PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ployandry versus polygyny versus parasites

Paul Schmid Schmid-Hempel and Ross H. Crozier

Phil. Trans. R. Soc. Lond. B 1999 **354**, 507-515
doi: 10.1098/rstb.1999.0401

THE ROYAL
SOCIETY

Polyandry versus polygyny versus parasites

Paul Schmid-Hempel[1] **and Ross H. Crozier**[2]

[1]*ETH Zürich, Experimental Ecology, ETH-Zentrum NW, CH-8092 Zürich, Switzerland* (*psh@eco.umnw.ethz.ch*)
[2]*Department of Biochemistry and Genetics, La Trobe University, Bundoora, Victoria 3083, Australia*

Although social insect colonies are most easily conceptualized as consisting of a single, once-mated queen and her worker progeny, the number of queens per colony and the number of times queens mate varies broadly in ants and other social insects. Various hypotheses have been suggested for the resulting range of breeding systems and social organizations, respectively; one set of hypotheses relating to both queen number and mate number at the same time is a need for genetic variation, especially in relation to disease resistance. We here carry out a comparative analysis using phylogenetic information and, contrary to one non-phylogenetic previous study, we find that polyandry and polygyny are not significantly associated. However, the level of relatedness within colonies, a quantity affected by both polyandry and polygyny, is significantly associated with parasite loads: species with colonies with low relatedness levels have lower parasite loads. Given that, under the variance-reduction principle, selection on queens for mating frequency ought to continue even in polygynous colonies, we suggest that while parasite loads indeed seem to correlate with intra-colony genetic variability, the relationship to polyandry and polygyny may be complex and requires considerably more experimental investigation.

Keywords: polygyny; polyandry; breeding system; genetic variation; parasitism; ants

1. INTRODUCTION

Polyandry (females mating with several males) and polygyny (the presence of several functional queens in the same colony) increase genetic variance within a colony and are widespread in social insects of all major taxa in ants, bees, wasps and termites (e.g. Boomsma & Ratnieks 1996; Crozier & Pamilo 1996). Genetic variance and the associated lower level of intra-colony worker relatedness is highly relevant to the biology of social insects, because of the effects on many major characteristics that shape the evolution and maintenance of sociality, such as sex allocation strategies, reproductive tactics, strategies of cooperation and conflict or the division of labour, (Moritz 1985; Ross 1988; Woyciechowski & Lomnicki 1987; Robinson 1992; Keller & Reeve 1994*a*; Ratnieks & Boomsma 1995).

But it is not obvious how females benefit, for example, from mating multiply. With several patri- or matrilines present in the colony, an increased potential for conflict results (e.g. Ratnieks 1988; Schmid-Hempel 1990; Crozier & Pamilo 1996; but see Keller 1997). Furthermore, additional matings require time and energy or expose the queen to predation risks (Moritz 1985). A number of adaptive hypotheses for the value of increased intra-colony genetic variability have therefore been suggested (for a review, see Crozier & Page 1985; Bourke & Franks 1995; Crozier & Pamilo 1996; Boomsma & Ratnieks 1996). For example, increased variation would allow the expression of a more complex colony phenotype to cope with a broader range of environmental conditions (Page *et al.* 1989, 1995; Robinson & Page 1989), reduce the variance in the production of diploid males (Page & Metcalf 1982; Pamilo *et al.* 1994) or reduce the queen–worker conflict over sex allocation to offspring (Pamilo 1991; Queller 1993*b*; Ratnieks & Boomsma 1995). More specifically, increased intra-colony genetic variability could militate against the effects of parasitism (Hamilton 1987; Sherman *et al.* 1988; Schmid-Hempel 1994). This subject in particular is a matter of ongoing controversy and debate (e.g. Kraus & Page 1998; Sherman *et al.* 1998).

Polyandry and polygyny affect intra-nest genetic variability differently, depending on the patterns of sperm use, relatedness among cohabitant queens and variance in dominance over egg production (e.g. Queller 1993*a*). Nevertheless, the two components interact to determine the level of variability and relatedness within the colony. Keller & Reeve (1994*b*) thus suggested that queen numbers may vary independently in function of ecological factors, e.g. the chances of successful dispersal and colony founding, while additional matings can be controlled by the queen and are costly. The number of matings and social organization (gyny) could thus balance one another so that a given level of genetic variation within a colony results. Polyandry should consequently be less frequent in polygynous colonies where a large amount of genetic variation already exists. In support, Keller & Reeve (1994*b*) assembled data on mating frequencies and levels of polygyny in ants, which is in fact the only group studied sufficiently for testing any such prediction. In their sample of 59 species, the authors indeed found the predicted association, i.e. polygynous species were less likely to be polyandrous. However, several criticisms can be made about this study. For example, phylogenetic contingencies were not appropriately accounted for, although their analysis at the generic level supported the hypothesis.

Received 3 June 1998 *Accepted* 5 August 1998

Here, we reanalyse the association between polygyny and polyandry in ants by taking into account phylogenetic contingencies to the degree possible. More specifically, we test whether the known distribution of parasite species in ants matches the expectations from the genetic variability hypothesis, either in relation to breeding system (monandry versus polyandry), social organization (monogyny versus polygyny) or intra-colony variability irrespective of both. We have restricted our analyses to ants because they are by far the best-investigated group for this kind of work. Furthermore, we use the number of parasite species per host species as a measure for the importance of parasitism in the host. This obviously leaves aside many intricacies of host–parasite interactions, but nevertheless seems a justifiable approach given the meagre database available to date. We will not discuss here how within-colony genetic variability affects the queen–worker conflict over sex allocation to offspring or split sex ratios (Boomsma & Grafen 1991; Ratnieks & Boomsma 1995), albeit these hypotheses have received some empirical support (Sundström 1994).

2. MATERIAL AND METHODS

Information on polyandry (grouped as monandrous versus polyandrous), on polygyny (monogynous versus polygynous), average genetic relatedness among workers within a colony and the occurrence of parasites were collated from the literature. We adopted a species-by-species approach, because the information on the breeding system, social organization and the data on parasites do not necessarily refer to the same populations. A species was classified as polyandrous or polygynous when it was classified as such by the authors of the source studies, independent of the number of actual matings or number of queens, respectively. In addition, we classified polymorphic species as polyandrous or polygynous. Socially parasitic ants, where habit is known, were excluded from the analysis because the relationship between genetic variability, mating system and selection on these characters in the case of their colonies is likely to differ from that of non-parasitic species. This is because many parasitic species typically do not contribute to the worker force of the host colony (which is under scrutiny here) but rather produce sexuals. Data for genetic relatedness, the breeding system and social organization are from Crozier & Pamilo ((1996), their table 4.7) and various other sources. The compilation of parasite species known for a given host is taken from Schmid-Hempel (1998, Appendix 2). These include viruses, bacteria, protozoa, fungi, nematodes, helminths (cestodes and trematodes) and parasitic insects (Hymenopteran, Diptera, Strepsiptera, Lepidoptera, etc.). Mites were excluded because their parasitic status is often unclear. The data are summarized in Appendix A.

Unfortunately, the phylogeny of ants is only poorly known and still subject to contending views. The phylogeny used here is shown in Appendix B. The statistical package provided by Purvis & Rambaut (1995, CAIC 2.0) was used to calculate independent contrasts to compare parasite richness in species with different breeding systems and social organization, respectively. All branch lengths were set equal. The association of polyandry with polygyny uses categorical variables (mono-/poly-). We therefore used Ridley's method of counting independent evolutionary events (Ridley 1983; Harvey & Pagel 1991) rather than CAIC for this part of the analysis. Ridley's method counts the number of branches along which transitions occurred (i.e. a change in the state of andry or gyny between the beginning and the end of a branch) and that ended in each of the four possible categories (poly- and mono- gyny, or andry). This two-by-two table can then be tested for association with the usual statistical techniques. We used MacClade3 (Maddison & Maddison 1992) to determine all changes, setting polytomies as hard. (This option reconstructs character evolution as if polytomies in the phylogenetic tree were real with multiple species arising independently from the ancestral species, rather than as if polytomies arose from ignorance despite an underlying dichotomous tree topology.)

More parasite species are described in well-investigated species of social insects, such as the wood ants or the fire ants, than in little-studied species (Schmid-Hempel 1998). Therefore, the number of recorded parasite species in a given host species was corrected for sampling effort using the 'study intensity' for the host species. As 'study intensity' we used the number of studies that were published for a given species during 11 years (1985–1995) and as obtained from checking *Biological Abstracts* on CD-ROM. The inclusion of all studies on the host species for this measure rather than using only studies on parasites seems justified, because the discovery of parasites is often reported as a casual aside in studies not designed for this purpose, while at the same time many declared studies on parasites often have a different scope than investigating the diversity or impact of parasites. Furthermore, the conclusions are virtually the same when the data are analysed without this study-intensity correction term. We used the data of Appendix A to calculate the residuals from the regression of ln(1+ number of reported parasite species) on ln(1+ number of studies) (with ln-transformations to normalize variances). These residuals are our measure of 'standardized parasite richness' for a given host species. If not stated otherwise, reported p-values are two-tailed. Power analysis was performed according to Cohen (1988). Statistical analyses were done with SPSS 6.1.

3. POLYANDRY, POLYGYNY AND PARASITISM

With the data listed in Appendix A, and with species taken as independent units, as in the study of Keller & Reeve (1994*b*), a significant association of polyandry with polygyny is indeed found (table 1). However, when the phylogenetic component according to Appendix B is taken into account (figure B1), this association becomes non-significant (table 2). Although the resulting sample sizes for independent evolutionary events are necessarily small, the test has a reasonable power of detecting a true difference (table 2). Furthermore, setting polytomies in the phylogenetic tree as hard biases towards a positive result because it increases the apparent number of independent contrasts; had a positive result been obtained in this case a conservative test would have been to resolve all polytomies in such a way as to minimize the number of transitions for one character or the other. Failure of the test involving hard polytomies to yield a positive result indicates that no association can be inferred from the data currently available.

Appendix A also gives the number of parasite species known per ant host species. When species are considered as independent, the average standardized parasite richness (see § 2) seems lower for polygynous than for monogynous, and lower for polyandrous than for monandrous species (table 3, see column 4 and footnote c),

Table 1. *The association of gyny with andry with species considered independent*

($\chi^2 = 6.227$, $p = 0.0126$.)

	monandry	polyandry
monogyny	8	26
polygyny	18	16

Table 2. *The number of independent evolutionary events, after Ridley's method*

(Fisher's exact test, one-tailed, $p = 0.128$. Power $(1-\beta) = 0.48$.)

	monandry	polyandry
monogyny	3	5
polygyny	12	5

Table 3. *Standardized parasite richness and comparison of independent contrasts for different social organizations and breeding systems*

	N^a	N^b	standardized parasite richness[c] (s.e.)	comparison of contrasts in richness[d]	significance[e]
social organization					
monogynous	63	40	0.0740 (0.092)	$t = 1.069$	$p = 0.30$
polygynous	54	36	−0.0884 (0.091)	$\Delta = -0.073$	d.f. = 15
breeding system					
monandrous	26	10	0.1616 (0.200)	$t = 0.300$	$p = 0.78$
polyandrous	42	20	−0.0007 (0.139)	$\Delta = 0.051$	d.f. = 15

[a] Number of species where social organization and breeding system is known.

[b] Number of species where parasite richness is also known.

[c] Comparing levels of gyny: $t = 1.25$, d.f. = 74, $p = 0.22$; of andry: $t = 0.67$, d.f. = 28, $p = 0.51$, ignoring phylogenetic relationships.

[d] Testing for zero difference of independent contrasts (CAIC) for parasite richness. Δ = difference of contrast for polygynous–monogynous, and polyandrous–monandrous, respectively.

[e] Power $(1-\beta) \leqslant 0.14$.

although these differences are not significant. These results are expected if breeding systems and social organizations with higher levels of genetic variation within colony were associated with fewer parasite species being present (on the assumption that lower relatedness levels indicate higher absolute levels of genetic variation). When the phylogenetic relationships are taken into account, the standardized parasite richness for polygynous species contrasted with monogynous species was again lower, but now higher for polyandrous contrasted with monandrous species (table 3). Again, none of these differences was significant. We also found that our conclusions are robust to minor changes in the database, e.g. counting some problematic species as monogynous rather than polygynous (Appendix A). Hence, the comparative analysis did not support the expectation that more variable breeding systems and social organizations are associated with fewer parasites. But a quick look at Appendix A shows that for very few species are data available for parasites, social organization and breeding system at the same time. For the time being, these conclusions are therefore limited in scope by the available sample size and the limited power of the associated tests.

In a final analysis, we related the chief quantity of main interest, i.e. the average genetic relatedness among workers within the colony, to the standardized parasite richness for the species. When species were considered independent, we found a positive correlation between these measurements (Spearman's $r = 0.690$, $p < 0.001$, $N = 24$ species). In this case, the analysis by independent contrasts (CAIC) produced the same result (figure 1). Such a positive correlation is expected from the variability versus parasitism hypothesis, where species with homogeneous colonies (high values of worker relatedness) should have more parasites than those with heterogeneous colonies (low values of worker relatedness).

4. DISCUSSION

The variation versus parasitism hypothesis suggests that genetic variation within the colony militates against the effects of parasitism. By implication, it assumes that the relationship between host and parasite is affected by genotype–genotype interactions (e.g. Clarke 1976; Anderson & May 1982). Genotypic variation for parasite susceptibility is indeed well-documented for the honeybee (e.g. Bamrick 1964; Taber 1982; Rinderer *et al.* 1975; Kulincevic 1986), and the bumblebee, *Bombus terrestris* (Shykoff & Schmid-Hempel 1991; Schmid-Hempel & Schmid-Hempel 1993; Schmid-Hempel & Loosli 1999). In ants, much less is known. However, a diverse array of glands is known to produce antibacterial or antifungal substances (e.g. Maschwitz 1974; Hölldobler & Engel-Siegel 1984; Beattie *et al.* 1985, 1986; Attygalle *et al.* 1989; Veal *et al.* 1992). For some of these glands, for example the metapleural gland of leaf-cutter ants (*Acromyrmex*), individual and between-colony variation in size has been documented (Bot & Boomsma 1996), suggesting a similar genotypic variation.

The importance of genotypic variation within colonies in relation to parasitism is here underpinned with the analysis of figure 1. As expected from the hypothesis, species with more variable colonies have fewer parasites (see also Schmid-Hempel 1998). Unfortunately, as with any such comparative analysis, the correlation could be

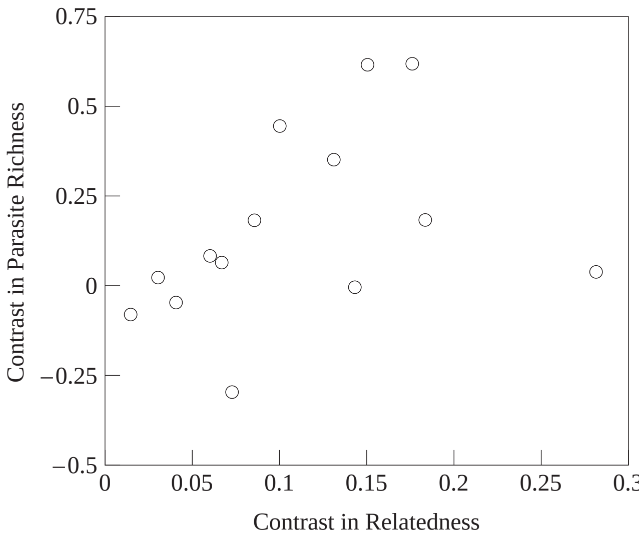

Figure 1. Correlation of independent contrasts in the standardized parasite richness (ordinate; ln-transformed) and contrasts in average genetic relatedness (abscissa; arcsin-transformed) within colony for $N=119$ ant species in the phylogeny (Appendix B), and $N=24$ species for which both quantities are known (Appendix A) ($r=0.622$, $F_{1,13}=8.199$, $p=0.013$, $n=14$ contrasts).

cause or effect, or reflect separate underlying factors. In fact, a number of additional characteristics correlate with polygyny. For example, polygynous species tend to have more closely related colonies (Keller 1995), which was also true in our sample (comparing mono- versus polygynous species without correction for phylogeny: $t=2.20$, d.f.$=35$, $n=37$ species, $p=0.035$). Queens of polygynous species often disperse less, have a dependent colony-founding mode and have larger colonies (Keller 1991; Rosengren *et al.* 1993; Ross & Keller 1995). Some of these factors may affect parasitism. But in the sample analysed here, there is no association of colony size and within-colony relatedness, nor did we find a difference in colony size between monogynous and polygynous, or between monandrous and polyandrous species (see also Schmid-Hempel 1998). It is nevertheless possible that the correlation shown in figure 1 is due to other factors than within-colony relatedness per se. However, such other factors, if they were able to explain our result without reference to intra-colony relatedness, should map on to the relatedness axis in the same way as relatedness does and should additionally not be eliminated by the phylogenetic correction.

We have also shown that for the data available so far, the degrees of polyandry and polygyny among social insects cannot be said to be correlated. In particular, polygynous species are not less likely to be polyandrous than monogynous ones, although the power allowed by the existing data is insufficient to rule out this effect emerging when further data become available. This result is in line with the preliminary analysis of Boomsma & Ratnieks (1996) and with a more detailed study in *Myrmica* ants where also no association, as claimed by Keller & Reeve (1994*b*), could be found in within-species as well as among-species comparisons (Pedersen & Boomsma 1999). The result of Keller & Reeve (1994*b*) is therefore likely to be an artefact of phylogenetic contingencies in the database or the particular set of species used. Similarly, we could not detect a difference in the known number of parasite species in polygynous versus monogynous ants, nor in polyandrous versus monandrous species. If anything, when phylogenetic dependencies are controlled for, the two breeding systems have different associations, in that the (non-significant) trends are for polygynous species to have fewer parasites when contrasted with monogynous ones and for polyandrous ants to have more than monandrous ones (table 3). These conclusions are unlikely to be affected by the inherent crudity of our species-by-species approach, since changes in assumptions about the breeding system or social organization of problematic species did not change the results.

Comparisons among populations within species, or experimental tests, are needed to further elucidate the causal link suggested by figure 1. Some experimental tests of this hypothesis have indeed been done, so far with somewhat ambiguous results. On the one hand, Woyciechowski *et al.* (1994) found no effect of multiple mating by honeybee queens on infection by a microsporidian parasite. Similar negative evidence is summarized by Kraus & Page (1998). However, three different studies in the bumblebee *B. terrestris* indicate that figure 1 reflects just such a general relationship. First, Shykoff & Schmid-Hempel (1991) showed that transmission and infection by a trypanosome parasite is lower in genetically heterogeneous than in homogeneous groups of workers. Second, in a field study comparing ‘polygynous’ (heterogeneous) versus ‘monogynous’ (homogeneous) colonies, Liersch & Schmid-Hempel (1998) found that homogeneous colonies had more parasites and smaller colony sizes at the end of their life cycle. Finally, in the study of Baer & Schmid-Hempel (1998), females were experimentally inseminated to be either monandrous or polyandrous. Again, over the course of a seasonal cycle in the field, polyandrous colonies had fewer parasites and higher fitness than monandrous colonies.

In conclusion, the comparative evidence presented here suggests that genetic variation within colony may indeed be important with respect to parasitism by whatever process variability comes about. In fact, neither polygyny or polyandry is associated with higher or lower parasite richness and these two are not correlated with each other in the way suggested by Keller & Reeve (1994*b*). It is likely that variation in polygyny is primarily driven by factors other than those responsible for variation in polyandry, but that both may be under selection by parasitism in ways more complex than generally appreciated until now. The exact processes still await more thorough investigation.

We thank Michel Chapuisat, Laurent Keller, Stella Koulianos, Pekka Pamilo and Graham J. Thompson for comments on the manuscript. The work was supported financially by grants from the Swiss National Science Foundation (3100-049040.95) and a European TMR-network (BBW no. 95.0575) to P.S.H., and from The Australian Research Council to R.H.C.

APPENDIX A. SPECIES AND DATA USED IN THE ANALYSES*

(Parasites are number of reported parasite species in this host (from Schmid-Hempel 1998). Values for breeding system, social organization (gyny, andry) and relatedness from Frumhoff & Ward (1992), Keller & Reeve (1994*b*), Bourke & Franks (1995), Boomsma & Ratnieks (1996), Crozier & Pamilo (1996) and various other sources. Gyny: 1, monogynous; 2, polygynous, or mixed mono-/polygynous. Andry: 1, monandrous; 2, polyandrous; Studies: number of studies for the species recorded in *Biological Abstracts* 1985–95.)

species	studies	gyny	andry	relatedness	parasites
Acromyrmex landolti	—	1	2	—	—
Acromyrmex versicolor [a]	—	1	2	—	—
Aphaenogaster rudis	—	1	1	0.75	—
Aphaenogaster subterranea	9	1	—	—	1
Atta cephalotes	48	1	2	—	2
Atta colombica	10	1	2	—	1
Atta laevigata	26	1	2	—	2
Atta sexdens	42	1	2	—	9
Atta texana	8	2	2	—	5
Brachymrmex depilis	—	1	2	—	—
Camponotus consobrinus	2	2	1	—	1
Camponotus floridanus	15	1	—	—	2
Camponotus herculaneus	1	1	—	—	10
Camponotus ligniperda	9	2	1	0.08	2
Camponotus novoboracensis	0	1	—	—	2
Camponotus pennsylvanicus	21	1	—	—	7
Camponotus sericeiventris	4	1	—	—	6
Cardiocondyla wroughtoni	—	2	2	—	—
Carebara vidua	—	1	2	—	—
Cataglyphis cursor	25	1	2	—	2
Cephalotes atratus	3	1	—	—	3
Colobopsis (*Camponotus*) *nipponicus*	—	1	1	0.75	—
Conomyrma bicolor	—	2	—	0.33	—
Conomyrma insana	—	1	2	0.65	—
Crematogaster lineolata	2	1	—	—	2
Crematogaster parabiotica	0	2	—	—	1
Crematogaster scutellaris	7	1	—	—	1
Eciton burchelli	10	1	2	—	10
Forelius pruinosus (*Iridomyrmex pruinosum*)	1	—	—	0.79	2
Formica aquilonia	—	2	2	0.2	—
Formica argentea	—	2	1	0.81	—
Formica bradleyi	—	1	2	—	—
Formica exsecta	13	2	2	0.6	1
Formica fusca[b]	25	2	—	0.57	12
Formica lugubris	29	—	—	0.5	5
Formica montana	—	2	2	—	—
Formica neogagates	6	2	—	—	1
Formica obscuripes	—	2	1	—	—
Formica opaciventris[c]	—	2	2	—	—
Formica podzolica	7	2	—	0.24	1
Formica polyctena[d]	75	2	—	0.29	3
Formica pratensis[e]	20	1	2	0.66	7
Formica pressilabris	—	2	2	0.3	—
Formica rufa	63	2	2	0.49	8
Formica rufibarbis	12	1	—	—	4
Formica transcaucasica	1	2	2	0.54	2
Formica truncorum	23	2	2	0.32	1
Formica yessensis	—	2	2	—	—
Iridomyrmex purpureus	—	2	1	0.75	—
Lasius alienus	14	1	2	—	6
Lasius flavus	32	2	2	0.72	10
Lasius neglectus	—	2	2	0.45	—
Lasius neoniger	9	1	—	—	2
Lasius niger	82	1	2	0.64	12
Linepithema humile (*Iridomyrmex humilis*)	60	2	1	0.015	5
Leptothorax acervorum	32	2	1	0.47	4
Leptothorax affinis	3	1	—	—	7
Leptothorax ambiguus	7	2	—	0.5	2
Leptothorax angustulus	1	1	—	—	1
Leptothorax corticalis	2	1	—	—	1
Leptothorax exilis	1	1	—	—	1
Leptothorax gredleri [f]	—	1	1	—	—
Leptothorax interruptus	0	2	—	—	2
Leptothorax lichtensteini	5	1	—	—	1
Leptothorax longispinosus	17	2	1	—	2

(*Cont.*)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

APPENDIX A *(Cont.)*

species	studies	gyny	andry	relatedness	parasites
Leptothorax muscorum	13	2	1	—	3
Leptothorax nigriceps	3	1	—	—	2
Leptothorax nylanderi	7	1	2	—	8
Leptothorax parvulus	1	1	—	—	4
Leptothorax pergandei[g]	—	1	1	0.76	—
Leptothorax rabaudi	0	1	—	—	3
Leptothorax recedens	4	1	—	—	1
Leptothorax tristis	0	1	—	—	2
Leptothorax tuberum	4	2	—	—	2
Leptothorax unifasciatus	14	1	—	—	7
Messor ebeninus	—	1	2	—	—
Monomorium pharaonis	—	2	1	—	—
Monomorium salmonis indicum	4	2	2	—	2
Mycocepurus goeldii	—	1	2	—	—
Myrmecia pilosula	24	2	—	0.17	2
Myrmecia pyriformis sp.I	5	1	—	—	1
Myrmecia tarsata	1	1	—	—	1
Myrmica americana	—	2	1	—	—
Myrmica lobicornis	0	2		0.64	1
Myrmica punctiventris[h]	—	1	1	0.77	—
Myrmica rubra	56	2	2	0.27	2
Myrmica ruginodis	34	2	2	0.63	4
Myrmica sabuleti	—	2	—	0.44	—
Myrmica scabrinodis	16	2	—	0.38	3
Myrmica schencki	14	2	—	—	1
Nothomyrmecia macrops	—	1	2?	0.25	—
Odontomachus haematoda	0	1	—	—	3
Oecophylla longinoda	11	1	—	—	1
Oecophylla smaragdina	—	1	1	—	—
Pheidole embolopyx	—	1	1	—	—
Pheidole pallidula	25	2	—	—	6
Pheidole sitarches campestris	0	1	1	—	1
Pheidole vinelandica	0	1	—	—	3
Plagiolepis pygmaea	14	2	—	—	2
Pogonomyrmex badius	12	1	2	—	2
Pogonomyrmex barbatus	—	1	2	—	—
Pogonomyrmex californicus	—	1	2	—	—
Pogonomyrmex desertorum	—	1	2	—	—
Pogonomyrmex maricopa	—	1	2	—	—
Pogonomyrmex occidentalis	—	1	2	—	—
Pogonomyrmex rugosus	—	1	2	—	—
Prenolepis imparis	—	1	2	—	—
Rhytidoponera chalybaea[i]	—	2	1	0.55	—
Rhytidoponera confusa[i]	—	2	1	0.48	—
Rhytidoponera enigmatica	—	2	1	—	—
Solenopsis geminata	60	2	1	0.79	16
Solenopsis invicta	302	2	1	0.71	21
Solenopsis richteri	32	2	1	0.74	19
Solenopsis texana	1	2	—	—	1
Tapinoma erraticum	10	2	—	—	1
Tapinoma minutum[k]	—	2	1	—	—
Tetramorium caespitum	39	1	—	—	9
Tetramorium simillimum	1	2	—	—	2
Wassmannia auropunctata	0	2	—	—	1

---

[a] Reichardt & Wheeler (1996).

[b] Monogynous according to Keller & Reeve's (1994*b*) database, but wrongly cited from Rosengren *et al.* (1993).

[c] Keller & Reeve (1994*b*), Crozier & Pamilo (1996).

[d] Also a social parasite of other species.

[e] According to Boomsma & Ratnieks (1996), *F. pratensis* is likely to be polyandrous (12 matings).

[f] *L. gredleri* is polygynous according to Heinze (personal communication, cited in Boomsma & Ratnieks (1996)).

[g] *L. pergandei* cited with single–double matings by Boomsma & Ratnieks (1996), but genetic relatedness data suggest full-sib families.

[h] *M. punctiventris* cited as polygynous (few queens) by Boomsma & Ratnieks (1996).

[i] Headed by single queen or several mated workers.

[k] *T. minutum* cited with single–double matings by Boomsma & Ratnieks (1996).

[*] The reported results remain the same if the following modifications to this Appendix are taken into account: *Formica argentea* ranked as monogynous (as in Bourke & Franks 1995), *Lasius flavus* ranked as monogynous, *Solenopsis invicta* having relatedness = 0.06.

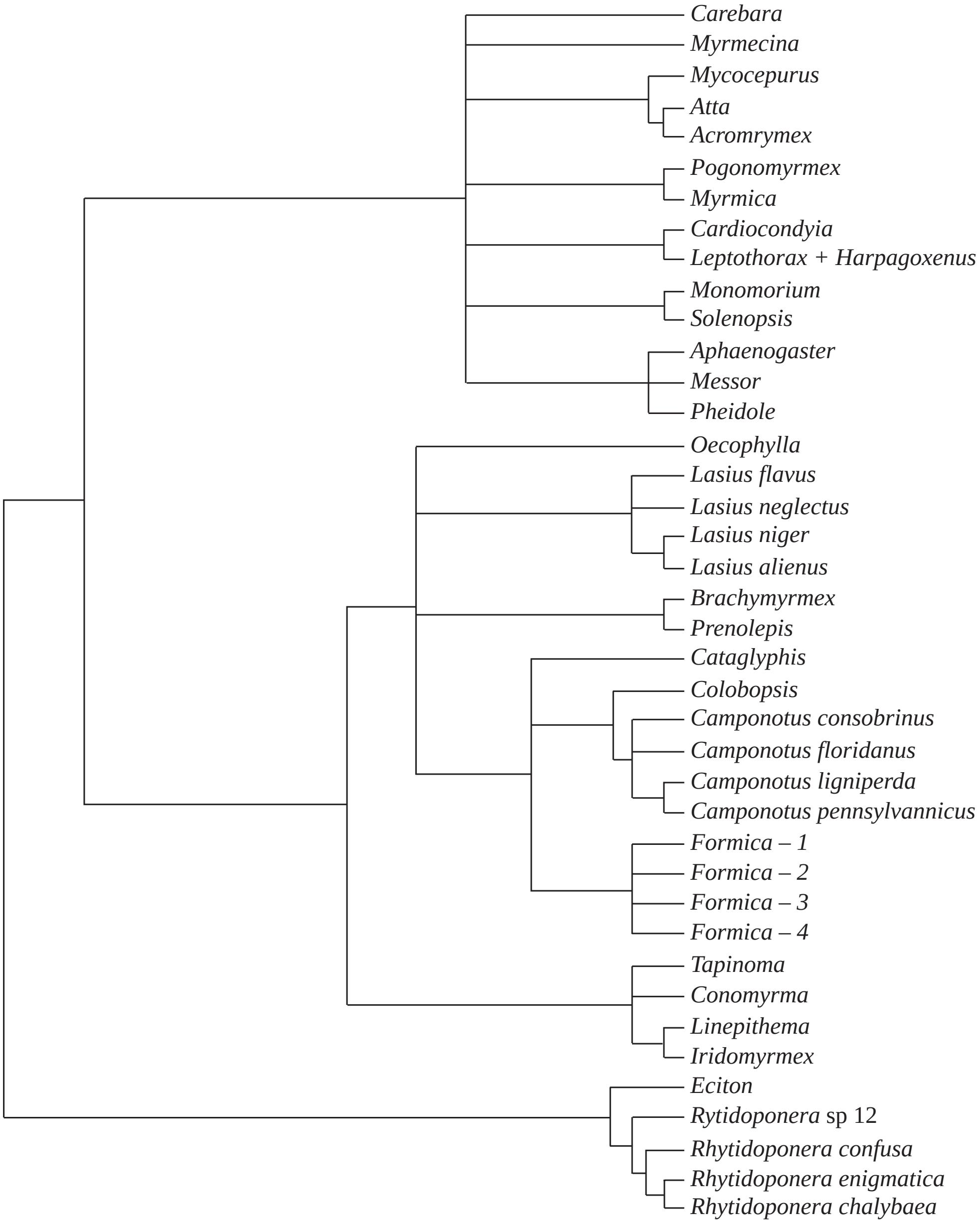

Figure B1. Ant phylogeny used for the comparative analysis.

APPENDIX B. PHYLOGENY OF ANTS

Main relationships among families according to Baroni-Urbani *et al.* (1992). Formicinae according to Agosti (1991). Attini according to Chapela *et al.* (1994) and Hinkle *et al.* (1994). Subgenera of *Formica* after Krombein *et al.* (1979) and P. Pamilo (personal communication). Subgenera of *Camponotus* after Bolton (1995). Dolichoderines after Shattuck (1995). Tribes of Myrmicinae after Bolton (1995) as are also the included genera, except for *Harpagoxenus* arising from *Leptothorax acervorum* (Baur *et al.* 1995, 1996). *Rhytidoponera* grouped after Ward (1980).

REFERENCES

Agosti, D. 1991 Revision of the oriental ant genus *Cladomyrma*, with an outline of the higher classification of the Formicinae (Hymenoptera: Formicidae). *Syst. Entomol.* **16**, 293–310.

Anderson, R. M. & May, R. M. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411–426.

Attygalle, A. B., Siegel, B., Vostrowsky, O., Bestmann, H. J. & Maschwitz, U. 1989 Chemical composition and function of the metapleural gland secretion of the ant, *Crematogaster deformis* Smith (Hymenoptera: Myrmicinae). *J. Chem. Ecol.* **15**, 317–329.

Baer, B. & Schmid-Hempel, P. 1998 Experimental variation in polyandry affects parasite loads and fitness in a bumblebee. *Nature.* (In the press.)

Bamrick, J. F. 1964 Resistance to American foulbrood in honeybees. V. Comparative pathogenesis in resistant and susceptible larvae. *J. Ins. Pathol.* **33**, 284–304.

Baroni-Urbani, C., Bolton, B. & Ward, P. S. 1992 The internal phylogenies of ants (Hymenoptera: Formicidae). *Syst. Entomol.* **17**, 301–329.

Baur, A., Chalwatzis, N., Buschinger, A. & Zimmermann, F. K. 1995 Mitochondrial DNA sequences reveal close relationships between social parasitic ants and their host species. *Curr. Genet.* **28**, 242–247.

Baur, A., Sanetra, M., Chalwatzis, N., Buschinger, A. & Zimmermann, F. K. 1996 Sequence comparisons of the internal transcribed spacer region of ribosomal genes support close relationships between parasitic ants and their respective host species (Hymenoptera: Formicidae). *Insect Soc.* **266**, 1691–6194.

Beattie, A. J., Turnbull, C. L., Hough, T., Jobson, S. & Knox, R. B. 1985 The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *Am. J. Bot.* **72**, 606–614.

Beattie, A. J., Turnbull, C. L., Hough, T., Jobson, S. & Knox, R. B. 1986 Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **79**, 448–450.

Bolton, B. 1995 *A new general catalogue of the ants of the world.* Cambridge, MA: Harvard University Press.

Boomsma, J. J. & Grafen, A. 1991 Colony-level sex ratio selection in the eusocial Hymenoptera. *J. Evol. Biol.* **3**, 383–407.

Boomsma, J. J. & Ratnieks, F. L. W. 1996 Paternity in eusocial hymenoptera. *Phil. Trans. R. Soc. Lond.* B **351**, 947–975.

Bot, A. N. M. & Boomsma, J. J. 1996 Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidae). *J. Kansas Entomol. Soc.* **69** (Suppl.), 375–383.

Bourke, A. F. G. & Franks, N. R. 1995 *Social evolution in ants.* Princeton University Press.

Chapela, I. H., Rehner, S. A., Schultz, T. R. & Mueller, U. G. 1994 Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* **266**, 1691–1694.

Clarke, B. C. 1976 The ecological genetics of host–parasite relationships. In *Genetic aspects of host–parasite interactions* (ed. A. E. R. Taylor & R. Muller), pp. 87–103. Oxford: Blackwell.

Cohen, J. 1988 *Statistical power analysis for the behavioral sciences.* Hillsdale, NJ: Lawrence Erlbaum Associates.

Crozier, R. H. & Page, R. E. 1985 On being the right size: male contributions and multiple mating in social hymenoptera. *Behav. Ecol. Sociobiol.* **18**, 105–116.

Crozier, R. H. & Pamilo, P. 1996 *Evolution of social insect colonies.* Oxford University Press.

Frumhoff, P. C. & Ward, P. S. 1992 Individual-level selection, colony-level selection, and the association between polygyny and worker monomorphism in ants. *Am. Nat.* **139**, 559–590.

Hamilton, W. D. 1987 Kinship, recognition, disease, and intelligence: constraints of social evolution. In *Animal societies: theory and facts* (ed. Y. Itô, J. L. Brown & J. Kikkawa), pp. 81–102. Tokyo: Japanese Scientific Society Press.

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology.* Oxford University Press.

Hinkle, G., Wetterer, J. K., Schultz, T. R. & Sogin, M. L. 1994 Phylogeny of the attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. *Science* **266**, 1695–1697.

Hölldobler, B. & Engel-Siegel, H. 1984 The metapleural gland of ants. *Psyche* **91**, 201–224.

Keller, L. 1991 Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera, Formicidae). *Ethol. Ecol. Evol.* **3**, 307–316.

Keller, L. 1995 Social life: the paradox of multiple queen colonies. *Trends Ecol. Evol.* **9**, 355–360.

Keller, L. 1997 Indiscriminate altruism: unduly nice parents and siblings. *Trends Ecol. Evol.* **12**, 99–103.

Keller, L. & Reeve, H. K. 1994*a* Partitioning of reproduction in animal societies. *Trends Ecol. Evol.* **9**, 98–102.

Keller, L. & Reeve, H. K. 1994*b* Genetic variability, queen number, and polyandry in social hymenoptera. *Evolution* **48**, 694–704.

Kraus, B. & Page, R. E. J. 1998 Parasites, pathogens, and polyandry in social insects. *Am. Nat.* **151**, 383–391.

Krombein, K. V., Hurd, P. D., Smith, O. R. & Burks, B. D. 1979 *Catalog of Hymenoptera in America north of Mexico* 2. *Symphyta and Apocrita* (*Aculaeata*). Washington, DC: Smithsonian Institution Press.

Kulincevic, J. M. 1986 Breeding accomplishments with honey bees. In *Bee genetics and breeding* (ed. T. E. Rinderer), pp. 391–414. Orlando, FL: Academic Press.

Liersch, S. & Schmid-Hempel, P. 1998 Genetic variability within social insect colonies reduces parasite load. *Proc. R. Soc. Lond.* B **265**, 221–225.

Maddison, W. P. & Maddison, D. R. 1992 *MacClade. Analysis of phylogeny and character evolution. Version 3.* Sunderland, MA: Sinauer Associates.

Maschwitz, U. 1974 Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. *Oecologia* **16**, 303–310.

Moritz, R. F. A. 1985 The effects of multiple mating on the worker–queen conflict in *Apis mellifera* L. *Behav. Ecol. Sociobiol.* **16**, 375–377.

Page, R. E. & Metcalf, R. A. 1982 Multiple mating, sperm utilization, and social evolution. *Am. Nat.* **119**, 263–281.

Page, R. E., Robinson, G. E., Calderone, N. W. & Rothenbuhler, W. C. 1989 Genetic structure, division of labor, and the evolution of insect societies. In *The genetics of social evolution* (ed. M. D. Breed & R. E. Page), pp. 15–29. Boulder, CO: Westview Press.

Page, R. E., Robinson, G. E., Fondrk, M. K. & Nasr, M. E. 1995 Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L). *Behav. Ecol. Sociobiol.* **36**, 387–396.

Pamilo, P. 1991 Evolution of colony characteristics in social insects. II. Number of reproductive individuals. *Am. Nat.* **137**, 83–107.

Pamilo, P., Sundström, L., Fortelius, W. & Rosengren, R. 1994 Diploid males and colony-level selection in *Formica* ants. *Ethol. Ecol. Evol.* **6**, 221–235.

Pedersen, J. S. & Boomsma, J. J. 1999 Positive association of queen number and queen mating frequency in *Myrmica* ants: a challenge to the genetic variability hypotheses. *Behav. Ecol. Sociobiol.* (In the press.)

Purvis, A. & Rambaut, A. 1995 Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comp. Appl. Biosci.* **11**, 247–251.

Queller, D. C. 1993*a* Genetic relatedness and its components in polygynous colonies of social insects. In *Queen number and sociality in insects* (ed. L. Keller), pp. 132–152. Oxford University Press.

Queller, D. C. 1993*b* Worker control of sex ratios and selection for extreme multiple mating by queens. *Am. Nat.* **142**, 346–351.

Ratnieks, F. L. W. 1988 Reproductive harmony via mutual policing by workers in eusocial hymenoptera. *Am. Nat.* **132**, 217–236.

Ratnieks, F. & Boomsma, J. J. 1995 Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. *Am. Nat.* **145**, 969–993.

Reichardt, A. K. & Wheeler, D. E. 1996 Multiple mating in the ant *Acromyrmex versicolor*: a case of female control. *Behav. Ecol. Sociobiol.* **38**, 219–225.

Ridley, M. 1983 *The explanation of organic diversity: the comparative method and adaptations for mating.* Oxford University Press.

Rinderer, T. E., Rothenbuhler, W. C. & Kulincevic, J. M. 1975 Responses of three genetically different stocks of the honey bee to a virus from bees with hairless-black syndrome. *J. Invert. Pathol.* **25**, 297–300.

Robinson, G. E. 1992 Regulation of division of labor in insect societies. *A. Rev. Entomol.* **37**, 637–665.

Robinson, G. E. & Page, E. R. 1989 Genetic basis for division of labor in an insect society. In *The genetics of social evolution* (ed. M. D. Breed & R. E. Page), pp. 61–80. Boulder, CO: Westview Press.

Rosengren, R., Sundström, L. & Fortelius, W. 1993 Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactics. In *Queen number and sociality in insects* (ed. L. Keller), pp. 308–333. Oxford University Press.

Ross, K. G. 1988 Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **23**, 341–355.

Ross, K. & Keller, L. 1995 Ecology and evolution of social organization insights from fire ants and other highly eusocial insects. *A. Rev. Ecol. Syst.* **26**, 631–656.

Schmid-Hempel, P. 1990 Reproductive competition and the evolution of work load in social insects. *Am. Nat.* **135**, 501–526.

Schmid-Hempel, P. 1994 Infection and colony variability in social insects. *Phil. Trans. R. Soc. Lond.* B **346**, 313–321.

Schmid-Hempel, P. 1998 *Parasites in social insects*. Princeton University Press.

Schmid-Hempel, P. & Loosli, R. 1999 A contribution to the knowlegde of *Nosema* infections in bumble bees, *Bombus* spp. *Apidologie* **29**. (In the press.)

Schmid-Hempel, P. & Schmid-Hempel, R. 1993 Transmission of a pathogen in *Bombus terrestris,* with a note on division of labour in social insects. *Behav. Ecol. Sociobiol.* **33**, 319–327.

Shattuck, S. O. 1995 Generic-level relationships within the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). *Syst. Entomol.* **20**, 217–228.

Sherman, P. W., Seeley, T. D. & Reeve, H. K. 1988 Parasites, pathogens, and polyandry in social Hymenoptera. *Am. Nat.* **131**, 602–610.

Sherman, P. W., Seeley, T. D. & Reeve, H. K. 1998 Parasites, pathogens, and polyandry in honey bees. *Am. Nat.* **151**, 392–396.

Shykoff, J. A. & Schmid-Hempel, P. 1991 Parasites and the advantage of genetic variability within social insect colonies. *Proc. R. Soc. Lond.* B **243**, 55–58.

Sundström, L. 1994 Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* **367**, 266–268.

Taber, S. 1982 Breeding for disease resistance. *Am. Bee J.* **122**, 177–179.

Veal, D. A., Trimble, J. E. & Beattie, A. J. 1992 Antimicrobial properties of secretions from the metapleural glands *Myrmecia gulosa* (the Australian bull ant). *J. Appl. Bacteriol.* **72**, 188–194.

Ward, P. S. 1980 A systematic revision of the *Rhytidoponera impressa* group (Hymenoptera. Formicidae) in Australia and New Guinea. *Rhytidoponera confusa*, *Rhytidoponera enigmatica*, new taxa. *Austr. J. Zool.* **28**, 475–498.

Woyciechowski, M. & Lomnicki, A. 1987 Multiple mating of queens and the sterility of workers among eusocial hymenoptera. *J. Theor. Biol.* **128**, 317–327.

Woyciechowski, M., Krol, E., Figurny, E., Stachowicz, M. & Tracz, M. 1994 Genetic diversity of workers and infection by the parasite *Nosema apis* in honey bee colonies (*Apis mellifera*). In *Proceedings of the 12th Congress of the International Union for the Study of Social Insects* (ed. A. Lenoir, G. Arnold & M. Lepage), pp. 347. Paris: Université Paris-Nord.