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Sociality and the Rate of rDNA Sequence Evolution in Wasps (Vespidae) and Honeybees (*Apis*)

J. Schmitz,[1] R.F.A. Moritz[2]

[1] Deutsches Primatenzentrum GmbH, Abteilung Primatengenetik, Kellnerweg 4, D-37077 Göttingen, Germany
[2] Institut für Zoologie, Martin-Luther-Universität Halle/Wittenberg, Kröllwitzer Str. 44, D-06099 Halle/Saale, Germany

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Abstract. Sequence data of mitochondrial 16S ribosomal DNA (mt-rDNA) and nuclear 28S ribosomal DNA (nuc-rDNA) were compared in two honeybee species (*Apis mellifera* and *Apis dorsata*) and a selection of 22 wasp species (Vespidae) with different levels of sociality. The averge substitution rates in mt-rDNA and nuc-rDNA were almost-equal in solitary species. In species with larger nests, however, the difference between the nuclear and the mitochondrial substitution rate significantly increased. The average substitution ratio, ψ (nucleotide substitutions in mt-rDNA/nucleotide substitutions in nuc-rDNA) was 1.48 ± 0.12 (SE) among the solitary Eumeninae, 3.70 ± 0.15 among five primitive social Stenogastrinae species, 3.24 ± 0.20 among five Polistinae species, 5.76 ± 0.33 among nine highly eusocial Vespinae, and 12.7 in the two *Apis* species. The high egg-laying rate and the effective population size skew between the sexes may contribute to the rise of the substitution ratio in the highly eusocial species. Drift and bottleneck effects in the mitochondrial DNA pool during speciation events as well as polyandry may further enhance this phenomenon.

Key words: Vespidae — *Apis* — Mitochondrial DNA — Nuclear DNA

Correspondence to: J. Schmitz; *e-mail:* jschmitz@www.dpz.gwdg.de

Introduction

Although there are plausible theories on the evolution of sociality in Hymenoptera (Breed and Page 1989; Crozier and Pamilo 1996), the consequences of social life for natural selection and evolutionary processes are less well understood. By definition, sociality clusters individuals into groups and causes characteristic patchy patterns in populations, typically called nests or colonies. Thus sociality causes dramatic changes in the population structure which are bound to have an impact on mechanisms of natural selection and evolution. In contrast to populations with solitary organisms, natural selection in social populations will operate on both the individual (outside and within the social group) and the society itself (Moritz and Southwick 1992). Moreover, there is extremely reproductive division of labor, with a single or few females (queens) producing extremely large numbers of eggs, whereas the infertile workers usually produce no offspring. This may have consequences for the inheritance of mitochondrial DNA. If mitochondrial DNA is inherited exclusively through the maternal lineage in social Hymenoptera [as shown for *Apis mellifera* (Meusel and Moritz 1993)], this will lead to a bias between the effective size of the nuclear and that of the mitochondrial DNA pool. This bias is particularly large with highly polyandrous mating systems, which are often found in social Hymenoptera. For example, queens of the giant honeybee (*Apis dorsata*) have been shown to mate with an average of more than 30 males (Moritz et al. 1995; Oldroyd et al. 1996). Thus, although the effective population size for nuclear genes is larger with multiple paternity than with single paternity (Sugg and Chesser

Table 1. Tajima and Nei (1984) distance of mitochondrial 16S rDNA (upper right) and nuclear 28S rDNA (lower left)[a]

	A. mellifera	*A. dorsata*	*V. crabro*	*V. tropica*	*P. anomala*	*P. nocturna*	*D. maculata*	*D. sylvestris*	*D. saxonica*	*V. vulgaris*	*V. germanica*	*P. dominulus*	*P. saggittarius*	*P. flavus*	*B. petiolata*
A. mellifera		0.080													
A. dorsata	0.006														
V. crabro				0.065	0.116	0.099	0.113	0.121	0.136	0.147	0.117				
V. tropica			0.007		0.132	0.119	0.151	0.177	0.175	0.175	0.139				
P. anomala			0.014	0.021		0.035	0.169	0.146	0.178	0.180	0.143				
P. nocturna			0.021	0.028	0.007		0.155	0.150	0.178	0.179	0.156				
D. maculata			0.021	0.029	0.021	0.029		0.111	0.134	0.147	0.142				
D. sylvestris			0.018	0.025	0.018	0.025	0.011		0.107	0.166	0.117				
D. saxonica			0.018	0.025	0.018	0.025	0.011	0.000		0.153	0.126				
V. vulgaris			0.032	0.040	0.032	0.040	0.040	0.036	0.036		0.085				
V. germanica			0.029	0.036	0.029	0.036	0.036	0.032	0.032	0.004					
P. dominulus													0.145	0.147	0.187
P. saggittarius												0.025		0.087	0.174
P. flavus												0.028	0.025		0.156
B. petiolata												0.062	0.058	0.070	
B. somereni												0.062	0.058	0.070	0.007
P. mellyi															
P. alternata															
L. vechti															
L. flavolineata															
E. fraterna															
E. sp.															
A. oviventris															
A. nigricornis															

[a] Typical nest sizes and the substitution ratio are listed. ψ_1 values, average distance data; ψ_2 values, branch length comparison. Ancestral distances correspond to the lengths of the terminal branches.

1994), the effective size for mitochondrial genes is not affected by this mechanism and remains constrained by the number of reproducing females. Because random drift is less effective in large populations (Ohta 1976; DeSalle and Templeton 1988), the polyandrous system maintains genetic diversity in populations, which in turn reduces the rate of genetic divergence between two separated populations during molecular evolution.

Mitochondrial genomes should thus diverge more quickly in social insets than nuclear genomes. The potential significance of sociality for molecular evolution was discussed by Crozier et al. (1989). He pointed out that the effective population size is rather low in social insects compared to *Drosophila* species, which might have consequences for evolutionary processes. Indeed, Crozier et al. (1989) found that the rate of nucleotide substitutions in the mitochondrial geneome of *Apis mellifera* was much higher than in *Drosophila,* matching the above predictions. However, as mentioned by Crozier et al. (1989), the two analyzed lineages have had extremely different life patterns in their evolutionary history and factors other than the differences in effective population sizes may have been the primary forces for their observation. Consequently, it seems rewarding to study more closely related taxa, which yet display the full range of sociality. The wasps in the family Vespidae are a group of Hymenoptera with morphologically, physiologically, and behaviorally extremely different taxa (Ross and Matthews 1991). They comprise solitary species in the univoltine Eumeninae as well as eusocial species with long-lived queens, colony fission, swarming, and colony sizes of up to 1.3 million adults in *Agelaia vicina* (Jeanne 1991). Therefore, the Vespidae form an ideal taxonomic group to study the impact of sociality on molecular evolutionary rates.

Here we analyze DNA sequences in a set of solitary and social Vespidae and two *Apis* species to determine the average substitution ratio, ψ, between mitochondrial nucleotide substitutions and nuclear substitutions represented in ribosomal DNA. A regression analysis of nest size (as a measure of the egg-laying capacity of the queens) versus ψ will indicate whether life in social groups can have impacts on evolutionary processes on the molecular level.

Materials and Methods

Samples. The samples were collected on several field trips in Germany (*Apis mellifera, Vespa crabro, Dolichovespula sylvestris, D. saxonica, Vespula germanica, V. vulgaris, Ancistrocerus oviventris, A. nigricornis*), Greece (*Polistes dominulus*), Malaysia (*Apis dorsata, Vespa tropica, Provespa anomala, P. nocturna, Polistes saggittarius,*

Table 1. Continued

	B. somereni	*P. mellyi*	*P. alternata*	*L. vechti*	*L. flavolineata*	*E. fraterna*	*E.* sp.	*A. oviventris*	*A. nigricornis*	Cells per nest	ψ_1	Ancestral distance: 16S rDNA	Ancestral distance: 28S rDNA	ψ_2
A. mellifera										25,000	12.70	0.0274	0.0031	8.84
A. dorsata										65,000	12.70	0.0490	0.0031	15.81
V. crabro										4,563	5.71	0.0171	*0.0035	4.89
V. tropica										3,130	5.39	0.0457	0.0070	6.53
P. anomala										2,500	6.88	0.0205	*0.0035	5.86
P. nocturna										4,946	5.09	0.0136	0.0070	1.94
D. maculata										1,500	5.71	0.0563	0.0070	8.04
D. sylvestris										940	6.69	0.0418	*0.0035	11.94
D. saxonica										1,521	7.24	0.0601	*0.0035	17.17
V. vulgaris										15,383	4.77	0.0536	0.0035	15.31
V.germanica										14,181	4.40	0.0278	*0.0035	7.94
P. dominulus	0.190									200	3.78	0.0693	0.0104	6.66
P. saggittarius	0.202									200	3.67	0.0382	0.0104	3.67
P. flavus	0.164									64	2.86	0.0555	0.0139	3.99
B. petiolata	0.046									100	2.85	0.0102	0.0034	3.00
B. somereni										128	3.05	0.0309	0.0034	9.09
P. mellyi			0.177	0.269	0.255	0.306				35	3.67	0.0789	0.0170	4.64
P. alternata		0.039		0.221	0.211	0.285				24	3.44	0.0749	0.0208	3.6
L. vechti		0.072	0.725		0.143	0.266				27	3.92	0.0762	0.0208	3.66
L. flavolineata		0.076	0.072	0.035		0.249				89	3.87	0.0645	0.0137	4.71
E. fraterna		0.088	0.077	0.050	0.039					9	4.38	0.0998	0.0207	4.82
E. sp.								0.212	0.217	4	1.24	0.0537	0.1194	0.45
A. oviventris							0.170		0.095	4	1.61	0.0345	0.0070	4.93
A. nigricornis							0.175	0.021		4	1.59	0.0562	0.0142	3.96

Parischnogaster alternata, P. mellyi, Liostenogaster flavolineata, L. vechti, Eustenogaster fraterna, Eumenes sp.), South Africa (*Belonogaster petiolata, B. somereni*), and the United States (*Dolichovespula maculata, Polistes flavus*). At least four individuals per species were stored in ethanol until further processing of the DNA. The nest sizes (number of cells) were obtained from the following sources: Ruttner (1988) for *Apis;* Matsuura and Yamane (1990) for *V. crabro, V. tropica, Provespa, Dolichovespula saxonica,* and *D. maculata;* Edwards (1980) for *Dolichovespula sylvestris, Vespula vulgaris,* and *V. germanica;* Ohgushi et al. (1983, 1986) for Stenogastrinae; Richards (1978) for *Polistes flavus;* Keeping and Crewe (1983) for *Belonogaster* and our own observations for other Polistinae species; and Blüthgen (1961) for Eumeninae.

DNA Analysis. DNA was extracted by routine techniques (Sambrook et al. 1989). The 5′ end of 28S nuc-rDNA [nucleotide positions 448 to 735, corresponding to the *Vespa crabro* sequence of Schmitz and Moritz (1994)] and the 5′ end of mitochondrial 16S rDNA [corresponding to positions 13,543 to 13,841 of the *Apis mellifera ligustica* mitochondrial genome (Crozier and Crozier 1993)] were amplified using the following primer pairs: for nuclear ribosomal DNA (nuc-rDNA), 5′-AAAGATCGAATGGGGAGATTC-3′ and 5′-CACCGGGTCCGTACCTCC-3′; and for mitochondrial ribosomal DNA (mt-rDNA), 5′-TTGACTGTACAAAGGTAGC-3′ and 5′-GATATTACGCTGTTATCCC-3′.

The PCR conditions were as follows: initial, 94°C for 3 min (denaturation); cycles, 30 times at 94°C for 30 s (denaturation), 55°C for 30 s (nuc-rDNA annealing) or 50°C for 30 s (mt-rDNA annealing), and 72°C for 30 s (polymerization). An elongation of PCR products by 72°C for 3 min completed the reaction. We used about 10 ng template DNA, 400 n*M* primer, 1.25 m*M* dNTPs, 1.5 m*M* $MgCl_2$ 10× reaction buffer, and 5 U/100 μl Taq polymerase.

The PCR products were purified, inserted in the pUC19-*Sma*I site, and cloned in *E. coli* DH5α. Sequence analysis was done with the Sequenase kit (U.S. Biochemical) using α-^{35}S-dATP for DNA labeling. The sequence reactions were electrophoresed in 7% acrylamide, 7 *M* urea gels and visualized by autoradiography. Sequences were multiply aligned using the Clustal V algorithm of Higgins and Sharp (1989).

Results

The sequence data were deposited in the EMBL data bank and can be retrieved under no. AF066893–AF066939 and AF067145. The sequences of the 28S rDNA and 16S rDNA were multiply aligned only within each subfamily. This avoided difficulties in the alignments due to insufficient homology. We determined the number of substitutions of a given species compared to each of the other species in the subfamily. The substitution frequency per nucleotide, *d*, was estimated using the method of Tajima and Nei (1984) for both nuclear and mitochondrial DNA (Table 1). We calculated the average substitution ratio, ψ_1, between mitochondrial and nuclear substitutions as follows:

$$\psi_{1_i} = \frac{\sum_{i \neq j}^{n} a_{ij}}{\sum_{i \neq j}^{n} b_{ij}}$$

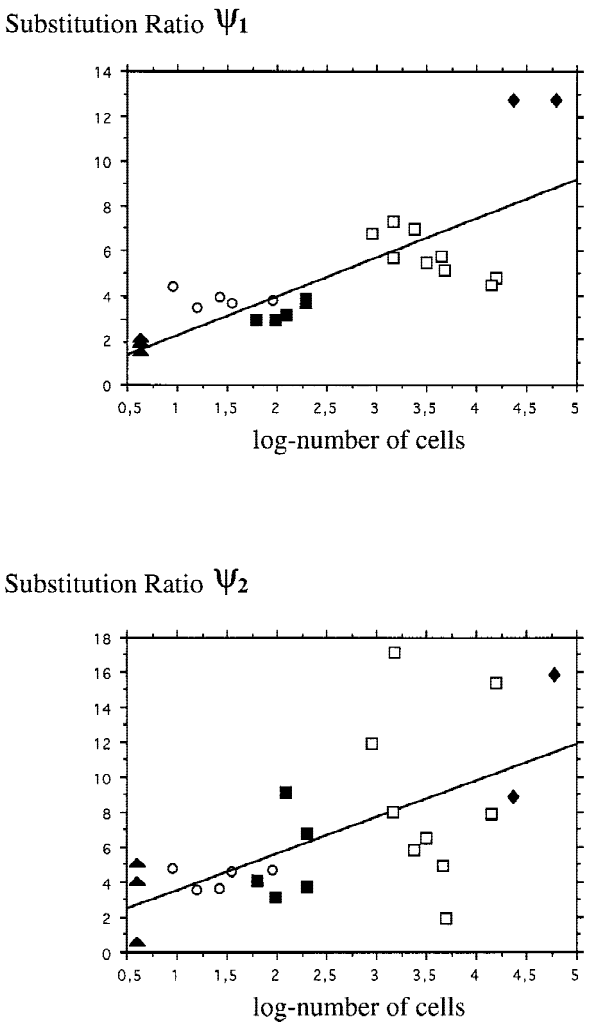

Fig. 1. Plot of the substitution ratio versus the nest size of various Vespidae and two *Apis* species. Species belonging to the same subfamily are marked with the same symbol. (♦) Apinae, (□) Vespinae, (■) Polistinae, (○) Stenogastrinae, and (▲) Eumeninae. **Top:** ψ_1 values (average distance data). **Bottom:** ψ_2 values (branch length comparison) (see Table 1).

where a_{ij} is the Tajima and Nei distance, *d,* in the mt-rDNA between species *i* and species *j;* b_{ij} is the Tajima and Nei distance, *d,* in the nuc-rDNA between species *i* and species *j;* and *n* is the number of species analyzed in the subfamily.

Irrespective of the absolute number of substitutions and the actual phylogenetic distances among the species within a subfamily, we obtained a measure for differential substitution rates in the mitochondrial and nuclear genomes. The solitary Eumeninae have ψ values close to 1, with an average of 1.48 ± 0.12 (SE). The primitive social Stenogastrinae have an average ψ_1 value of 3.70 ± 0.15, Polistinae wasps an average of 3.24 ± 0.20, the eusocial Vespinae an average of 5.76 ± 0.33, and the two honeybee speices of ψ_1 value of 12.7 (Fig. 1, top).

The analysis suffers from the lack of independence of the data points resulting from averaging the ψ_1 values within each subfamily. To avoid this problem, we chose an alternative statistical analysis: using the branch and bound search of the PAUP package (Swofford 1991), we developed a parsimony tree based on the combined data sets of mt-rDNA and nuc-rDNA for each subfamily. The resulting topologies were taken as user-defined trees to reconstruct phylogenetic trees from the separate data sets.

The hypothetical sequence of the nearest ancestor to a terminal taxon was determined by the PAUP option ''states for interior nodes,'' and the Tajima and Nei (1984) distance was calculated. These distances (a_i for mt and b_i for nuclear DNA), which reflect the terminal branch lengths, were used to calculate ψ_2 separately for each species *i.*

$$\psi_{2_i} = \frac{a_i}{b_i}$$

where a_i is the Tajima and Nei distance of species *i* to a hypothetical ancestor for mt-rDNA and b_i is the Tajima and Nei distance of species *i* to a hypothetical ancestor for nuc-rDNA.

In some eusocial wasps (labeled with an asterisk in Table 1) we found no substitutions in nuc-rDNA between the hypothetical ancestor and the species in question. In these cases we made the assumption that one more sequenced nucleotide would have yielded a substitution to avoid a division by zero. Hereby we overestimated the nuclear substitution rate. Thus we underestimated ψ_2 to various extents in the Vespinae, which may have weakened the observed correlation between nest size and ψ_2. Since this technique provides only one data point per species, the variance is much larger than in the first procedure. Nevertheless, after plotting these independent substitution ratios versus nest sizes (Fig. 1, bottom), we found that, with increasing nest size, ψ_2 also increases. In spite of the large variance, we obtained a highly significant correlation between ψ and nest size ($r^2 = 0.61$, $P < 0.0001$, for the average distance data and $r^2 = 0.38$, $P < 0.002$, for branch length comparison). This value gains in significance in the light of the very roughly estimated colony sizes, which were obtained from various literature sources (see Fig. 1) and might have distorted the observed correlation.

Discussion

Our results clearly indicate an increase in the substitution ratio ψ with increasing nest size. Data available for other species show that substitution ratios between mitochondrial 16S rDNA and nuclear 28S rDNA are usually close to unity. For example, a multiple sequence alignment among three species of mammals [*Mus musculus, Rattus* sp., and *Homo sapiens* (Hassouna et al. 1984; Gutell and Fox 1988; Gonzales et al. 1990)] leads to ψ values of 0.83, 0.79, and 0.7, respectively, similar to those ob-

Table 2. Mean genetic distances (Tajima and Nei 1984) between the investigated subfamilies for mitochondrial 16S rDNA (upper right) and nuclear 28S rDNA (lower left)

	Apinae	Vespinae	Polistinae	Stenogastrinae	Eumeninae
Apinae		**0.353**	0.462	**0.493**	**0.344**
Vespinae	**0.297**		0.270	**0.426**	**0.268**
Polistinae	0.348	0.123		0.449	0.284
Stenogastrinae	**0.253**	**0.286**	0.363		**0.379**
Eumeninae	**0.305**	**0.143**	0.204	**0.290**	

[a] Boldface numbers are used in the relative rate test in Fig. 2.

tained for the solitary Eumeninae wasps in our study. The ψ values are obviously higher for social Vespinae and the two *Apis* species than for more primitively social and solitary species. The significant correlation of ψ and nest size holds also after omitting the bees from the analysis in the distance comparison ($r^2 = 0.58$, $P < 0.0001$) and remains significant by consulting the branch length comparison ($r^2 = 0.28$, $P < 0.02$). The branch length comparison of terminal taxa is based on autapomorphic characters which results in a severe reduction of the data set. This explains the extensive variation of the ψ_2 values (see Fig. 1, bottom) and the weaker correlation.

What are the potential reasons for the increasing substitution ratios? The investigated taxa are phylogenetically relatively closely related. Therefore, there are no reasons to assume principal differences in evolutionary mechanisms (repair efficiency, hitchhiking effects, concerted evolution of nuc-rDNA, AT content etc.) forcing a substitution bias with increasing social complexity. High substitution ratios can result either from a high nucleotide substitution rate in the mitochondrial genome or from a low rate in the nuclear genome. We calculated a relative rate test (Avise et al. 1994) using the mean genetic distances of the members of each subfamily (Table 2). Both effects (increasing of substitutions in mt-DNA and decreasing of substitutions in nuc-DNA with increasing social complexity) may be involved to a varying extent, depending on which three-subfamily combination was used (Fig. 2). Since the relative rate test requires comparison of the DNA sequences between the subfamilies, sequence similarities for mitochondrial DNA were lower than 70% in several cases. Therefore, the results of the tree taxon test are of only limited value. The comparisons of the within-subfamily parameters ψ are not prone to this problem.

Enhanced Substitution Rate in mt-DNA with Increased Sociality

At first glance it may seem counterintuitive to expect any impact on the substitution rate in the mitochondrial genome as a consequence of sociality. However, Crozier et al. (1989) argued that the effective population size in social bees is reduced compared to solitary insects. If this holds true in a graduated manner for different levels of sociality, we expect an accelerated rate of evolution for the per se reduced mitochondrial gene pool compared to the larger nuclear gene pool. Wilson et al. (1985) demonstrated very strikingly the different effects of the reduction of population sizes for mitochondrial and nuclear DNA, respectively. Furthermore, Hale and Singh (1991) argued that small population sizes in combination with low levels of migration could affect nuclear DNA and mitochondrial DNA variability differently.

The number of offspring produced by a social reproductive dominant female is much larger than that by a solitary female. In general, a queen of a large nest will produce thousands of eggs which develop into infertile workers before a new female reproductive (gyne) is reared. The mitochondrial haplotype is prone to drift during each cell division in the female germ line. Potential heteroplasmy is lost rapidly until it reaches the drift-mutation equilibrium. If speciation events coincide with strong bottlenecks, the equilibrium is distorted and it requires many generations to reestablish the new equilibrium frequency of heteroplasmic individuals (Wade et al. 1994). At equilibrium ancestral alleles are lost due to mutation alone and no longer through drift effects. This drift-mutation equilibrium for mitochondrial alleles in a matrilineage can be reached in the social species within a single generation. The same process can take thousands of generations in a solitary species with a low egg-laying rate. As a consequence, the loss of ancestral haplotypes due to drift should be less pronounced in solitary but more severe in the social species.

Reduced Substitution Rate in nuc-DNA with Increased Sociality

Polyandry counteracts the effects of genetic drift by increasing the effective population size. This favors maintenance of ancestral genomes in diverging populations and thus reduces genetic distances between these populations. Cole (1983) found that polyandry in social Hymenoptera increases with increasing nest size. This hypothesis may hold true for wasps, and polyandry has been shown to be high in Vespinae wasps. Ross (1986) and Ross and Carpenter (1991) found effective male numbers of 3.3 and 7.1 for *Vespula squamosa* and *V. maculiformis*, respectively. The degree of polyandry

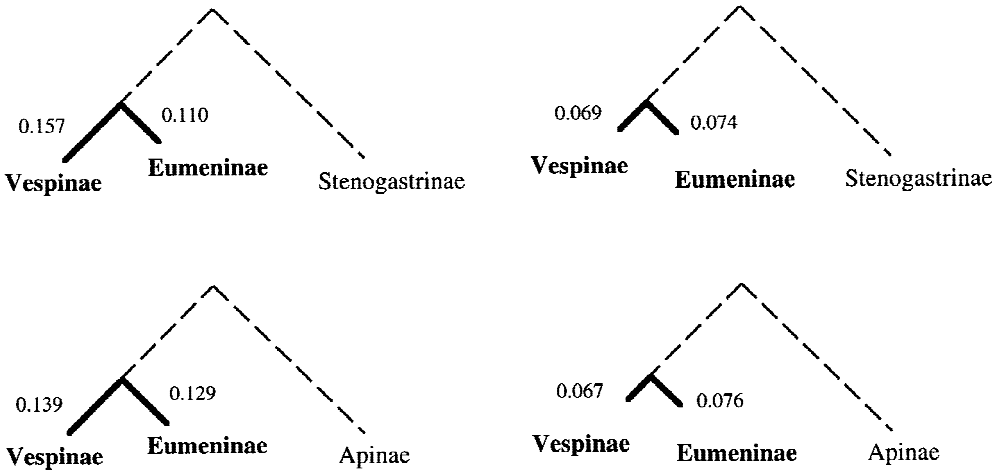

Fig. 2. Relative rate test of Vespinae and Eumeninae. *Left trees* correspond to mitochondrial 16S rDNA; *right trees* correspond to nuclear 28S rDNA. The outgroups were chosen according to Schmitz and Moritz (1998). The inferred branch lengths (Tajima–Nei distances) are shown.

seems to be less for Politinae wasps. Metcalf (1980) found only single- or double-mated queens in *Polistes variatus* and *P. metricus.* Muralidharan et al. (1986) reported on single or a low degree of multiple mating in primitively eusocial *Ropalidia marginata.* We are unaware of any data on multiple mating for the Stenogastrinae and Eumeninae, but in light of Cole's (1983) observations, it would be surprising to find a high degree of polyandry in these species. An extremely high degree of polyandry has been reported for the two *Apis* species (Estoup et al. 1994; Moritz et al. 1995, Oldroyd et al. 1996, 1997), with average effective male numbers between 17 for *A. mellifera* and 23 for *A. dorsata.* This supports the significance of polyandry for the different substitution rates in nuclear and mitochondrial DNA.

Given that the above theoretical considerations are true, the correlation between the substitution ratio and the nest size should hold also within the subfamilies. However, this analysis is hampered by the current database. Most nest size estimates stem from singular observations. Yet nest size depends strongly on ecological constraints and life history conditions of the colonies. Potential effects at the evolutionary level are therefore difficult to detect. As long as we have little information on mean and variance of nest sizes for most species, the data do not sensibly allow for an analysis at this fine scale.

We conclude that life in social groups with reproductive hierarchies seems to have a differential effect on the molecular evolutionary rates at the mitochondrial and nuclear DNA level. Although we have presented some potential mechanisms, it must remain open how this is achieved and which genetic and evolutionary processes ultimately determine these effects. At the present stage we will not exclude either of the two outlined possibilities for the increased substitution ratio in social wasps. However, both mechanisms are nonexclusive and they seem to operate easily synergistically, the one increasing the substitution rate in mitochondria and the other reducing the substitution rate in the nuclear genome. Further studies, e.g., determining the degree of polyandry in social wasps using microsatellite variability like those already published for honeybees (Estoup et al. 1994; Moritz et al. 1995), may contribute further to clearing the issue. Comparative analyses, studying nest size in detail and further potentially relevant parameters such as population sizes and dispersal rates of males and females, should further improve our understanding of the impact of sociality on evolution at the molecular level.

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References

Avise JC, Nelson WS, Sugita H (1994) A speciation history of ''living fossils'': Molecular evolutionary patterns in horseshoe crabs. Evolution 48:1986–2001

Blüthgen P (1961) Die Faltenwespen Mitteleuropas (Hymenoptera, Diploptera). Akademie-Verlag, Berlin

Breed MD, Page RE (1989) The genetics of social evolution. Westview Press, Boulder, CO

Cole BJ (1983) Multiple mating and the evolution of social behavior in the Hymenoptera. Behav Ecol Sociobiol 12:191–201

Crozier RH, Crozier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera:* Complete sequence and genome organization. Genetics 133:97–117

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Sex allocation and kin selection. Oxford University Press, Oxford

Crozier RH, Crozier YC, Mackinlay AG (1989) The CO-I and CO-II region of honeybee mitochondrial DNA: Evidence for variation in insect mitochondrial evolutionary rates. Mol Biol Evol 6:399–411

DeSalle R, Templeton AR (1988) Founder effects and the rate of mitochondrial DNA evolution in Hawaiian *Drosophila*. Evolution 42: 1076–1084

Edwards R (1980) Social wasps, their biology and control. Rentokil, East Grinstead, UK

Estoup A, Solignac M, Cornuet JM (1994) Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. Proc R Soc Lond B 258:1–7

Gonzales IL, Sylvester JE, Smith TF, Stambolian D, Smickel RD (1990) Ribosomal RNA gene sequence and hominoid phylogeny. Mol Biol Evol 7:203–219

Gutell RR, Fox GE (1988) A compilation of the large subunit RNA sequences presented in a structural format. Nucleic Acids Res 16: 175–313

Hale RL, Singh RS (1991) Contrasting patterns of genetic structure and evolutionary history as revealed by mitochondrial DNA and nuclear gene-enzyme variation between *Drosophila melanogaster* and *Drosophila simulans.* J Genet 70:79–90

Hassouna N, Michot B, Bachellerie J-P (1984) The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Res 12:3563–3583

Higgins DG, Sharp PM (1989) Fast and sensitive multiple sequence alignment on a microcomputer. CABIOS 5:151–153

Jeanne RL (1991) The swarm founding Polistinae. In: Ross KG, Matthews RW (eds) The social biology of wasps. Cornell University Press, Ithaca, NY, pp 191–231

Keeping MG, Crewe RM (1983) Parasitoids, commensals and colony size in nests of Belonogaster (Hymenoptera: Vespidae). J Entomol Soc S Afr 46:309–323

Matsuura M, Yamane S (1990) Biology of the vespine wasps. Springer-Verlag, Berlin

Metcalf RA (1980) Sex ratios, parent-offspring conflict, and local competition for mates in the social wasps *Polistes metricus* and *Polistes variatus.* Am Nat 116:642–654

Meusel MS, Moritz RFA (1993) Transfer of paternal mitochondrial DNA during fertilization of the egg of honeybees, *Apis mellifera* L. Curr Genet 24:539–543

Moritz RFA, Southwick EE (1992) Bees as superorganisms. Springer-Verlag, Berlin

Moritz RFA, Kryger P, Koeniger G, Koeniger N, Estoup A, Tingek S (1995) High degree of polyandry in *Apis dorsata* queens detected by DNA microsatellite variability. Behav Ecol Sociobiol 37:357–363

Muralidharan K, Shaila MS, Gadagkar R (1986) Evidence for multiple mating in the primitively eusocial wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae). J Genet 65:153–158

Ohgushi R, Sakagami SF, Yamane S, Abbas ND (1983) Nest architecture and related notes of Stenogastrine wasps in the province of Sumatra Barat, Indonesia (Hymenoptera, Vespidae). Sci Rep Kanazawa Univ 28:27–58

Ohgushi R, Yamane S, Abbas ND (1986) Additional descriptions and records of Stenogastrine nests collected in Sumatra Barat, Indonesia, with some biological notes (Hymenoptera, Vespidae). Kontyû 54:1–11

Ohta T (1976) Role of slightly deleterious mutations in molecular evolution and polymorphisms. Theoret Popul Biol 10:254–275

Oldroyd BP, Smolensky AJ, Cornuet J-M, Wongsiri S, Estoupt A, Rinderer TE, Crozier RH (1996) Levels of polyandry and intracolonial relationships in Apis dorsata (Hymenoptera: Apidae). Ann Entomol Soc Am 89:276–283

Oldroyd BP, Clifton MJ, Wongsiri S, Estoupt A, Rinderer TE, Sylvester HA (1997) Polyandry in the geneus Apis, particularly Apis andreniformis. Behav Ecol Sociobiol 40:17–26

Richards OW (1978) The social wasps of the Americas excluding the Vespinae. British Museum, London

Ross KG (1986) Kin selection and the problem of sperm utilization in social insects. Nature 323:798–800

Ross KG, Carpenter JM (1991) Population genetic structure, relatedness, and breeding system. In: Ross KG, Matthews RW (eds) The social biology of wasps. Cornell University Press, Ithaca, NY, pp 451–479

Ross KG, Matthews RW (1991) The social biology of wasps. Cornell University Press, Ithaca, NY

Ruttner F (1988) Biogeography and taxonomy of honeybees. Springer-Verlag, Berlin

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a labortory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schmitz J, Moritz RFA (1994) Sequence analysis of the D1 and D2 regions of 28S rDNA in the hornet (*Vespa crabro*) (Hymenoptera, Vespidae). Insect Mol Biol 3:273–277

Schmitz J, Moritz RFA (1998) Molecular phylogeny of Vespidae (Hymenoptera) and the evolution of sociality in wasps. Mol Phy Evol 9:183–191

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. Genetics 137:1147–1155

Swofford DL (1991) PAUP: Phylogenetic analysis using parsimony, Version 3.0s. Illinois Natural History Survey, Champaign

Tajima F, Nei M (1984) Estimation of evolutionary distance between nucleotide sequences. Mol Biol Evol 1:269–285

Wade MJ, McKnight ML, Shafter HB (1994) The effects of kin structured colonization on nuclear and cytoplasmic diversity. Evolution 48:1114–1120

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biol J Linn Soc 26:375–400