

Research



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A split sex ratio in solitary and social nests of a facultatively social bee

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A classic prediction of kin selection theory is that a mixed population of social and solitary nests of haplodiploid insects should exhibit a split sex ratio among offspring: female biased in social nests, male biased in solitary nests. Here, we provide the first evidence of a solitary–social split sex ratio, using the sweat bee *Megalopta genalis* (Halictidae). Data from 2502 offspring collected from naturally occurring nests across 6 years spanning the range of the *M. genalis* reproductive season show that despite significant yearly and seasonal variation, the offspring sex ratio of social nests is consistently more female biased than in solitary nests. This suggests that split sex ratios may facilitate the evolutionary origins of cooperation based on reproductive altruism via kin selection.

1. Background

Reproductive altruism, with a breeding female assisted by her non-reproductive worker daughters, has evolved multiple times in the insect order Hymenoptera (bees, ants and wasps). Trivers & Hare [1] predicted that a split sex ratio should favour the evolution of reproductive altruism among the Hymenoptera through kin selection by increasing the indirect fitness benefits to non-reproductive workers. This logic is based on the fact that workers of the social Hymenoptera are female, and due to their haplodiploid genetic system, they are more closely related to their sisters than to their brothers that develop from unfertilized eggs. In colonies headed by a singly mated queen, sister–sister relatedness is 0.75, while sister–brother relatedness is 0.25. If a species has both solitary and social reproductive behaviour, as expected during the initial stages of social evolution, the social nests should bias their sex ratio toward female reproductives in order to enhance the indirect kin selection benefits to the workers. If there is then an overabundance of female reproductives from these social nests, the solitary reproductive females (who are equally related to their sons and daughters, $r = 0.5$ for each) would benefit from producing the rarer sex and thus produce a male-biased offspring sex ratio [1–3]. The result would be a split sex ratio: female biased in social nests, and male biased in solitary nests.

Subsequent studies of small-colony social bees demonstrated that colony offspring sex ratio among social nests changed adaptively depending on whether workers were sisters or daughters to the dominant reproductive female [2,4–7]. However, the hypothesis proposed by Trivers & Hare [1]—a mixed population made up of social nests with a female-biased offspring sex ratio, and solitary nests with a male-biased sex ratio—has never been tested [8].

Here, we use the facultatively social or solitary sweat bee *Megalopta genalis* to test the split sex ratio hypothesis. *M. genalis* can nest solitarily or socially [9,10]. Social nests contain a reproductive female and typically 1–2

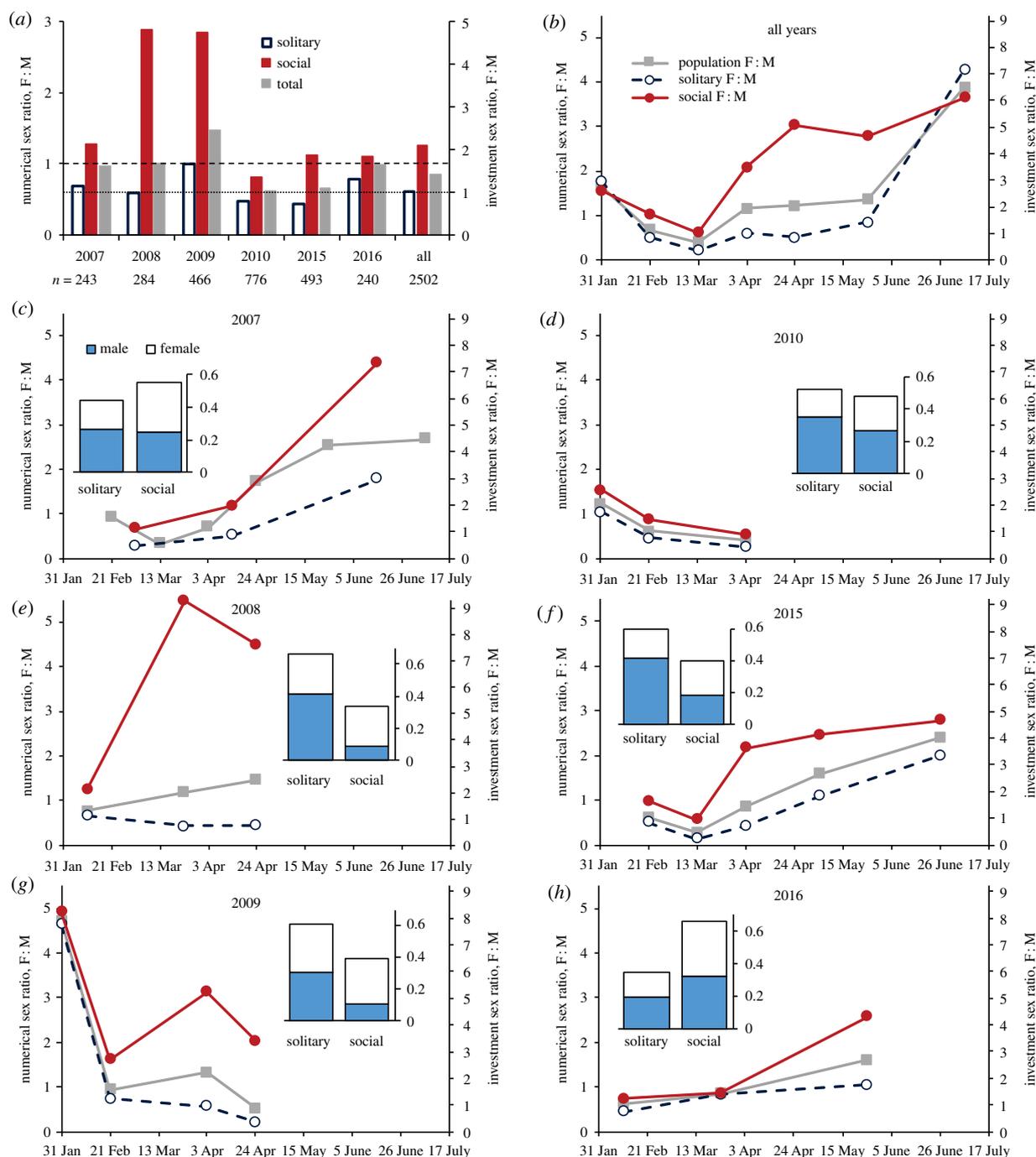


Figure 1. For all panels, the left vertical axis shows the observed numerical F:M sex ratio, and the right vertical axis shows the estimated investment ratio. (a) Yearly sex ratios. Numbers under each year represent the total number of offspring reared that year. Social nests are represented by filled red columns, solitary nests by open blue columns, and the total population by filled grey columns. (b) Sex ratios by season. Data were grouped into three-week periods for presentation. Labels on the horizontal axis represent the midpoint of each period. Where sample size was not sufficient in a given period, adjacent periods were pooled (see electronic supplementary materials for details). In this and subsequent panels, social nests are represented by filled red circles, solitary nests by open blue circles and the total population by filled grey squares. C-H: Sex ratio by season for each year. Insets show the proportion of each year's sample consisting of offspring from social or solitary nests, and the proportion of those that were male (blue fill) or female (white fill).

non-reproductive workers [11]. *Megalopta genalis* reproductives are monogamous and worker reproduction is rare, so social workers typically raise full siblings [10]. In standardized observation nests, solitary females produced a more male-biased sex ratio than social nests [10], but that study did not include the full range of nest ages and seasonal variation exhibited by *M. genalis*. Here we use sex ratio data of offspring reared from field-collected nests of mixed ages across six years in both the wet and dry seasons to test the prediction that social nests will have a more female-biased offspring sex ratio than solitary nests.

2. Methods

We collected nests from the forest in Barro Colorado Island, (9°09' N, 79°51' W), Panama, across the reproductive season (January–July) and reared offspring to adulthood as described in [12]. We reared 2502 offspring in total, in 2007, 2008, 2009, 2010, 2015 and 2016 (see figure 1 and electronic supplementary material for yearly and seasonal sample sizes). We characterized nests as solitary (a single female) or social (more than one female) based on the number of adult females present in the nest at the time of collection. We have never observed nests initiated by multiple foundresses, so multi-female nests consist of close

relatives, confirmed with genetic markers [10]. To calculate investment sex ratio, we multiplied the number of male offspring by 0.59 to account for the difference in mean body dry mass between females (38.28 ± 8.91 mg) and males (22.62 ± 6.56 mg) [13]. We analysed sex of each reared offspring (a binary response variable) using a generalized linear mixed model (GLMM) with binomial distribution and logit link function in SPSS version 25. We used year, social status (social or solitary) of the natal nest, and date as fixed effects, and nest identity as a random effect to account for the non-independence of offspring from the same nest. Date, measured as days since 1 January for each year, in order to compare across years, served as a proxy for the effect of season. The dry season on BCI generally lasts from mid-December to mid-April, before giving way to the wet season [14].

3. Results

We found a split offspring sex ratio between social and solitary nests. Social nests were more female biased than solitary nests (GLMM social status $F_{1,2494} = 54.03$, $p = 0.001$) even though sex ratio also varied across seasons ($F_{1,2494} = 11.27$, $p < 0.001$) and years ($F_{5,2494} = 10.85$, $p < 0.001$; figure 1). The effect of nest identity was also significant ($Z = 6.18$, $p < 0.001$) meaning that offspring from the same nest tend to be the same sex. The effect of social status on offspring sex was also significant in each year analysed individually; the effect of season was significant in every year except 2008 (table 1).

We collected 605 solitary nests from which we reared 852 male and 511 female offspring (avg. \pm s.d. males per nest = 1.41 ± 1.30 ; females = 0.84 ± 0.95). We collected 371 social nests from which we reared 508 male and 631 female offspring (males per nest = 1.37 ± 1.49 ; females = 1.70 ± 1.63). The numerical sex ratio for all reared offspring was 0.84 F:M (46% female, 54% male). In social nests the F:M ratio was 1.24, and in solitary nests it was 0.60; figure 1). The overall investment sex ratio was 1.42 F:M (2.11 in social nests, 1.02 in solitary nests). Overall sex ratio varied from year to year (numerical F:M sex ratio range = 0.62–1.44, avg. = 0.94 ± 0.30 ; investment sex ratio range = 1.05–2.44, avg. = 1.59 ± 0.51 ; figure 1). The yearly numerical offspring F:M sex ratio from social nests averaged 2.51 ± 1.24 times greater than that of solitary nests (range = 1.38–4.79).

Sex ratios also varied seasonally (figure 1). At the beginning of the year, which is also the beginning of the local dry season, sex ratio was female biased, then male biased in February and March, and then again female biased in May–July, which is the wet season (brood provisioning largely stops between August and December [9]).

4. Discussion

Here we show a split offspring sex ratio between solitary and social nests of the same species in the same population as predicted by kin selection theory. Social nests produce a female-biased brood, while solitary nests produce either male-biased or less female-biased broods (depending on the year and season). The bias towards females at the beginning of the year, which is also the beginning of the bees' provisioning season when most nests are being initiated, suggests that many of the female offspring we collected then were destined to be non-reproductive workers in social nests. The rise in

Table 1. Results of a GLMM for each year. See text for details, see figure 1a for yearly sample sizes.

	social status		season		nest	
	F	p	F	p	Z	p
2007	5.57	0.019	15.44	<0.001	2.17	0.030
2008	19.04	<0.001	0.19	0.67	2.12	0.034
2009	21.59	<0.001	17.31	<0.001	2.81	0.005
2010	7.36	0.007	29.17	<0.001	3.16	0.002
2015	10.98	0.001	21.07	<0.001	2.44	0.015
2016	3.92	0.049	10.74	0.001	1.52	0.129

female production at the end of the provisioning season (June–July) may represent investment in future reproductives for the subsequent dry season. Our sampling effort was not evenly distributed across seasons or years (figure 1; electronic supplementary materials), so our overall sex ratio results are not an unbiased estimate of population sex ratio. However, the consistently more female-biased sex ratios in social nests relative to solitary nests confirm expectations from split sex ratio theory [1–3].

Some immature females we collected would have developed into non-reproductive workers. Thus, our sex ratio estimates are an overestimate of the number of reproductive females, due to the inclusion of some daughters that would have developed into non-reproductive workers. Most social nests have only one or two workers [11], and for a nest to be designated as social in our study, there must already be at least a second adult female present. It is therefore reasonable to assume that most immature females collected from social nests were not destined to be non-reproductive workers, as these nests already had at least one worker.

It is likely that our results overestimate female production in solitary nests. A foundress pursuing a social nesting strategy, rearing her first, not-yet-hatched, female worker would count in our study as a solitary reproductive with female offspring, since there was only one adult female present at collection. Thus our estimates of the split between social and solitary sex ratios are conservative because our calculation of sex ratio in solitary nests include some non-reproductive female offspring in nests that would have become social if they had not been collected. Additionally, if a social nest lost its worker(s) before collection ('failed social' of [10]), it would be counted as solitary in this study. Again, this would bias our conclusions toward assigning an excess of female offspring to solitary nests. Despite the considerable yearly and seasonal population-level variation, and the unavoidable biases in our collection methods, our data show that social nests consistently invested more reproductive effort in females than solitary nests did.

In previous research on *M. genalis*, we showed that workers had lower inclusive fitness than reproductives who successfully initiate a nest. This suggested that fitness benefits to the social reproductive female resulting from her manipulating daughters to stay as workers appear to be more important in selecting for social behaviour than do indirect fitness benefits to workers [11]. However, the simulation models of [11] only led to the evolution of sociality when

relatedness was high, suggesting that a maternal manipulation route to an evolutionary origin of sociality would be facilitated by higher relatedness among nest-mates resulting from split sex ratios, although [11] did not explicitly test the effect of sex ratio variation.

Female-biased sex ratios in social species with permanently differentiated worker castes are often described in terms of ‘worker control’ of sex ratios because this bias increases worker inclusive fitness (reviewed by Gardner *et al.* [8]). Our data are consistent with enhanced worker inclusive fitness, but we do not know how or if *M. genalis* workers control the sex ratio. Social reproductive females lay eggs, choosing to fertilize them or not, and aggressively dominate the workers, which are smaller-bodied than the reproductives [10,15–18]. However workers have access to cells as they are provisioned [18]—and sealed cells are often later opened [19]—so they may be aware of the sex of developing offspring and the eventual sex ratio of their natal nest and thus be able to enhance any kin selection advantage by choosing to remain and help in female-biased colonies. Solitary nest sex ratio bias must be due to the choice of the reproductive female, because no workers are present and solitary reproductives are mated [11] so male bias does not arise due to unmatedness [20]. Also, split sex ratios can be adaptive for all individual bees [2]. For instance, theory predicts that with the increased resources provided by worker(s) foraging effort, social reproductives should invest more in female offspring [21,22]. Likewise, if an increase in offspring condition leads to a greater increase in fitness for female than for male offspring, reproductives who can afford to produce better-condition offspring should specialize on producing females [23,24].

References

- Trivers RL, Hare H. 1976 Haplodiploidy and the evolution of the social insect. *Science* **191**, 249–263. (doi:10.1126/science.1108197)
- Boomsma J. 1991 Adaptive colony sex ratios in primitively eusocial bees. *Trends Ecol. Evol.* **6**, 92–95. (doi:10.1016/0169-5347(91)90182-W)
- Boomsma J, Grafen A. 1991 Colony-level sex ratio selection in the eusocial Hymenoptera. *J. Evol. Biol.* **4**, 383–407. (doi:10.1046/j.1420-9101.1991.4030383.x)
- Yanega D. 1989 Caste determination and differential diapause within the first brood of *Halictus rubicundus* in New York (Hymenoptera: Halictidae). *Behav. Ecol. Sociobiol.* **24**, 97–107. (doi:10.1007/BF00299641)
- Mueller UG. 1991 Haplodiploidy and the evolution of facultative sex ratios in a primitively eusocial bee. *Science* **254**, 442–444. (doi:10.1126/science.254.5030.442)
- Boomsma J, Eickwort G. 1993 Colony structure, provisioning and sex allocation in the sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). *Biol. J. Linn. Soc.* **48**, 355–377. (doi:10.1111/j.1095-8312.1993.tb02097.x)
- Packer L, Owen RE. 1994 Relatedness and sex ratio in a primitively eusocial halictine bee. *Behav. Ecol. Sociobiol.* **34**, 1–10. (doi:10.1007/BF00175452)
- Gardner A, Alpedrinha J, West SA. 2011 Haplodiploidy and the evolution of eusociality: split sex ratios. *Am. Nat.* **179**, 240–256. (doi:10.1086/663683)
- Wcislo WT, Arneson L, Roesch K, Gonzalez V, Smith A, Fernández H. 2004 The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? *Biol. J. Linn. Soc.* **83**, 377–387. (doi:10.1111/j.1095-8312.2004.00399.x)
- Kapheim KM, Smith AR, Nonacs P, Wcislo WT, Wayne RK. 2013 Foundress polyphenism and the origins of eusociality in a facultatively eusocial sweat bee, *Megalopta genalis* (Halictidae). *Behav. Ecol. Sociobiol.* **67**, 331–340. (doi:10.1007/s00265-012-1453-x)
- Kapheim KM, Nonacs P, Smith AR, Wayne RK, Wcislo WT. 2015 Kinship, parental manipulation and evolutionary origins of eusociality. *Proc. R. Soc. B* **282**, 20142886. (doi:10.1098/rspb.2014.2886)
- Smith A, Harper C, Kapheim K, Simons M, Kingwell C, Wcislo W. 2018 Effects of social organization and resource availability on brood parasitism in the facultatively social nocturnal bee *Megalopta genalis*. *Insectes Soc.* **65**, 85–93. (doi:10.1007/s00040-017-0590-7)
- Kapheim KM, Bernal SP, Smith AR, Nonacs P, Wcislo WT. 2011 Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). *Behav. Ecol. Sociobiol.* **65**, 1179–1190. (doi:10.1007/s00265-010-1131-9)
- Leigh EG. 1999 *Tropical forest ecology: a view from Barro Colorado island*. Oxford, UK: Oxford University Press.
- Smith AR, Kapheim KM, O'Donnell S, Wcislo WT. 2009 Social competition but not subfertility leads to a division of labour in the facultatively social sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Anim. Behav.* **78**, 1043–1050. (doi:10.1016/j.anbehav.2009.06.032)
- Smith A, Simons M, Bazarko V, Seid M. 2018 The influence of sociality, caste, and size on behavior in a facultatively eusocial bee. *Insectes Soc.* **66**, 153–163. (doi:10.1007/s00040-018-00679-4)
- Kapheim KM, Smith AR, Ihle KE, Amdam GV, Nonacs P, Wcislo WT. 2012 Physiological variation as a mechanism for developmental caste-biasing in a

5. Conclusion

Patterns of sex ratio investment in *M. genalis* match predictions made more than 40 years ago by Trivers & Hare [1] and extended by Boomsma [2] and Boomsma & Grafen [3]: workers gaining increased indirect fitness by helping to produce more female offspring, while solitary reproductives specialize on males. The fact that this classic prediction of kin selection is met in a species in which maternal manipulation appears to select for social behaviour highlights the inter-play between genetic and socio-environmental processes, and the need to consider both in future studies of social evolution.

Ethics. Research on BCI was conducted with permission from the Autoridad Nacional del Ambiente in accordance with the laws of the Republic of Panama.

Data accessibility. Data are available from the Dryad Digital Repository at: <https://doi.org/10.5061/dryad.62dt334> [25].

Authors' contributions. A.S., K.K. and C.K. collected nests; A.S. analysed data. All authors contributed to project design and writing the manuscript. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. The authors have no competing interests to declare.

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- facultatively eusocial sweat bee. *Proc. R. Soc. B* **279**, 1437–1446. (doi:10.1098/rspb.2011.1652)
18. Kapheim KM, Chan T, Smith A, Wcislo WT, Nonacs P. 2016 Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. *Insectes Soc.* **63**, 185–191. (doi:10.1007/s00040-015-0454-y)
 19. Quiñones A, Wcislo W. 2015 Cryptic extended brood care in the facultatively eusocial sweat bee *Megalopta genalis*. *Insectes Soc.* **62**, 307–313. (doi:10.1007/s00040-015-0409-3)
 20. Godfray H, Grafen A. 1988 Unmatedness and the evolution of eusociality. *Am. Nat.* **131**, 303–305. (doi:10.1086/284791)
 21. Frank S, Crespi B. 1989 Synergism between sib-rearing and sex ratio in Hymenoptera. *Behav. Ecol. Sociobiol.* **24**, 155–162. (doi:10.1007/BF00292098)
 22. Rosenheim JA, Nonacs P, Mangel M. 1996 Sex ratios and multifaceted parental investment. *Am. Nat.* **148**, 501–535. (doi:10.1086/285937)
 23. Trivers RL, Willard DE. 1973 Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**, 90–92. (doi:10.1126/science.179.4068.90)
 24. Veller C, Haig D, Nowak MA. 2016 The Trivers–Willard hypothesis: sex ratio or investment? *Proc. R. Soc. B* **283**, 20160126. (doi:10.1098/rspb.2016.0126)
 25. Smith AR, Kapheim KM, Kingwell CJ, Wcislo WT. 2019 Data from: A split sex ratio in solitary and social nests of a facultatively social bee. Dryad Digital Repository. (<https://doi.org/10.5061/dryad.62dt334>)