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A simple method for repeated measurements and storage of ant (Hymenoptera: Formicidae) heads in studies of polymorphism

Harold G. Fowler
Marcelo N. Schlindwein

Departamento de Ecologia, Instituto de Biociências - UNESP - 13506-900 - Rio Claro - São Paulo, Brasil

Summary

Worker polymorphism in ants has long been a concern of myrmecologists, and, given the evolutionary significance of worker size variation, quantification has become a major concern. Due to allometric relationships among body parts, most studies focus upon worker head dimensions, and repeatable measurements are major sources of experimental error. We present a simple method for obtaining repeatable measurements and for storing measured head capsules, based upon decapitation and flush mounting on microscopic slides, which are used for measurements with microscope ocular micrometers. Using student labor, we have reduced repeated measurement error in 50%.

Key words: Ant, Allometry, Method, Head capsule, Polymorfism

Resumo

O polimorfismo de operárias em formigas sempre foi um tema de estudo de mirmecólogos, e, pelas suas implicações evolutivas, exige métodos quantitativos. Devido as relações alométricas entre partes corporais, a maioria dos estudos usan as dimensões da cabeça da operária. Porém, é difícil obter medidas repetidas, sendo uma fonte potencial de erro experimental. Apresentamos um método simples para obter medidas repetidas e para manter as cápsulas cefálicas em forma permanente. As cabeças são removidas e montadas sobre lâminas de microscópio, as quais podem ser medidas com um micrômetro ocular. Usando alunos de graduação, o erro de medição cai 50% com esse método.

Unitermos: Formiga, Alometria, Método, Cápsula cefálica, Polimorfismo.

Introduction

Worker polymorphism in ants has long interested myrmecologists since initial studies of Wheeler (1910), and following recent theoretical treatments (Kennedy, 1961; Oster and Wilson, 1978; Lumsden, 1982; Fowler *et al.* 1991) has moved to the forefront of evolutionary ecology. Although a number of studies documenting worker polymorphism have been performed (Fowler, 1986), a major limitation in these studies is obtaining repeatable measurements. Because of the allometric relationships among hard body parts in ants (Lumsden, 1982), researchers have generally quantified worker size variation through head capsule measurements (Zacharov, 1976). However, because of the fractal morphology of the head capsule of the majority of ant species, difficulties arise in obtaining repeatable measurements of chosen parameters. Here, we present a method for obtaining repeatable measurements of parameters of worker size variation using head capsules, and demonstrate that this method reduces measurement error. Also, our method permits storage of measured head capsules for future reference and documentation.

Material and methods

All ant species we have tested have a concave curvature of the head capsule in the occipital region. However, if decapitated, heads can be mounted flush on microscope slides, due to the symetry of the head capsule, and affixed using transparent nail polish. This method permits a large number of head capsules to be mounted on the same slide, which can then be measured with an ocular micrometer. The prepared slides can then be stored in slide boxes for future reference or measurement.

To evaluate the reliability of our method, we mounted 50 worker head capsules of each of *Camponotus blandus* (Fr. Smith), *Labidus praedator* (Fr. Smith), *Atta sexdens rubropilosa* Forel, *Pheidole oxyops* Forel, *Dolichoderus decollatus* Fr. Smith, and *Solenopsis saevissima* (Fr. Smith). Fifty head capsules of each species not mounted but left attached to the worker bodies were also used. Using an ocular micrometer mounted on a Wild dissecting microscope, 5 students measured each head capsule 5 times, but not in succession. Each head had its own reference code, known only to us. Measurement reliability was established through comparisions of the coefficient of variation, both among students and among species. Maximal occipital head width was the only parameter used for measurements. Differences in coefficients of variation were tested with analysis of variance between species and students.

Results and discussion

Although our method is initially time consuming, with training 50 ants can be decapitated and mounted within a 30 minute period. Measurement times also rapidly decline with training, and a microscope stage greatly accelerates measurements. Prepared slides have shown no deterioration of head structure over a 15 year period (1980-1995), if stored in sealed microscope slide holders.

Table 1 - Coefficients of variations with respect to maximal occipital width measured with an ocular micrometer among university students for different ant species. Mean student coefficients of variation are calculated from 250 measurements of each mounted and unmounted head capsules.

		Mean coefficient of variation associated with: student				
Species	Type	1	2	3	4	5
A. sexdens r.	Mounted	3.4	3.9	2.2	2.0	1.8
	Unmounted	7.7	6.3	5.4	6.2	4.8
S. saevissima	Mounted	2.1	1.8	2.3	1.7	0.9
	Unmounted	8.5	9.2	7.3	6.6	5.2
P. oxyops	Mounted	1.4	1.0	1.3	1.0	1.6
	Unmounted	5.2	4.3	4.5	1.2	3.7
D. decollatus	Mounted	1.4	1.7	2.4	4.2	1.9
	Unmounted	6.8	5.4	7.0	4.3	5.5
C. blandus	Mounted	2.8	3.1	2.0	0.9	2.2
	Unmounted	6.1	6.7	5.6	3.8	6.3

Moreover, the repeated reliability of our method can be verified by examining coefficients of variation associated with repeated head measurements (Table 1). For all species measured with students, mounted heads were characterized by significantly lower levels of measurement error than unmounted heads using Student's t test (all values $<0,05$). Moreover, mounted heads produced at least a 50% reduction of measurement error (Table 1). Significant differences in mean coefficients of variation of maximal occipital width were found associated with species and students through analysis of variance (Table 2). In general, the more polymorphic the species, the larger the associated coefficients of variation, irrespective of the method employed (Table 1). However, a larger amount of variation was attributable to individual student measurer, highlighting the often undiscussed aspect of measurement error in worker polymorphism studies.

Table 2 - Two-way analysis of variance of coefficients of variation among individual species and student measurers of mean values listed in Table 1.

Source of variation:	D. F.	S. S.	M.S.	F. value
ant species	4	1695.08	423.77	4.763*
type of measurement	1	19443.92	19443.92	218.545*
species type	4	1033.88	258.47	2.905*
error	40	3558.80	88.97	
measurer	4	1377.08	344.27	2.932*
type of measurement	1	19443.92	19443.92	165.621*
measurer type	4	214.68	53.76	0.457
error	40	4696.00	117.400	

* $p < 0,05$

Given these results, we strongly urge that ants be decapitated and head capsules be mounted on microscope slides for measurements, if ants are not to be studied live. Repeated measurement is urged even in these cases.

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