was expressed as %/h.

Table 1. Group influence on net water loss rate (%/h, 0% r.h., 22–24 °C) and water pool of adult female lady beetles, *Hippodamia convergens*. Values (mean ± SE) followed by the same letter within a column do not differ significantly (ANOVA; P>0.05). Observations were made on individuals in each of the experimental groups until 45 beetles had been examined; there were 45 groups for each test size with one designated beetle per group

		Initial water content			Water loss rate	(%/h, 0% r.h.)
Group size	N	Initial mass (mg)	Water mass (mg)	Body water (%)	Still air	Moving air (42.8 ml/min)
1	45	18.84 ± 1.15^a	11.83 ± 0.65^a	62.79 ± 2.1^a	0.396 ± 0.006^a	0.392 ± 0.005^a
5	45	18.76 ± 1.12^a	11.91 ± 0.53^a	63.49 ± 2.4^a	0.381 ± 0.005^b	0.401 ± 0.004^a
10	45	18.92 ± 1.16^a	11.74 ± 0.69^a	62.05 ± 1.9^a	0.237 ± 0.003^c	0.394 ± 0.007^a
20	45	18.88 ± 1.19^a	11.76 ± 0.61^a	62.29 ± 2.3^a	0.229 ± 0.004^d	0.397 ± 0.005^a

To determine dehydration tolerance, individual beetles were weighed, held at 33% r.h., 40 °C, and every 30 min a subset (N=20) was removed, reweighed to calculate percent change in weight $(w_t - w_0/w_0)$ (100%); w_t = weight at time t and w_0 = initial weight) and then placed upside down in a Petri plate (9 cm i.d.) that contained 6 droplets of water. The point where 50% of the test population was moribund (unable to right themselves, crawl to water droplets and drink) after a day served to denote an irreversible level of dehydration.

The ability by beetles to drink water was tested by offering adult female beetles six droplets of Evans blue-stained water (10%) in a Petri plate (9 cm i.d.); 1 beetle per plate. We checked for the presence of blue coloration in the gut by light microscopy.

Results were compared by an analysis of variance (ANOVA), using the arcsin transformation for percentage data and the test for the equality of several slopes (Sokal & Rohlf, 1981).

Results and discussion

Beetles in the experiment were approximately the same size and had similar initial water mass (Table 1). In all cases, dry mass was a positive correlate of water mass ($r^2 \leq 0.93$; $P<0.001$). These considerations indicate that the differences we noted between treatments (group sizes) are likely to be group-related.

Individual beetles in the largest group examined (20 in the cluster) retained water nearly two-times more effectively than isolated beetles; net water loss rates for individuals in groups of 5 and 10 were between both extremes (Table 1). Beetles tolerated losing only 27.81 ± 2.7% of their body weight (20 per replicate; N = 3), but this modest tolerance for weight loss during dehydration can be countered by aggregation, a feature that makes this limit unreached by suppressing net water loss rates. In fact, we have observed a 2–4 day increase in survival at 0% r.h. (22–24 °C) for beetles in clusters of 20 than when kept individually (data not shown). Beetles offered droplets of Evans blue dyed-water drank the colored liquid and stain was evident in their gut (20 per replicate; N = 3, and in large bulk colonies beetles were seen drinking water from droplets and moist cotton.

Until now it was not known that adult females of the convergent lady beetle, *H. convergens* could regulate water loss behaviorally by forming clusters. Only *S. rotundus* is the other beetle known to have this ability (Yoder et al., 1992). Like most arthropods (reviewed by Hadley, 1994), the lady beetle's water content (approximately 62%) is close to the mean water content (70%) of many insects; their dehydration tolerance (about 30%) is within the range (17–89%) of other arthropods and seems to be a typical limit; and net water losses through the cuticle and respiration are balanced mainly by drinking free water. The 'group effect' in *H. convergens* beetles is the distinctive feature, and we anticipate that other aggregation beetles in this taxon will extend this trend.

Modifications made by the 'group' on the avenues that contribute to net water loss rate (namely, integumental plus respiratory water loss; Wharton, 1985) are not completely known. One possibility is that the aggregation behaves like a 'superorganism' with regard to water balance (Yoder et al., 1992); small bodied, otherwise dehydration-prone, beetles reap the enhanced water conservation features of a large arthropod by surface area to volume properties (Schmidt-Nielsen, 1984). Alternatively, aggregation may raise the relative humidity for beetles inside the cluster, or extra cuticular wax may be acquired by beetles being in direct physical contact. Denlinger, Tanaka and Wolda's work on *S. rotundus* (reviewed by Denlinger, 1994) suggests that lower O_2 consumption rates with increasing group size may reduce net water loss by decreasing respiratory water loss, and we are presently investigating this in *H. convergens*.

The following experiment was conducted to help clarify how group-size might affect net water loss rates of individual beetles. If lowering net water loss is due to increased relative humidity in the beetle cluster, we would anticipate little effect of the 'group' by removing the humidified boundary layer over the beetle's cuticular surface. Indeed, dry air pumped continuously over clusters of beetles in an open-flow system (flow rate of 42.8 ml/min; modified from Wharton & Knülle, 1966) ablated the 'group effect' on net water loss (Table 1). In absence of a buildup of vapor pressure within the cluster net water loss is unaltered by gains. We have assumed that increased relative humidity is an important component of the 'group effect', but this is the first evidence that this is occurring.

Our experiments with adult female lady beetles showed enhanced water conservation in group sizes of 5, 10 and 20 beetles, with striking progression as group size increased. Whether water loss rates would continue to drop for lady beetles in a group of 'thousands' is not known. Alternatively, an equilibrium point may be reached; that is, at some upper limit of beetles no further reduction in water loss rates would be achieved. Certainly, defense and access to mates are major benefits of aggregation (Hodek, 1973), and it is now apparent that aggregation plays an additional role in water conservation.

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