Mitochondrial DNA variation in social wasps (Hymenoptera, Vespidae)

J. Schmitz and R. F. A. Moritz

Bayerische Landesanstalt für Bienenzucht Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Burgbergstrasse 70, D-8520 Erlangen (Federal Republic of Germany) Received 23 February 1990; accepted 26 June 1990

Summary. Patterns of restriction fragment length polymorphisms (RFLP) of European Vespinae were more similar within genera than between them. Distance trees were constructed that support the hypothesis of monophyly of the genera Vespula and Dolichovespula. Within the genus Vespula, V. germanica was more closely related to V. rufa than to V. vulgaris. The position of the genus Vespa remained uncertain due to the precision limits of the RFLP technique. Key words. Vespinae; mitochondrial DNA; restriction fragment length polymorphism (RFLP).

Research Articles

Linnaeus¹ classified the social wasps in one genus which he called *Vespa*. Since then many authors have dealt with the problem of classification and phylogeny of the social wasps. Frequently the opinions about monophyly and the evolutionary steps of the individual genera differ substantially.

De Beaumont² suggested a Vespinae phylogeny based on morphological characters where the genera Dolichovespula and Vespula are two separate monophyletic groups. He divided the Vespula into Paravespula (P. vulgaris and P. germanica) and Vespula (V. rufa and V. austriaca). Yamane³ compared morphological and behavioral larval characters. Sticking to the monophyly of Vespula and Dolichovespula, he suggested a phylogenetic tree with Vespula as the yellowiacket genus with the most primitive features, and Dolichovespula with more derived features. Varvio-Aho et al.⁴ investigated the genetic relationship of eight northern European yellowjacket species in an isozyme study. In their tree, D. media shows a greater distance from D. saxonica than from V. vulgaris. V. vulgaris was only weakly related to V. rufa. Carpenter ⁵ constructed a phylogenetic tree based on seventeen morphological characters. This tree supports the monophyly of Vespula and Dolichovespula. The Vespula group is divided into Paravespula and Vespula, corresponding to De Beaumont's² system. Carpenter⁵ obtained a phylogenetic sequence with Vespa as a sister-group of the remaining yellowjackets. A common basic assumption is that the genus Vespa has the most primitive features in both morphology and behavior^{2, 3, 6}. This suggests that a Vespa-like ancestor forms the phylogenetic basis of the Vespinae. The monophyly of the subfamily Vespinae was strongly supported by Carpenter 7.

In this study we used restriction fragment length polymorphisms (RFLP) of Vespinae mitochondrial DNA (mtDNA) to obtain more information about phylogenetic relationships within the Vespinae.

The molecular basis of mtDNA polymorphism has been described by numerous authors⁸⁻¹⁰. MtDNA is a powerful tool for reconstructing the phylogenetic relationships between and within species¹¹.

Material and methods

Wasp nests were collected within an area of 50 km around Erlangen in southern Germany. We used three nests each of six wasp species of the three genera: *Vespula* (*V. vulgaris, V. germanica, V. rufa*), *Dolichovespula* (*D. saxonica, D. media*), and *Vespa* (*V. crabro*). From each nest about 1 g of larvae were taken and analyzed.

The intestine of each individual larva was excised and the tissue homogenized, using a method similar to that of Moritz et al.¹², in 0.25 M sucrose-TE-buffer (0.25 M sucrose, 0.03 M Tris, 0.01 M EDTA, pH 8) in a Potter-Elvehjem homogenizer with a teflon pestle. In a first centrifugation ($700 \times g$, 4 °C for 20 min) cell debris and nuclear DNA was pelleted. The supernatant was cen-

trifuged again at $11,000 \times g$, $4 \degree C$ for 20 min, to isolate the mitochondria.

The pellet was resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and treated with SDS (1 % in final concentration) and proteinase K (50 μ g/ml) for 2 h at 40 °C. Proteins were extracted with phenol and subsequently with chloroform and ether as described by Maniatis et al.¹³.

Ether was removed by heating the sample to 60 °C for 15 min. 5 M NaCl was added to a final concentration of 0.1 M and the mtDNA precipitated in ethanol at -70 °C. The mtDNA was pelleted at 12,000 × g (30 min, 0 °C) and washed in 70 % ethanol. The sample was dried under vacuum for 15 min and resuspended in TE-buffer (10 mM Tris, 1 mM EDTA, pH 8).

We used 1–5 units of enzyme (BRL Gibco: Acc I, Bcl I, Bgl II, EcoR I, Hind III, Mbo I and Xba I,) for each restriction digest of about 50 ng mtDNA in a total suspension volume of 20 µl. The buffer and temperature conditions used were as recommended by the supplier. The fragments were end-labelled by adding 1 µCi α -³²P ATP, unlabelled deoxy-nucleotide triphosphates (dCTP, dGTP, dTTP to a final concentration of 0.5 mM), and 0.4 U Klenow-fragment (large fragment of DNA polymerase I). After incubation for 20 min at room temperature the reaction was terminated by adding EDTA to a final concentration of 20 mM.

The samples were stained with a color marker (0.08% bromophenol blue, 60% sucrose, 0.05 M EDTA) and loaded into a 1% submarine horizontal agarose gel. Electrophoresis was carried out with 50 mA/31 V for 13 h in TAE electrophoresis buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8). The fragments were visualized after drying by autoradiography on Cronex 4 X-ray film for 3 h at -70 °C using DuPont Cronex Hi Plus intensifying screens.

Results

Two of the restriction patterns produced by Hind III, Mbo I and Bcl I, EcoR I are shown in figure 1 and figure 2. We used a bacteriophage lambda DNA digest as a size standard (EcoR I + Hind III). For further evaluation we used the restriction patterns produced by the 6-basepair enzymes Acc I, Bcl I, EcoR I, Hind III and Xba I. The fragments produced by these and other restriction endonucleases are shown in the table. There were no RFLPs between the three nests of the same species.

The genetic similarity between various pairs of organisms can be estimated by the portion of shared fragments (F-value) in their restriction pattern. This fragment method of Nei and Li¹⁴ is based on the following equation:

$$F = \frac{2 N_{xy}}{N_x + N_y}$$
(1)

1070



Figure 1. Hind III, Mbo I, digestion of mtDNA from social wasps. A lambda-EcoR I/Hind III digestion is used as length standard: 21,226-5,148-4,973-4,277-3,530-2,027-1,904-1,584-1,330-983-831 and 564 bp. V.v. = Vespula vulgaris, V.r. = V. rufa, V.g. = V. germanica, D.s. = Dolichovespula saxonica, D.m. = D. media, V.c. = Vespa crabro. Lanes 13 and 14 not interpretable.



Figure 3. The genetic distance estimated by the equations given by Nei and Li^{14} and Upholt¹⁵ with standard error between pairs of species (lower half of the matrix). The same values represented as pie charts (upper half). Shaded area = distance.





Figure 2. Bcl I, Eco RI digestion of mtDNA from social wasps. A lambda-EcoR I/Hind III digestion is used as length standard: 21,226-5,148-4,973-4,277-3,530-2,027-1,904-1,584-1,330-983-831 and 564 bp. Lanes 4 and 8 not interpretable. Species as in figure 1.

Figure 4. Distance tree based on RFLPs of six European social wasps, with the standard error produced by the UPGMA method. The values above the horizontal lines represent the genetic distances.

Where N_x and N_y are the numbers of fragments in organism X and Y. N_{xy} is the number of fragments shared between the two organisms.

The proportion of fragments shared between two organisms (F) can be used to estimate the average number of nucleotide substitutions per restriction site (δ) for each pair of organisms. We estimated the genetic distance (δ) using Upholt's¹⁵ equation:

$$\delta = 1 - \left[\frac{-F + (F^2 + 8F)^{1/2}}{2}\right]^{1/r}$$
(2)

Where r is the number of base pairs recognized per cleavage site.

To estimate standard errors for the δ -values we used the jackknife procedure developed by Tukey¹⁶, done over restriction enzymes (fig. 3).

The genetic distances were used to construct a phylogenetic tree. Because the distance matrix showed some violations of the triangle inequality, we used the UPGMA

Research Articles

Size (in base pairs) of mtDNA fragments obtained after digestion by restriction enzymes from the six European wasps. The arithmetic means (\bar{X}) of the mt genome size and standard errors (s_a) are given below.

	V. vulgaris	V. rufa	V. germanica	D. saxonica	D. media	V. crabro
EcoR I	16788 1585	6026 4266 3631 1905 1585	7413 4467 1850 1738 1413	17783 4677	7810 3631 3020 2188 1738	10000 7810 1862 1400
Hinf I				12589 2818 1585 977 832		
Hind III	14900 3548 2570	10000 3548 2630 1659	16050 3548	15382 4266 2630 708	15382 3508 2700 933	16503 4786 4266
Mbo I	8128 2042 1738 1549 1479 1148 678	4786 4074 4000 2239 1862 1698	7080 7000 2239 1950 1622 631	6310 3467 2692 2291 1549 1122 741		
Bcl I	13962 4786 3598	12589 4786 3548	12589 4786 3548	8318 7413 5129	8710 7413 3631 1641	15031 6166 3631
Acc I	14125 3891 2754	10000 7586	10000 7073 3090	11220 6607 1349	16982 1349	
Bgl II		12023 5888				
Xba I	17783 2344 1072	7943 5248 2455	14125 5248 1182	14125 5012 3981	9120 4842 3713 1109 692	17370 1907
X s _x	$\begin{array}{c} 20078 \\ \pm 850 \end{array}$	17996 ± 599	19774 ±607	20796 ± 794	$\begin{array}{c} 20022 \\ \pm 836 \end{array}$	$\begin{array}{c} 22683 \\ \pm 1501 \end{array}$

method of Sokal and Michener^{17, 18}. With this method the principle of triangle inequalities is not required¹⁸. The resulting distance tree for the six social wasps analysed is shown in figure 4. Other parsimonious trees (Fitch-Magoliash, mixed Wagner-Sokal, PHYLIP 3.2 package, provided by Dr Felsenstein) yielded similar topologies.

Discussion

The RFLP method used in this study is a useful tool for estimating the relationship between species which are relatively closely related, i.e. between species belonging to the same genus. To estimate the relationship between species which are very divergent, i.e. *V. crabro* and *Vespula* species, the fragment method is not very accurate, and the restriction site method ^{14, 18} estimates with a higher precision. Nevertheless, our data fit nicely into some of the previously published models of wasp phylogeny, though not into others.

The topology of the distance tree deduced from the distance matrix indicates a close relationship between *Vespa crabro* and the *Dolichovespula* group. This is mainly determined by the low genetic distance estimated between *V. crabro* and *D. media*. A more detailed comparison of mtDNA cleavage maps may be necessary to derive more exact estimates.

Furthermore, RFLPs in this study are mainly produced by 6-base enzymes which only yield large fragments. It is a problem to estimate the exact length of large fragments in agarose gels. Therefore our estimates of the total mt genome length suffer from large standard errors. By using restriction endonucleases with 4-base recognition sites the mt genome length could be estimated more exactly.

The phylogenetic tree supports the hypothesis of the monophyly of the genera *Vespula* and *Dolichovespula*. This is in agreement with studies of De Beaumont² and more recently Carpenter⁵. Varvio-Aho et al.⁴ questioned the monophyly of *Vespula* and *Dolichovespula* by comparing isozyme polymorphisms. However, Carpenter¹⁹ pointed out that the heterogeneity within the genera was the result of a misinterpretation of their isozyme data. In contrast to models which divide the genus *Vespula* into *Paravespula* (*P. vulgaris* and *P. germanica*) and *Vespula*

1072 Experientia 46 (1990), Birkhäuser Verlag, CH-4010 Basel/Switzerland

 $(V. rufa)^{2, 5}$, we found that V. germanica was the closest to V. rufa. Yamane³ concluded on the basis of larval characters that Dolichovespula species share some derived characters and are less closely related to Vespa than to Vespula species. In our study we found, in accordance with Green²⁰, that the ancestral genus of Vespa is more related to the genus of Dolichovespula than to the genus of Vespula. This is in agreement with the thesis that the Vespula group represents the most recently derived group.

The present study gives further evidence for the phylogenetic relationship of six European social wasps, based on genetic data. The results are largely in agreement with morphological studies^{2, 20}, with the exception of dividing *Paravespula* and *Vespula* into distinct groups. The next step in the study of wasp mtDNA should be the analysis of restriction site maps, which will give us further details of Vespinae phylogeny.

Acknowledgments. This work was supported by the Deutsche Forschungsgemeinschaft (Grant No. Mo 373/4-1; RFAM).

- 1 Linnaeus, C., Systema Naturae, 10th edn. Holmiae Laur Salvii (1785).
- 2 Beaumont, J. De, Mitt. schweiz. ent. Ges. 31 (1958) 168.
- 3 Yamane, S., Ins. Matsumurana New Ser. 8 (1976) 1.

- 4 Varvio-Aho, S. L., Pamilo, P., and Pekkarinen, A., Insectes soc. 31 (1984) 375.
- 5 Carpenter, J. M., Syst. Ent. 12 (1987) 413.
- 6 Richards, O. W., Biol. Rev. 46 (1971) 483.
- 7 Carpenter, J. M., Syst. Ent. 7 (1981) 11.
- 8 Densmore, L. D., Wright, J. W., and Brown, W. M., Genetics 110 (1985) 689.
- 9 Bermingham, E., Lamb, T., and Avise, J. C., J. Hered. 77 (1986) 249.
- 10 Merril, C. R., and Harrington, M. G., Trend genet. 5 (1985) 150.
- 11 Solignac, M., Monnerot, M., and Mounolou, J. C., J. molec. Evol. 23 (1986) 31.
- 12 Moritz, R. F. A., Hawkins, C. F., Crozier, R. H., and Mackinlay, A. G., Experientia 42 (1986) 322.
- 13 Maniatis, T., Fritsch, E. F., and Sambrook, J., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1982.
- 14 Nei, M., and Li, W. H., Proc. natl Acad. Sci. USA 76 (1979) 5269.
- 15 Upholt, W. B., Nucl. Acid Res. 4 (1977) 1257.
- 16 Tukey, J. W., Exploratory Data Analysis. Addison-Wesley, Reading, Massachusetts 1977.
- 17 Sokal, R. R., and Michener, C. D., Univ. Kansas Sci. Bull. 28 (1958) 1409.
- 18 Nei, M., Molecular Evolutionary Genetics. Columbia University Press, New York 1987.
- 19 Carpenter, J. M., Insectes soc. 34 (1987) 58.
- 20 Green, A., Ann. ent. Soc. Am. 72 (1979) 614.

0014-4754/90/101068-05\$1.50 + 0.20/0 © Birkhäuser Verlag Basel, 1990