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Original article

The reliability of morphological traits in the differentiation of *Bombus terrestris* and *B. lucorum* (Hymenoptera: Apidae)*

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Abstract – The bumblebees of the subgenus *Bombus* sensu strictu are a notoriously difficult taxonomic group because identification keys are based on the morphology of the sexuals, yet the workers are easily confused based on morphological characters alone. Based on a large field sample of workers putatively belonging to either *B. terrestris* or *B. lucorum*, we here test the applicability and accuracy of a frequently used taxonomic identification key for continental European bumblebees and mtDNA restriction fragment length polymorphism (RFLP) that are diagnostic for queens to distinguish between *B. terrestris* and *B. lucorum*, two highly abundant but easily confused species in Central Europe. Bumblebee workers were grouped into *B. terrestris* and *B. lucorum* either based on the taxonomic key or their mtDNA RFLP. We also genotyped all workers with six polymorphic microsatellite loci to show which grouping better matched a coherent Hardy-Weinberg population. Firstly we could show that the mtDNA RFLPs diagnostic in queens also allowed an unambiguous discrimination of the two species. Moreover, the population genetic data confirmed that the mtDNA RFLP method is superior to the taxonomic tools available. The morphological key provided 45% misclassifications for *B. lucorum* and 5% for *B. terrestris*. Hence, for studies on *B. terrestris* we recommend to double check species identity with mtDNA RFLP analysis, especially when conducted in Central Europe.

bumblebee / identification / morphology / mtDNA / population genetics

1. INTRODUCTION

Currently approximately 250 bumblebee species (tribe: Bombini, genus: *Bombus*) are globally recognised (Michener, 2000). Most species of the genus can be easily separated by distinctive morphological features and pronounced body colorations. However, like in most genera, some species bear a strong resemblance in appearance, impeding species identification. In particular species of the subgenus *Bombus* sensu strictu are difficult to discriminate by classical determination keys due to a lack of prominent interspecific variation (von Hagen, 1991). This resulted in persistent taxonomic problems and Williams (1998) reports on 186 synonyms for *B. lucorum* alone. In Central Europe there are four sympatric species of the *Bombus* s. str. group: *B. cryptarum*, *B. lucorum*, *B. magnus* and *B. terrestris*, of which *B. terrestris* and *B. lucorum* are by far the most common (von Hagen, 1991). Particularly in Central Europe workers of these two bumblebee species almost perfectly resemble each other and only the queens and males allow for reliable identification (Williams, 1994; Bertsch, 1997; Bertsch et al., 2005).

This taxonomic problem is particularly critical because *B. terrestris* in Central Europe has become an intensively studied model organism in behavioural ecology (e.g. Goulson and Stout, 2001; Duchateau et al., 2004;

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Gerloff and Schmid-Hempel, 2005; Raine and Chittka, 2008; Wolf and Moritz, 2008; Raine et al., 2009), population genetics (e.g. Widmer et al., 1998; Chapman et al., 2003; Darvill et al., 2005; Herrmann et al., 2007; Kraus et al., 2009) and pollination ecology (e.g. Waser et al., 1996; Chittka et al., 1997; Kwon and Saeed, 2003; Hanley et al., 2008; Osborne et al., 2008; Wolf and Moritz, 2009). Many researchers therefore very much appreciated the highly comprehensive identification key of Mauss (1994) (shortened version also incorporated in von Hagen (1991)), which is based on coloration and claims to provide several discriminating characters that separate Central European *B. lucorum* and *B. terrestris*. Mauss (1994) refers to differences in the width of the collar, which should be narrow in *B. terrestris* and broad in *B. lucorum* workers. The colour of the hairs on tergite II is supposed to be "golden yellow" in *B. terrestris* in contrast to "lemon yellow" in *B. lucorum*. In addition an aggregation of very fine dots in the ocellus-orbital-area and in the surface structure of tergite II rim is exclusively present in *B. terrestris*. All these differences are, however, very subtle and require considerable experience in their evaluation, which may affect the accuracy of species identification.

More recently DNA tools using mitochondrial DNA (mtDNA) variation have been developed for bumblebee species identification. For example, Ellis et al. (2005) applied mtDNA-markers to confirm the species status of *B. ruderatus* and *B. hortorum*, two sympatrically occurring and phenotypically similar bumblebee species that lack any diagnostic morphological characteristics in workers and males. Using two restriction enzymes (*EcoN* I (= *Xag* I), *Hinf* I) Murray et al. (2008) found restriction length polymorphism (RFLP) in the mitochondrial cytochrome oxidase subunit 1 (cox) specific to *B. terrestris* and *B. lucorum* queens, which can be unambiguously separated by morphological traits. These species specific genetic fingerprints may therefore distinguish workers of *B. terrestris* and *B. lucorum* overcoming the identification problems resulting from the morphological resemblance.

DNA techniques do not help if one is in the field, however, and they involve considerable costs and require specific infrastructure. It would be extremely useful if Mauss' (1994) key were able to discriminate between the two species despite the above caveats. In this study we tested the applicability and accuracy of both the morphological identification key and mtDNA polymorphisms (species specific in queens) in discriminating workers of both species. Accuracy of species separation was tested by population genetic analyses using microsatellite DNA markers (Estoup et al., 1993, 1995) following population genetic principles. Thus, we compare the grouping of worker genotypes based on the Mauss (1994) key with that obtained by mtDNA RFLP. If the mtDNA-approach exceeds the morphological key in precision we predict that under correct species assignment (Hartl and Clark, 1997): (1) allelic richness per species should be less; (2) linkage disequilibrium (LD) should be weaker; and (3) population differentiation between species samples should be stronger, as compared to analyses based on a less accurate (e.g. morphology based) species assignment.

2. MATERIALS AND METHODS

2.1. Sampling

Workers belonging to either *B. terrestris* or *B. lucorum* were sampled from May 30th to July 12th 2005 in the Botanical Garden of Halle/Saale (Germany; 51° 29.20' N, 11° 57.40'E) (n = 62, 124 chromosomal sets). Individuals were caught from flowers or in flight using an insect net. After initial species identification in the field, all sampled individuals were sacrificed and immediately stored in ethanol (99%) until DNA processing. In the laboratory each individual was again microscopically scrutinized for species identity and sex using the identification key of Mauss (1994).

2.2. mtDNA analysis

One leg of each individual was used for DNA extraction following a *Chelex*-extraction as described by Walsh et al. (1991). Following

the protocol of Murray et al. (2008), a part of mitochondrial COX1 gene (1064 bp) was amplified by PCR using a primer, which was originally developed for *Apis mellifera* (Tanaka et al., 2001):
Forward 5'- ATAATTTTTTTTATAGTTATA – 3'
Reverse 5'- GATATTAATCCTAAAAAATG-TTGAGG – 3'
PCRs were performed following standard protocols (e.g. Kraus et al., 2009).

2.3. Digestion with restriction enzymes

Amplified mtDNA fragments were digested using the two restriction enzymes *Xag*1 (*Eco*N1) and *Hinf*1 (Murray et al., 2008). Each reaction contained 10 μL PCR product, 1U of both enzymes and 1 μL of NE-buffer No 2 (Fermentas). HPLC grade water was added to obtain a total reaction volume of 20 μL. The samples were gently mixed and briefly centrifuged to ensure that the sample was at the bottom of the reaction tube. The samples were then incubated at 37 °C for 4 h. Restriction fragments were electrophoretically separated on 2% agarose gels and visualized over UV light after staining in ethidium bromide solution (5 μg/mL in water).

2.4. Microsatellite analysis

All individuals were genotyped at seven microsatellite loci (B100, B118, B119, B121, B124, B131 and B132; Estoup et al., 1993, 1995) following standard PCR protocols using fluorescent labelled forward primers (e.g. Kraus et al., 2009). The amplified DNA fragments were separated in an automated DNA capillary sequencer (MegaBACE 1000) according to the instructions of the manufacturer. Allele sizes were scored using the Fragment profiler® software (GE Healthcare).

2.5. Colony assignment

In social insects the colony (i.e. the mated queen) rather than the individual workers represent the population genetically. Accordingly, we first needed to infer the mother-queen genotypes from the sampled worker genotypes to allow for unbiased population genetic estimates (Kraus et al., 2009). Moreover, the use of the queens rather then the workers compensates for the different numbers of workers from the various colonies in the sample set. The queen-genotypes were inferred using the program COLONY 1.3 (Wang, 2004), which takes advantage of the high intracolonial relatedness of bumblebee workers (supersisters with G = 0.75). Based on a maximum-likelihood algorithm the worker genotypes are assigned to the minimal number of putative mother queens (queen genotypes), that could have produced all the detected worker genotypes.

Because we wanted to test for the discriminatory power of the morphological and the mtDNA based methods, we grouped the total sample in two ways for the colony assignments of the workers: (1) according to the morphological characters suggested by Mauss (1994) into *B. terrestris* and *B. lucorum* (hereafter referred to as "morph"); and (2) according to the mtDNA RFLPs (Murray et al., 2008) (hereafter referred to as "mtDNA"). Maternal genotypes were obtained by performing four replicate COLONY runs with different seed numbers separately for each of those samples classified as *B. terrestris* and *B. lucorum* ("morph" and "mtDNA" grouping per species, respectively) (overall 4 × 4 runs). Queen genotypes inferred with the highest *log* probability (1 of 4 runs per group) were chosen for further analyses.

2.6. Population genetic parameters

The classical population genetic parameters: number of alleles (A_n), and observed and expected heterozygosity (H_o, H_e) were calculated with the software FSTAT v 2.9.3.2. (Goudet, 1995). Allelic richness (A_r) and Private allele richness (pA_r) were calculated correcting for different sample-sizes using the software *HP-rare* (Kalinowski, 2005). Again using FSTAT, we computed the pairwise F_{ST} values and linkage disequilibrium (nominal level: 1/1000) in *B. terrestris* and *B. lucorum* for both the "morph" and the "mtDNA" grouping.

2.7. Population assignment

We reconstructed the population composition for the morph and the mtDNA groupings entirely based on microsatellite data of the inferred queens using the program STRUCTURE 2.2 (Pritchard et al., 2000; Falush et al., 2003). Since the two species do not interbreed, we based the analysis on two discrete populations (k = 2) with independent allele frequencies and no common ancestors (no admixture model). The output was assumed to be

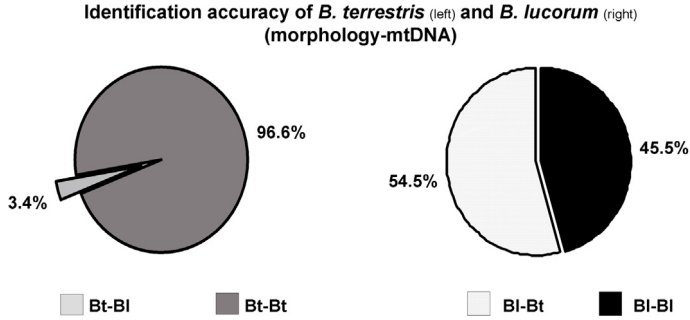

Figure 1. Accuracy of the identification key (Mauss, 1994) in comparison to mtDNA based species assignment. Whereas morphological traits allowed a correct identification of *B. terrestris* in 96.6% of all cases only 45.5% of the putative *B. lucorum* were identified correctly by means of morphological traits.

representative once the highest log-probability became stable.

3. RESULTS

3.1. Species identification

The amplification and restriction of the mtDNA of *B. terrestris* and *B. lucorum* workers resulted in two clearly distinguishable and species specific RFLP patterns matching those found in queens of both species by Murray et al. (2008).

Both identification techniques, the morphological key (Mauss, 1994) and mtDNA RFLPs, however, yielded different results in several cases. Using the identification key of Mauss (1994), we classified 28 workers as *B. terrestris*, which could be assigned to 18 colonies and 34 workers as *B. lucorum*, which were assigned to 20 colonies.

The mtDNA RFLP classification yielded 27 *B. terrestris* colonies (n = 46 workers) and 13 *B. lucorum* colonies (n = 16 workers). Given that the classification based on the mtDNA technique is the correct one, we found that 96.6% of all *B. terrestris* workers were correctly identified with the morphological traits of the Mauss key. In contrast only 45.5% of the *B. lucorum* workers were correctly identified using the morphological key and the majority of the samples were actually *B. terrestris* workers as shown by their mtDNA RFLP pattern (Fig. 1).

3.2. Population genetic parameters

We used a population genetic approach using microsatellite DNA loci to independently verify which of both identification techniques is superior for the true taxonomic classification. Based on the queen genotypes inferred from the worker samples, we derived classical population genetic parameters (H_o, H_e, A_n, A_r, pA_r) (Tab. Ia, b) to test which classification better supports the case of two species and hence genetically fully separated populations.

In spite of the different mtDNA and morphological classification neither allelic richness (A_r) nor private allelic richness (pA_r) differed significantly between morphology and mtDNA derived populations.

In accordance with our hypothesis we found linkage disequilibria for two pairs of loci in the morphological grouping of both species (*B. lucorum* (morph): LD B100 × B124: P = 0.048); *B. terrestris* (morph): LD B100 × B119: P = 0.044). The linkage disequilibrium did not reach the significance levels required for a Bonferroni correction (5% nominal level: P_{adj} = 0.00012), thus we could not exclude these to be stochastic effects. Nevertheless, we could not detect significant LD for any pair of loci in the mtDNA RFLP grouped population.

Irrespective of the identification approach, the putative *B. terrestris* populations were significantly differentiated from the putative *B. lucorum* populations (test for population differentiation: $P < 0.05$). However, mtDNA derived classification showed a more pronounced

Table I. Genetic diversity (number of alleles (A_n), allelic richness (A_r), private allele richness (pA_r)) and between-population sub-structure (F_{ST}) of the putative *B. terrestris* and *B. lucorum* populations (i.e. inferred queen genotypes) determined according to (a) morphological traits and (b) mtDNA polymorphisms. Sample sizes are given in both number of workers (n_W) per group and number of inferred queens (n_C). No significant differences in allelic richness between morphological and mtDNA-based species discrimination could be detected. Genetic sub-structure between the two populations was substantially higher between the non-intermixed populations obtained by species assignment based on mtDNA-polymorphisms as compared to the morphology based approach.

(a) species identification based on morphological traits (Mauss, 1994)

	B. terrestris (n_c = 18, n_w = 28)			*B. lucorum* (n_c = 20, n_w = 34)			overall (n_w = 62)		F_{ST} (Bt-Bl)
Locus	A_n	A_r	pA_r	A_n	A_r	pA_r	A_n	A_r	
B100	8	6.92	1.03	11	9.05	0.35	14	14.00	
B118	6	5.64	0.09	8	6.07	0.38	8	7.89	
B119	7	6.07	1.02	4	3.77	0.00	7	6.72	
B121	6	4.90	0.20	9	6.52	0.86	9	8.65	
B124	10	8.58	1.64	11	8.19	0.99	14	13.42	
B131	3	2.22	0.43	2	1.80	0.19	3	2.87	
B132	8	6.29	0.55	13	10.01	1.28	16	15.22	
mean	6.86	5.80	0.71	8.29	6.49	0.58	10.14	9.83	
SE	0.83	0.74	0.21	1.51	1.11	0.18	1.77	1.71	0.13

(b) species identification based on mtDNA RFLPs (Murray et al., 2008)

	B. terrestris (n_c = 27, n_w = 46)			*B. lucorum* (n_c = 13, n_w = 16)			overall (n_w = 62)		F_{ST} (Bt-Bl)
Locus	A_n	A_r	pA_r	A_n	A_r	pA_r	A_n	A_r	
B100	13	8.32	0.82	9	9.00	1.98	16	15.43	
B118	8	6.08	0.24	3	2.85	0.01	8	7.82	
B119	7	5.34	0.41	2	2.00	0.00	7	6.67	
B121	8	5.56	0.64	4	3.99	1.44	10	9.60	
B124	11	8.07	0.51	5	4.54	0.48	12	11.38	
B131	3	1.81	0.19	1	1.00	0.00	3	2.65	
B132	11	7.72	0.93	12	11.14	3.62	18	16.83	
mean	8.71	6.13	0.54	5.14	4.93	1.08	10.57	10.06	
SE	1.25	0.85	0.11	1.50	1.42	0.52	1.97	1.88	0.25

sub-structure (F_{ST} = 0.25) than the morphological classification (F_{ST} = 0.13).

The allele frequency distributions of the mtDNA derived *B. terrestris* and *B. lucorum* populations differed significantly at six out of seven loci (R × C Fisher exact test) in contrast to only four loci based on the morphological classification (Fig. 2).

3.3. Population assignment

Using STRUCTURE as an analytical tool, the individual assignment of the derived diploid queen genotypes to k = 2 discrete populations clearly showed a higher precision of the molecular approach in comparison to the morphological key. Five individuals with "*terrestris*" genotypes were grouped into the *lucorum* group if the morphological key was used (Fig. 3a). In contrast all individuals were correctly assigned with the mtDNA RFLP method (Fig. 3b).

4. DISCUSSION

Our results suggest that the morphological key is not reliable tool to separate between *B. terrestris* and *B. lucorum* workers in the tested

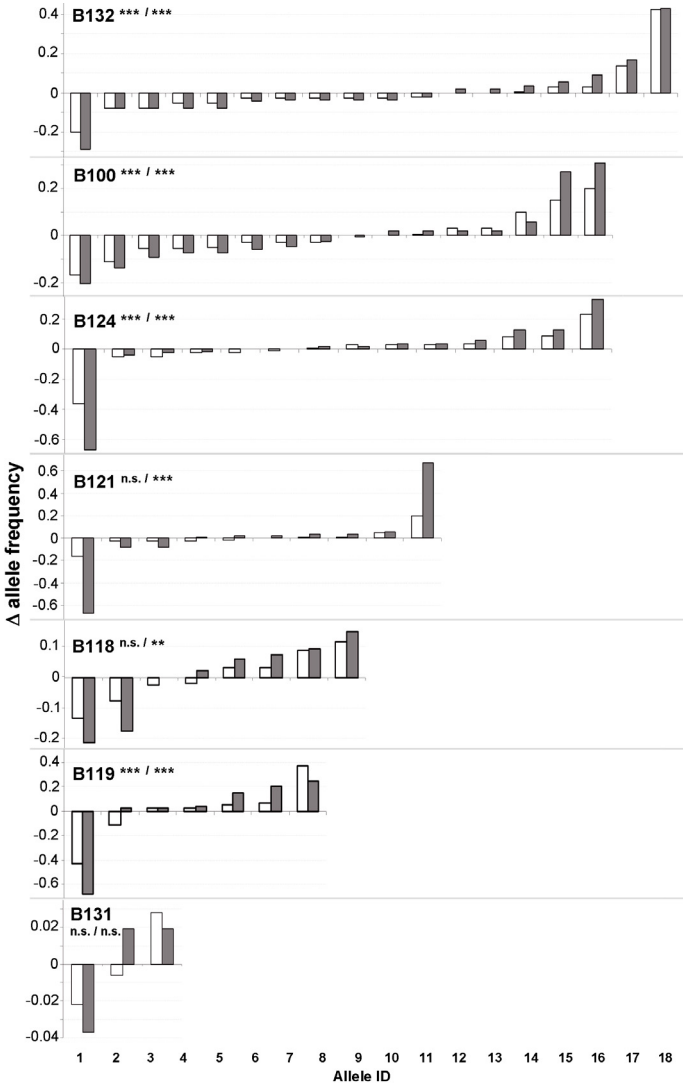

Figure 2. Difference between the allele frequencies (*Δ* allele frequency) of the *B. terrestris* and *B. lucorum* populations derived from morphology (white) and mtDNA RFLP (grey) groupings. The alleles are coded with a number and sorted in ascending order for the difference allele frequency. Morphology-derived populations differed significantly in the allele distribution (population wide frequency) in four out of seven loci (first significance indicators: $^{***/}$ = $P < 0.001$, $^{**/}$ = $P < 0.01$), whereas populations from the mtDNA grouping differed significantly at six of seven loci (second significance indicators $^{/***}$ = $P < 0.001$, $^{/**}$ = $P < 0.01$).

population. One might argue that more experienced taxonomists might be better able to discriminate the characters but it is difficult to assess when experience is sufficient. A field guide should help in the field and our study shows that the key of Mauss (1994) has serious limitations it the case of *B. terrestris / B. lucorum*. In particular, the precision for identifying *B. lucorum* was rather unsatisfactory with an almost even chance to confuse them with *B. terrestris* workers. However, over 95% of the individuals that were identified as *B. terrestris*

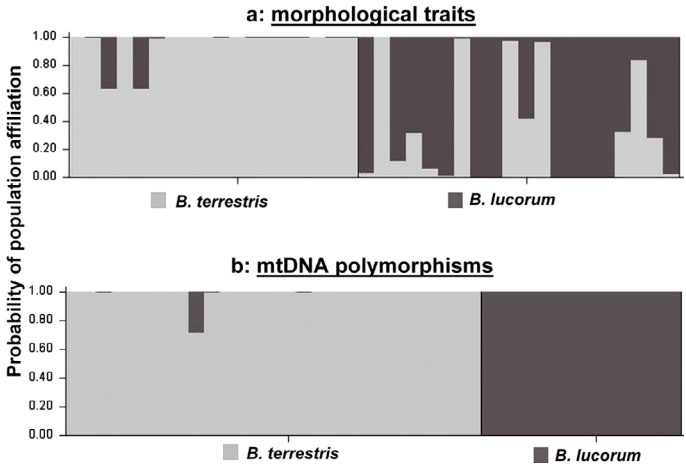

Figure 3. STRUCTURE 2.2 - Barplot of the individual species assignment based on microsatellite data. Using morphological traits for species identification (a) misclassifications occurred in the putative *B. lucorum* population but not in the putative *B. terrestris* population. Based on mtDNA species discrimination (b) no misclassified individuals could be detected.

were correctly assigned, showing that the key works much better for *B. terrestris* than for *B. lucorum.* If a worker has been identified as *B. terrestris* with the Mauss (1994) key it very likely is the Bufftailed Bumblebee. The problem is that many workers identified as *B. lucorum* are nevertheless *B. terrestris*. Though the differences between the two species may be more pronounced in other Central European populations, our data is in line with the literature, where in field studies in various parts of Central Europe workers of the two species could not be separated (e.g. Schmid-Hempel and Durrer, 1991; Dramstad and Fry, 1995; Dramstad, 1996; Saville et al., 1997; Meek et al., 2002; Dramstad et al., 2003; Westphal et al., 2006).

This problem is particularly nagging when it comes to issues addressing conservation biology, which aim at the evaluation of gene-flow among populations in fragmented landscapes. Miss-classifications will generate artefacts, which would make all conclusions drawn from population genetic studies obsolete. We therefore strongly recommend the inclusion of mtDNA RFLP analyses to confirm the species identity in any population genetic or molecular ecological study in the *Bombus* sensu strictu group.

If these often very abundant species can only be reliably identified with molecular markers (Murray et al., 2008), any study on these species, especially in Central Europe, will either require the knowledge of the sexuals or an mtDNA RFLP analysis. Since mtDNA RFLP analyses involve considerable costs, expertise, appropriate facilities and sampling, this may seem as a serious constraint today. However, the decreasing expenses for DNA studies and the user friendly services provided by specialized companies are likely to remove this obstacle to further implement *B. terrestris* as a model system in biological research.

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Fiabilité des caractères morphologiques dans la différenciation de *Bombus terrestris* et *B. lucorum* (Hymenoptera : Apidae).

bourdons / identification / morphologie / ADN mitochondrial / génétique des populations

Zusammenfassung – Die Verlässlichkeit von morphologischen Merkmalen bei der Unterscheidung von *Bombus terrestris* und *B. lucorum* (Hymenoptera: Apidae). Die Artbestimmung bei Hummelarbeiterinnen der Untergattung *Bombus sensu strictu* mittles morphologischer Merkmale hat sich wiederholt als schwierig erwiesen, da morphologische Bestimmungsmerkmale zwar bei Geschlechtstieren (Königinnen und Drohnen) eine gute Unterscheidung zulassen, bei Arbeiterinnen jedoch häufig uneindeutig sind.
Basierend auf einer grossen Freilandstichprobe von *B. terrestris / B. lucorum* Arbeiterinnen, zwei häufige, aber schwer zu unterscheidende mitteleuropäische Arten, testen wir hier die Anwendbarkeit und diagnostische Verlässlichkeit zweier Bestimmungsmethoden. Zum Einen, die eines häufig genutzten Bestimmungsschlüssels für mitteleuropäische Hummeln, zum Anderen, mtDNA Restriktions-Fragmentlängen-Polymorphismen (RFLP), die eine Artunterscheidung bei Königinnen von *B. terrestris* und *B. lucorum* erlauben. Die Hummelarbeiterinnen wurden dabei basierend auf entweder morphologischen Merkmalen oder anhand ihrer mtDNA RFLPs zu *B. terrestris* oder *B. lucorum* zugeordnet. Alle Individuen wurden an sechs Mikrosatelliten-Loci genotypisiert um zu testen, welche der beiden Artgruppierungen (Morphologie- oder mtDNA-basiert) besser mit einer zu erwartenden Hardy-Weinberg Population übereinstimmt.
Zum Ersten konnten wir zeigen, dass die für Königinnen diagnostischen mtDNA RFLPs auch bei Arbeiterinnen eine eindeutige Artbestimmung zulassen. Darüber hinaus konnten wir durch unsere populationsgenetischen Analysen bestätigen, dass die Artbestimmung mittels mtDNA RFLPs der durch morphologische Merkmale in Präzision deutlich überlegen ist. Die Artbestimmung mittels des Bestimmungsschlüssels führte zu 45% Fehlbestimmungen bei *B. lucorum*, Fehlbestimmungen bei *B. terrestris* wurden in 5% aller Fälle gefunden. Folglich empfehlen wir die verlässliche Artbestimmung mittels genetischer Methoden bei Studien an *B. terrestris*, vor allem, wenn diese in Mitteleuropa durchgeführt werden und populationsgenetische Untersuchungen beinhalten.

Hummel / Artbestimmung / Morphologie / mtDNA / Populationsgenetik

REFERENCES

Bertsch A. (1997) Abgrenzung der Hummel-Arten *Bombus cryptarum* und *B. lucorum* mittels männlicher Labialdrüsen-Sekrete und morphologischer Merkmale (Hymenoptera, Apidae), Entomol. Gen. 22, 129–145.

Bertsch A., Schweer H., Titze A., Tanaka H. (2005) Male labial gland secretions and mitochondrial DNA markers support species status of *Bombus cryptarum* and *B. magnus* (Hymenoptera: Apidae), Insect. Soc. 52, 45–54.

Chapman R.E., Wang J., Bourke A.F.G. (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumblebee pollinators, Mol. Ecol. 12, 2801–2808.

Chittka L., Gumpert A., Kunze J. (1997) Foraging dynamics of bumble bees: correlates of movements within and between plant species, Behav. Ecol. 8, 239–249.

Darvill B., Knight M.E., Goulson D. (2005) Use of genetic markers to quantify bumblebee foraging range and nest density, Oikos 107, 471–478.

Dramstad W.E. (1996) Do bumblebees (Hymenoptera, Apidae) really forage close to their nests? J. Insect Behav. 9, 163–182.

Dramstad W.E., Fry G. (1995) Foraging activity of bumblebees (*Bombus*) in relation to flower resources on arable land, Agr. Ecosyst. Environ. 53, 123–135.

Dramstad W.E., Fry G.L.A., Schaffer M.J. (2003) Bumblebee foraging – in closer really better? Agr. Ecosyst. Environ. 95, 349–357.

Duchateau M.J., Velthuis H.H.W., Boomsma J.J. (2004) Sex ratio variation in the bumblebee *Bombus terrestris*, Behav. Ecol. 15, 71–82.

Ellis J.S., Knight M.E., Goulson D. (2005) Delineating species for conservation using mitochondrial sequence data: the taxonomic status of two problematic *Bombus* species (Hymenoptera: Apidae), J. Insect Conserv. 9, 75–83.

Ellis J.S., Knight M.E., Darvill B., Goulson D. (2006) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae), Mol. Ecol. 15, 4375–4386.

Estoup A., Solignac M., Harry M., Cornuet J.-M. (1993) Characterization of (GT)n and (CT)n microsatellites in two insect species *Apis mellifera* and *Bombus terrestris*, Nucleic Acids Research 21, 1427–1431.

Estoup A., Taillez C., Cornuet J.-M., Solignac M. (1995) Size homoplasy and mutationalprocesses of interrupted microsatellites in Apidae species, *Apis mellifera* and *Bombus terrestris*, Mol. Biol. Evol. 12, 1074–1084.

Falush D., Stephens M., Prichard J.K. (2003) Interference of population structure using multilocus genotype data: linked loci and correlated allele frequencies, Genetics 164, 1567–1587.

Fussell M., Corbet S.A. (1992) Flower usage by bumblebees: A basis for forage plant management, J. Appl. Ecol. 29, 451–465.

Gerloff C.U., Schmid-Hempel P. (2005) Inbreeding depression and family variation in a social insect,

Bombus terrestris (Hymenoptera, Apidae), Oikos 111, 67–80.

Goudet J. (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics, J. Hered. 86, 485–486.

Goulson D., Stout J.D. (2001) Homing ability of the bumblebee *Bombus terrestris* (Hymenoptera, Apidae), Apidologie 32, 105–111.

Hanley M.E., Franco M., Pichon S., Darvill B., Goulson D. (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants, Funct. Ecol. 22, 592–598.

Hartl D.L., Clarke A.G. (1997) Principles of population genetics, 3rd ed., Sinauer Associates, Inc.

Herrmann F., Westphal C., Moritz R.F.A., Steffan-Dewenter I. (2007) Genetic diversity and mass resources promote colony size and forager densities of a social bee (*Bombus pascuorum*) in agricultural landscapes, Mol. Ecol. 16, 1167–1178.

Kalinowski S.T. (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness, Mol. Ecol. Notes 5, 187–189.

Kraus F.B., Wolf S., Moritz R.F.A. (2009) Male flight distance and population sub-structure in the bumblebee *Bombus terrestris*, J. Anim. Ecol. 78, 247–252.

Kwon Y.J., Saeed S. (2003) Effect of temperature on the foraging activity of *Bombus terrestris* L. (Hymenoptera, Apidae) on greenhouse hot pepper (*Capsicum annuum* L.), Appl. Entomol. Zool. 38, 275–280.

Mauss V. (1994) *Bestimmungsschlüssel für Hummeln*, Deutscher Jugendbund für Naturbeobachtungen (DJN), Hamburg.

Meek B., Loxton D., Sparks T., Pywell R., Pickett H., Nowakowski M. (2002) The effect of arable field margin composition on invertebrate biodiversity, Biol. Conserv. 106, 259–271.

Michener C.D. (2000) Bees of the world, John Hopkins University Press, Maryland, USA.

Murray T.E., Fitzpatrick U., Brown M.J.F., Paxton R.J. (2008) Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs, Conserv. Gen. 9, 653–666.

Osborne J.L., Martin A.P., Carreck N.L., Swain J.L., Knight M.E., Goulson D., Hale R.J., Sanderson R.A. (2008) Bumblebee flight distances in relation to the forage landscape, J. Anim. Ecol. 77, 406–415.

Pritchard J.K., Stephens M., Donnelly P. (2000) Interference of population structure from multilocus genotype data, Genetics 155, 945–959.

Raine N.E., Chittka L. (2008) The correlation of learning speed and natural foraging success in bumblebees, Proc. R. Soc. London B 275, 803–808.

Raine N.E., Rossmo D.K., Le Comber S.C. (2009) Geographic profiling applied to testing models of bumblebee foraging, J. R. Soc. Interface 6, 307–319.

Saville N.M., Dramstad W.E., Fry G.L.A., Corbet S.A. (1997) Bumblebee movement in a fragmented agricultural landscape, Agr. Ecosyst. Environ. 61, 145–154.

Schmid-Hempel P., Durrer S. (1991) Parasites, floral resources and reproduction in natural populations of bumblebees, Oikos 62, 342–350.

Tanaka H., Roubik D.W., Kato M., Liew F., Gunsalam G. (2001) Phylogenetic position of *Apis nuluensis* of northern Borneo and phylogeography of *A. cerana* as inferred from mitochondrial DNA sequences, Insect. Soc. 48, 44–51.

von Hagen E. (1991) Hummeln: bestimmen, ansiedeln, vermehren, schützen, 3rd ed., Augsburg: Natur-Verlag.

Walsh P.S., Metzger D.A., Higuchi R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, Biotechniques 10, 506–512.

Wang J. (2004) Sibship reconstruction from genetic data with typing errors, Genetics 166, 1963–1979.

Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems, and why it matters, Ecology 77, 1043–1060.

Westphal C., Steffan-Dewenter I., Tscharntke T. (2006) Bumblebees experience landscapes at different spatial scales: possible implications for coexistence, Oecologia 149, 289–300.

Widmer A., Schmid-Hempel P., Estoup A., Scholl A. (1998) Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymenoptera: Apidae) from the Canary Islands and Madeira, Heredity 81, 568–572.

Williams P.H. (1994) Phylogenetic relationships among bumble bees (*Bombus* Latr.): a reappraisal of morphological evidence, Syst. Entomol. 19, 327–344.

Williams P.H. (1998) An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini), Bull. Nat. Hist. Museum (Entomology) 67, 79–152.

Wolf S., Moritz R.F.A. (2008) Foraging distance in *Bombus terrestris* (Hymenoptera: Apidae), Apidologie 39, 419–427.

Wolf S., Moritz R.F.A. (2009) Pollination potential of bumblebee (*Bombus spec*.) drones (Hymenoptera: Apidae), submitted.