

The Evolution of Worker Caste Diversity in Social Insects

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Submitted April 28, 2005; Accepted November 10, 2005;
Electronically published January 30, 2006

Online enhancement: appendix.

ABSTRACT: Morphological diversification of workers is predicted to improve the division of labor within social insect colonies, yet many species have monomorphic workers. Individual-level selection on the reproductive capacities of workers may counter colony-level selection for diversification, and life-history differences between species (timing of caste determination, colony size, genetic variation available) may mediate the strength of this selection. We tested this through phylogenetically independent contrast analyses on a new data set for 35 ant species. Evidence was found that early divergence of queen-worker developmental pathways may facilitate the evolution of worker diversity because queen-worker dimorphism was strongly positively associated with diversity. By contrast, risks for colonies that invest in specialized workers and colony size effects on costs of worker reproduction seem unlikely to strongly affect the evolution of worker diversity because there was no significant association between colony size and diversity when controlling statistically for queen-worker dimorphism. Finally, worker diversity was greater in species with multiple lineages per colony, and it was negatively associated with relatedness in monogynous species. This could be due to high intracolony genetic variance favoring the expression and evolution of great worker diversity or to diversity evolving more easily when there is selection for repression of worker reproduction (worker policing).

Keywords: colony, kin selection, policing, polyandry and polygyny, polymorphism.

Eusocial insects (ants, termites, some bees, wasps, thrips, and aphids) constitute one of the most impressive examples of social organization in the animal world. Tens to millions of individuals live together in colonies, with some females specializing in reproduction (queens, often large bodied) while others (workers, generally related to queens) devote themselves mostly or exclusively to collecting food and taking care of the queens' broods (Hamilton 1964; Wilson 1971; Engels and Buschinger 1990; Bourke and Franks 1995; Crozier and Pamilo 1996; males die shortly after mating, except in termites). Classic theory predicts that variation in worker shapes and sizes should evolve because of selection for an efficient division of labor within colonies (West-Eberhard 1975; Oster and Wilson 1978; Wheeler 1986; Hölldobler and Wilson 1990; Frumhoff and Ward 1992), similar to the common queen-worker size and shape dimorphism (Peeters 1997). Consistent with this, workers in a number of social insects, particularly some ants and termites, are greatly diversified, and this generally influences the efficiency of colony labor and defense (Wilson 1953, 1984; Oster and Wilson 1978; Wilson and Hölldobler 1985; Walker and Stamps 1986; Hölldobler and Wilson 1990; Detrain and Pasteels 1992; Schmid-Hempel 1992; Hasegawa 1993, 1997; Beshers and Traniello 1994; Ferster and Traniello 1995; O'Donnell and Jeanne 1995; Dejean and Feneron 1996; Passera et al. 1996; Whitehouse and Jaffe 1996; Anderson and Ratnieks 1999a, 1999b; Stapley 1999; Wetterer 1999; Yang et al. 2004; but see Walker and Stamps 1986; Traniello and Beshers 1991; Schmid-Hempel 1992).

Great worker diversity is, however, a rarity in social insects (Oster and Wilson 1978; Wheeler 1986; Hölldobler and Wilson 1990; Schmid-Hempel 1992; Bourke and Franks 1995), and some of the most successful species in terms of ecological expansion (e.g., Argentine ants *Linepithema humile* and the little fire ant *Wasmannia auropunctata*) possess workers that vary little in size and shape (Frumhoff and Ward 1992). There are two types of possible explanations. Low levels of worker diversity may be retained by colony-level selection in some species. Species differ in ecology and life history, and the number and kinds of tasks carried out by workers vary, while the degree of behavioral plasticity (Wilson 1984, 1985; Calabi and

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Traniello 1989a, 1989b; Detrain and Pasteels 1991; Brown and Traniello 1998; Tschinkel et al. 2003; Yang et al. 2004) probably also differs among species. Also, colony-level selection for a diverse worker force may be countered by individual-level selection on workers to retain a morphology allowing a high personal reproduction (Oster and Wilson 1978; Frumhoff and Ward 1992). Workers of many ant, bee, and wasp species are capable of laying viable eggs despite not mating, and these eggs turn into viable, normal males (normal males are haploid in hymenopterans, arising from unfertilized eggs). Several studies have found that the reproductive potential of workers does co-vary with their degree of morphological specialization, with small workers often having a low reproductive potential (Oster and Wilson 1978, p. 102; Breed and Harrison 1988; Frumhoff and Ward 1992; Ito et al. 1994; Heinze et al. 1999; Dietemann et al. 2002; Gobin and Ito 2003; Dijkstra et al. 2005). For example, ovariole number correlates positively with worker size in *Myrmecia gulosa* bulldog ants (Dietemann et al. 2002), and in *Acanthomyrmex ferox* ants, only major workers lay reproductive eggs after queen death (Gobin and Ito 2003). Hence worker diversity may be affected simultaneously by factors acting at the colony level and factors affecting the personal reproductive chances of workers (see also Tsuji 1995). Life-history differences could affect this balance by affecting the strength of selection on the reproductive potentials of workers and could also have a direct impact on the ease with which worker diversity can evolve.

Three leading sets of hypotheses suggest how life history may constrain or facilitate the evolution of worker diversity. The first is based on the fact that in most social Hymenoptera (ants, bees, wasps), all female larvae have the potential to develop into queens, with caste fate being determined only by quantitative differences in the amounts of food received or qualitative differences in nutrition at some sensitive period during larval life (Hölldobler and Wilson 1990). Wheeler (1986) suggested that early caste determination would make more time available for worker-destined larvae to develop along potentially diverse pathways. Also, once larvae become worker destined, they should be under weaker selection to retain a morphology permitting a high reproductive potential because they can no longer become queens. Hence the evolution of worker diversification should be easier in species where caste determination happens early, as it generally should in species with great queen-worker size dimorphism. Queen-worker dimorphism will correlate with the timing of caste determination even if queen-destined larvae speed up their developmental rate after the point of caste determination. This is because we consider "time" as time available for growth and differentiation. If, however, workers in some species cease growth immediately

after caste determination (late caste determination and low worker diversity, in accordance with Wheeler 1986) while queens keep growing (high queen-worker dimorphism), it would simply make it more difficult to detect an overall positive association between queen-worker dimorphism and worker diversity. Environmental (e.g., nutritional) determination of caste is assumed here (as is believed to generally hold for ants; Wheeler 1986), but we note that the genetic determination of caste found in a few species (Winter and Buschinger 1986; Fersch et al. 2000) can simply be considered very early caste determination.

The second set of hypotheses is based on colony size effects on worker diversity evolution. First, producing large specialized workers could be too risky for small-colony species because it would tie down a substantial amount of resources (Oster and Wilson 1978; Wheeler 1991). Consistent with this view, younger and smaller ant colonies of a given species usually show less worker diversity than do older colonies (Tschinkel 1988, 1993; Wetterer 1994). Second, colony size is predicted to influence whether workers are selected to reproduce (producing sons; Wenseleers et al. 2004b). This is because costs of worker reproduction for colonies will be affected by colony size if a worker that turns reproductive ceases to work (1 nonworking worker out of 100,000 in a large-colony species will diminish colony productivity less than 1 out of 500 in a small-colony species, more so when colony productivity is a concave function of the number of workers). Also, large-colony species may have a smaller queen-worker fecundity ratio because queens are greatly outnumbered by workers. Workers in such species may therefore mainly compete for reproduction with other workers, not the queen, which reduces selection against worker reproduction (Wenseleers et al. 2004b). Selection for workers to retain a reproductive morphology is hence expected to increase with species-specific colony size, and the evolution of great worker caste diversity should therefore be more difficult in large-colony species (contrary to Bourke's [1999] prediction).

The third set of hypotheses proposes that mating/breeding system evolution may be linked with caste evolution. If worker size and shape vary because of genotypic differences (Fraser et al. 1999; Hughes et al. 2003), then the presence of more than one lineage per colony should lead to a greater morphological diversity of workers and possibly permit the evolution of lineages specializing on different types of workers (Crozier and Page 1985; Fuchs and Moritz 1999; Crozier and Fjerdingstad 2001). Also, we note that worker diversity may evolve more easily in multi-lineage species (species with multiple queens per colony or multiple mates per queen) because worker policing (Ratnieks 1988; Pamilo 1991) should be more favored in such cases (all other things being equal) because of the decreased relatedness of a worker to an average worker-

produced male. Once worker policing is installed there should be weaker selection on workers to resist morphological specialization because they would only rarely succeed in reproducing (Wenseleers et al. 2004a, 2004b). A third factor lowering selection on workers of polygynous (multiqueen) species to retain a reproductive morphology is the fact that loss of all queens should happen only very rarely (Crozier and Pamilo 1996).

Explicit tests on factors constraining or facilitating the evolution of worker caste diversity have been scarce and incomplete. Frumhoff and Ward (1992) analyzed the association of gyny levels (number of queens per colony) and worker diversity but could not control for closely related species being similar due to common descent and not due to independent evolutionary events (so-called phylogenetic effects). Also, they did not take into account variation in andry levels (number of mates per queen). Brown and Schmid-Hempel (2003) analyzed worker diversity, controlling for phylogenetic effects, but only considered variation in andry levels, not gyny levels. Finally, actual tests on the importance of the timing of caste determination (Wheeler 1986), colony size (Oster and Wilson 1978; Bourke 1999; Wenseleers et al. 2004b), and mating/breeding system have not yet been carried out. Therefore it remains unknown whether these hypotheses can help explain the evolution of variable worker diversity levels in social insects.

We here test the three above hypothesis sets in a comparative study on 35 ant species from around the world. Comparative studies do not permit establishing causal relationships, only correlational ones, but have the advantage of examining issues at the evolutionary timescale. We collected new data on worker diversity for all species and examined the covariance of worker diversity with queen-worker size dimorphism, colony size, and colony socio-genetic structure (polygyny, monogyny, monoandry, or polyandry, mean nestmate relatedness), using queen-worker dimorphism as an estimator of the timing of caste determination. Our tests used the methods of independent contrasts to circumvent the problem of phylogenetic effects (Felsenstein 1985), as implemented in the program CAIC (Purvis and Rambaut 1995). Our data were plotted onto a composite phylogeny that we derived from published morphological and molecular studies.

Methods

Samples

We obtained new samples of workers from the 35 ant species listed in table 1; for most species, queens were also obtained. Samples were collected from mature colonies of the size that produce sexuals, by us or by colleagues who

previously worked on the species in question. Sampling consisted of collecting all or a large part of the nest and ants outside in order to obtain as random as possible a sample of all worker types. Collected workers and queens were stored at $\leq -20^{\circ}\text{C}$. For samples collected overseas, ants were wrapped in cotton-wool to avoid fragmentation and shipped to Australia in a liquid nitrogen dryshipper or (a few species) in 70% ethanol. For each species, we selected at random up to 70 individual workers from one colony. For species with colony sizes < 70 , all individual workers were examined (for exact numbers of workers examined, see table 1).

For each worker, we took the body weight. For the ethanol-preserved samples, we standardized the drip-off time before weighing on the basis of tests on local species (correlation between fresh/frozen weight and ethanol dripped-off weight > 0.95 , $n = 10$ workers). Also, we measured the maximal width of the head (after cutting it off) and the maximal length of the hindmost right femur (leg segment; also cut off). Measurements were taken using a dissecting microscope equipped with a micrometer platform and a video camera connected to a Macintosh computer running the public domain program NIH Image (developed at the U.S. National Institutes of Health and available at <http://rsb.info.nih.gov/nih-image/>). Samples were always oriented in the same way for measurements, and repeatability was very high ($r^2 > 0.92$ for all measurements and species, $P < .0005$). For queens, we measured frozen/fresh body weight or obtained relevant data from personal communications or literature for the species for which no queen samples were available.

Quantification of Worker Diversity

Morphological diversity of workers was quantified in two ways. First, we estimated worker diversity by overall size variation as quantified by the coefficient of variation (CV) of fresh body weight of workers. Body weight is a variable of particular interest because it is directly connected to the cost of producing a worker and because worker size and the type of tasks carried out tend to co-vary (see introduction). Second, we analyzed the relationship of head width and femur length in workers through curvilinear regressions (as in Feener et al. 1988), testing whether polynomial regressions including linear, quadratic, or cubic terms fit the data best. To estimate worker diversity in a species, we then used the highest exponent whose term was significant (i.e., 1 for linear, 2 for quadratic, and 3 for cubic). Our two estimators of species-specific worker diversity were positively correlated across species ($r = 0.60$, $n = 35$, $P < .0005$), but the CV should allow for more powerful tests because it is a continuous variable.

We did not carry out classic allometric analyses where

Table 1: Data set used for the comparative analyses

Species	N	Log CV of body weight	Highest exponent head-femur ^a	Colony size ^b	Log Q-W dimorphism ^c	Relatedness ^d	Polyandry	Polygyny	Multilineage
<i>Acromyrmex echinator</i>	70	-.108	1	4	1.079	.4	Yes	Yes	Yes
<i>Acromyrmex octospinosus</i>	68	.103	1	4	1.187	.33	Yes	No	Yes
<i>Aphaenogaster rudis</i>	70	-.949	1	2		.75	No	No	No
<i>Camponotus consobrinus</i>	65	-.308	3	3	.505	.61	No	Yes	Yes
<i>Colobopsis nipponicus</i>	63	-.355	1	2	.400	.75	No	No	No
<i>Cyphomyrmex longiscapis</i>	22	-1.001	1	1	.357	.75	No	No	No
<i>Dorymyrmex bicolor</i>	68	-.804	1	4		.33	No	Yes	Yes
<i>Forelius mccoeki</i>	61	-.630	1	4	.689			Yes	Yes
<i>Formica exsecta</i>	70	-.565	1	4	.513	.64	Yes	Yes	Yes
<i>Formica sanguinea</i>	69	-.399	1	4		.19	Yes	Yes	Yes
<i>Iridomyrmex purpureus</i>	64	-.820	1	5	1.043		No	Yes	Yes
<i>Lasius niger</i>	70	-.704	1	4	1.123	.70	Yes	No	Yes
<i>Leptothorax acervorum</i>	45	-.953	1	2	.007	.65	No	Yes	Yes
<i>Leptothorax ambiguus</i>	19	-.463	1	1	.487	.50	No	Yes	Yes
<i>Leptothorax longispinosus</i>	66	-.334	0	1	.359	.57	No	Yes	Yes
<i>Linepithema humile</i>	58	-.577	1	5	1.315	.02	No	Yes	Yes
<i>Myrmecia pilosula</i>	66	-.645	1	3					
<i>Myrmecia pyriformis</i>	70	-.382	1	3	.664	.52	Yes	No	Yes
<i>Myrmica punctiventris</i>	46	-.811	1	2	.282	.77	No	No	No
<i>Myrmica ruginodis</i>	70	-.795	1	2	.345	.59	Yes	Yes	Yes
<i>Myrmica scabrinodis</i>	70	-.701	1	3	.507	.42	No	Yes	Yes
<i>Myrmica tahoensis</i>	37	-.713	1	2	.359	.59	No	Yes	Yes
<i>Nothomyrmecia macrops</i>	45	-.823	1	1	.198	.61	Yes	No	Yes
<i>Oecophylla smaragdina</i>	62	-.433	3	5	1.215	.70	Yes		Yes
<i>Pheidole megacephala</i>	68	-.004	3	5				Yes	Yes
<i>Pheidole pallidula</i>	70	.050	3	4	1.346	.65	No	Yes	Yes
<i>Pogonomyrmex barbatus</i>	19	-.864	1	4	.470		Yes	No	Yes
<i>Polyrhachis australis</i>	63	-.959	1	2	.398	.42	No	Yes	Yes
<i>Proformica longiseta</i>	39	-.140	3	2	.857	.35	Yes	Yes	Yes
<i>Rhytidoponera metallica</i>	40	-.789	1	2	0	.08	No	Yes	Yes
<i>Rhytidoponera victoriae</i>	69	-.783	1	2	0				
<i>Sericomyrmex amabilis</i>	62	-.855	1	3		.75	No	No	No
<i>Solenopsis geminata</i>	63	.181	3	5	.879	.79	No	Yes	Yes
<i>Solenopsis invicta</i>	64	-.131	3	5	1.089	.71	No	No	No
<i>Trachymyrmex zeteki</i>	65	-.662	2	3		.75	No	No	No

Note: N represents the number of workers analyzed per colony. One colony was analyzed per species.

^a Highest significant exponent from a curvilinear regression of head width on femur length.

^b Colony size is given by order of magnitude: 1 = dozens of workers, 2 = hundreds, 3 = thousands, 4 = ten thousands, and 5 = more than 100,000.

^c Logarithm of queen-worker body weight dimorphism.

^d Represents the average nestmate relatedness determined through genetic analyses of workers.

worker diversity is estimated by the number of different allometric curves that can be fitted to a log-log plot of two morphological variables (see Oster and Wilson 1978; Hölldobler and Wilson 1990). We chose not to do so because such analyses require choosing a cutoff point merely on a belief that the cloud of data points starts following a different allometric curve there (Feener et al. 1988).

Quantification of Queen-Worker Size Dimorphism

Queen-worker dimorphism was estimated by the ratio of queen body weight over mean worker body weight, using weight data for one to five queens per species. All data

were either for gynes (young, mature queens still in their natal nest) or colony queens with established, mature colonies. Hence no data came from starved queens. For the two queenless species (see Peeters 1997), the green-headed ants *Rhytidoponera metallica* and *Rhytidoponera victoriae*, “queen”-worker dimorphism was assumed to be 1 because all workers could potentially mate and become reproductives (gamergates). Real queens do exist in *R. metallica* and alate females of *R. victoriae* occur occasionally, as recorded, for example, in an article by Haskins and Whelden (1965), but almost all colonies found in nature are headed by gamergates (Ward 1986). Whether these two species were included in analyses (tests presented below) did not affect

test results, changing only very slightly the degrees of significance.

Literature Data on Sociogenetic Structure and Colony Size

Colony size was estimated by order of magnitude based on literature, personal estimations by the authors, or personal communications from specialist colleagues (see appendix in the online edition of the *American Naturalist*). Information on number of queens per colony, number of mates per queen, and average nestmate relatedness came from published literature or in a few cases from unpublished studies by colleagues (appendix). All estimates of andry levels and nestmate relatedness were based on genetic analyses of offspring (using microsatellite DNA or allozyme markers). All analyses involving nestmate relatedness were performed both including and excluding four species with important among-population variation in relatedness (the wood ant *Formica exsecta*, the Argentine ant *Linepithema humile*, and the fire ants *Solenopsis invicta* and *Solenopsis geminata*). Including (results below) or excluding these species changed test results very little.

Comparative Analyses

All comparative analyses were based on independent contrasts using the program CAIC (Purvis and Rambaut 1995). Calculation of contrasts was made possible by plotting our morphological data onto a composite phylogeny (fig. 1) that we constructed from published molecular and morphological trees using Schmid-Hempel and Crozier's (1999) work as a basis and including information from Chiotis et al. (2000), Johnson et al. (2003), and Ward and Brady (2003). Because no information on branch lengths could be assigned to our composite phylogeny, we set all branch lengths equal (see Purvis and Rambaut 1995). Branch length information is not indispensable for independent contrast analyses, which are generally robust (Garland et al. 1992; Diaz-Uriarte and Garland 1998).

Contrasts for continuous variables were obtained using the CAIC "crunch" option and were then subjected to regression analyses through the origin; for categorical predictor variables, we used the "brunch" option and then applied sign tests (see Purvis and Rambaut 1995). The number of informative contrasts was often substantially smaller than the number of species and nodal pairs for which data on predictor and dependent variables were available. This was because only contrasts that differ from 0 are informative (Purvis and Rambaut 1995), contrasts can generally not be estimated for higher nodes for categorical variables because of lack of models of categorical trait evolution, and only one contrast is calculated at

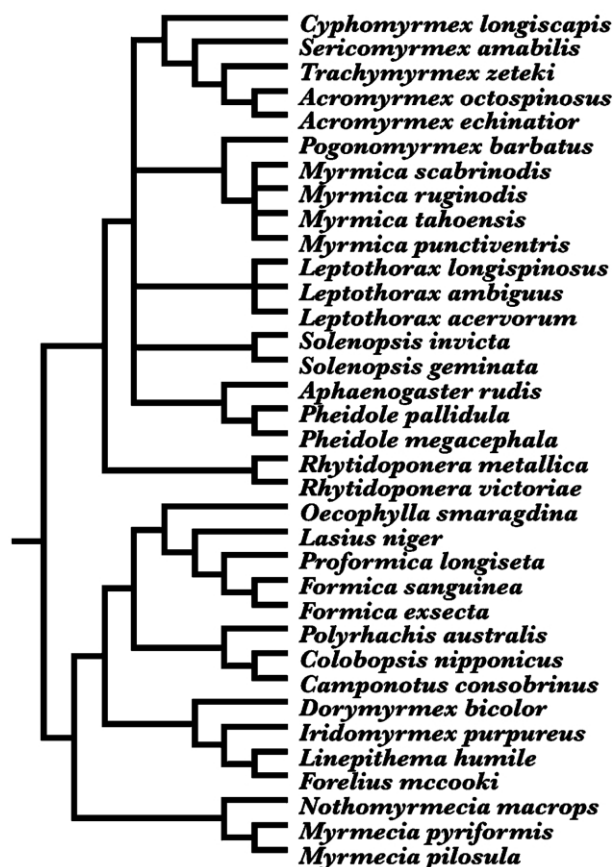


Figure 1: Composite phylogeny of the species studied (see text for derivation).

polytomous nodes regardless of the number of taxa involved. All continuous variables were logarithmically transformed before analyses, to suit the null model of CAIC, which says that different lineages are equally likely to show the same proportional change in the dependent variable (Felsenstein 1985). We verified that contrasts were appropriately standardized by applying the tests proposed by Garland et al. (1992) and given in the CAIC package. Thus we verified that residuals from the regression of contrast y on contrast x were not associated with contrasts, that standard deviations of contrasts were not associated with the values of contrasts, and that contrasts were not associated with their nodal values. The majority of tests were based on a priori hypotheses, and tests given below are hence one tailed unless otherwise stated.

Results

Species with greater queen-worker size dimorphism or larger colonies displayed greater morphological diversity

among workers. This was highly significant for queen-worker dimorphism (fig. 2) and marginally significant for colony size (fig. 3) when estimating worker diversity by the CV of body weight. Colony size and queen-worker dimorphism were also positively associated with worker diversity when this was estimated by the greatest significant exponent from curvilinear regression of worker head width on femur, and this was significant for queen-worker dimorphism (19 contrast sets, $t = 1.9$, $P = .04$) but non-significant for colony size (26 contrast sets, $t = 1.5$, $P = .08$).

Queen-worker size dimorphism and colony size were closely correlated across species (19 contrast sets, $t = 3.7$, $P = .002$, two-tailed test), but this did not confound the above analyses. When controlling for colony size in a partial regression analysis using CAIC, queen-worker dimorphism remained significantly associated with worker diversity estimated by CV of body weights ($t = 2.9$, $P = .005$) and showed a trend for a positive association with diversity estimated by the highest curvilinear exponent ($t = 1.3$, $P = .11$). By contrast, colony size, which was only weakly associated with worker diversity in the direct tests (see above), was no longer significantly related to diversity when queen-worker dimorphism was controlled for (CV: $t = -0.82$, $P = .22$; exponent: $t = 0.20$, $P = .43$; 19 contrast sets for all tests).

Average relatedness among nestmates was negatively associated with worker diversity estimated by CV of body weight, but this was not significant (20 contrast sets, $t = -1.1$, $P = .14$). There was no significant relation between diversity estimated by the highest curvilinear ex-

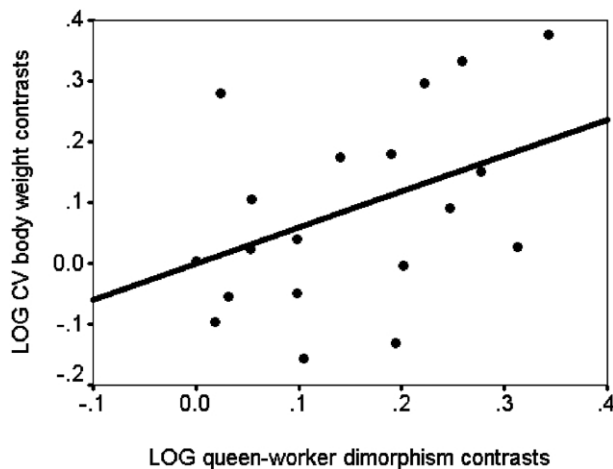


Figure 2: Species with greater queen-worker body weight dimorphism had greater diversity (CV) of worker body weight (19 contrast sets, $t = 3.4$, $P = .002$). When controlling for colony size, the association remained significant.

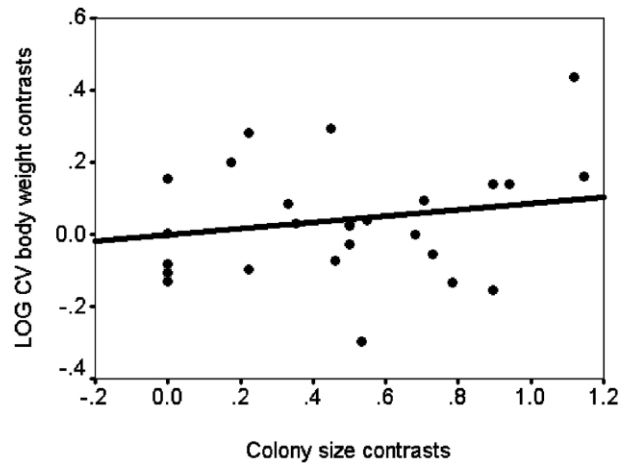


Figure 3: Species with larger colonies had a marginally significant greater diversity (CV) of worker body weight (26 contrast sets, $t = 1.7$, $P = .05$).

ponent and nestmate relatedness (20 contrast sets, $t = 0.43$, $P = .34$). Within monandrous species that were mono- or polygynous, nestmate relatedness was not significantly associated with worker diversity (for CV: $t = 0.22$, $P = .42$; for curvilinear exponent: $t = 0.38$, $P = .35$, NS; 13 contrast sets for both tests). In monogynous species that were mono- or polyandrous, nestmate relatedness was, however, significantly negatively associated with worker diversity estimated by the CV of body weight (fig. 4) and also when controlling for colony size (test result unchanged) but not with diversity estimated by curvilinear regression ($t = 1.02$, $P = .18$, seven contrast sets).

When dividing the whole data set into species with single or multilineage colonies (multiple queens or multiple mates per queen), species with more genetically diverse colonies showed significantly greater worker diversity as estimated by the CV of body weight (fig. 5). There was no trend when diversity was estimated by the curvilinear exponent ($n = 4$ informative contrast sets, two negative and two positive, $P = .69$, one-tailed sign test), but the power of the test was very low because of few informative contrasts. When controlling statistically for colony size, a tendency for a greater worker diversity (CV of body weight) in species with multilineages remained, although this was no longer significant (five out of six contrast sets positive, $P = .11$, one-tailed sign test; but with low power because of few informative contrasts). Also, monandrous species with polygynous colonies had greater worker diversity than those with monogynous colonies when diversity was estimated by the CV of body weight (fig. 6; for the curvilinear exponent, only three informative contrasts were available and therefore no test was performed).

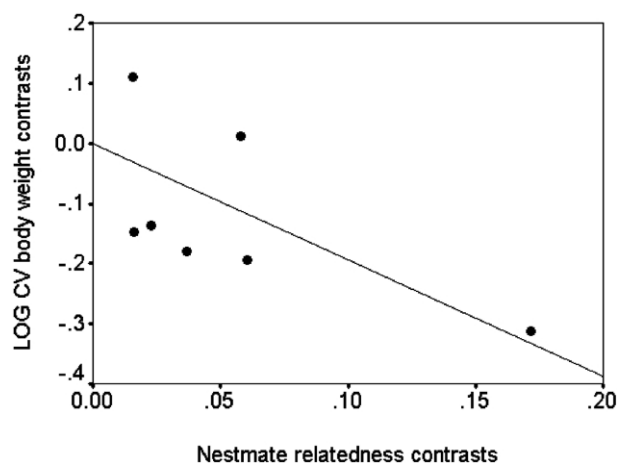


Figure 4: Within monogynous species, nestmate relatedness was significantly negatively associated with worker diversity (CV) of body weight (seven contrast sets, $t = -3.4$, $P = .008$). This was not changed by controlling for colony size.

Controlling for colony size did not change this result. Worker diversity in monogynous species with mono- or polyandrous colonies did not differ (CV: 4 informative contrasts of which 2 were positive and 2 negative, $P = .69$, one-tailed sign test, NS; exponent: only 2 informative contrasts available and hence no test performed). However, the power of the test was low because so few informative contrasts were available.

Discussion

Our study provides evidence that the evolution of worker caste diversity in social insects may be constrained by life history directly or by life history mediating the strength of selection on workers to retain reproductive morphologies. The first piece of evidence was support that worker diversity evolves more easily in species where queen and worker developmental pathways probably diverge early during larval development, as predicted by Wheeler (1986). Worker caste diversity and mean queen-worker size dimorphism were strongly positively associated in our comparative data set. This association was not primarily due to species with larger colonies (which often have large queens due to fecundity selection; Wilson 1971; see also Tschinkel 1987) having more diverse workers (Oster and Wilson 1978). This conclusion follows because controlling for colony size did not eliminate the significant positive association between queen-worker dimorphism and worker diversity.

Our study does not permit identifying the causal factor for the association between queen-worker dimorphism

and worker diversity. Hence we cannot say whether the greater worker diversity in species with high queen-worker dimorphism is due to there being more time for worker-destined larvae to follow different developmental pathways in such species or to larvae in such species experiencing weaker selection to resist morphological specialization because they lose the chance to become queens early on (Wheeler 1986). Both factors could be acting simultaneously. More information on the genes expressed differentially in larvae turning into queens and workers in social insects (see, e.g., Evans and Wheeler 1999, 2000, 2001; Cnaani et al. 2000; Abouheif and Wray 2002) would be of value. Also, early caste determination might occur not only in species with great queen-worker dimorphism but also in some of the species in which queens and workers are similar in size (low dimorphism). It would therefore be useful to apply genomic expression analyses (references above) to verify that the developmental pathways of larvae becoming queens and larvae turning into workers do diverge later in species with low dimorphism.

The second finding of our study was that colony size seems unlikely to strongly affect the evolution of worker diversity because these two variables were only marginally positively associated in our comparative data set. Also, this weak association did not remain significant when controlling statistically for queen-worker size dimorphism. The loss of significance could in part be due to the lower power of the tests that examined simultaneously the effect of colony size and queen-worker dimorphism (19 contrast

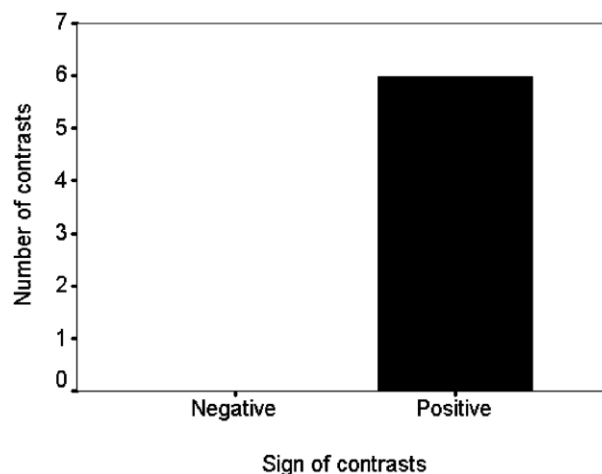


Figure 5: Species with multilineage colonies (>1 queen per colony or >1 mate per queen) had a greater diversity (CV) of worker body weight (six informative contrast sets, all of which were positive; $P = .016$, one-tailed sign test). When statistically controlling the test for colony size, the association between lineage number and worker diversity remained close to significant.

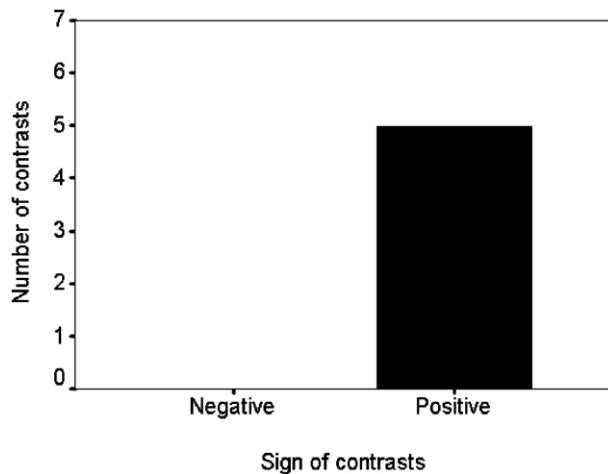


Figure 6: Within monandrous species, those with more than one queen per colony showed a greater diversity (CV) of worker body weight (five contrasts, all positive; $P = .03$, one-tailed sign test). This result remained the same when controlling for colony size.

sets vs. 26 in the test treating only colony size), but if there is any genuine association between colony size and worker diversity, our results show that it must be quite weak. The absence of a clear positive association between colony size and worker diversity implies little support for the hypothesis that it may be too risky for small-colony species to tie up resources in the production of specialized workers (Oster and Wilson 1978). Also, the lack of a negative association between colony size and worker diversity gives no support for selection on workers to retain a reproductive morphology being greater in large-colony species where colony-level costs of worker reproduction were expected to be smaller (Wenseleers et al. 2004b). Finally, the positive association between colony size and queen-worker dimorphism in our study does not fit the prediction that workers should evolve more easily toward extreme (here small) sizes in small-colony species (cf. Wenseleers et al. 2004b). Instead, queen size likely increases over evolutionary time to allow space for more ovarioles, eggs (Wilson 1971; see also Tschinkel 1987), and body reserves (Keller and Passera 1989) when great queen fecundity is selectively favored.

Independent empirical evidence, moreover, exists that colony size only weakly affects selection on worker reproductive potentials in social insects. This evidence comes from a recent comparative study in which no association was found between colony size and realized reproduction by ant workers (in species where workers have ovaries; Hammond and Keller 2004). Deviations from the theoretical expectations of stronger selection for worker reproduction in large-colony species (Wenseleers et al.

2004b) could arise in at least two ways. First, a reproductive worker could allocate only a part of its efforts to personal reproduction (as found in queenless ants; see, e.g., Monnin and Peeters 1999), and hence colony-level costs in terms of lost productivity per reproductive worker could vary among species in a manner less tightly tied to colony size. Second, the fecundity of queens relative to workers, which affects the degree to which worker reproduction is favored, may be less tightly negatively correlated with colony size in ants than Wenseleers et al. (2004b) assumed. For example, queen to worker fecundity ratios should remain very high in species with only a single queen per colony, because colonies in such species can only become large when the queen has a very great fecundity (see also Tschinkel 1987). Hence, selection on workers to retain a capacity for high personal reproduction could become at least partially decoupled from colony size. The absence of clear relationships between colony size and worker diversity (our study) and between colony size and worker reproduction (Hammond and Keller 2004) accord with such a decoupling.

Our third main finding was that the evolution of worker diversity is linked to a core component of life history, the mating/breeding system. Species with multilineage colonies (multiple queens or multiple mates per queen) displayed a greater diversity of workers, and diversity was also associated with nestmate relatedness across monogynous species and with the number of queens per colony in monandrous species. These results stand in contrast to the findings of previous comparative studies. Frumhoff and Ward (1992) found a negative relationship between queen number and worker diversity, Bourke (1988) found that more worker reproduction took place in polygynous colonies under queen-right conditions (but see Crozier and Pamilo 1996; Hammond and Keller 2004), while Brown and Schmid-Hempel (2003) found no significant relationship between andry levels and worker diversity. Our tests are, however, analytically more powerful and more appropriate than previous tests. Bourke (1988) and Frumhoff and Ward (1992) did not take andry levels into account and could not control for phylogenetic effects, while Brown and Schmid-Hempel (2003) did not take gyny levels into account. Differences in the type of diversity estimators used might also have had some influence (they examined allometric data from a series of published studies).

We cannot determine whether the greater worker diversity of more genetically diverse species in our study is directly due to increased intracolony genetic variance or to worker policing. If worker policing is the causal factor then we would expect nestmate relatedness and frequency of worker reproduction to correlate positively across species. Two recent comparative studies have tested for such

a correlation (Hammond and Keller 2004; Ratnieks et al. 2006). The study with the largest sample size ($n = 66$ species) and the most extensive coverage of relatedness variation (Ratnieks et al. 2006; referring to T. Wenseleers and F. L. W. Ratnieks, unpublished manuscript) found a significant relationship between average nestmate relatedness and worker reproduction (estimated through genetic analyses of males). This finding supports that nestmate relatedness affects whether reproduction by social insect workers is favored and hence is likely to affect selection on worker reproductive capacities and morphology. Considerable unexplained variation in worker reproduction, however, remained in Wenseleers and Ratnieks's (unpublished manuscript) data set (see also Hammond and Keller 2004). This suggests that varying costs of worker reproduction and policing (lowered colony efficiency) in different species (Wenseleers et al. 2004b) may introduce some noise into the relationship between nestmate relatedness and worker reproduction. Overall, the information available allows no conclusions regarding the relative importance of genetic variance per se and of worker policing (favored by high intracolony genetic diversity) for the evolution of worker caste diversity. Behavioral studies could, however, help resolve this issue because worker policing behavior should occur in multilineage species if suppression of worker reproduction is what favors caste diversification in these species, whereas this is not required if greater intracolony genetic variance directly increases caste diversification.

Overall, our findings suggest that the timing of caste determination, mating/breeding system, and possibly colony size should be under selective pressure in species where ecological factors favor great worker diversity, within the constraint that these traits also affect the adaptiveness of other aspects of a species' ecology (e.g., timing of caste determination may be constrained by the need to vary adaptively late in larval life the number of queens produced; mating system evolution may be constrained by costs for queens that mate multiply; and colony size may be limited by the microhabitat occupied, such as a twig or a nut).

Acknowledgments

We warmly thank J. J. Boomsma, A. F. G. Bourke, C. J. DeHeer, T. Ebersole, J. D. Evans, E. I. Fernandez, V. S. Fraser, K. Gartlan, P. J. Gertsch, D. M. Gordon, R. L. Hammond, J. M. Herbers, R. N. Johnson, L. Keller, U. G. Mueller, P. Pamilo, J. S. Pedersen, S. K. Robson, K. G. Ross, M. Sanetra, T. Satoh, M.-A. Schneider, P. Seppä, P. Sjödin, A. V. Suarez, L. Sundström, W. R. Tschinkel, and G. J. Umphrey for collecting or advising on samples; P. Sjödin and W. R. Tschinkel for shipping assistance; G.

Gilroy for assistance in the laboratory; B. E. Kaufmann and M. Sanetra for use of unpublished data; A. A. Hoffmann for discussion; J. D. Evans, R. L. Hammond, E. Hasegawa, M. Molet, K. Tsuji, T. Wenseleers, D. E. Wheeler, and an anonymous reviewer for constructive comments on earlier versions of this article; and J. Clobert for endorsing E.J.F.'s stay in Paris. Our work was funded by the Australian Research Council (grant A19925028).

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