Social conditions facilitate water conservation in a solitary bee

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Abstract

Climatic stressors are important drivers in the evolution of social behavior. Social animals tend to thrive in harsh and unpredictable environments, yet the mechanisms underlying social advantages in these contexts are often unclear. Here, we explore water conservation in forced associations of a solitary bee to test the hypothesis that grouping can generate synergistic physiological benefits in an incipient social context. Paired bees displayed mutual tolerance and experienced reduced water loss relative to singleton bees when exposed to acute low humidity stress. Social advantages like these can facilitate the evolution of cooperation among non-relatives and offer important insights into the social consequences of climate change.

Introduction

Group living can arise as an adaptive strategy for coping with environmental challenges. For insects, as small-bodied terrestrial organisms, conservation of body water is principal among these challenges [1]. Temporary grouping is known to facilitate water conservation in insects, particularly during seasonal dry periods or vulnerable life history stages [2–4]. These same physiological benefits of grouping may also play a role in the evolution of more stable societies [5,6], though empirical evidence for these effects is scarce. Here, we artificially induce social conditions in a typically solitary bee (*Melissodes tepidus timberlakei* Cockerell, 1926) to test the hypothesis that grouping can generate water conservation benefits even in the absence of a phylogenetic history of social behavior.

Water availability shapes the distributions of social organisms by shaping the costs and benefits of grouping in a given environmental context [7–11]. Complex social organization can generate novel strategies for regulating nest humidity and collective water balance, particularly among the eusocial insects [12–15]. Likewise in simpler societies, such as ant foundress associations and the facultatively social bees and wasps, water balance considerations can shape social decisions [5,6,16–20]. Particularly for soil-nesting species, these effects may be exacerbated by increased drought under climate change, which is causing rapid drying of soils in many regions [21]. These shifts in water availability and predictability will likely have profound but poorly understood consequences for animal social organization and social evolution [22,23].

Communal nesting strategies are often present at low levels in otherwise solitary populations [24–26], providing a useful empirical context for examining the selective factors underlying transitions from solitary to group living. These societies typically consist of unrelated individuals, wherein mutualistic benefits of grouping compensate for the intrinsic costs of cooperating with non-kin [6,27]. We investigated water conservation in a solitary bee (*Melissodes tepdius timberlakei* Cockerell, 1926) nesting in water-saturated soil. Communal nesting has not been described for *M. tepidus* , but is known in the genus [28]. We exposed single and artificially paired bees to a low-humidity stress assay to explore social impacts on water balance. These

1

interactions between grouping and physiological stress resistance have implications for social evolutionary change in changing environments.

Methods

Study population, nesting biology, and field collections

We sampled bees from an aggregation of *M. tepidus timberlakei* nesting along the shoreline of a coastal lagoon on the University of California, Santa Barbara campus (34.410358 N, -119.850442 W). This nesting aggregation hosts several hundred nests annually and has been observed at this location since at least 2019. Nests are predominantly concentrated along a 40 m stretch of the lagoon shoreline, within 2 m of the water, and are found both in bare soil and beneath patches of Alkali Heath (*Frankenia salina* (Molina) I.M. Johnst.). Nest initiation began in mid-May 2023, and flight activity continued through July 2023. We periodically measured soil moisture throughout the nesting site using a TDR soil moisture meter (FieldScout TDR 300, Spectrum Technologies, Inc., Aurora, IL) between June 2022 and May 2023 and found that the soil was consistently at its saturation point (52% volumetric water content).

We collected adult female *M. tepidus timberlakei* exiting their nests between late June and early July 2023, by placing plastic cups over nest entrances to capture bees departing on morning foraging trips. We transported bees back to the lab within one hour of collection, where they were weighed using a microbalance (0.001g precision). All bees were paint-marked on the thorax using an oil-based paint pen to facilitate identification during data collection and to prevent resampling on subsequent days. After assays, bees were rehydrated and released at their nesting site.

Water loss assays

We used a gravimetric water loss assay to compare water loss in single and paired bees at roughly 0% humidity, at 24 ± 1°C. Bees were placed in 15 mL falcon tubes containing 5 mL desiccant (silica gel), from which they were separated by 2 mL of cotton. Bees were randomly assigned to a social condition treatment, as either singletons (1 bee per tube, $n = 31$ bees) or pairs (2 bees per tube, $n = 28$ bees). We kept bees in the 0% humidity tubes for 3 hours before re-weighing. We assumed that mass loss equaled water loss over this time interval [1] and estimated total water loss as the difference in mass before and after the 3 hour assay. We estimated water loss as a percentage of total body mass as follows:

% body mass lost = [(initial mass – final mass) / (initial mass)] x 100

Additionally, to estimate the total body water content of *M. tepidus timberlakei* females, we dried a subset of females ($n = 6$) in a drying oven for 3 days at 60°C, then subtracted dry mass from initial live mass.

Behavioral observations

To estimate the activity level of bees as a possible predictor of water loss, we performed behavioral scan samples of each individual every 20 minutes throughout the 3-hour assay. We recorded behaviors according to a standard ethogram and observed three unique behaviors during the assays: 1) idle, 2) walking/climbing, and 3) self-grooming. No social behaviors, including agonistic interactions, were observed. We estimated the proportion of active time as the number of scan samples where an active behavior (walking/climbing) was recorded divided by the total number of scan samples. Following assays, bees were rehydrated and returned to their nest site, excluding those sacrificed to estimate dry mass.

Statistical analysis

To understand how social condition influences water conservation, we fit two linear regression models, one with the absolute mass lost as the response variable, and one with the proportional mass lost as the response variable. As predictor variables, we included factors we expected to influence water conservation, including: 1) the social condition (singleton vs. paired), 2) the initial body mass, 3) the proportion of active time, and 4) the interaction between the social condition and the proportion of active time. The interaction term tested the hypothesis that social condition influences active time, i.e., if proximity to another bee stimulates

activity. We confirmed that models met assumptions of normality and homoscedasticity by evaluating QQ-plots and plots of fitted values versus residuals. We then performed Type-II ANOVAs on fitted models to evaluate the significance of our predictor values. We excluded from our analysis any bees that died over the course of the assay, as well as any bees that were paired with bees that died. Results are presented as mean $\pm$ standard error. All analyses were performed in R version 4.2.1 using the "car" package [29,30].

Results

Social condition significantly predicted both absolute mass lost and proportion of mass lost at 0% humidity (Table 1; Figure 1). Paired bees lost 1.892 ± 0.181 mg ($4.22 \pm 1.81\%$) of body mass, while singleton bees lost 2.452 ± 0.173 mg ($5.17 \pm 0.04\%$) of body mass. The proportion of active time also significantly predicted absolute and proportional mass loss, but initial body mass did not significantly predict either (Table 1). Likewise, the interaction between social condition and proportion of active time did not predict absolute or proportional mass loss. On average, *M. tepidus* females sampled in our study contained 26.0 ± 1.86 mg ($62.2 \pm 0.94\%$) body water.

Table 1. ANOVA tables showing the effects of evaluated predictor variables (social condition, activity time, initial mass, and the interaction between social condition and activity time) on two response variables (top: total mass lost, and bottom: the proportion of mass lost).

Total mass lost (mg)

	Sum Sq.	Df	F-Value
Social condition (singleton vs. paired)	5.076×10^{-6}	1	5.785
Proportion of time spent active	4.143×10^{-6}	1	4.722
Initial mass	1.253×10^{-6}	1	1.428
Social condition:Prop. active time	4.190×10^{-6}	1	0.560
Residuals	4.651×10^{-5}	53	

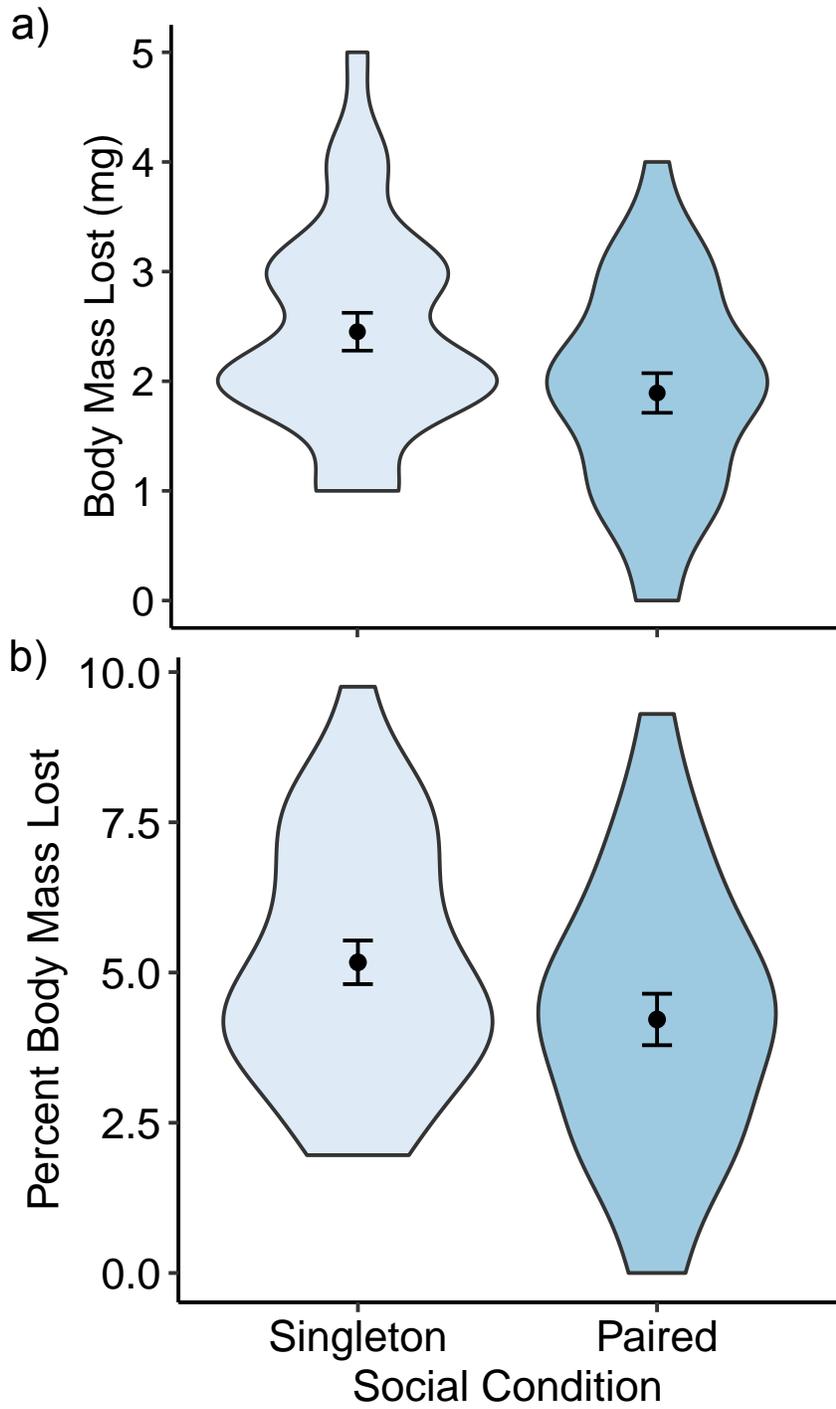| **Proportion of mass lost**	**Proportion of mass lost**	**Proportion of mass lost**	**Proportion of mass l**
	Sum Sq.	**Df**	**F-Value**
Social condition (singleton vs. paired)	2.275×10^{-3}	1	5.535
Proportion of time spent active	2.055×10^{-3}	1	4.999
Initial mass	8.761×10^{-4}	1	2.131
Social condition:Prop. active time	2.785×10^{-4}	1	0.678
Residuals	2.179×10^{-2}	53	

3

Figure 1: Figure 1. Water loss in singleton ($n = 31$) vs. paired ($n = 28$) bees estimated as the (a) absolute and (b) percent mass lost over 3 hours at 0% humidity. The width of the violin indicates the density of the data at a given value. The black dots and error bars show mean and standard error, respectively.

Discussion

Group living fundamentally alters an organism's microclimatic niche [31–35]. The group niche can offer refuge from stressful environmental conditions, particularly under climate change. Here, we demonstrated that social conditions facilitate water conservation in typically solitary bees. When exposed to low humidity, paired bees retained more water than bees kept alone, with no differences in activity level. These advantages of social living offer insights into the ecological conditions that can give rise to incipient communal societies.

Several mechanisms have been proposed to account for social water conservation advantages. These benefits have been described across arthropod taxa, including beetles [36–38], cockroaches [4], bed bugs [2], woodlice [39,40], and larval Lepidoptera [3,41]. These animals aggregate in the tens to thousands of individuals, greatly reducing their collective surface-area-to-volume ratio and thereby reducing evaporative water loss. Interestingly, we found similar benefits for groups of just two individuals. Paired bees in our study generally stood adjacent to one another rather than forming a tight huddle, indicating that their effective surface-area-to-volume ratios were not substantially changed in the social treatment. Instead, it may be that groups benefitted from altered microclimates, perhaps through the creation of a humidified boundary layer via mutual transpiration [2]. Simultaneously, paired bees may benefit from reduced respiratory water loss if metabolic rate decreases with increasing group size, as in other insects [38,42]. Metabolic studies of paired and single solitary or communal bees have the potential to clarify the mechanisms underlying this social advantage.

Bees in the social treatment experienced a modest but significant reduction in water loss relative to singletons (~2% of average body water content) in just three hours at 0% humidity. Over longer time periods in natural contexts, accumulated differences in water loss could account for differential mortality and/or fitness outcomes for solitary vs. social individuals. Similarly, we found no effect of body size on water loss in our study, though longer exposures to low-humidity stress could reveal size effects on water balance, as in other systems [1,43]. Importantly, our study design allowed for non-destructive sampling with minimal observed harm to study subjects. Bees were rehydrated and returned to their nest sites within five hours of capture, and observed foraging on subsequent days. Many common insect physiological stress assays are lethal or inflict severe sublethal injuries, limiting their usefulness for large-scale studies of non-model insect systems. Our study provides a template for future studies aimed at expanding our understanding of physiological stress responses in rare and declining bee species while minimizing impacts on source populations.

Under climate change, increasing drought will restrict soil moisture in many regions, with unknown consequences for the behavior and distributions of ground-nesting bees, many of which exhibit preferences for particular soil abiotic conditions [44,45]. Our study population of *M. tepidus timberlakei* nests in water-saturated soil, suggesting a possible preference for moisture-rich soils. Species-level data on bees' soil moisture preferences are scarce, and the nesting preferences of *M. tepidus* are largely unknown. More data on soil conditions at *M. tepidus* nesting aggregations could shed light on the breadth of soil moisture conditions they tolerate. Under unfavorable conditions, joining a nest with other females could increase humidity in nesting tunnels and help mitigate challenges of maintaining water homeostasis in dry soils.

Combined, our behavioral and physiological data suggest both a capacity for group living as well as a physiological advantage of grouping in dry conditions. Water loss increased significantly with active time, in line with previous studies [1,46], yet paired bees were no more active than singleton bees, suggesting that proximity to another bee did not stimulate activity (e.g., avoidance). Furthermore, we did not observe any instances of aggression in pairs. The lack of aggressive or avoidant behaviors observed in our study mirrors observations of other communal and solitary bees [47,48], and indicates a capacity for mutual tolerance of unrelated conspecifics, an important pre-adaptation to the formation of stable communal groups. Contexts like these that combine behavioral plasticity with selective advantages for social individuals may broadly resemble conditions at the evolutionary origins of group living.

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Data Accessibility

All data and associated code are available at: https://doi.org/10.5281/zenodo.8237366.

References

1. Hadley N. 1994 *Water Relations of Terrestrial Arthropods* . New York: Academic Press.

2. Benoit JB, Grosso N, Yoder J, Denlinger DL. 2007 Resistance to dehydration between bouts of blood feeding in the bed bug, *Cimex lectularius* , is enhanced by water conservation, aggregation, and quiescence. *Am J Trop Med Hyg* **76** , 987–993.

3. Klok CJ, Chown SL. 1999 Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. *Funct. Ecol.* **13** , 417–427.

4. Yoder JA, Grojean NC. 1997 Group influence on water conservation in the giant Madagascar hissing-cockroach, *Gromphadorhina portentosa*(Dictyoptera: Blaberidae). *Physiol. Entomol.* **22** , 79–82. (doi:10.1111/j.1365-3032.1997.tb01143.x)

5. Johnson R. 2021 Desiccation limits recruitment in the pleometrotic desert seed-harvester ant *Veromessor pergandei* . *Ecol. Evol.* **11** , 294–308. (doi:10.1002/ece3.7039)

6. Ostwald M, Haney B, Fewell J. 2022 Ecological Drivers of Non-Kin Cooperation in the Hymenoptera. *Front. Ecol. Evol.* **10** , 768392. (doi:doi: 10.3389/fevo.2022.768392)

7. Jetz W, Rubenstein DR. 2011 Environmental Uncertainty and the Global Biogeography of Cooperative Breeding in Birds. *Curr. Biol.***21** , 72–78. (doi:10.1016/j.cub.2010.11.075)

8. La Richelière F *et al.* 2022 Warm and arid regions of the world are hotspots of superorganism complexity. *Proc. R. Soc. B Biol. Sci.* **289** . (doi:10.1098/rspb.2021.1899)

9. Lukas D, Clutton-Brock T. 2017 Climate and the distribution of cooperative breeding in mammals. *R Soc Open Sci* **4** , 160897.

10. Purcell J. 2011 Geographic patterns in the distribution of social systems in terrestrial arthropods. *Biol. Rev.* **86** , 475–491. (doi:10.1111/j.1469-185X.2010.00156.x)

11. Purcell J, Avilés L. 2008 Gradients of precipitation and ant abundance may contribute to the altitudinal range limit of subsocial spiders: insights from a transplant experiment. *Proc R Soc B***275** , 2617–2625. (doi:10.1098/rspb.2008.0582)

12. Bollazzi M, Roces F. 2010 Leaf-cutting ant workers (Acromyrmex heyeri) trade off nest thermoregulation for humidity control. *J. Ethol.* **28** , 399–403. (doi:10.1007/s10164-010-0207-3)

13. Lindauer M. 1954 Temperaturregulierung und Wasserhaushalt im Bienenstaat. *Z Vergl Physiol* **36** , 391–432.

14. Nicolson SW. 2009 Water homeostasis in bees, with the emphasis on sociality. *J. Exp. Biol.* **212** , 429–434. (doi:10.1242/jeb.022343)

15. Ostwald M, Smith ML, Seeley TD. 2016 The behavioral regulation of thirst, water collection and water storage in honey bee colonies.*J. Exp. Biol.* **219** , 2156–2165. (doi:10.1242/jeb.139824)

16. Bohart G, Youssef N. 1976 The Biology and Behavior of *Evylaeus galpinsiae* . *Wasmann J. Biol.* **34** , 185–234.

17. Danforth B. 1991 Female Foraging and Intranest Behavior of a Communal Bee, *Perdita portalis* (Hymenoptera: Andrenidae).*Ann. Entomol. Soc. Am.* **84** , 537–548.

18. McCorquodale DB. 1989 Soil softness, nest initiation and nest sharing in the wasp, *Cerceris antipodes* (Hymenoptera: Sphecidae).*Ecol. Entomol.* **14** , 191–196.

19. Ostwald M, Fox TP, Hillery WS, Shaffer Z, Harrison JF, Fewell JH. 2022 Group-living carpenter bees conserve heat and body mass better than solitary individuals in winter. *Anim. Behav.* **189** , 59–67. (doi:10.1016/j.anbehav.2022.04.012)

20. Pfennig W. 1995 Absence of joint nesting advantage in desert seed harvester ants: evidence from a field experiment. *Anim Behav***49** , 567–575.

21. Berg A, Sheffield J. 2018 Climate Change and Drought: the Soil Moisture Perspective. *Curr. Clim. Change Rep.* **4** , 180–191. (doi:10.1007/s40641-018-0095-0)

22. Blumstein DT, Hayes LD, Pinter-Wollman N. 2022 Social consequences of rapid environmental change. *Trends Ecol. Evol.* , S0169534722002853. (doi:10.1016/j.tree.2022.11.005)

23. Ostwald MM, da Silva CRB, Seltmann K. 2023 How does climate change impact social bees and bee sociality? *Authorea Preprints*(doi:10.22541/au.169175894.41597921/v1)

24. Heinze J, Kellner K, Seal J. 2017 Sociality in Ants. In*Comparative Social Evolution* (eds DR Rubenstein, P Abbot), pp. 21–49. Cambridge: Cambridge University Press.

25. Michener C. 2007 *The Bees of the World* . Baltimore, MD: Johns Hopkins University Press.

26. Ross K, Matthews R, editors. 1991 *The Social Biology of Wasps* . Ithaca, NY: Comstock Publishing Associates.

27. Clutton-Brock T. 2009 Cooperation between non-kin in animal societies. *Nature* **461** , 51–57. (doi:10.1038/nature08366)

28. Hurd P, Linsley E. 1959 Observations on the nest-site behavior of*Melissodes composita* Tucker and its Parasites, with notes on the communal use of nest entrances. *Entomol. News* **LXX** , 141–146.

29. R Core Team. 2023 R: A Language and Environment for Statistical Computing. *R Found. Stat. Comput.*

30. Fox J, Weisberg S. 2019 *An {R} Companion to Applied Regression, Third Edition* . Thousand Oaks, CA: Sage.

31. Boratynski JS, Willis CKR, Jefimow M, Wojciechowski S. 2015 Comparative Biochemistry and Physiology, Part A Huddling reduces evaporative water loss in torpid Natterer's bats, Myotis nattereri.*Comp. Biochem. Physiol. Part A* **179** , 125–132. (doi:10.1016/j.cbpa.2014.09.035)

32. Gilbert C, Robertson G, Le Maho Y, Naito Y, Ancel A. 2006 Huddling behavior in emperor penguins: Dynamics of huddling. *Physiol. Behav.* **88** , 479–488. (doi:10.1016/j.physbeh.2006.04.024)

33. Jones JC, Oldroyd BP. 2006 Nest Thermoregulation in Social Insects. In *Advances in Insect Physiology* , pp. 153–191. Elsevier. (doi:10.1016/S0065-2806(06)33003-2)

34. Krause J, Ruxton GD. 2002 *Living in Groups* . Oxford: Oxford University Press.

35. Yahav S, Buffenstein R. 1991 Huddling Behavior Facilitates Homeothermy in the Naked Mole Rat Heterocephalus glaber. *Physiol. Zool.* **64** , 871–884.

36. Bong L-J, Neoh K-B, Yoshimura T. 2018 Comparison of Water Relation in Two Powderpost Beetles Relative to Body Size and Ontogenetic and Behavioral Traits. *Environ. Entomol.* **47** , 990–996. (doi:10.1093/ee/nvy062)

37. Rasa OAE. 1997 Aggregation in a Desert Tenebrionid Beetle: A Cost/Benefit Analysis. *Ethology* **103** , 466–487. (doi:10.1111/j.1439-0310.1997.tb00161.x)

38. Yoder JA, Denlinger DL, Wolda H. 1992 Aggregation promotes water conservation during diapause in the tropical fungus beetle, Stenotarsus rotundus. *Entomol. Exp. Appl.* **63** , 203–205.

39. Broly P, Devigne L, Deneubourg J-L, Devigne C. 2014 Effects of group size on aggregation against desiccation in woodlice (Isopoda: Oniscidea). *Physiol. Entomol.* **39** , 165–171. (doi:10.1111/phen.12060)

40. Derhé M, Moss A, Edwards D, Carmenta R, Hassall M. 2010 Predicting the effect of climate change on aggregation behaviour in four species of terrestrial isopods. *Behaviour* **147** , 151–164. (doi:10.1163/000579509X12512861455834)

41. Willmer PG. 1980 The effects of a fluctuating environment on the water relations of larval Lepidoptera. *Ecol. Entomol.* **5** , 271–292. (doi:10.1111/j.1365-2311.1980.tb01150.x)

42. Southwick EE, Roubik DW, Williams JM. 1990 Comparative energy balance in groups of africanized and european honey bees: Ecological implications. *Comp. Biochem. Physiol. A Physiol.* **97** , 1–7. (doi:10.1016/0300-9629(90)90713-3)

43. Addo-bediako A, Chown SL, Gaston KJ. 2001 Revisiting water loss in insects: a large scale view. *J. Insect Physiol.* **47** , 1377–1388.

44. Antoine CM, Forrest JRK. 2021 Nesting habitat of ground-nesting bees: a review. *Ecol. Entomol.* **46** , 143–159. (doi:10.1111/een.12986)

45. Cane JH. 1991 Soils of Ground-Nesting Bees (Hymenoptera: Apoidea): Texture, Moisture, Cell Depth and Climate. *J. Kans. Entomol. Soc.* **64** , 406–413.

46. Lighton JRB, Bartholomew GA. 1988 Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus* : Effects of temperature, body mass, group size, and humidity. *Proc. Natl. Acad. Sci.* **85** , 4765–4769. (doi:10.1073/pnas.85.13.4765)

47. McConnell-Garner J, Kukuk PF. 1997 Behavioral Interactions of Two Solitary, Halictine Bees with Comparisons among Solitary, Communal and Eusocial Species. *Ethology* **103** , 19–32. (doi:10.1111/j.1439-0310.1997.tb00003.x)

48. Packer L. 2006 Use of artificial arenas to predict the social organisation of halictine bees: Data for fourteen species from Chile. **53** , 307–315. (doi:10.1007/s00040-006-0873-x)