



Ancestral Monogamy Shows Kin Selection Is Key to the Evolution of Eusociality

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Science **320**, 1213 (2008);
DOI: 10.1126/science.1156108

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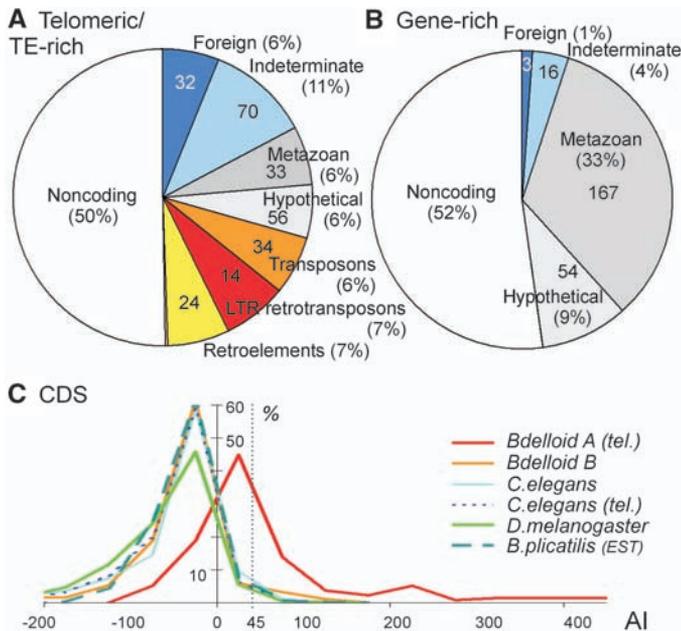
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Fig. 3. Comparison of bdelloid telomeric/TE-rich regions (A) with bdelloid gene-rich regions (B) and with other model invertebrates (C). Pie charts were built with 921,903 base pairs (bp) (A) and 661,316 bp (B) of genomic DNA, respectively (excluding one of the two colinear partners). For TE count, ~1.3 Mb of genomic DNA in each data set was analyzed (including both colinear partners). The size of each sector corresponds to the percentage of the total length (bp) occupied by each category in a data set. Numbers correspond to the total count of entries from each category in a



data set: genes (foreign; indeterminate, including repetitive proteins with AI > 45; metazoan; hypothetical ORFs) and TEs (DNA TEs; retrovirus-like LTR retrotransposons; telomere-associated *Athena* retroelements). The numbers of telomeric repeat stretches in data sets A and B are 67 and 1, respectively. (C) Histogram showing the distribution of the AI value in bins of 50 for CDS from the bdelloid telomeric/TE-rich and gene-rich data sets and from *C. elegans*, *D. melanogaster*, *C. elegans* telomers, and *B. plicatilis* EST (24).

mon universal ancestor and subsequent loss in other kingdoms and in at least four major metazoan branches preceding Rotifera on the evolutionary tree (28), and is inconsistent with our finding of genes representing fusions between domains of prokaryotic and eukaryotic origin. It may be that HGT is facilitated by membrane disruption and DNA fragmentation and repair associated with the repeated desiccation and recovery experienced in typical bdelloid habitats, allowing DNA in ingested or other environmental material to enter bdelloid genomes (12, 29). Whether there may also be homologous replacement by DNA segments released from related individuals remains to be seen. If there is, bdelloid rotifers may experience genetic exchange resembling that in sexual populations (30). Although the adaptive importance of such massive HGT remains to be elucidated, it is evident that such events have frequently occurred in the genomes of bdelloid rotifers, probably mediated by their unusual lifestyle.

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31. We thank W. Reznikoff, M. Belfort, and D. Mark Welch for comments and J. Mark Welch, K. van Doninck, and J. Hur for communicating results before publication. Supported by NSF grant MCB-0614142 (M.M. and I.R.A.) and NIH grant GM072708 (M.M.). Sequences obtained in this study were deposited in GenBank (accession numbers EU643473 to EU643504).

Supporting Online Material

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12 February 2008; accepted 22 April 2008
10.1126/science.1156407

Ancestral Monogamy Shows Kin Selection Is Key to the Evolution of Eusociality

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Close relatedness has long been considered crucial to the evolution of eusociality. However, it has recently been suggested that close relatedness may be a consequence, rather than a cause, of eusociality. We tested this idea with a comparative analysis of female mating frequencies in 267 species of eusocial bees, wasps, and ants. We found that mating with a single male, which maximizes relatedness, is ancestral for all eight independent eusocial lineages that we investigated. Mating with multiple males is always derived. Furthermore, we found that high polyandry (>2 effective mates) occurs only in lineages whose workers have lost reproductive totipotency. These results provide the first evidence that monogamy was critical in the evolution of eusociality, strongly supporting the prediction of inclusive fitness theory.

Eusocial behavior, exemplified by social insects, represents one of the pinnacles of sociality and is characterized by individuals altruistically helping to rear siblings rather than their own offspring (1). The established paradigm, based on inclusive fitness (kin selection) theory, is that eusociality evolves be-

cause of a combination of the direct benefits of altruism (i.e., helpers increase the number of individuals reared) and close relatedness between group members, such that the inclusive fitness of helpers exceeds that achievable through a solitary lifestyle (2–5). High relatedness, arising from the delayed dispersal of offspring [possibly

but not necessarily strengthened by genetic factors such as inbreeding or haplodiploidy (5)], has therefore long been thought to be critical to the evolution of eusociality. However, relatedness in colonies of some extant species is relatively low, and this has led E. O. Wilson, the founder of sociobiology (1), to propose recently that eusociality instead evolves because of direct benefits alone, with relatedness being unimportant (6–8). Under this alternative hypothesis, individuals that share a “eusociality allele,” and which may be related or unrelated, cooperate because their direct fitness is greater when in a social group than when alone (for example, if independent nest founding is extremely risky). Limited dispersal may then tend to lead to groups being made up of kin, but, in contrast to kin selection theory, this increased relatedness is hypothesized to be a consequence, rather than a cause, of the evolution of eusociality (6–8).

Comparative patterns of female mating frequencies may help to resolve this controversy, because mating with multiple males (polyandry) reduces relatedness among siblings (2). If kin selection is important for the evolution of eusociality, then monandry, which maximizes relatedness, should be ancestral (2, 4, 5). Alternatively, if high relatedness is unnecessary for the evolution of eusociality (6–8), then there should be no such relationship. The mating systems present when eusociality originated can be inferred by ancestral state reconstruction on the basis of the phylogeny and mating systems of extant species. The eusocial Hymenoptera provide the ideal group for such an analysis, as they are well studied and comprise nine lineages in which eusociality has independently evolved: once in sphecid wasps (9, 10), three times in halictid bees (11), once in allodapine bees (12), once in corbiculate bees (13), twice in vespine wasps (in the Stenogastrinae and the Polistinae+Vespininae) (14), and once in ants. The eusocial Hymenoptera also exhibit a wide range of mating systems, from obligate monandry to extreme polyandry (15–17).

We compiled a data set of female mating frequencies for 267 species of eusocial bees, wasps, and ants (table S1), covering all of the eusocial lineages except allodapine bees. Most species are monandrous, about a quarter show facultative low polyandry (<2 effective mates), and nine clades within the eusocial lineages have high levels of polyandry (>2 effective mates) (Fig. 1). We mapped these data onto a phylogeny (Fig. 1 and fig. S1) and carried out ancestral state reconstruction (18).

Our analysis shows that monandry was the ancestral state for all eight of the independent origins of eusociality (Fig. 1 and Table 1). All females are monandrous in the only eusocial sphecid wasp (maximum likelihood model $P < 0.0001$ of monandry not being the ancestral state). Females in the three eusocial lineages of halictid bees mate either singly or very rarely doubly ($P = 0.001$, $P = 0.001$, and $P = 0.003$ for the three lineages). Among the corbiculate bees, all stingless bees are monandrous, all honeybees are polyandrous, and bumblebees are ancestrally monandrous with facultative low polyandry being derived in, principally, the *Pyrobombus* clade ($P = 0.003$) (fig. S2). Overall, monandry is the ancestral state for the eusocial corbiculate bees ($P = 0.015$). Monandry is also the most probable ancestral state for both the stenogastrine ($P = 0.003$) and Polistinae+Vespininae ($P = 0.043$) lineages of wasps. The ants show the most evolutionary transitions in mating system, but again monandry was the ancestral state ($P = 0.034$). Thus, in all the eusocial lineages of Hymenoptera where polyandry occurs (Fig. 1), it is derived. Furthermore, most polyandrous species have very low effective mating frequencies. The phylogenetic analysis shows that high levels of polyandry (>2 effective mates) are even more clearly derived ($P < 0.001$ in all cases) (Fig. 1 and Table 1).

The data do not allow us to determine whether monandry was already present in the solitary ancestors or whether monandry and eusociality evolved concurrently, but they are clearly linked. These findings of ancestral monandry and derived polyandry strongly support the prediction of kin selection theory that high relatedness was important in the evolution of eusociality. The results are incompatible with the idea that high relatedness is derived (6–8). Data on mating systems in non-hymenopteran eusocial lineages are far less extensive than for the Hymenoptera, but these data indicate that monandry and high relatedness are also the norm in these groups (Table 1) (4, 19–23). Furthermore, in the one group in which the comparative relationship has been examined (the thrips), high relatedness resulting from inbreeding was inferred at the origins of eusociality (24).

We also used our data for a second, independent test of the role of kin selection in the evolution of eusociality. In some eusocial taxa (25, 26), social evolution has reached the point where workers have lost the ability to mate and reproduce sexually (that is, they are no longer reproductively totipotent). As a result, workers in these taxa cannot found colonies independently or replace the breeder queen and so are irreversibly eusocial. Inclusive fitness theory predicts that polyandry in eusocial taxa will normally evolve only after workers have lost reproductive totipotency (2, 4), whereas there should be no relationship if Wilson’s hypothesis is correct (6–8). For each of the species in

our data set, we scored worker reproductive totipotency as 1 or 0 from the literature (18). The loss of the physiological and behavioral ability to mate almost always results from the evolution of morphologically distinct workers, so where explicit data were unavailable, we assumed that species with queen/worker dimorphism had non-totipotent workers. We analyzed the relationship between totipotency and polyandry across the eusocial Hymenoptera using the method of phylogenetically independent contrasts (18). Both the proportion of females mating multiply (least-squares regression $F_{1,184} = 7.53$, $P = 0.007$) and their effective mating frequency ($F_{1,174} = 6.42$, $P = 0.012$) were significantly lower in species with totipotent workers than in species where workers had lost reproductive totipotency (Fig. 2A and table S1). Worker totipotency was significantly rarer in polyandrous species than in those with monandry, and none of the species with high levels of polyandry had totipotent workers ($F_{1,217} = 5.91$, $P = 0.016$) (Fig. 2B).

In five of the eusocial lineages (sphecid wasps, the three halictid lineages, and stenogastrine wasps), all females are totipotent. However, in the three eusocial lineages in which some taxa have non-totipotent workers (corbiculate bees, polistine+vespine wasps, and ants), ancestral state reconstruction indicated that totipotency was lost prior to or concurrently with the evolution of polyandry. Non-totipotency is ancestral in the eusocial corbiculate bees (maximum likelihood model $P = 0.015$ of totipotency being the ancestral state), whereas polyandry is derived. The clade of polistine+vespine wasps is ancestrally totipotent ($P = 0.969$). However, non-totipotency is ancestral in the vespines ($P = 0.015$), so totipotency was lost before the subsequent evolution of polyandry in this clade. Finally, in the ants, totipotency is ancestral overall ($P = 0.983$) but was lost early in the evolution of polyandrous *Pachycondyla* ($P = 0.015$). Non-totipotency is the ancestral state for non-ponerine ants ($P = 0.03$), indicating that it was lost before the multiple evolutions of polyandry in these ants.

In addition to polyandry, relatedness in eusocial colonies is also decreased if colonies have multiple, simultaneously breeding females (functional polygyny), as occurs in some extant species of eusocial Hymenoptera (27). If functional polygyny had been the ancestral state in eusocial lineages, then this would weaken the support for high relatedness being important in the evolution of eusociality. Although our data set was collected primarily to examine polyandry, we also performed an ancestral state reconstruction for functional polygyny (18, 27). Colonies of eusocial sphecids normally have only a single reproducing female at any one time, as do colonies of the only *Augochlora* and *Halictus* species included in our analyses. In the third eusocial lineage of halictid bees, *Lasioglossum*, the ancestral state was uncertain ($P = 0.069$). Func-

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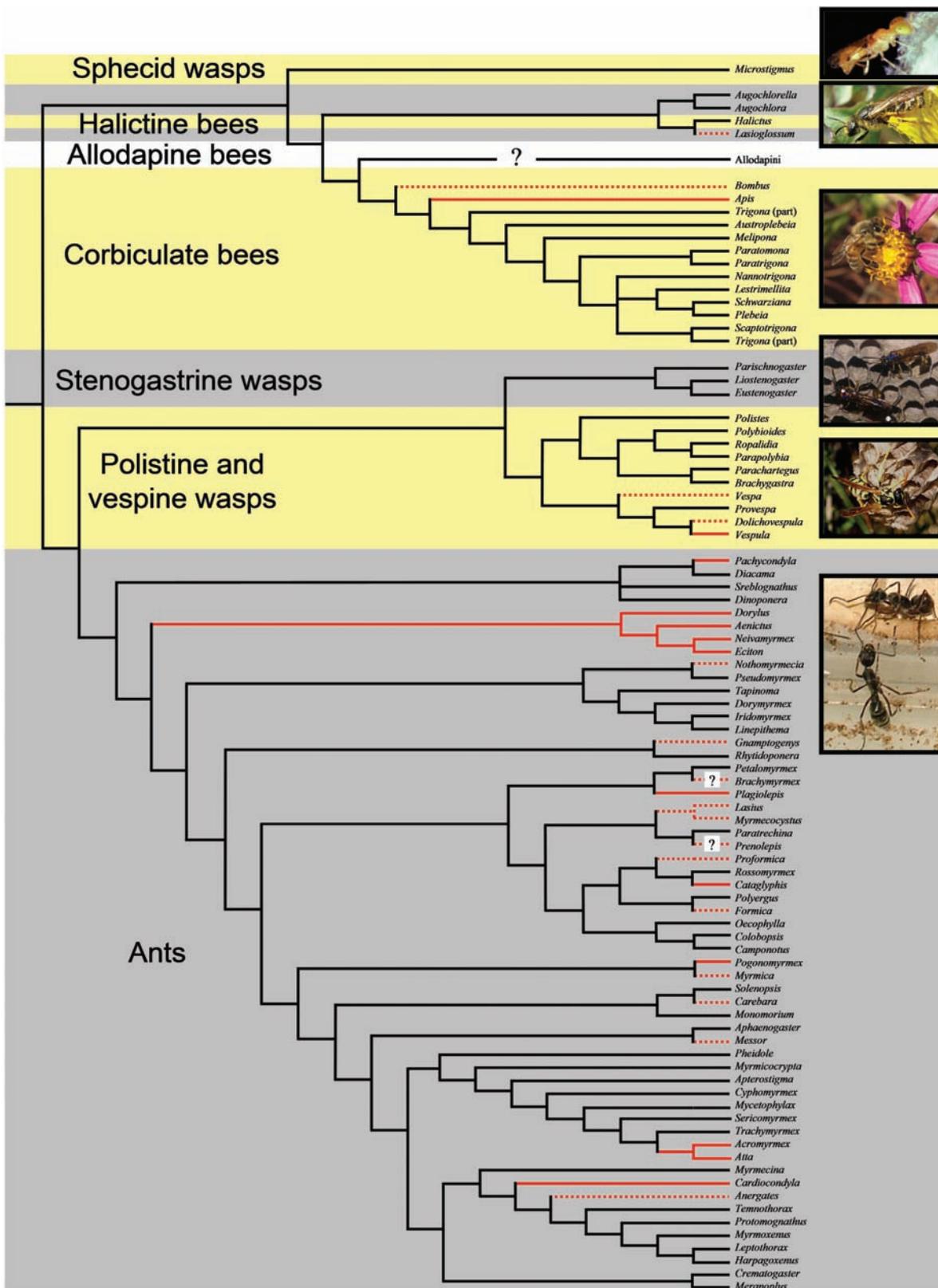


Fig. 1. Phylogeny of genera of eusocial Hymenoptera (ants, bees, and wasps) for which female mating-frequency data are available. See fig. S1 for the species-level phylogeny used in the analyses. Each independent origin of eusociality is indicated by alternately colored clades. Clades exhibiting high polyandry (>2 effective mates) have solid red branches, those exhibiting facultative low polyandry (>1 but <2 effective mates) have dotted red branches,

and entirely monandrous genera have solid black branches. Mating-frequency data are not available for the allodapine bees. [Photographs from top to bottom are of (and reproduced with permission from): *Microstigmus comes* (R. Matthews), *Lasioglossum malachurum* (C. Polidori), *Apis mellifera* (F.L.W. Ratnieks), *Liostenogaster flavolineata* (J. Fields), *Polistes dominulus* (W.O.H. Hughes), and *Diacamma* sp. (W.O.H. Hughes)]

tional monogyny was found to be ancestral in the eusocial corbiculate bees ($P = 0.001$), stenogastrine wasps ($P = 0.0005$), polistine+vespine wasps ($P = 0.006$), and ants ($P = 0.0018$). Colonies of some primitively eusocial species, such as *Polistes* wasps (28) and the stenogastrine wasp *Liostenogaster* (29), may have several mated females. However, these females reproduce consecutively, reducing the impact on relatedness relative to concurrent reproduction. Therefore, the evidence indicates that functional monogyny is ancestral in the eusocial Hyme-

noptera, again in keeping with inclusive fitness theory.

Our study comes to the simple conclusion that monandry was the ancestral state when eusociality arose in ants, bees, and wasps, something that also appears generally true in the non-hymenopteran eusocial taxa. Additionally, we show that worker reproductive totipotency is associated with monandry. Obligate or high levels of polyandry are invariably derived and occur only in species whose workers lack the ability to become the primary breeders. Thus, polyandry seems to have

evolved after social evolution had reached a point at which reversion from eusociality was impossible. Similarly, functional polygyny is also derived. Our conclusions strongly indicate that kin selection and high relatedness have played a decisive role in the evolution of eusociality.

Table 1. Summary of evidence for monandry in the independent origins of eusociality in the Hymenoptera (ants, bees, and wasps; this study) and other eusocial lineages. P values relate to ancestral state reconstructions by maximum likelihood. The first value indicates the probability of monandry not being the ancestral state, whereas the second value indicates the probability of high polyandry (>2 effective mates) being the ancestral state.

Taxa	Eusocial origins	Eusocial species	Evidence
Sphecoid wasps	1	1	This study; $P < 0.0001$; $P < 0.0001$
Halictid bees (<i>Augochlorella/Augochlora</i>)	1	Many of 140	This study; $P = 0.0014$; $P < 0.0001$
Halictid bees (<i>Halictus</i>)	1	Most of 217	This study; $P = 0.0014$; $P < 0.0001$
Halictid bees (<i>Lasioglossum</i>)	1	Most of 544	This study; $P = 0.003$; $P < 0.0001$
Allodapine bees	1		No data
Corbiculate bees	1	≈1000	This study; $P = 0.015$; $P = 0.0007$
Stenogastrine wasps	1	≈50	This study; $P = 0.0026$; $P < 0.0001$
Polistine and vespine wasps	1	≈860	This study; $P = 0.043$; $P < 0.0001$
Ants	1	≈12,000	This study; $P = 0.034$; $P = 0.0007$
Ambrosia beetle	1	1	Monoandry thought probable but no data (19)
Aphids	≈17	≈50	Eusocial colonies produced parthenogenetically by single female (4, 23)
Termites	1	≈2800	Generally monoandrous, with only a few species exhibiting low polyandry (4)
Thrips	1	7	Normally monoandrous (4, 23, 24)
Snapping shrimps	3	6	Monoandrous (20)
Mole rats	2	2	Facultative low polyandry (21, 22)

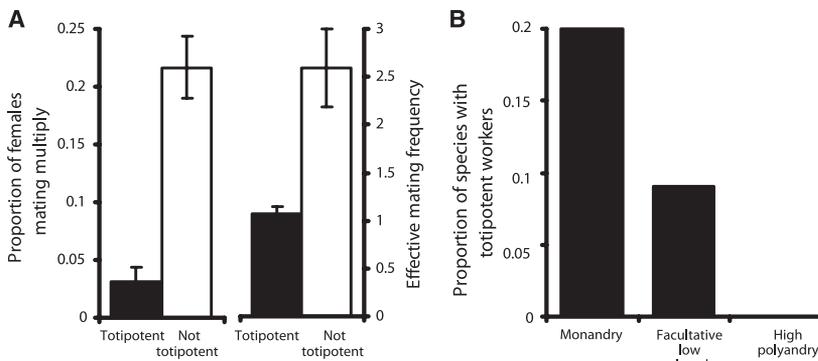


Fig. 2. (A) Mean (\pm SE, indicated by error bars) proportion of females mating multiply and effective mating frequency for species with and without totipotent workers. (B) Occurrence of totipotent workers in species with monandry, facultative low polyandry (>1 but <2 effective mates), or high polyandry (>2 effective mates).

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30. We thank H. Helanterä, T. Wenseleers, A. Bourke, R. Hammond, J. Boomsma, and the anonymous reviewers for comments on the manuscript. This work was supported by the European Commission via a Marie Curie Outgoing International Fellowship to W.O.H.H. B.P.O. and M.B. were supported by the Australian Research Council.

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5 February 2008; accepted 22 April 2008
 10.1126/science.1156108