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MORPHOMETRICS AS A TOOL IN IDENTIFICATION : A CASE STUDY ON A MYRMICA FROM FRANCE (Hymenoptera, Formicidae)

par
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Résumé: Morphométrie comme méthode dans l'identification: un example d'un *Myrmica* de France.

Des populations de trois espèces de *Myrmica* ont été étudiées dans une région de la Haute Savoie francaise, qui est caractérisée par la présence de deux espèces du papillon rare *Maculinea*. L'identification de *Myrmica rubra* et *M. scabrinodis* ne posait aucun problème, tandis que la troisième espèce qui a été considérée comme *M. vandeli* Bondroit ne pouvait être discernée que par ses femelles et mâles. Les ouvrières de cette espèce sont difficiles à séparer de *M. scabrinodis*, d'autant plus que les différences entre les mâles de *M. vandeli* et *M. sabuleti* sont assez faibles. Les femelles, au contraire, sont faciles à identifier par leur taille beaucoup plus grande et leur couleur plus foncée par rapport aux femelles de *M. scabrinodis*. Cette contribution confirme l'identification d'une espèce par une étude morphométrique, et nous permet ensuite de déterminer les ouvrières avec un certitude relative.

Mots-clés: *Formicidae, morphométrie, distinction, Myrmica vandeli, Myrmica scabrinodis.*

Summary: Populations of 3 species of *Myrmica* were examined at a site in the Haute Savoie region of France; the site is notable for the presence of 2 species of the rare butterfly genus *Maculinea*. *M. rubra* and *M. scabrinodis* were easily identified but, initially, the third species could be separated only by differences in the gynes and males. The species was assumed to be *M. vandeli* Bondroit. The workers of this species are difficult to separate from *M. scabrinodis* and the males can easily be mistaken for *M. sabuleti*. Only the queens are readily recognisable, being very similar to *M. scabrinodis*, but generally much larger and darker. This paper shows how an examination of morphometrics confirmed the identification of the species and enabled us to determine workers with a known reliability.

Key-words: *Formicidae, morphometry, discrimination, Myrmica vandeli, Myrmica scabrinodis.*

INTRODUCTION

People who work on the ecology and population biology of ants often encounter forms that appear to be different from the normal species. The reasons for considering them to be different are often a qualitative assortment of observations on behaviour, nest sites, *etc.* The scientist is then faced with 2 related but separate problems: is the form really different, using the accepted morphological criteria for determining speciation within the genus?; and, if so, what name should be given to it? The first question is of primary importance to the field biologist, but the second becomes important when he wishes to publish his observations. We were faced with these problems when working on a site near Yenne, in the Haute Savoie region of France.

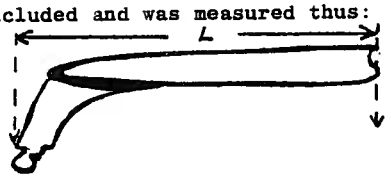
This site was a small marsh at the bottom of old hay-meadows, and it supported colonies of 2 species of the rare butterfly genus *Maculinea*: *M. nausithous* Bgstr. and *M. teleius* Bgstr. (Thomas 1984). The larvae of these butterflies are parasitic on colonies of *Myrmica* ants; we found that each *Maculinea* had a specific host, and that *Maculinea nausithous* used *Myrmica rubra* L. whilst *Maculinea teleius* used *Myrmica scabrinodis* Nyl. (Thomas 1983). It was therefore important to estimate the abundance and distribution of the 2 *Myrmica* species and to know the average nest population of each, so that we could estimate the carrying capacity of the site for the butterfly populations. In some of the nests of "*scabrinodis*" we found very large, dark, atypical queens. Subsequently, we began to notice small behavioural differences between the workers in the nests of "*scabrinodis*", and this led us to suspect that we might in fact be dealing with 2 distinct forms or species. If this was the case, obviously it would have an important bearing on our study of the butterfly populations.

For the first year we had only workers and queens available, and because Elmes (1978) had already studied queens of the *M. scabrinodis* group using morphometrics, we decided to use this approach on these ants. Using the key of Kutter (1977), which is the best current guide to the identification of *Myrmica*, we suspected that the large queens were either *Myrmica vandeli* Bondroit or some unknown species. However, we were misled by his descriptions of the workers and males, and the suggestion that *M. vandeli* might be a parasite did not fit our observations of the nest structure or the behaviour of the workers. The following illustrates the morphometric approach to this problem. In fact, it was more piecemeal than is suggested here because new material, including males and the types, were obtained as the study progressed. Here, we start with the detailed examination of the queens, as we did in fact, then examine the males, which were found clearly to be distinct from *M. scabrinodis*, and finally we consider the workers, which are the hardest to distinguish but which are all that are available in most field studies.

METHODS

Eleven measurements were made on queens and workers, and 10 on males: headwidth, headlength, minimum frons width, scape length, thorax width, epinotal spine length (females only), petiole width, post-petiole width, post-petiole height, and the number of hairs on the petiole; all were measured as indicated by Elmes (1978). The length of a head bristle was not measured for this study, but the length of the female scape was included and was measured thus:

Scape length: L



All measurements were recorded to the nearest 100th of a mm.

Canonical variate analysis was carried out on the groups of samples. For a detailed explanation of the method see Blackith & Reyment (1971), and for its application to *Myrmica* ants see Elmes (1978). In simple terms, this method attempts to maximise the between-/within-group variance ratio; in other words, it looks for a linear combination of the original morphometrics that emphasise differences which exist between the distinct groups while condensing the differences between the individuals within those groups. If there is no discrimination between 2 groups, they cannot be separated by this method. For convenience, the canonical variate (Cv) scores are standardised to have unit average within-group variance and an overall mean of zero.

The method assumes that the original measurements are normal and that the within-group variances are homogenous, so theoretically the within-group canonical variate scores should all have the same spread and the confidence limits can be calculated as the square root of χ^2 with the degree of freedom equal to the number of canonical variates that are considered togather. In practice, the data never conform exactly to the assumptions, so we consider it best to show confidence limits that are calculated from the actual scores. When 2 canonical variates are considered together, as is usual in this study, the confidence limits are ellipses whose major axis lies along the correlation between the 2 scores. If the assumptions about the data were perfectly true, these would be equal-sized circles!

As a starting point, we have taken the comparison of the queens of *Myrmica scabrinodis*, *Myrmica sabuleti* Meinert and *Myrmica hirsuta* Elmes (Elmes 1978). Here the analysis is repeated with scape included instead of bristle length (Fig. 1). In the case of 3 groups, only 2 canonical variates are obtained: Cv1 accounting for 83% of the between-/within-group variation and Cv2 the remaining 17%.

We can illustrate the discriminating power of the method by including in the analysis 2 more groups from each of the 3 species. These were:

M. scabrinodis, 10 queens from Bindon Hill, Dorset, GB, and 10 queens from Yenne, France (the same site as the unknown queens).

M. sabuleti, 10 queens from Oland, Sweden, and 10 queens from miscellaneous sites in France.

M. hirsuta, 10 queens from Bindon Hill, Dorset, GB, and 9 queens from the DDR.

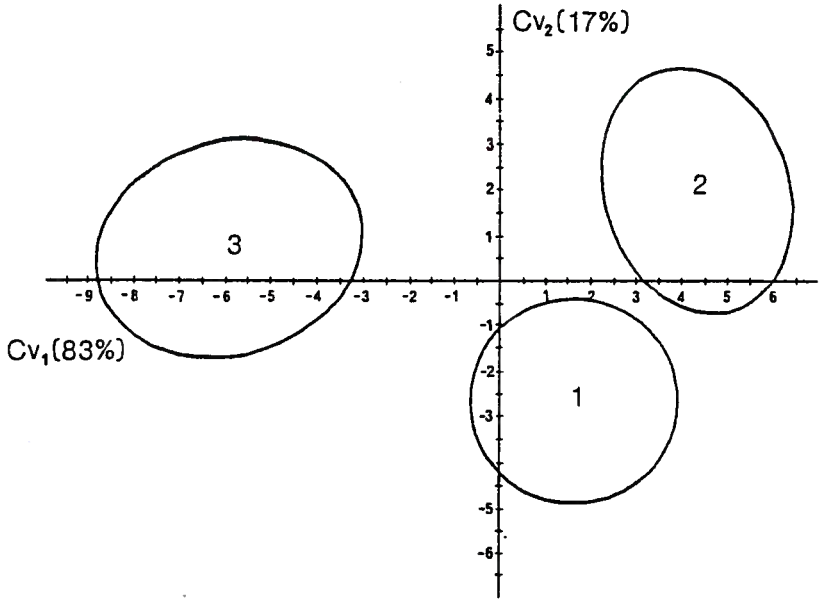


Figure 1: Distribution of the Cv scores for 40 queens of each of M. scabrinodis (1), M. sabuleti (2), M. hirsuta (3). The ellipses are the 95% confidence limits. These are the same individuals used by Elmes (1978).

In every case, treating these as separate groups resulted in them overlying the correct species, being indistinguishable from the base samples. For clarity of illustration, the extra queens of each species have been pooled to give groups of 20, 20, 19, respectively (Fig. 2). Thus, for determining these species by the use of the 11 morphometrics and comparison with the base groups (Fig. 1), the method is quite robust.

RESULTS

QUEENS

The putative *M. vandeli* from Yenne were included and compared with the groups obtained by combining the groups 1 and 1a, *etc.*, from Fig. 2. This produced a clear separation of the *M. vandeli*. Two further samples of 9 suspected *M. vandeli* queens collected from the Massif Central and 10 queens from St Bonnet, Hautes Alpes, were added, and as expected, these were not discriminable from the Yenne sample. For clarity, these 19 queens are illustrated as one group (4a) in Fig. 3.

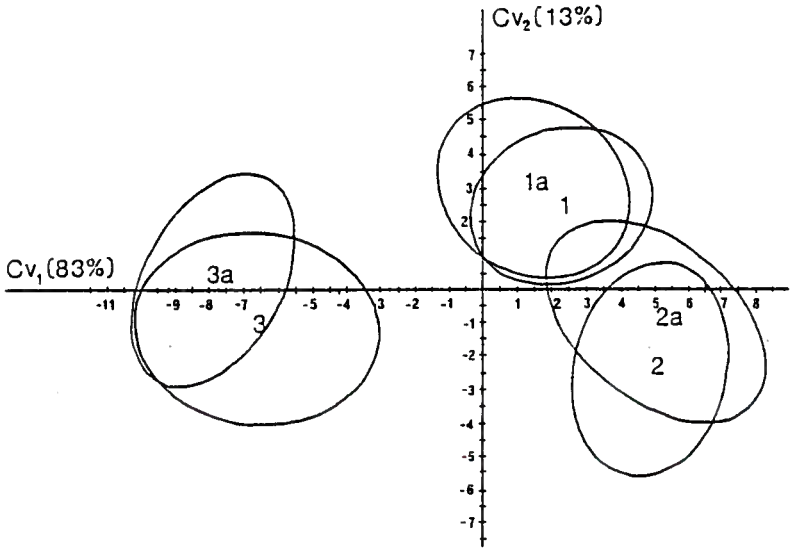


Figure 2: Distribution of the Cv scores for base samples 1, 2 and 3 and three new groups of 20 M. scabrinodis (1a), 20 M. sabuleti (2a), and 19 M. hirsuta (3a). The ellipses are the 95% confidence limits. Note that the scores for Cv2 have been 'flipped' compared to Figure 1; this is arbitrary and has no significance.

This convinced us that we were truly dealing with a fourth species that differed mophologically from the original 3 by at least the same amount as these differ from each other. The final step was to confirm the name. The type specimens of *M. vandeli* were then obtained and measured, and their Cv scores were found to be indistinguishable from our unknown species (Fig. 4, groups 4 and 4b), confirming our original visual impression that we were dealing with *M. vandeli*.

If we wish to use these measurements to identify a specimen between *M. scabrinodis* and *M. vandeli*, then a Cv analysis on just these 2 groups gives only one Cv. If the average of the 2 group mean scores, weighted for the actual within-group SD, is used to discriminate between them, then there is only a 1 in 10,000 chance of misidentifying an individual. If the variables that are measured are reduced from 11 to 3, then the odds of misidentification rise only slightly to 3 in 10,000; this is adequate for most purposes. The 3 best variables for discrimination between queens of these species are headwidth (W), post-petiole width (PPW) and petiole hair number (H). In order to calculate the Cv score, the measurements in mm. are put in the following equation:

Cv score = 11.913 W + 13.458 PPW - 25.889 + 0.192 H

M. scabrinodis **< -0.91 >** *M. vandeli*. **Confidence 99.97%**

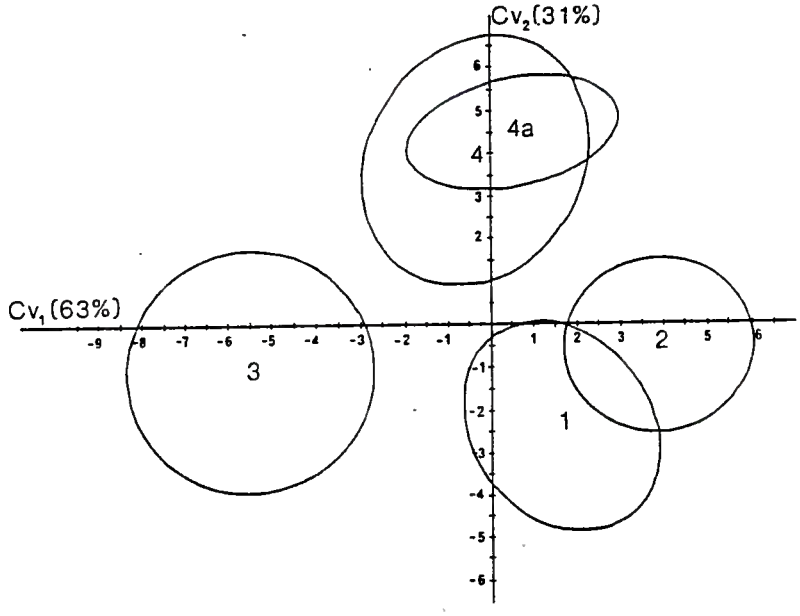


Figure 3: Distribution of the Cv scores for 4 groups of queens: 60 M. scabrinodis (1), 60 M. sabuleti (2), 59 M. hirsuta (3) and 40 "M. vandeli" (4). 19 M. vandeli (4a) from 2 other French sites are included: these cannot be distinguished from (4). The ellipses represent the 95% confidence limits.

With only 3 measurements and a pocket calculator, this becomes a practicable method of identification for non-specialists. Obviously, if a different pair is considered (*eg M. vandeli/M. sabuleti*) then the components of the reduced variable set would be different. As a general rule, when more species are discriminated between together, more measurements must be used.

MALES

Males of the putative *M. vandeli* were caught at all 3 sites in France: the Hautes Alpes, Haute Savoie and Massif Central. Cv analysis was used to compare these with the sets of males of *M. scabrinodis*, *M. sabuleti* and *M. hirsuta* that were used by Elmes (1978). Nine males of *M. hirsuta* from DDR were also included in the analysis. This confirmed the visual impression that *M. scabrinodis* is quite distinct from the other 3 species; its most obvious

distinction is a short antennal scape. For clarity of presentation, *M. scabrinodis* is omitted from the subsequent analyses, although its inclusion should have no effect on the discrimination between the other species; this would be done by lower level canonical variates (*ie* Cv3, Cv4, *etc.*).

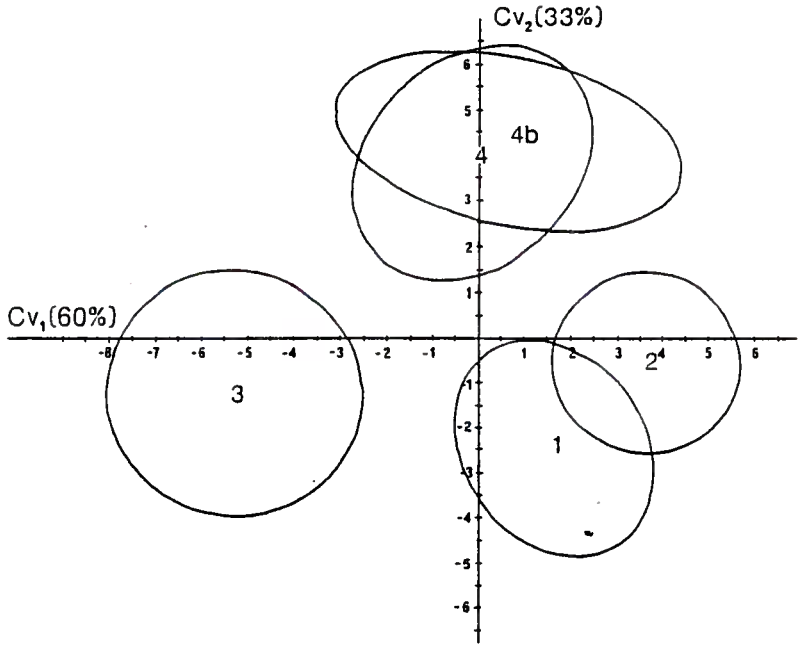


Figure 4: Distribution of the Cv scores for M. scabrinodis 60 queens (1), M. sabuleti 60 queens (2), M. hirsuta 59 queens (3), and M. vandeli 6 type specimens (4b) and combined sample of 59 queens from 3 sites in France (4). The ellipses are the 95% confidence limits.

Note: the large apparent variance of the type specimens is due to the small sample size.

Somewhat unexpectedly, the *M. hirsuta* from the DDR were discriminable from those caught by Elmes (1978) (but remember that no discrimination could be made between the females from DDR and UK). The most obvious difference is the DDR specimens' greater pilosity and wider post-petiole - the characters most often associated with parasitic ants. This is not so surprising if one accepts the theory of the independent occurrence or evolution of *M. hirsuta* parasites within *M. sabuleti* populations, as proposed by Elmes (1978). The haploid males might be expected to show greater individual variation than the diploid females in all species, and this might be exaggerated if the various geographical races of *M. hir-*

suta are in fact of different genetic origins. Further investigation of this problem will be made elsewhere. Here, the 9 males from the DDR are ignored in subsequent analyses.

When samples of *M. vandeli* from the 2 French sites were compared with *M. sabuleti*, each discriminated well from that species, but no discrimination could be made between the various *M. vandeli* samples. Therefore, these were combined and compared again with *M. sabuleti*, and with 14 type and paratype specimens of *M. vandeli* from the Bondroit Collection (Fig. 5). It is seen that no real discrimination can be made between our *M. vandeli* and Bondroit's types; this, again, confirms out opinion that the putative *M. vandeli* are indeed that species.

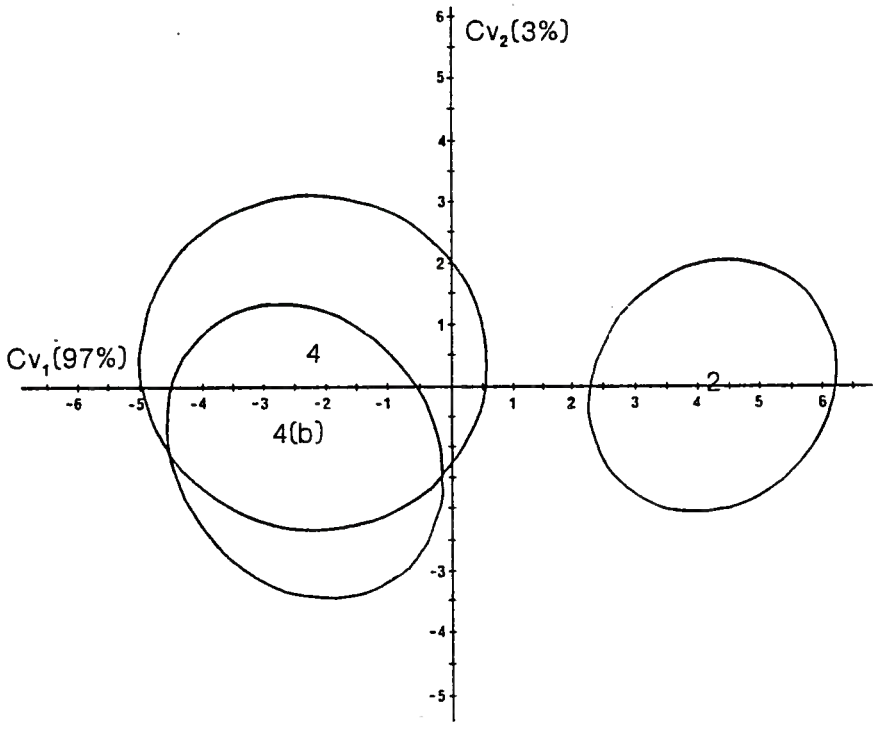


Figure 5: Distribution of the Cv scores for 3 groups of males: 28 M. sabuleti (2), 39 M. vandeli from 3 sites (4), and 14 type specimens of M. vandeli (4b). The ellipses represent the 95% confidence limits.

When all the *M. vandeli*, including the type specimens, are compared against *M. sabuleti* and *M. hirsuta* from the UK, it is seen that *M. vandeli* males are quite similar to *M. hirsuta* males (Fig. 6). This might lead to problems if *M. vandeli* males were caught apart from their related females. A simple comparison has

been made between the pairs of species. *M. sabuleti* can be separated satisfactorily from *M. vandeli* using only 3 measurements: post-petiole width (PPW), post-petiole height (PPH) and the number of hairs on the petiole (H).

Cv score = -25.23 PPW + 30.63 PPH - 0.34 H + 1.96

M. vandeli < 1.32 > *M. sabuleti*. Confidence 99.93%

But a similar comparison of *M. hirsuta* with *M. vandeli* produced a less clear separation. Most of the original measurements contribute to the discrimination to some extent, although the best 4 are: minimum frons width (F), petiole width (PW), post-petiole width (PPW) and post-petiole height (PPH).

Cv score = 15.49 F + 27.81 PW + 1.32 PPW + 3.64 PPH - 16.51

M. vandeli < 0.471 > *M. hirsuta* (GB). Confidence 83.7%.

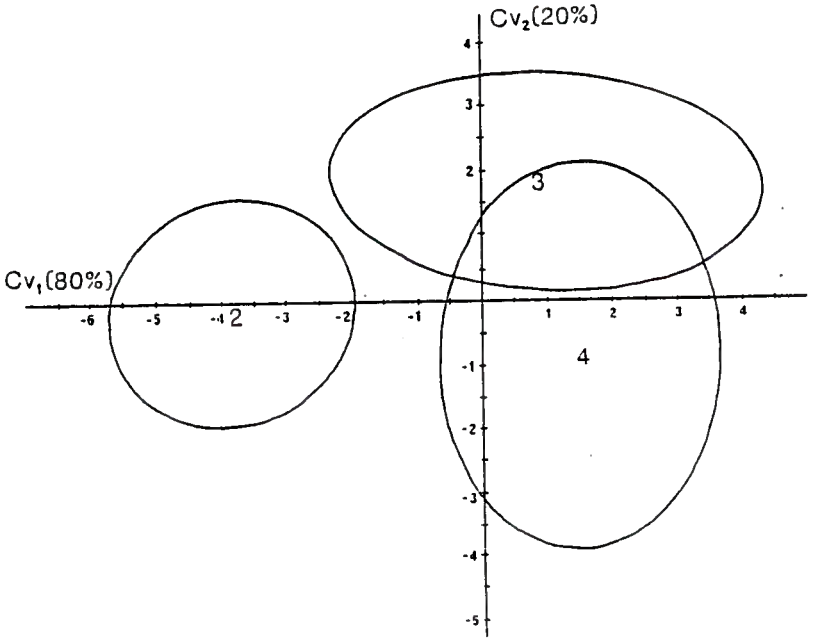


Figure 6: Distribution of the Cv scores for 3 groups of males: 28 M. sabuleti (2), 27 M. hirsuta from GB (3), and 53 M. vandeli (4). The ellipses represent the 95% confidence limits.

WORKERS

Whereas the males and females of *M. vandeli* were previously known from the Bondroit Collection, the workers were descr-

ibed from only 3 rather dubious specimens by Kutter (1977). It was essential for us to be able to separate workers of the species from those of *M. scabrinodis* from the site at Yenne with a high degree of confidence, because we did not wish to disturb the nests too much when we assessed the populations of ants that were available for parasitisation by *Maculinea* butterflies. Consequently, only workers were available for most identifications.

We compared a sample of 40 workers of *M. vandeli* from 8 nests at Yenne with similar samples of *M. scabrinodis* and *M. sabuleti* that were obtained within a few hundred metres of each other at that site. Where possible, only those colonies which had either queens or males available for confirmation were used to obtain the worker samples. A good discrimination was obtained between *M. vandeli* and the other 2 species, although, interestingly, a much poorer discrimination was obtained between *M. scabrinodis* and *M. sabuleti* at Yenne than is normal for other sites (Fig. 7).

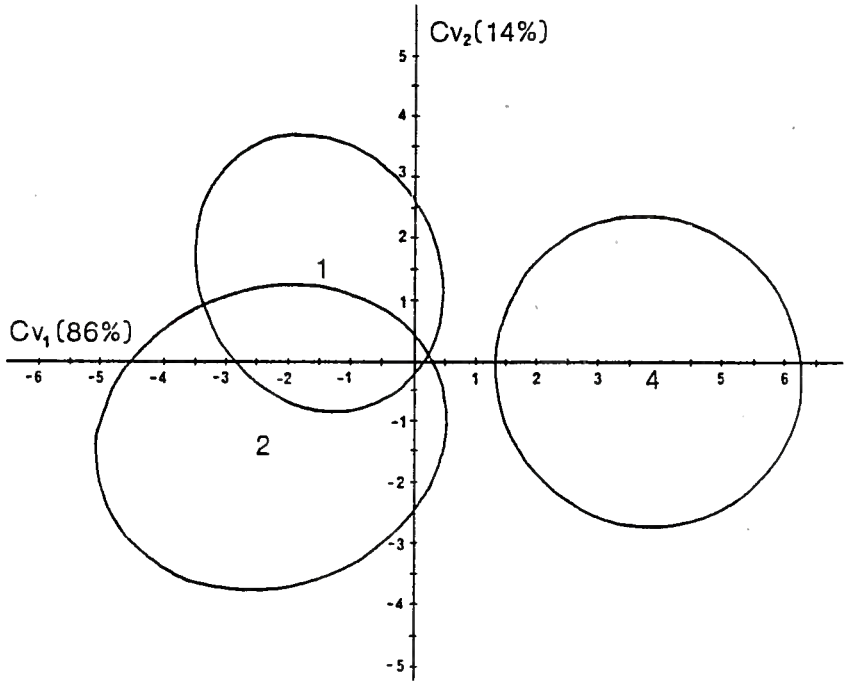


Figure 7: The distribution of Cv scores for 3 species of workers: 40 M. scabrinodis (1), 40 M. sabuleti (2) and 40 M. vandeli (4). The ellipses represent the 95% confidence limits.

When *M. scabrinodis* and *M. vandeli* workers are considered as a pair, we find that only 3 measurements are needed to obtain a satisfactory discrimination. It should be remembered that

this applies to workers from the Yenne site, although our experience indicates that the overall discrimination for specimens from elsewhere would not be much different from this. The required measurements are: minimum frons width (F), post-petiole width (PPW) and the number of hairs on the petiole (H).

Cv score = 53.10 F - 33.09 PPW + 0.42 H - 9.69

M. scabrinodis < -0.20 > *M. vandeli*. Confidence 99.53%

These measurements were used as a practical aid to our field study. Because of the small amount of variation among workers within colonies, it can be demonstrated that if 3 workers from the same nest are available, then that colony can be identified between *M. scabrinodis* and *M. vandeli* using these 3 measurements, with a probability of error so small as to be almost a certainty!

DISCUSSION

The method of canonical variate analysis of morphometrics has enabled us, who are not specialist taxonomists, to recognise and assign a name to specimens from a species that had been previously poorly described. Furthermore, we have been able to isolate those measurements that are of most use for the separation of *M. vandeli* workers from *M. scabrinodis*, and *M. vandeli* males from *M. sabuleti* - two sets of discriminations that are impossible to make with confidence by a casual inspection of the ants.

Apart from the larger size, darker coloration, and coarser sculpture of the queens, we find that *M. vandeli* females (both workers and queens) are generally larger than *M. scabrinodis*, with relatively wider heads and post-petioles and much more hair in the peduncle region. These last 2 features are characters of social parasites and led Bondroit (who had only seen a few queens and males, caught flying during a nuptial flight) to speculate that *M. vandeli* might also be parasitic. All our evidence is that the possession of these characters is coincidental; we have excavated many discrete nests that contained only *M. vandeli*, and conclude that this is a perfectly good free-living species.

The males are easily separated from *M. scabrinodis* and *M. sabuleti* by this method, but are rather difficult to separate from *M. hirsuta*, both having long scapes. high pilosity and a wide post-petiole. The workers, like the queens, are fairly easily separated from *M. scabrinodis*, with pilosity and post-petiole width again being important in the discrimination. This general association of so-called parasitic characters in an apparently free-living species is interesting. In our opinion, it tends to support Elmes' view that while these characters are often linked with a set of behavioural characteristics that give the individual a proclivity towards a parasitic mode of life. Parasites without these morphological characters might also occur by chance, as might the morphological characters in non-parasites (Elmes 1978).

We hope to publish a detailed assessment of the ecology of *M. vandeli* elsewhere, but here we take the opportunity to report that it lives in wet grassland. It tends to nest in tussocks of

fine grass that are themselves quite dry even if they are surrounded by water, although it seems likely that nests may be temporarily submerged when the snows melt in Spring. In our experience, nests of *M. vandeli* are always sympatric with *M. scabrinodis*, with the latter nesting in wetter tussocks of coarser vegetation. There is thus a distinct partition of habitat on a fine level but not on a wider scale; no doubt this, combined with the similarity of the workers to *M. scabrinodis*, has led to the failure to recognise this species. Having seen *M. vandeli*'s habitat at Yenne, we soon found it on similar-looking sites in two areas of France, over 100 km apart, and on one site in Switzerland. We have also been told of its occurrence in the German/Austrian Alps and in Czechoslovakia (Petr Werner pers. comm.). We suspect that it is in fact a widespread species, possibly confined to the mountains of Central and Southern Europe. Judging by its habitat requirements, it is likely to be more common than *M. scabrinodis* in some parts of the Alps.

ACKNOWLEDGEMENTS

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