

Ecology, Not the Genetics of Sex Determination, Determines Who Helps in Eusocial Populations

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Summary

In eusocial species, the sex ratio of helpers varies from female only, in taxa such as the social Hymenoptera (ants, bees, and wasps) [1], to an unbiased mixture of males and females, as in most termites [2]. Hamilton suggested that this difference owes to the haplodiploid genetics of the Hymenoptera leading to females being relatively more related to their siblings [3]. However, it has been argued that Hamilton's hypothesis does not work [4–9] and that the sex of helpers could instead be explained by variation in the ecological factors that favor eusociality [10]. Here we test these two competing hypotheses, which focus on the possible importance of different terms in Hamilton's rule [2, 11], with a comparative study across all sexual eusocial taxa. We find that the sex ratio of helpers (1) shows no significant correlation with whether species are haplodiploid or diploid and (2) shows a strong correlation with the ecological factor that had favored eusociality. Specifically, when the role of helpers is to defend the nest, both males and females help, whereas when the role of helpers is to provide brood care, then helpers are the sex or sexes that provided parental care ancestrally. More generally, our results confirm the ability of kin selection theory to explain the biology of eusocial species, independently of ploidy, and add support to the idea that haplodiploidy has been more important for shaping conflicts within eusocial societies than for explaining its origins [6, 12–19].

Results and Discussion

Hamilton [3] argued that the genetics of sex determination explained variation across eusocial species in the sex ratio of the helper castes (Figures 1 and 2A and Figure S2 available online). In haplodiploid species, such as the social Hymenoptera, females are more related to their full sisters (life-for-life $r = 3/4$) than to their own daughters ($r = 1/2$), and Hamilton suggested that this predisposes females to helping. In contrast, in diploid species, such as termites, individuals are as related to their full siblings as they are to their own offspring, independently of sex, and so both sexes are under equal selection to

help. However, other authors have argued that Hamilton's "haplodiploidy hypothesis" does not explain variation in the sex of the helper caste, because haplodiploidy also leads to females being less related to their full brothers ($r = 1/4$) than to their sons ($r = 1/2$; see the [Supplemental Experimental Procedures](#) and [Figure S3](#)) [4–9].

An alternative explanation for the variation in the sex ratio of the workers across species is variation in the ecological factors that favored the evolution of eusociality [10]. If the main advantage of having helpers is to provide care for the young, then we would expect the helpers to be drawn from the sex or sexes that provided parental care in that species' solitary ancestor, which is usually females [10, 20–27] (Figure 2B). In contrast, if the main advantage of having helpers is to help defend the colony and neither sex is preadapted to soldiering, then we would expect the helpers to be drawn equally from both sexes [10, 28, 29] (Figure 2B). While this ecological explanation provides an alternative to Hamilton's haplodiploidy hypothesis, it is fully compatible with the theory of kin selection more generally.

Genetics versus Ecology

Here, we provide the first formal test of the two competing hypotheses for which sex should help, with a comparative study across all sexual (i.e., nonparthenogenetic) eusocial taxa. We start by considering all taxa that fit Wilson's [1] broader definition of eusociality, with cooperative care of young, reproductive division of labor, and overlapping generations (Figure 3). We utilized published DNA sequences to construct a phylogeny of these taxa (Figures 3 and S1). We collected data on the helper sex ratio of representative species within each origin of eusociality from published sources (see [Table S2](#) for data and references) and classified the sex ratio of each species as factor with two levels, either female biased or unbiased (Figure 3). No species have a strong male biased helper sex ratio.

We found, with a phylogeny based mixed model [30], that the genetics of sex determination is not significantly correlated with the sex ratio of the helpers ($p_{\text{mcmc}} = 0.416$; Figures 3 and 4 and [Table S4](#)). Considering haplodiploid species, the Hymenoptera and haplodiploid ambrosia beetles have predominantly female helpers, but the thrips have a mixture of male and female helpers (Figure 3). Considering the diploid species, the termites, sponge-dwelling shrimps, and mole rats have relatively unbiased helper sex ratios, but the spiders and diploid ambrosia beetles have predominantly female helpers (Figure 3).

In contrast, we found that the ecological advantage of social living is strongly correlated with the sex ratio of the helpers ($p_{\text{mcmc}} = 0.001$; Figures 3 and 4 and [Table S4](#)). For each of the species included, we determined whether the role of helpers is either defense only (soldiers) or also includes brood care (workers). In cases where helpers provide brood care, we then determined whether this has evolved from species where brood care was either maternal or biparental ([Table S2](#)). In all species where the primary function of helpers is brood care and care was ancestrally provided by mothers—ambrosia beetles, Hymenoptera, and spiders—the helpers are exclusively female. In all other species, where either brood care evolved from biparental care or the primary function of helpers

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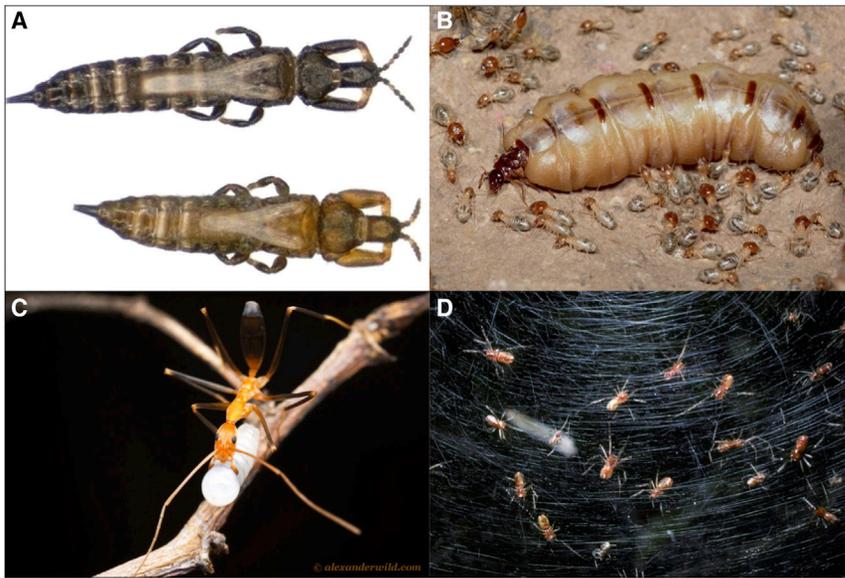


Figure 1. Helper Sex Ratios in Eusocial Societies
Some species have an equal number of male and female helpers, such as in (A) the thrips *Kladothrips intermedius* (picture by Holly Caravan) and (B) the termite *Macrotermes gilvus* (picture by Mitsuhiro Imamori). Other species only have female helpers, such as in (C) the ant *Leptomyrmex darlingtoni* (picture by Alexander Wild) and (D) the spider *Anelosimus eximius* (picture by Ken Preston-Mafham).

is colony defense—thrips, termites, sponge-dwelling shrimps, and mole rats—the helpers are a relatively unbiased mixture of males and females.

We obtained the same results when we repeated our analyses based on the taxa that fit Crespi and Yanega's [3, 29] and Boomsma's [16, 18, 19] more strict definition of eusociality. These researchers restrict eusociality to cases in which some or all individuals become irreversibly fixed into castes, prior to reproductive maturity [16, 18, 19, 29, 31–35]. This excludes the mole rats, the sponge-dwelling shrimp, and one of the ambrosia beetles. Without these taxa, we still found that the genetics of sex determination showed no correlation with the sex of helpers ($p_{\text{mcmc}} = 0.504$), whereas the ecological advantage of group living did ($p_{\text{mcmc}} < 0.001$; Table S5). The same pattern holds when we further restrict our analyses to the obligate eusocial species, where the breeding and helper castes are mutually dependent and helpers provide brood care [18, 19, 29]: the Hymenoptera evolved female helpers from ancestral maternal care, while the higher termites evolved mixed-sex helpers from ancestral biparental care. However, these transitions to obligate eusociality have occurred too infrequently for a useful formal comparative analysis. Taken together, these results suggest that ecology is more important than the genetics of sex determination in shaping the sex ratio of helpers, however eusociality is defined. The importance of ecology in at least some Hymenoptera is further supported by recent experiments and gene expression studies [26, 36], which suggest that the nursing behavior of workers in bees and wasps evolved directly from maternal care, which came to be directed toward siblings rather than offspring.

We have focused on the helper sex ratios and not whether, in species with helpers of both sexes, there is sex-specific division of labor. There are too few data at present for a formal analysis of division of labor. Among the termites, where the best data are available, it appears that some differences can be explained by ecological factors. For example, in those termites where females are more involved in defensive tasks [37–40], they are often larger, and hence better suited to defend the nest [39]. However, in other termite species, sex-linked genes have been shown to be involved in caste determination and helper behavior [41].

Conclusions

We have provided clear support for the hypothesis that the sex of the helping caste in eusocial species is driven by ecology and not the genetics of sex determination. Why would the genetics of sex determination not matter from a kin selection perspective? While females in monogamous haplodiploid populations are more related to their sisters than to their daughters, they are

less related to their brothers than to their sons. Overall, these factors exactly cancel, so that female helpers are equally related, on average, to their siblings and their offspring [4–9] (Supplemental Experimental Procedures). Similarly, while males in haplodiploid populations are less related to their sisters than they are to their mate's daughters, they are more related to their brothers than they are to their mate's sons. Again, this cancels, so that male helpers are equally related, on average, to their siblings and their mate's offspring [4–9] (Supplemental Experimental Procedures). Consequently, haplodiploidy does not, by itself, predispose either sex to altruistic helping. Haplodiploidy could still matter, if helping was correlated with sex ratio variation across colonies, but the empirical data suggest that this is not common [13–16]. More generally, this adds to the growing theoretical and empirical literature that suggests that the major role of haplodiploidy-mediated relatedness asymmetries has been to shape conflicts in existing eusocial societies, such as over the sex ratio and who produces males, rather than the evolution of cooperation itself [12–19].

Experimental Procedures

Data Collection

The number of times that eusociality has evolved depends upon how it is defined. Following Wilson's [1] definition, eusociality has evolved in five orders of insects [2, 16, 32, 35, 42], one order of crustaceans [34], one order of spiders [43], and one order of mammals [44]. In most cases, multiple transitions have taken place within an order. Figure 2 shows the number of independent transitions in each of these orders (based on the published literature; see Table S2 for references), as well the hypothetical evolutionary relationship between the orders where eusociality occurs (based on our phylogenetic reconstruction described below). Following Crespi and Yanega's [29] and Boomsma's [18] definitions, eusociality is restricted to the Arthropods and has evolved only in the Hymenoptera, Thysanoptera, Araneae (only according to Crespi and Yanega), Isoptera, and Coleoptera.

We collected data from the literature on representative species for each origin of eusociality. These data included (1) helper sex ratio (including both "soldiers" and "workers") with "unbiased" and "female only" as the two levels (as few taxa display male biased helper sex ratios), (2) the type of help (defense, brood care, or both, where we define brood care by the presence of direct provisioning of the young, or related behaviors such as cleaning the larva), and (3) genetic system (haplodiploidy versus diploidy) for all obligate and facultative eusocial taxa.

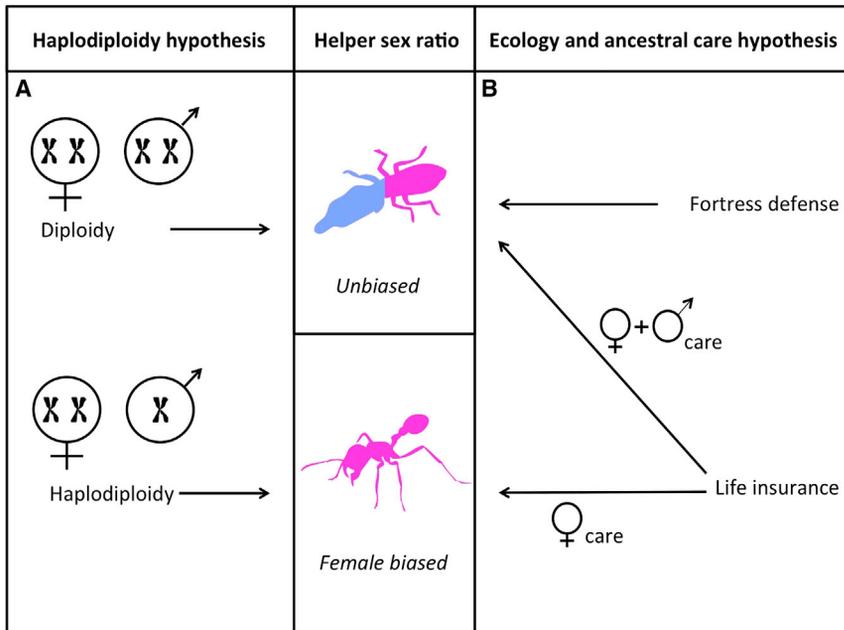


Figure 2. Competing Hypotheses for Who Helps (A) The haplodiploidy hypothesis suggests that, in haplodiploid species, a higher relatedness between sisters ($r = 0.75$, life-for-life relatedness) favors female helpers. In contrast, in diploid species, there is no relatedness asymmetry, and so both sexes are equally favored to help. This hypothesis focuses on the possible importance of variation in the relatedness (r) term in Hamilton's rule [3, 11]. (B) The ecology and ancestral care hypothesis suggests that the sex of helpers depends upon the ecological (economic) benefit of cooperation and eusociality. Specifically, either the cooperative rearing of brood when there is a significant chance that the parent will die before parental care is completed (life insurance) or living in a defendable food resource (fortress defense) [10]. If helpers rear brood, then we would expect the helpers to be drawn from the sex or sexes that provided parental care in the ancestral solitary species, which is usually females. In contrast, if helpers defend the colony, and neither sex is preadapted to be a soldier, then we would expect the helpers to be drawn from both sexes. This hypothesis focuses on the possible importance of variation in the benefit (b) and cost (c) terms in Hamilton's rule [3, 11]. See also Figure S3 and Table S6.

We analyzed the sex ratio as a dichotomous variable with a single data point per origin of eusociality, because the continuous estimates of the helper sex ratio in the literature are both relatively rare and tend to be biased. Specifically, researchers tend to report when sex ratios are biased away from equality or only one sex, and not when they are these more usual values. For example, in termites, there are many estimates of biased helper sex ratios (Table S3), while in fact most termites are unbiased [2, 45]. We included the assignment for each origin with their references in the Supplemental Information (Table S2). We also give more detail on the way the data were collected, as well as a table with raw data, in the Supplemental Experimental Procedures and Table S3.

Most traits were not variable between species within an origin—in those cases, the data in Table S2 represent a randomly selected representative

species for which phylogenetic data were available (see below) within that origin (we use a single data point per origin, when the traits considered do not differ between closely related species). We reconstructed the most likely ancestral type of parental care by collecting data from the closest noneusocial sister groups, where sister groups were assigned by studying recent molecular phylogenies of the relevant taxonomic groups.

We excluded three taxa, classified as facultatively eusocial by Crespi and Yanega [29], from our analysis. These are (1) aphids, where the soldiers are produced parthenogenetically and thus are female by default; (2) polyembryonic wasps, where the role of the soldiers is between sex sibling conflict [46]; and (3) parasitic Trematodes, as both soldiers as well as reproductives are clones of one diploid larva [47].

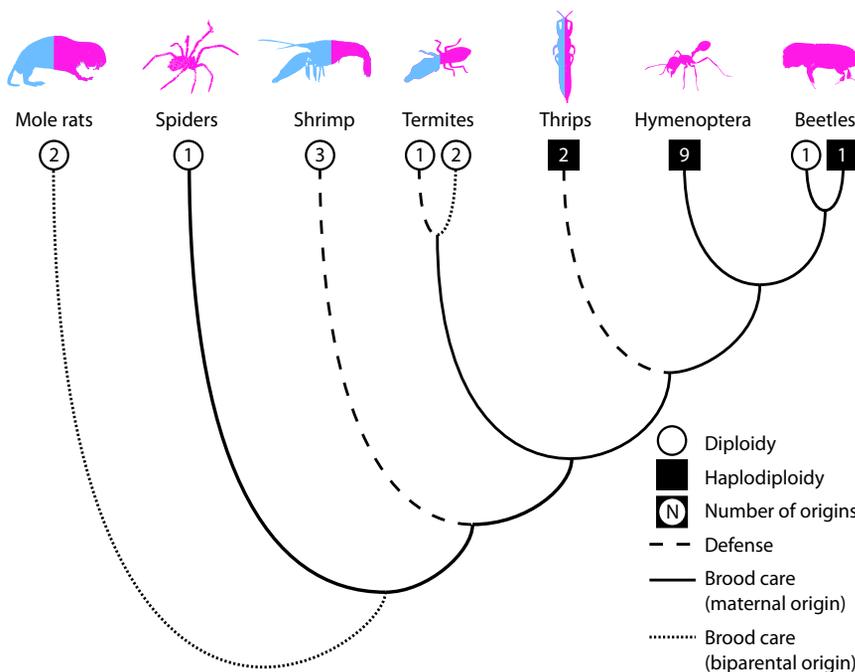


Figure 3. The Helping Sex The figure shows a schematic cladogram of the taxa included in our analysis. Open circles and filled squares represent diploid versus haplodiploid taxa, respectively, while the number within each square/circle shows the number of independent origins of eusociality (according to Wilson's [1] definition of eusociality) within each clade. The color of the icon represents the sex ratio of workers—half blue, half pink for unbiased and pink for female only. See also Figure S1 and Tables S1–S3.

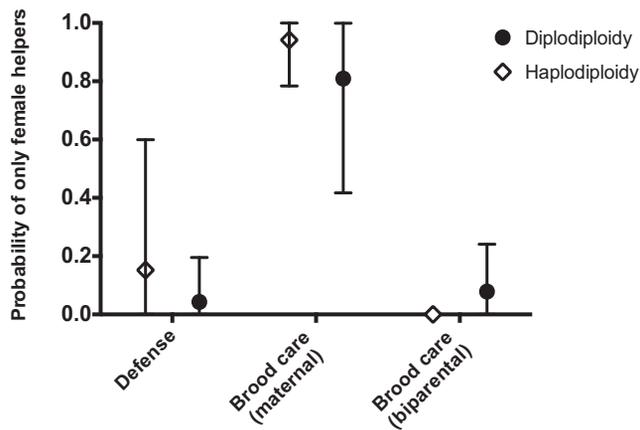


Figure 4. Helper Sex Ratios

The probability of having female only helpers, as opposed to an unbiased mixture of males and females (where 0 represents an unbiased helper sex ratio), for haploidy and diploidy taxa in the different ecological scenarios. Predicted values are derived from the binary phylogenetic mixed model and were generated using the “predict” function in the R package MCMCglmm. Error bars represent 95% confidence intervals. The probability of female-biased helpers does not depend on genetic system but differs strongly between the different role of helpers: brood care derived from maternal care leads to female biased helpers, while brood care derived from biparental care and colony defense leads to an unbiased mixture of male and female helpers. See also Figures S1 and S2 and Tables S1–S5.

Phylogenetic Reconstruction

We estimated phylogenetic relationships among eusocial lineages to control for nonindependence caused by shared ancestry in the comparative analysis. In our analysis, there was imperfect overlap between the set of species with social plus genetic system data and the set of species with phylogenetic data. We therefore chose to reconstruct a molecular phylogeny based on a single representative taxon for each origin of eusociality (see above and Table S2). The only group for which we included multiple taxa per origin is the termites: we include the ancestral termites that have soldiers, as well as each termite lineage in which a true worker cast has evolved independently [48, 49]. We wrote a Python script to retrieve published DNA sequences from GenBank, targeting data from nine loci: *12S*, *16S*, *18S*, *28S*, *COI*, *Cytb*, *elongation factor 1 alpha*, *wingless*, and *long-wavelength rhodopsin*. These loci have been sampled unevenly across eusocial lineages. So that DNA sampling of each eusocial lineage could be maximized, several terminal nodes in the phylogeny estimate were composite samples that we constructed from multiple species in a genus (same genus as the representative species in Table S2). For genera in which eusociality has evolved repeatedly, terminal nodes are based on sequences of a single species. We give the NCBI sequence identifier numbers in Table S2. The final alignment had 4,154 positions.

We used MAFFT [50] to align sequence from each locus and a Bayesian approach (BEAST v1.7.4; [51]) to estimate a time-scaled phylogeny of eusocial lineages under a HKY nucleotide substitution model [52], with gamma-distributed among-site rate heterogeneity. We estimated model parameters independently across genomes and codon positions. We modeled among-lineage variation in nucleotide substitution rates using a relaxed-clock uncorrelated log-normal model [53]. We used a pure-birth model of phylogenetic branching and imposed exponential prior probability distributions on the ages of termites [54], thrips [55], and bees and wasps [56]. In addition to the topological constraints required to set node age priors, we enforced the monophyly of Pancrustacea [57] and bees [58]. These relationships are well established, but were not recovered in preliminary unconstrained analyses.

Statistical Analysis

We analyzed the data using a phylogenetic mixed model approach [30] in the R package MCMCglmm [59], assuming a Brownian model on the logit probability scale for the phylogenetic effects [30]. We included each of the recognized origins of eusociality as a single data point, thereby correcting for pseudoreplication with individual origins. We corrected for phylogenetic

nonindependence between origins by using the reconstructed molecular phylogeny describe above. In order to correct for uncertainty in the phylogenetic reconstruction, we marginalized over the posterior distribution of trees [30] by sampling a tree from the posterior at iteration t , running the MCMC comparative analysis for 1,000 iterations, and saving the last MCMC sample. The values of the latent variables and variance components were passed as starting values to the analysis at iteration $t + 1$ for which a new tree from the posterior sample was taken. The process was repeated for 1,300 iterations (i.e., $t = 1,300$), where we disposed of the first 300 runs as burn-in. We used a mixed model with a binary error structure with helper sex ratio as the response variable (“unbiased” and “female biased”). As predictors, we included genetic system (haploidy versus diploidy) and the assumed “route towards eusociality”: brood care (either evolved from maternal care or from biparental care) or defense. We used a weakly informative Gelman prior [60, 61] for the fixed effects, an inverse Wishart prior for random effects, and fixed the residual variance to 1 (as this cannot be estimated from binary data). We provided our R code (including the prior specification) in the Supplemental Experimental Procedures. We report the significance of our fixed effects in terms of p_{MCMC} , which is twice the posterior probability that the estimate is negative or positive (whichever probability is smallest). This value can be interpreted as a Bayesian equivalent to the traditional p value [30, 61]

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and six tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2013.10.013>.

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References

- Wilson, E.O. (1975). *Sociobiology* (Harvard: Belknap Press).
- Thorne, B.L. (1997). Evolution of eusociality in termites. *Annu. Rev. Ecol. Syst.* 28, 27–54.
- Hamilton, W.D. (1972). Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* 3, 193–232.
- Charlesworth, B. (1978). Some models of the evolution of altruistic behaviour between siblings. *J. Theor. Biol.* 72, 297–319.
- Charnov, E.L. (1978). Evolution of eusocial behavior: offspring choice or parental parasitism? *J. Theor. Biol.* 75, 451–465.
- Trivers, R.L., and Hare, H. (1976). Haplodiploidy and the evolution of the social insect. *Science* 191, 249–263.
- Craig, R. (1982). Evolution of male workers in the Hymenoptera. *J. Theor. Biol.* 94, 95–105.
- Pamilo, P. (1991). Evolution of the sterile caste. *J. Theor. Biol.* 149, 75–95.
- Bourke, A.F.G., and Franks, N.R. (1995). *Social Evolution in Ants* (Princeton: Princeton University Press).
- Queller, D.C., and Strassmann, J.E. (1998). Kin Selection and Social Insects. *Bioscience* 48, 165–175.
- Hamilton, W.D. (1964). The genetical evolution of social behaviour. I. *J. Theor. Biol.* 7, 1–16.
- Ratnieks, F.L., Foster, K.R., and Wenseleers, T. (2006). Conflict resolution in insect societies. *Annu. Rev. Entomol.* 51, 581–608.
- Gardner, A., Alpedrinha, J., and West, S.A. (2012). Haplodiploidy and the evolution of eusociality: split sex ratios. *Am. Nat.* 179, 240–256.
- West, S. (2009). *Sex Allocation* (Princeton: Princeton University Press).
- Alpedrinha, J., West, S.A., and Gardner, A. (2013). Haplodiploidy and the evolution of eusociality: worker reproduction. *Am. Nat.* 182, 421–438.
- Boomsma, J.J. (2009). Lifetime monogamy and the evolution of eusociality. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 3191–3207.

17. Hughes, W.O.H., Oldroyd, B.P., Beekman, M., and Ratnieks, F.L.W. (2008). Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* 320, 1213–1216.
18. Boomsma, J.J. (2013). Beyond promiscuity: mate-choice commitments in social breeding. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368, 20120050.
19. Boomsma, J.J. (2007). Kin selection versus sexual selection: why the ends do not meet. *Curr. Biol.* 17, R673–R683.
20. Gadagkar, R. (1991). Demographic predisposition to the evolution of eusociality: a hierarchy of models. *Proc. Natl. Acad. Sci. USA* 88, 10993–10997.
21. Queller, D.C. (1994). Extended parental care and the origin of eusociality. *Proc. R. Soc. Lond. B* 256, 105–111.
22. Queller, D.C. (1989). The evolution of eusociality: reproductive head starts of workers. *Proc. Natl. Acad. Sci. USA* 86, 3224–3226.
23. Queller, D.C. (1997). Why do females care more than males? *Proc Biol Sci.* 264, 1555–1557.
24. Kokko, H., and Jennions, M.D. (2012). Sex differences in parental care. In *The Evolution of Parental Care*, N.J. Royle, P.T. Smiseth, and M. Kolliker, eds. (Oxford: Oxford University Press), pp. 101–116.
25. Tallamy, D.W. (2001). Evolution of exclusive paternal care in arthropods. *Annu. Rev. Entomol.* 46, 139–165.
26. Field, J., and Brace, S. (2004). Pre-social benefits of extended parental care. *Nature* 428, 650–652.
27. Crozier, R.H. (2008). Advanced eusociality, kin selection and male haploidy. *Aust. J. Entomol.* 47, 2–8.
28. Crespi, B.J. (1994). Three conditions for the evolution of eusociality: are they sufficient? *Insectes Soc.* 41, 395–400.
29. Crespi, B.J., and Yanega, D. (1995). The definition of eusociality. *Behav. Ecol.* 6, 109–115.
30. Hadfield, J.D., and Nakagawa, S. (2010). General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. *J. Evol. Biol.* 23, 494–508.
31. Rypstra, A.L. (1993). Prey size, social competition, and the development of reproductive division of labor in social spider groups. *Am. Nat.* 142, 868–880.
32. Kent, D.S., and Simpson, J.A. (1992). Eusociality in the beetle *Austroplatypus incompertus* (Coleoptera: Curculionidae). *Naturwissenschaften* 79, 86–87.
33. Crespi, B.J. (1992). Eusociality in Australian gall thrips. *Nature* 359, 724–726.
34. Duffy, J.E. (1996). Eusociality in a coral-reef shrimp. *Nature* 381, 512–514.
35. Biedermann, P.H.W., and Taborsky, M. (2011). Larval helpers and age polyethism in ambrosia beetles. *Proc. Natl. Acad. Sci. USA* 108, 17064–17069.
36. Amdam, G.V., Csondes, A., Fondrk, M.K., and Page, R.E., Jr. (2006). Complex social behaviour derived from maternal reproductive traits. *Nature* 439, 76–78.
37. Matsuura, K. (2006). A novel hypothesis for the origin of the sexual division of labor in termites: which sex should be soldiers? *Evol. Ecol.* 20, 565–574.
38. Muller, H., and Korb, J. (2008). Male or female soldiers? An evaluation of several factors which may influence soldier sex ratio in lower termites. *Mar. Biol.* 55, 213–219.
39. Bourguignon, T., Hayashi, Y., and Miura, T. (2012). Skewed soldier sex ratio in termites: testing the size-threshold hypothesis. *Insectes Soc.* 59, 557–563.
40. Crespi, B.J., and Mound, L.A. (1997). Ecology and evolution of social behavior among Australian gall thrips and their allies. In *The Evolution of Social Behavior in Insects and Arachnids*, J.C. Choe and B.J. Crespi, eds. (Cambridge: Cambridge University Press), pp. 166–180.
41. Hayashi, Y., Lo, N., Miyata, H., and Kitade, O. (2007). Sex-linked genetic influence on caste determination in a termite. *Science* 318, 985–987.
42. Foster, W.A. (1990). Experimental evidence for effective and altruistic colony defence against natural predators by soldiers of the gall-forming aphid *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). *Behav. Ecol. Sociobiol.* 27, 421–430.
43. Vollrath, F. (1986). Eusociality and extraordinary sex ratios in the spider *Anelosimus eximius* (Araneae: Theridiidae). *Behav. Ecol. Sociobiol.* 18, 283–287.
44. Sherman, P.W., Jarvis, J.U.M., and Alexander, R.D. (1991). *The Biology of the Naked Mole-Rat* (Princeton: Princeton University Press).
45. Abe, T., Bigness, D.E., and Higashi, M. (2000). *Termites: Evolution, Sociality, Symbioses, Ecology* (New York: Springer).
46. Gardner, A., Hardy, I.C., Taylor, P.D., and West, S.A. (2007). Spiteful soldiers and sex ratio conflict in polyembryonic parasitoid wasps. *Am. Nat.* 169, 519–533.
47. Hechinger, R.F., Wood, A.C., and Kuris, A.M. (2011). Social organization in a flatworm: trematode parasites form soldier and reproductive castes. *Proc. Biol. Sci.* 278, 656–665.
48. Legendre, F., Whiting, M.F., Bordereau, C., Canello, E.M., Evans, T.A., and Grandcolas, P. (2008). The phylogeny of termites (Dictyoptera: Isoptera) based on mitochondrial and nuclear markers: Implications for the evolution of the worker and pseudergate castes, and foraging behaviors. *Mol. Phylogenet. Evol.* 48, 615–627.
49. Inward, D.J., Vogler, A.P., and Eggleton, P. (2007). A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Mol. Phylogenet. Evol.* 44, 953–967.
50. Katoh, K., and Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* 9, 286–298.
51. Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.
52. Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22, 160–174.
53. Drummond, A.J., Ho, S.Y.W., Phillips, M.J., and Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, e88.
54. Kukalová-Peck, J. (2009). Carboniferous protodonatoid dragonfly nymphs and the synapomorphies of Odonatoptera and Ephemeroptera (Insecta: Paleoptera). *Paleodiversity* 2, 169–198.
55. Martynov, A.V. (1935). A find of Thysanoptera in the Permian deposits. *Compte Rendus (Doklady) de l'Academie des Sciences de l'URSS* 3, 333–335.
56. Rasnitsyn, A.P. (1969). Origin and evolution of lower Hymenoptera. *Trudy Paleontologicheskii Instituta* 1969, 1–196.
57. Regier, J.C., Shultz, J.W., and Kambic, R.E. (2005). Pancrustacean phylogeny: hexapods are terrestrial crustaceans and maxillopods are not monophyletic. *Proc. Biol. Sci.* 272, 395–401.
58. Danforth, B.N., Fang, J., and Sipes, S. (2006). Analysis of family-level relationships in bees (Hymenoptera: Apiformes) using 28S and two previously unexplored nuclear genes: CAD and RNA polymerase II. *Mol. Phylogenet. Evol.* 39, 358–372.
59. Hadfield, J.D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *J. Stat. Softw.* 33, 1–22.
60. Gelman, A., Jakulin, A., Pittau, M.G., and Su, Y.-S. (2008). A weakly informative default prior distribution for logistic and other regression models. *Ann. Appl. Stat.* 2, 1360–1383.
61. Hadfield, J.D., Heap, E.A., Bayer, F., Mittell, E.A., and Crouch, N.M. (2013). Disentangling genetic and prenatal sources of familial resemblance across ontogeny in a wild passerine. *Evolution* 67, 2701–2713.