

Small worker bumble bees (*Bombus impatiens*) are hardier against starvation than their larger sisters

M. J. Couvillon · A. Dornhaus

Received: 6 October 2009 / Revised: 9 December 2009 / Accepted: 24 December 2009 / Published online: 12 January 2010
© Birkhäuser Verlag, Basel/Switzerland 2010

Abstract In bumble bees (*Bombus* spp.), where workers within the same colony exhibit up to a tenfold difference in mass, labor is divided by body size. Current adaptive explanations for this important life history feature are unsatisfactory. Within the colony, what is the function of the smaller workers? Here, we report on the differential robustness to starvation of small and large worker bumble bees (*Bombus impatiens*); when nectar is scarce, small workers remain alive significantly longer than larger workers. The presence of small workers, and size variation in general, might act as insurance against times of nectar shortage. These data may provide a novel, adaptive explanation, independent of division of labor, for size polymorphism within the worker caste.

Keywords Polymorphism · Robustness · Social insects · Bumble bees · *Bombus impatiens*

Introduction

For organisms that are indisputable ecological successes, biologists have devoted decades to examining what traits might contribute fitness advantages. Have those traits evolved for specific purposes or were they successfully

co-opted at a later time? How might characteristics confer a positive fitness consequence on the evolutionary lifespan of a genus? One of the most successful groups of organisms is the social Hymenoptera. Ants, bees, and wasps are ecologically dominant, species-rich, and contribute a very large proportion to the terrestrial biomass (Oster and Wilson, 1978; Wilson, 1987; Hölldobler and Wilson, 1990; Michener, 2000; Chapman and Bourke, 2001). In addition, social insects frequently display many interesting and unique life history traits whose adaptive function remains unclear.

For example, a bumble bee (*Bombus* spp.) worker may be up to tenfold greater in mass than her sister (Fig. 1) from the same colony (Cumber, 1949; Plowright and Jay, 1968; Alford, 1975; Goulson et al., 2002). This size polymorphism, symmetric about a single mean, is present throughout the colony cycle and independent of resource availability (Couvillon et al., submitted). Its presence is linked with division of labor within a bumble bee colony; the larger workers tend to forage, whereas smaller workers concentrate on intranidal tasks like nursing and incubating (Cumber, 1949; Goulson et al., 2002; Foster et al., 2004; Gardner et al., 2007; Jandt and Dornhaus, 2009). However, is effective division of labor the evolutionary reason for size polymorphism? There are good data to demonstrate that larger workers are better at foraging compared to smaller workers; larger foragers bring back more nectar per unit time (Goulson et al., 2002; Spaethe and Weidenmüller, 2002), fly at cooler temperatures (Free and Butler, 1959), probe deeper flowers (Peat et al., 2005), and may be less prone to predation (Goulson et al., 2002). However, if size polymorphism in bumble bees is an adaptation for division of labor, we would predict that smaller workers should also be better at their tasks. This has not yet been shown. In fact, there is even evidence to suggest that smaller workers are

M. J. Couvillon · A. Dornhaus
Department of Ecology and Evolutionary Biology,
University of Arizona, Tucson, AZ 85721, USA

M. J. Couvillon (✉)
Laboratory of Apiculture and Social Insects,
Department of Biology and Environmental Science,
University of Sussex, Falmer,
Brighton BN1 9QG, UK
e-mail: M.Couvillon@sussex.ac.uk



Fig. 1 Though bumble bee (*Bombus impatiens*) workers are highly related ($r = 0.75$), there may be a tenfold difference in mass between sisters of the same nest

also less efficient at nursing compared to larger workers (Cnaani and Hefetz, 1994). Why then is size polymorphism such a consistent feature in *Bombus*?

Bumble bees evolved in temperate and alpine climates (Hines, 2008), which can experience rainy spells of many days or even weeks in which foraging is impossible. Bumble bee colonies are annual (Cumber, 1949) and do not store sufficient food to last for extended periods; thus, during such periods, a colony could potentially starve. However, if even a fraction of the workers remains alive, this could have positive fitness consequences, as they may raise remaining brood and also lay male eggs, ensuring a genetic contribution for the next generation.

Here we ask whether an individual worker's physiology might preferentially predispose her to survive a nectar shortage. Specifically, we tested whether differential survival to starvation might be correlated with worker body size.

Methods

We obtained four queenright bumble bee colonies (*B. impatiens*, colonies 1–4) from a commercial breeder (Koppert Biological Systems, Romulus, MI, USA). At the start of the experiment, colonies typically had 15–30 workers plus queen with brood; each colony at its peak size had approximately 350 individual workers with natural body size variation (Cnaani et al., 2002) plus brood and honey stores (Fig. 2). Colonies were housed in wooden boxes (22 × 22 × 11 cm) with screened ventilation holes and a Plexiglas cover, which allowed us visible access to the entire nest. The colonies were connected by plastic tubing to a foraging chamber (58 × 36 × 40 cm), where sugar

solution (“BeeHappy”, provided by Koppert) was presented in feeders ad libitum for 3 months. Defrosted pollen (1 rounded tsp, approximately 5 g) was delivered directly to the nest through a round opening in the Plexiglas cover daily. Colonies were kept at constant room temperature (25°C) and ambient humidity. The bumble bees behaved normally with the overhead lights (12:12 L:D) that were on during the day, as reported in previous studies (Couvillon and Dornhaus, 2009; Jandt and Dornhaus, 2009).

We did not control for the age of the workers; however, recent data demonstrate that all sizes of bees are produced throughout the entire colony cycle (Couvillon et al., submitted). Therefore, we do not expect large and small bees to differ in age, so any variation in lifespan is evenly distributed across the bees.

After 3 months, we discontinued feeding the colonies. For all colonies, the queen was still alive at the time of food discontinuation. Colonies survived for 3–6 days on stored honey; the honeypots were easy to see, as our colony nests are flat and spread out in the boxes. Once all visible honeypots were empty (of both honey and stored pollen, although pollen does not contain enough sugar to keep an adult alive), we collected dead bees every day at the same time (10:00 a.m.) and measured their body size using thorax width, which is a standard measurement of bumble bee size (Goulson et al., 2002). In total, we measured 1,432 dead bees from the four colonies.

Results

Body size was not normally distributed (Kolmogorov–Smirnov, $P < 0.05$ for all colonies), and the size distribution in all four colonies was positively skewed (Colony 1: skew = 0.59, Colony 2: skew = 0.5, Colony 3: skew = 0.49, Colony 4: skew = 0.30). Based on the Central Limit Theorem, our sample sizes were sufficient to justify the use of parametric statistics for our regression analysis (Sokal and Rohlf, 1995); however, we also analyzed using non-parametric statistics and obtained the same results.

The mean worker body size differed significantly among the colonies (Colony 1 ($n = 361$): 3.54 mm, Colony 2 ($n = 623$): 2.99 mm, Colony 3 ($n = 243$): 3.80 mm, Colony 4 ($n = 205$): 3.85 mm, One-way ANOVA, $F = 125.00$, $P < 0.001$; Fig. 2). This significance was maintained using non-parametric statistics (Kruskal–Wallis, $H = 307.42$, $P < 0.001$).

The average size of worker bees that died from starvation significantly decreased in all colonies the longer the colonies went without food, demonstrating that the larger bees died earlier. Thus, smaller workers are more robust to

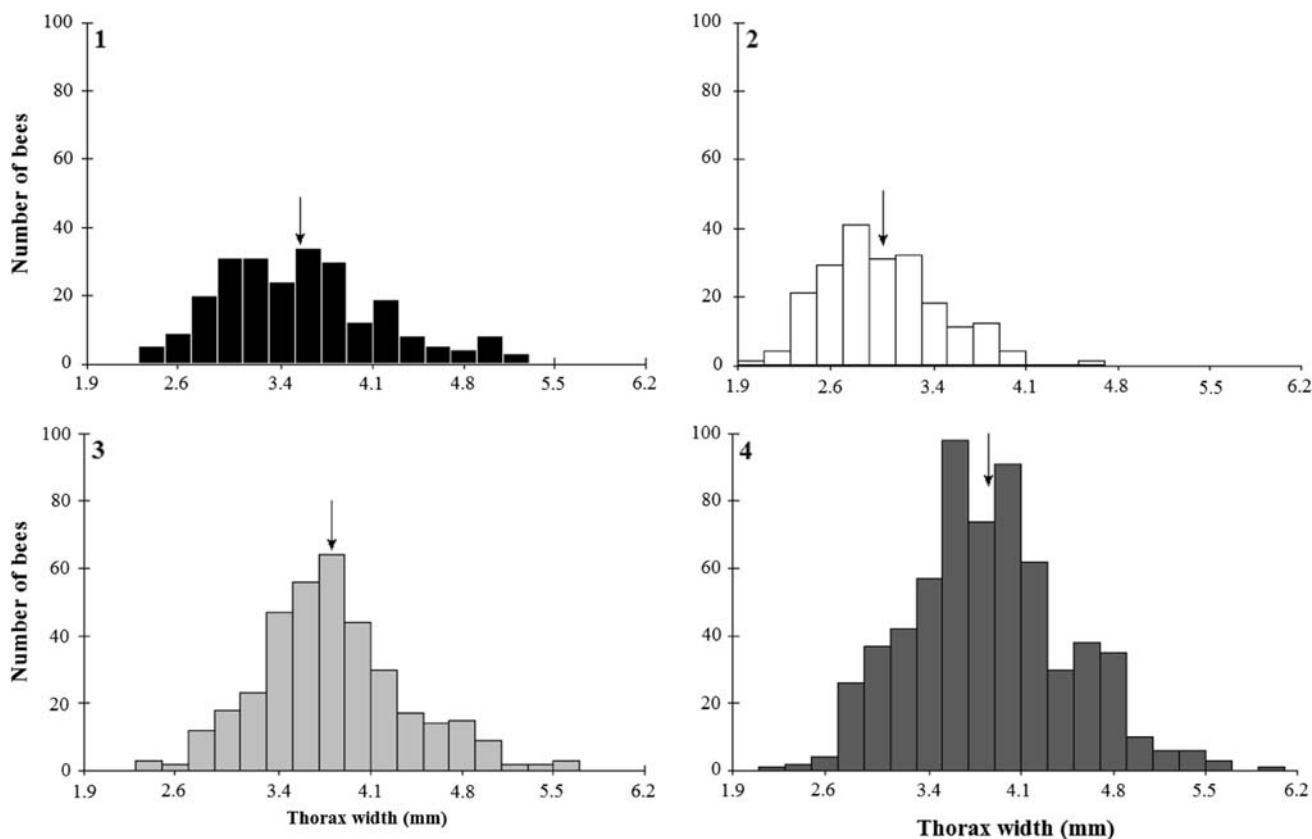


Fig. 2 Mean (arrows) worker body size differed significantly among the colonies. Body size was not normally distributed (Kolmogorov–Smirnov, $P < 0.05$ for all colonies). Size distribution in all four

colonies was positively skewed (Colony 1: $n = 361$, skew = 0.59, Colony 2: $n = 623$, skew = 0.5, Colony 3: $n = 243$, skew = 0.49, Colony 4: $n = 205$, skew = 0.30)

starvation compared to larger workers (Regression analyses—Colony 1: $R^2 = 0.12$, $F_{1,1} = 31.72$, $P < 0.001$; Colony 2: $R^2 = 0.21$, $F_{1,1} = 53.01$, $P < 0.001$; Colony 3: $R^2 = 0.19$, $F_{1,1} = 81.52$, $P < 0.001$; Colony 4: $R^2 = 0.10$, $F_{1,1} = 71.84$, $P < 0.001$) (Fig. 3). This significance was maintained using non-parametric statistics (Spearman’s Rank Correlation—Colony 1: -0.34 , $P < 0.001$; Colony 2: -0.41 , $P < 0.001$; Colony 3: -0.41 , $P < 0.001$; Colony 4: -0.40 , $P < 0.001$).

Discussion

We found that smaller worker bees were significantly more resilient to starvation than larger workers bees. This supports the hypothesis that individual worker physiology, specifically body size, might be correlated with ability to survive nectar dearth.

Why might smaller worker bumble bees better withstand starvation? One simple hypothesis would be that small workers need less food per individual per day to survive. Alternatively, smaller workers may store more nutrients in their body. If the latter is the case, starvation resistance

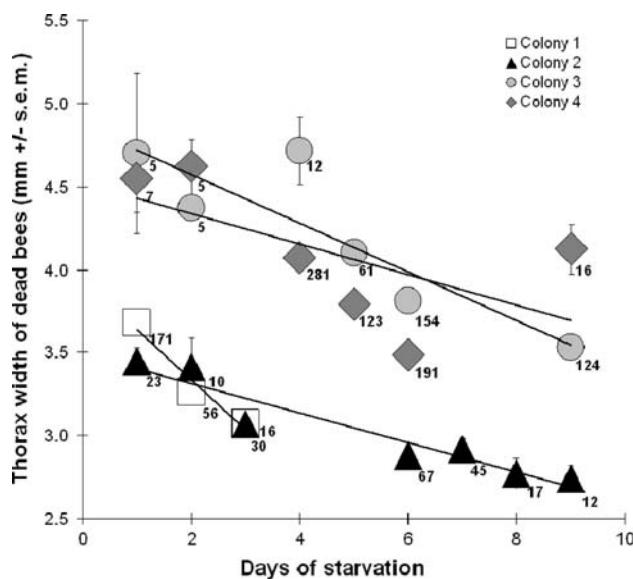


Fig. 3 Smaller worker bees lived significantly longer than larger worker bees in all four colonies (Regression analysis, all $P < 0.001$; mean values \pm SEM are shown). Different symbols represent different colonies, and the numbers beside each symbol are how many bees died and were measured on that day for that colony. “Days of starvation” is the time since honey stores were emptied

may be a result of task specialization in the colony instead of body size per se. In particular, nurse bee physiology may better withstand starvation; in social hymenoptera, there is a general trend for nurses to carry a proportionally higher lipid content (Tschinkel, 1998; Markiewicz and O'Donnell, 2001; Toth and Robinson, 2005). Smaller bees tend to be nurses more often than larger bees (Jandt and Dornhaus, 2009). It was suggested that fatter nurses and, conversely, leaner foragers are adaptive because foraging typically occurs toward the end of a worker's life, and keeping them lean is cheap as foragers experience higher mortality (i.e., a "disposable" caste) (Porter and Jorgensen, 1981). It is important to note, however, that the foragers in our study did not have very far to forage (<30 cm); therefore, it is unlikely that the bees disproportionately expended their stored energy, although this would be an interesting idea to test in both laboratory and field. Finally, bumble bee workers do not necessarily forage toward the end of their lives (Yerushalmi et al., 2006). Thus, whether or not bumble bee nurses also store more lipids or other nutrients, and how this relates to task specialization and age, remains unknown; we are now investigating this issue.

Mortality in our study may also have been affected by water loss in addition to starvation because we did not provide bees with separate water feeders; however, smaller bees, with a higher surface area:volume ratio, would be more susceptible to desiccation through the cuticle. In fact, there are much previous data to suggest that larger size in organisms reduces water loss (Chown and Gaston, 1999; Hoffmann and Harshman, 1999; Gilchrist et al., 2001; Telonis-Scott et al., 2006; Stillwell et al., 2007). Instead, smaller bees survived longer, which runs counter to previous work on starvation and desiccation resistance in other insects (Heinze et al., 2003; Stillwell et al., 2007). It might be interesting to model the optimal size distribution under the assumption of particular cost/benefit ratios in different conditions like starvation or desiccation, which would allow us to make predictions regarding the evolution of size distribution.

Of course, even if size polymorphism imparts fitness through increased survival to starvation, it may or may not also be an adaptation for division of labor (Couvillon and Dornhaus, 2009), as these hypotheses are not mutually exclusive. In division of labor, workers specialize, either permanently or temporarily, on a subset of tasks. This is thought to increase the efficiency (fitness) of a system as compared to one where all workers perform all tasks indiscriminately (Oster and Wilson, 1978; Beshers and Fewell, 2001; O'Donnell and Foster, 2001). Indeed, division of labor has been cited as a major reason for the incredible success of social insects (Wilson, 1985; Robinson, 1992). However, if this were the case in bumble bees, a difference in benefit/cost ratio of producing small and

large workers for different tasks would have to be shown (Dornhaus, 2008). In addition, when thinking about adaptive explanations, it is important to note that efficiency, which describes superior performance under normal conditions, is not the only measure of biological fitness. Robustness is concerned with continued performance under non-ideal conditions (Bonabeau et al., 2000). When nectar is scarce, small workers remain alive significantly longer than larger workers. The presence of small workers, which might be less efficient at "normal" colony work of foraging and nursing, and size variation in general, might impart robustness, but not necessarily efficiency, to bumble bee colonies, allowing them to survive during times of high colony stress. Total colony fitness may be maximized by balancing the trade-off between the expensive, non-robust but very efficient large workers and the inexpensive, non-efficient but very robust small workers. As such, these data may provide a novel, adaptive explanation for size polymorphism that is independent of division of labor and open up a new area of investigation. Identifying what exactly in the physiology of smaller bees confers starvation robustness would be an interesting next step in studying the fitness benefits of size polymorphism.

Acknowledgments We thank Jennifer Bonds for her help with data collection and Duncan Jackson and Dan Papaj for comments on the manuscript. This work was funded by a NIH PERT fellowship to MJC through the Center for Insect Science.

References

- Alford D.V. 1975. *Bumblebees*, Davis-Poynter. London.
- Beshers S.N. and Fewell J.H. 2001. Models of division of labor in social insects. *Annu. Rev. Entomol.* **46**: 413–440
- Bonabeau E., Dorigo M. and Theraulaz G. 2000. Inspiration for optimization from social insect behaviour. *Nature* **406**: 39–42
- Chapman R.E. and Bourke A.F.G. 2001. The influence of sociality on the conservation biology of social insects. *Ecol. Lett.* **4**: 650–662
- Chown S.L. and Gaston K.J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biol. Rev.* **74**: 87–120
- Cnaani J. and Hefetz A. 1994. The effect of workers size frequency-distribution on colony development in *Bombus terrestris*. *Insect. Soc.* **41**: 301–307
- Cnaani J., Schmid-Hempel R. and Schmidt J.O. 2002. Colony development, larval development and worker reproduction in *Bombus impatiens* Cresson. *Insect. Soc.* **49**: 164–170
- Couvillon M.J. and Dornhaus A. 2009. Location, location, location: larvae position inside the nest is correlated with adult body size in worker bumble bees (*Bombus impatiens*). *Proc. R. Soc. B* **276**: 2411–2418
- Cumber R.A. 1949. The biology of humble-bees, with special reference to the production of the worker caste. *Trans. R. Ent. Soc. Lond.* **100**: 1–45
- Dornhaus A. 2008. Specialization does not predict individual efficiency in an ant. *PLoS* **6**: e285
- Foster R.L., Brunskill A., Verdirame D. and O'Donnell S. 2004. Reproductive physiology, dominance interactions, and division of labour among bumble bee workers. *Physiol. Entomol.* **29**: 327–334

- Free J.B. and Butler C.G. 1959. *Bumblebees*, Collins. London.
- Gardner K., Foster R. and O'Donnell S. 2007. Experimental analysis of worker division of labor in bumblebee nest thermoregulation (*Bombus huntii*, Hymenoptera: Apidae). *Behav. Ecol. Sociobiol.* **61**: 783–792
- Gilchrist G.W., Huey R.B. and Serra L. 2001. Rapid evolution of wing size clines in *Drosophila subobscura*. *Genetica* **112**: 273–286
- Goulson D., Peat J., Stout J.C., Tucker J., Darvill B., Derwent L.C. and Hughes W.O.H. 2002. Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Anim. Behav.* **64**: 123–130
- Heinze J., Foitzik S., Fischer B., Wanke T. and Kipyatkov V.E. 2003. The significance of latitudinal variation in body size in a holarctic ant, *Leptothorax acervorum*. *Ecography* **26**: 349–355
- Hines H.M. 2008. Historical biogeography, divergence times, and diversification patterns of bumble bees (Hymenoptera: Apidae: *Bombus*). *Syst. Biol.* **57**: 58–75
- Hoffmann A.A. and Harshman L.G. 1999. Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. *Heredity* **83**: 637–643
- Hölldobler B. and Wilson E.O. 1990. *The Ants*, Harvard University Press. Cambridge, Massachusetts. 732 pp
- Jandt J. and Dornhaus A. 2009. Spatial organization and division of labor in the bumble bee, *Bombus impatiens*. *Anim. Behav.* **77**: 641–651
- Markiewicz D. and O'Donnell S. 2001. Social dominance, task performance and nutrition: implications for reproduction in eusocial wasps. *J. Comp. Physiol. A* **187**: 327–333
- Michener C.D. 2000. *The Bees of the World*, Johns Hopkins University Press. Baltimore.
- O'Donnell S. and Foster R.L. 2001. Thresholds of response in nest thermoregulation by worker bumble bees, *Bombus bifarius nearcticus* (Hymenoptera: Apidae). *Ethology* **107**: 387–399
- Oster G.F. and Wilson E.O. 1978. *Caste and Ecology in the Social Insects*, Princeton University Press. Princeton, New Jersey. 352 pp
- Peat J., Tucker J. and Goulson D. 2005. Does intraspecific size variation in bumblebees allow colonies to efficiently exploit different flowers? *Ecol. Entomol.* **30**: 176–181
- Plowright R.C. and Jay S.C. 1968. Caste differentiation in bumblebees (*Bombus* Latr.: Hym.) .1. Determination of female size. *Insect. Soc.* **15**: 171–192
- Porter S.D. and Jorgensen C.D. 1981. Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? *Behav. Ecol. Sociobiol.* **9**: 247–256
- Robinson G.E. 1992. Regulation of division-of-labor in insect societies. *Annu. Rev. Entomol.* **37**: 637–665
- Sokal R.R. and Rohlf F.J. 1995. *Biometry*, W.H. Freeman and Company. New York. 850 pp
- Spaethe J. and Weidenmuller A. 2002. Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insect. Soc.* **49**: 142–146
- Stillwell R.A.C., Morse G.A.E. and Fox C.A. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *Amer. Nat.* **170**: 358–369
- Telonis-Scott M., Guthridge K.M. and Hoffmann A.A. 2006. A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance: response to selection, physiology and correlated responses. *J. Exp. Biol.* **209**: 1837–1847
- Toth A.L. and Robinson G.E. 2005. Worker nutrition and division of labour in honeybees. *Anim. Behav.* **69**: 427–435
- Tschinkel W.R. 1998. Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insect. Soc.* **45**: 385–410
- Wilson E.O. 1985. The sociogenesis of insect colonies. *Science* **228**: 1489–1495
- Wilson E.O. 1987. Causes of ecological success: the case of the ants. *J. Anim. Ecol.* **56**: 1–9
- Yerushalmi S., Bodenheimer S. and Bloch G. 2006. Developmentally determined attenuation in circadian rhythms links chronobiology to social organization in bees. *J. Exp. Biol.* **209**: 1044–1051