

# Effect of mating stage on water balance, cuticular hydrocarbons and metabolism in the desert harvester ant, *Pogonomyrmex barbatus*

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## Abstract

Water-loss rates increase after mating in queens of the harvester ant *Pogonomyrmex barbatus* (Formicidae: Myrmicinae), then increase again after the mated queens excavate an incipient nest. We determined the mechanistic basis for these increased water-loss rates by examining cuticular permeability, respiratory water loss, metabolic rates, and cuticular hydrocarbons for queens at three stages in the mating sequence: unmated alate queens, newly mated dealate queens, and mated queens excavated from their incipient nest. Both total water loss and cuticular transpiration increased significantly following mating, with cuticular transpiration accounting for 97% of the increased water loss. In contrast, metabolic rate and respiratory water loss were unaffected by mating stage. The total quantity of cuticular hydrocarbons did not vary by mating stage. However, relative amounts of four of the most abundant cuticular hydrocarbons did vary by mating stage, as did quantities of *n*-alkanes and methylalkanes. The general pattern was that percent composition of *n*-alkanes decreased through the mating sequence, while percent composition of methylalkanes increased over the same sequence. We discuss three mechanisms that might cause these post-mating increases in cuticular permeability. Our data support the hypothesis that part of this increase results from soil particles abrading the cuticle during the process of nest excavation.

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**Keywords:** Seed-harvester ant; Cuticular hydrocarbon; Queen mating stage; *Pogonomyrmex*; Water loss

## 1. Introduction

The abiotic environment exerts strong selective pressure on the water balance of terrestrial invertebrates (Edney, 1977; Hadley, 1994). The evolutionary effect of these abiotic factors is evidenced by comparative studies showing that water-loss rates are often positively associated with increasingly mesic conditions in insects (e.g., Massion, 1983; Hadley and Schultz, 1987; Hood and Tschinkel, 1990; Studier and Lavoie, 1990; Gibbs and Matzkin, 2001; Gibbs et al., 2003; Klok and Chown, 2003) and other arthropods (Toolson and

Hadley, 1977; Hadley et al., 1981; Worland and Block, 1986). The adaptive basis of these interspecific differences involves mechanisms that reduce water loss through the cuticle and respiratory loss via the spiracles, which together comprise the primary avenues of water loss. These mechanisms include epicuticular lipids that function to waterproof the cuticle, and discontinuous gas exchange, which has been proposed as a mechanism to reduce respiratory water loss (Lighton, 1996).

Abiotic factors can also act at the proximate level to affect water-loss rates. In addition to the dramatic effects of temperature on cuticular permeability (Gibbs, 2002), activities such as excavating a burrow expose individuals to soil particles that can abrade the cuticle (Holdgate and Seal, 1956; Nel, 1965; Johnson, 2000a, b). Abrasion could increase cuticular

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transpiration by: (1) decreasing the quantity of epicuticular lipids, (2) displacing epicuticular lipids and thereby decreasing their waterproofing properties, or (3) causing physical damage to the cuticle (Hadley, 1994). Unfortunately, little information is available on mechanisms by which abrasion affects water-loss rates, because of the difficulty in partitioning cuticular water loss and respiratory water loss for individuals that do not use discontinuous gas exchange (for a review see Hadley, 1994). This study examines the mechanistic basis for increased water-loss rates by female reproductive ants (queens) of the seed-harvester ant *Pogonomyrmex barbatus* (F. Smith) (subfamily Myrmicinae) as they progress from unmated alate (winged) queens to newly mated dealate queens to dealate queens excavated from their incipient nests. Ant queens provide an ideal system to examine changes in the contribution of cuticular versus respiratory water loss and the mechanistic basis of these changes, because mating and initiating a new nest result in a 2–3-fold increase in total water-loss rates (Johnson, 2000b). An additional benefit of this system is that water-loss rates increase in a stepwise fashion through the mating sequence. For live individuals, water-loss rates are lowest for unmated alate queens, intermediate for newly mated dealate queens, and highest for mated queens two days after beginning to excavate their incipient nest. The pattern differs slightly for dead individuals, because water-loss rates are similar for alate queens and newly mated dealate queens, but both groups have significantly lower water-loss rates than those of dealate queens excavated from their incipient nest 2 days after mating. Cuticular abrasion was suggested to cause these post-mating increases in water loss (Johnson 2000b), but other mechanisms could be involved. For example, changes in metabolic rate or in the composition of cuticular lipids could also increase water-loss rates.

*P. barbatus* is a soil-nesting species, whose annual colony cycle begins with producing a cohort of reproductive sexuals from mid-June through early July. Summer rains trigger reproductive queens and males to fly from their nests to mating aggregations that contain many thousands of individuals. Mating is a scramble competition, with numerous males continuously vying to mate with each queen. Each queen mates with multiple males (Hölldobler, 1976; Hölldobler and Wilson, 1990), then leaves the mating aggregation, tears off her wings, and searches for a site to excavate her new nest. The queen then remains in her nest, and metabolizes body reserves to support herself and rear her first brood of workers, which emerge after 4–5 weeks at 30 °C (Johnson, 1998, 2002).

We used flow-through respirometry to measure water-loss rate and metabolic rate for queens of each mating stage, and then we analyzed cuticular hydrocarbons from these same individuals. We partitioned cuticular

transpiration and respiratory water loss using a regression technique that can be used for all modes of gas exchange (see Gibbs and Johnson, 2004). Thus, we could determine which of these two routes effected post-mating increases in water loss. Cuticular abrasion should result in an increased cuticular transpiration and possibly changes in the amount or composition of cuticular hydrocarbons. Additionally, cuticular abrasion should not affect metabolic rate, i.e., metabolic rate may be correlated with respiratory water loss, but it should not be correlated with cuticular water loss.

## 2. Methods and materials

### 2.1. Collections

We collected *P. barbatus* queens at three stages of mating and colony founding in July and August near Rodeo, New Mexico, USA; (1) unmated alate queens were excavated from their nests, (2) newly mated, dealate queens were collected as they walked from the mating aggregation, but before beginning to excavate their new nests, and (3) mated, dealate queens were excavated from their incipient nests two days after mating. Each ant was placed in a 1.5 ml microcentrifuge tube that had been punctured for ventilation, along with a small piece of moist paper towel to maintain hydration. Ants were then shipped via overnight express to the laboratory. Individuals were maintained in microcentrifuge tubes until respirometry measurements, which were performed within 1 week.

### 2.2. Respirometry

We measured water-loss rate and metabolic rate for each ant using a TR-2 flow-through respirometry system (Sable Systems, Las Vegas, Nevada, USA). Ants were weighed to the nearest 0.1 mg, then placed in 5-ml glass-aluminum chambers inside a darkened incubator at 30 °C. Dry, CO<sub>2</sub>-free air was pumped through the chambers at 100 ml min<sup>-1</sup> to a Li-Cor (Lincoln, Nebraska, USA) LI-6262 infrared CO<sub>2</sub> and water vapor sensor. Chambers containing the ants were in the respirometer for an acclimation interval of approximately 3 h; then recorded for 30 min. Time-averaged data were recorded every 5 s and analyzed using Datacan V software (Sable Systems; Las Vegas, Nevada USA). Ants were reweighed after each run; estimates of water loss during the run, as calculated from mass loss, were consistent with flow-through measurements. Immediately after respirometry, the ants were frozen at –75 °C for later extraction of cuticular hydrocarbons.

A primary goal of this study was to assess changes in the contribution of cuticular water loss and respiratory water loss in these queens. This goal was facilitated

by our ability to partition cuticular water loss and respiratory water loss using a statistical technique that involved regressing water-loss rate against CO<sub>2</sub> release for each ant; the regression used the 5 s time-averaged values over the 30 min respirometry run. This regression yielded a positive, statistically significant linear relationship for all ants (Gibbs and Johnson, 2004). Water-loss rate (mg h<sup>-1</sup>) at the intercept, where CO<sub>2</sub> release (μl h<sup>-1</sup>) equals zero, was assumed to represent cuticular transpiration. Respiratory water loss was calculated as the difference between total water loss and cuticular water loss. This technique involved extrapolating the regression line down to the point of spiracular closure for individuals that used continuous gas exchange. Our confidence in this extrapolation is based on the fact that individuals that displayed discontinuous gas exchange demonstrated a straight line relationship down to spiracular closure. We then tested the effect of mating stage (independent variable) on total water-loss rate, cuticular water loss, respiratory water loss, and metabolic rate (dependent variables) using one-way ANCOVAs, with initial wet mass as the covariate in each model. The capacity of our respirometer limited us to measuring 12 individuals per day, such that respirometry occurred over 6 days. Consequently, we also included assay date as a second covariate in each model.

### 2.3. Hydrocarbon analyses

We analyzed cuticular hydrocarbons (HCs) from 51 ants for which we had respirometry data. Individual ants were extracted for 10 min in HPLC-grade hexane and then extracted again for 1 min. The combined extracts were evaporated under nitrogen and then 2.5 μg of *n*-icosane in a small volume of hexane were added to serve as an internal standard for lipid analyses. The extracted lipids were applied to a small silica gel column in a pasteur pipet, and HCs were eluted with approximately 5 ml of hexane (Toolson, 1982). The hexane was evaporated under a stream of nitrogen, and HCs were stored at -20 °C under nitrogen.

We used a Hewlett-Packard HP5890A gas chromatograph with flame-ionization detector and DB-1 column (JW Scientific, Sacramento, CA, USA) to analyze HC amounts and composition. The run conditions were 5 min at 150 °C, followed by a ramp at 5 °C min<sup>-1</sup> to 310 °C, then 10 min at 310 °C. Hydrocarbons were initially identified by comparison to retention times of *n*-alkane standards and literature data for *P. barbatus* (Nelson et al., 2001; Wagner et al., 1998, 2000). These identifications were confirmed using gas chromatography-mass spectrometry (GC-MS) at the University of Arizona's Mass Spectrometry facility. Quantities of HCs were determined by comparing peak areas to those of *n*-icosane standards.

We examined the effect of mating stage on both the quantity and composition of HCs. Total quantities of HCs and quantity per unit surface area were compared across mating stages using a one-way ANOVA; surface area (SA, cm<sup>2</sup>) of each individual was calculated using Meeh's formula (Edney, 1977):  $SA = 12M^{0.67}$ , where *M* is mass in grams. We then assessed changes in individual HCs across the three mating stages using multiple analysis-of-covariance (MANCOVA), with the data being percent composition of each HC for each individual. The MANCOVA included only HCs that averaged >5% of the total HC composition; time in the laboratory was included as a covariate. Running a MANCOVA using percentage data can result in statistical problems associated with multicollinearity and normality. We minimized multicollinearity by analyzing only data from the six most abundant hydrocarbons, which accounted for about 75% of the entire HC profile. Potential deviations from normality were assessed using quantile probability plots for the residuals of each variable. We also compared our raw percentage data for each variable to a normal distribution using a Kolmogorov-Smirnov statistic. None of these analyses showed strong systematic deviation from a normal distribution, indicating that our percentage data met the assumptions of MANCOVA. Additionally, all individual variables fit the univariate homogeneity of variance assumption. A posteriori univariate *F* tests were used to determine which HCs contributed to overall differences in the MANCOVA. We used Tukey-Kramer pairwise comparisons to assess patterns of variation for HCs that displayed significant *F* values. We also examined the effect of mating stage on changes in total quantities of the two major classes of HCs (*n*-alkanes and methylalkanes) using one-way ANOVA. For all statistical analyses, variables were transformed, as necessary, to meet the assumptions of ANOVA. For the MANCOVA, the assumption of homogeneity of covariance matrices was not met (Box's *M* test,  $P < 0.001$ ) when using either transformed or untransformed variables. Lastly, we used multiple regressions to investigate the relationship between HC amounts and cuticular water loss, after correcting quantity of HCs of each individual for body size of the individual (see above). Analyses were performed using JMP software (Sall et al., 2001).

## 3. Results

### 3.1. Patterns of gas exchange

Individual ants released CO<sub>2</sub> in one of two general patterns, cyclic or continuous (Table 1; Fig. 1). Cyclic gas exchange was performed by 34 queens, with peaks in water loss corresponding to simultaneous CO<sub>2</sub> release

Table 1  
Number of queens of *P. barbatus* that exhibited cyclic and continuous gas exchange at each of the three stages of mating

Mating Stage	Pattern of gas exchange		Total
	Cyclic	Continuous	
Unmated	13	6	19
Mated pre-dug	10	13	23
Mated post-dug	11	9	20
Total	34	28	62

For mating stage: unmated = alate queen collected from her nest; mated pre-dug = dealate queen captured as she walked from the mating aggregation; mated post-dug = dealate queen excavated from her incipient nest two days after mating.

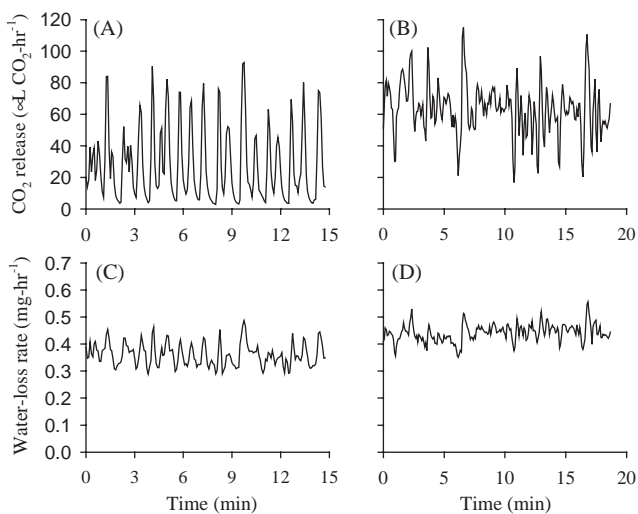


Fig. 1. Representative recordings of carbon dioxide release (top panels) and water loss (bottom panels) from two newly mated, dealate queens of the ant *P. barbatus*.

(Fig. 1A and C). The other 28 ants released CO<sub>2</sub> continuously with no evidence of cycles (Fig. 1B). Overall, the pattern of gas exchange did not differ as a function of mating stage (Table 1; 3 × 2 contingency test,  $G = 2.51$ ,  $P > 0.1$ ,  $n = 62$ ) (Sokal and Rohlf, 1995). Only one ant produced a large burst of water loss that appeared to represent excretion.

### 3.2. Effect of mating stage on water loss

A one-way ANOVA indicated that initial wet mass (mean = 48.2 mg, range 43.2–53.3 mg) did not vary across the three mating stages ( $F_{2,46} = 0.14$ ,  $P = 0.87$ ) or time in the laboratory ( $F_{10,38} = 0.91$ ,  $P = 0.53$ ). Total water loss (TWL) varied by mating stage (one-way ANCOVA,  $F_{2,52} = 55.8$ ,  $P < 0.0001$ ), and increased by 68% after mating, and by an additional 40% 2 days after beginning to excavate their new colony (a poster-

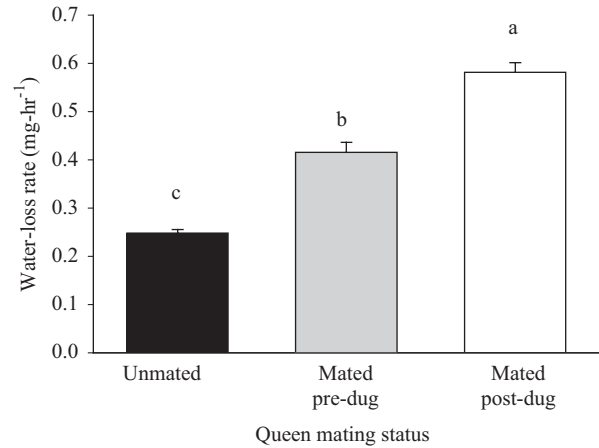


Fig. 2. Effect of mating stage on water-loss rates for queens of *P. barbatus*. For queen mating stage: unmated = alate queen collected from her nest; mated pre-dug = newly mated, dealate queen captured as she walked from the mating aggregation; mated post-dug = dealate queen excavated from her incipient nest 2 days after mating. Significant differences ( $P < 0.05$ ) are denoted by the letters a–c: a > b > c. Groupings are based on a one-way ANOVA followed by Tukey–Kramer pairwise comparisons.

iori Tukey–Kramer pairwise comparisons,  $P < 0.05$  for all comparisons; Fig. 2). Overall, total water-loss rates were 2.36 times higher for queens excavated 2 days after beginning to excavate their nest than for unmated queens.

Fig. 3 illustrates the method used to calculate cuticular water loss. The pattern of cuticular transpiration, as calculated using our regression method (Fig. 3), mimicked that of total water loss, nearly doubling after queens mated, then increasing by another 50% 2 days after queens began to excavate their new nest ( $F_{2,52} = 53.2$ ,  $P < 0.0001$ ; Fig. 4A). Across all individuals, cuticular water loss (CWL) accounted for an average of 83% (range = 48–95%) of the total water loss. In contrast, respiratory water loss (RWL) and metabolic rate did not vary with mating stage ( $F_{[RWL]2,52} = 0.096$ ,  $P = 0.91$ ; Fig. 4B;  $F_{[metabolic\ rate]2,52} = 0.58$ ,  $P = 0.61$ ). The two covariates, initial mass and assay date, were not significant in any model ( $P > 0.16$  in all cases).

The similar pattern of post-mating increases in both cuticular water loss and total water loss suggested a strong relationship between these two variables. We examined this relationship by regressing total water loss (dependent variable) against cuticular water loss (independent variable) for all individuals; the slope of the regression indicates the proportionate increase in total water loss that occurs through the cuticle. Because both variables were measured with similar error, we used Model II regression (Sokal and Rohlf, 1995). Across all individuals, cuticular water loss was positively correlated with total water loss (TWL =  $0.969 [\pm 0.022]CWL + 0.072 [\pm 0.009]$ ,  $n = 62$ ,  $P < 0.0001$ ;

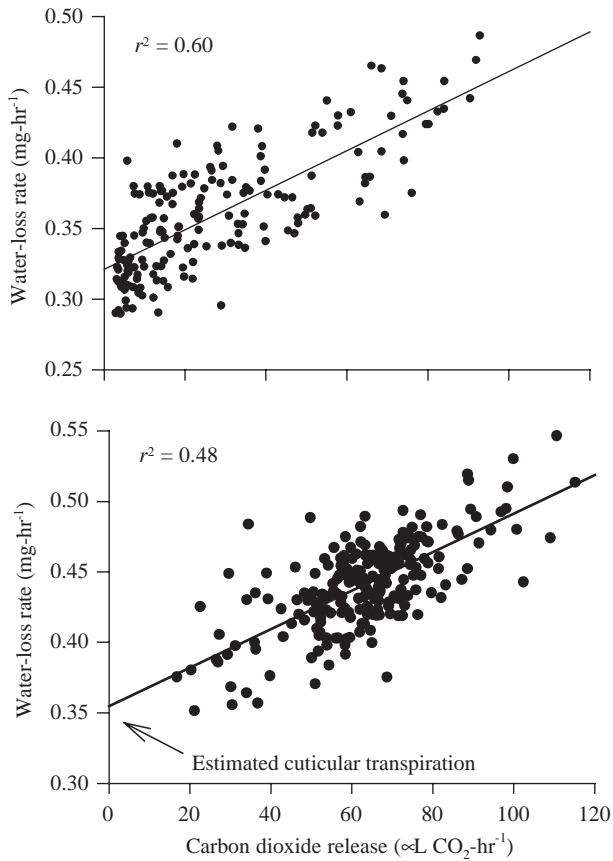


Fig. 3. Plots of water-loss rate versus CO<sub>2</sub> release for newly mated dealate queens of *P. barbatus* using different patterns of gas exchange (cyclic in upper panel, continuous in lower panel). Plots are for the same individuals as shown in Fig. 1. The arrow in the lower panel denotes the Y-intercept of the regression line, corresponding to the point at which CO<sub>2</sub> release is absent, and the spiracles are completely closed. The value of the Y-intercept thus represents cuticular water loss. Respiratory water loss was the difference between total water loss and cuticular water loss.

Fig. 5). The slope of the regression line indicates that cuticular water loss accounted for 97% of the post-mating related increase in total water loss. Analysis of metabolic rate and respiratory water loss corroborated these results. Using a stepwise regression, only cuticular water loss was correlated with TWL ( $P < 0.0001$ ), whereas TWL was not correlated with respiratory water loss ( $P > 0.45$ ) or metabolic rate ( $P > 0.09$ ).

### 3.3. Cuticular hydrocarbons

The cuticle contained a mixture of hydrocarbons that included *n*-alkanes, methylalkanes, and alkenes; 15 GC peaks accounted for >90% of the total HCs. All HCs were 23–31 carbons long, with the most abundant being *n*-pentacosane (33.2%). Total quantity of HCs per individual did not vary by mating stage (ANCOVA,

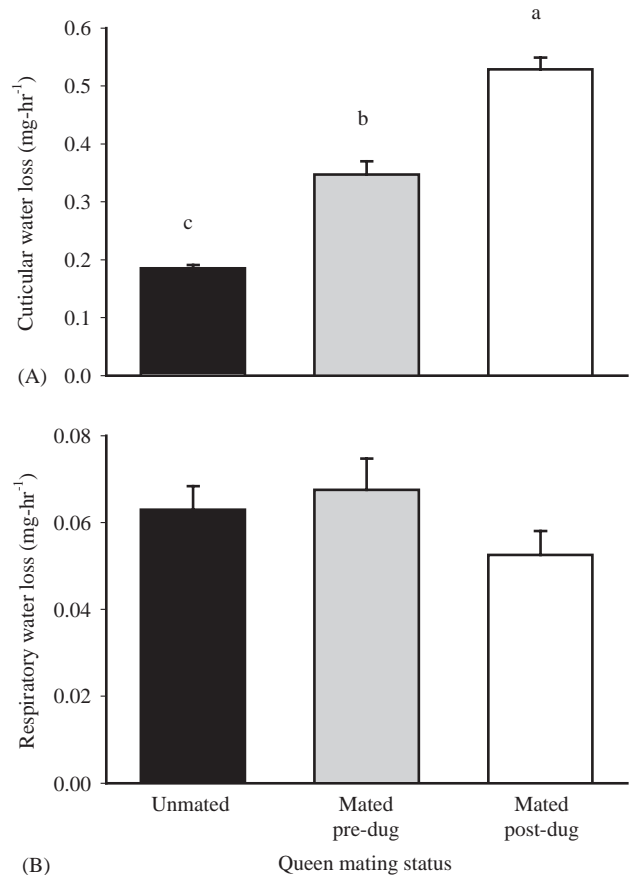


Fig. 4. Effect of mating stage on cuticular transpiration (A) and respiratory water loss (B) in queens of *P. barbatus*. Significant differences ( $P < 0.05$ ) within each panel are denoted by the letters a–c: a > b > c. Groupings are based on a one-way ANOVA followed by Tukey–Kramer pairwise comparisons. See Fig. 2 for information on queen mating stage.

$F_{2,44} = 2.58$ ,  $P > 0.08$ ; Fig. 6) or time in the laboratory ( $F_{1,44} = 0.73$ ,  $P = 0.40$ ). Results were similar when comparing the quantity of HCs per unit surface area (not shown).

Each of six HCs accounted for >5% of the total HC composition, and they collectively accounted for about 76% of the total HCs (Table 2). Three of these were *n*-alkanes containing 23–27 carbons. The other three HCs were multiple peaks of methylalkanes that were not routinely distinguishable from each other. Composition of the two classes of HCs (*n*-alkanes and methylalkanes) varied significantly over the three mating stages, with *n*-alkanes decreasing and methylalkanes increasing through the sequence (one-way ANOVA, *n*-alkanes;  $F_{2,48} = 5.1$ ,  $P < 0.009$ ; methylalkanes;  $F_{2,48} = 15.7$ ,  $P < 0.001$ ; Tukey–Kramer pairwise comparisons,  $P < 0.05$  for both tests; Fig. 7). The overall composition of the six predominant HCs varied by mating stage (Wilks'  $\lambda = 0.287$ ,  $F_{12,84} = 6.07$ ,  $P < 0.0001$ ; Table 2), but not by time in the laboratory (Wilks'  $\lambda = 0.764$ ,



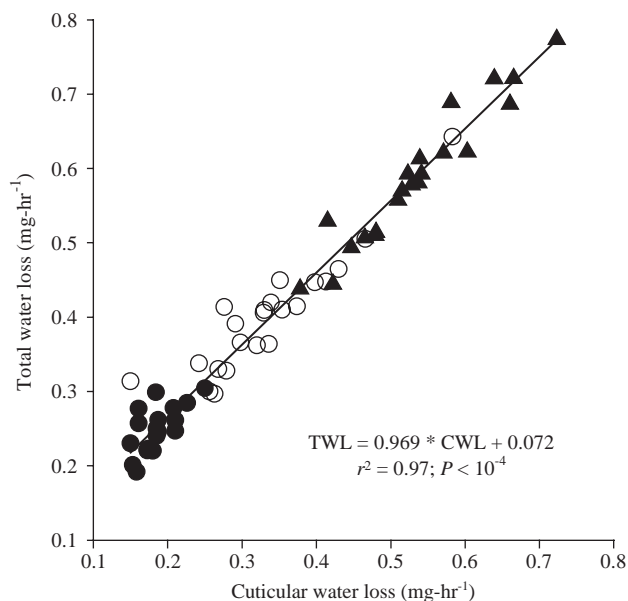


Fig. 5. Relationship between cuticular water loss and total water loss in queens of *P. barbatus*. Symbols denote queen mating stage: filled circles = unmated; open circles = mated pre-dug; filled triangles = mated post-dug. See Fig. 2 for information on queen mating stage.

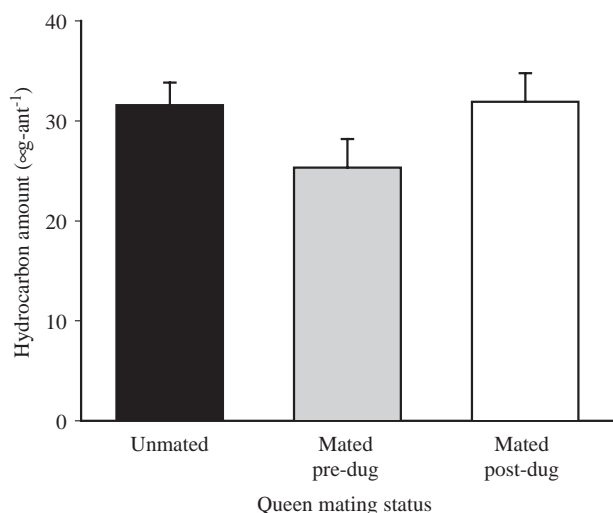


Fig. 6. Effect of mating stage on the quantity of cuticular hydrocarbons in queens of *P. barbatus*. See Fig. 2 for information on queen mating stage.

$F_{6,42} = 2.16$ ,  $P = 0.067$ ; Table 2). Percent composition varied by mating stage for four of these HCs (Tukey Kramer pairwise comparisons,  $P < 0.001$ ; Table 2, Fig. 7). The general pattern was that percentage composition of *n*-tricosane decreased through the mating sequence, while percentage composition of the three methylalkanes increased over the same sequence. We investigated the relationship between quantities of

these four HCs and cuticular water loss, after correcting CWL and HC amounts for surface area of each ant. A multiple regression indicated that *n*-tricosane levels were negatively correlated with CWL ( $r = -0.50$ ,  $P < 0.01$ ), whereas quantities of methylheptacosane were positively correlated with CWL ( $r = 0.60$ ,  $P < 0.0001$ ). Both regressions remained significant after adjusting *P* values using the Bonferonni sequential correction.

#### 4. Discussion

##### 4.1. Effect of mating stage on cuticular water loss and respiratory water loss

Our regression method enabled us to determine post-mating changes in the relative contribution of cuticular transpiration and respiratory water loss, despite the fact that 28 of 62 (45%) of the *P. barbatus* queens displayed continuous gas exchange (Table 1). Cuticular transpiration was the primary avenue of water loss for queens of *P. barbatus*, accounting for an average of 83% of the total water loss across all individuals. Additionally, increased cuticular water loss accounted for essentially all (an estimated 97%) of the increase in total water loss displayed by newly mated queens and those excavated from their incipient nest relative to that of alate queens (Fig. 5).

Respiratory water loss was a minor component of water loss in queens of *P. barbatus*, accounting for an average of 17% of the total water loss across all individuals. In comparison, in the closely related *P. rugosus*, respiratory water loss was estimated at 13% and 5% for alate queens and workers, respectively (Lighton et al., 1993; Quinlan and Lighton, 1999). Some authors (e.g. Hadley, 1994) have argued that variation in respiratory water loss is a relatively unimportant part of water balance because it comprises such a small fraction of the overall water budget. Our data demonstrate that status of an individual can cause significant changes in percent respiratory water loss despite a lack of variation in amount of water lost through the spiracles. Variation in cuticular transpiration, along with similar levels of respiratory water loss across the three mating stages resulted in percent respiratory water loss decreasing from 26% in alate queens to 18% in newly mated queens, to 9.5% in queens excavated from their incipient nests. We therefore concur with the argument (Chown, 2002; Chown and Davis, 2003) that the percentage of water lost via respiration has little to do with its significance to overall water balance. The nearly three-fold difference in percent respiratory water loss between alate queens and workers of *P. rugosus* (see above) probably has a similar basis given that cuticular abrasion appears to increase water-loss rates in both castes (Johnson, 2000b).

Table 2

Summary statistics for the six predominant cuticular hydrocarbons in queens of the seed-harvester ant *P. barbatus* (see text)

Overall model	Wilks' $\lambda$	df	F	P
Mating stage	0.287	12, 84	6.07	<0.000001
Time in lab	0.764	6, 42	2.16	0.067
<i>Between-subjects effects</i>				
		Mating stage		
Hydrocarbon name (# Cs)	% Total HCs	df	F	P
Alkanes				
<i>n</i> -Tricosane (23)	10.5	2	5.94	0.005
<i>n</i> -Pentacosane (25)	33.2	2	2.39	0.102
<i>n</i> -Heptacosane (27)	5.3	2	2.78	0.072
Monomethylalkanes				
7- and 13-methylpentacosane (25)	7.0	2	4.12	0.023
7- and 13-methylheptacosane (27)	12.8	2	14.96	<0.001
7-, 9- and 15-methylnonacosane (29)	7.7	2	4.96	0.011
Error		47		
Total	76.5	51		
$R^2$		0.247		

The overall model is based on the Wilks'  $\lambda$  value from a multivariate analysis-of-covariance; mating stage was the dependent variable and time in the laboratory was the covariate. See Fig. 7 for percent composition of each hydrocarbon in the three mating classes.

In this study, queens from all three mating stages exhibited cyclic and continuous gas exchange, and the proportion of ants using these modes of gas exchange did not vary with mating stage (Table 1). Thus, these queens retained spiracular control after mating and excavating their incipient nest. Our findings contrast with studies indicating that alate queens of other desert ants, e.g., *P. rugosus* (Lighton et al., 1993) and *Messor pergandei* and *M. julianus* (Lighton and Berrigan, 1995), consistently use discontinuous gas exchange. It would be interesting to examine post-mating changes in cuticular water loss and respiratory water loss in these species along with possible concomitant changes in patterns of gas exchange, especially given that mating and excavating the incipient nest effect increased water-loss rates for queens of these species (Johnson, 2000b). As one example, preliminary data indicate that most alate queens of *P. californicus* used discontinuous gas exchange, whereas almost all queens collected from their incipient nests used continuous gas exchange (M.C. Quinlan and R.A. Johnson, unpublished data).

The progressive increase in water loss from unmated alate queen to newly mated dealate queen to mated queens excavated from their incipient nest, parallels the pattern documented by Johnson (2000b). The primary difference between the two studies is that total water-loss rates in this study were approximately 25% lower than those measured by Johnson (2000b). This difference presumably reflects our use of flow-through techniques, whereas Johnson (2000b) measured water loss gravimetrically. The weighing procedure may have disturbed the ants, thereby increasing metabolic rate and

respiratory water loss (Addo-Bediako et al., 2001; Lighton and Wehner, 1993) or caused them to defecate.

Excretory water loss is easy to detect in flow-through respirometry, because it is associated with a burst of humidity as the excreted water evaporates. We observed only one such burst in 62 individuals, indicating that excretory water loss is a minor component of the total water budget for queens of *P. barbatus*.

#### 4.2. Effect of mating stage on cuticular lipids

This study documents progressive changes in the HC profile of queen harvester ants over the two day period from unmated queens to mated queens in their incipient nests. The general trend was a decrease in the proportion of straight-chain *n*-alkanes, and an increase in the proportion of methylalkanes. Because surface lipids provide the primary barrier to cuticular water loss, these changes may have been responsible for the progressive increase in permeability. Two factors determine the effectiveness of the lipid barrier: its thickness (i.e. the amount of lipid) and its composition. Lipid quantity was not affected by mating status; so increased CWL cannot have resulted from decreased HC levels. However, changes in composition are potentially consistent with increased barrier permeability.

Solid-phase lipids are less permeable than melted ones (Rourke and Gibbs, 1999), and melting points of HCs depend on their structure. Longer chain hydrocarbons increase melting points, while methyl groups and unsaturation decrease melting points (Gibbs and Pomonis, 1995). Queens excavated from their incipient

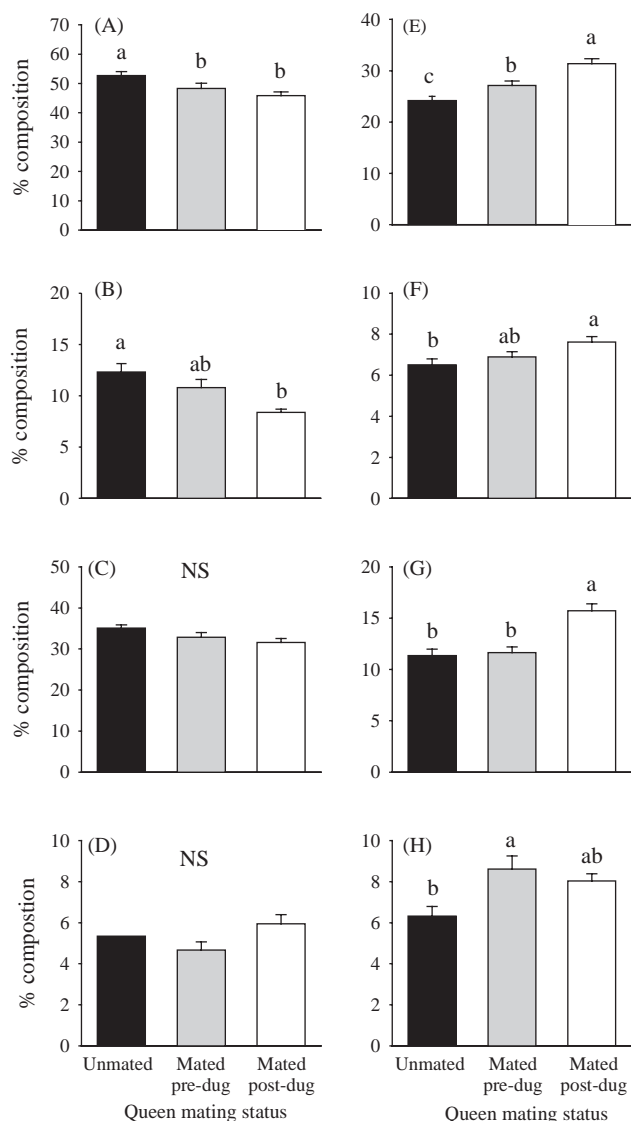


Fig. 7. Effect of mating stage on percentage composition of the six predominant cuticular hydrocarbons in queens of *P. barbatus*. For panels; all *n*-alkanes (A), *n*-tricosane (B), *n*-pentacosane (C), *n*-heptacosane (D), all methylalkanes (E), 7- and 13-methylpentacosane (F), 7- and 13-methylheptacosane (G), and 7-, 9-, and 15-methylnonacosane (H). Significant differences ( $P < 0.05$ ) within each panel are denoted by the letters *a–c*:  $a > b > c$ . Groupings are based on a one-way ANOVA followed by Tukey–Kramer pairwise comparisons. See Fig. 2 for information on queen mating stage.

nests contained the lowest levels of *n*-tricosane, which melts at 49 °C (Gibbs, 1995). We did not measure melting points in this study, but other work indicates that HCs melt between 25 and 40 °C in unmated queens of *P. barbatus* (A.G. Gibbs and R.A. Johnson, unpublished data). Thus, lower levels of *n*-tricosane would tend to lower the melting point. Queens excavated from nests also contained higher levels of methylalkanes, which tend to reduce melting points (Gibbs and Pomonis, 1995). We therefore predict that

HC melting points would tend to decrease after mating and nest founding. Because our experimental temperature of 30 °C was within the melting range for unmated queens (see above), these changes in HC composition would likely have made the surface lipids more fluid in mated queens, which would likely increase cuticular permeability (Rourke and Gibbs, 1999). We also note that HCs are not the only surface lipids on *P. barbatus*. Wax esters comprise ~13% of the total surface lipids for *P. barbatus* workers (Nelson et al., 2001); most of these waxes melt above 40 °C (Patel et al., 2001) and so would tend to raise melting points. Thus, differences in the abundance or composition of wax esters could also affect cuticular permeability across the three mating stages.

#### 4.3. Mechanisms effecting increased cuticular water loss

Mating and colony founding by queens of *P. barbatus* involve a series of activities that include flying from their nest to the mating aggregation, mating with multiple males, tearing off their wings, and excavating a nest. Three mechanisms could increase cuticular permeability along this sequence: (1) wing loss by mated queens, (2) changes in amount or composition of cuticular hydrocarbons, and (3) abrasion that scratches and/or displaces protective layers of the cuticle. We evaluate these mechanisms by comparing water-loss rates of live and dead (HCN-killed) queens (Fig. 8; see Johnson, 2000b), using the assumption that direct support for a mechanism requires that it increase cuticular permeability regardless of vital state. Note that comparison of water loss rates for live versus dead queens used the same population of individuals, with the individuals randomly assigned to the live/dead treatment; all individuals were then run simultaneously in the same experimental setup (see Johnson 2000b). We also note that age does not cause any of the observed differences in water loss rates across the three mating stages. Queens used in the current experiments were produced as a cohort, and individuals from all three mating stages were collected and run over the same time interval.

Our first mechanism posits that increased cuticular permeability of newly mated dealate queens results from water escaping through a temporary opening in the cuticle following the queen tearing off her wings. We reject this mechanism because water-loss rates of dead individuals do not increase following wing loss (Johnson, 2000b).

The total amount and/or composition of HCs can also affect cuticular water loss (Hadley, 1977; Toolson, 1982, 1984). Total quantity of HCs did not vary by mating stage, indicating that this variable did not affect cuticular permeability for queens of *P. barbatus*. However, HC composition varied across the three mating stages in a pattern consistent with that predicted



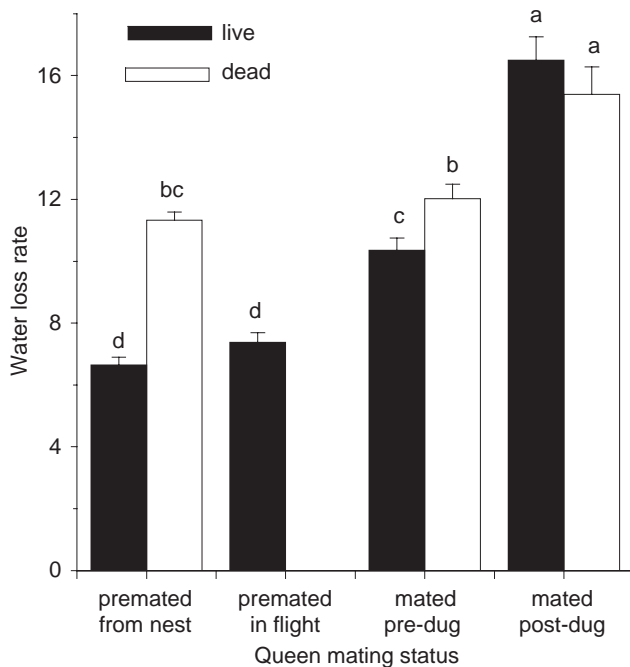


Fig. 8. Water loss rate ( $\mu\text{g h}^{-1} \text{cm}^{-2} \text{Torr}^{-1}$ ) over 8 h at  $30^\circ\text{C}$  for live and dead (HCN-killed) queens of *P. barbatus* (data from Johnson 2000b). Water loss rates for this figure were calculated gravimetrically, while this study calculated water loss rates using flow-through respirometry. Significant differences ( $P < 0.05$ ) are denoted by the letters *a–d*:  $a > b > c > d$ . Groupings are based on a one-way ANOVA across all cells followed by a Duncan's multiple range test (see Johnson 2000b).  $N = 15\text{--}45$  individuals per cell. See Fig. 2 for information on queen mating stage; predated in flight individuals were unmated queens captured as they flew into the mating aggregation.

to increase cuticular permeability, i.e., decreases in *n*-alkanes and increases in methylalkanes. Additionally, quantities of two of these HCs were highly correlated with cuticular permeability across all individuals (positive correlations for methylheptacosane; negative for *n*-tricosane; see above). Despite these strong correlations, two lines of evidence suggest that changes in HC composition did not significantly affect cuticular permeability. First, water-loss rates of live queens increased after mating and again after nest excavation, whereas water-loss rates of dead queens increased only for those excavated from their incipient nests (Johnson, 2000b). We doubt that progressive changes in HC composition would increase cuticular permeability of dead queens at one stage (following nest excavation), but not another (following mating). Second, quantities of individual HCs and of the two classes of HCs (*n*-alkanes and methylalkanes) varied in a statistically significant manner across the three mating stages, but overall changes in composition were small (approximately, 7% for both *n*-alkanes and methylalkanes; Fig. 7). It seems unlikely that these minor changes in HC composition could cause 2–2.5-fold increases in cuticular permeability, when much more dramatic differences in other

species have no effect (Gibbs et al., 1998; Montooth and Gibbs, 2003).

The third possible mechanism, cuticular abrasion, could occur during mating and/or colony excavation. In the first case, the cuticle could be damaged by the numerous males surrounding and attempting to mate with each queen. However, mating does not cause such abrasion, given that cuticular permeability increased in live but not dead newly mated dealate queens. Similarly, the increased cuticular permeability of newly mated dealate queens did not result from metabolic effects related to flying to the mating aggregation, because water-loss rates of unmated queens entering the mating aggregation are similar to those of unmated queens captured from their natal nest, and rates for both groups are significantly lower than those of newly mated dealate queens (Fig. 8; Johnson, 2000b). Moreover, we believe that the increased cuticular permeability of live but not dead newly mated dealate queens results from physiological changes associated with mating, rather than from mechanical damage by males (Johnson, 2000b). In *Pogonomyrmex* queens, mating results in changes such as increased aggression that are probably mediated by hormones. We suggest that mating related changes in hormone levels may also decrease active control of water loss in live queens independent of the effects of cuticle abrasion.

Cuticular abrasion could also result from queens excavating their incipient nests. That water-loss rates increased for both live and dead queens at this stage supports the hypothesis that abrasion is the causal mechanism. Such abrasion likely occurs by soil particles scratching and/or displacing cuticular hydrocarbons and causing furrows that enhance transpiration. The cuticle itself might also be damaged. That cuticular abrasion occurs is also supported by data on workers, because both live and dead workers reared in the absence of soil lose water more slowly than field-collected workers (Johnson, 2000b). Scanning electron microscope photographs might provide direct evidence of cuticular abrasion.

Overall, this study links avenues of water loss to causative mechanisms in queens of the harvester ant *P. barbatus*. Comparing water-loss rates and cuticular hydrocarbon profiles on the same individuals provided a powerful technique to assess the association between these two variables (Toolson, 1984), especially given that cuticular permeability caused essentially all mating related increases in water loss. However, these data also demonstrate the problem of using correlative rather than experimental data. Hydrocarbon profiles changed in the predicted pattern, but changes in composition were so slight that they may not have significantly affected cuticular permeability. Cuticular abrasion was the only mechanism that we could document to increase cuticular permeability, and this occurred only as a result

of nest excavation. The mechanism that increased water-loss rates for live but not dead newly mated dealate queens remains unclear. Moreover, rapid increases in water-loss rates induced by mating and colony founding, along with the different patterns exhibited by live and dead individuals, indicate that these queens provide an ideal system to examine multiple aspects of water balance.

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