Sociality and the Rate of Molecular Evolution

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The molecular clock does not tick at a uniform rate in all taxa but may be influenced by species characteristics. Eusocial species (those with reproductive division of labor) have been predicted to have faster rates of molecular evolution than their nonsocial relatives because of greatly reduced effective population size; if most individuals in a population are nonreproductive and only one or few queens produce all the offspring, then eusocial animals could have much lower effective population sizes than their solitary relatives, which should increase the rate of substitution of "nearly neutral" mutations. An earlier study reported faster rates in eusocial honeybees and vespid wasps but failed to correct for phylogenetic nonindependence or to distinguish between potential causes of rate variation. Because sociality has evolved independently in many different lineages, it is possible to conduct a more wide-ranging study to test the generality of the relationship. We have conducted a comparative analysis of 25 phylogenetically independent pairs of social lineages and their nonsocial relatives, including bees, wasps, ants, termites, shrimps, and mole rats, using a range of available DNA sequences (mitochondrial and nuclear DNA coding for proteins and RNAs, and nontranslated sequences). By including a wide range of social taxa, we were able to test whether there is a general influence of sociality on rates of molecular evolution and to test specific predictions of the hypothesis: (1) that social species have faster rates because they have reduced effective population sizes; (2) that mitochondrial genes would show a greater effect of sociality than nuclear genes; and (3) that rates of molecular evolution should be correlated with the degree of sociality. We find no consistent pattern in rates of molecular evolution between social and nonsocial lineages and no evidence that mitochondrial genes show faster rates in social taxa. However, we show that the most highly eusocial Hymenoptera do have faster rates than their nonsocial relatives. We also find that social parasites (that utilize the workers from related species to produce their own offspring) have faster rates than their social relatives, which is consistent with an effect of lower effective population size on rate of molecular evolution. Our results illustrate the importance of allowing for phylogenetic nonindependence when conducting investigations of determinants of variation in rate of molecular evolution.

Introduction

Social animals have reproductive division of labor: a small number of individuals produce all the offspring, and all others are nonreproductive helpers that contribute to raising of offspring and maintaining the colony. Sociality has evolved multiple times, most notably in the Hymenoptera (bees, wasps, ants) and Isoptera (termites) and also in a wide range of taxa including spiders, beetles, aphids, shrimp, and mammals (Keller and Chapuisat 2002). Because of the dramatic impact on reproductive dynamics, sociality could influence molecular evolution in a number of ways, including reduction in the effective population size and an increase in the number of DNA replications per unit time. Higher substitution rates in eusocial lineages were reported in an earlier study (Schmitz and Moritz 1998), but because they included only two independent origins of sociality in their study, they were not able to demonstrate whether the effect was general to all social taxa nor were they able to test the possible causes of this effect.

The most widely discussed influence of sociality on molecular evolution is through the reduction in effective population size (N_e: the number of individuals that contribute alleles to each generation), which determines the relative strengths of selection and drift. In a large population with many reproductive individuals, the effect of random sampling on allele frequencies is minimal, so selection can cause alleles with relatively small selective advantage to go to fixation. Small populations are more greatly affected by stochastic fluctuations in allele frequencies, so drift can overpower selection for alleles with small selection coefficients. Therefore, the fixation of "nearly neutral" alleles by drift is expected to be the greatest in small populations (Kimura 1983; Ohta 1987). Under the nearly neutral theory, the fate of an allele is expected to be determined by drift if its selection coefficient is less than the reciprocal of twice the effective population size ($s < 1/2N_e$) (Ohta 1987). It is worth noting that the effect of population size on substitution rate depends on the type of substitution—the fixation of advantageous mutations should be faster in large populations, but nearly neutral mutations will become fixed faster in small populations—so the magnitude and direction of the population size effect is not clear.

Effective population size is determined not only by the number of individuals present in a population but also by population structure and reproductive dynamics. Social animals provide an interesting case, for while there may be thousands of sterile workers in a colony, only a small number of reproductive individuals produce the offspring that make up the next generation. In the most extreme cases, a single queen who has mated with only one male may produce hundreds of sexual (reproductive) offspring. Social animals are therefore expected to have much lower values of N_e than solitary species (Crozier 1979). So, if effective population size influences substitution rate, social lineages could have different rates of molecular evolution than their nonsocial relatives. However, there are currently no empirical estimates of N_e available for social species, so it is difficult to test this prediction directly. Furthermore, because social species may differ from their nonsocial relatives in a number of ways that could influence rates of molecular evolution, observing faster rates in social taxa does not necessarily implicate N_e.

Key words: eusocial, substitution rate, nearly neutral theory, molecular clock, effective population size, comparative method.

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To test for an effect of sociality on the rate of molecular evolution, Schmitz and Moritz (1998) analyzed DNA sequence data from the eusocial honeybees *Apis mellifera* and *Apis dorsata* and from 22 species of vespid wasps that showed various degrees of sociality. They predicted that the ratio of mitochondrial to nuclear substitution rates would increase with the degree of sociality because mitochondrial genes, passed only through the female line, should have a more dramatically reduced N_e than nuclear genes which can be passed through both male and female lines. They supported this prediction by comparing branch lengths in the social species to their nonsocial relatives. However, the study was limited by restricted taxon sampling and the use of overlapping pairwise comparisons, which were not statistically independent. Furthermore, the inclusion of only two independent origins of sociality (in honeybees and vespid wasps) limited the conclusions that could be drawn concerning the effect of sociality on molecular evolution. Evidence that levels of intraspecific heterozygosity vary with the level of eusociality has also been controversial (Berkelhamer 1983; Owen, 1985; Reeve, Shellman Reeve, and Pfenning 1985).

Because sociality has arisen independently in a variety of lineages, it is possible to make phylogenetically independent comparisons across a wide range of taxa. We have tested for an effect of sociality on rate of molecular evolution using a comparative analysis that spans a wide range of taxa (bees, wasps, ants, termites, shrimps, and mole rats) and many types of DNA sequences (mitochondrial, nuclear, RNA coding, protein coding, and introns). We find no consistent effect of sociality on the rate of molecular evolution nor do we support Schmitz and Moritz's prediction that mitochondrial genes should show a more marked effect than nuclear genes. However, the "deeper" contrasts, between highly eusocial lineages and related nonsocial tribes or families, do conform to the predicted patterns, so it may be that extreme levels of sociality over long evolutionary periods increase overall rates of molecular evolution. In addition, we find that social parasites have consistently faster substitution rates than their social relatives.

Materials and Methods

Comparative Analysis of Substitution Rates

Species cannot be treated as independent estimators of the relationship between biological traits because those traits may be associated by phylogenetic inertia (inherited together from a common ancestor) rather than by any causal link. Methods for testing hypotheses in a phylogenetic context have been developed (Harvey and Pagel 1991; Harvey and Rambaut 2000; Martins 2000) but are not easily applied to investigating correlates of substitution rates (Slowinski and Arbogast 1999). Absolute measures of substitution rate, calculated by inferring the number of changes over a period of evolutionary time, cannot be regarded as instantaneous measures at the tips (or nodes) of phylogenies. Absolute rates may also be subject to systematic measurement biases (Slowinski and Arbogast 1999).

Calculation of absolute substitution rates can be avoided by comparing relative branch lengths (see fig. 1). The difference in branch lengths for any pair of lineages can be compared to the difference in the species' characteristics, either as a continuous variable analyzed by general linear models such as correlation (Mooers and Harvey 1994; Bromham, Rambaut, and Harvey 1996) or simply by a sign test (Barraclough, Harvey, and Nee 1996). Branch lengths can be estimated for the pair from distance matrices or estimated directly from sequence data (Bromham, Rambaut, and Harvey 1996). Because the comparison is between lineages of the same age, the same measurement biases (such as saturation or degree of site-to-site rate variation) are likely to apply to both lineages (Bromham 2002).

Studies of determinants of variation in rate of molecular evolution across phylogenies have commonly been statistically flawed due to the inclusion of the same phylogenetic branches many times in a single analysis (e.g., Omland 1997; Bleiweiss 1998; Nunn and Stanley 1998; Schmitz and Moritz 1998). An alternative approach is to compare rates estimated for each branch (edge) of the phylogeny with the inferred value of a trait along that branch (Schmitz and Moritz 1998; Bromham et al. 2002), although this approach begs caution; if each lineage inherits both its initial substitution rate and the initial value of the trait of interest, then closely related lineages are likely to share similar values of both rates and traits, potentially creating a spurious association between the two. This complication is avoided by comparing phylogenetically independent pairs of species at the tips (terminal lineages) of the phylogeny (Bromham et al. 2002).

Data

We defined a social species as one with reproductive division of labor, indicated by the presence of nonreproductive workers. We then identified molecular phylogenetic studies that included both social and nonsocial taxa and from these selected phylogenetically independent pairs of taxa, each consisting of a social lineage and a close nonsocial relative (table 1). Comparisons are phylogenetically independent if the branches that connect each comparison do not overlap on a phylogeny (Harvey and Purvis 1991). This ensures that the difference in sociality (and N_e) between the compared species has arisen independently of any other pair in the analysis. Phylogenetic independence of species comparisons is essential to avoid the problem of effectively including the same observations (branches) multiple times in a single statistical analysis (Felsenstein 1985; Harvey and Pagel 1991). Selection of statistically independent comparisons, therefore, relies on some knowledge of phylogenetic relationships. We have chosen each of the comparisons from published phylogenies (see table 1); if these phylogenies are shown in future to be incorrect, then the data may need to be reanalyzed.

Where multiple species were available for a given comparison, species were chosen to maximize the difference in lifetime reproductive output per sexual female between the pair. Estimates of lifetime reproductive output per sexual (reproductive) female were based on data in the literature and by consulting experts on the taxa (see table 1), including information on the lifetime reproductive output of reproducing females. For species where these data were not

Table 1
Phylogenetically Independent Comparisons Between Social and Nonsocial Lineages

Taxa	Pair	Social Species (A)	$repr_A$[a]	Nonsocial Species (B)[b]	$repr_B$	Sequences[c]	References[d]
Bees	1	*Bombus pratorum*	<500	*Psithyrus vestalis**	<80	COI	a, b, c
	2	*Apis mellifera*	1,000,000	*Anthophora pacifica*	<10	rhod, cytb, 28S	a, d, e, f, g
	3	*Lasioglossum* (*Dialictus*) *umbripenne*	~20	*Lasioglossum* (*Hemihalictus*) *lustrans*	<10	COI, EF1α	h
	4	*Lasioglossum* (*Dialictus*) *zephyrum*	18 (63 max)	*Lasioglossum* (*Paralictus*) *asteris**	<10	COI, EF1α	h, i
	5	*Lasioglossum* (*Evylaeus*) *truncatum*	~20	*Lasioglossum* (*Sphecodogastra*) *noctivagum*	<10	COI, EF1α	h
	6	*Lasioglossum* (*Evylaeus*) *subtropicum*	~20	*Lasioglossum* (*Evylaeus*) *fulvicorne*	<10	COI, EF1α	h
	7	*Lasioglossum* (*Evylaeus*) *albipes* (Dordogne)	~20	*Lasioglossum* (*Evylaeus*) *albipes* (Vosges)	<10	COI, EF1α	h
	8	*Lasioglossum* (*Evylaeus*) *marginatum*	2,180	*Lasioglossum* (*Lasioglossum*) *athabascense*	<10	COI, EF1α	h, j
	9	*Halictus* (*Halictus*) *farinosus*	~20	*Halictus* (*Halictus*) *quadricinctus*	<10	COI, EF1α	H
	10	*Halictus* (*Halictus*) *ligatus*	22	*Thrincohalictus prognathus*	<10	COI, EF1α	h, k
	11	*Augochlorella pomoniella*	14–21	*Neocorynura discolor*	<10	COI, EF1α	h, z
	12	*Exoneura robusta*	<10	*Inquilina dawsoni**	<10	cytb, COI, EF1α	x
	13	*Exoneurella tridentate*	80	*Exoneurella setosa*	<10	cytb, COI, EF1α	x, y
Wasps	14	*Vespula germanica*	14,181	*Eumenes coarctatus*	<10	28S, 16S	l
Ants	15	*Leptothorax acervorum*	471	*Doronomyrmex kutteri**	150	COI, 18S	m, n, o, p
	16	*Myrmica sabuleti*	—	*Myrmica hirsuta**	—	COI, COII, cytb	+
	17	*Myrmica scabrinodis*	—	*Myrmica karavajevi**	—	COI, COII, cytb	+
	18	*Myrmica rubra* (Finland)	—	*Myrmica microrubra* (Finland)*	—	COI, COII, cytb	+
	19	*Myrmica rubra* (England)	—	*Myrmica microrubra* (England)*	—	COI, COII, cytb	+
Termites	20	*Microhodotermes viator*	1,000,000	*Periplaneta americana*	<1,000	COII, 16S	q
Shrimps	21	*Synalpheus chacei*	243	*Synalpheus* "*bousfieldi* A"	7	COI, 16S	r
	22	*Synalpheus regalus*	356	*Synalpheus* "*rathbunae* A"	80	COI, 16S	r
	23	*Synalpheus* "*paraneptunus* small"	23	*Synalpheus paraneptunus*	18	16S	r
Mole rats	24	*Heterocephalus glaber*	1,000	*Heliophobius argenteocinereus*	30	cytb, 12S, TTR	s, t, u, v
	25	*Cryptomys damarensis*	132	*Cryptomys hottentotus natalenis*	60	cytb, 12S, TTR	s, t, u, v, w

[a] Estimated lifetime reproductive output per sexual female: some are averages, others are rough estimates, and some are based on related taxa; see *Materials and Methods* for explanation or the cited references for further information.

[b] Solitary species, except for social parasites which are marked with an asterisk.

[c] Mitochondrial genes: cytochrome b (cytb), cytochrome oxidase subunits one (COI) and two (COII), 12S ribosomal RNA (12S), and 16S ribosomal RNA (16S). Nuclear genes: long-wavelength rhodopsin (rhod), elongation factor 1 alpha (EF1α), 28S ribosomal RNA (28S), 18S ribosomal RNA (18S), and GPPH2 transthyretin intron 1 (TTR).

[d] a, Bourke (1997); b, Michener (1974); c, Pedersen (1996); d, Cameron (1993); e, Roubik (1989); f, Mardulyn and Cameron (1999), Cameron and Mardulyn (2001); g, Koulianos et al. (1999); h, Danforth (1999, 2002), Danforth and Ji (2001), Danforth, Conway, and Ji (2003); i, Greenberg (1982); j, Plateau-Quénu (1959); k, Richards and Packer (1998); l, Schmitz and Moritz (1998); m, A. Buschinger (personal communication); n, Baur, Buschinger, and Zimmermann (1993); o, Douwes et al. (personal communication); p, Chan, Hingle, and Bourke (1999); q, Thompson et al. (2000); r, Duffy, Morrison, and Ríos (2000); s, Jarvis and Bennet (1991); t, Walton, Nedbal, and Honeycutt (2000); u, Faulkes et al. (1997); v, Allard and Honeycutt (1992); w, N. C. Bennet (P. Douwes, B. Stille, and M. Stille, personal communication); x, Bull, Schwarz, and Cooper (2003); y, Hurst (2001); z, Mueller, Eickwort, and Aquadro (1994); +, Savolainen and Vepsäläinen (2003).

available, we extrapolated from related species (for *Lasioglossum*, *Bombus*, *Psithyrus*, and *Heliophobius*) or used data on colony size as a conservative estimate of reproductive output (*Synalpheus*: these estimates will underestimate true reproductive output per sexual female if longevity of reproductive females is higher in the social species). We were unable to obtain estimates of reproductive output for all the *Myrmica* species.

Phylogenetic Analysis

DNA sequences were obtained from GenBank, aligned by eye in Se-Al (Rambaut 1996). Maximum likelihood (ML) branch lengths were calculated under an HKY + Γ model (Hasegawa, Kishino, and Yano 1985; Yang 1993). To estimate parameter values for transition/transversion ratio (ti/tv) and gamma shape parameter (α) for each alignment, an initial topology was estimated from the data using neighbor-joining (HKY + Γ, $\alpha = 0.5$), then branch lengths and parameter values were optimized on this topology by ML (using PAUP*; Swofford 1999). To obtain ML branch lengths for the comparisons, taxa were pruned from the data to remove any nontarget taxa occurring in the in-group; this was done to avoid any bias due to node density effect (Bromham et al. 2002). ML branch lengths were then optimized on the target taxa and out-groups using the parameter values (ti/tv, α) estimated for the whole data set.

Where several genes were available for the same comparison, the genes were concatenated into a multigene alignment and analyzed together (table 2). Because the hypothesis being tested concerns genome-wide rates of substitution, concatenation of sequences is an appropriate analysis of multigene data. Different genes can have different average substitution rates, just as sites within a gene can have different substitution rates (such as the different codon positions of a protein-coding sequence). We allow for variation in rates across all sites in the alignments by incorporating a gamma parameter into the substitution model used and estimating the gamma shape parameter for each alignment, whether single gene or concatenated, using ML.

Concatenated alignments were used for this analysis not only because we are concerned with average genomic substitution rates but also because analyzing genes separately would introduce a number of statistical problems. First, including more than one data point for a given comparison (i.e., one value for each gene) would compromise the phylogenetic independence of data points and would therefore be likely to produce a biased result. Second, branch lengths estimated from single genes would have larger errors because each represents a smaller sample of substitutions. Third, combining single-gene estimates to give an average branch length difference for each comparison would give equal weight to estimates with different errors (particularly, short sequences or slow-changing sequences with few substitutions would be given as much weight as longer sequences or those with more substitutions).

Because Schmitz and Moritz (1998) predicted that the effect of sociality on substitution rate should be stronger for mitochondrial genes than for nuclear sequences, we have analyzed mitochondrial and nuclear sequences separately (where more than one mitochondrial or nuclear sequence was available for a comparison, a concatenated alignment was used; table 2).

We estimated genetic distance separately for synonymous and nonsynonymous substitutions using the program PAML (Yang 2001), available at http://abacus.gene.ucl.ac.uk/software/paml.html. We then estimated branch lengths for each comparison using a relative-rates test, by comparing the distance between the pair to the distance between each member of the pair and an out-group taxon (Sarich and Wilson 1973; Li, Tanimura, and Sharp 1987; Bromham, Rambaut, and Harvey 1996). Although the relative-rates test can be problematic when used as "clock tests" to detect significant levels of rate variation (Scherer 1989; Robinson et al. 1998; Bromham et al. 2000), it remains as a useful tool for comparing rates across lineages (e.g., Bromham, Rambaut, and Harvey 1996; Woolfit and Bromham 2003).

Statistical Analysis

Two statistical tests were performed on the data. First, a sign test was conducted to see if there was a bias toward the social lineage having a faster rate of molecular evolution. If sociality had no influence on rate of molecular evolution, then we would expect that the social lineage would be faster in roughly half the comparisons and slower in roughly half. A significant excess of positive or negative contrasts would indicate that sociality influenced the rate of molecular evolution.

Second, we performed regression analyses using the reproductive data as a continuous measure of degree of sociality. For each pair of taxa in table 1, we calculated contrasts that reflect the difference in sociality and the difference in branch length for each independent comparison. Each contrast was calculated as the difference of the logged values of the species characteristic (i.e., contrast value for degree of sociality as measured by reproductive output, $\text{repr} = \ln(\text{repr}_A/\text{repr}_B)$, contrast for rate of molecular evolution as measured by ML branch lengths, $\text{BL} = \ln(\text{BL}_A/\text{BL}_B)$). Inspection of the distribution of contrast values revealed that the branch length contrasts were approximately normally distributed, but the reproductive output contrasts were strongly right skewed. Consequently, we performed both parametric (linear regressions) and nonparametric (Spearman rank correlations) tests on the contrast, both forced through the origin (see Harvey and Pagel 1991). In addition to reproductive rate, we divided the contrasts into those between a social species and a social parasite (parasite = 1; pairs marked with an asterisk in table 1) and those between social and nonsocial species (parasite = 0). Due to lack of reproductive data, comparisons between species of *Myrmica* (pairs 16–19) could not be included in the regression analysis.

Results and Discussion

A plot of the contrasts for the set of all sequences is given in figure 1. The sign test did not reveal a significant association between rates of molecular evolution and degree of sociality for the set of all sequences or for the mitochondrial or nuclear sequences when analyzed separately. The linear regression analyses did not provide

Table 2
ML Branch Lengths for Comparisons

	All Sequences						Mitochondrial		Nuclear		Nonsynonymous		Synonymous	
Pair	N[a]	L[b]	α[c]	ti/tv[d]	BL_A	BL_B	BL_A	BL_B	BL_A	BL_B	dN_A	dN_B	dS_A	dS_B
1	11	531	0.16	0.99	0.049	0.121	0.049	0.121			0.011	0.028	0.164	0.382
2	17	2,162	0.62	1.05	0.170	0.128	0.146	0.209	0.176	0.090	0.096	0.151	0.302	0.306
3	21	2,742	0.22	2.03	0.062	0.051	0.095	0.105	0.022	0.049	0.004	0.001	0.157	0.096
4	21	2,742	0.22	2.03	0.008	0.021	0.017	0.049	0.001	0.000	0.000	0.000	0.008	0.000
5	21	2,742	0.22	2.03	0.034	0.028	0.074	0.072	0.006	0.006	0.000	0.000	0.032	0.015
6	21	2,742	0.22	2.03	0.026	0.029	0.052	0.072	0.007	0.002	0.000	0.000	0.016	0.008
7	21	2,742	0.22	2.03	0.005	0.013	0.025	0.007	0.002	0.000	0.000	0.000	0.000	0.000
8	21	2,742	0.22	2.03	0.055	0.076	0.131	0.126	0.018	0.049	0.000	0.002	0.175	0.089
9	21	2,742	0.22	2.03	0.010	0.006			0.008	0.004	0.000	0.002	0.041	−0.002
10	21	2,742	0.22	2.03	0.090	0.013			0.071	0.011	0.000	0.000	0.298	0.045
11	21	2,742	0.22	2.03	0.053	0.049			0.037	0.036	0.002	0.000	0.097	0.055
12	30	1,502	0.26	0.74	0.023	0.029	0.028	0.049	0.011	0.000	0.000	0.000	0.041	0.001
13	30	1,502	0.26	0.74	0.048	0.068	0.079	0.106	0.007	0.017	0.000	0.000	0.041	0.069
14	12	599	0.69	0.72	0.079	0.076	0.160	0.040	0.029	0.119				
15	5	1,572	0.01	1.68	0.005	0.009	0.000	0.029	0.007	0.002	0.003	0.000	−0.044	0.154
16	16	2,769	0.24	2.66	0.006	0.011	0.006	0.011			0.000	0.005	0.015	0.026
17	16	2,769	0.24	2.66	0.050	0.095	0.050	0.095			0.006	0.022	0.254	0.216
18	16	2,769	0.24	2.66	0.001	0.000	0.001	0.000			0.000	0.000	0.005	0.002
19	16	2,769	0.24	2.66	0.000	0.001	0.000	0.001			0.000	0.001	0.001	0.004
20	4	1,394	0.58	1.09	0.170	0.126	0.170	0.126			0.048	0.078	0.896	0.638
21	5	1,095	0.24	3.01	0.081	0.096	0.081	0.096			0.008	0.008	0.200	0.757
22	5	1,095	0.24	3.01	0.058	0.163	0.058	0.163			0.012	0.015	0.375	0.778
23	9	519	0.28	2.42	0.016	0.016	0.016	0.016						
24	7	3,118	0.31	2.42	0.158	0.194	0.319	0.315	0.045	0.089	0.063	0.061	−0.464	−0.536
25	7	3,118	0.31	2.42	0.062	0.077	0.096	0.114	0.017	0.026	0.026	0.028	0.469	0.455

[a] Number of species in the phylogeny.
[b] Length of the alignment.
[c] Estimated gamma shape parameter.
[d] Estimated transition/transversion ratio.

any support for a relationship between relative reproductive output and rate of molecular evolution for all sequences (slope = −0.152, *P* value: 0.91), mitochondrial (slope = 0.054, *P* value: 0.76) or nuclear sequences (slope = −0.696, *P* value: 0.58), or when parasite was added as a covariable (slope = 0.99, *P* = 0.66). There were too few comparisons to assess patterns in nonsynonymous rates because the nonsynonymous branch lengths were frequently zero (see table 2). For the synonymous branch lengths, the sign tests showed no increase in rates in social taxa compared to their nonsocial relatives nor did the regression analysis (slope = 0.002, *P* = 0.99). None of the nonparametric Spearman rank correlation tests revealed a significant relationship. Seven out of eight of the comparisons between a social species and a social parasite were negative (all sequences: sign test, *P* = 0.035), indicating that, for these comparisons, the parasites have consistently faster rates of molecular evolution. Because four of the parasites versus social comparisons do not have reproductive data, we are unable to conduct a regression analysis on these comparisons.

The lack of a significant trend across the data suggests that there is no general relationship between sociality and rate of molecular evolution. Furthermore, these results do not support the prediction that the increase in rate of molecular evolution due to sociality will be stronger for mitochondrial genes (Schmitz and Moritz 1998). Separate analysis of mitochondrial and nuclear genes does not reveal any difference in the pattern of rates of social and nonsocial species nor does calculating the ratio of rates in mitochondrial and nuclear genes.

Why do the comparisons used in this study fail to support the predicted increase in rates in social lineages? One possible interpretation is that the effect of N_e on rates of molecular evolution is not as simple as predicted. Although the increase in substitution rates in small populations is one of the central tenets of the nearly neutral theory, there have been relatively few empirical tests of the effect, virtually none of which have used phylogenetically independent comparisons (see Woolfit and Bromham 2003). The population size effect is used to explain the observation of a molecular clock (constant rate of molecular change over time) for amino acid (nonsynonymous) substitutions, despite the apparent generation time effect on the rate of neutral (synonymous) substitutions (Ohta and Kimura 1971): because small-bodied species with rapid generation turnover will tend to have large populations (Chao and Carr 1993), the generation time effect might be counterbalanced by the reduction in drift, leaving a roughly even rate of amino acid substitutions (Ohta 1993). But until the magnitude of the effect of population size on substitution rate is determined, it cannot be known whether it cancels out the generation time effect as neatly as predicted. More empirical tests, comparing substitution rates in taxa that differ consistently in effective population size, are an essential step toward verifying the effect of N_e on the rate of molecular evolution.

Alternatively, it may be that, despite the dramatic reduction in the number of sexual females contributing alleles to each generation, the N_e of social species is not reduced enough to affect their substitution rates. Because many factors could influence the diversity of alleles passed to each generation in a social species, it is difficult to make a direct estimate of N_e. For example, Crozier's (1979) formula for calculating N_e for social hymenopterans includes not only the number of breeding females but also the degree of polyandry (the number of males each queen has mated with) and the number of colonies. In social hymenopterans, male haploidy further reduces effective population size (Crozier 1979). N_e might also be influenced by the relatedness of the queens and drones, the incidence of renegade reproduction by workers, the movement of reproductive individuals between colonies, and the success of queens founding new colonies. Furthermore, the predicted reduction in N_e as a consequence of sociality might be partially countered by the ability to maintain larger populations over many generations, in contrast to solitary species that may suffer repeated population reductions (Crozier 1979). These complicating factors make a quantitative comparison of N_e impractical. More direct measures of N_e would allow the influence of degree of sociality and effective population size to be tested separately.

Sociality could have effects on substitution rate beyond the expected reduction in N_e. For example, the reproduction of social hymenopterans is likely to introduce another change that may influence substitution rates: alteration of the average number of cell divisions in the germ line per generation. Queens of highly eusocial species may live twice as long (or more) as females from related solitary species, and because they start to produce sexual (reproductive) offspring usually only after 1–2 years, there may be a twofold difference in generation time between highly eusocial species and their nonsocial relatives. Oogenesis in Hymenoptera is continuous (Büning 1994), such that the more eggs are produced, the greater the average number of cell divisions taken to produce each egg. This is in contrast to oogenesis in mammals, for example, where all eggs are produced by the same average number of cell divisions, but is more similar to mammalian spermatogenesis, where mutation rate increases with paternal age due to the increasing number of cell divisions in the germ line (Crow 1997). So if a social hymenopteran female produces a large number of workers before laying eggs that become reproductive offspring, then the average number of cell divisions taken to produce sexual offspring would be much higher than in nonsocial species. In this case, for a given rate of DNA copy error, the sexual offspring of the social female will have a much higher chance of inheriting mutations than the sexual offspring of a nonsocial female. This effect could be compounded by the much higher fecundity of social females: a single queen can produce hundreds of sexual offspring (Crozier and Pamilo 1996; Bourke 1997). Compared to species that produce reproductive offspring directly, social hymenopteran species may have many more germ-line DNA replications per reproductive generation and, therefore, might be expected to accumulate more mutations per generation through DNA copy errors.

The effect of number of DNA replications on substitution rate is supported by the observation of "male-driven evolution": in mammals, genes that spend more time in males appear to accumulate more DNA copy errors, because it takes more cell divisions to make sperm than

eggs (Shimmin, Chang, and Li 1993; Chang et al. 1994), a result supported by results from birds where the females are of heterogametic sex (Ellegren and Fridolfsson 1997). This copy error effect may also underlie the generation time effect observed for some vertebrate DNA sequences: species with shorter generation turnover times are assumed to have more DNA replications per unit time and will therefore accumulate more replication errors (Ohta 1993; Mooers and Harvey 1994; Bromham, Rambaut, and Harvey 1996; Li et al. 1996; Bromham 2002). However, while production of more eggs is expected to increase the average number of germ-line replications in Hymenoptera, this is not necessarily the case for all taxa—for example, because oogenesis in mammals is not continuous, the number of offspring produced by a social female (such as naked mole rats—see table 1) would not be expected to increase the average number of cell divisions. In addition, while there is strong support for a generation time effect in tetrapod vertebrates (Mooers and Harvey 1994; Bromham, Rambaut, and Harvey 1996; Bromham 2002), it is not yet known if this pattern holds true for invertebrate taxa, which make up most of the comparisons in this study.

Figure 1 reveals two patterns that may be worthy of further investigation. First, the deeper contrasts, those where the social lineage and its nonsocial relative are from different tribes, subfamilies, or families, are all positive. There are several possible explanations of this pattern. First, it may be an ascertainment bias: only for deep contrasts has there been sufficient time for the accumulation of a significantly different number of substitutions in the social and nonsocial lineages. However, branch length is a product of both time and rate of molecular evolution, and rate varies between genes. The phylogenetic studies from which the sequences were taken will have targeted genes with an appropriate rate of molecular evolution to give a sufficient number of genetic differences. So although the average number of substitutions per site for the deeper comparisons is greater than the average for all comparisons, there are many "shallow" comparisons which have longer branch lengths but are not consistently positive, for example, the comparisons between shrimp species or naked mole rat genera (table 2). Second, these contrasts have the greatest difference in reproductive output between highly eusocial and nonsocial females. For example, a honeybee queen (*Apis mellifera*) has five orders of magnitude more offspring than a solitary *Anthophora* female, and termites (*Microhodotermes*), vespid wasps (*Vespula*), and *Tetragona* bees have greater than a thousand times more offspring than their nonsocial relatives. So it may be that extreme sociality can bias rates, but the effect is not apparent for the majority of social taxa. Note that this group of deep, highly eusocial, positive contrasts includes the two social lineages, honeybees and vespid wasps, included in the analysis of Schmitz and Moritz (1998), which accounts for their support for a positive relationship between sociality and rate of molecular evolution. This highlights the importance of including many phylogenetically independent contrasts if a general conclusion about determinants of rate variation is to be made.

The other group of comparisons that may reveal a pattern for further investigation is the eight contrasts between a social species and a social parasite (marked with an asterisk

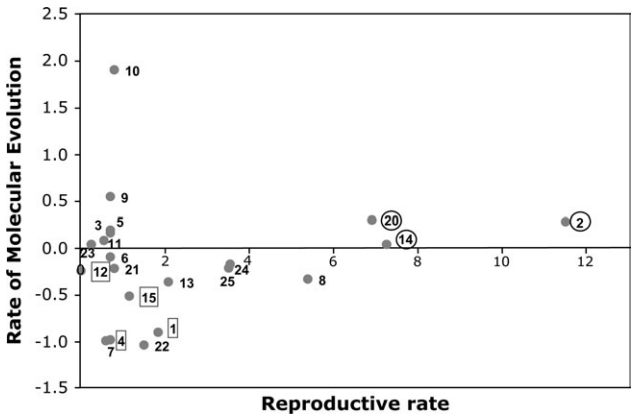

FIG. 1.—Ratio of logged branch lengths plotted against ratio of logged reproductive outputs for the contrasts (see tables 1 and 2). A positive ratio indicates that the social species has the longer branch. Contrasts are identified by pair number. Deep comparisons (those between tribes and families) are circled. Contrasts between a social species and a social parasite are marked with a square. Regression analyses on these data do not support a significant relationship between relative reproductive output and rate of molecular evolution (slope = −0.152, *P* value: 0.91). Regression with parasite added as a covariable was also not significant (slope = 0.99, *P* value: 0.66).

in table 1). Seven of the social parasites included in this study have a faster rate of molecular evolution than the related social lineage (see table 2). There are two key biological differences between the social parasites and their social relatives which might influence molecular rates. Rather than establishing their own colonies, the parasitic females enter a colony of the host species, kill the queen (or coexist with her), and use the colony to produce their own sexual offspring. Because they rely on a host colony to reproduce, social parasites might be expected to maintain a lower N_e than their social hosts (though this may not be true for facultative parasitic species). If this is true, then social parasites might be expected to have a faster rate of nearly neutral substitutions than their closely related social hosts. Social parasites may also have a lower average number of genome replications per generation if they produce only sexual offspring without first producing a large number of sterile workers, so they may acquire fewer DNA copy errors per unit time, reducing the mutation rate in these lineages. This effect might be evident in a lower number of synonymous mutations in social parasite lineages.

Therefore, it may be possible to use social parasite species to distinguish between two alternative hypotheses—population size or DNA copy number effect—for faster rates of molecular evolution in social lineages. Thus, we have two different predictions for the rate of molecular evolution in social parasites: (1) if N_e is the predominant influence on rates of molecular evolution, then social parasites that have a lower N_e than their hosts should have a faster rate of molecular evolution; (2) if the DNA copy error effect (a.k.a. generation time effect) is the predominant influence, then the social parasites that do not produce nonreproductive workers before their sexual offspring should have lower rates (fewer DNA copy errors per unit time). We only have eight comparisons between social species and their nonsocial parasites, so we are unable to test this prediction rigorously. However, our data show that most of the social

parasites have faster rates than their social relatives, which is consistent with an effect of reduced population size. If more sequence data for social parasites become available, this pattern could be tested more thoroughly.

Potentially, examining synonymous and nonsynonymous substitutions could separate the effects of population size and DNA copy frequency. Because generation time effect is predicted to increase the mutation rate by increasing the rate of accumulation of DNA copy errors, it is expected to be reflected primarily in the synonymous substitution rate. Population size, by influencing the relative strength of selection and drift, should affect all substitutions, both synonymous and nonsynonymous. We found no increase in the rate of synonymous substitution rate in social lineages, but there was insufficient data to reliably test the nonsynonymous substitution rate because for most of the comparisons the nonsynonymous genetic distance was zero. In any case, this test is not as clear-cut as predicted from theory because the generation time effect has been noted for nonsynonymous changes (and total genetic distance) for vertebrates (Martin and Palumbi 1993; Mooers and Harvey 1994; Bromham 2002). Furthermore, if synonymous codon usage is not random but is biased toward particular codons, then synonymous substitutions may behave as nearly neutral mutations and thus be affected by population size (Akashi 1997). Ideally, comparisons between taxa that differ in population size but do not vary in average number of DNA replications per unit time would help to distinguish these two hypotheses. Unfortunately, there is insufficient life-history data available for the taxa used in this study to allow direct assessment of the effect of generation time (and covarying traits such as body size and metabolic rate: see Martin and Palumbi 1993; Mooers and Harvey 1994; Bromham, Rambaut, and Harvey 1996). Similarly, there are insufficient sequence data currently available to test for differences in synonymous codon usage between social and nonsocial lineages (the sample of codons is too small, given that only half of the available sequences are protein coding and most are less than a kilobase in length). Increase in available data may make these analyses possible in future.

Elucidating the influence of social structure, reproductive dynamics, and other population processes on rates of molecular evolution is essential to the use of DNA data as a record of evolutionary history, particularly for the reliability of molecular clock studies. If rates of molecular evolution are influenced by species characteristics such as sociality, then we must expect substitution rates to evolve along phylogenies as species evolve, and this complicates the use of branch length from molecular phylogenies to predict lineage divergence times (Bromham and Hendy 2000). If the effect of population size does not counterbalance the DNA copy (generation time) effect as simply as predicted, the result would be residual variation in substitution rate between lineages (Gillespie 1991). This would be consistent with empirical evidence for an effect of life history on substitution rate for some gene sequences for a range of vertebrate taxa (Martin and Palumbi 1993; Mooers and Harvey 1994; Bromham, Rambaut, and Harvey 1996; Bromham 2002). The upshot of the observed variation in rates of molecular evolution between lineages is that the molecular clock is expected to run at different rates in even closely related species, making inference of divergence times from molecular data problematic and prone to systematic errors (Bromham et al. 2000). It is therefore critical that the effect of population dynamics on the rate of molecular evolution is closely examined and that empirical evidence is sought for the predictions of molecular evolutionary theory.

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online (www.mbe.oupjournals.org).

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