Evidence for alternative trapping strategies in two forms of the pitcher plant, *Nepenthes rafflesiana*

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Supplementary data:

Testing the feasibility of replacing *N. rafflesiana* pitcher fluid with water

Background:

We experimentally 'knocked out' individual components of pitcher traps to determine their contribution to natural prey capture (see main article, *Experiments on the contribution of individual trap components towards natural capture success*). In this context, we planned to replace the pitcher fluid with filtered rain water to compare the retention efficiency of both liquids. As the pitchers secrete a polysaccharide into the digestive fluid which makes it viscoelastic (Gaume & Forterre 2007), we performed a test to ensure that the properties of the water were not altered by the pitchers.

Materials and Methods:

For each form of *N. rafflesiana*, we selected five one-week old pitchers from five different plants in the field. Using a 20 mL syringe with attached silicon tube, we removed the fluid and all prey from each pitcher and rinsed the pitcher twice with filtered rain water. We then filled the pitcher with filtered rain water up to the natural fluid level. The water was sampled after 10 min (sample 1) and the pitchers were refilled. The water was sampled again after 3 days (sample 2). Similarly treated fluid samples from the same *N. rafflesiana* form were combined to measure insect retention efficiency in glass vials, using the same three ant species and method as described in the main article (*Retention experiments*).

Results:

We found that pitchers indeed changed the properties of the added rainwater. The tested water samples had a significantly higher retention efficiency (hierarchical log-linear analysis, $n = 150$; treatment × outcome: $df = 12$, partial $\chi^2 = 50.84$, $P < 0.001$; ant × outcome: $df = 6$, partial $\chi^2 = 90.89$, $P < 0.001$; Fig. S1). Post-hoc Chi-square tests with Bonferroni-Holm correction revealed that for *Crematogaster* sp. ants, the retention efficiency of both the 10-min and the 3-days sample was significantly higher than the control (rain water). The other two ant species showed similar trends, but the effects were not significant.

Conclusion:

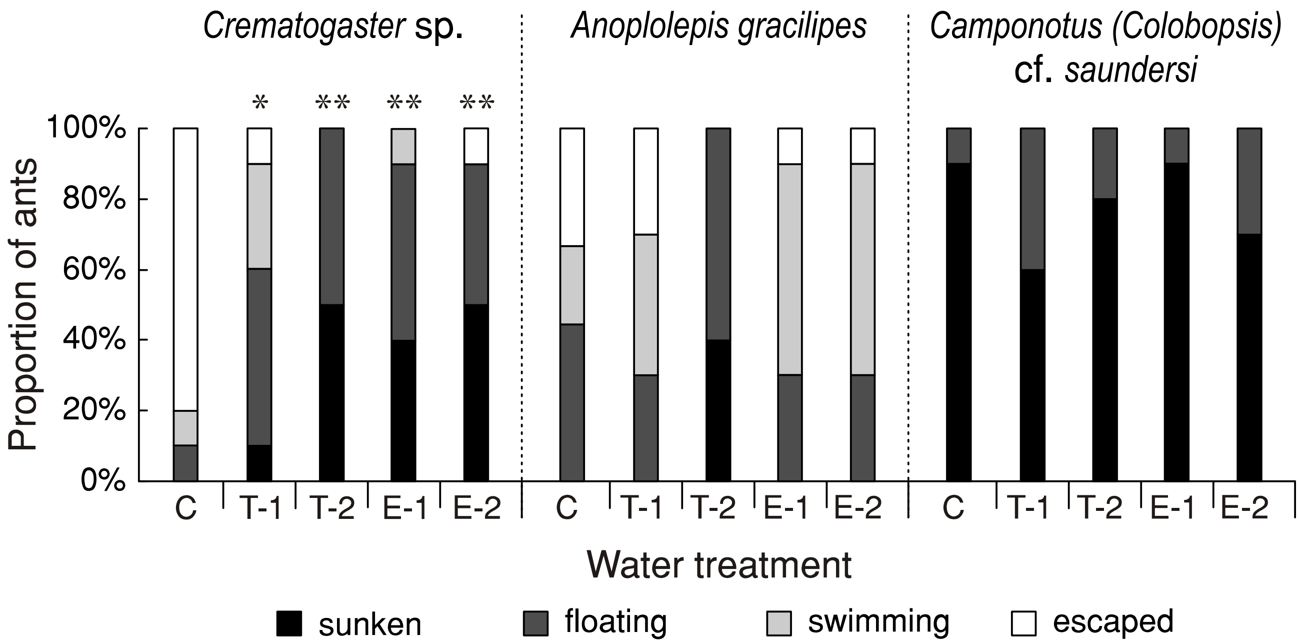
Consistent with Gaume & Forterre (2007), our results indicate that specific components of the pitcher fluid increase the retention efficiency for prey insects. It is likely that very low concentrations of polysaccharide still present in the pitchers (despite thorough washing, 10-min sample) or secreted anew (3-day sample) are sufficient to make the pitcher fluid viscoelastic and thereby increase its retention efficiency. Thus, comparing the retention efficiency of pitcher fluid and water inside life pitchers in the field was not possible using this approach. Instead, we performed retention experiments on pitcher fluids and water in glass vials.

Supplementary figure legends

Fig. S1. Retention rate for three ant species dropped into glass vials filled with different fluids (C = rain water; T1 = water after 10 min in typical form pitchers, T2 = water after 3 days in typical form pitchers, E1 = same as T1, but elongate form, E2 = same as T2, but elongate form). Each bar represents 10 ants. Asterisks indicate significant differences between treatments and the control (*: $P < 0.05$, **: $P < 0.01$).

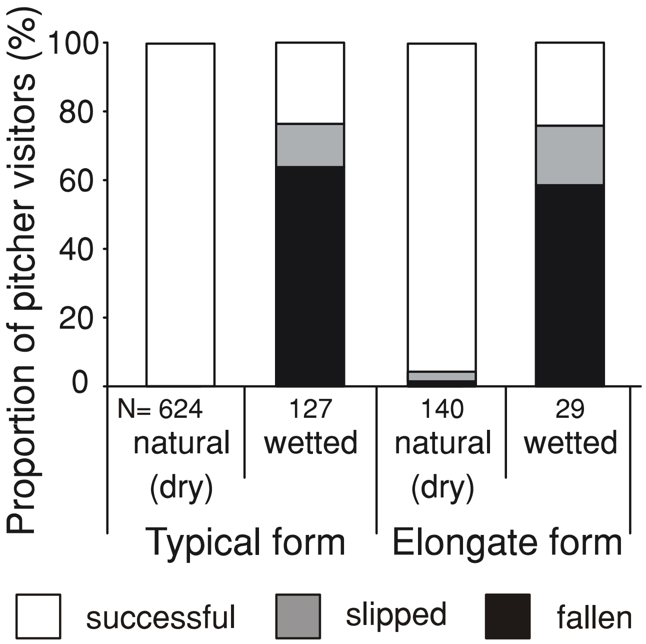
Fig. S2. Comparison of the peristome trapping efficiency of both forms of *N. rafflesiana* under dry and wet conditions (running experiment with *Camponotus (Colobopsis)* cf. *saundersi* ants, cf. Bauer *et al.*, 2008, 2009). Pitchers were first tested dry and then wet after spraying with rain water.

Figure S1



Crematogaster sp.
Anoplolepis gracilipes
Camponotus (Colobopsis) cf. saundersi
*
**
**
**
100%
80%
60%
40%
20%
0%
Proportion of ants
C
T-1
T-2
E-1
E-2
C
T-1
T-2
E-1
E-2
C
T-1
T-2
E-1
E-2
Water treatment
sunken
floating
swimming
escaped

Figure S2



Proportion of pitcher visitors (%)
100
80
60
40
20
0
N= 624
natural
(dry)
127
wetted
140
natural
(dry)
29
wetted
Typical form
Elongate form
successful
slipped
fallen