

Convergent Reversion to Single Mating in a Wasp Social Parasite

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ABSTRACT: While eusociality arose in species with single-mating females, multiple mating by queens has evolved repeatedly across the social ants, bees, and wasps. Understanding the benefits and costs of multiple mating of queens is important because polyandry results in reduced relatedness between siblings, reducing kin-selected benefits of helping while also selecting for secondary social traits that reduce intracolony conflict. The leading hypothesis for the benefits of polyandry in social insects emphasizes advantages of a genetically diverse workforce. Workerless social parasite species (inquilines) provide a unique opportunity to test this hypothesis, since they are derived from social ancestors but do not produce workers of their own. Such parasites are thus predicted to evolve single mating because they would experience the costs of multiple mating but not the benefits if such benefits accrue through the production of a genetically diverse group of workers. Here we show that the workerless social parasite *Dolichovespula arctica*, a derived parasite of wasps, has reverted to obligate single mating from a facultatively polyandrous ancestor, mirroring a similar reversion from obligate polyandry to approximate monandry in a social parasite of fungus-farming ants. This finding and a comparison with two other cases where inquilinism did not induce reversal to monandry support the hypothesis that facultative polyandry can be costly and may be maintained by benefits of a genetically diverse workforce.

Keywords: mating frequency, eusociality, Vespidae, social parasitism, paternity, inquilinism.

Introduction

The evolution of multiple mating by females (polyandry) has been a major focus of research in evolutionary biology (Taylor et al. 2014). The mating systems of social insects have attracted particular interest, given the wide variation in

queen mating frequencies across the ants, bees, and wasps; the importance of multiple mating in determining kin-selected benefits of altruism to workers; and the remarkable repeated origins of extreme polyandry in a few phylogenetically diverse, highly eusocial taxa (Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Strassmann 2001; Hughes et al. 2008a; Boomsma et al. 2009; Boomsma 2013; Jaffé 2014). A variety of hypotheses have been proposed to explain the selective benefits of polyandry in social insects (Crozier and Page 1985; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001). Such benefits are an interesting problem given the likely costs of polyandry, including increased energy expenditure (Hayworth et al. 2009), elevated exposure to predators and sexually transmitted diseases (Crozier and Page 1985; Roberts et al. 2015), and higher immunity costs associated with the storage of sperm (Baer et al. 2006). The hypotheses receiving the most support are those that involve the benefits of a genetically diverse colony: that elevated intracolony diversity (i) improves pathogen resistance (Hamilton 1987; Sherman et al. 1988; Schmid-Hempel 1998), (ii) improves the organization of work or division of labor (Page et al. 1989), or (iii) reduces the variance in the production of diploid males as a result of single-locus sex determination (Page 1980; Crozier and Page 1985). A fourth hypothesis, not based on genetic diversity, is that queens mate with more males to provide sufficient sperm to produce the queen's many daughters (Cole 1983).

There is experimental support for hypotheses suggesting that a genetically diverse workforce improves colony-level disease resistance (Baer and Schmid-Hempel 1999; Hughes and Boomsma 2004; Seeley and Tarpy 2007) and increases colony productivity by improving the organization of work (Jones et al. 2004; Mattila and Seeley 2007; Mattila et al. 2008). While experimental approaches to understanding the evolution of polyandry are powerful, they are limited to those few species amenable to manipulation. Given the diversity of life histories and mating syndromes across the social Hymenoptera, it seems possible that different benefits may apply in different taxa (Crozier and Fjerdingstad 2001), many

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of which are not easily studied experimentally. A complementary approach is to compare the mating biology of closely related taxa that differ in key life-history traits that could influence the costs and benefits of polyandry (Boomsma et al. 2009). Such studies, particularly when coupled with a phylogenetic perspective, have provided strong evidence that mating frequency is negatively associated with queen number (Kronauer and Boomsma 2007; Hughes et al. 2008b) and paternity skew (Jaffé et al. 2012; Barth et al. 2014; Loope et al. 2014) but positively correlated with colony size (Kronauer et al. 2010; Loope et al. 2014), all generally supporting arguments for a genetic diversity benefit to polyandry.

An important but understudied life-history shift predicted to influence the costs and benefits of polyandry is the evolution of social parasitism. In the social insects, obligate inquiline social parasite females invade colonies of a different species to reproduce and remain dependent on the host species for the entire reproductive period (Buschinger 2009). This extreme form of social parasitism has evolved repeatedly from social ancestors in the ants (Buschinger 2009), bees (Hines and Cameron 2010), and wasps (Cervo 2006; Lopez-Osorio et al. 2015). Females that join or take over a host colony can acquire through parasitism a genetically diverse workforce without paying the costs of polyandry and thus are predicted to evolve single mating (Sumner et al. 2004b). To date, the only support for this link between mating system and parasitism comes from a clade of obligately polyandrous leafcutter ants with a striking reversion to approximate monandry in a derived inquiline social parasite (Sumner et al. 2004b). The association between social parasitism and single mating in *Acromyrmex insinuator* suggests that socially parasitic inquilines do not experience the benefits that otherwise maintain polyandry in closely related taxa, and it also implies that multiple mating has costs. However, the generality of this result is unclear, as the only other tests of this association found no similar reversion to monandry in two other social parasite species (Hoffman et al. 2008; Thurin and Aron 2011). Using a comparative approach, we tested the hypothesis that the production of a large workforce underlies the evolutionary benefits of polyandry in social insects using the workerless inquiline wasp *Dolichovespula arctica*, a derived social parasite of aerial-nesting wasps.

Dolichovespula arctica is an obligate inquiline social parasite with a natural history similar to the handful of other derived obligate social parasites of eusocial vespine wasps (Jeanne 1977; Greene et al. 1978; Greene 1991; Lopez-Osorio et al. 2015). Females emerge from hibernation in the spring and invade young colonies of a related species, *Dolichovespula arenaria* (fig. 1). The parasite female coexists with the host queen for several days before killing her and beginning to lay eggs. The inquiline produces no worker offspring, and the host workers rear the parasite brood as if they were sib-



Figure 1: The host queen (*Dolichovespula arenaria*; black and yellow markings) grooms herself on the nest envelope of a preemergence colony, while an inquiline social parasite (*Dolichovespula arctica*; black and ivory markings) inspects cells on the comb inside the nest. We modified shadowing and highlighting levels with Adobe Photoshop to make the parasite more visible. Photo by Robert Jeanne.

lings, although aggression between the inquiline and workers often escalates, eventually resulting in her death. Recent phylogenetic analyses (Lopez-Osorio et al. 2015; Perrard et al. 2015) place *D. arctica*, like its host, within the subgenus *Boreovespula*. Most members of the genus, including the host species, exhibit facultative polyandry (Foster et al. 2001; Bonckaert et al. 2011b; Loope 2015), a mating system in which queens frequently mate singly but also sometimes mate more than once (Boomsma and Ratnieks 1996; Boomsma 2009, 2013). It has been argued that facultative polyandry represents a mating syndrome distinct from obligate polyandry (always multiple queen mating), with possibly very different selective pressures maintaining it (Boomsma and Ratnieks 1996; Boomsma 2013). Importantly, the facultative polyandry of *Dolichovespula* allows insight into the costs and benefits of this understudied mating syndrome. Here we investigate the evolution of facultative polyandry by describing the mating biology of *D. arctica* and comparing it, in a phylogenetic framework, to the mating biology of close relatives.

Methods

We collected colonies of *Dolichovespula arenaria* parasitized by *Dolichovespula arctica* in the summers of 2012

and 2013 in Tompkins County, New York, freezing specimens at -20°C until genotyping. We used nine colonies with female *D. arctica* adults or brood, inferring paternity from offspring genotypes, and six colonies with only the mother inquiline present, inferring mating frequency from stored sperm genotypes.

Genotyping Adults

We used four microsatellite loci—*Rufa05*, *Rufa13*, *Rufa15*, and *List2004*—that are highly variable in other *Dolichovespula* species (Foster et al. 2001; Loope 2015). Adult genotypes were obtained using methods described in our previous work on wasp paternity (Loope et al. 2014). DNA was extracted from a single antenna or leg in 100 μL of a 10% Chelex solution by incubating at 55°C for 20 min. We used dye-labeled primers (Applied Biosystems) to perform multiplex polymerase chain reaction (PCR). Reaction volumes were 5.5 μL per sample (0.1 μL of each forward and reverse primer, 1.2 μL of water, 3.0 μL of Qiagen Type-It Microsatellite Master Mix, and 0.5 μL of DNA). PCR conditions were 95°C for 15 min; 35 cycles of 95°C for 30 s, 50°C for 90 s, and 72°C for 60 s; and then 60°C for 30 min. Fragment analysis was performed on an ABI-3730xl sequencer using 0.5 μL of PCR product combined with 15 μL of HiDi formamide and 0.15 μL of LIZ 500 internal size standard (Applied Biosystems). Allele sizes were called using GeneMarker (SoftGenetics) and checked twice by eye.

Paternity Estimation in Colonies with Female Brood

We genotyped 23–25 daughters from each of the nine parasitized colonies containing *D. arctica* daughters ($n = 9$). Maternal genotypes were determined by direct genotyping ($n = 4$) or inferred from the genotypes of seven or eight male pupae ($n = 5$). The minimum number of patriline required to explain daughter genotypes was determined by comparison of maternal genotypes and daughter genotypes. Maternal and daughter genotypes were also used to estimate paternity using the full likelihood method of Colony2 software, with no sibship size prior and error rates of 0.01 (ver. 2.0.5.7 for Mac; Jones and Wang 2010). When estimating paternity from offspring genotypes, there are two sources of error: nonsampling and nondetection (Boomsma and Ratnieks 1996). Nonsampling error is the chance that the sampled daughters do not include a patriline present in the colony, and it was calculated according to the method of Boomsma and Ratnieks (1996). Nondetection error occurs when a male mate is not detected due to genetic similarity either to another male or to the queen (e.g., when two males mated to the same female share alleles at all genotyped loci; Pamilo 1982; Foster et al. 1999). We calculated the population-level probability that two males have identi-

cal observed genotypes by means of the formula in Boomsma and Ratnieks (1996), and we calculated the colony-specific probability of nondetection error by means of the formula in Foster et al. (1999).

In one colony, a paternal null allele was detected at *Rufa05*; all offspring appeared homozygous for one of each of the maternal alleles. Before analyzing paternity, this was coded as a novel allele. Mendelian inheritance patterns in other colonies and at other loci indicated no additional null alleles.

Genotyping Sperm

For an additional six inquiline females collected in June 2012, before they had produced pupal or adult offspring, we genotyped sperm stored in the spermatheca to infer the number of mates (Chapuisat 1998). The orange-red sperm clump was dissected out of each inquiline's spermatheca in molecular-grade water using insect pins and transferred to 20 μL of 5% Chelex solution for DNA extraction. The protocol described above was then followed for genotyping sperm clumps. Each adult female was also genotyped using methods described above, and these genotypes were compared with stored sperm genotypes to detect contamination with maternal tissue. Nondetection errors can still occur in these estimates of mate number, since two male mates could possess identical genotypes at all typed loci. We thus calculated the probability of two males sharing the detected sperm genotype for each female.

Inbreeding

We calculated the average relatedness of males to their female mates as a measure of inbreeding (Liautard and Sundström 2005) using the female genotypes and inferred male genotypes and the program Relatedness (ver. 5.0.8; Goodnight and Queller 1998). We also calculated the regression relatedness among female offspring within the nine colonies with daughter genotypes. If inbreeding occurs under single mating, average pairwise relatedness values should be higher than the expected 0.75 for haplodiploid full sisters.

Phylogenetic Comparison

Although the phylogenetic position of *D. arctica* was recently reported (Lopez-Osorio et al. 2015), we performed similar phylogenetic analyses to include new genetic data from *Dolichovespula norwegica*, one of the few *Dolichovespula* species with known paternity. This analysis thus reveals the phylogenetic relationships among the seven *Dolichovespula* species for which there are paternity data.

We generated sequence data for *D. norwegica* and retrieved DNA sequences from GenBank (accession numbers

are provided in Lopez-Osorio et al. 2014, 2015). We extracted DNA using the DNeasy Blood and Tissue Kit (Qiagen) and amplified gene fragments using PCR with primers listed in previous studies (Lopez-Osorio et al. 2014, 2015). We sequenced fragments from the mitochondrial genes *12S*, *16S*, *COI*, *COII*, *CYTB*, and *ATP6* and from the nuclear genes *28S*, *CAD*, *EF1a-F2*, *Pol II*, and *wg*. Each PCR included 22 μL of nuclease-free water, 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, and 1 μL of DNA extract. The 25- μL total volume in each reaction was added to illustra PuReTaq Ready-to-Go PCR beads (GE Healthcare Life Sciences). PCR cycling conditions were 4 min of initial denaturation at 94°C; followed by 35–40 cycles of 30 s at 94°C, 45 s of annealing at 43°–58°C, and 45 s of elongation at 72°C; and then 6 min of final elongation at 72°C. We assembled contigs using Geneious 6 (Biomatters) and performed multiple sequence alignments in MAFFT (ver. 7; Katoh and Standley 2013) with the Auto option.

Protein-coding genes partitioned on the basis of codon positions and ribosomal DNA genes were submitted to PartitionFinder (ver. 1.0.1; Lanfear et al. 2012) under the following settings: branchlengths = unlinked, models = mrbayes, and model_selection = corrected Akaike information criterion. Phylogenetic analyses were carried out on CIPRES (Miller et al. 2010) using MrBayes (ver. 3.2; Ronquist et al. 2012) with nruns = 2, nchains = 8, samplefreq = 1,000, brlenspr = unconstrained:Exp(100), 40 million generations, and unlinking parameters across partitions. We assessed convergence in Tracer (ver. 1.6; Rambaut et al. 2013) by examining effective sample size values.

To see whether species quantitatively differ in mating frequency, we used the Fisher exact test to compare the frequency of single mating in *D. arctica* to its three closest relatives in the *Boreovespula* clade (sensu Perrard et al. 2015): *D. saxonica*, *D. norwegica*, and *D. arenaria*. We similarly compared the average pedigree sister relatedness within families as a measure of genetic diversity, using the Welch unequal-variances *t*-test. Average pedigree relatedness was estimated from reported effective paternity (m_e) of colonies using the formula $0.25 + 0.5/m_e$ (Pamilo 1993).

Ancestral State Reconstruction

We used ancestral state reconstruction analysis to estimate the mean mate number (a continuous trait) and mating system type (a discrete trait; monandry, facultative polyandry, or obligate polyandry) of the most recent social ancestor of *D. arctica*. Our Bayesian phylogenetic analysis (above) agreed with the conventional placement of the genus *Vespula* as sister to *Dolichovespula*, but this disagrees with a recent phylogenomic analysis of these groups (Lopez-Osorio et al. 2017), which places *Vespa* as sister to *Dolichovespula*. We wanted to account for both possibilities in our ancestral

state reconstructions, so we used a constraint tree in a maximum likelihood (ML) phylogenetic analysis to generate two trees representing these two scenarios. Using the alignments from the same 11 genes described above, we conducted partitioned phylogenetic analyses using RAxML (ver. 8; Stamatakis 2014) with 100 alternative runs and the general time-reversible model with gamma correction, and we estimated branch support with 100 bootstrap pseudoreplicates. The optimal ML tree recovered *Dolichovespula* and *Vespula* as sister taxa. We then performed an additional ML analysis using a constraint tree enforcing a sister relationship between *Dolichovespula* and *Vespa*. Both the optimal and the constrained ML trees were considered for inference of ancestral states. After dropping tips without trait data using the treedata function in the R package geiger (Pennell et al. 2014), we inferred ancestral states for the continuous and discrete characters using the functions fastAnc and rerootingMethod in the R package phytools (Revell 2012), respectively. Ancestral states for continuous data were visualized using the contMap function in phytools.

New sequence data are available on GenBank (accession nos. KY452445–KY452453). Microsatellite genotypes, error rate calculations, GenBank accession numbers for sequences used in phylogenies, and comparative data on mating behavior taken from the literature are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.k6r4v> (Loope et al. 2017).

Results

A single male mate was sufficient to explain paternity in each of the nine sets of genotyped daughters. The Colony2 analysis agreed: the ML configuration of paternities for each colony was a single father (table 1). Given our sampling of ~24 daughters per colony, the probability of not sampling a second father with 50% or 20% of paternity occurs with probabilities 5.9×10^{-9} and 0.0047, respectively. Given the allele frequencies estimated from the parental generation (i.e., reproductive females and their inferred mates), the probability of two randomly selected males sharing genotypes at all four loci was 0.003. The average colony-specific nondetection error was 0.0057 (range: 0.024–0.0004). Given these low error rates, we can conclude that our estimates of paternity based on these loci are accurate.

The stored sperm genotypes from five of six analyzed inquiline females suggested a single mate: one allele was present at each locus, and each sperm sample lacked shared alleles with the female at some loci, meaning that contamination with female spermathecal tissue did not occur. The (colony-level) probability of male nondetection error was very low for these females (mean: 0.0012; range: 0.0034–0.0004), meaning that these estimates of mating frequency are also reliable.

Table 1: Observed mating frequencies and sibling regression relatedness for *Dolichovespula arctica* females

Female	Analysis type ^a	No. daughters typed	Inferred no. fathers/mates	Regression relatedness ^b
Arc1	Daughters	24	1	.76
Arc2	Daughters	25	1	.78
Arc3	Daughters	23	1	.82
Arc4	Daughters	24	1	.68
Arc5	Daughters	24	1	.70
Arc6	Daughters	24	1	.68
Arc7	Daughters	24	1	.65
Arc8	Daughters	23	1	.72
Arc9	Daughters	24	1	.72
Arc10	Sperm	...	1	...
Arc11	Sperm	...	1	...
Arc12	Sperm	...	1	...
Arc13	Sperm	...	1	...
Arc14	Sperm	...	1	...

^a Female mating frequency was determined either by genotyping daughters or by genotyping sperm stored in spermatheca.

^b Relatedness among daughters.

In one of six stored sperm genotypes, two alleles were present at two loci. However, in this sample all alleles present in the female were also present in the sperm genotype, suggesting contamination from female tissue. Given the frequency of the female alleles, the probability that a randomly selected male shares an allele with this female at all loci is 0.005, suggesting that contamination is a more likely explanation than actual double mating. If such contamination occurred, the resulting genotypes are consistent with a single male mate, but because of the ambiguity the sample was removed from further analyses. Thus, of 14 females with reliable estimates of paternity or mating frequency, all indicated a single male mate.

The average relatedness between the 14 inferred males and their mates was -0.0041 (95% confidence interval: -0.21 to

0.20) from jackknifing over loci. The average sibling relatedness for the nine colonies with genotyped daughters was 0.73 with the 95% confidence interval (0.68–0.79) overlapping 0.75, further suggesting no inbreeding in these colonies (table 1).

Our phylogeny agreed with previously published trees (Lopez-Osorio et al. 2015; Perrard et al. 2015) and places *Dolichovespula norwegica* in the *Boreovespula* clade (fig. 2; fig. A1), which otherwise consists of facultatively polyandrous species. Only two nodes had Bayesian support values of less than 1.0 (fig. 2; fig. A1).

Quantitatively, the mating system of *Dolichovespula arctica* was distinct from closely related species. The frequency of single mating in our sample of *D. arctica* females was significantly higher than that in *D. arctica*'s three closest rela-

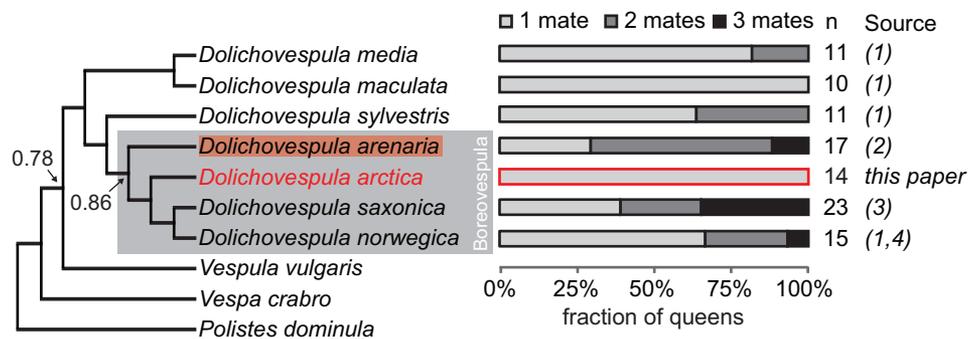


Figure 2: Evolution of mating frequency in *Dolichovespula* wasps. The cladogram was pruned to include only species with known paternity/mating frequency (for full tree, see fig. A1). All unlabeled nodes have Bayesian support values of 1.0. *Dolichovespula arctica* (red text) is a social parasite of *Dolichovespula arenaria* (highlighted). Bars depict the distribution of mating frequencies as a proportion of females analyzed. All mating frequencies were estimated from the paternity of daughters except for the five *D. arctica* stored sperm samples presented in this article (see “Methods”). Sample size (*n*) refers to the number of queens or females analyzed. Source data are from (1) Foster et al. (2001), (2) Loope (2015), (3) Bonckaert et al. (2011b), and (4) Bonckaert et al. (2011a).

tives (fig. 2; Fisher exact test; *Dolichovespula arenaria*: 4 of 17 monandrous, $P < .001$; *Dsaxonica saxonica*: 9 of 23 monandrous, $P < .001$; *D. norwegica*: 10 of 15 monandrous, $P = .042$). Similarly, pedigree relatedness of daughters within colonies of eusocial species, an estimate of genetic diversity that accounts for paternity frequency and paternity skew, was significantly different from that estimated for all *D. arctica* families (Welch unequal-variances *t*-test; *D. arenaria*, mean = 0.58 ± 0.11 SD, $t = 6.27$, $df = 16$, $P < .001$; *D. saxonica*, mean = 0.59 ± 0.14 SD, $t = 5.40$, $df = 22$, $P < .001$; *D. norwegica*, mean = 0.70 ± 0.08 SD, $t = 2.36$, $df = 14$, $P = .033$). All six P values are still significant after controlling for multiple comparisons with a false discovery rate of 0.05 and the method of Benjamini and Hochberg (Benjamini and Hochberg 1995; Verhoeven et al. 2005) in the `p.adjust` function in R.

Ancestral state reconstructions suggested that the most recent social ancestor of *D. arctica* was facultatively polyandrous (figs. A2, A3). For this ancestor under the traditional phylogenetic scenario with *Vespula* as the sister genus to *Dolichovespula*, the marginal probability for the three mating syndromes was 0.96 for facultative polyandry, 0.02 for monandry, and 0.01 for obligate polyandry (fig. A2A). The estimated mean queen mate number from the fastAnc analysis was 1.52 (95% CI: 1.23–1.83; fig. A2B). The same analyses with a constraint tree forcing *Vespa* as the sister genus to *Dolichovespula* yielded a nearly identical result (fig. A3). The inferred mean mate number was 1.53, and the marginal probability of a facultatively polyandrous mating system was 0.96.

Discussion

Although most species in the genus *Dolichovespula* are facultatively polyandrous, all analyzed females of the social parasite *Dolichovespula arctica* were singly mated (fig. 2). In contrast, most queens of the host species, *Dolichovespula arenaria*, mate multiply in our study population (~71% polyandrous; fig. 2; Loope 2015). Thus,inquilines that take

over *D. arenaria* colonies may inherit the benefits of a genetically diverse workforce without paying the costs of mating multiply. The three most closely related species, all members of the subgenus *Boreovespula*, all have facultative multiple mating, suggesting that their most recent common ancestor with *D. arctica* was also facultatively polyandrous (fig. 2). *Dolichovespula arctica* is the only of these species that is completely monandrous and has a mating system that is quantitatively distinct from that of its host and related species. Other than its socially parasitic habit, *D. arctica* is similar in many respects to *D. arenaria* and congeners: reproductive females mate in the fall, store sperm during diapause over winter, and only live for a single year. This inquiline species is common in our study population: the host *D. arenaria* is abundant, and ~40% of *D. arenaria* colonies are infected (Keyel 1983). Males are frequently seen on flowers in the late summer and patrol low vegetation, aggregating in a manner similar to other vespine wasps (K. J. Loope, personal observation), making limited male availability an unlikely explanation for the reversion to monandry. Instead, our data suggest that the reversion resulted from the adoption of an obligately parasitic lifestyle. Single mating in *D. arctica* supports the hypothesis that facultative multiple mating is costly and that the benefits maintaining it can be connected to the production of a genetically diverse workforce (Sumner et al. 2004b). Once worker production is lost, it appears that the benefits of polyandry likely disappear, and the costs drive social parasites to monandry.

Comparisons to Other Taxa

Evolutionary reductions in polyandry appear to be rare. Other than the two social parasite examples (*D. arctica* and *Acromyrmex insinuator*; table 2), only two such transitions are known. A polygynous army ant evolved facultative polyandry from obligate polyandry (Kronauer and Boomsma 2007). This reduction in polyandry neatly reflects the trade-off between the two sources of within-colony genetic diversity (multiple queens or multiple mates) that has also been

Table 2: Mating systems and life-history traits of social parasites in the eusocial Hymenoptera

Species	Family	Reversion to monandry?	Ancestor	Worker production?	Inbreeding?	Reference(s)
<i>Acromyrmex insinuator</i>	Formicidae	Yes	Obligate polyandry	Reduced	No	Sumner et al. 2004a, 2004b
<i>Dolichovespula arctica</i>	Vespidae	Yes	Facultative polyandry	No	No	This article
<i>Plagiolepis xene</i>	Formicidae	No	Obligate polyandry	No	Yes	Thurin and Aron 2011
<i>Vespula squamosa</i>	Vespidae	No	Obligate polyandry	Yes	No	Hoffman et al. 2008

demonstrated in a large-scale comparative analysis (Hughes et al. 2008b). Several desert ants (*Cataglyphus* spp.) have reverted from obligate to facultative polyandry as well, although no ecological or life-history factors associated with these transitions are apparent (Aron et al. 2016; Boomsma 2016). Together with *A. insinuator* (Sumner et al. 2004b), all reversions to date have been from obligate polyandry to mating systems with at least some residual multiple mating; *D. arctica* is the first reversion documented from a facultatively polyandrous ancestor and the first to reevolve obligate single mating.

Comparing our findings in *D. arctica* to other studies suggests that monandry is probably adaptive only for particular types of social parasites within the social Hymenoptera (table 2). The ant *Plagiolepis xene*, like *D. arctica*, is a workerless inquiline, yet this species maintains the ancestral polyandry of its congeners (Thurin and Aron 2011). Unlike *D. arctica* and *A. insinuator*, this species and its host (Trontti et al. 2007) are highly inbred: females mate with siblings inside the nest. Females thus avoid mating flights and presumably the associated costs of finding multiple mates, which could explain the persistence of polyandry in this species (Thurin and Aron 2011). In this scenario, polyandry may be maintained because it has low costs for queens but benefits males (Trontti et al. 2007). In another exception to the association between social parasitism and monandry, *Vespula squamosa* queens, like *D. arctica* females, frequently invade and take over young colonies of another species of vespine wasp, but they are always polyandrous (Hoffman et al. 2008). A critical difference between this species and *D. arctica* is that the socially parasitic females of *V. squamosa* go on to produce a large, free-living workforce of their own (MacDonald and Matthews 1984); such colonies only initially rely on host workers, making them temporary rather than permanent social parasites (Buschinger 2009). Because they must produce a large, free-living colony of workers, *V. squamosa* queens presumably experience the same selection pressures that maintain polyandry in other free-living *Vespula* species that are not socially parasitic in the founding stage (Loope et al. 2014).

The Benefits of Facultative Polyandry

While the reversion to monandry in the leafcutter ant social parasite was from a highly polyandrous ancestor (Villesen et al. 2002; Sumner et al. 2004a), species in the *Boreovespula* group exhibit facultative polyandry (fig. 2), indicating that *D. arctica* evolved monandry from facultative polyandry. Most studies addressing the benefits of polyandry in the eusocial Hymenoptera have focused on obligately polyandrous species, and the benefits maintaining facultative polyandry may be different and are currently unknown (Boomsma 2013). The reversion to monandry suggests female rather than male

control over mating frequency: *D. arctica* females differ from queens of closely related species in that they no longer produce workers, while selection on male mating traits is presumably similar in both social and inquiline species. The reversion to monandry by the social parasite therefore implies that the facultative, relatively low levels of polyandry of other *Dolichovespula* are adaptive for females and are not exclusively the result of male strategies that result in multiple matings that females would otherwise avoid (i.e., convenience polyandry; Thornhill and Alcock 1983; Trontti et al. 2007).

How might females benefit from facultative polyandry? It is possible that this mating system results from a balanced polymorphism, wherein females with monandrous and polyandrous strategies obtain equal fitness in the population (Crozier and Fjerdingstad 2001). Alternatively, facultative polyandry may be a conditional strategy that results in single or multiple mating depending on local conditions, such as mate availability, local mating risks, or the quality or compatibility of the first mate (Crozier and Fjerdingstad 2001; Boomsma 2013). The reversion to obligate monandry in *D. arctica* implies that queens of facultatively polyandrous *Dolichovespula* benefit from their second mates via their production of workers rather than as a follow-up to an incompatible or undesirable first mate (Boomsma 2013), since the latter would apply for inquiline species as well as social species. There is some evidence that polyandrous *Dolichovespula* queens benefit as a result of conflict with workers over male parentage. In the host species *D. arenaria*, workers preferentially kill queens that mate singly but generally spare polyandrous ones (Loope 2015). Queen killing takes place in the reproductive stage and is therefore probably costly to queens (Bourke 1994; Loope 2016). In *D. saxonica*, workers may prevent worker reproduction more in colonies headed by polyandrous queens (Foster and Ratnieks 2000; but see Bonckaert et al. 2011b), which would presumably benefit polyandrous queens. Alternatively, increasing genetic diversity by sometimes mating multiply could provide benefits due to pathogen resistance or improved division of labor, as has been hypothesized for obligate polyandry, but direct tests of this hypothesis are lacking in any facultatively polyandrous species. However, small increases in genetic diversity, such as those provided by double mating, have been shown to confer disease resistance benefits in groups of bumblebees and ants (Baer and Schmid-Hempel 1999; Hughes and Boomsma 2004). In contrast, such benefits were not found in wild or laboratory-reared colonies of the facultatively polyandrous ant, *Lasius niger* (Fjerdingstad et al. 2003). In the large-colony, obligately polyandrous yellowjacket wasp *Vespula maculifrons*, paternity is positively associated with the number of queens produced, suggesting a productivity advantage to polyandry in a species where effective mating frequency ranges from ~3 to 9 (Goodisman et al. 2007). This increase in fitness is consistent with diversity benefits of poly-

andry but not with the aforementioned conflict-reduction benefits, since conflicts over male parentage should be absent once effective paternity exceeds 2.0 (Ratnieks 1988). Interspecific comparisons across the Vespinae show that mating frequency is positively associated with colony size and negatively associated with paternity skew, both predictions of the disease resistance and division-of-labor versions of the diversity hypothesis (Loope et al. 2014). However, these patterns are driven mostly by the contrasts caused by species with obligate polyandry, and colony size does not appear to be correlated with paternity in the facultatively polyandrous *Dolichovespula* or the slightly more obligately polyandrous *Vespula rufa* group (i.e., the subgenus *Vespula* sensu stricto; Perrard et al. 2015).

Several features of vespine wasp biology argue against the remaining two leading hypotheses for facultative or obligate polyandry in this clade. The diploid male load hypothesis suggests that polyandry reduces the variance across colonies in the proportion of colony offspring that are (costly) diploid males. This variance reduction could be beneficial to queens if colonies invest in few valuable reproductive units, such as swarms; if most daughter colonies fail before reproduction, monandry may be favored instead (Page 1980; Crozier and Fjerdingstad 2001; Rueppell et al. 2008). Similar demographic models suggest that monandry is more beneficial in the early colony stage, when colonies are small and the costs of a small (evenly distributed) diploid load resulting from polyandry will be amplified by exponential growth; in contrast, polyandry is advantageous if reproduction is delayed until colonies reach their mature size (Pamilo et al. 1994). This hypothesis has been suggested to explain the convergent obligate polyandry in the swarm-founding army ants and honey bees and the monandry of most bumble bees and other independent-founding species, since swarm founding means that most reproductive units exceed the threshold for colony survival and reproduction and avoid the small-colony growth phase (Kronauer et al. 2007; Rueppell et al. 2008). Although diploid males have been reported in vespine wasps (Foster et al. 2000; Darrouzet et al. 2015),

this hypothesis cannot explain the observed variation across species. Vespine species of all three mating syndromes (monandry, facultative polyandry, and obligate polyandry) are independent founding, and the vast majority of colonies in species of all three types fail early, prior to the reproductive stage (Matsuura 1984; Archer 2010). Thus, the prediction that polyandry is associated with colony founding strategy or colony mortality schedule (Rueppell et al. 2008) is not supported in these wasps. Similarly, colony size and complexity, but not founding strategy, predict the low mating frequency of a small-colony ant with a convergently evolved army-ant syndrome (Kronauer et al. 2010).

Finally, the sperm limitation hypothesis (Cole 1983) is not likely to explain facultative polyandry in *Dolichovespula*. Although *D. arctica* females produce fewer female offspring than do queens of their polyandrous social relatives, as predicted by the sperm limitation hypothesis, they store as much sperm in their spermatheca as the facultatively polyandrous *Dolichovespula* and (near) obligately polyandrous *Vespula vidua* and *Vespula consobrina* (Stein and Fell 1994; Loope et al. 2014). We are left, then, with the possibility of conflict reduction benefits or diversity benefits from disease resistance or division of labor as the most likely to apply to facultative polyandry in *Dolichovespula*. Furthermore, more direct tests of these hypotheses in this group are surely warranted.

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APPENDIX

Untrimmed Bayesian Tree and Full Ancestral State Reconstructions

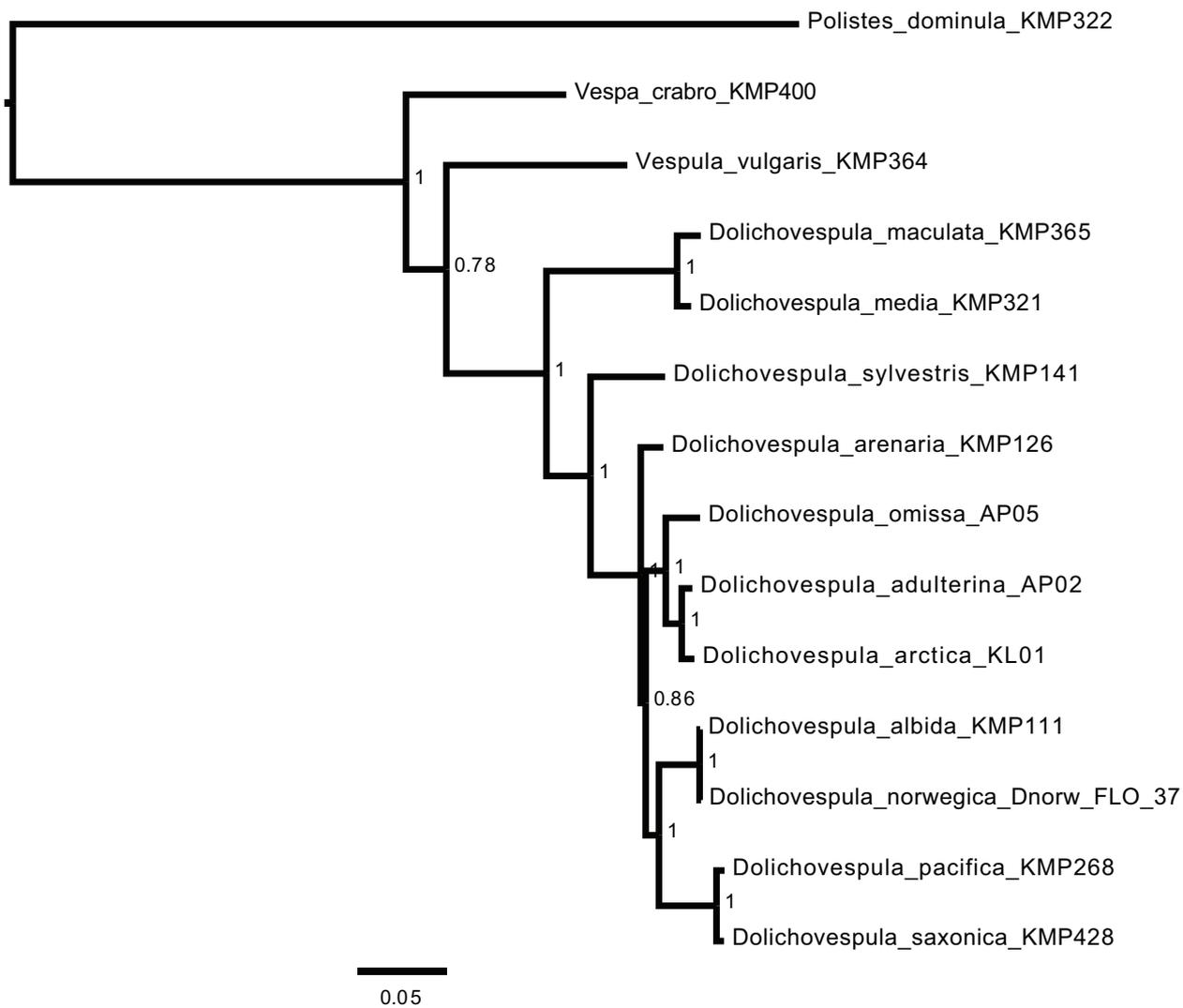


Figure A1: Phylogenetic tree for all *Dolichovespula* species with sequence data available, created using MrBayes (see “Methods”).

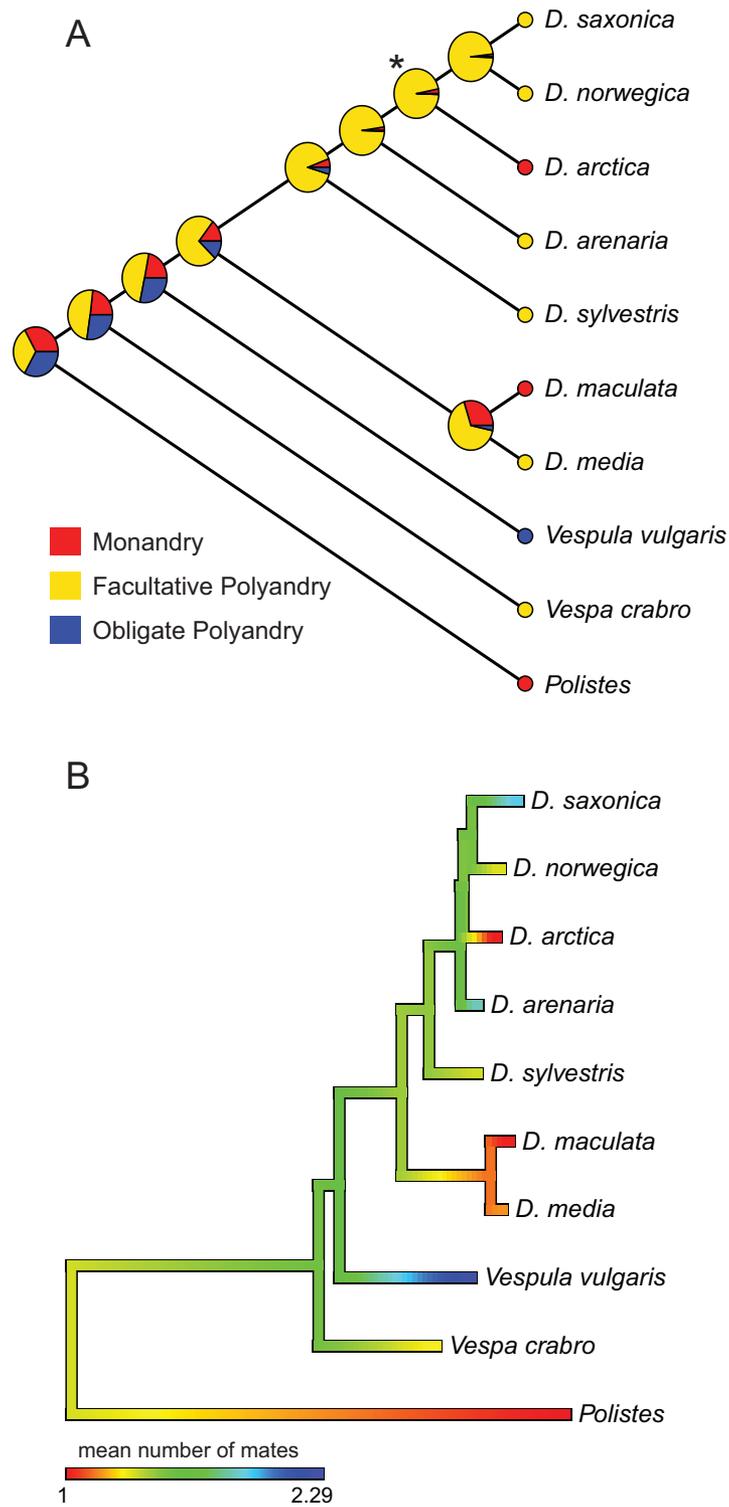


Figure A2: Ancestral state reconstructions for type of mating system (A) and mean mate number (B). Pie charts on nodes reflect the marginal probabilities of the three possible mating systems. Estimated probabilities for the social ancestor of *Dolichovespula arctica* (node marked with an asterisk) are reported in “Results.”

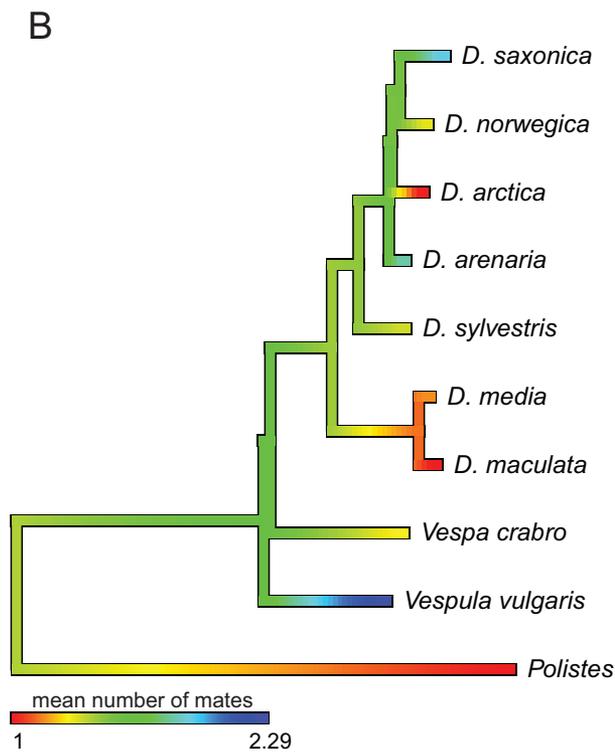
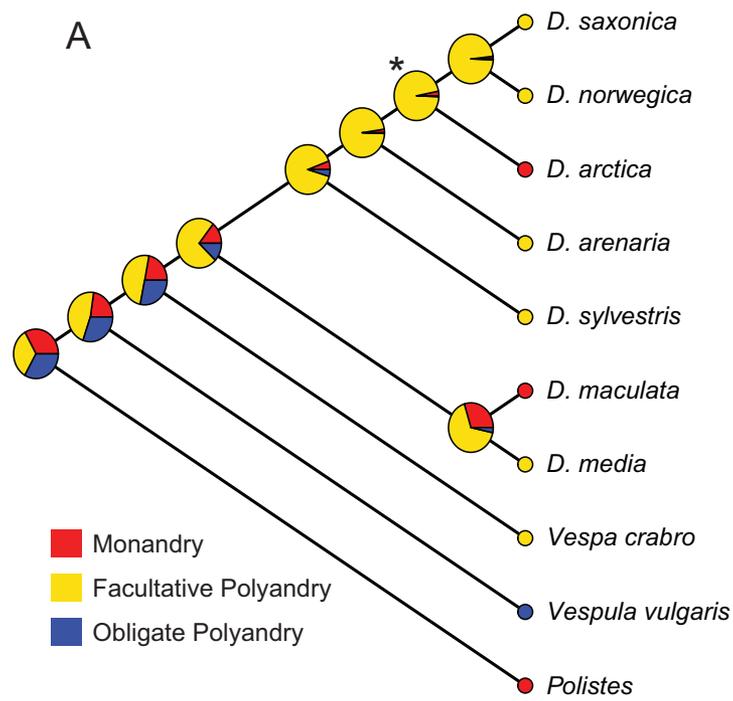


Figure A3: Ancestral state reconstructions for type of mating system (A) and mean mate number (B). Pie charts on nodes reflect the marginal probabilities of the three possible mating systems. Estimated probabilities for the social ancestor of *Dolichovespula arctica* (node marked with an asterisk) are reported in “Results.” This analysis is based on an alternative phylogenetic hypothesis reflecting the uncertainty in the relative positions of the genera *Dolichovespula*, *Vespula*, and *Vespa*.

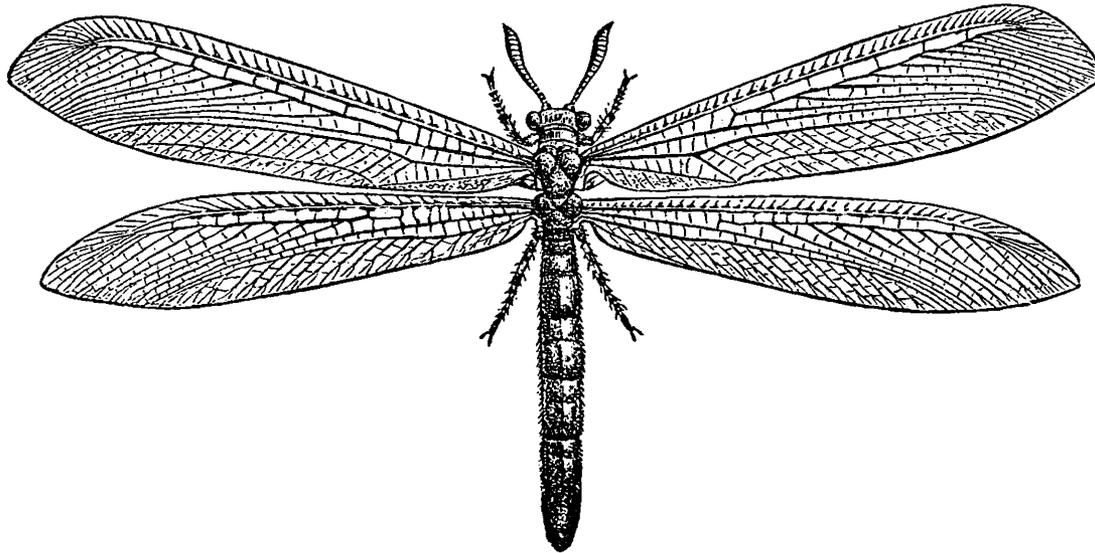
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“On the twenty-ninth of August, while hunting spiders on the hill north of Bartholomew’s pond in South Danvers, Mass., I unexpectedly found the pit of an ant-lion (*Myrmeleo immaculatus* De Geer), in a clear space under the shade of a large boulder. The pit . . . was about two inches in diameter and one deep. The insect himself was hid at the bottom, but when I dropped bits of earth into the hole he showed his position by throwing up sand.” From “The Ant Lion” by J. H. Emerton (*The American Naturalist*, 1871, 4:705–708).