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Clustering of bumblebee subgenera based on interspecific genetic relationships (Hymenoptera, Apidae: *Bombus* and *Psithyrus*)

Pekka Pamilo, Antti Pekkarinen & Sirkka-Liisa Varvio

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Enzyme gene differentiation was studied in 21 *Bombus* and five *Psithyrus* species from northern Europe. With the exception of the species pair *Subterraneobombus subterraneus* and *S. distinguendus*, all species can be distinguished from each other on the basis of the eleven loci examined. Species-specific staining patterns of two enzymes in *Pyrobombus lapponicus* (Fabricius) and *P. monticola* (Smith) from sympatric populations confirm the specific status of the two forms.

Four algorithms were used to reconstruct the phylogenetic tree from the electrophoretic data: UPGMA, Fitch-Margoliash, Wagner parsimony and restricted maximum likelihood methods. The reliability of the UPGMA tree was evaluated by estimating variances of the branch lengths and branching points. The main results are: (1) *Psithyrus* species form a single cluster, supporting the monophyletic origin of the group. (2) The species of the conventional subgenera cluster together, except in the subgenus *Pyrobombus*. (3) The clustering of the genus *Bombus* does not follow the division into sections *Odontobombus* and *Anodontobombus*. The results support the previous suggestion that the subgenus *Bombus* is divergent from the other subgenera and indicate a phyletic relationship of the subgenera *Alpigenobombus*, *Alpinobombus* and *Kallobombus* with the subgenera of the section *Odontobombus*. Further studies are required to establish statistical significance of the emerging patterns.

Pekka Pamilo and Sirkka-Liisa Varvio, Department of Genetics, Univ. of Helsinki, Arkadiankatu 7, SF-00100 Helsinki.
Antti Pekkarinen, Department of Zoology, Univ. of Helsinki, P.Rautatiekatu 13, SF-00100 Helsinki, Finland.

1. Introduction

Bumblebees are mainly Holarctic insects comprising about 250 species in the genus *Bombus* ('true' bumblebees) and 44 in the genus *Psithyrus* (cuckoo bumblebees) (Williams 1985). The *Bombus* species are eusocial having, with a few tropical exceptions, annual colonies. Some *Bombus* species are known to be facultative, or even obligatory, social parasites of other bumblebees. All *Psithyrus* species are social parasites of 'true' bumblebees and lack the worker caste. The host specificity of *Psithyrus* is variable; reports of the Palaearctic species indicate specialization in one to several, usually closely related, host species (Pouvreau 1973), whereas the Nearctic *Psithyrus* seem to be less host specific (Richards 1975).

The morphological distinction between *Bombus* and *Psithyrus* is clear and the monophyletic origin of *Psithyrus* has been generally accepted, although many characters specific to *Psithyrus* could be explained as resulting from parallel evolution caused by the parasitic life style. Monophyly of *Psithyrus* has been confirmed by studies of characters not associated with social parasitism (Plowright & Stephen 1973, Pekkarinen et al., 1979, Obrecht & Scholl 1981). The subgeneric division of *Bombus* summarized by Richards (1968) and that of *Psithyrus* suggested by Popov (1931) are widely used. Studies of several characters not used in defining the subgenera have supported the subgeneric division of *Bombus* (Plowright & Stephen 1973, Pekkarinen 1979). Some groupings of subgenera within the genus *Bombus* have been suggested on both morphological (Krüger 1917, 1920, Milliron 1961, 1971) and behavioural (Sladen 1899) basis, but such clusterings have not been well established (Sakagami 1976).

Although the basic life history is very similar in different bumblebee species, many important differences concerning the details of the social organization of colonies, queen-worker differentiation etc. exist (Sakagami 1976). Knowledge of the phylogenetic relationships of the species would give a valuable background for understanding the evolution of social life patterns within *Bombus* and the coevolution of *Psithyrus* and their hosts. Relationships of *Psithyrus* and the *Bombus* subgenera have recently been examined on the basis of male genitalia by Ito (1985; 32 *Bombus* subgenera) using phenetic clustering and by Williams (1985; 33 *Bombus* subgenera) who proposed a cladistic tree. The topologies of these morphologically derived trees disagree with each other. An independent clustering can be based on interspecific genetic relationships. It is well known that the genetic divergence between two existing species is approximately proportional to the time of separation of the two evolutionary lineages (Nei 1987). Therefore, it is possible to use pairwise genetic differences for studying the phylogenetic relationships. In the present study, we apply this approach and examine the genetic relationships of bumblebees by means of enzyme electrophoresis. Especially, we examine how the affinities of the *Bombus* subgenera agree with the morphological clusterings mentioned above. The phylogenetic estimates based on genetic data necessarily include sampling errors due to a small number of gene loci examined. Therefore, we stress the need to test the statistical significance of the observed clustering. One test procedure is proposed here. Preliminary results have been reported by Pekkarinen et al. (1979) and Pamilo et al. (1981).

2. Materials and methods

The species studied and the sample sizes are listed in Table 1. Subgeneric names are used below (see Table 1). The nomenclature is according to Løken (1973, 1985) except that we consider *Bombus wurflenii* to be the correct original spelling (Radoszkowski 1859) under article 32 of the code (ICZN 1985). The material originates mainly from Finland, but some specimens from Scandinavia and Denmark have been included.

Horizontal starch gel electrophoresis (Varvio-Aho et al. 1984) was used to examine the following enzyme systems (the Enzyme Commission numbers in parentheses): aldehyde oxidase (AO, 1.2.3.1), esterases (EST, 3.1.1), glucosephosphate isomerase (GPI, 5.3.1.9), alpha-glycerophosphate dehydrogenase (GPD, 1.1.1.8), hexokinase (HK, 2.7.1.1), isocitrate dehydrogenase (IDH, 1.1.1.42), leucine aminopeptidase (LAP, 3.4.11.1), malate dehydrogenase (MDH, 1.1.1.37), malic enzyme (ME, 1.1.1.40), and phosphoglucomutase (PGM, 2.7.5.1). There are two loci encoding each of GPD, LAP and MDH (only one is used here), and multiple esterase loci. The esterases are not used in our phylogenetic analyses because it is not possible to tell the homologous loci, or even the number of loci expressed.

The electrophoretically characterized isozymes can be divided into distinct mobility classes or electromorphs. We denote the mobility of the commonest allozyme of each enzyme in *B. lucorum* by 100. The mobility difference of the other electromorphs from this reference is measured in millimetres (rounded to the nearest one) and this difference is added to (or subtracted from) 100 to provide a measure of the relative mobility of the given electromorph under our standard electrophoretic conditions. The commonest electromorphs of each species are listed in Table 1. Each electromorph is here considered to represent one allelic class of the gene in question. Enzyme gene studies have indicated two putative sibling species in *B. lucorum*, identifiable on the basis of PGM (Scholl & Obrecht 1983, Pamilo et al. 1984); the species used here as the reference is the one with the slow PGM variant. There are some differences between the present scorings and those given in our preliminary report (Pekkarinen et al. 1979). These differences are mainly due to improved resolution by the buffer system of Varvio-Aho & Pamilo (1980) and partly due to possible intraspecific geographic variation (Pamilo et al. 1984).

A number of methods for estimating phylogenetic trees from genetic data exist. These can be divided into several categories depending on the principle used in clustering, the main categories being genetic distance methods, maximum parsimony methods and maximum likelihood methods. The methods have been reviewed by Felsenstein (1982) and Nei (1987). The distance methods are based on the matrix of pairwise genetic distances which is then reduced to a tree form. We use both UPGMA clustering and the Fitch-Margoliash (FM) method for analysing the distance matrix based on Nei's standard genetic distances (Nei 1987). The UPGMA method clusters species using the arithmetic averages, and the FM method minimizes the differences between the original genetic distances (D_i) and those calculated along the realized branches of the tree (the patristic distances). The parsimony method infers the ancestral allelic states and the criterion is to minimize the total number of mutational changes in the tree. For this purpose, we code our data in 0, 1 form depending on whether the given allele is present (1) or absent (0) in a species (see Varvio-Aho et al. 1984). The restricted maximum likelihood (REML) method is based on the assumption that the gene frequencies change according to a Brownian motion process. The algorithm evaluates the likelihood that the gene frequencies have the observed values under this probability model. The tree with the highest likelihood is selected as the best one. Because the level of electrophoretic variation in bumblebees is low (Pamilo et al. 1984), we use only the commonest alleles at each locus in our analyses. The REML algorithm does not allow missing data, and we omit the loci *Lap-1*, *Lap-2* and *Mdh-2* and the species *Bombus terrestris* and

Table 1. Prevalent electromorphs of the enzymes studied. A bar indicates that the enzyme was not examined. n is the sample size examined.

	n	PGM	GPI	GPD-1	GPD-2	IDH	ME	HK	MDH-2	AO	LAP-1	LAP-2
Bombus												
lucorum (L.)	343	100	100	100	100	100	100	100	100	100	100	100
terrestris (L.)	28	100	—	100	100	100	100	100	—	100	98	100
sporadicus Nylander	6	100	100	100	100	100	99	96	—	100	100	100
Pyrobombus												
hypnorum (L.)	54	98	96	103	100	100	100	96	101	102	101	99
cingulatus Wahlberg	21	96	96	103	100	100	100	96	101	102	101	99
jonellus (Kirby)	40	96	96	103	100	104	100	96	101	102	102	99
pratorum (L.)	62	96	96	103	100	100	100	100	101	102	101	98
lapponicus (Fabr.)	42	94	98	103	100	102	100	100	—	103	100	98
monticola (Smith)	25	94	96	103	100	102	100	100	—	103	—	98
Melanobombus												
lapidarius (L.)	278	98	99	103	100	100	100	100	100	102	100	99
Alpinobombus												
balteatus Dahlbom	36	100	100	103	100	102	102	96	—	102	98	98
Alpigenobombus												
wurflenii Radoszk.	8	98	98	103	100	102	99	96	100	102	—	—
Kallobombus												
soroeensis (Fabr.)	5	100	98	103	103	102	96	98	101	102	102	98
Megabombus												
hortorum (L.)	51	100	98	103	100	100	101	98	100	102	100	99
consobrinus Dahlbom	11	100	96	103	100	100	101	98	101	102	—	—
Subterraneobombus												
subterraneus (L.)	19	100	98	103	100	104	102	104	100	102	101	98
distinguendus Morawitz	5	100	98	103	100	104	102	104	100	102	—	—
Thoracobombus												
pascuorum (Scopoli)	248	98	98	103	100	104	99	98	101	101	101	98
ruderarius (Müller)	30	98	98	103	100	104	99	100	101	103	101	98
veteranus (Fabr.)	18	98	98	103	100	106	99	100	100	102	101	98
sylvarum (L.)	1	98	98	103	100	106	99	100	101	103	—	—
Psithyrus												
rupestris (Fabr.)	15	97	95	103	105	102	99	98	100	98	100	—
Fernaldaepsithyrus												
sylvestris Lepeletier	50	100	100	103	105	102	99	100	100	99	101	99
flavidus (Eversmann)	18	100	100	103	103	102	99	100	—	99	101	99
Ashtonipsithyrus												
bohemicus (Seidl)	105	97	100	103	103	102	99	98	101	99	101	99
Metapsithyrus												
campestris (Panzer)	8	100	99	103	105	102	—	—	100	—	100	—

Metapsithyrus campestris from that analysis. The Wagner parsimony analysis was repeated 50 times and the FM (allowing only non-negative branch lengths) and REML analyses ten times using Felsenstein's PHYLIP program package. We base our conclusions chiefly on the UPGMA clustering because that allows tests of statistical significance. In other words, the different clustering algorithms are used to evaluate the reliability of the results in relation to the underlying assumptions of clustering, whereas the statistical tests of the UPGMA tree examine the reliability of the observed patterns in relation to the data set used.

The statistical significance of clustering in the UPGMA tree is tested as follows. Let i and j refer to two hierarchically located branching points in the tree, and the estimated mean distances at these points are $\hat{D}_i$ and $\hat{D}_j$, respectively. In order to say that the inner cluster (connected at j) is a good phyletic unit, we should show that $\hat{D}_i - \hat{D}_j$ is significantly greater than zero. For this we need the variance

$$\mathrm{Var}\ (\hat{D}_i - \hat{D}_j) = \mathrm{Var}\ (\hat{D}_i) + \mathrm{Var}\ (\hat{D}_j) - 2\mathrm{Cov}\ (\hat{D}_i, \hat{D}_j)$$

(Mueller & Ayala 1982, Nei et al. 1985). We use the jackknife technique to estimate this variance. When one locus, say k, is omitted from the data, we can recalculate all pairwise distances associated to the two branching points. Let us denote the mean distances calculated without locus k by $\hat{D}_{i,k}$ and $\hat{D}_{j,k}$ and $\hat{d}_k = \hat{D}_{i,k} - \hat{D}_{j,k}$. It can be shown that the jackknife method of Mueller & Ayala (1982) gives

$$\mathrm{Var}\ (D_i - D_j) = [\Sigma\hat{d}_k^2 - (\Sigma\hat{d}_k)^2/n](n-1)/n$$

where n is the total number of loci examined and the summations are done by eliminating one locus at a time. We have compared some variances with those obtained by the method of Nei et al. (1985), and the values were close to each other. We test the significance of the differences $d = D_i - D_j$ using t-test. This is done for all pairs of hierarchically located branching points in order to see at which level a significantly defined outgroup is found for a given inner cluster. The distance matrices obtained by omitting one locus at a time were also used to construct new UPGMA trees (jackknife trees) to see what kind of changes take place in the topology when one locus is omitted. We also estimate the variances of the branching points of the UPGMA tree using the method of Nei et al. (1985).

In order to compare the estimated phylogenetic trees with morphologically-based clusterings of the *Bombus* subgenera, we have re-examined several morphological characters.

3. Results and discussion

3.1. Species-specific allozymes

Based on the commonest alleles at each locus, all the species can be distinguished from each other, except the pair *Subterraneobombus subterraneus* and *S. distinguendus* (Table 1). The species *Pyrobombus lapponicus* and *P. monticola* were considered conspecific until distinguished by Svensson (1979) (see also Pekkarinen 1982). The two species are largely allopatric and they show some overlap in the morphological characters, but our present results confirm the specific status of the two forms. The individuals from sympatric populations in Norway (Tri: Skibotn) and Finland (Le: Kilpisjärvi) show species-specific allozymes of GPI and EST (Table 1).

Obrecht & Scholl (1981) reported allozyme patterns in some Central European *Bombus* and *Psithyrus* species. Of these, 15 species and three enzymes (GPI, IDH and PGM) are included in our study. Five of the 45 electrophoretic characters which can be compared give differing results. According to Obrecht & Scholl, GPI of *Metapsithyrus campestris* is identical to that in most other *Psithyrus* and *Bombus lucorum*, whereas we detected a slight difference in the mobility (Table 1). Obrecht & Scholl found unique electromorphs of PGM in *Psithyrus rupestris* and *Metapsithyrus campestris* and of IDH in *Pyrobombus pratorum* and *P. monticola* (= their *P. lapponicus*), whereas we could not detect such species-specific allozymes.

3.2. Clustering of the *Bombus* subgenera

The main features of the UPGMA tree (Fig. 1) can be summarized as follows. (1) The genus *Psithyrus* forms a well defined, although not statistically significant cluster. (2) There is a relatively good clustering at the subgeneric level, except in *Pyrobombus* from which the species pair *lapponicus* and *monticola* is detached. (3) The

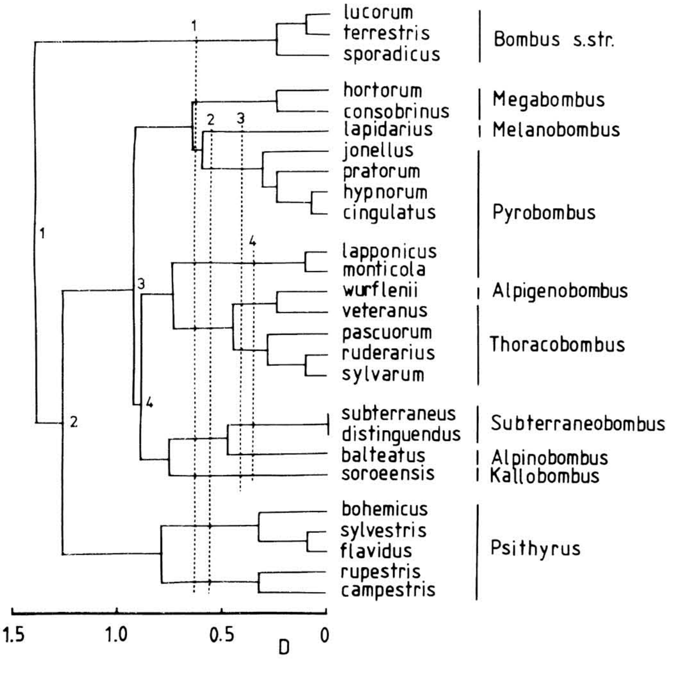

Fig. 1. The UPGMA phenogram based on the electrophoretic data of the species studied. D = the average genetic distance as detected by electrophoresis. The vertical (broken) lines indicate that the clusters to the right of a given line are significant at 95% level with reference to the branching point indicated by the same number.

Table 2. Proposed groupings of the *Bombus* subgenera. Note that the clusters from the present study are not statistically significant.

Krüger 1917	Sladen 1899 (from Sakagami 1976)	Ito 1985	Present results
Anodontobombus	Non-pocket makers	*Bombus* group	Cluster I
Bombus s.str.	*Bombus* s.str.	*Bombus* s.str.	*Bombus* s.str.
Melanobombus	*Melanobombus*	*Pyrobombus* group	Cluster II
Pyrobombus	*Pyrobombus*	*Melanobombus*	*Melanobombus*
Alpigenobombus	*Kallobombus*	*Pyrobombus*	*Pyrobombus*
Alpinobombus	Pocket makers	*Alpigenobombus*	*Megabombus*
Kallobombus	*Alpigenobombus*	*Alpinobombus* group	Cluster III
Odontobombus	*Alpinobombus*	*Alpinobombus*	*Pyrobombus* (2 species)
Megabombus	*Megabombus*	*Kallobombus*	*Alpigenobombus*
Subterraneobombus	*Subterraneobombus*	*Subterraneobombus*	*Alpinobombus*
Thoracobombus	*Thoracobombus*	*Megabombus* group	*Kallobombus*
		Megabombus	*Subterraneobombus*
		Thoracobombus	*Thoracobombus*
		(Psithyrus)	

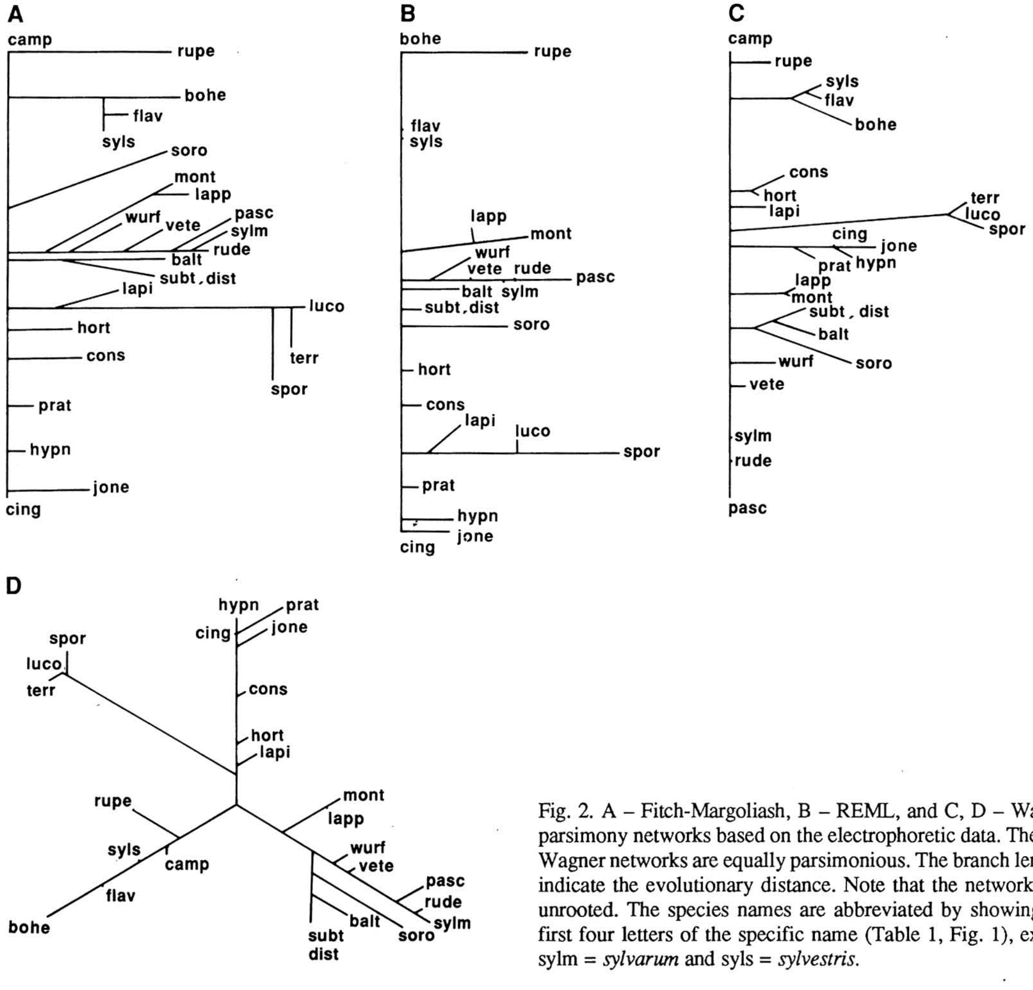

Fig. 2. A – Fitch-Margoliash, B – REML, and C, D – Wagner parsimony networks based on the electrophoretic data. The two Wagner networks are equally parsimonious. The branch lengths indicate the evolutionary distance. Note that the networks are unrooted. The species names are abbreviated by showing the first four letters of the specific name (Table 1, Fig. 1), except sylm = *sylvarum* and syls = *sylvestris*.

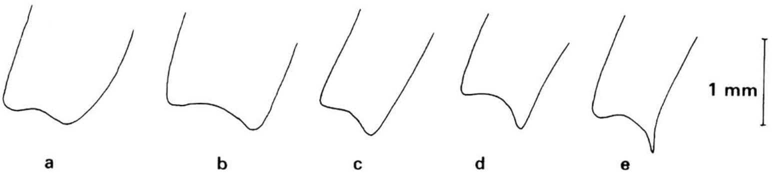

Fig. 3. Apical margins of the left middle basitarsus in *Bombus* queens. a – *Bombus* s.str. *lucorum*, b – *Alpinobombus balteatus*, c – *Kallobombus soroeensis*, d – *Alpigenobombus wurflenii*, e – *Thoracobombus pascuorum.*

Bombus subgenera form three major groups: the subgenus *Bombus* s.str., a group with the subgenera *Pyrobombus* (except *lapponicus* and *monticola*), *Melanobombus* and *Megabombus*, and a group with *Thoracobombus, Subterraneobombus, Alpinobombus, Alpigenobombus, Kallobombus* and the *Pyrobombus* species *lapponicus* and *monticola* (Table 2). These groups get support also from the trees produced by the other algorithms, although only one of the two equally parsimonious Wagner trees shows all three groups as good clusters (Fig. 2). We want to emphasize that not all of these associations are statistically significant.

Four of the branching points are numbered in the UPGMA tree and associated to each of these points we show a vertical (broken) line (Fig. 1). The species clusters to the right of any such line are statistically significant at the 95% level with reference to the outgroup species indicated by the given branching point. For instance, *Melanobombus lapidarius* and the subgenus*Pyrobombus* (excluding *lapponicus* and *monticola*) are signficantly more closely related to each other than either is to the subgenus *Bombus* s.str. (but cf. Fig. 2), and the subgenus *Bombus* s.str. forms a statistically significant cluster of its own. On the other hand, it is not possible to conclude on the basis of the present results that the single cluster of *Psithyrus* would be statistically significant, and the same holds for many other patterns of the UPGMA tree (Fig. 1). We next compare these findings with various morphological classifications of the *Bombus* subgenera (Table 2).

Above the subgeneric level, Krüger (1917, 1920) divided the genus *Bombus* in two sections, *Odontobombus* and *Anodontobombus* (Table 2). The most distinctive character in this division is the presence or absence of a spinous structure of the middle basitarsus in females. The character state in *Alpigenobombus wurflenii* and *Kallobombus soroeensis* can be considered intermediate (Fig. 3), which makes their placement in the division difficult. *A. wurflenii* and *K. soroeensis* differ from the other *Anodontobombus* species also by having two instead of three joints of the maxillary palps. Krüger (1920) also noted that the head of the *Odontobombus* species is relatively longer than that of *Anodontobombus*.

Sladen (1899) divided *Bombus* into two groups, pouch makers and pollen storers, on the basis of the pollen feeding of the larvae in the nests (Table 2). The pouch-makers feed the larvae through a pocket made at the side of the larval cell and the pollen storers perforate the wall of the cell when feeding the larvae. Sakagami (1976) advocated more appropriate names, pocket makers and non-pocket makers, for the two groups and noted that this is bionomically a very basic division. The homogeneity of *Odontobombus* (they are all pocket makers) led him further to suggest that this section might be a monophyletic group, whereas *Anodontobombus* might "involve some different phyletic lines" (see also Richards 1927). On the other hand, Cumber (1949) and Hobbs (1964) pointed out that pocket making is a kind of mass provisioning and could represent the ancestral character state (see also Plowright 1977). In that case, pocket making could not be used to define monophyly of *Odontobombus*.

Our results show several features which indicate that neither *Odontobombus* nor *Anodontobombus* may be a well defined monophyletic entity. (1) The subgenus *Bombus* s.str. is only remotely related to any other subgenus. (2) The subgenus *Megabombus* is detached from other *Odontobombus* species. (3) The species *Alpigenobombus wurflenii, Alpinobombus balteatus* and *Kallobombus soroeensis* do not cluster with the other *Anodontobombus* species. These features are shared by all of our trees (Fig. 1, 2). We next discuss these points in greater detail.

The UPGMA tree places the subgenus *Bombus* s.str. as a sister-group to all other bumblebees, including *Psithyrus*, but its outgroup position is not statistically significant. The trees produced by the other algorithms are unrooted networks but they indicate that *Bombus* s.str. would be amongst the other *Bombus* s.l. subgenera, although always separated by a long branch (Fig. 2). The numerical taxonomic analysis of wing venation (Plowright &

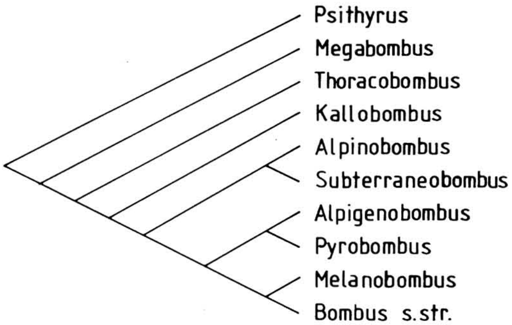

Fig. 4. The cladogram of the *Bombus* subgenera proposed by Williams (1985) on the basis of male genitals. Only those subgenera present in our own material have been included here.

Stephen 1973) and the cladistic analysis of male genital characters (Williams 1985, Fig. 4) do not support the outgroup position of *Bombus* s.str.

Alpigenobombus wurflenii and *Alpinobombus balteatus* cluster tightly with the *Odontobombus* subgenera *Thoracobombus* and *Subterraneobombus* in our phylogenetic trees, suggesting a true evolutionary relationship. The cluster of *A. wurflenii* and *T. veteranus* is statistically significant in relation to the branching point 4 of Fig. 1. All these species are also pocket-makers (Table 2). This supports Sakagami's (1976) view of the phyletic unity of the pocket making species, although the cladistic analysis of Williams (1985) is in a sharp contrast with it. The positions of *Megabombus* and the species pair *Pyrobombus lapponicus* and *P. monticola* in our trees disagree with the above interpretation, but the large standard errors associated with the branching points leading to these species in the UPGMA tree indicate that their positions in the reconstructed phylogenetic tree are not well settled.

As mentioned above, *Kallobombus soroeensis*, although not a pocket maker, shares some morphological similarities with *Odontobombus*. However, its position among the other subgenera can not be firmly established, and it even shows some tendency to cluster with *Psithyrus* in the jackknifed UPGMA trees (see also Pamilo et al. 1981).

Based on the morphology of male genitalia, Ito (1985) divided bumblebees in seven major groups, four of which are represented in our study (Table 2). This clustering disagrees with the cladistic tree proposed by Williams (1985) on the basis of the male genitalia (Fig. 4), although the results can be consistent with each other if the clustering of Ito is partly based on preserved ancestral similarities (e.g. in his *Megabombus* group). Our results have several features which favour the views of Ito (1985): the subgenera *Alpinobombus* and *Kallobombus* cluster with *Subterraneobombus* and this cluster is then attached to *Thoracobombus; Melanobombus* is related to *Pyrobombus*; and *Bombus* s.str. is separated from the other subgenera. However, our results do not support the *Megabombus* group of Ito (1985), and they also indicate different affinites for the subgenus *Alpigenobombus*. Williams (1985) also recognizes the relatedness of *Alpinobombus* and *Subterraneobombus*, but our results do not support the suggested affinity of *Alpigenobombus* and *Pyrobombus* or that of *Melanobombus* and *Bombus* s.str. In both cases our UPGMA tree provides a different topology with statistical significance (Fig. 1), but the FM and REML methods cluster *Bombus* s.str. and *Melanobombus* closer to each other (Fig. 2A, B). Clearly, more studies are needed to settle the relationships above the subgeneric level.

3.3. Evolution of social parasitism

Both intra- and interspecific nest usurpation by some *Bombus* s.l. queens is known to occur: among 93 recorded cases in Alberta (Canada), Richards (1975) observed 83% intraspecific and 17% interspecific nest usurpations. This type of parasitism is temporary and generally takes place between closely related species. It is possibly induced by lack of available nest sites (Richards 1975). Two species with regular social parasitism have also been found in the genus *Bombus*.

The social parasitism in closely related *Bombus* s.l. species is clearly scattered in various phylogenetic lineages and has originated independently in different subgenera. This is analogous to the evolution of social parasitism in the yellowjackets (*Vespula*) (de Beaumont 1958, Varvio-Aho et al. 1984).

The large genetic distance between *Bombus* and *Psithyrus* and the apparent synapomorphies of *Psithyrus* confirm the monophyletic origin of *Psithyrus*. The remarkable colour resemblance between a given *Psithyrus* parasite and its *Bombus* host (Reinig 1935) is thus a consequence of convergent evolution, probably caused by Müllerian mimicry (Plowright & Owen 1980).

We have previously argued that a phylogenetic tree should show coevolution between *Psithyrus* and their hosts (Pamilo et al. 1981). The background of this hypothesis is that the recognition of a host nest and probably the success of nest usurpation depend on species-specific odours (Cederberg 1983, Fisher 1984, 1985), and it might be difficult for a social parasite to cross a barrier between two host species with very different pheromones. Unfortunately, there is too much uncertainty in the estimated phylogenetic trees to test this hypothesis, but partial support comes from the clustering of the *Psithyrus* species within subgenera (see also Obrecht & Scholl 1981) and the suggested host specificity at the subgeneric level of *Bombus* and *Psithyrus* (Popov 1931). We can also note that the species parasitizing *Odontobombus* hosts, *Metapsithyrus campestris* and *Allopsithyrus barbutellus*, cluster together in the results of Obrecht & Scholl (1981).

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