BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Research

Cite this article: Brahma A, Mandal S, Gadagkar R. 2018 Current indirect fitness and future direct fitness are not incompatible. *Biol. Lett.* **14**: 20170592.
http://dx.doi.org/10.1098/rsbl.2017.0592

Received: 22 September 2017
Accepted: 16 January 2018

Subject Areas:
behaviour, ecology, evolution

Keywords:
social behaviour, direct fitness, indirect fitness, reproductive potential, primitively eusocial wasp, *Ropalidia marginata*

Author for correspondence:
Raghavendra Gadagkar
e-mail: ragh@iisc.ac.in

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3985308.

THE ROYAL SOCIETY PUBLISHING

Evolutionary biology

Current indirect fitness and future direct fitness are not incompatible

Anindita Brahma, Souvik Mandal and Raghavendra Gadagkar

Centre for Ecological Sciences, Indian Institute of Science, Bangalore, Karnataka 560012, India

AB, 0000-0002-9275-9051; SM, 0000-0002-9552-5613; RG, 0000-0002-0328-9378

In primitively eusocial insects, many individuals function as workers despite being capable of independent reproduction. Such altruistic behaviour is usually explained by the argument that workers gain indirect fitness by helping close genetic relatives. The focus on indirect fitness has left open the question of whether workers are also capable of getting direct fitness in the future in spite of working towards indirect fitness in the present. To investigate this question, we recorded behavioural profiles of all wasps on six naturally occurring nests of *Ropalidia marginata*, and then isolated all wasps in individual plastic boxes, giving them an opportunity to initiate nests and lay eggs. We found that 41% of the wasps successfully did so. Compared to those that failed to initiate nests, those that did were significantly younger, had significantly higher frequency of self-feeding behaviour on their parent nests but were not different in the levels of work performed in the parent nests. Thus ageing and poor feeding, rather than working for their colonies, constrain individuals for future independent reproduction. Hence, future direct fitness and present work towards gaining indirect fitness are not incompatible, making it easier for worker behaviour to be selected by kin selection or multilevel selection.

1. Introduction

A striking feature of primitively eusocial insects is the presence of functionally sterile workers who assist their queens to produce offspring. Inclusive fitness theory has been the dominant paradigm to resolve this apparent paradox. Because workers usually help genetic relatives, the evolution of such altruistic behaviour is suggested to be primarily driven by the indirect fitness gained by workers [1]. However, intra-colony relatedness can sometimes be low and workers may sometimes be completely unrelated [2,3], suggesting that indirect fitness might not always be sufficient to promote the evolution of worker behaviour. Parental manipulation and differential nutrition have also been suggested to bias development of some individuals into queens and others into workers [2,4,5]. Another factor that might promote worker behaviour is the possibility that workers also gain some direct fitness in the future. It is known however that the reproductive potential of the workers decreases with their age [6]. The question of interest is whether ageing workers can nevertheless retain their potential for future independent reproduction. One way of retaining such potential might be to conserve energy for future reproduction by working less, as has been shown in some species in the context of becoming future queens [7–10]. However, the question of whether an individual can revert back to egg-laying by leaving the parent nest and founding its own nest after being a worker remains open.

Here, we attempted to answer this question with the primitively eusocial wasp *Ropalidia marginata*, a tropical, perennial species in which workers help the queen to rear her offspring. All colonies consist of a single queen who monopolises reproduction during her tenure. Most workers are related to the queen to varying degrees and spend their whole lives working for the colony, and thus

gaining indirect fitness [2]. Workers of *R. marginata* can also sometimes gain direct fitness either by overthrowing the current queen to become the new queen or by leaving the natal nest and initiating their own nests [2]. In this experiment, we provided opportunities to every female wasp in a colony to initiate her own nest and examined which factors affected their potential for such future independent reproduction.

2. Material and methods

We used six naturally occurring, post-emergence colonies of *R. marginata* located inside the Indian Institute of Science campus, Bangalore (12.97° N, 77.59° E). We monitored the nests every night for at least two months, uniquely marked every newly eclosed wasp with quick-drying Testors© enamel paint and recorded the date of eclosion. When the ages of most of the wasps were known, we collected quantitative behavioural data for 20 h over a span of 4 days for each of the six nests in their natural habitat (see electronic supplementary materials for details). Following this, we isolated each wasp inside an aerated plastic box containing soft wood as building material and maintained them at 30°C until the end of the experiment. We provided each wasp with *ad libitum* food consisting of larva of *Corcyra cephalonica*, dilute honey and water, replenished on alternate days. We monitored each box until the wasp initiated a nest and laid an egg, or died, whichever was earlier.

We fitted a generalized linear mixed effects model with binomial error structure to identify the variables that significantly affected the probability of nest initiation in the wasps. We excluded the queens of the six nests from the analysis because they had prior experience of laying eggs. The global model was

$$\text{global model (model 1)} = \text{glmer(initiation} \sim \text{age} + \text{db} + \text{work} + \text{fe} + \text{age} \times \text{work} + (1|\text{nest ID}),$$

$$\text{data} = \text{data.q, family} = \text{binomial(link} = '\text{ logit}')),$$

where 'db', 'work' and 'fe' refer to frequencies per hour of dominance, total work done (intranidal and extranidal) and self-feeding behaviours, respectively. Next, we dropped the insignificant interaction term from model 1 to simplify it.

Our final model was

$$\text{final model (model 2)} = \text{glmer(initiation} \sim \text{age} + \text{db} + \text{work} + \text{fe} + (1|\text{nest ID}),$$

$$\text{data} = \text{data.q, family} = \text{binomial(link} = '\text{ logit}')).$$

For more details of the methods and models used, see electronic supplementary material.

3. Results

We isolated all female wasps from six nests (a total of 123 wasps, including 6 queens), out of which 41% (49 workers and 1 queen) initiated nests (hereafter, egg layers) and the remaining 59% (68 workers and 5 queens) died without initiating nests (hereafter, non-egg layers). The range of ages for the 87 wasps for which we had data was 2–61 days. The mean age at which the egg layers laid their first egg was 66.1 ± 39.1 days after eclosion, and the latency to initiate nests was 49.6 ± 35.2 days after isolation.

We found that age at isolation (table 1) and frequency per hour of self-feeding behaviour during its residence on the parent nest (table 1) had significant effects on the probability of initiating nests and laying eggs in isolation. The probability of egg-laying significantly decreased with increasing age of the wasps (GLM; estimate = −0.059, z = −2.901, $p = 0.0037$; effect size = 0.75; figure 1*a*) and increased significantly with the increase in self-feeding behaviour (GLM; estimate = 0.996, $z = 2.021$, $p = 0.043$; effect size = 0.36; figure 1*b*). These results are also corroborated by pairwise comparisons using the Mann–Whitney U test (see electronic supplementary material).

We did not find any significant effects of other potential predictor variables such as frequencies per hour of work done or dominance behaviours (table 1). The probability of nest initiation did not change significantly with increasing frequency of work done (GLM; estimate = −0.08, z = −0.95, $p = 0.34$; effect size = 0.18) and it also did not significantly change with increasing frequency per hour of dominance behaviour (GLM; estimate = 0.02, z = 0.17, $p = 0.87$; effect size = 0.03).

Table 1. Parameter estimates of global model (model 1) and final model (model 2). One (*) and two asterisks (**) signify statistically significant difference at $\alpha = 0.05$ and $\alpha = 0.01$, respectively.

predictors	estimate	*z*-value	*p*-value	effect size
global model (model 1)				
age	0.002	0.06	0.95	0.75
db	0.07	0.25	0.80	0.03
work	0.18	0.72	0.47	0.18
fe	3.72	3.28	0.001**	0.36
age × work	−0.01	−1.47	0.14	NA
final model (model 2)				
age	−0.05	−2.31	0.02*	0.75
db	0.05	0.19	0.85	0.03
work	−0.12	−0.78	0.43	0.18
fe	2.97	3.15	0.002**	0.36

4. Discussion

Here, we investigated whether working for the parent nest and gaining indirect fitness reduces the possibility of leaving the parent nest to initiate a new nest and gaining direct fitness by reproducing independently in the future. Forty-one per cent of the wasps tested retained the ability to reproduce and gain direct fitness when isolated from their parent nests. We found that the frequency per hour of self-feeding behaviour on the parent nest increased the probability of founding nests and laying eggs upon isolation. This is not surprising because it is well-known that feeding is important to maintain nutritional reserves and facilitate reproduction [2,5,11]. Feeding-self behaviour involves mastication of food by adult wasps and swallowing the juices; this is the way in which adults of 'thread-waist' wasp species acquire nutrition [12].

We also find that increasing age of the wasps decreased the probability of founding nests and laying eggs upon isolation, as has also been seen in *Polistes canadensis* [6]. Previous work on *R. marginata* has shown that there was a significant positive correlation between age and the probability of becoming the next queen of the colony [13]. Although this seems contradictory to the current results, the present study deals with future independent reproduction while the previous study

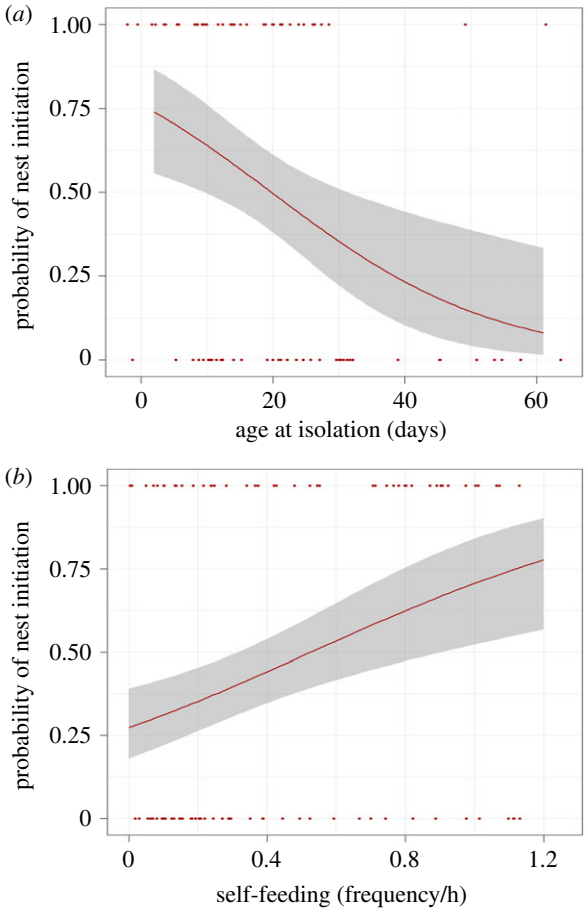

Figure 1. Probability of nest initiation with (*a*) increasing age at isolation ($N = 87$), and (*b*) increasing self-feeding behaviour on nest ($N = 117$). All comparisons were done using generalized linear models at $\alpha = 0.05$. The lines and confidence intervals are based on the model predictions, while the raw data (binary) are plotted above and below with the jitter function in *R* to help visualize the data. (Online version in colour.)

dealt with overthrowing the existing queen of the colony. Older wasps of a colony are lined up in the queue for becoming the next queen [13] and have invested heavily in terms of time and energy to help in rearing the queen's offspring; this makes independent nest foundation a less attractive option as opposed to attempting to become future queen in the same nest. Young wasps are towards the lower end of the reproductive hierarchy and the chances of becoming the egg layer of the colony in their lifetime is low, thus making independent nest foundation a more attractive option to gain direct fitness. It is therefore not surprising that age positively affects the probability of overthrowing the queen and negatively affects the probability of initiating new nests.

Interestingly, the rate of work performed on the parent colony had no influence on the probability of initiating nests and laying eggs upon isolation. It should also be noted that work × age interaction term was also not significant (table 1). Energy-intensive work like foraging is predicted to result in reduced reproductive capacity in social insects [7]. This might seem contradictory to our results, but we speculate that although *R. marginata* workers worked hard to gain indirect fitness, those that were capable of future direct fitness through independent reproduction were the individuals who had compensated by feeding more than the rest.

We did not include mating status of wasps in our analysis because mating is not essential for female wasps to found nests, develop their ovaries and lay viable eggs [2,14]. Frequency per hour of dominance behaviour had no effect on the probability of nest foundation and egg-laying. This might be because dominance behaviour appears to be unrelated to reproductive regulation or competition [2,15,16] in *R. marginata*. Somewhat surprisingly, only one out of six queens initiated nests, while the remaining five died without initiating nests, although they lived for 42–209 days after isolation and had been observed to lay eggs just before isolation. This may well be because initiating a nest without help from workers for a wasp that has already experienced being the queen of an established colony may not be required in nature, but is certainly worthy of more detailed future investigations.

5. Conclusion

Current work towards gaining indirect fitness is not incompatible with future reproduction for gaining direct fitness. This result has obvious implications for the evolution of social behaviour because altruistic working behaviour can be more easily selected as it does not preclude future direct fitness. While making it easier for kin selection to operate, our results suggest that individual selection and multilevel selection may also be relevant in the evolution of social behaviour.

Ethics. Wasps in this study were collected and euthanized humanely, according to standard collection protocols.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. R.G. and A.B. designed study, A.B. conducted the study and analysed data, S.M. assisted with collecting age data and maintaining wasps and A.B. and R.G. co-wrote the manuscript, which was reviewed by S.M. All authors agree to be held accountable for the content and approve the final version of the manuscript.

Competing interests. Authors declare no competing interests.

Funding. This work was supported by grants (to R.G.) from the Science and Engineering Research Board (SERB), Department of Science and Technology, Department of Science and Technology (including DST-FIST program), Department of Biotechnology (including DBT-IISc Partnership Program), Council of Scientific and Industrial Research, and Ministry of Environment, Forests and Climate Change, Government of India.

Acknowledgements. We are thankful to Dr Kavita Isvaran and Dr Anjan Nandi for their valuable help with statistical analysis.

References

1. Bourke AFG. 2011 *Principles of social evolution*. Oxford, UK: Oxford University Press.
2. Gadagkar R. 2001 *The social biology of Ropalidia marginata: toward understanding the evolution of eusociality*. Cambridge, MA: Harvard University Press.
3. Leadbeater E, Carruthers JM, Green JP, Rosser NS, Field J. 2011 Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science* **333**, 874–876. (doi:10.1126/science.1205140)
4. Alexander RD. 1974 The evolution of social behavior. *Annu. Rev. Ecol. Syst.* **5**, 325–383.
5. Berens AJ, Hunt JH, Toth AL. 2015 Nourishment level affects caste-related gene expression in *Polistes* wasps. *BMC Genomics* **16**, 235. (doi:10.1186/s12864-015-1410-y)
6. Sumner S, Kelstrup H, Fanelli D. 2010 Reproductive constraints, direct fitness and indirect fitness

benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*. *Proc. R. Soc. B* **277**, 1721–1728. (doi:10.1098/rspb.2009.2289)

7. O'Donnell, S. 1996 Reproductive potential and division of labor in wasps: are queen and worker behavior alternative strategies? *Ethol. Ecol. Evol.* **8**, 305–308. (doi:10.1080/08927014.1996.9522921)
8. Cant MA, Field J. 2001 Helping effort and future fitness in cooperative animal societies. *Proc. R. Soc. Lond. B* **268**, 1959–1964. (doi:10.1098/rspb.2001.1754)
9. Field J, Cronin A, Bridge C. 2006 Future fitness and helping in social queues. *Nature* **441**, 214–217. (doi:10.1038/nature04560)
10. Bridge C, Field J. 2007 Queuing for dominance: gerontocracy and queue-jumping in the hover wasp *Liostenogaster flavolineata*. *Behav. Ecol. Sociobiol.* **61**, 1253–1259. (doi:10.1007/s00265-007-0355-9)
11. Molina Y, O'Donnell S. 2008 A developmental test of the dominance-nutrition hypothesis: linking adult feeding, aggression, and reproductive potential in the paperwasp *Mischocyttarus mastigophorus*. *Ethol. Ecol. Evol.* **20**, 125–139. (doi:10.1080/08927014.2008.9522533)
12. Hunt JH. 1991 Nourishment and the evolution of the social vespidae. In *The social biology of wasps* (eds KG Ross, RW Matthews), pp. 426–450. Ithaca, NY: Cornell University Press.
13. Bang A, Gadagkar R. 2012 Reproductive queue without overt conflict in the primitively eusocial wasp *Ropalidia marginata*. *Proc. Natl Acad. Sci. USA* **109**, 14 494–14 499. (doi:10.1073/pnas.1212698109)
14. Shukla S, Shilpa MC, Gadagkar R. 2013 Virgin wasps develop ovaries on par with mated females, but lay fewer eggs. *Insectes Soc.* **60**, 345–350. (doi:10.1007/s00040-013-0299-1)
15. Bruyndonckx N, Kardile SP, Gadagkar R. 2006 Dominance behaviour and regulation of foraging in the primitively eusocial wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae). *Behav. Processes* **72**, 100–103. (doi:10.1016/j.beproc.2005.11.013)
16. Lamba S, Chandrasekhar K, Gadagkar R. 2008 Signaling hunger through aggression—the regulation of foraging in a primitively eusocial wasp. *Naturwissenschaften* **95**, 677–680. (doi:10.1007/s00114-008-0369-9)