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A preliminary study of the usefulness of morphometric tools for splitting the *Monomorium antarcticum* (Smith) complex (Hymenoptera: Formicidae), New Zealand's most common native ants

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Abstract

The *Monomorium antarcticum* complex includes the most common and ubiquitous of New Zealand ants. This group displays a high degree of variability, causing considerable confusion in its taxonomy. Using classical methods, previous authors have divided this complex into five or more species. However it is possible that most of the characters used are intraspecific rather than interspecific, and that there might be only one or two viable species in the complex. It is therefore important to examine new approaches that have the potential to address *M. antarcticum* systematics more effectively. This study explores the possibility of employing multivariate morphometric tools towards a classification of the *M. antarcticum* complex. Thirty-two *M. antarcticum (sensu lato)* colonies were randomly collected between April 28 and May 29, 2003, in the Wellington region. Principal component analysis and discriminant analysis were applied to examine five morphometric variables of the workers. The results of these statistical analyses show the existence of three distinct morphospecies in samples collected, indicating multivariate morphometric analyses as useful tools for discriminating *M. antarcticum s.l.* species. The next step will be to use these tools to examine the *M. antarcticum* complex from throughout New Zealand and combine them with molecular analyses.

Keywords: *Monomorium antarcticum*, Formicidae, morphological characters, principal component analysis, discriminant analysis.

Introduction

The *Monomorium antarcticum* complex includes what are probably the most common and most ubiquitous of New Zealand ants, with a distribution ranging from subtropical forest in the far north to beech forests and mountain tussock grassland in the south (Brown 1958; Don 1974). They also show high morphological variability, such as substantial size difference among workers within each colony and between colonies, causing considerable confusion in the taxonomy of the complex (Brown 1958). In most literature and collections, this complex has been divided into five or more species, corresponding to trends in colour, size, structure of clypeus, structure of propodeum, and so on (Brown 1958). However, intergrades exist for each of these characters, calling the validity of these species into doubt. Brown (1958) accordingly suggested that there might be only one or two species in this group. This state of poorly resolved taxonomy indicates the necessity of finding alternative approaches that can discriminate *M. antarcticum* species more effectively. To that end, our aim in this paper was to explore the potential of multivariate morphometric tools.

When multiple morphometric characters are used, principal component analysis and discriminate analysis, which is also referred to as canonical variate analysis, are powerful statistical tools in taxonomy (Quicke 1993). Both techniques generate new scores that are representative of all original variables, and not linearly correlated with one another. Usually the first two or three principal components or discriminant functions explain most of the useful variations in the data, easing the task of interpreting multiple variables. One or other of these techniques has been used to elucidate ant taxonomic issues such as evolutionary patterns in the Australian *Rhytidoponera* (Crozier *et al.* 1986); unresolved problems in the *Formica rufa* group (Douwes 1981, Czechowski & Douwes 1995); distinguishing castes in the polymorphic ant *Camponotus rugipes* (Diniz-Filho *et al.* 1994); delineating *Myrmica* species (Elmes 1978); and delimiting sibling species in the *fulva-rudis-texana* complex of the ant genus *Aphaenogaster* (Umphrey 1996).

Having encountered no morphometric study on ants employing both principal component and discriminant analyses, we decided to attempt such an analysis on the *M. antarcticum* complex. We wanted to assess the usefulness of such an approach on local variants of this complex in the Wellington region prior to investing resources in a nationwide analysis.

Materials and methods

Thirty-two colonies were randomly collected from the Wellington region between 28 April and 29 May, 2003. By digging with hand shovel and spoon, while collecting disturbed ants with aspirator, we managed to excavate all but three colonies nearly intact. Apart from these three colonies where it was physically impossible to reach deep into the nests, we left no remnants of the nests and only small numbers of stray workers. All samples were frozen immediately after collecting and were later counted and preserved in 70% ethanol until the time of measuring. The samples were divided into two groups: 5 detailed samples and 27 non-detailed colonies. We randomly selected 100 workers from each detailed sample, and 10 workers from each non-detailed sample, as subjects of this study.

Based on previous morphometric studies (eg. Czechowski & Douwes 1995, Diniz-Filho *et al.* 1994, Umphrey 1996) and the relative ease of accurate measuring, we selected five continuous morphological characters, which were measured under a stereomicroscope at 40x power with an eyepiece micrometer. These characters are: Head Length (HL) - length of the head measured dorsally, from the middle point of an anterior transverse line across the clypeal border to the middle point of the posterior margin; Head width (HW) - width of the head measured in dorsal view immediately behind the eyes; Scape length (SL) - maximum linear measurement of the length of the antennal scape, excluding the basal condyle; Weber's length (WL) - diagonal measurement from the anterior margin of the pronotum to the posterior extremity of the metapleural lobe, taken with specimen in approximate lateral view but slightly tilted so that both reference points were sharply focused; Hind tibia length (HTL) - maximum length of the hind tibia. The last three characters were all measured on the left side of the body. Each sample was also labelled by the coloration of the workers.

With morphometric data thus collected, we performed principal component and discriminate analyses using SPSS for Windows (SPSS 2002). By using mean measurements of 10 bees per colony, rather than individual bees, Daly and Balling (1978) obtained better results for identifying Africanized honey bees. Thus aside from measurements of individual ants, we also introduced mean measurements of 10 consecutively measured ants into the analyses. The principal component analyses used varimax with Kaiser normalization as the rotation method. All discriminant analyses used the numeric expression Rv. Bernoulli (1.0).

Table 1. Summary of classification from discriminant analysis with cross validation.

Grouping	Percentage of correct classification with cross validation
Five detailed samples	
Five forms corresponding to five colonies	79.40%
Four forms corresponding to four worker colorations (black, dark brown, brown, yellow)	86.80%
Three forms corresponding to three distinct clusters (black, brown, yellow)	94.60%
Five detailed and 27 non-detailed samples.	
32 forms corresponding to 32 colonies	42.20%
Four forms corresponding to four worker colorations (black, dark brown, brown, yellow)	86.00%
Three forms corresponding to three distinct clusters (black, brown, yellow)	94.30%

Results

The median number of worker ants in the *M. antarcticum* complex colonies that we sampled was 1800 (range: 28 to approximately 11400). One colony of 2800 ants had a total of 66 wingless queens, while nine of the colonies had more than one wingless queen. Three colonies had winged queens, which was surprising as the sampling was preformed late in the summer.

Plotting head width against hind tibia length indicates that the samples belong to at least two distinct morphometric forms (Fig. 1). In order to minimize the effect of size variation, all variables were divided by hind tibia length, which was chosen because it was easy to measure accurately. Using ratios thus obtained instead of the raw data, principal component analysis of the five detailed samples yielded two very distinct morphological clusters (Fig. 2). The second cluster shows a degree of separation, suggesting the existence of more than one morphospecies within that cluster. Thus it is possible that: 1) the five samples belong to five different morphs; 2) there are four morphs, distinguishable by colour; 3) there are three morphs, two of them overlapping to form the second cluster.

We then applied discriminant analysis on all three possible groupings to evaluate the robustness of the classification based on each of them. The resulting plots are largely indistinguishable. Three distinct clusters can be clearly recognized (Fig. 3), supporting the previous conclusion that there are three morphospecies in our collection of *M. antarcticum* ants. The third type of grouping yielded the highest percentage of correct classification (Table 1), further strengthening this argument.

Subsequently, we introduced data from the 27 non-detailed samples into the statistical analyses. This addition proved to have little impact on the results (Figs 4 & 5). In discriminant analyses, the third type of grouping again yielded the best classification (Table 1).

Finally, we introduced the mean measurements, resulting in a clearer separation of 3 groupings, particularly with the discriminant analysis (Figs 6-9).

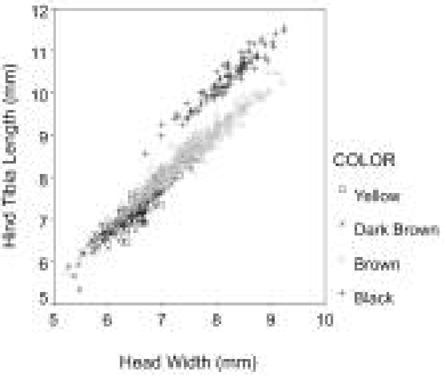

Fig. 1. Bivariate plot of head width versus hind tibia length, 100 workers from each of the 5 detailed samples, 10 workers from each of the 27 non-detailed samples.

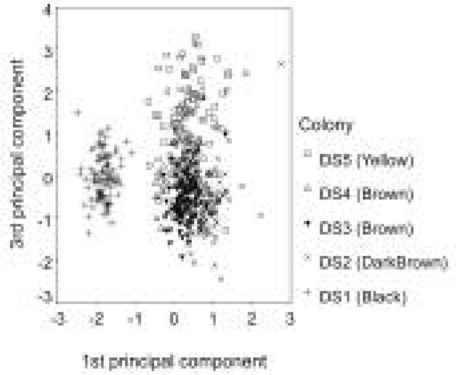

Fig. 2. Scatter plot of principal component 1 (63.9%) and 3 (10.6%) from PCA based on continuous variables (in ratio) of workers from the five detailed samples. Rotation Method: Varimax with Kaiser Normalization. DS- detailed samples.

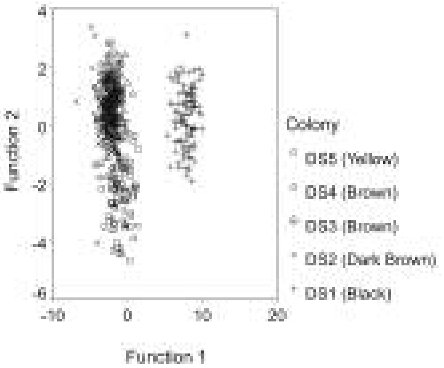

Fig. 3. Scatter plot of functions 1 (93.3%) and 2 (6.7%) from discriminant analysis, the 5 detailed samples are divided into 3 groups: 1) black ants; 2) dark brown and brown ants; 3) yellow ants. DS- detailed samples.

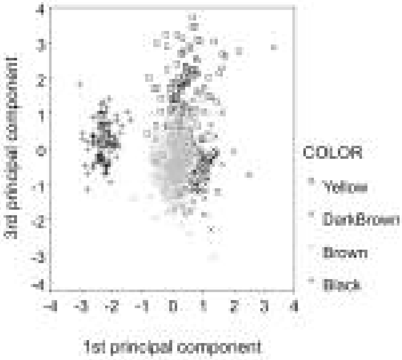

Fig. 4. Scatter plot of principal component 1 (62.8%) and 3 (10.3%) from PCA based on continuous variables (in ratio) of workers from all 32 samples. Rotation Method: Varimax with Kaiser Normalization.

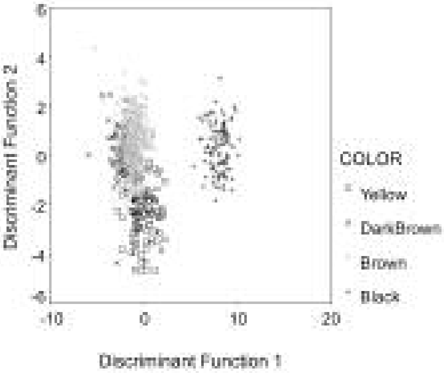

Fig. 5. Scatter plot of function 1 (92.1%) and 2 (7.9%) from discriminant analysis, 32 samples are divided into 3 groups: 1) black ants; 2) dark brown and brown ants; 3) yellow ants.

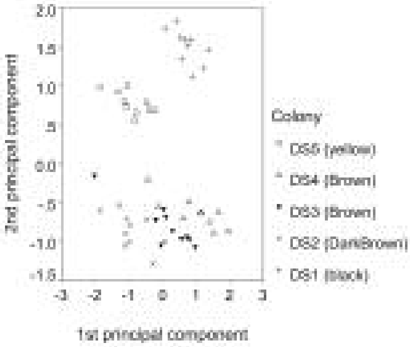

Fig. 6. Scatter plot of principal component 1 (99%) and 2 (0.7%) from PCA based on mean measurements (raw data) of every 10 workers from 5 detailed samples. Rotation Method: Varimax with Kaiser Normalization. DS- detailed samples.

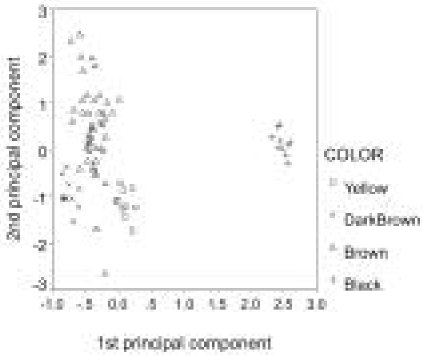

Fig. 7. Scatter plot of principal component 1 (97.8%) and 2 (1.8%) from PCA based on mean measurements (raw data) of every 10 workers from all 32 samples. Rotation Method: Varimax with Kaiser Normalization.

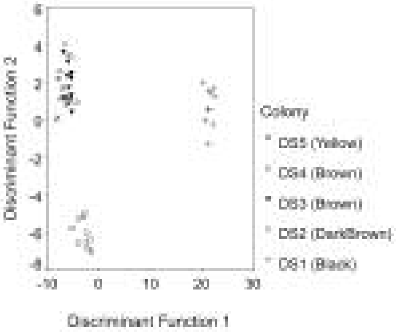

Fig. 8. Scatter plot of function 1 (92.6%) and 2 (7.4%) from discriminant analysis using mean measurements of every 10 workers, 5 detailed samples divided into three groups: 1) black ants; 2) dark brown and brown ants; 3) yellow ants. Percentage of correct classification with cross validation: 100%. DS- detailed samples.

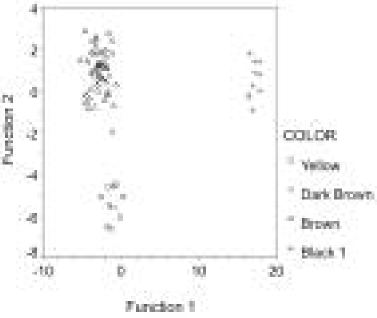

Fig. 9. Scatter plot of function 1 (90.9%) and 2 (9.1%) from discriminant analysis using mean measurements of every 10 workers, 32 samples divided into three groups: 1) black ants; 2) dark brown and brown ants; 3) yellow ants. Percentage of correct classification with cross validation: 98.7%.

Discussion

To evaluate the effectiveness of multivariate morphometric methods in discriminating *M. antarcticum* species, we compared delineations based on a few different approaches. Applying body size as a criterion, the vast majority of the black ants can be separated from most of the dark brown and yellow ants (Fig. 1). However, these brown ants distribute normally across the whole range of body size (Fig. 1), invalidating this size-based delineation. Moreover, in each colour form other than that of the brown ants, the range of body size may be under-represented due to the small number of available samples. In some cases bivariate plots are adequate for the purpose of discriminating species. By plotting workers' petiole heights against petiole lengths, Lucas *et al.* (2002) were able to differentiate three taxa from the species complex *Pachycondyla villosa*, which was previously regarded as a single species. Similarly Ward (1985, 1989) used scatter plots to good measures in his studies on pseudomyrmecine ants, differentiating a number of species. In our study, this method was able to separate the black ants from the other colour forms neatly. It was unable to address the relationship among the other three colour forms, however, indicating its inherent limits. Applying worker coloration as a criterion, our samples can be divided into four colour forms: black, dark brown, brown, and yellow. However, the validity of each form as a species is highly suspect without other supporting evidence. Brown (1958) suggested that the yellowish ants might just be brown ants of incipient colonies. Following his reasoning, the other two forms may well represent brown ants of aged colonies.

Using multivariate morphometric tools enabled us to discriminate the *M. antarcticum s.l.* ants in our collection into three clusters. These are readily recognizable, although two of them overlap, indicating the existence of three morphospecies. Unfortunately, due to the lack of replication for black and yellow ants, we cannot say that the two colour morphs are representative of two corresponding morphospecies. Discriminant analysis provided slightly more compact and distinct clusters than principal component analysis. Given that every colour form was monomorphic (plotting each pair of morphological characters selected gave results similar to Fig. 1), there were distinct advantages in adopting the technique used by Daly and Balling (1978). The results support the previous indication that there are three morphospecies. The neatness of these results suggests substituting raw data with mean measurements is a highly attractive technique for discriminating monomorphic species that form intermingling clusters. In our study, using this technique clearly favoured discriminant analysis over principal component analysis. Applying discriminant analysis with mean measurements yielded clusters so clear-cut and widely separated (Figs 8, 9) that we can provide a quantified definition for each of the three morphospecies with great confidence. The same certainly cannot be said of principal component analysis (Figs 6, 7).

Multivariate morphometric techniques nevertheless have their own limits. The existence of social parasitism, for example, may cause artificial interpenetration of morphometric clusters. Umphrey (1996) demonstrated that, although morphometric analyses improved the ability to recognize most members of the *fulva-rudis-texana* complex, sometimes only genetic evidence was definitive. Lucas *et al.* (2002) used biochemical as well as morphometric methods to examine the *Pachycondyla villosa* species complex. The biochemical technique yielded more definitive and better quantified results. Moreover, the present study, apart from general limits of multivariate morphometric studies, also suffers from its limited geographical scope.

Logically, therefore, the next step will be to use morphometric techniques to examine the *M. antarcticum* complex from throughout New Zealand and combine them with molecular or biochemical analyses. Some work of the latter type has already been conducted. Jones *et al.* (1988) analyzed venom alkaloidal compositions of New Zealand *M. antarcticum*; their results suggesting the presence of at least four "species" in this complex. By analyzing a chemical compound in venom of New Zealand *M. antarcticum*, Don and Jones (1993) identified two "species" that were also distinguishable by colour. These biochemical analyses thus appear to support the findings of our morphometric study. Combining our technique with other methods such as biochemical analysis therefore might provide useful means for delineating *Monomorium antarcticum s.l.* ants of New Zealand.

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