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Analyzing large-scale and intranidal phenotype distributions in eusocial Hymenoptera – a taxonomic tool to distinguish intraspecific dimorphism from heterospecificity

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Abstract

Ant and termite nests are long-term stable, semi-closed systems constantly producing conspecific worker populations of related individuals over many generations. Accordingly, nests of these eusocial insects, as they are found in nature, offer free of cost an analysis situation that has to be generated in other groups of organisms by controlled rearing experiments. A test system based on analyzing intranidal and large-scale phenotype distributions and comparing the observed distributions with predictions for different scenarios of heterospecificity and intraspecific dimorphism is introduced by a case study on ants. The test system, named DIMORPH test, allows a taxonomist to distinguish if discrete character syndromes represent separate species or an intraspecific phenomenon. One of the most important parameters within the test system is the abundance and distribution of phenotypically mixed nest populations. Five biological explanations are possible for ant nests with a mixture of discrete phenotypes: They may represent (1) genetically determined intraspecific morphs, (2) intraspecific modifications induced by environmental factors, (3) the association of a temporary social parasite with a host species, (4) the association of a permanent social parasite with a host species, and (5) a parabiotic association of two basically independent (self-sustaining) species. The paper explains the biological background of the scenarios (1) to (5) and presents mathematical models and generalizations from empirical data to predict phenotype distributions for each scenario under variable conditions. Four cases of intraspecific dimorphism and five cases of taxonomically recognized pairs of cryptic or similar species are presented and analyzed. The observed intranidal phenotype distribution was most similar to the predicted scenario of intraspecific dimorphism in *Camponotus lateralis* (OLIVIER, 1792), *Lasius umbratus* (NYLANDER, 1846), *Formica lugubris* ZETTERSTEDT, 1838, and *Cardiocondyla elegans* EMERY, 1869. In three of these examples, intraspecific morphs had been considered previously as different species. Heterospecificity was confirmed for four pairs of cryptic species and one pair of closely related species: *Formica pressilabris* NYLANDER, 1846 vs. *F. foreli* BONDROIT, 1918, *Temnothorax crassispinus* (KARAVAJEV, 1926) vs. *T. crasecundus* SEIFERT & CSÖSZ, 2015, *Temnothorax luteus* (FOREL, 1874) vs. *T. racovitzai* (BONDROIT, 1918), two cryptic species of the *Pheidole pallidula* (NYLANDER, 1849) complex, and *Myrmica vandeli* BONDROIT, 1920 vs. *M. scabrinodis* NYLANDER, 1846. The phenotype-based DIMORPH test can be applied to the large worldwide collections of mounted museum material or private collections of ants independent from age or DNA degradation and can thus operate in fields where genetic investigation faces analytical and logistic problems and where controlled rearing experiments are not possible. The system can be adapted, with some modification, to other groups of eusocial organisms.

Key words: Numeric morphology-based alpha-taxonomy, pleiotropy, intraspecific polymorphism, parabiosis, temporary social parasitism, permanent social parasitism.

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Introduction

There is some automatism for taxonomists to suppose heterospecificity for clearly separable phenotypes when they show a degree and type of differences as they are usual between species in the group of organisms under study. Such expectations will prove true in the majority of cases. Yet, the task of taxonomists is not finished at this stage. They should develop a sense for the unexpected, and sometimes the unexpected is not the very exception. The Pragmatic Species Concept (SEIFERT 2014) recommends as a taxonomic working routine to check if distinct phenotypes could represent an intraspecific polymorphism. Morphology-based taxonomists cannot a priori fulfill this requirement when studying a group of organisms living solitarily, in temporary pairs or in random instable associations such as swarms of fishes or herds of antelopes. A taxonomist cannot a priori distinguish between heterospecificity and intraspecific dimorphism if there are a number of more thickset fishes with more greenish iridescence and more pointed pectoral fins in a swarm of normal herrings. In such groups of organisms, the decision process essentially needs information from sources other than morphology. These are in the best case a combination of nuDNA genetics with

long-term observation of mating and reproduction behavior in nature or in laboratory.

Eusocial insects, in contrast, are an ideal object for taxonomic a priori assessment. They provide sources of information not given in other groups of organisms. Nests of ants, as they are found in nature, offer "free of charge" an analysis situation that has to be generated in other groups of organisms by controlled rearing experiments. That is, ant nests are long-term stable, semi-closed systems constantly producing conspecific worker populations of related individuals over many generations. Furthermore, collecting workers in sufficient numbers is possible at any time of the field season. If clean nest samples with several workers per nest are collected and if these samples are available from a reasonably large geographic area, a particular analysis becomes possible. The taxonomist can analyse within-nest and large-scale phenotype distribution and can compare the observed distribution with predictions for different scenarios of heterospecificity and intraspecific dimorphism.

These analyses are introduced in this paper. They aim to prevent taxonomists from misinterpreting discrete intraspecific character syndromes as separate species. This goal of distinguishing intraspecific dimorphism from heterospecificity is realistic when the taxonomist has access to a sufficiently large number of samples from a sympatric area. Beyond that, the paper wants to develop a sense among ant taxonomists which scenarios have to be considered but it will not claim that the precise nature of a heterospecific intranidal association could be determined by analyses of within-nest and overall phenotype distributions alone. The analyses and considerations of biological backgrounds presented here refer to ants but are likely to be translated with some modification to other groups of eusocial organisms.

Background knowledge and the development of predictions

The taxonomists' view on syndromes of discrete characters and their supposed genetic background: The danger of a taxonomic misinterpretation of intraspecific phenotype polymorphism varies with the form in which it occurs. If we consider syndromes of multiple characters and not a single character – such as a white, pink and red color of Gregor Mendel's flowers which represent three discrete phenotypes – intraspecific polymorphism based on codominant inheritance of phenotypic characters does not present a serious problem. The high frequency of intermediate forms in multi-character cases and the poorly defined borders between phenotypes in attempts to cluster these by exploratory and hypothesis driven analyses will reduce the probability of considering the homozygous genotypes as different species. More dangerous are discrete characters controlled by dominant-recessive inheritance or other mechanisms. If there is a discrete difference in only a single character, such as a simple dark / light color difference, cautious taxonomists more readily consider the possibility of intraspecific dimorphism. They are usually aware that a single point mutation may cause such a change as shown, for example, by LUS (1932), BARRION & SAXENA (1987), MAJERUS (1998), ANDRES & CORDERO (1999), MAJUMDAR & al. (2008). They know that intraspecific morphs, either directly inherited or environment-induced, have frequently been described as good species. Famous textbook examples are *Panthera melas* (CUVIER, 1809) which is a taxonomic name for the black mutant of the Leopard *Panthera pardus* (LINNAEUS, 1758) or *Araschnia prorsa* (LINNAEUS, 1758) that is a name for an environment-induced modification of the butterfly *Araschnia levana* (LINNAEUS, 1758).

The largest risks for taxonomists are presented by systems of correlated, discrete characters which occur as a syndrome. This can be explained by an example. There is a Mediterranean *Camponotus* MAYR, 1861 carpenter ant in which profuse scape pilosity, more linear frontal carinae, a weakly developed scape base extension, a shorter scape, a shorter propodeum and a broader head occur in a fixed combination and there is another, apparently closely related *Camponotus* ant in which missing scape pilosity, sinusoidally formed frontal carinae, a strongly developed scape base extension, a longer scape, a longer propodeum and a broader head occur in another fixed combination (Figs. 1, 2). Discrete phenotypes formed as a syndrome of multiple, seemingly independent characters should require mutually compatible changes in many different gene loci and each ant taxonomist with some experience would immediately assume here two clearly evolved species. This conclusion was drawn in this case by three different authors: In the determination key of SEIFERT (2007) the hairy form was separated from *Camponotus lateralis* (OLIVIER, 1792) under the provisional designation "*Camponotus lateralis* sp. 2" and BOROWIEC & SALATA (2014) ascribed the hairy form of *C. lateralis* to the species *C. honaziensis* KARAMAN & AKTAÇ, 2013 (see also the pictures coded CASENT0914262 in www.antweb.org). This paper will show, among other similar examples, that the two phenotypes are provided by the same gene pool and represent an example of a treacherous intraspecific dimorphism of the ant *C. lateralis.*

This result may appear surprising for a taxonomist with a view centered on morphology. Discrete character syndromes provided by the same gene pool are preferentially explained by the pleiotropic action of a single regulatory gene encoding a transcription factor. The genetic literature provides a lot of examples for monogenic regulation of character syndromes (e.g., GRUENEBERG 1938, JERKADZIADOSZ & al. 1992, MCKONE & al. 2003, MARCELLINI & SIMPSON 2006, PRUD'HOMME & al. 2006, MCGREGOR & al. 2007, PRUD'HOMME & al. 2007, WAYNE & OSTRANDER 2007, STANKE & al. 2013, SCHWITZGEBEL 2014). In ants, monogenic dominant-recessive control of gyne polymorphism has been credibly shown in long-term rearing experiments by WINTER & BUSCHINGER (1986) for the slave-hunting ant *Harpagoxenus sublaevis* (NYLANDER, 1849) and by BUSCHINGER (2005) for *Myrmecina graminicola* (LATREILLE, 1802). A similar mechanism was suggested for a Nearctic *Leptothorax* MAYR, 1855 species (HEINZE & BUSCHINGER 1987).

Discrete character syndromes may also be controlled by the combination of several genes with low crossing-over frequency in a linkage group on the same chromosome. This "supergenic" mechanism is supposed to be rarer than monogenic control. However, such cases have been documented recently for ants and are possibly more frequent than currently assumed. WANG & al. (2013) and PURCELL & al. (2014) demonstrated for two ant subfamilies that alternate syndromes of gyne morphology and be-

havior, associated with either monogynous or polygynous colony organization, are controlled by a single Y-like, non-recombinant chromosome that is not homologous in the investigated *Solenopsis* WESTWOOD, 1840 and *Formica* LINNAEUS, 1758 ants.

Both monogenic and supergenic control are likely to have statistically similar effects on distribution of phenotypes over the nests and populations when they follow a Mendelian inheritance. Within the taxonomic context, the question of the underlying genetic mechanism is of lower importance and simulating both ways by a simple Mendelian model of monogenic dominant-recessive inheritance appears an acceptable simplification.

Five explanations for ant nests with mixtures of discrete phenotypes: If a taxonomist collected material in nature and demonstrated the existence of discrete phenotypes forming clusters that can be identified with a minimum error or are separated by wide gaps, he has to make credible that the clusters do not represent an intraspecific phenomenon. It was explained above that satisfying this requirement finds ideal conditions in eusocial insects with perennial societies of defined kinship. Of particular interest is here the abundance and distribution of phenotypically mixed nest populations. There are five possible scenarios for ant nests with a mixture of discrete phenotypes. The phenotypes may represent

Scenario (1): genetically determined intraspecific morphs,
Scenario (2): intraspecific modifications induced by environmental factors,
Scenario (3): the association of a temporary social parasite with a host species,
Scenario (4): the association of a permanent social parasite with a host species, and
Scenario (5): a parabiotic association of two basically independent (self-sustaining) species.

I introduce here an analytical method named the DIMORPH test. The basic rationale of this test system to distinguish between heterospecificity and intraspecific dimorphism is comparing the observed nest-type frequencies (pure nests of phenotype 1 vs. mixed nests of phenotype 1 and 2 vs. pure nests of phenotype 2) with predictions for the five scenarios. These predictions are derived from a mathematical model (scenario 1) and generalizations from empirical data (scenarios 2 to 5). The consideration of nest type frequencies is restricted here to the worker population because this is the only caste permanently available in sufficient sample sizes. In the following I explain the biological background of the scenarios (1) to (5) and present methods to predict the corresponding nest-type frequencies. It will be shown below that predicting scenarios (2) to (5) frequently lacks a precisely defined basis but we should bear in mind that the intention of this paper is distinguishing heterospecificity from intraspecific dimorphism. The analyses presented have the explicit aim to avoid the production of taxonomic synonyms. Within the taxonomic context, it does not matter if the analyses confuse a parabiotic association with an association of a temporary social parasite with a host.

In the following I present the principles of the prediction of nest-type frequencies. The individual worker morphologies are neutrally termed throughout the paper as "phenotypes" irrespective if they are finally identified as morphs or species. The consideration restricts in all predictions to the geographic area where both phenotypes occur sympatrically. Important variables to be considered in the analyses are within-nest sample size and the recognition rate R.

Taxonomic data sets usually have an unequal within-nest sample size distribution. This depends on the heterogenous individual preferences of ant collectors and how much material was available at all in historic and recent collections. There may be, for example, in a certain observation sample ten nest samples with two workers, 15 samples with three, 25 samples with four, one sample with five, and eight samples with six workers. Direct calculation of Hardy-Weinberg frequencies appeared too difficult for such irregular data sets. Accordingly, the prediction was done in scenario (1) by a simulation that exactly repeated the unequal nest-sample size distribution in the observation samples.

The ratio of individuals with reliably determined phenotypes within the total of the observation sample is called the recognition rate R. A "reliably determined" phenotype was assumed if the linear discriminant analysis of multiple morphometric data of an individual showed a posterior probability of $p > 0.95$ when testing a hypothesis previously formed by an exploratory data analysis. All nest samples containing at least one reliably determined individual of the alternate phenotype were considered as mixed and the remaining samples to contain only a single morph irrespective if they contained individuals with $p > 0.95$ or not.

A low recognition rate and a low mean within-nest sample size will reduce the frequency of identified mixed nests and increase the number or supposed pure nests in the observation sample. These changes have to be considered in the simulation of the monogenic scenario (1) and the predictions for the three scenarios of heterospecificity (3), (4) and (5).

Scenario (1) – modeling "monogenic" intraspecific dimorphism: I supposed above that a simple Mendelian model of monogenic dominant-recessive, pleiotropic inheritance is a reasonable way to describe the distribution of discrete character syndromes independent if there is really such a mechanism underlying or if a linkage group of *n* non-recombinant structural genes has caused this effect. Firstly we consider which traits of ant biology are important for the model.

(a) Ants are haplo-diploid, the males are hemizygous.

(b) Ant nests are perennial. Their worker populations show comparably low annual fluctuations.

(c) Ant nests contain worker populations of closely related individuals. Relatedness varies between two extremes: The population may represent the offspring of a single mother and a single father in monogynous-monoandrous nests but may have thousands of mothers and fathers in polygynous-polyandrous nests. The social type of an ant nest affects the intranidal genotypic and phenotypic diversity. Polygynous nests may have more phenotypical diversity than monogynous nests (SEIFERT 1991). Considering that we aim here at taxonomic sampling in larger geographic areas with several local populations, small-scale deviations should be statistically equalized in a sufficiently large sample even in socially polymorphic ant species. The problem is more thoroughly treated in Results and Discussion.

(d) Populations of monogynous ants with nuptial-flight and strong dispersal capacity will approach panmixis (SEPPÄ & PAMILO 1995, BOOMSMA & VAN DER HAVE 1998, VAN DER HAVE & al. 2011). Yet, strong deviations from panmictic behavior may occur in polygynous societies on a small geographical scale due to population viscosity (LIAUTARD & KELLER 2001, CHAPUISAT & al. 1997). This is caused by dominance of intranidal mating, weak long-range dispersal of alates and differential queen acceptance according to kinship value. Bearing in mind that our taxonomic view includes samples from a larger geographical area, highly polygynous ant species can be expected to behave on the metapopulation level not too different from monogynous / monoandrous species. In other words, polygyny should not affect the presented test system dramatically which is supported by the following considerations. The critical parameter in the test is the frequency of nests containing both phenotypes. High frequencies of mixed nests indicate intraspecific dimorphism and low frequencies heterospecificity. Polygyny will increase the chance that two phenotypes may occur within the same nest. Reduced exchange between viscose populations of highly polygynous ants, on the other hand, will violate the panmixis condition and reduce the number of mixed nests. These opposing trends should cause some kind of compensation on the metapopulation level.

Which are the basic assumptions and procedures in the simulation of scenario (1)? The "monogenic" model assumes panmictic behavior in the sympatric zone and discrete dimorphism. As ant males are hemizygous, a worker offspring results from the following mating combinations: *AA* × *A*, *AA* × *a*, *Aa* × *A*, *Aa* × *a*, *aa* × *A*, and *aa* × *a*. If p_A is the frequency of allele *A* and p_a that of allele *a*, the genotypes distribute according to $p_A^2 + 2\,p_A p_a + p_a^2$. The simulation averages the data of two runs – the first run assumed the rarer phenotype and the second run the more abundant phenotype to be homozygous recessive. Allele frequencies were calculated alone from individual phenotype frequencies as they occurred in the observation sample in the sympatric zone. If there was in the analysis a total of 40 workers of phenotype 1 (genotype *aa*), and 120 workers of phenotype 2 (genotypes *Aa* and *AA*), the frequency of allele *a* was the square root of (40 / 160) = 0.5. Genotypes of parents and the resulting worker offspring were simulated by random number generation. Then, using the recognition rate R, it was determined at random if the applied morphological classification system could positively identify a genetically determined phenotype. If there was, for instance, a genotypic prediction of 100 workers of phenotype 1 in the total data set and the recognition rate R was 90%, only 90 workers in the simulation were identified as phenotype 1 with the individual decisions determined by random numbers. As a consequence from recognition rates below 1.0 and the low number of workers analysed within the nest samples, the frequency of predicted mixed nests will be reduced and the number of pure nests increased in deviation from the ideal Hardy-Weinberg situation. The prediction of scenario (1) was calculated as mean of 1000 repeats and compared with the really observed situation.

Scenario (2) – intraspecific dimorphism induced by environmental factors: Environmental modification (EM) may occasionally cause morphs with distinct character syndromes. EM may be mediated by most different environmental factors: temperature, day length, water supply, quantity and quality of nutrition, pheromones and many others. Physical and chemical factors may have multiple effects. Sometimes an environmental factor may act analogous to a transcription factor: Soy genistein in the diet of mice, for instance, both changes the coat color and protects from obesity (DOLINOY & al. 2006).

Assessment of EM is very difficult. It is not clear in the first instance if a polymorphism is due to EM or direct genetic inheritance. Mathematically predicting the frequency of environmentally induced morphs in a metapopulation of ants or in a particular ant nest is a priori impossible. This would require intimate knowledge of the environmental history of nest sites and on reasons, mechanisms and parameters of the modifying process. Furthermore, rearing experiments under controlled conditions and parallel genetic investigations are needed.

The perennial nature of ant nests, the long life expectancy of workers and the fact that they are reared in most species from both spring and summer broods (or in the tropics throughout the year) implies a high probability that preimaginal worker ontogenesis is subject to strongly varying environmental influences. Accordingly there should be quite many nests with phenotype mixtures. The problem caused by the impossibility of mathematically describing EM appears rather harmless if we consider which types of possible errors occur and if these matter within the taxonomic context. The central question is: May EM cause phenotype distributions which are not separable from those indicating heterospecificity? Confusion with the distribution of two separate species of self-sustaining species in which mixed nests occur in less than 2% of all nests observed – see point (5) below – is almost impossible. This type of error requires intraspecific separation in two very different environments with very constant conditions throughout the season and EM to cause a very rigid switch from one phenotype to the other without generating intermediate examples. There is not known any example for a very eurypotent ant species in Europe or worldwide with such a rigid intraspecific habitat segregation. The same arguments exclude a confusion with the distribution for temporary social parasitism in which mixed nest form less than 1% of the total – see background information for scenario (3) given below. Confusion with permanent social parasites requires EM to generate many pure nests of one phenotype 1 ("host"), fewer or much fewer nests with phenotype mixtures ("host" + "parasite") but never to generate pure nests with phenotype 2 ("parasite"). Such a scenario is highly unlikely for conventional EM. Yet, infestion with endoparasites may generate similar distributions. These cases, at least those which are sufficiently studied today (e.g., infestions by *Microsporidium* or *Mermis*, see CSŐSZ & MAJOROS 2009), are not really dangerous for experienced taxonomists. Good taxonomists usually recognize different types of pathogenic phenotype deformations. Furthermore, "uncontrolled" variability or intermediacy of pathogenic phenotypes may additionally signalize an intraspecific phenomenon.

The remaining, more likely, type of error, confusing EM with direct genetic inheritance, is regrettable from the perspective of understanding ant biology but does not matter from a taxonomic point of view. Apart from using the prediction formulae presented below, I recommend to fol-

low a simple rule of thumb: If there are more than 4% of all nest samples in a larger geographic area mixed with two discrete phenotypes and if there are no indications for a social parasite to be involved, an ant taxonomist should refrain in the first place from considering them as different species and should investigate what is behind the phenomenon.

Scenarios (3) and (4) – association of a temporary or permanent social parasite species with a host: Intranidal co-occurrence of ants with distinct phenotypes is also given in nests mixed of temporary or permanent social parasites and their host species. Morphologies of these social parasites differ from their hosts in a way that heterospecificity is no question. Hence, there should normally not exist a taxonomic problem on the host vs. parasite level. Here, we ask for the hypothetical case of social parasitism where host and parasite are very similar and where no special morphological characters have been developed indicating a parasite identity. Such a situation will occur in initial stage of parasite evolution and should be generally rare. There is no question that such cases are cleared up in the most conclusive way by ethological and genetic investigations. However, as a taxonomist frequently has only series of dead and very old museum material at hand but no deeper biological information, the distribution of pure and mixed nest samples may provide a hypothesis on a possible biological background.

Scenario (3) – predicting the distribution of temporary social parasites and host: In case of species always founding their nests by temporary social parasitism and considering a larger geographic range, mixed host-parasite nests are much rarer then pure parasite nests and the latter themselves are much rarer than pure host nests. The situation in temporary social parasites belonging to the subgenera *Chthonolasius* RUZSKY, 1913 and *Austrolasius* FABER, 1967 which found colonies in hosts of the subgenera *Lasius* RUZSKY, 1913 and *Cautolasius* WILSON, 1955 provides an exemplary picture. A survey of collection material and data files present in SMN Görlitz discovered only 21 nest samples mixed of social parasites and host but 505 pure samples of the social parasites when all fifteen parasite and nine host species are considered collectively. This is a ratio of 1 : 24 or 0.0416. The general rarity of mixed parasite-host nests is explained by the fact that the parasite needs the host only for the initial stage of colony foundation. After the host queen has been killed by the parasite or her own children, the *Lasius* parasite queen consumes the host eggs. When a sufficient population of temporary parasite workers has been reared by the host workers, the parasite workers begin to kill the remaining host workers. Consequently, the transition to a pure parasite colony is frequently completed in *Lasius* as soon as 12 - 14 months after colony foundation (CRAWLEY 1909, HÖLLDOBLER 1953; B. Seifert, unpubl.). The relation between established nests of the temporary parasite and host nests can be derived from nest density data from 232 study plots over all habitat types in Central Europe (B. Seifert, unpubl.). Over all *Lasius* species, the mean collective density of *Chthonolasius* and *Austrolasius* social parasites was 0.32 nests / 100 m² and 27.3 nests / 100 m² in the host species. This is a relation of 1 : 86 or 0.012. It must be noted that this figure was calculated from a survey aiming at ecological data, containing a significant fraction of marginal habitats or such with strong anthropogenic impact where hosts but no parasites are present. Considering that average myrmecologists avoid collecting in such poor habitats with low species diversity and that ant taxonomists evaluate just these collections, it seems adequate to correct in the prediction the parasite-host ratio to 1 : 40 (0.025).

Temporary social parasitism in the genus *Formica* LINNAEUS, 1758 differs in some aspects from the situation in *Lasius*. In the Palaearctic zone, temporary social parasites parasitizing ants of the subgenus *Serviformica* FOREL, 1913, belong to the subgenera *Coptoformica* FOREL, 1913, *Raptiformica* FOREL, 1913 and *Formica* s.str. (i.e., the red mound-building wood ants). The latter three subgenera colonize a new site by temporary social parasitism in *Serviformica*, may then propagate by colony fission and will subsequently reduce the populations of the subordinate *Serviformica* species by aggressive interference, competition, predation or active raiding. The extremes of such local cycles may vary between no social parasites present on a site and almost complete displacement of host species. As particular local situations are not relevant for our taxonomic considerations, we have to ask what are typical or average figures for a larger geographic area. Within the system of 232 test plots in Central Europe, the collective density of all species of *Formica* s.str., *Raptiformica* and *Coptoformica* was 0.231 nests / 100 m² while the collective density of all species of *Serviformica* was 4.67 / 100 m². This means a ratio of temporary parasites versus hosts in the genus *Formica* of about 1 : 20, precisely 0.0495. This is twice the value of 0.025 assumed for *Lasius*. Accordingly we may fix as generalization for both genera a value 0.0372. An obvious figure for the relation of mixed host + parasite nests vs. pure parasite nests cannot be given for temporary social parasitism in *Formica*. In the subgenera *Formica* s.str. and *Coptoformica* this ratio is much lower than the *Lasius* figure of 0.0416 because of frequent nest foundation by colony-fission and in the subgenus *Raptiformica* much higher because of facultative slave raids. For a generalization we can only use the figure of *Lasius*.

For testing if the nest-type frequencies could indicate temporary social parasitism, a prediction was built up with the following assumptions. The observed number of pure nests of the rarer phenotype determines the assumed number of pure parasite nests P. According to the data described above, the assumed number M of mixed parasite-host nests should then be M = 0.0416*P and the number of pure host nests H should be H = P*26.9. The precise algorithm of calculating the prediction for the scenario of temporary social parasitism is presented in Table 1. The data are finally standardized in a way that the sum of samples in the prediction and observation are equal.

The empiric data in *Lasius* and *Formica* described above were collected under nearly ideal conditions: Host and parasite could be identified with recognition rates of 1.0 and the within-nest sample size of these field collections was much higher than the within-nest sample size of NUMOBAT-evaluated workers. As it was done in the simulation for monogenic dimorphism, we have to consider in the predictions for scenarios (3), (4) and (5) that a low mean sample size and recognition rates below 1 will reduce the frequency of recognized mixed nests which are defined as pure if a mixed status cannot be shown on the $p > 0.95$ level. Conversely, the frequency of pure nests of

Tab. 1: Variables and algorithms to calculate predictions for nest-type frequencies of three scenarios of heterospecific intranidal associations in ants.

Observation sample:		
P1	= frequency of pure nests of the rarer phenotype 1	
M	= frequency of mixed nests with both phenotype 1 and 2	
P2	= frequency of pure nests of the more abundant phenotype 2	
T	= total number of samples	→ $T = P1 + M + P2$
Correction for influence of low recognition rates and low and unequal within-nest sample size:		
M_{RE}	= number of mixed nests predicted in the simulation of scenario (1) operating with the really found recognition rate and really observed nest sample size distribution	
M_{ID}	= number of mixed nests predicted in the simulation of scenario (1) assuming "ideal" conditions: a recognition rate of 1.0 and a sample size of always 10 workers per nest.	
C_1	= correction factor describing the reduction of mixed nest frequency	→ $C_1 = M_{RE} / M_{ID}$
Algorithm for prediction of frequencies in the parabiosis scenario:		
M_1	= interim value of mixed (parabiotic) nests	→ $M_1 = 0.01 * T$
$P1_1$	= interim value of pure nests of phenotype 1	→ $P1_1 = P1 / (P1 + P2) * 0.99 * T$
$P2_1$	= interim value of pure nests of phenotype 2	→ $P2_1 = P2 / (P1 + P2) * 0.99 * T$
M'	= corrected (final) value of mixed nests	→ $M' = M_1 * C_1$
D	= difference caused by correction	→ $D = M_1 – M'$
P1'	= final value of pure phenotype 1 nests	→ $P1' = P1_1 / (P1_1 + P2_1) * D + P1_1$
P2'	= final value of pure phenotype 2 nests	→ $P2' = P2_1 / (P1_1 + P2_1) * D + P2_1$
Algorithm for prediction of frequencies in the temporary social parasitism scenario:		
P_1	= 1st interim value of pure parasite nests	→ $P_1 = P1$
M_1	= 1st interim value of mixed parasite-host nests	→ $M_1 = 0.0416 * P1$
H_1	= 1st interim value of pure host nests	→ $H_1 = P1 * 26.9$
C_2	= correction factor adjusting $P_1 + M_1 + H_1$ to T	→ $C_2 = T / (P_1 + M_1 + H_1)$
P_2	= 2nd interim value of pure parasite nests	→ $P_2 = C_2 * P_1$
M_2	= 2nd interim value of mixed parasite-host nests	→ $M_2 = C_2 * M_1$
H_2	= 2nd interim value of pure host nests	→ $H_2 = C_2 * H_1$
M"	= final value of mixed parasite-host nests	→ $M" = M_2 * C_1$
D	= difference caused by correction	→ $D = M_2 - M"$
P1"	= final value of pure parasite nests	→ $P1" = P_2 / (P_2 + H_2) * D + P_2$
P2"	= final value of pure host nests	→ $P2" = H_2 / (P_2 + H_2) * D + H_2$
Algorithm for prediction of frequencies in the permanent social parasitism scenario:		
P1'"	= final value of pure parasite nests	→ $P1'" = 0$
H_1	= 1st interim value for pure host nests	→ $H_1 = P2$
M_1	= 1st interim value for mixed parasite-host nests	→ $M_1 = 0.02 * P2$
C_2	= correction factor adjusting $P1'" + M_1 + H_1$ to T	→ $C_2 = T / (M_1 + H_1)$
M_2	= 2nd interim value for mixed parasite-host nests	→ $M_2 = C_2 * M_1$
H_2	= 2nd interim value for pure host nests	→ $H_2 = C_2 * H_1$
M'"	= final value of mixed parasite-host nests	→ $M'" = M_2 * C_1$
D	= difference caused by correction	→ $D = M_2 - M'"$
P2'"	= final value of pure host nests	→ $P1'" = H_2 + D$

the alternate phenotypes will increase. In order to estimate the influence of lower recognition rates and lower within-nest sample size of a particular case, the simulation of the dimorphism scenario (1) was repeated assuming near to ideal conditions: A recognition rate of 1.0 and all nest samples containing 10 workers. The frequencies obtained in these runs were then compared with the frequencies from runs under real conditions with recognition rates ranging between 0.820 and 0.993 and a mean sample size between 2.44 and 10.0 (see Tab. 2). These simulations showed clear

differences between the ten cases reported here. The strongest reduction of the number of identified mixed nests, down to 80.8% of the ideal value, occurred in the *Pheidole* case under a recognition rate of 0.829 and a mean sample size of 2.80. The weakest reduction, down to 99.3% of the ideal value, occurred in the *Formica lugubris* case under a recognition rate of 0.914 and a mean sample size of 6.21. These reduction factors were used to finally correct the nest-type frequencies in all three predictions for heterospecificity. Details of the algorithms are presented in Table 1. However, these corrections had only a minute effect on frequencies and test parameters – mainly because the frequency of mixed nests in the three heterospecificity scenarios was already very small before the correction.

Scenario (4) – predicting the distribution of permanent social parasites and hosts: Permanent social parasitism includes dulotic species and inquilines. All obligatory dulotic species known so far differ morphologically from the host species in a way that a taxonomist can safely conclude on heterospecificity. Genus-level differences are the rule. Yet, some species of less evolved inquilines may be rather similar to the host species. Important for the prediction is that a permanent social parasite cannot live alone and pure nests of the rarer phenotype will not occur. As a consequence, only mixed host-parasite and pure host nests are possible with the latter being much more abundant over larger territories. These facts alone exclude a confusion with temporary social parasitism and parabiosis. Yet, we have to consider and describe this scenario because nest-type frequencies of monogenic dimorphism may be inseparable from those of permanent social parasitism when the allele frequency of the rarer morph in the dimorphism scenario is about 0.1.

Data of four parasite species are available. These indicate strong variation of the ratio of mixed parasite-host against pure host nests depending upon geographical scale and population dynamics. The parasitation ratio of *Tetramorium* nests by the inquiline *Strongylognathus testaceus* is about 4% in some parts of the German countries Brandenburg and Sachsen (SEIFERT 2007) whereas it was only 0.33% in 900 checked *Tetramorium* nests in southern France (BERNARD 1968). The inquiline *Myrmica hirsuta* may locally parasitize 40 - 50% of *Myrmica sabuleti* nests (SEIFERT 1988, MÜLLER 2003) but the Central European average over all *M. sabuleti* populations is surely not above 2% (SEIFERT 2007). The ratio of nests of the dulotic species *Harpagoxenus sublaevis* (NYLANDER, 1849) against the *Leptothorax* MAYR, 1855 host nests was 1.5% against 98.5% in 232 test plots in Central Europe but at local spots *Harpagoxenus* grew to 5% (B. Seifert, unpubl.). The relation of nests of the dulotic species *Polyergus rufescens* (LATREILLE, 1798) against *Serviformica* host nests was 1.5% against 98.5% (accidentally the same figure as in *Harpagoxenus*) in 16 study plots in limestone grasslands of Thüringen and Sachsen-Anhalt but locally *Polyergus* achieved 3.3% (B. Seifert, unpubl.). From these data and referring to a larger geographical scale it seems a reasonable generalization to assume 2% of mixed parasite-host nests and 98% of pure host nests.

The precise algorithm of calculating the prediction for the scenario of permanent social parasitism is presented in Table 1. The algorithm firstly assumes that the number of pure nest samples of the more abundant morph in the observation sample determines the number of pure host nests H in the prediction. The number of mixed parasite-host nests M is then M = 0.02*H and the number of pure parasite nests is P = 0. The sum of nest-type frequencies in the prediction is equalized to the sum of nest-type frequencies in the observation sample and finally corrected to for frequency distortions caused by recognition rates < 1 and small within-nest sample size.

Scenario (5) – parabiotic associations of two independent species: Intranidal co-occurrence of ants with distinct phenotypes may also represent a parabiosis. Parabiotic species share nest space, foraging trails and food sources but keep their brood chambers separate. It is important to note that all long-lived parabiotic associations known so far in ants involve very distantly related, immediately separable species, belonging to different subfamilies. Most frequent are parabioses between *Crematogaster* LUND, 1831 (subfamily Myrmicinae) and *Camponotus* (subfamily Formicinae). At least seven species of *Crematogaster* and six species of *Camponotus* are reported worldwide to live in a stable parabiosis (KAUDEWITZ 1955, BERNARD 1968, SWAIN 1980, SEIFERT 2007, VANTAUX & al. 2007, MENZEL & al. 2008, MENZEL & al. 2014; F. Menzel, unpubl.). In northern Italy and the Balkans, mixed nests of three *Crematogaster* species with *Camponotus lateralis* made up 12% of 113 nests found (data and collection material provided by courtesy of H.C. Wagner & A. Vesnic). Other examples of stable parabioses are those of *Crematogaster* with *Dolichoderus* LUND, 1831 (SWAIN 1980) and of *Pseudomyrmex* LUND, 1831 with *Camponotus* (GALLEGO-ROPERO & FEITOSA 2014). Furthermore, the inquiline relations of *Polyrhachis* SMITH, 1857 with *Diacamma* MAYR, 1862 (MASCHWITZ & al. 2000) and of *Polyrhachis* with *Rhytidiponera* MAYR, 1862 (MASCHWITZ & al. 2003) most probably evolved from parabiotic associations.

The fact that all long-term stable parabioses known worldwide are between members of different subfamilies is intriguing and a short excursus on the possible reasons should be allowed here. Contemporary evolution theories assume reinforcement to be a significant segregating factor during speciation (DOBZHANSKY 1940, SERVEDIO & NOOR 2003, COYNE & ORR 2004). Reinforcement occurs when natural selection strengthens behavioral discrimination to prevent costly interspecies matings, such as when matings produce sterile hybrids. For instance, reinforcement in *Drosophila* for female mating discrimination is enhanced by natural selection. Accordingly, it seems plausible to assume that selection should act to avoid too intimate contacts of reproductive cycles between closely related ant species. Development of parabiotic associations is more likely when there is no longer any danger for interspecific hybridization as it is given between members of different subfamilies. Another, may be the more powerful, component of explanation should be an ecological one: Overlap of ecological niches and interspecific aggression grow with increasing relatedness. This leads to mutual spacial exclusion or active displacement of closely related species (for ants shown by SEIFERT 1987).

Mixed nest populations of closely related ant species that are self-sustaining in mature colonies are exceptional and short-lived. They have been observed, for example, in several *Formica rufa* group ants (HAGEMANN & SCHMIDT 1985, SEIFERT 1991, CZECHOWSKI 1996), *Temnothorax*

MAYR, 1861 (PUSCH & al. 2006), *Messor* FOREL, 1890 (STEINER & al. 2011) or in *Myrmica* LATREILLE, 1804 (BAGHERIAN YAZDI & al. 2012). The majority of such parabiotic cases represent a transitionary stage of a nest shifting from one species population to another after colony usurpation by a heterospecific queen, peaceful adoption of a queen by an orphaned nest or a stage after allopleometrotic colony foundation. Raiding of heterospecific brood could also explain temporarily mixed societies as it was shown for *Pogonomyrmex* MAYR, 1868 and *Messor* (HÖLLDOBLER & MARKL 1990; RISSING & POLLOCK 1991). The frequency of parabiotic associations of closely related species is generally < 2% of all nests observed for both involved species – here I fix 1% in the prediction algorithm. Possible genetic and ecological reasons leading to this small figure were explained above.

For our taxonomic purpose, we need only to consider the temporary parabioses of closely related species. If this type of association is confirmed by phenotype distributions, an argument for heterospecificity is given. It is not relevant in this context if 1% of interspecifically mixed nests represent true parabioses or a determination error and it does also not matter within the taxonomic context if a parabiosis is confused with temporary social parasitism. The algorithm predicting nest-type frequencies for temporary parabioses of closely related species is presented in Table 1 and includes an equalization of the total number of samples in the observation and prediction and a final correction for frequency distortions caused by recognition rates < 1 and small within-nest sample size.

Material and methods

Analysis methods: In a first analytical step phenotyping was done in material both from sympatric and allopatric ranges. There were two alternative ways, a direct and an indirect one, to demonstrate discrete phenotypes. The direct approach started on the level of worker individuals and formed initial hypotheses on discrete phenotypes by the exploratory data analyses principal component analysis (PCA) and k-means clustering. The hypotheses provided by exploratory data analyses were then tested by a single run of a linear discriminant analysis (LDA). All phenotypes with posterior probabilities of $p > 0.95$ were considered as "reliably recognized" and their proportion from the total is the recognition rate R. This direct way of analysis was applied when phenotypes differed more obviously.

Alternatively, in more hidden cases of polymorphism such as in *Cardiocondyla elegans*, when starting the analysis on the individual level provided no clear cluster separation, the analysis began by running the exploratory data analyses NC-Ward clustering and NC-K-means clustering (SEIFERT & al. 2014b) which operate on the nest-sample level. Clusters resolved in this way on the nest-sample level are likely to be correlated with phenotypes on the individual level even if a considerable fraction of nest samples was a mixture of phenotypes. These NC clusters were accepted as hypothesis on individual phenotypes and tested in a first LDA in all worker individuals. If there are mixed nests in the material, this initial LDA will give a first impression of the situation. Accepting the classifications of the first LDA as hypothesis, a second LDA was run and the analysis terminated. As in the direct way, all phenotypes with posterior probabilities of $p > 0.95$ were considered as "reliably classified".

Phenotyping included all material from both the sympatric and allopatric ranges, but the prediction scenarios, including calculation of allele frequencies, considered only material from geographic areas for which sympatric occurrence of the alternate phenotypes was directly confirmed or appeared probable according to the large scale distribution pattern. Phenotype frequencies and the resulting allele frequencies are based not only on reliably determined individuals but on any individual with posterior probability of $p > 0.5$. LDA, PCA and k-means clustering were run with the software package SPSS 15.0 and NC-Ward and NC-K-means in the software package R freely available under the GNU / GPL license from http://sourceforge.net/projects/agnesclustering/. Removal of allometric variance (RAV) was performed in strongly allometric species according to the method of SEIFERT (2008).

Observed and predicted distributions of nest types were compared by the X^2 test of independence according to SOKAL & ROHLF (1995). There is a problem in X^2 statistics with very small samples relevant for the data sets used in this paper – frequently the number of mixed nests in the observation data set and in the predictions for heterospecificity is below five in a certain cell (see Tab. 2). Small frequencies are supposed to result in disproportionately increased X^2 values and thus in a false rejection of the null hypothesis. Another unsolved problem occurs with possible differences in the degrees of freedom (df) because the three predictions for heterospecificity were generated with data from the observation sample. This should result in a reduction of df. The 3 × 2 table used in all the tests basically has df = 2. The observation sample has three singular parameters P1, M, P2 and one collective parameter T = P1 + M + P2. The prediction (simulation) for genetically mediated dimorphism does not use any data of the observation sample. Accordingly, the basic figure of df = 2 should remain unchanged. The prediction for parabiosis used P1 and P2, the prediction for temporary social parasitism P1 and T and that for permanent social parasitism P2 and T. There is no rationale or algorithm available telling us in which way df has to be reduced in the heterospecificity scenarios and we have to leave the question open. However, the strong differences in the basic structure of the predictions should provide a powerful differentiation of the scenarios and probably make the df question less important.

These two problems can be circumvented if we follow alternative approaches (e.g., AKAIKE 1973, BURNHAM & ANDERSON 2002) which aim to estimate the quality of a model, relative to each of the other proposed models without assessing the quality of a model in an absolute sense. Here I select which of a range of possible predictions best fits the observation data rather than testing whether observations differ from predictions of a particular scenario more than expected by chance. This solution is allowed by the following observation. If sample size is multiplied in all three nest-type frequencies by a factor that the smallest sample becomes a value of 10 or 100, there is a full correlation of the X^2 values with those of the primary data set. In other words, the ranking of X^2 values is independent from absolute sample size. This ranking has a linear correspondence with the Akaike Information Criterion as

long as we use the same total number of nests in all four scenarios. Accordingly, the smallest X^2 values always indicate the most likely of the four scenarios.

Four cases of intraspecific dimorphism are presented here – three of these have produced (or are likely to produce) erroneous descriptions of species while the fourth case is more hidden. I also present four cases of taxonomically recognized pairs of cryptic species and one example of more easily separable related species is added to show the difficulty to distinguish between parabiosis and temporary social parasitism.

Case 1: intraspecific dimorphism in *Camponotus lateralis* (OLIVIER, 1792): The primary analysis included 64 nest samples containing 86 workers of phenotype 1 and 64 workers of phenotype 2 from Spain, southern France, Italy, Croatia, Montenegro, Macedonia, Bulgaria, Greece, Cyprus, Asia Minor and Georgia. Thirteen morphometric characters including shape, microsculpture and pilosity characters were numerically recorded (see supplementary file Appendix S1, available as digital supplementary material to this article, at the journal's web pages).

Removal of allometric variance was performed by diphasic functions because there is a major-minor dimorphism in this species superimposing the shape-pilosity dimorphism. The observation sample to be compared with the predictions considered 53 samples from sympatric range.

Case 2: intraspecific dimorphism in *Lasius umbratus* (NYLANDER, 1846): The primary analysis included 113 nest samples from Europe with 57 workers of phenotype 1 and 289 of phenotype 2. The observation sample to be compared with the predictions was reduced to 104 samples from the assumed sympatric range of both phenotypes that included Austria, Czechia, Great Britain, Germany, Poland, Slovakia, Slovenia, Sweden, and Switzerland. Fourteen morphometric characters including shape, pilosity and pubescence characters were numerically recorded (see Appendix S1).

Case 3: intraspecific dimorphism in *Formica lugubris* ZETTERSTEDT, 1838: The primary analysis included 77 nest samples from Fennoscandia with 108 workers of phenotype 1 and 369 workers of phenotype 2. Since phenotype 1 was not found in Norway, the predictions were restricted to Sweden and Finland with a total of 67 nest samples. Seven morphological characters including four shape and six pilosity characters were numerically recorded (see Appendix S1).

Case 4: intraspecific dimorphism in *Cardiocondyla elegans* EMERY, 1869: The analysis included 48 nest samples with 56 workers of phenotype 1 and 63 workers of phenotype 2 from Iberia, France, and Italy. This is the whole known range of *Cardiocondyla elegans.*

Fourteen morphological characters – head size, eleven shape and two pubescence characters –were numerically recorded (see Appendix S1).

Case 5: the cryptic species *Formica pressilabris* NYLANDER, 1846 vs. *F. foreli* BONDROIT, 1918: The primary analysis included 252 nest samples from the whole Palaearctic range with 955 workers – 481 workers of phenotype 1 (= *F. pressilabris*) and 476 workers of phenotype 2 (= *F. foreli*). Excluding samples from north temperate regions of Fennoscandia and Siberia where phenotype 1 occurs alone and samples from the Apennine, Asia Minor and the Central Asian Mountains where phenotype 2 occurs alone, the predictions were restricted to 206 nest samples with 772 workers from the supposed sympatric range in Europe – in detail 343 workers of phenotype 1 and 429 workers of phenotype 2. Absolute size, two shape and three setae characters were numerically recorded (see Appendix S1).

Case 6: the cryptic species *Temnothorax crassispinus* (KARAVAJEV, 1926) and *T. crasecundus* SEIFERT & CSŐSZ, 2015: The primary analysis included 203 nest samples with 603 workers from the whole Westpalaearctic range of both phenotypes (SEIFERT & CSŐSZ 2015). The predictions were restricted to 84 nest samples from the sympatric range in Europe (Greece, Bulgaria, Romania, and Moldova) containing 93 workers of phenotype 1 (= *Temnothorax crassispinus*) and 159 workers of phenotype 2 (= *T. crasecundus*). Absolute size and 29 shape characters were numerically recorded (for details see SEIFERT & CSŐSZ 2015).

Case 7: the cryptic species *Temnothorax luteus* (FOREL, 1874) and *T. racovitzai* (BONDROIT, 1918): The primary analysis included 64 nest samples from the whole European range with 178 workers of both phenotypes (SEIFERT & al. 2014a). The predictions were restricted to 53 nest samples from the sympatric range in Spain and France containing 73 workers of phenotype 1 (= *Temnothorax racovitzai*) and 80 workers of phenotype 2 (= *T. luteus*). Absolute size and 17 shape characters were numerically recorded (for details, see SEIFERT & al. 2014a).

Case 8: two cryptic species of the *Pheidole pallidula* (NYLANDER, 1849) complex: The primary analysis included 72 nest samples from the whole Palaearctic range of the two species from Portugal to Middle Asia with a total of 191 major workers analysed. The predictions were restricted to 43 nest samples from the sympatric range in the Balkans and Asia Minor containing 58 workers of phenotype 1 (= *P.* sp. BALC) and 65 workers of phenotype 2 (= *P.* sp. KOSH). Absolute size, 15 shape characters and one seta character were numerically recorded (see Appendix S1).

Case 9: the related species *Myrmica scabrinodis* NYLANDER, 1846 and *M. vandeli* (BONDROIT, 1920): *Myrmica scabrinodis* and *M. vandeli* are similar but no cryptic species. Experienced researchers can separate the two species with a low error rate by simple eye inspection using a stereomicroscope. I used a data set of Wolfgang Münch who inspected about ten workers per nest in 3763 nest samples of both species collected in Baden-Württemberg / Germany. This area is in the centre of the sympatric range. The case is presented to show the difficulty to separate between the predictions for parabiosis and temporary social parasitism.

Results and discussion

Table 2 shows the results of the DIMORPH test for all nine cases treated here. Remember that the X^2 test statistic in these 3 × 2 tables correctly indicates the ranking of similarities when the total number of samples is equal in each case. Lowest X^2 values indicate which prediction among the four presented is most similar to the observation. The DIMORPH test indicated intraspecific dimorphism in the first four cases and heterospecificity in the last five cases. Note that the presented parabiosis scenario refers to intranidal associations of closely related species in which higher frequencies of mixed nests have not been observed so far.

Tab. 2: Data of the DIMORPH test. Comparison of observed nest-type frequencies with predicted frequencies for parabiosis of closely related species, temporary social parasitism, permanent social parasitism and intraspecific dimorphism. P1 = number of nests with only phenotype 1, M = number of nests with phenotype mixtures, P2 = number of nests with only phenotype 2, R= recognition rate on the p > 0.95 level, m = mean within-nest sample size. X^2 is the test statistic of a Chi-squared test of independence according to SOKAL & ROHLF (1995). X^2 data correctly show the ranking of similarity between observation and prediction with lowest values (printed in heavy type) indicating which prediction is the most probable.

Case		Observed	Prediction heterospecificity			Prediction dimorphism
			Parabiosis	Temporary social parasite	Permanent social parasite	
Camponotus lateralis, morph 2 (P1) vs. morph 1 (P2)	P1 M P2 R, m X^2, p	20 8 25 0.993, 2.68	23.32 0.52 29.16 7.14, 0.028	1.90 0.08 51.02 31.63, 0.001	0 1.03 51.97 34.83, 0.001	15.41 15.99 21.59 **3.51**, 0.170
Lasius umbratus, Compacta morph (P1) vs. normal morph (P2)	P1 M P2 R, m X^2, p	9 19 76 0.974, 3.01	10.91 0.97 92.12 18.01, 0.001	3.72 0.14 100.13 24.08, 0.001	0 1.90 102.10 26.82, 0.001	8.76 17.19 78.04 **0.05**, 0.744
Formica lugubris, Hippie morph (P1) vs. normal morph (P2)	P1 M P2 R, m X^2, p	15 10 42 0.914, 6.21	17.46 0.67 48.87 8.86, 0.012	2.40 0.10 64.50 23.58, 0.001	0 1.30 65.70 26.89, 0.001	9.34 15.24 42.42 **2.41**, 0.283
Cardiocondyla elegans, slender morph (P1) vs. thick morph (P2)	P1 M P2 R, m X^2, p	19 5 24 0.916, 2.44	21.03 0.41 26.56 4.13, 0.126	1.57 0.06 42.37 24.55, 0.001	0 0.80 47.20 29.60, 0.001	16.92 11.85 19.23 **3.43**, 0.177
Formica pressilabris (P1) vs. *F. foreli* (P2)	P1 M P2 R, m X^2, p	85 7 114 0.868, 3.76	87.22 1.80 116.98 **3.14**, 0.202	7.36 0.27 198.36 94.28, 0.001	0 3.54 202.46 110.90, 0.001	65.26 53.35 87.39 41.71, 0.001
Temnothorax crassispinus (P1) vs. *T. crasecundus* (P2)	P1 M P2 R, m X^2, p	30 2 52 0.849, 3.05	30.47 0.70 52.83 **0.64**, 0.605	3.01 0.11 80.88 30.03, 0.001	0 1.37 82.63 37.09, 0.001	21.83 19.35 42.82 16.28, 0.001
Temnothorax luteus (P1) vs. *T. ravovitzai* (P2)	P1 M P2 R, m X^2, p	26 1 26 0.938, 2.98	26.24 0.52 26.24 **0.15**, 0.734	1.90 0.08 51.02 29.73, 0.001	0 1.06 51.94 34.64, 0.001	18.19 14.80 20.01 14.21, 0.001
Pheidole sp. BALC (P1) vs. *Pheidole* sp. KOSH (P2)	P1 M P2 R, m X^2, p	21 1 22 0.829, 2.80	21.31 0.36 22.32 **0.31**, 0.691	1.57 0.06 42.37 23.21, 0.001	0 0.70 43.29 28.00, 0.001	16.60 10.11 18.28 8.32, 0.016
Myrmica vandeli (P1) vs. *M. scabrinodis* (P2)	P1 M P2 R, m X^2, p	225 38 3500 0.900, 10.0	225.02 37.63 3500.35 **0.00**, 0.779	134.67 5.60 3622.73 48.88, 0.001	0 73.78 3689.22 241.43, 0.001	93.38 292.61 3377.02 252.69, 0.001

More detailed comments on each case are given in the following.

Case 1: Intraspecific shape-setae-pubescence dimorphism in *Camponotus lateralis*

The separation of the phenotypes in a PCA was complete (Fig. 3) and the LDA confirmed this clustering in 150 individuals by 100% with a recognition rate of 100% for posterior probabilities of p > 0.95. The discriminant values were separated by a wide gap, being -2.712 ± 0.704 [-5.26, -0.85] in 86 workers of phenotype 1 and 3.644 ± 1.196 [0.77, 6.14] in 64 workers of phenotype 2. Phenotype 1 is a fixed combination of missing or reduced scape pilosity, of a strongly developed scape base extension, a more elon-

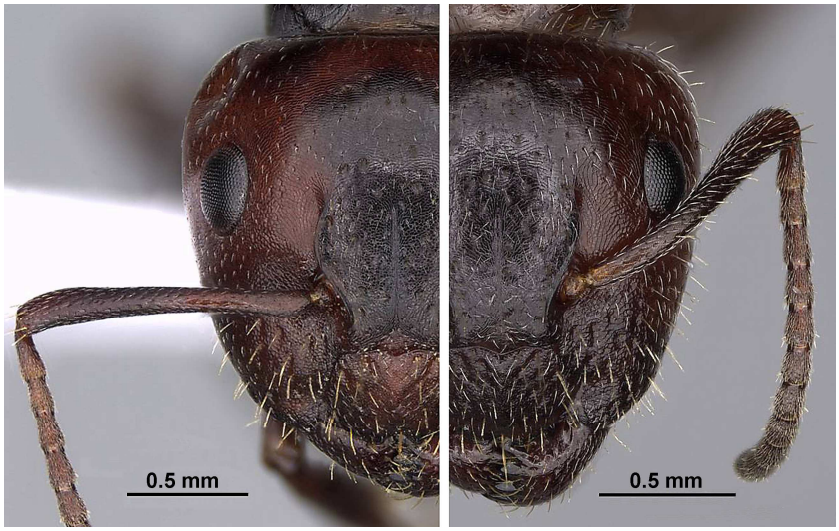

Fig. 1: Intraspecific dimorphism in *Camponotus lateralis*, head of two major workers shown. Left: Phenotype 1 with reduced pilosity of scape and posterior head, a strongly developed scape base extension and more sinusoidally curved frontal carinae compared to phenotype 2 (right). Photos from antweb.org, taken by Michele Esposito. Right: CASENT0914262, specimen misidentified by L. Borowiec as *Camponotus honaziensis*. Left: CASENT0914267.

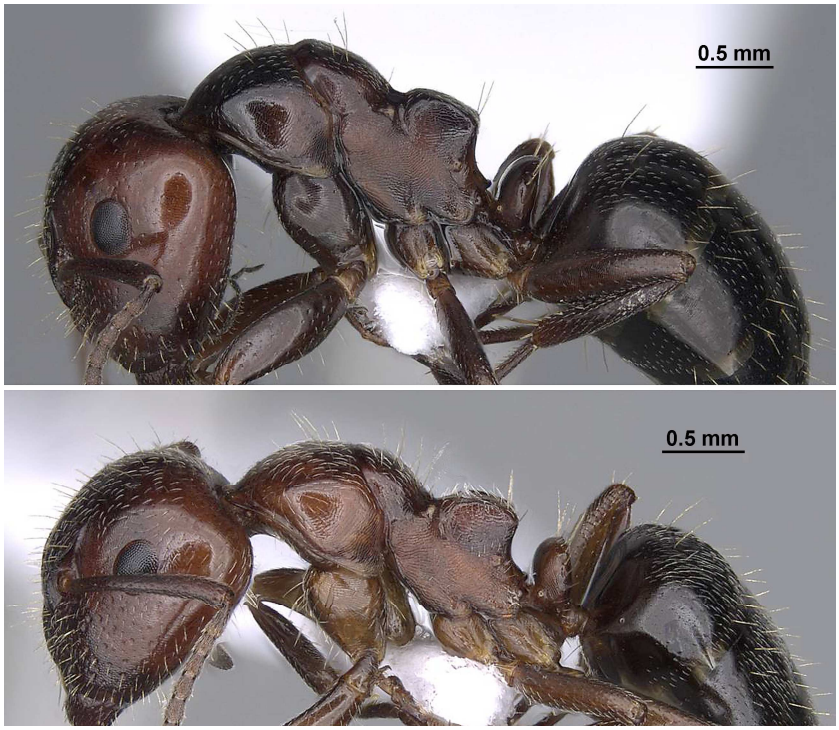

Fig. 2: Intraspecific dimorphism in *Camponotus lateralis*, mesosoma of two major workers shown. Upper: Phenotype 1 with a longer, weakly convex dorsal plane of propodeum, shorter pubescence and more developed pilosity compared to phenotype 2 (lower). Photos from antweb.org, taken by Michele Esposito. Lower: CASENT0914262, specimen misidentified by L. Borowiec as *Camponotus honaziensis*. Upper: CASENT0914267.

gated scape, a longer, weakly convex dorsal plane of propodeum, shorter pubescence, sinosoidally formed frontal carinae and a broader head (Figs. 1, 2). Phenotype 2 combines profuse scape pilosity with a weakly developed scape base extension, a shorter scape, a shorter, strongly convex dorsal plane of propodeum, much longer pubescence, more linear frontal carinae and a broader head (Figs. 1, 2). Both phenotypes provide the appearance of closely related but clearly separable species and they were misinterpreted as such in the past. Having at hand two clean samples of phenotype 2, SEIFERT (2007) hypothesized phenotype 2 to represent a species "*Camponotus lateralis* sp. 2". Recently, BOROWIEC & SALATA (2014) misidentified phenotype 2 as *C. honaziensis* KARAMAN & AKTAÇ, 2013 (see CASENT0914262 in www.antweb.org, and Figs. 1 and 2). Investigation of three workers from the holotype nest of *C. honaziensis* from Honaz Dagi National Park, 37.467° N, 29.217° E, 1195 m, leg. Karaman 2007.07.15, No 07 / 2344 clearly confirmed the heterospecificity from *C. lateralis* phenotype 2. Table 3 shows a number of striking differences in RAV-corrected shape variables.

A proportion of 15% mixed nests for an average within-nest sample size of only 2.68 is a very high figure. Intraspecific monogenic dimorphism is by far the most probable scenario for phenotypes 1 and 2 indicated by much lower X^2 values compared to the three scenarios for heterospecificity (Tab. 2). In this context it should be mentioned that nest samples from Bulgaria, where only phenotype 1 was observed, were included into the sympatric range. An exclusion of this area would increase the percentage of mixed samples in the observation sample and would make the fitting to the dimorphism scenario even stronger and not change the rejection of the other models. There is no clear approach to exclude environmental modification of having generated this dimorphism but the absence of intermediate morphs strongly suggests a direct genetic mechanism. Yet, from a taxonomical perspective the true reason for the development of this dimorphism does not matter.

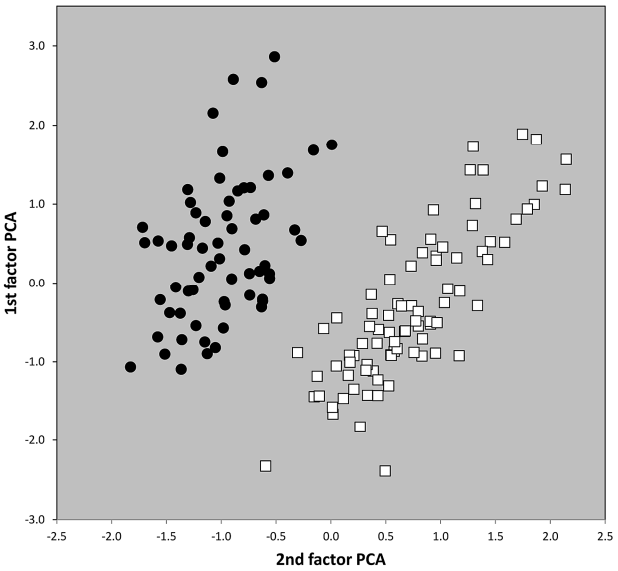

Fig. 3: Principal component analysis of phenotype 1 (white squares, n = 86) and phenotype 2 (black discs, n = 64) of *Camponotus lateralis* considering eight shape characters, three pilosity characters and head size.

Tab. 3: Comparison of five diagnostic shape variables and a seta character between phenotype 2 workers of *Camponotus lateralis* and three paratype workers from the holotype nest of *C. honaziensis* after removal of allometric variance.

	***C. lateralis*, phenotype 2 (n = 64)**	***C. honaziensis*, workers from holotype nest (n = 3)**
SL / $CS_{1.25}$	0.874 ± 0.020 [0.822, 0.920]	0.974 ± 0.004 [0.970, 0.978]
$SCI_{1.25}$	1.023 ± 0.037 [0.955, 1.122]	1.375 ± 0.012 [1.361, 1.384]
MW / $CS_{1.25}$	0.709 ± 0.015 [0.669, 0.745]	0.754 ± 0.008 [0.749, 0.763]
PRW / $CS_{1.25}$	0.252 ± 0.017 [0.218, 0.291]	0.313 ± 0.005 [0.308, 0.317]
PRL / $CS_{1.25}$	0.326 ± 0.018 [0.272, 0.371]	0.386 ± 0.013 [0.377, 0.400]
$nSc_{1.25}$	8.51 ± 2.06 [4.0, 12.3]	1.10 ± 1.35 [0.0, 2.6]

I conclude that phenotype 1 and 2 are the expression of a panmictic, conspecific gene pool.

Case 2: Intraspecific shape-setae-pubescence dimorphism in *Lasius umbratus*

The LDA confirmed the PCA clustering (Fig. 4) in 346 individuals by 100%. The recognition rate in the re-classified sample was 97.4% for posterior probabilities of p > 0.95. The discriminant values were separated by a gap and were -2.314 ± 1.164 [-5.45, -0.37] in 57 workers of phenotype 1 and 2.865 ± 0.966 [0.37, 5.32] in 289 workers of phenotype 2. Most remarkably, the separation of these intraspecific morphs was much stronger than several interspecific separations between related species in the subgenus *Chthonolasius* where error rates of up to 10% may occur on worker individual level (B. Seifert, unpubl.).

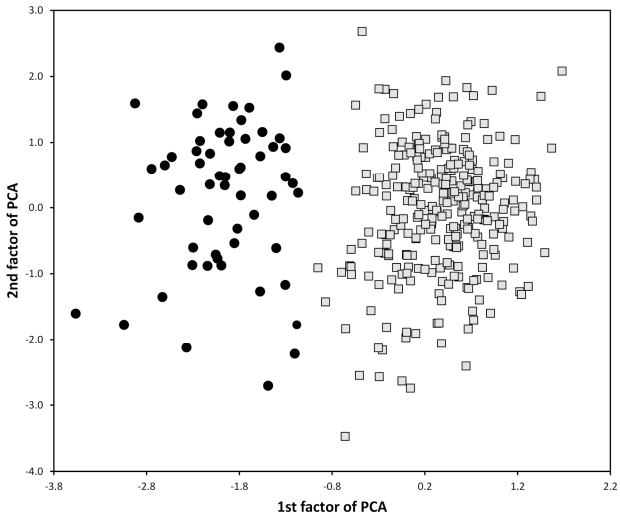

Fig. 4: Principal component analysis of phenotype 1 (Compacta morph, black discs, n = 57) and phenotype 2 (normal morph, grey squares, n = 289) of *Lasius umbratus* considering eight shape characters, four pilosity characters and head size.

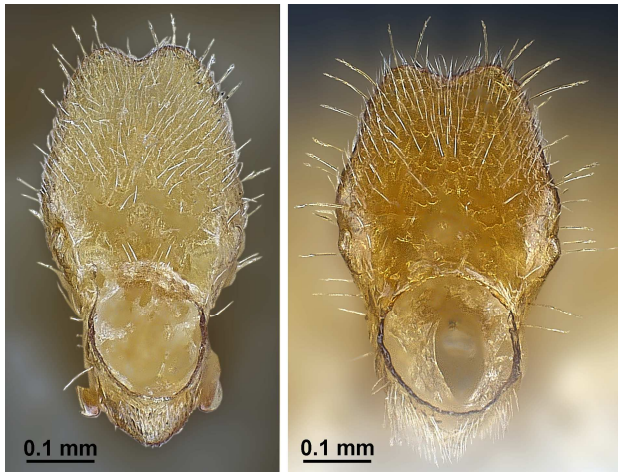

Fig. 5: Petiole scale in frontal view of phenotype 1 (Compacta morph, right) and phenotype 2 (normal morph, left) of *Lasius umbratus*. Note the larger width and lower height of petiole scale and longer setae in the Compacta morph.

A proportion of 18.3% mixed nests is a very high value for an average within-nest sample size of only 3.0. Intraspecific dimorphism is by far the most probable explanation in this case indicated by much lower X^2 values compared to the three scenarios for heterospecificity. Phenotype 1 differs from phenotype 2 in particular by a shorter scape, reduced ratios of the maximum vs. minimum diameters of scape and hind tibia, a smaller height of petiole scale, more dense pubescence on frontal head and gaster tergites, a larger width of base and crest of petiole scale and longer setae on dorsal face of first gaster tergite. Figure 5 shows typical petiole scales of both morphs. I name this syndrome herewith "Compacta morph" due to the reduced slenderness of several body parts, the more cylin-

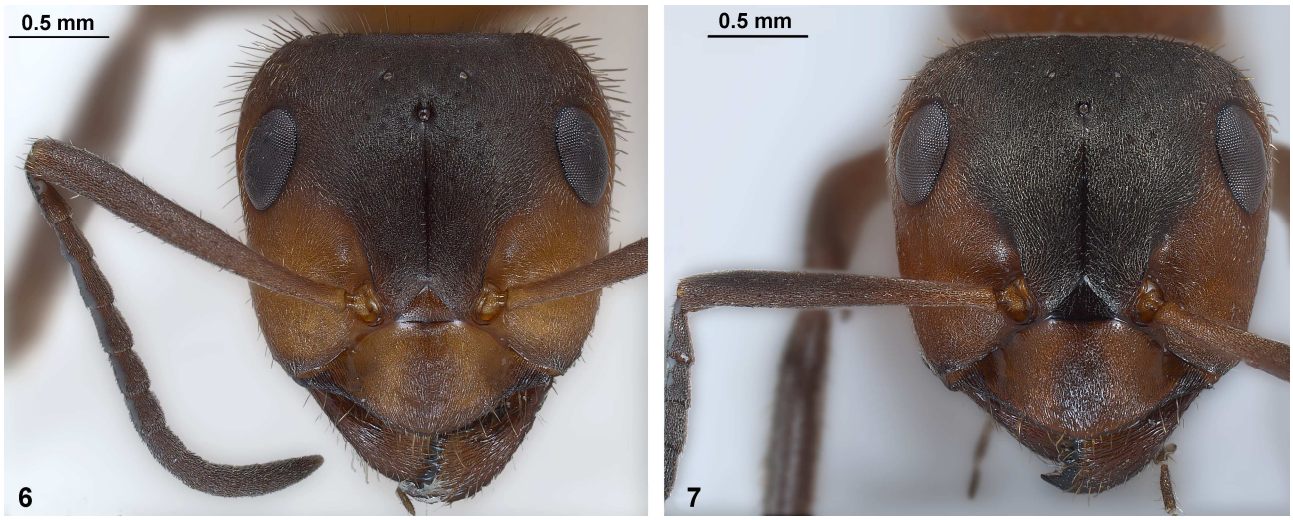

Fig. 6 - 7: Head of *Formica lugubris*, (6) the Hippie morph and (7) the normal morph.

drical appendage diameters and the denser pubescence. It should be mentioned in this context that I intended to describe the Compacta morph as species in about 2006 having at hand in that time two pure nest samples of this well-different ant. Hastily translating this strong impression into a taxonomic act would have meant the production of a synonym.

There is no clear argumentation to exclude environmental modification to have caused this dimorphism and we have to leave this question open. The Compacta syndrome probably occurs also in the related *Lasius distinguendus* (EMERY, 1916), but the number of reliably determined samples available for an analysis was too low due to unsolved discrimination of workers from *Lasius balcanicus* SEIFERT, 1982. A variant of the Compacta syndrome seems to represent the morph B of *Cardiocondyla mauritanica* FOREL, 1890 which is distinguished from the normal morph by significantly reduced head length and shorter postocular head, shorter spines, more thickset petiole and postpetiole and longer gastral pubescence (SEIFERT 2003b).

Case 3: intraspecific shape-setae dimorphism in *Formica lugubris*

A PCA considering head size and the two most diagnostic characters – number of setae on dorsal plane of scape and on rear margin of vertex – separated the phenotypes less clearly than in the previous cases. A LDA considering 11 characters confirmed the PCA clustering (Fig. 8) in 477 individuals by 99.6%. The recognition rate in the re-classified sample was 91.4% for posterior probabilities of $p > 0.95$. A ratio of 14.9 % mixed nests is a significant number for a mean within-nest sample size of 6.2 (Tab. 2). The observed nest-type frequencies for the sympatric area in Sweden and Finland are most similar to the prediction for monogenic dimorphism. The next similar scenario, though having a clearly larger X^2 value, is parabiosis. An observation sample of a real dimorphism case would show some trend to a nest-type distribution of the parabiosis scenario if the panmixis condition is incompletely fulfilled. Maybe this is the case here.

Phenotype 1 has been described by SEIFERT (2003a) as the "Hippie" morph of the Fennoscandian population of *Formica lugubris*. The Hippie morph is characterized by

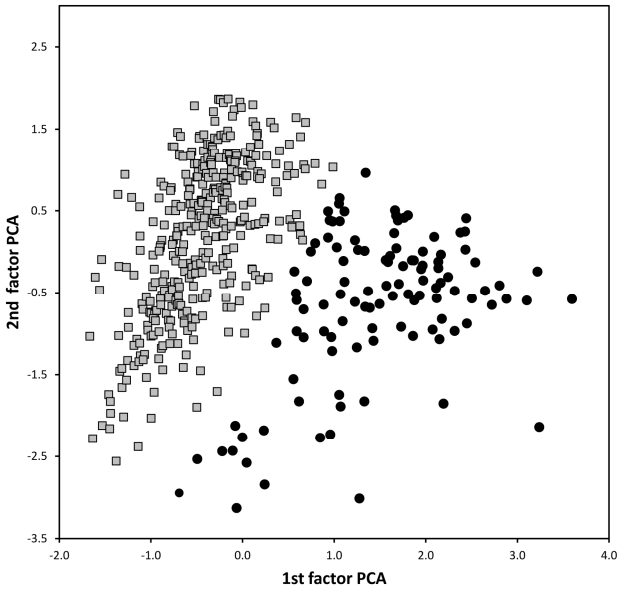

Fig. 8: Principal component analysis of phenotype 1 (Hippie morph, black discs, n = 108) and phenotype 2 (normal morph, grey squares, n = 369) of Fennoscandian *Formica lugubris* considering two pilosity characters and head size.

a very strong pilosity. It differs from the normal morph by longer and more numerous setae on dorsum of scape, posterior head margin and metapleuron. These characters are correlated with a more elongated head as well as a longer and narrower scape (Figs. 6, 7). The Hippie morph was supposed in the 1980s to represent a new Fennoscandian wood ant species (C.A. Collingwood, pers. comm.).

Case 4: intraspecific shape-pubescence dimorphism in *Cardiocondyla elegans*

NC-Ward clustering, which operates on the nest sample level, showed two clear clusters which provided the hypotheses for running an LDA on worker individual basis. The classification of this initial LDA served as hypothesis for the final LDA which indicated five mixed nests on the $p > 0.95$ level (Fig. 9, Tab. 2). These are 10.4% mixed nests within a sample of 48 nests. This figure is very high considering that mean within-nest sample size was only

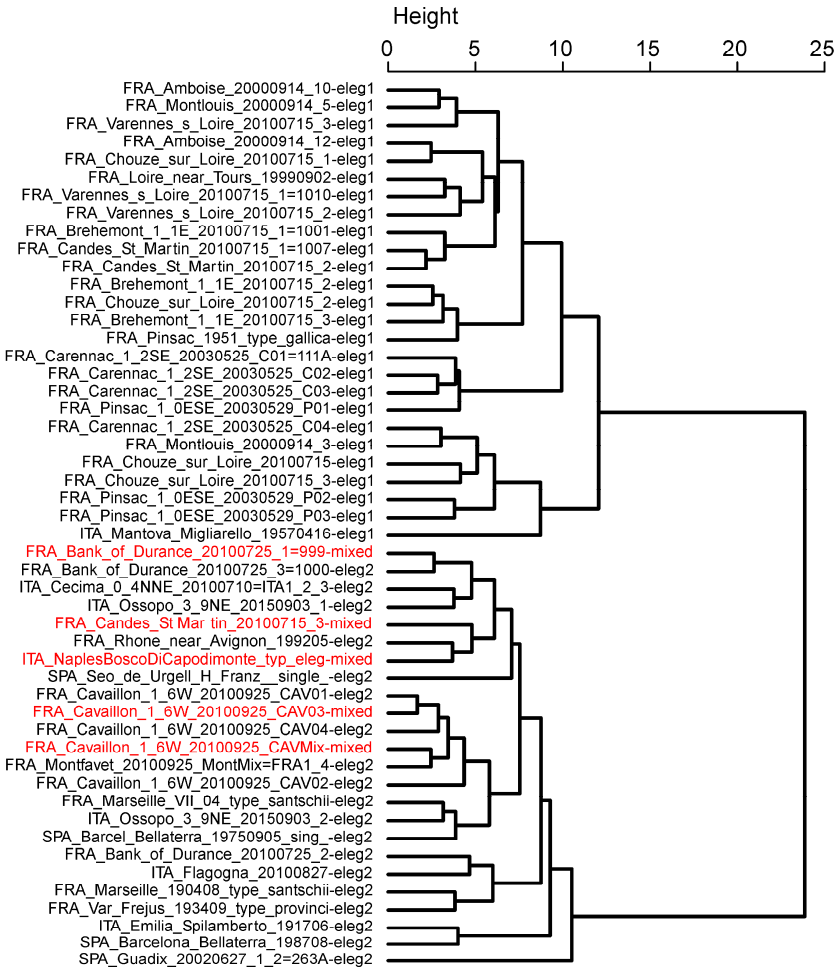

Fig. 9: NC-Ward clustering of intraspecific dimorphism in *Cardiocondyla elegans*. Nests containing both phenotypes are pronounced by red label texts.

2.44 – a reduced chance to detect mixed nests. Among the four scenarios there is the highest similarity of the observed nest-type frequencies with the prediction for dimorphism (X^2 3.43) but very close to this is the prediction for parabiosis (X^2 4.13) whereas both social parasitism scenarios are clearly rejected. The biology of *Cardiocondyla elegans* is well known and just those populations being the basis of this study have been extensively examined (LAFRÉCHOUX & al. 1999, 2000; LENOIR 2006, LENOIR & al. 2007, MERCIER & al. 2007). *Cardiocondyla elegans* is a strongly thermophilous ant, typically occurring and being most abundant on open riverine sand-gravel banks with very sparse herb layer. At French rivers it is a pioneer species and indicative for the first stage of stabilization of sand banks. It is in densely populated patches best considered as polydomous-oligogynous on the colony level with a high ratio of inbreeding: 70.4% of the copulations of winged gynes are with a brother (LENOIR & al. 2007). It is clear from these studies that the phenotypes distinguished here do not represent different species. Furthermore, it is most probable that the insular distribution and rarity of habitats in connection with strong inbreeding should result in considerable deviations from the panmixis condition required for running the dimorphism model. The two morphs can be termed as slender and thick morphs. Compared to the slender morph, the thick morph is a syndrome of larger absolute size, relatively higher and wider petiole and a more dense pubescence of first gaster tergite. The example is instructive. It shows that a very clear clustering by an exploratory data analysis on the nest sample level must not necessarily indicate two species, that mixed nests may be hidden within the clusters and that a strong violation of the panmixis condition increases the probability of confusing parabiosis and dimorphism.

Case 5: the cryptic species *Formica pressilabris* and *F. foreli*

The cryptic species *Formica pressilabris* and *F. foreli* have been separated by phenotype throughout their large Palaearctic ranges on the basis of nest sample means which is supported by data on ecology and altitudinal distribution in Central Europe (SEIFERT 2000).

Heterospecificity has been recently confirmed by NC clustering for almost the same data set used here: the error relative to the controlling LDA was 1.7% in NC-Ward and 0.0% NC-k-means clustering (SEIFERT & al. 2014b). In the material investigated here, NC-k-Means clustering, which classifies on the nest sample level, provided the hypothesis for the initial LDA that classified (phenotyped) individual workers. The phenotyping of this initial LDA served as hypothesis for the final LDA which indicated 7 mixed nests on the $p > 0.95$ level (Tab. 2). These 3.4% mixed nests within a sample of 206 nests from the sympatric zone are not a high value but may be understood as a warning signal possibly indicating intraspecific dimorphism. Among the four scenarios considered here, parabiosis is clearly the most probable one (X^2 3.14) whereas all other scenarios including dimorphism (X^2 41.74) are extremely unlikely. These data show that the phenotypes of *Formica pressilabris* and *F. foreli* represent clearly separate species. The

supposed intranidal phenotype mixtures might either indicate occasional local hybridization, temporary parabiosis and / or a weakness of the parsimonious 6-character morphological identification system.

Case 6: the cryptic species *Temnothorax crassispinus* and *T. crasecundus*

A separate species identity of the cryptic species *Temnothorax crassispinus* and *T. crasecundus* has been shown by SEIFERT & CSŐSZ (2015) who also rejected intraspecific dimorphism without providing an elaborated test system. The error of exploratory data analyses relative to the controlling LDA classification was 1.5% in NC-Ward and 2.0% in NC-k-means clustering. I investigated here the same data set as SEIFERT & CSŐSZ (2015) and used the nest-sample level classification of NC-Ward clustering as hypothesis for the initial LDA that classified (phenotyped) individual workers. The phenotyping of this initial LDA served as hypothesis for the final LDA which indicated two mixed nests on the $p > 0.95$ level (Tab. 2). These 2.3% mixed nests within a sample of 84 nests from the sympatric zone are below the critical threshold. Among the four scenarios considered here parabiosis is clearly the most probable (X^2 0.631) whereas all other scenarios including dimorphism (X^2 16.28) are very unlikely. The supposed intranidal phenotype mixtures are either short-term temporary parabioses or classification errors.

Case 7: the cryptic species *Temnothorax luteus* and *T. racovitzai*

A separate species identity of the cryptic species *Temnothorax luteus* and *T. racovitzai* has been shown by SEIFERT & al. (2014a). They got an error of exploratory data analyses relative to the controlling LDA classification of both 0% in NC-Ward and NC-k-means clustering when the character set was reduced to the seven most diagnostic characters. SEIFERT & al. (2014a) found no mixed nests under this condition. I reinvestigated here exactly the same material but considered the full set of 18 characters. The nest-sample level classification of SEIFERT & al. (2014a) was used as hypothesis for the initial LDA that phenotyped individual workers. The phenotyping of this initial LDA served as hypothesis for the final LDA which indicated one mixed nest on the $p > 0.95$ level (Tab. 2). These 1.9% mixed nests within a sample of 53 nests from the sympatric zone are below the critical threshold. Among the four scenarios considered here, parabiosis is clearly the most probable (X^2 0.154) whereas all other scenarios including dimorphism (X^2 14.21) are very unlikely. The supposed case of an intranidal phenotype mixture is probably a due to a phenotyping error.

Case 8: two cryptic species of the *Pheidole pallidula* complex

The Westpalaearctic *Pheidole pallidula* complex contains four cryptic species which are distributed around the Mediterranean Basin (B. Seifert, unpubl.). Two of these, one described and one still undescribed species, are most similar and show a broad geographic range overlap in the Balkans and Asia Minor. The error of exploratory data analyses relative to the controlling LDA classification was 2.8% in NC-Ward and 0% in NC-k-means clustering when the character set was reduced to the seven most diagnostic characters. I used the nest-sample level classification of NC-k-means as hypothesis for the initial LDA to phenotype the individual workers but considered all 17 characters. The phenotyping of this initial LDA served as hypothesis for the final LDA which indicated 1 mixed nest on the $p > 0.95$ level (Tab. 2). This means 2.3% of mixed nests within a sample of 44 nests from the sympatric zone. Among the four scenarios considered here, parabiosis is clearly the most probable one (X^2 0.306) whereas all other scenarios including dimorphism (X^2 8.52) are very unlikely. It was not confirmed by the collectors if the single mixed sample was really collected from the same nest. If so, the only explanations are a temporal parabiosis or a phenotyping error.

Case 9: the related species *Myrmica scabrinodis* and *M. vandeli*

There are no NUMOBAT data available for the 3763 nest samples investigated but only subjective determination of about ten workers per nest by simple eye inspection. Apart from difficulties with the extremely rare interspecific hybrids (BAGHERIAN YAZDI & al. 2012), *Myrmica scabrinodis* and *M. vandeli* are well-separable by experienced researchers on a subjective basis and the recognition rate should have been about 90% during processing of such masses of specimens in this case. W. Münch found in Baden-Württemberg 225 pure nest samples of *M. vandeli*, 38 mixed nests and 3500 pure nests of *M. scabrinodis*. This observation is inseparable from the prediction for parabiosis (X^2 0.01) whereas all other scenarios have a much higher test statistics (Tab. 2). Important from the taxonomic point of view is the clear rejection of intraspecific dimorphism (X^2 252.69) and the confirmation of heterospecificity. The prediction for temporary social parasitism ranks next (X^2 48.88) and seems a possible option. *Myrmica vandeli* has been supposed to be a temporary social parasite of *M. scabrinodis* (SEIFERT 2007, RADCHENKO & ELMES 2010). The observations from Baden-Württemberg show a ratio of pure *M. vandeli* nests against mixed *vandeli-scabrinodis* nests of about 6 : 1. The higher frequency of mixed nests compared to the figure of *Lasius* (24 : 1) was explained by a hypothesized weaker tendency of the *M. vandeli* queen for early disabling or killing the host queens (BAGHERIAN YAZDI & al. 2012). A clear biological interpretation of the mixed nests can only be given by long-term observation of nest populations in laboratory.

Error sources of the DIMORPH test and recommendations to avoid these

Having a comparative look on the nine cases presented in Table 2, the fixation of the relative nest-type frequencies in the three heterospecificity scenarios based on empirical data does not seem to be a substantial problem. These scenarios have such different nest-type frequency distributions that imprecise estimation of a particular nest-type frequency should not matter too much. Furthermore, a confusion of these scenarios does not matter from a taxonomic point of view.

More dangerous is obviously a violation of the panmixis condition leading to a reduction of the mixed nest frequency in the observation sample as seen most clearly in the *Cardiocondyla elegans* case. The nest-type frequency distributions may then approach to the parabiosis condi-

tion. The same effect, namely a reduction of the proportion of mixed nests, would be caused by a geographic differentiation in phenotype frequencies. In all four presented cases of intraspecific dimorphism, parabiosis ranks on the second place in the range of scenarios. This suggests that the ideal panmixis condition and / or homogenous geographic distribution of phenotypes is always violated to a certain degree (as one may expect). As a consequence, when running the DIMORPH test, it is necessary for a researcher to consider the mating scenarios in a species group (if known), the distribution type of a species caused by the availability of adequate habitats and the geographic distribution of phenotypes as it appears from the data. The fact that dimorphism ranked first even in the *Cardiocondyla* case indicates a certain robustness of the system proposed here.

The presented genetic dimorphism model assumes the presence of only a single and monoandrous queen. This should apply to the majority of ant species worldwide. Yet, polygyny is quite frequent in ants of the temperate zone – as much as 28% of Central European species may occasionally or frequently form polygynous societies (SEIFERT 2007). Polyandry is much less abundant: mating numbers below 1.4 clearly predominate in ants on a worldwide scale whereas mating numbers of up to 12 are only known in very evolved societies of leaf-cutter, driver and harvester ants (STRASSMAN 2001). A violation of the monogyny-monoandry condition will act in just the opposite direction compared to violating the panmixis and homogenous geographic distribution condition: An increased frequency of mixed nests and a reduced proportion of nests with only recessive phenotypes. In other words, polygyny and polyandry are suspected to cause an erroneous rejection of heterospecificity by the DIMORPH test. However, as already explained above, polygyny is connected with two opposing trends being likely to cause some kind of compensation on the metapopulation level. Polygyny will increase the chance that two phenotypes may occur within the same nest but reduced exchange between viscose populations of highly polygynous ants will simultaneously violate the panmixis condition and reduce the number of mixed nests. Within the cases analysed here strong polygyny is given only in the case of *Formica foreli* vs. *F. pressilabris* (SEIFERT 2007) where heterospecificity was clearly confirmed. Fennoscandian *Formica lugubris* are monodomous and monogynous to weakly polygynous (PAMILO & al. 1994). Accordingly, the rejection of heterospecificity and acceptance of intraspecific dimorphism is not likely of being caused by a violation of conditions required in the DIMORPH test. No disturbance of the heterospecificity indication was also visible in *Myrmica scabrinodis* and *M. vandeli* which are weakly polygynous (SEIFERT 2007). The remaining six cases refer to monogynous / monoandrous to weakly polyandrous species and do not present a problem in this respect.

A collecting bias by the taxonomist may also represent an error source. Due to limitations in working capacity and storage space, a taxonomist cannot afford collecting and maintaining thousands of samples of an abundant species – maybe he stops after ten years when having gathered some 100 samples. If he suddenly recognizes a certain problem, such as a rare intraspecific dimorphism or a rare species, he may be biased to increase the sample size in just this rare phenomenon disproportionately. An investigator running the DIMORPH test should assess the relevance of this error source. In the cases presented here, a sampling bias was excluded because it was impossible to determine the phenotypes in the field (cases 2 - 8). In the more easier separable phenotypes of case 1, each available sample was used without any bias because the material of both phenotypes was generally rare. In case 9 a sampling bias was also not given because the collector generally took each sample he found independent from his prejudice in the field.

A rare source of error occurs when the frequency of the recessive allele is very low. Assume an observation sample of 100 nests with zero pure nests of phenotype 1, four nests with both phenotype 1 and 2, and 96 pure nests of phenotype 2, a recognition rate of 1.0 and a mean within-nest sample size of 10. Assuming the rarer phenotype being homozygous recessive, the frequency of the recessive allele is then 0.10. The simulation of genetically mediated dimorphism will then predict the following frequencies: 0.06% pure nests of the rarer phenotype 1, 1.92% nests with both phenotype 1 and 2 and 98.03% pure nests of phenotype 2. This frequency distribution of nest types of dimorphism is inseparable from the prediction for permanent social parasitism that is 0 : 2 : 98. Assuming for the same scenario a recognition rate of 0.9 and a mean within-nest sample size of 2.5, the prediction for dimorphism does not change strongly: 0.34% pure nests of the rarer phenotype, 1.60% nests with both phenotype 1 and 2 and 98.07% pure nests of phenotype 2. This frequency distribution is also inseparable from the prediction for permanent social parasitism. However, such cases should occur rarely and there is only one procedure to minimize this error: The taxonomist has to look if some characters of the supposed social parasite are in agreement with those normally observed in that life form type.

Conclusion

The data confirm that the proposed DIMORPH test is a valuable taxonomic tool for eusocial insect taxonomists to assess the critical question if a phenotype constitutes a species or an intraspecific morph. Violation of the panmixis, homogenous geographic distribution and monogyny / monoandry conditions required for the test system may produce errors and the investigator should consider this with care. At least in the nine cases presented here, the DIMORPH tests lead to reasonable conclusions. The value of the test becomes apparent when considering which types of error matter within a taxonomic context. It seems also possible that the apparently good performance, or robustness, is in some cases supported by compensating effects – e.g., a violation of the panmixis condition reduces the number of mixed nests whereas a simultaneous violation of the monogyny / monoandry condition increases the number of mixed nests. The DIMORPH test has to be run in further cases before a reliable assessment of its performance can be done.

There is no doubt that only those rather few researchers will use the DIMORPH test who "automatically" produce the data needed for the test because they are engaged in Numeric Morphology-Based Alpha-Taxonomy. In other words, the test is a purposeful analysis of a byproduct of NUMOBAT research and researchers should seize this op-

portunity. Terminating a NUMOBAT study by a DIMORPH test constitutes a new form of multi-source taxonomy. Another dimension is added to our system of vision: morphology is projected on a series of templates indicating certain phenomena. It should become a routine process in ant taxonomy to assess if two separate clusters are heterospecific or intraspecific.

There is also no doubt that ethological or genetic investigation may lead to much deeper insights in the nature of a phenomenon. Yet, those who emphasize the leading position of genetics should also recognize the advantages of non-destructive morphological phenotyping and its performance in delimiting cryptic species. NUMOBAT can make use of the large worldwide collections of mounted museum material or private collections of ants independent from molecular degradation or curatorial ban of damage and can thus operate in fields where genetic investigation faces practical and logistic problems (FRANZ 2005, BROWER 2006, SCHLICK-STEINER & al. 2007, STEINER & al. 2009). The following excursus will explain this in more detail. It is an enormous advantage of morphology-based taxonomy to make use of any collection material existing in the natural history collections of the world beginning with the type specimens of Linnaeus from 1758. An estimate of the costs to re-collect this material today in order to obtain fresh material for DNA analysis would end in billions of dollar. The logistic problem of contemporary morphology-based taxonomists is mainly arranging loans of already existing collection material and to a lesser degree of supplementing this by recent sampling. They have a lower pressure for spending time, money, and manpower in organizing and carrying out expeditions to remote regions of the globe several of which are developing today into dangerous areas. For example, my own NUMOBAT investigations included material collected by the expeditions of Przewalski and Kozlov in North Tibet in 1876 - 1902, of Forel in Libya in 1889 / 1893, or of Klapperich in Afghanistan in 1952 - 1953. Few scientists should seriously intend at the moment to collect in these regions. Even if a research project does not require expeditions to remote and dangerous parts of the globe, collecting fresh material of species with hidden nest sites and low population density (such as *Lasius umbratus*) or of species being on the verge of extinction (such as *Formica foreli* and *pressilabris* in Central Europe) remains time consuming, costly and is sometimes even prohibited by law. Nobody can expect that collectings done by naturalists over 150 years can be repeated within the time frame of a recent research project of gene-based taxonomy. The recently enacted Nagoya Protocol on Access and Benefit-Sharing added further difficulties for collecting of specimens for genetic investigations.

The non-destructive investigation of type material is perhaps the most deciding advantage of morphology-based taxonomy. Taking a small tissue sample for DNA analysis from the ball of toes of a monkey-eating eagle or from the skin of a quagga does not significantly affect the value of the particular type specimen. However, if one requires to take a DNA sample from the primary type specimen of a small insect, responsibly thinking museum curators should prohibit this damage. These restrictions result in a helplessness of gene-based taxonomy in linking gene clusters to zoological nomenclature (SCHLICK-STEINER & al. 2007, STEINER & al. 2009). The frequent failure of genomics, transcriptomics and proteomics in evaluation of type material due to molecular degradation and / or curatorial prohibition of damage is reflected in a recent position paper of about thirty German molecular taxonomists published by the German National Academy of Sciences Leopoldina. They proposed to degrade the taxonomic supremacy of primary type specimens (NATIONALE AKADEMIE DER WISSENSCHAFTEN LEOPOLDINA 2014).

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