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Removal of allometric variance improves species separation in multi-character discriminant functions when species are strongly allometric and exposes diagnostic characters

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Abstract

Removal of allometric variance (RAV) based upon regression of character ratios (indices) against body size may considerably improve species discrimination in ants when only single or very few characters are available or considered. This clear advantage of RAV is lost in multi-character discriminant analyses (DA) in the moderately allometric ant genera *Hypoponera*, *Temnothorax*, *Cardiocondyla*, *Myrmica*, *Lasius*, and *Formica*. In order to asses if this result is repeated in strongly allometric organisms, two sister species of the ant genus *Camponotus*, *C. ligniperda* (LATREILLE, 1802) and *C. herculeanus* (LINNAEUS, 1758), were investigated. Several DAs using primary absolute data, simple ratios against body size, log-transformed and RAV-corrected data were run. On the single-character level, the interspecific overlap ratio was reduced from 71.2 % in primary ratios to 21.0 % in RAV-corrected ratios. In comparison to any other data type tested, RAV-corrected data showed an improved performance also in multi-character DAs. The advantage of RAV-corrected against log-transformed data is significant with $p < 0.005$ for combinations between two and five characters but is very small in a six-character DA. RAV is expected to constantly loose its benefits with increasing number of characters computed. This does not exclude residual advantages when the species considered are extremely similar and strongly allometric. Apart from their variable contribution to species discrimination, RAV-transformed data have the constant advantage of exposing diagnostic characters and of unmasking pseudo-characters, i.e., to show interspecific differences rather independent from environmental factors that may have influenced worker body size. For this reason, RAV is always recommended in the taxonomic practice. Procedures of the RAV technique are explained in more detail and working routines of how to treat monophasic and diphasic allometries are presented. It is recommended to start any RAV analysis with a graphical analysis. Predictions that measurement error and natural variation of CS (cephalic size) significantly reduce regression slopes in index-formulae of the type $Y / CS = a * CS + b$ were confirmed by simulations. This slope reduction, however, is meaningless because the second step of the RAV technique completely compensates for this. Predictions of absolute measurements Y by the index-formula and by logarithmic descriptions of the type $\log Y = \log b + a * \log x$ are nearly equal in 13 *Temnothorax* and *Formica* species with monophasic allometries.

Key words: Species separation, morphometry, graphical analysis, piecewise regression.

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Introduction

Allometries are usually understood as differential change of a quantitative character with variation in overall body size. For example, head size is negatively allometric in *Homo sapiens* with adults having relatively smaller heads than children. Such an allometry that is the expression of a true, dynamic growth is termed ontogenetic. On the other hand, head size is positively allometric in adult workers of many ant genera and such a relation is termed static allometry (GOULD 1966), i.e., it refers to the pattern of variation within the same instar of the ontogenetic cycle in which true growth no longer occurs. Several scientists also use the term evolutionary or phylogenetic allometry but a clear distinction of this category from static allometry is complex (CHEVERUD 1982).

Changes in shape that are unrelated to size, i.e., changes that are not explained by allometries, have been attributed to independent adaptive processes (HUXLEY 1932, GOULD 1975). Other workers (e.g., RENSCH 1959, JERISON 1973, PILBEAM & GOULD 1974, MCHENRY 1975, MARSHALL & CORRUCCINI 1978, WOOD & STACK 1980) have assumed that if differences in shape among populations can be attributed to size differences alone, then these shape differences are merely the secondary effects of evolutionary size change and are of no taxonomic significance. SEIFERT (2002) produced similar arguments: Since worker body size in ants is largely influenced by nutrition and physical factors during larval growth, interspecific differences occurring independently from body size are the best indicators for heterospecificity, reflecting the "genetic core" of the species. In ants, allometries occur not only in metric measurements of basic body morphology but also in characters such as intensity of sculpture, density of pubescence hairs, setae numbers, and pigmentation scores (SEIFERT 1988a, b, 1992, 1997, 2000, 2003a, b, 2004b, 2005, 2006a, SCHLICK-STEINER & al. 2003, SEIFERT & GOROPASHNAYA 2004).

Attempts to correct biological data for body-size-dependent (allometric) variation are not new. McCoy & al. (2006) considered the main techniques that have been applied in ecological research in the past. They distinguished three different procedures for size-correction of data – residuals analysis, shearing and analysis of covariance (ANCOVA) – and concluded that all three techniques have flaws that limit their use in morphological analyses in some respect. The technique of removal of allometric variance (RAV) introduced by Seifert (2002) is another variant. It has some similarities to ANCOVA by using a univariate descriptor of body size as a covariate and estimating a common slope for multiple groups.

One purpose of this paper is to consider the index question in the taxonomic context. The problem with the use of simple, primary indices Y / X in biological studies has been very early recognized by Pearson (1897) and in the time after 1945 it has been addressed by several authors on twenty occasions at least (for a review see Packard & Boardman 1999). The main objections against simple indices are that they may produce pseudo-differences or, inversely, conceal real differences rather than disclosing them. These objections, however, were mostly ignored as the current practice of data presentation and evaluation by a large number of contemporary taxonomists shows. The simplicity of indices and the advantage they may have under certain conditions make it unlikely that they will cease to be used. This prompted me to recommend a procedure in which indices are used as primary data input, then to describe them as a function of body size and finally to remove the influence of body size (Seifert 2002). In the time since then, this method has been extensively used (e.g., Seifert 2003a, b, c, 2004a, b, 2005, 2006a, Schlick-Steiner & al. 2003, Seifert & Goropashnaya 2004).

The RAV method may considerably improve species discrimination when single characters are considered (Seifert 2002). However, I also argued that RAV should improve the performance of multi-character discriminant functions. In the time since then, this idea was tested in some 200 discriminant analyses (DAs) computed to distinguish closely related species of *Hypoponera, Temnothorax, Cardiocondyla*, *Myrmica*, *Lasius* and *Formica* (Seifert 2003a, 2003b, 2003c, 2004a, 2004b, 2005, 2006a, Schlick-Steiner & al. 2003, Seifert & Goropashnaya 2004). These analyses showed that RAV did not measurably improve species discrimination in these genera. Canonical discriminant functions can evidently compensate for moderate allometric effects when more than three or four characters are computed. The remaining advantage of RAV data was the demonstration of true, size-independent interspecific differences in comparative tables.

The main purpose of this paper is to address the question, if RAV significantly improves the performance of multi-character discriminant functions when very strong allometries are involved. Strongly allometric relations are obvious in ant genera such as *Pheidole*, *Messor* or *Camponotus*, with small workers appearing as "normally" shaped ants, whereas in the largest workers relative head size is increased to such a degree that they appear to be "walking heads" in some species. I investigated the mentioned question in workers of two sister species of the genus *Camponotus*: *C. ligniperda* (Latreille, 1802) and *C. herculeanus* (Linnaeus, 1758). Both species are widely distributed and locally abundant in temperate or boreo-montane woodland biomes of the Western Palaearctic. Determination by subjective assessment of pigmentation characters and surface structures (e.g., Foerster 1850, Forel 1874, Bondroit 1918, Stitz 1939, Pisarski 1961, Agosti & Collingwood 1987) had led to a frequent confusion of both species over some 150 years, before the problem became solved by numeric description of diagnostic characters (Seifert 1989). There is only one character known which allows a complete species separation without special treatment of data: PLG, the mean absolute length of pubescence hairs on the dorsum of the first gaster tergite. According to Seifert (2007), PLG ranges between 47 and 72 µm in *C. ligniperda* but from 77 to 120 µm in *C. herculeanus*.

Summing up, the paper wants to consider the index-question in the taxonomic context, to redescribe the RAV method of Seifert (2002), to propose a working routine of how to treat biphasic allometries and to investigate advantages and putative risks of the RAV method.

The selected material included 19 ant species and represented the complete range of size variation known in each of these species. Only the *Camponotus* species were suitable test organisms to address the question whether RAV improves DAs in strongly allometric species. The *Formica* and *Messor* species were used here as examples for showing the features of scatter plots of different data systems and the *Temnothorax* and *Formica* species to investigate if the suggested slope bias of the RAV method results in any misindication compared to a logarithmic description. The latter three genera were no suitable test organisms to investigate the mentioned question because *Formica* and *Temnothorax* showed too weak allometries and the *Messor* species too strong interspecific differences.

Material

I investigated: 50 workers of *C. ligniperda* from 29 samples and 23 localities in Bulgaria, Finland, Germany, and Sweden; 54 workers of *C. herculeanus* from 30 samples and 23 localities in Germany, Slovakia and Sweden; 215 workers of *Formica polyctena* Foerster, 1850 from 41 samples and 25 localities in Bulgaria, Finland, Germany, Poland, Russia, and Switzerland; 243 workers of *Formica rufa* Linnaeus, 1761 from 48 samples and 26 localities in the Czech Republic, Finland, France, Germany, Greece, Poland, Sweden, and Switzerland; 64 workers of *Formica candida* Smith, 1878 from China, Kyrgyzstan, Mongolia, and Russia; 64 workers of *Formica clara* Forel, 1886 from Azerbaijdzhan, Cyprus, Iran, Lebanon, Pakistan, Syria, and Turkey; 168 workers of *Formica clarissima* Emery, 1925 from China, Mongolia and Russia; 250 workers of *Formica cunicularia* Latreille, 1798 from England, France, Georgia, Germany, Greece, Hungary, Italy, Kazakhstan, Kyrgyzstan, Portugal, Switzerland, Turkey, and the Ukraine; 69 workers of *Formica fusca* Linnaeus, 1758 from France, Germany, Italy, and Sweden; 94 workers of *Formica lemani* Bondroit, 1917 from the Czech Republic, England, Finland, France, Germany, Poland, and Sweden; 194 workers of *Formica litoralis* Kuznetzov-Ugamsky, 1926 from China, Kazakhstan and Kyrgyzstan; 570 workers of *Formica lusatica* Seifert, 1997 from Bulgaria, China, the Czech Republic, Finland, Georgia, Germany, Greece, Hungary, India, Iran, Italy, Kazakhstan, Kyrgyzstan, the Netherlands, Pakistan, Russia, Slovakia, Switzerland, and Turkey; 103 workers of *Formica pamirica* DLUSSKY, 1965 from China and Kyrgyzstan; 191 workers of *Formica rufibarbis* FABRICIUS, 1793 from Austria, Bosnia, Bulgaria, Finland, France, Germany, Hungary, Kazakhstan, Russia, Sweden, and Switzerland; 159 workers of *Formica subpilosa* RUZSKY, 1902 from Afghanistan, Azerbaijdzhan, Iran, Kazakhstan, Kyrgyzstan, Turkey, and Uzbekistan; 60 workers of *Messor capitatus* (LATREILLE, 1798) from 11 samples and 4 localities in Italy; 54 workers of *Messor* cf. *wasmanni* KRAUSSE, 1910 from 7 samples and 2 localities in Italy; 116 workers of *Temnothorax crassispinus* (KARAVAJEV, 1926) from 52 samples and 38 localities in Austria, Bosnia, Czech Rep., Germany, Italy, Poland, Slovenia, and the Ukraine; 109 workers of *Temnothorax nylanderi* (FOERSTER, 1850) from 48 samples and 36 localities in Austria, France, Germany, Italy, Poland, Sweden, and Switzerland.

Methods

Morphology of *Camponotus*

Each worker specimen was evaluated for seven numeric characters at magnifications of 32× - 320×. All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around x, y, and z axes. A Wild M10 high-performance stereomicroscope equipped with a 1.6× planapochromatic front lens was used. A Schott KL 1500 cold-light source equipped with two flexible, focally mounted light-cables, providing 30°-inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimum resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the two cold-light sources depending upon the required illumination regime was quickly provided by regulating voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks ranging over 65 % of the visual field was used. To avoid parallax error, the measuring line was constantly kept vertical within the visual field. A mean measurement error of ± 1 µm was calculated for small and well-defined structures such as PLG, but one of ± 10 µm for large structures that are difficult to position and visualise, such as mesosoma length. To avoid rounding errors, all measurements were recorded in µm even for characters for which a precision of ± 1 µm is impossible. The following seven characters were evaluated:

CL	Maximum cephalic length in median line; the head must be carefully tilted to the position yielding the true maximum. Excavations of hind vertex and / or clypeus reduce CL. Thus, in *Camponotus*, CL is often smaller than the commonly used "overall head length in full face view", which includes the occipital corners and cannot be measured precisely without special devices.
CS	Cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
CW	Maximum cephalic width; this is either across, behind, or in front of the eyes.
EYE	Eye-size index: the arithmetic mean of the large (EL) and small diameter (EW) of the elliptic compound eye is divided by CS, i.e., EYE = (EL+EW) / (CL+CW). All structurally visible ommatidiae are considered. EW is always taken perpendicular to EL, i.e., it is not necessarily the smallest eye diameter.
FRS	Distance of the frontal carinae immediately caudal of posterior intersection points between frontal carinae and lamellae dorsal of torulus. If these dorsal lamellae do not laterally surpass the frontal carinae, the deepest point of scape corner pits may be taken as reference line. These pits take up the inner corner of scape base when the scape is fully switched caudad and produce a dark triangular shadow in the lateral frontal lobes immediately posterior of the dorsal lamellae of the scape joint capsule.
HTL	Length of hind tibia measured from its distalmost point to a proximal point A. Point A is marked by the maximum constriction of the inner side of tibia immediately before its junction with the femur (figure A265 in SEIFERT 2007).
MH	In workers: maximum mesosoma height at mesopleural level measured perpendicular to longer axis of mesosoma. The longer axis is defined in lateral view as straight line from the centre of propodeal lobe to the border point between anterior pronotal shield and propleuron.
ML	Maximum length of mesosoma from anteriormost point of neck shield to caudalmost median point near petiolar junction. If the anterior margin of the neck shield is concealed by head structures, ML may be estimated by taking the transition point of anterior pronotal slope to neck shield as the anterior landmark and multiplying the resulting length by the factor 1.03.
MW	Maximum mesosoma width.
PEH	Maximum petiole height measured perpendicular to a reference line, which is, in *Leptothorax* and *Temnothorax*, the chord spanning between caudal corner of ventral petiole profile and the caudal end of the subpetiolar process.
PEL	Diagonal petiolar length in lateral view; measured from anterior corner of subpetiolar process to dorsocaudal corner of caudal cylinder. Do not erroneously take as reference point the dorsocaudal corner of the helcium which is sometimes visible.
PEW	Maximum width of petiole.
PLG	Average length of pubescence hairs on dorsum of first gaster tergite. Six measurements are averaged at least.
PPW	Maximum width of postpetiole.
SL	Maximum straight-line scape length excluding articular condyle.
SP	Maximum length of propodeal spines; measured in dorsofrontal view along long axis of spine, from spine tip to a line, orthogonal to the long axis, that touches bottom of interspinal meniscus. Left and right spine lengths are averaged. This mode of measuring is less ambiguous than other methods but results in some spine length in species with spines reduced to blunt corners.
SPBA	Smallest distance of lateral margins of propodeal spines at their base. This should be measured in dorsofrontal view, since the wider parts of the ventral propodeum do not interfere the measurement in this position. If the lateral margins of spines diverge con-

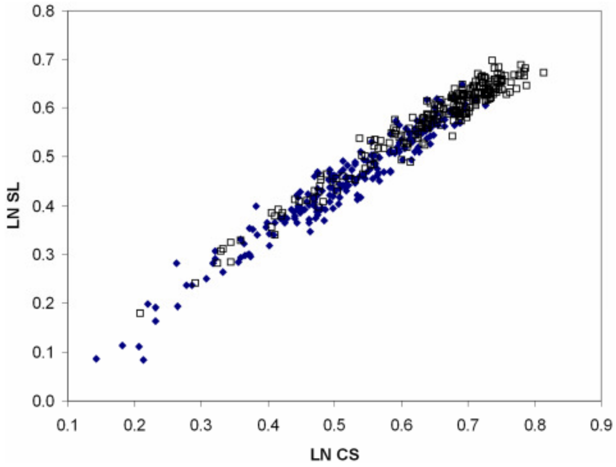

Fig. 1: Double-logarithmic plot of scape length SL against cephalic size CS in 215 workers of *Formica polyctena* (filled blue rhombi) and 243 workers of *F.rufa* (empty black squares). Condensing of data in the right-upper part of the graphics does not allow distinction of species.

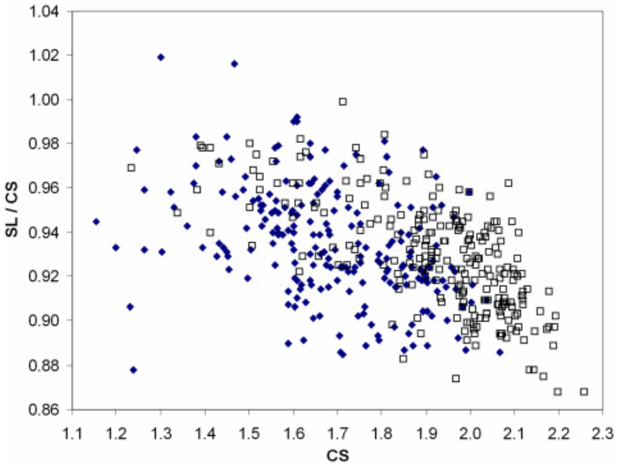

Fig. 2: Scape length index SL / CS plotted against cephalic size CS in 215 workers of *Formica polyctena* (filled blue rhombi) and 243 workers of *F. rufa* (empty black squares). Interspecific differences are much better resolved.

tinuously from the tip to the base, a smallest distance at base is not defined. In this case SPBA is measured at the level of the bottom of the interspinal meniscus.

SPST Distance between centre of propodeal stigma and spine tip. The stigma centre refers to the midpoint defined by the outer cuticular ring but not to the centre of stigma opening which may be positioned excentrically.

SPTI The distance of spine tips in dorsal view; if spine tips are rounded or thick, take the centres of spine tips as reference points.

sqPDG Square root of pubescence distance on dorsomedian part of first gaster tergite. The number of pubescence hairs n crossing a transverse measuring line of length L is counted, hairs just touching the line are counted as 0.5. The pubescence distance PDG is then given by L / n. Exact counting is promoted by clean surfaces and flat, reflexion-reduced illumination. The counting was performed at magnifications of 320×. Areas with torn-off pubescence were excluded. Four countings along four differently positioned measuring lines of ± 400 µm length are averaged. In order to normalize the positively skewed distributions, the square root of PDG is calculated.

Computation methods and reasons for their application

The allometric relation of two characters y and x is traditionally described by a power function or its logarithmic transformation (Huxley 1924, 1932; in ants used for instance by Wilson 1953 and Sudd & Franks 1987):

$$y = b * x^a \quad \text{or} \quad \log y = \log b + a * \log x \qquad [1]$$

Positive and negative allometries correspond to the case of a > 1 and a < 1, respectively, and isometry occurs when a = 1. Suggested advantages of log-transformation are reduction of heteroscedasticity and that weak monophasic allometry can often be described by a single linear function. However, in diphasic or triphasic allometry, as for instance in *Camponotus*, *Dorylus* and *Oecophylla*, in which the line "breaks" into two or three segments (Wilson 1953), a description by a single set of parameters is no longer possible.

Deviating from this tradition, Seifert (2002) described allometries as a linear function of the ratio of two characters y and x against a measure of body size (see also Wardiatno & Tamaki 2001). Cephalic length CL and cephalic width CW are measurements always taken in any morphometric study, irrespectively of which ant group is considered, and their direct measurement is usually not affected or hindered by other body structures in dry, mounted worker specimens, whereas this is more frequently the case with mesosoma length. I use the arithmetic mean of CL and CW, cephalic size CS, as a general, less-variable size measure. The linear relationship considered is:

$$y / x = a * CS + b \qquad [2]$$

This type of computation has been chosen for the following reasons:

(i) Positive, negative or absent allometries are indicated by positive, negative or zero slopes (Figs. 2, 4, 6) so that the form of allometry can directly be detected by visual inspection of scatter plots. This is only possible in logarithmic presentations (Figs. 1, 3, 5) when a subsidiary line with slope 1 is drawn.

(ii) In logarithmic descriptions, the correlation coefficient may be close to 1 also in fully isometric systems. Hence, it provides only information how well a certain data set is fitted. In formula [2] the correlation coefficient provides an estimate how significant an allometric relation is.

(iii) The use of simple, primary ratios y / x is most problematic because they may feign or conceal interspecific differences (Packard & Boardman 1999, Seifert 2002). However, the simplicity of forming them and the quite frequent cases in which they are not misleading will lead to their continued use at least in determination keys. Therefore it makes sense to continue to use indices – but only in a way that removes their principal disadvantage, which is size-dependent variance.

(iv) When comparing scatter plots of several ant species, the index / size plot has a better optical resolution than the double logarithmic plot because it avoids condensation

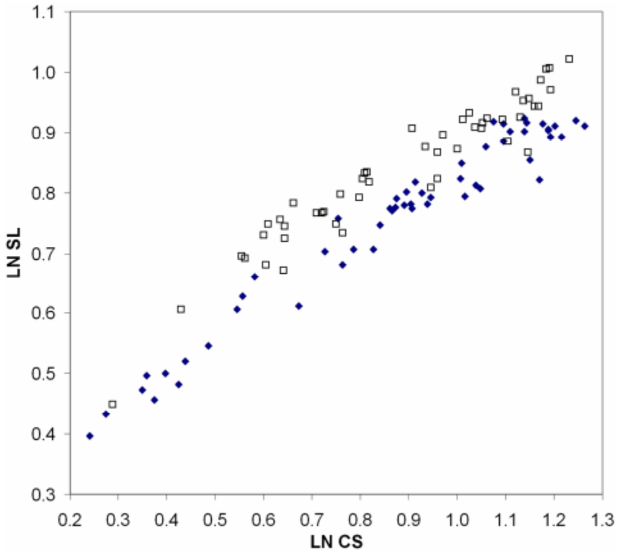

Fig. 3: Double-logarithmic plot of scape length SL against cephalic size CS in 54 workers of *Camponotus herculeanus* (filled blue rhombi) and 50 workers of *C. ligniperda* (empty black squares).

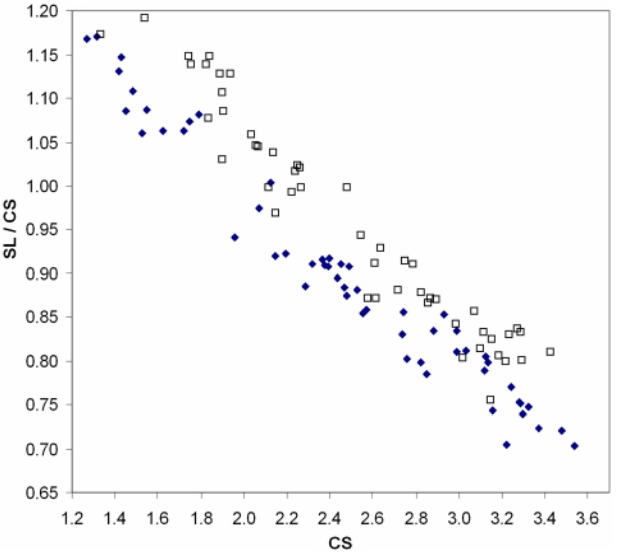

Fig. 4: Scape length index SL / CS plotted against cephalic size CS in 54 workers of *Camponotus herculeanus* (filled blue rhombi) and 50 workers of *C. ligniperda* (empty black squares).

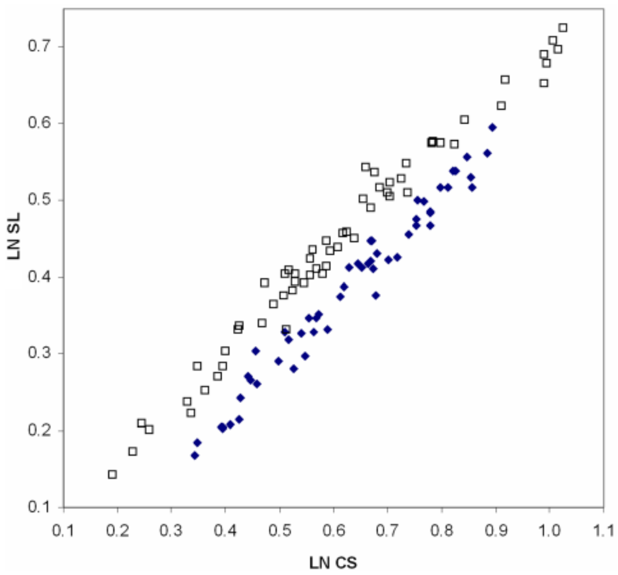

Fig. 5: Double-logarithmic plot of scape length SL against cephalic size CS in 60 workers of *Messor capitatus* (empty black squares) and 54 workers of *Messor* cf. *wasmanni* (filled blue rhombi).

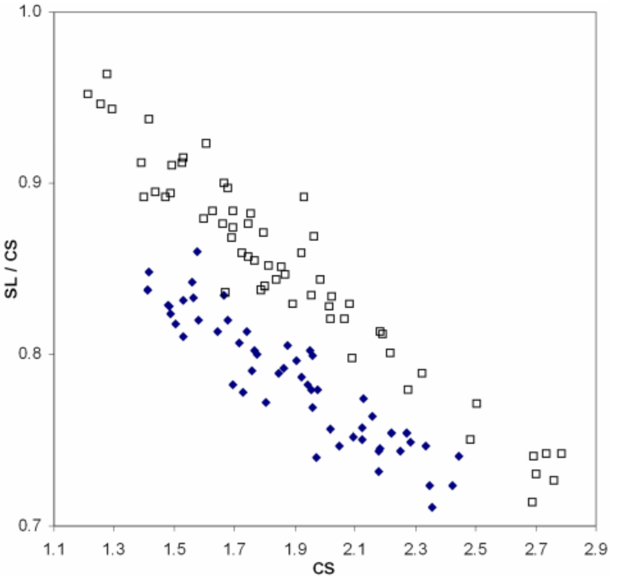

Fig. 6: Scape length index SL / CS plotted against cephalic size CS in 60 workers of *Messor capitatus* (empty black squares) and 54 workers of *Messor* cf. *wasmanni* (filled blue rhombi).

of data points in the right half of x-axis and upper half of y-axis. This advantage becomes obvious when rather many data points are presented: In the *F. polyctena* / *rufa* example the logarithmic presentation (Fig. 1) fails to show that *F. polyctena* has a lower average scape length than *F. rufa* in equally-sized individuals (Fig. 2).

(v) If the index denominator x is identical with the body size indicator CS, this "double" computation leads (as in the logarithmic approach) to linearisation of allometric functions in ant genera that do not show extreme allometries.

(vi) A number of metric (e.g., angles) or non-metric characters (e.g., seta numbers) may show zero values. Describing the allometries of such characters by logarithmic functions would necessitate to transform zero values to values above zero. This manipulation is not necessary in formula [2].

However, a fundamental objection may be presented against the unusual mathematic construction in formula [2]: The correlation between the error of the predictor and dependent variable could possibly produce damage when computing regression functions. Explicitly, measurement and natural errors of CS should reduce the slope of regression. Slope reduction is elucidated in more detail in the first part of the Results and discussion section and shown to be meaningless in the context of the RAV technique.

Box 1: Functions to remove allometric variance in diphasic allometries in the species *Camponotus ligniperda* and *C. herculeanus.*

$CL / CW_{2.5} = CL / CW / (-0.2016 * CS + 1.4832) * 0.9792$ for $CS \leq 2.605$
$CL / CW_{2.5} = CL / CW / (-0.0883 * CS + 1.1880) * 0.9792$ for $CS \geq 2.605$
$SL / CS_{2.5} = SL / CS / (-0.2622 * CS + 1.5294) * 0.9085$ for $CS \leq 2.137$
$SL / CS_{2.5} = SL / CS / (-0.1667 * CS + 1.3253) * 0.9085$ for $CS \geq 2.137$
$HTL / CS_{2.5} = HTL / CS / (-0.2841 * CS + 1.7389) * 1.0287$ for $CS \leq 2.534$
$HTL / CS_{2.5} = HTL / CS / (-0.1468 * CS + 1.3909) * 1.0287$ for $CS \geq 2.534$
$ML / CS_{2.5} = ML / CS / (-0.2330 * CS + 2.0496) * 1.4672$ for $CS \leq 2.591$
$ML / CS_{2.5} = ML / CS / (-0.1342 * CS + 1.7936) * 1.4672$ for $CS \geq 2.591$
$sqPDG / CS_{2.5} = sqPDG / CS / (-0.2284 * CS + 0.8540) * 0.3232$ for $CS \leq 2.259$
$sqPDG / CS_{2.5} = sqPDG / CS / (-0.0612 * CS + 0.4764) * 0.3332$ for $CS \geq 2.259$
$PLG / CS_{2.5} = PLG / CS / (-1.7521 * CS + 7.3026) * 2.9223$ for $CS \leq 2.531$
$PLG / CS_{2.5} = PLG / CS / (-0.5610 * CS + 4.28078 * 2.9223$ for $CS \geq 2.531$

When the allometries of two species A and B have been described according to function [2], a cumulative descriptor D is calculated by a function the parameters of which are the arithmetic mean of the species-specific parameters:

$$D = 1/2 * (a_A + a_B) * CS + 1/2 * (b_A + b_B) \quad [3]$$

This function offers a good description for both species when their allometries show a similar basic behaviour. This situation almost always applies because DAs are only used to separate most similar, closely related species with comparable morphological traits. However, similar basic behaviour is not required for each character considered in a DA. If, in the worst case, a character would show a negative allometry in species A and a positive one in species B, the cumulative descriptor function would occupy a neutral position and as result no RAV would be performed.

If the arithmetic mean of mean head sizes of species A and B is defined as standard size CS_S, a calibration constant C_{cal} is defined as:

$$C_{cal} = 1/2 * (a_A + a_B) * CS_S + 1/2 * (b_A + b_B) * CS_S \quad [4]$$

An allometrically corrected index value I_{CSS} is then computed for an individual ant i by division of its primary index value by the descriptor value and multiplication by the calibration constant:

$$I_{CSS} = [(Y_i / X_i) / D] * C_{cal} \quad [5]$$

I_{CSS} can be understood as an allometrically corrected value predicted for a situation in which each individual would have the standard head size CSS. The calibration constant does not change species discrimination but it offers a direct side-by-side comparison of primary and allometrically corrected ratios in synoptic tables. The misleading effects of primary indices are then visible at a quick glance.

Computation of diphasic allometries

Any description of allometries and any RAV intended to transform data for use in a discriminant process should start with a scatter plot of data points. This is recommended to avoid severe mistakes. Visual inspection of these scatter plots will provide indications whether there is an allometry at all, whether a single linear function is a sufficient descriptor or whether there is a diphasic or triphasic allometry requiring different functions to describe different size intervals (which is called piecewise regression). A scatter plot will also show at a quick glance which size intervals possibly have too few data points and which data points should be checked for measurement or recording errors.

A lot of differing approaches to perform piecewise regression have been introduced and examined (e.g., TISHLER & ZANG 1981, TOMS & LESPERANCE 2003, RYAN & PORTH 2007). These piecewise regression models are usually "broken-stick" models, where two or more lines are joined at unknown points, called "breakpoints" or "knots". Existing programs are either fully automatic or need the intuitive fixation of reference points which may be the value of the x variable at an assumed breakpoint. I wrote an own program for piecewise regression which is run after visual inspection of the scatter plot. It calculates both a single descriptor function for the whole size interval and two descriptor functions when the graphics suggests a diphasic behaviour. The program needs two intuitively determined input values defining the range of both regressions: the upper limit of the left and the lower limit of the right regression. A broader overlap range of the regressions is chosen when the number of data is not sufficient or when some strongly detached data points are expected to alter regression function in an abnormous way. On the other hand, the upper limit of the left regression range may coincide with the lower limit of the right regression range (no overlap) when number and distribution of data point allows this. When the two regression functions have been defined, the upper limit of the validity range of the left and the lower limit of the validity range of the right regression is given by the CS value at the intersection point of the two functions. The calibration constant C_{cal} is calculated by that function in which CS_S is situated.

I_{CSS} data in *C. herculeanus* and *ligniperda* were computed with CS_S fixed at 2.5 mm which is very close to the mean head size of both species (2.487 and 2.496 mm). The explicit functions are given in Box 1.

PLG allows a 100 % distinction of the sister species – both as primary absolute value or transformed into $PLG / CS_{2.5}$. Because of this overwhelming discriminative power,

Tab. 1: Description of allometries of a character ratio Y / CS by the formula Y / CS = a * CS + b in a set of 116 worker specimens of *Temnothorax crassispinus*. Given are arithmetic mean and standard deviation of a character ratio, its slope (a), ordinate intersection (b) and correlation coefficient (r) when regressing it against CS. Left columns show the unchanged primary data set and right columns data of 30 regressions in which an accessory error of ± 226 % was imposed on CS by random allocation of error values from a normally distributed data matrix.

character ratio	**primary regression**				**simulations with an accessory measurement error of 226 % of the primary error of CS**			
	mean SD	a	b	r	mean SD	a mean SD	b mean SD	r mean
CS	641.6 38.1				641.6 38.0			
SL / CS	0.7566 0.0152	-0.15629	0.85688	0.3920	0.7566 0.0152	-0.15736 0.00421	0.85757 0.00273	0.3927
FRS / CS	0.3629 0.0109	-0.00058	0.36324	0.0020	0.3629 0.0109	-0.00109 0.00273	0.36358 0.00178	0.0038
MW / CS	0.6017 0.0149	0.15579	0.50171	0.3992	0.6017 0.0148	0.15476 0.00482	0.50238 0.00311	0.3948
SPBA / CS	0.2982 0.0147	0.11791	0.22252	0.3052	0.2982 0.0147	0.11722 0.00283	0.22297 0.00183	0.3029
SPTI / CS	0.3521 0.0162	0.09429	0.29162	0.2218	0.3521 0.0161	0.09301 0.00306	0.29244 0.00198	0.2185
PEW / CS	0.2596 0.0110	0.04846	0.22855	0.1681	0.2596 0.0109	0.04783 0.00205	0.22895 0.00132	0.1656
PPW / CS	0.3683 0.0133	0.02767	0.35054	0.0792	0.3683 0.0133	0.02688 0.00250	0.35105 0.00162	0.0767
SP / CS	0.2622 0.0184	0.16186	0.15839	0.3363	0.2622 0.0183	0.16111 0.00271	0.15887 0.00175	0.3344
SPST / CS	0.3259 0.0178	0.04204	0.29890	0.0903	0.3259 0.0177	0.04179 0.00270	0.29906 0.00175	0.0896
MH / CS	0.5311 0.0141	0.01393	0.52219	0.0375	0.5311 0.0141	0.01319 0.00366	0.52267 0.00236	0.0354
ML / CS	1.1895 0.0185	0.07235	1.14312	0.1493	1.1895 0.0186	0.00704 0.00784	1.14436 0.00509	0.1434
PEL / CS	0.4831 0.0174	0.15398	0.38429	0.3371	0.4831 0.0173	0.15394 0.00167	0.38432 0.00107	0.3370
PEH / CS	0.3638 0.0111	0.01105	0.35669	0.0380	0.3638 0.0110	0.01100 0.00077	0.35889 0.00050	0.0380

inclusion of PLG into the study would equalize the results of the discriminant analyses quite independently from considering primary data or data sets generated by different transformation models. As a consequence, it was excluded from the discriminant analysis.

Discriminant analysis and performance testing

Four systems with different types of data (models) were tested:

(A) primary ratios CL / CW, SL / CS, ML / CS, HTL / CS, sqPDG / CS, and CS. Note: CS was introduced to include information about absolute size which otherwise would have been lost and CL / CS and CW / CS were excluded because of being reciprocal repeats of each other. Instead, CL / CW was introduced to compensate for this information loss.

(B) absolute data of CL, CW, SL, ML, HTL, and sqPDG.

(C) logarithmic transformations of CL, CW, SL, ML, HTL, and PDG.

(D) the RAV-corrected ratios CL / $CW_{2.5}$, SL / $CS_{2.5}$, HTL / $CS_{2.5}$, ML / $CS_{2.5}$, sqPDG / $CS_{2.5}$, and CS. Note the introduction of CS and CL / $CW_{2.5}$ and exclusion of CL / $CS_{2.5}$ and CW / $CS_{2.5}$ for the same reasons as stated in (A).

A canonical DA with a "Leave-One-Out Cross-Validation" analysis (LOOCV, LACHENBRUCH & MICKEY 1968, LESAFFRE & al. 1989) using a SPSS statistical package was run for all six single characters and all possible combinations of 2, 3, 4, 5, and 6 characters. The discriminative

Tab. 2: Description of allometries of a character ratio Y / CS by the formula Y / CS = a * CS + b in a set of 116 worker specimens of *Temnothorax crassispinus*. Given are arithmetic mean and standard deviation of a character ratio, its slope (a), ordinate intersection (b) and correlation coefficient (r) when regressing it against CS. Left columns show the unchanged primary data set and right columns data when the inclusive error of CS is removed.

character ratio	**primary regression including the inclusive error of CS**				**"ideal" regression with inclusive error of CS removed**			
	mean SD	a	b	error	mean SD	a	b	r
CS	641.6 38.1				641.6 37.7			
SL / CS	0.7566 0.0152	-0.15629	0.85688	0.00086 = 5.7 %	0.7565 0.0141	-0.13436	0.84271	0.3588
FRS / CS	0.3629 0.0109	-0.00058	0.36324	0.00055 = 5.0 %	0.3628 0.0105	0.01385	0.35389	0.0496
MW / CS	0.6017 0.0149	0.15579	0.50171	0.00171 = 11.5 %	0.6017 0.0120	0.20074	0.47288	0.6288
SPBA / CS	0.2982 0.0147	0.11791	0.22252	0.00093 = 6.3 %	0.2981 0.0141	0.14242	0.20674	0.3804
SPTI / CS	0.3521 0.0162	0.09429	0.29162	0.00094 = 5.8 %	0.3520 0.0155	0.11870	0.27588	0.2893
PEW / CS	0.2596 0.0110	0.04846	0.22855	0.00073 = 6.6 %	0.2596 0.0102	0.06768	0.21618	0.2491
PPW / CS	0.3683 0.0133	0.02767	0.35054	0.00083 = 6.2 %	0.3682 0.0124	0.04945	0.33651	0.1498
SP / CS	0.2622 0.0184	0.16186	0.15839	0.00056 = 3.0 %	0.2622 0.0181	0.17665	0.14891	0.3668
SPST / CS	0.3259 0.0178	0.04204	0.29890	0.00059 = 3.3 %	0.3258 0.0174	0.05760	0.28888	0.1247
MH / CS	0.5311 0.0141	0.01393	0.52219	0.00183 = 13.0 %	0.5310 0.0107	0.06212	0.49113	0.2186
ML / CS	1.1895 0.0185	0.07235	1.14312	0.00224 = 12.1 %	1.1894 0.0133	0.13101	1.10547	0.3705
PEL / CS	0.4831 0.0174	0.15398	0.38429	0.00129 = 7.4 %	0.4830 0.0161	0.18799	0.36236	0.4389
PEH / CS	0.3638 0.0111	0.01105	0.35669	0.00097 = 8.7 %	0.3637 0.0098	0.03640	0.34033	0.1403

performance of the models was indicated by the mean error, i.e., the degree of rejection of the a-priori hypothesis by the LOOCV analysis. Significance testing was done by a Mann-Whitney (U) test comparing the distribution of error rates for combinations of 1 - 5 characters and of a-posteriori probabilities for 6 characters where only one solution in each model exists.

Results and discussion

Effect of measurement error and natural variation of CS in regressions of the type Y / CS = a * CS + b

The major objection against formula [2] should be that it entails a correlation between the error of the predictor and dependent variable which could produce havoc when computing regression functions. The theory predicts that errors of CS should reduce the slope. I have tested if this effect is of practical significance.

116 workers of the ant *Temnothorax crassispinus* and 109 of *T. nylanderi* were selected. *Temnothorax* species show isometry or moderate monophasic allometries which allows a description by a single regression line, and they are small-sized which results in a larger relative measurement error. Expressed as standard deviation, the measurement error of CL and CW in this genus is about 1.0 µm, and that of CS is ± 0.7 µm (SEIFERT 2006b). Imposing an accessory mean measurement error of ± 2.26 µm on the data of CL and CW by random selection from a table of normally distributed error values should represent a hard

Tab. 3: Description of allometries of a character ratio Y / CS by the formula Y / CS = a * CS + b in a set of 109 worker specimens of *Temnothorax nylanderi*. Arrangement of data as in Tab. 2.

character ratio	**primary regression including the inclusive error of CS**				**"ideal" regression with inclusive error of CS removed**			
	mean SD	a	b	error	mean SD	a	b	r
CS	649.4 33.7				649.4 33.2			
SL / CS	0.7501 0.0141	-0.19153	0.87447	0.00114 = 8.1 %	0.7501 0.0123	-0.15771	0.85248	0.4279
FRS / CS	0.3615 0.0084	-0.03058	0.38135	0.00051 = 6.1 %	0.3614 0.0079	-0.01549	0.37148	0.0651
MW / CS	0.6036 0.0119	0.18247	0.48512	0.00126 = 10.6 %	0.6036 0.0105	0.21997	0.46076	0.6991
SPBA / CS	0.2688 0.0114	0.03835	0.24385	0.00065 = 5.7 %	0.2688 0.0110	0.05758	0.23136	0.1742
SPTI / CS	0.3120 0.0152	0.05926	0.27353	0.00035 = 2.3 %	0.3120 0.0152	0.06950	0.26689	0.1524
PEW / CS	0.2555 0.0092	0.08176	0.20240	0.00044 = 4.8 %	0.2555 0.0092	0.09477	0.19396	0.3438
PPW / CS	0.3757 0.0126	0.13262	0.28953	0.00044 = 3.5 %	0.3756 0.0126	0.14552	0.28114	0.3842
SP / CS	0.2062 0.0169	0.14758	0.11040	0.00005 = 0.3 %	0.2062 0.0171	0.14668	0.11094	0.2848
SPST / CS	0.2757 0.0163	0.08207	0.22238	0.00027 = 1.7 %	0.2757 0.0164	0.09003	0.21721	0.1835
MH / CS	0.5301 0.0138	-0.06037	0.56929	0.00165 = 12.0 %	0.5299 0.0111	-0.01130	0.53727	0.0334
ML / CS	1.1807 0.0212	0.17516	1.06690	0.00235 = 11.1 %	1.1805 0.0175	0.24489	1.02149	0.4640
PEL / CS	0.4785 0.0154	0.20509	0.34527	0.00072 = 4.7 %	0.4784 0.0151	0.22648	0.33133	0.4977
PEH / CS	0.3663 0.0098	0.05103	0.33317	0.00050 = 5.1 %	0.3663 0.0096	0.06579	0.32354	0.2275

test of the system. Table 1 shows for *T. crassispinus* that even this treatment did not change slope, ordinate intersection and regression coefficients of 13 character ratios. The slopes were very slightly decreased and ordinate intersection very slightly raised in each case and the average reduction of regression coefficient was only 0.18 %. This shows that even larger measurement errors of CS are unimportant when allometries in ants are described by formula [2].

But is this also the case when the natural "error" of CS adds to the measurement error? The natural error of CS can be understood as genetically or environmentally caused deviation from an average situation that is a function of "overall body size". It is to some degree a matter of philosophy which absolute metric measures are considered as most adequate indicators of overall body size. The selected characters anyway should have a highly positive correlation. Among the 18 metric characters measured the following six characters showed the highest mean linear correlation coefficient to each other in both species: ML (0.948), CW (0.945), CL (0.937), MW (0.934), MH (0.908) and SL (0.908). It is probably a good choice also to include the latter two less correlated characters because they compensate for the "optimism" introduced by CL and CW which inevitably must have a high correlation with CS (here 0.991). When defining overall body size (SIZE) as geometric mean of these six characters, a standard value of head size CS_{sta} can be described in *T. crassispinus* by the following function:

$$CS_{sta} = 1.18380 * SIZE + 0.02464 \quad (r = 0.9877,\ n = 116) \qquad [6a]$$

The corresponding function in *T. nylanderi* is:

$CS_{sta} = 1.19094 * SIZE + 0.02274$ (r = 0.9872, n = 109) [6b]

The inclusive error dCS, combining measurement and natural error of CS, is then given by:

$dCS = CS - CS_{sta}$ [7]

The inclusive error was normally distributed in the given data set in both species with a mean of ± 5.97 µm in *T. crassispinus*, and ± 5.38 µm in *T. nylanderi*. Subtracting dCS from CS results in an "ideal" CS. When all ratios are calculated anew with this ideal CS as denominator, we have a data set for testing how the inclusive error should affect the slopes of regression lines in formula-[2]-type functions. Tables 2 and 3 show that the effect of inclusive error on decreasing the slopes is much more obvious than that of the measurement error. Compared to the ideal data set, expressed as percentage of the primary standard deviation and as mean for 13 character ratios y / CS, the primary regression increases the standard deviation by 7.3 % in *T. crassispinus*, and by 5.8 % in *T. nylanderi*. This seems substantial. However, would an increased noise compared to an ideal data set really translate into an average prediction error? During the revising process of this manuscript several referees suggested that a logarithmic description of allometries according to formula [1] should be better because no slope bias is expected here. We can assess the practical effect of slope reduction if we calculate absolute measurements both with a transformation of formula [1]:

$y = \exp(\log b + a * \log x)$ [8]

and a transformation of formula [2]:

$y = (a * CS + b) * CS$ [9]

Absolute measurements y predicted by the parameters of the index formula [2] should then be larger in very small workers and lower in very large workers compared to those predicted by parameters of the log-formula [1]. The data presented in Table 4 (*Temnothorax*) and Table 5 (*Formica*) show that this is definitely not the case. The predictions are practically coincident and deviations more likely to represent rounding errors. In *Temnothorax*, formula [9] predicts very small workers to have on average 0.20 % larger values and very large workers to have 0.09 % larger values. Almost the same result was achieved in a data set with eleven *Formica* species: small workers were predicted to have 0.02 % smaller and large workers 0.22 % smaller values. This is an almost negligible slope reduction.

As a consequence, there are no indications for a prediction error by formula [2]. Even if somebody continues to doubt this: the important point in the present context is that character ratios Y / CS are divided by the descriptor value (function [5]). This process in itself means a correction against any real or hypothetic slope reduction. The RAV technique primarily aims to improve the performance of discriminant procedures by reducing interspecific character overlap and the question of slope reduction is of only theoretic interest in this context.

Tab. 4: Prediction of absolute measurements Y by the transformed log-formula $Y = \exp(\log b + a * \log x)$ and by the transformed index-formula $Y = (a * CS + b) * CS$ in *Temnothorax crassispinus* and *T. nylanderi.*

	CS = 0.520 mm		**CS = 0.640 mm**		**CS = 0.710 mm**	
	log	index	log	index	log	index
Temnothorax nylanderi (n = 116)						
SL	0.404	0.403	0.484	0.484	0.530	0.530
FRS	0.189	0.189	0.232	0.232	0.257	0.258
MW	0.302	0.303	0.385	0.385	0.434	0.435
SPBA	0.147	0.148	0.191	0.191	0.217	0.217
SPTI	0.176	0.177	0.225	0.225	0.254	0.255
PEW	0.132	0.132	0.166	0.166	0.186	0.187
PPW	0.190	0.190	0.236	0.236	0.263	0.263
SP	0.125	0.126	0.167	0.168	0.194	0.194
SPST	0.166	0.167	0.208	0.209	0.233	0.233
MH	0.275	0.275	0.340	0.340	0.378	0.378
ML	0.614	0.614	0.761	0.761	0.848	0.848
PEL	0.241	0.241	0.309	0.309	0.350	0.350
PEH	0.188	0.188	0.233	0.233	0.259	0.259
Temnothorax crassispinus (n = 109)						
SL	0.404	0.403	0.481	0.481	0.525	0.524
FRS	0.190	0.190	0.231	0.232	0.255	0.255
MW	0.301	0.302	0.385	0.385	0.436	0.436
SPBA	0.137	0.137	0.172	0.172	0.192	0.192
SPTI	0.158	0.158	0.199	0.199	0.224	0.224
PEW	0.127	0.127	0.163	0.163	0.185	0.185
PPW	0.186	0.186	0.240	0.240	0.272	0.272
SP	0.096	0.097	0.131	0.131	0.152	0.153
SPST	0.137	0.138	0.176	0.176	0.199	0.199
MH	0.280	0.280	0.339	0.340	0.374	0.374
ML	0.601	0.602	0.755	0.755	0.845	0.846
PEL	0.234	0.235	0.305	0.305	0.348	0.349
PEH	0.187	0.187	0.234	0.234	0.262	0.262

Camponotus: Removal of allometric variance strongly improves discriminative power at the single-character level

Table 6 shows arithmetic mean, standard deviation, minimum and maximum values, the coefficient of variation and ratio of individuals placed within the empiric interspecific overlap range for 7 characters and their transformations. Indexing of primary characters against CS increases the mean within-species coefficient of variation from 13.0 % in

Tab. 5: Prediction of absolute measurements Y by the transformed log-formula Y = exp (log b + a * log x) and by the transformed index-formula Y = (a * CS + b) * CS in eleven *Formica* species.

	CS = 1.1 mm		**CS = 1.7 mm**	
	log	index	log	index
Formica candida (n = 64)				
CL	1.171	1.172	1.762	1.753
SL	1.158	1.158	1.700	1.686
PEW	0.452	0.453	0.776	0.784
EYE	0.321	0.321	0.449	0.440
Formica clara (n = 64)				
CL	1.186	1.185	1.794	1.793
SL	1.217	1.215	1.793	1.790
PEW	0.481	0.484	0.820	0.823
EYE	0.341	0.340	0.472	0.470
Formica clarissima (n = 168)				
CL	1.182	1.182	1.780	1.774
SL	1.177	1.176	1.693	1.676
PEW	0.457	0.457	0.809	0.820
EYE	0.323	0.323	0.451	0.445
Formica cunicularia (n = 250)				
CL	1.190	1.189	1.776	1.773
SL	1.229	1.225	1.755	1.748
PEW	0.473	0.474	0.845	0.853
EYE	0.348	0.346	0.490	0.488
Formica fusca (n = 69)				
CL	1.195	1.194	1.788	1.784
SL	1.220	1.218	1.774	1.766
PEW	0.452	0.454	0.767	0.771
EYE	0.349	0.348	0.492	0.489
Formica lemani (n = 94)				
CL	1.199	1.199	1.786	1.780
SL	1.159	1.158	1.701	1.694
PEW	0.461	0.462	0.758	0.762
EYE	0.331	0.331	0.472	0.468
Formica litoralis (n = 194)				
CL	1.175	1.175	1.799	1.797
SL	1.181	1.181	1.728	1.715
PEW	0.446	0.447	0.792	0.799
EYE	0.324	0.324	0.452	0.446
Formica lusatica (n = 570)				
CL	1.183	1.182	1.789	1.788
SL	1.231	1.227	1.781	1.778
PEW	0.479	0.482	0.839	0.843
EYE	0.339	0.337	0.469	0.467
Formica pamirica (n = 103)				
CL	1.183	1.183	1.796	1.792
SL	1.187	1.186	1.754	1.746
PEW	0.415	0.417	0.755	0.762
EYE	0.328	0.327	0.453	0.447
Formica rufibarbis (n = 191)				
CL	1.188	1.186	1.788	1.786
SL	1.211	1.207	1.754	1.750
PEW	0.497	0.498	0.834	0.837
EYE	0.341	0.339	0.472	0.470
Formica subpilosa (n = 159)				
CL	1.190	1.190	1.803	1.800
SL	1.208	1.207	1.785	1.779
PEW	0.424	0.425	0.670	0.672
EYE	0.322	0.321	0.448	0.445

primary data to 15.0 % and the mean interspecific overlap ratio from 56.9 % to 72.3 %. These data illustrate that simple indexing of characters does not increase the discriminative power on single-character level in genera with very strong allometries. This contrasts with the situation in genera with weak or moderate allometries such as *Temnothorax*, *Myrmica*, *Lasius* and *Formica* where indexing reduces interspecific overlap (SEIFERT 1992, 1997, 2003a, 2003b, 2004a, 2005, 2006a).

Removal of allometric variance (RAV), however, reduces the mean within-species coefficient of variation to 4.7 % and the mean empiric interspecific overlap ratio to 21.0 %. The latter figure indicates a 2.7fold mean improvement in interspecific discrimination by RAV on the single-character level when compared to primary data.

Camponotus: Removal of allometric variance significantly improves discriminative power in a multi-character discriminant analysis

Table 7 shows the mean error rate in a LOOCV discriminant analysis for all possible combinations of different character numbers. Compared to computations with primary data, the discriminative power is reduced by indexing against CS but clearly increased in log-transformed and RAV data. The difference of log-transformed and RAV data on one side against primary and CS-indexed data on the other side is significant with $p < 0.0005$ for each possible character combination.

RAV has a significantly higher performance against log-transformation for all possible combinations of 1 to 5 characters ($p < 0.005$). However, the performance practically equalises at 6 characters: the error rate is zero in both systems and the distributions of a-posteriori probabilities are not significantly different, although a weak residual advantage of RAV is indicated by the higher a-posteriori probability in the most uncertain individual – 0.993 in RAV-corrected versus 0.896 in log-transformed data.

Tab. 6: Morphometric data of worker specimens of *Camponotus* sister species for seven characters and three different types of data: primary (absolute) measures (yellow background), index ratios (pink background) and index ratios with removed allometric variance as prediction for the condition that each specimen has a head size of 2.5 mm (blue background). Given are arithmetic mean, standard deviation, minimum and maximum values, coefficient of variation and ratio of individuals placed within the empiric interspecific overlap range.

character ratio	***Camponotus herculeanus*** (n = 54)		***Camponotus ligniperda*** (n = 50)		**empiric overlap**
	mean ± SD [min, max]	coeff. var.	mean ± SD [min, max]	coeff. var.	
CS [mm]	2.487 ± 0.646 [1.272, 3.537]	26.0	2.496± 0.557 [1.336, 3.428]	22.3	96.2 %
CL [mm]	2.418 ± 0.530 [1.364, 3.277]	21.9	2.536 ± 0.459 [1.467, 3.274]	18.1	97.1 %
CW [mm]	2.555 ± 0.764 [1.180, 3.797]	29.9	2.456 ± 0.658 [1.205, 3.582]	26.8	96.2 %
SL [mm]	2.151 ± 0.303 [1.487, 2.516]	14.1	2.325 ± 0.258 [1.568, 2.777]	11.1	85.6 %
HTL [mm]	2.389 ± 0.359 [1.586, 2.901]	15.0	2.767 ± 0.340 [1.877, 3.325]	12.3	70.2 %
ML [mm]	3.498 ± 0.702 [2.059, 4.584]	20.1	3.778 ± 0.578 [2.334, 4.672]	15.3	92.3 %
sqPDG [µm]	7.38 ± 0.72 [5.39, 8.83]	9.8	9.00 ± 0.84 [7.81, 11.40]	9.3	36.5 %
PLG [µm]	97.5 ± 12.0 [76.6, 123.0]	12.3	62.7 ± 6.4 [46.7, 72.6]	10.2	0.0 %
CL / CW	0.975 ± 0.098 [0.848, 1.168]	10.1	1.059 ± 0.104 [0.911, 1.242]	9.8	71.1 %
SL / CS	0.898 ± 0.131 [0.703, 1.171]	14.6	0.958 ± 0.123 [0.756, 1.192]	12.8	89.4 %
HTL / CS	0.994 ± 0.135 [0.761, 1.272]	13.6	1.136 ± 0.131 [0.945, 1.409]	11.5	68.3 %
ML / CS	1.432 ± 0.106 [1.272, 1.677]	7.4	1.539 ± 0.123 [1.363, 1.778]	8.0	73.1 %
sqPDG / CS [* 10^{-2}]	0.315 ± 0.081 [0.198, 0.567]	25.7	0.375 ± 0.069 [0.276, 0.594]	18.4	78.8 %
PLG / CS [* 10^{-2}]	4.16 ± 1.03 [2.65, 3.54]	24.8	2.64 ± 0.67 [1.34, 3.43]	25.4	51.9 %
CL / $CW_{2.5}$	0.949 ± 0.017 [0.909, 0.978]	1.8	1.035 ± 0.025 [0.983, 1.090]	2.4	0.0 %
SL / $CS_{2.5}$	0.882 ± 0.024 [0.813, 0.938]	2.7	0.947 ± 0.032 [0.858, 1.004]	3.4	63.5 %
HTL / $CS_{2.5}$	0.960 ± 0.031 [0.897, 1.028]	3.2	1.102 ± 0.031 [1.029, 1.166]	2.8	0.0 %
ML / $CS_{2.5}$	1.410 ± 0.033 [1.318, 1.476]	2.3	1.517 ± 0.032 [1.405, 1.589]	2.1	39.4 %
sqPDG / $CS_{2.5}$ [* 0^{-2}]	0.289 ± 0.023 [0.225, 0.333]	8.0	0.351 ± 0.024 [0.308, 0.416]	6.8	23.1 %
PLG / $CS_{2.5}$ [* 0^{-2}]	3.78 ± 0.41 [3.04, 4.82]	10.8	2.41 ± 0.24 [1.80, 2.79]	10.0	0.0 %

Conclusions

The results show that RAV based upon regression of character ratios (indices) against body size may considerably improve species discrimination in ants when only single or very few characters are available or when a reduction of character number is indicated for reasons of limited working capacity or for mathematical reasons such as indicated by MODER & al. (2007). An advantage of RAV-corrected data in multi-character DAs becomes only evident when no characters of very strong discriminative power are known, when allometries are very strong and when the number of considered characters is comparably low. The superior performance of RAV-transformed data is probably lost in DAs computing > 10 characters even when most similar organisms are considered but this prediction remains to be checked. This would require as test organisms a pair of highly similar cryptic species with very strong allometries. At the moment there are no data sets of such test organisms available but the Central European sister species of *Messor* cf. *structor* (SCHLICK-STEINER & al. 2006) could make a good study system.

Tab. 7: Mean error rates ± standard deviation of error after LOOCV discriminant analysis and generation of all possible character combinations for sets of 1, 2, ... 6 characters.

number of characters	1	2	3	4	5	6
number of possible character combinations	6	15	20	15	6	1
(a) primary data indexed against CS	33.32 ± 4.53	19.23 ±11.92	11.00 ±10.63	4.69 ± 6.72	2.07 ± 0.92	1.0
(b) primary data	38.12 ±11.46	10.71 ± 8.77	3.42 ± 3.00	1.49 ± 1.29	1.30 ± 0.46	1.0
(c) log-transformation of primary data	37.80 ±12.26	9.82 ± 7.93	3.21 ± 2.90	1.35 ± 1.61	0.50 ± 0.55	0.0
(d) RAV-corrected data	8.35 ± 8.78	3.01 ± 2.98	1.44 ± 1.51	0.65 ± 1.19	0.00 ± 0.00	0.0
one-sided U test system (c) vs. (d)	0.005	0.0005	0.0001	0.0001	0.0001	0.20

Apart from the question of how RAV contributes to species discrimination, which varies from significant improvement to neutrality, RAV-transformed index data have one constant advantage: they expose diagnostic characters and unmask pseudo-characters, i.e., they show interspecific differences rather independent from environmental factors that may have influenced larval growth and resulted in different worker body size. For this reason, execution of RAV according to the working routine described above and side-by-side presentation of data from different species in synoptic tables is clearly recommendable in taxonomic practice. It must be pointed out that any RAV analysis should start with a graphical analysis.

In spite of theoretical predictions for a negative slope bias in the RAV-formula based upon regression of indices against body size, it was not possible to find any practical disadvantage or misindication compared to the traditional allometry formula of HUXLEY (1924, 1932). Some of the advantages of the RAV-index-regression over the double logarithmic regression, as they were expressed under the points (i) to (vi) in the section "The computation methods and reasons for their application", may be gradual or relative but others are clearly given and the inclusive advantage of the presented RAV-methodology is evident.

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Zusammenfassung

Das Entfernen allometrischer Varianz (RAV) morphologischer Daten, das auf der Regression von Indices gegen die Körpergröße basiert, kann die Artunterscheidung beträchtlich verbessern, wenn nur einzelne oder wenige Merkmale zur Verfügung stehen oder berücksichtigt werden können. Dieser klare Vorteil von RAV geht bei viele Merkmale berücksichtigenden Diskriminanzanalysen innerhalb der moderat allometrischen Ameisengattungen *Hypoponera*, *Temnothorax*, *Cardiocondyla*, *Myrmica*, *Lasius* und *Formica* verloren. Um nachzuprüfen, ob sich dieses negative Ergebnis auch bei stark allometrischen Organismen wiederholt, wurden zwei Schwesterarten des Ameisengenus *Camponotus* untersucht: *C. ligniperda* (LATREILLE, 1802) und *C. herculeanus* (LINNAEUS, 1758). Es wurden die Ergebnisse von Diskriminanzanalysen verglichen, die Absolutmaße, einfache Indices, logarithmisch transformierte Absolutmaße und RAV-korrigierte Indices verrechneten. Auf der Ebene der Einzelmerkmale war die mittlere interspezifische Überlappungsrate am größten bei einfachen Indices (71.2 %) und am geringsten bei RAV-korrigierten Indices (21.0 %). In multiplen, zwei bis fünf Merkmale berücksichtigenden Diskriminanzanalysen zeigten RAV-korrigierte Indices im Vergleich zu logarithmisch transformierten Absolutmaßen eine signifikant höhere Auftrennung ($p < 0.005$). Bei Verrechnung von sechs Merkmalen war der Vorteil schon sehr gering geworden, und man muss erwarten, dass er bei noch höheren Merkmalszahlen ganz verloren geht. Für extrem ähnliche und stark allometrische Arten, die als Testsystem nicht vorlagen, kann nicht ausgeschlossen werden, dass RAV auch bei Merkmalszahlen um 10 noch Gewinne bringt. Abgesehen von ihrem schwankenden Beitrag zu einer verbesserten Artunterscheidung haben RAV-Daten den konstanten Vorteil, dass sie diagnostische Merkmale hervortreten lassen bzw. Pseudomerkmale demaskieren, d.h. sie zeigen interspezifische Unterschiede unabhängig von Umwelteinflüssen, die die Körpergröße von Arbeiterinnen beeinflusst haben können. Daher ist die vergleichende Darstellung von RAV-korrigierten Daten in der taxonomischen Praxis immer zu empfehlen. Die Arbeit erläutert Einzelschritte der RAV-Methode und macht Vorschläge zur Behandlung diphasischer Allometrien. Es wird empfohlen, jede RAV-Prozedur mit einer graphischen Analyse zu beginnen. Voraussagen, dass Messfehler und die natürliche Variation des Körpergrößenmaßes CS den Anstieg der RAV-Index-Funktion vom Typ $Y / CS = a * CS + b$ senken, wurden durch Simulationen bestätigt. Diese Reduzierung des Anstieges bleibt jedoch folgenlos, weil der zweite Schritt der RAV-Prozedur diesen Fehler vollständig kompensiert. Zusätzlich wurde für 13 monophasische Allometrien zeigende *Temnothorax*- und *Formica*-Arten festgestellt, dass Voraussagen von Absolutmaßen durch die RAV-Index-Funktion bzw. durch die traditionelle logarithmische Allometriebeschreibung vom Typ $Y = \log b + a * \log x$ nahezu identische Ergebnisse bringen.

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