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Bumblebee (Hymenoptera: Apidae) sample storage for a posteriori molecular studies: Interactions between sample storage and DNA-extraction techniques

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Abstract. A global decline in pollinator abundance and diversity has demanded increased research attention to the ecology and genetics of bumblebees. However, as progressively more restrictions are placed on sampling for insects, researchers are increasingly obliged to use archival specimens collected for purposes other than genetic analyses. In this study we assessed the suitability, for population genetic studies, of popular, low-cost methods for preservation and storage of bumblebee specimens. Specimens of *Bombus terrestris* L. were held under six storage regimes for up to two years. DNA was extracted from the samples using three extraction protocols and the quality of the DNA was examined using PCR amplification of a mitochondrial and a nuclear gene. All extraction and storage methods provided sufficient DNA for successful PCR amplification. However, samples preserved in acetone or at freezing temperatures yielded the highest DNA concentrations. DNA yields from pinned specimens at room temperature declined over time, particularly when using standard extraction techniques. DNA concentrations were significantly lower from specimens preserved in 70% ethanol compared to all other extraction techniques and declined linearly over the two years of storage. These results indicate that two of the most popular insect storage methods (pinning and storage in ethanol) should be avoided for the long-term preservation of genetic material for future studies. We suggest that optimal insect preservation methods should be incorporated into research protocols in order to best capitalise on limited collection opportunities.

INTRODUCTION

Bumblebees (Hymenoptera: Apidae) have become a focal taxon for numerous studies aimed at monitoring and mitigating the current global pollinator decline. This has increased research support and funding for research on bees, their symbionts and associated diseases (Kluser & Peduzzi, 2007; Potts et al., 2010). Many of these studies employ genetic analyses for species identification (RFLP: Murray et al., 2008), disease diagnostics (RT-PCR: Genersch et al., 2006), behaviour (Lepais et al., 2010), and phylogeography (mtDNA and microsatellite analyses: Widmer et al., 1998; Darvill et al., 2006). For example, pre- and post-trade comparisons of bumblebee population genetics can be used to address concerns about a growing international trade in bumblebees for the horticultural industry (Goulson et al., 2008; Williams & Osborne, 2009). Biologists interested in insect conservation are often challenged due to tighter legislation around issues of specimen collection and field experiments (Finley, 1988; Dessauer et al., 1996; Braby, 2007). Although these requirements are implemented to protect biodiversity in vulnerable habitats and regions, they often discourage the research attention of experts. Some researchers respond by increasing the objectives of permitted collecting expeditions (i.e., including extra groups for colleagues: Dessauer et al., 1996) or by using specimens and collections from colleagues for previously unplanned or novel purposes (i.e., using emerging molecular applications on archival specimens: Mahunnah & Mshigeni, 1996; Mehrotra, 1996). Furthermore, the use of archival specimens is gaining importance where the genetic make-up of populations has potentially been affected by hybridisation events or by major landscape changes in the past (Franck et al., 2000; Buckley et al., 2008).

The principal problems faced by researchers wishing to use DNA from stored specimens in molecular studies result from DNA shearing and inter-strand cross-linking (Lindahl, 1993; Mandrioli et al., 2006). Shearing, the degradation of DNA into smaller fragments, is caused by a variety of agents, including UV radiation, high temperatures, pH and salinity (Lindahl, 1993). Shearing can affect both the extraction and amplification of DNA because short fragments precipitate less than long ones (Lindahl, 1993; Dessauer et al., 1996). Inter-strand cross-linking is due to chemical changes in the DNA (including the addition of alkyl groups) that inhibit denaturation and, consequently, amplification (Mandrioli et al., 2006). Proper storage conditions prevent or delay DNA degrada-

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tion and maintain larger DNA fragments that aid in PCR experiments (Mandrioli et al., 2006). However, the comparative efficiencies of different storage methods for maintaining DNA integrity have received relatively little attention, with most studies using previously collected specimens for analyses which restricts their ability to conduct more detailed experiments of optimal storage (Post et al., 1993; Reiss et al., 1995; Koch et al., 1998; Fukatsu, 1999; Quicke et al., 1999; Dean & Ballard, 2001; Mandrioli et al., 2006).

A variety of storage techniques are available to entomologists. Quicke et al. (1999) present a detailed list of some common storage methods with their associated benefits and practical considerations. These include cryopreservation (freezing in liquid nitrogen with storage at –70°C or –80°C), direct drying (pinning), critical point drying and chemical drying. Insects can also be preserved in a variety of chemical solvents. Fukatsu (1999) and Mandrioli et al. (2006) have investigated the efficiency of a range of chemical storage conditions, including acetone and ethanol at various concentrations, methanol, diethyl ether, ethyl acetate, chloroform, 2-propanol, and Carnoy's solution. However, during field expeditions, entomologists predominantly use entomological boxes or ethanol to store collected specimens. Our experience indicates that many researchers still use low concentration alcohol solutions: for example, store-bought ethanol, which is often used during distant collection trips (because of restrictions on carrying pure alcohol during air-travel) is typically 70% (Schauff, 2001). For entomologists, collection pinning has been traditionally popular as a storage method: pinning, where specimens are carefully pinned to a board and dried (either naturally or in an oven) has the advantage that the specimens can be easily examined by taxonomists for key morphological features (Bisanti et al., 2009; Frantsevich & Wang, 2009). Furthermore, pinned specimens can be exhibited for museum patrons and are aesthetically pleasing to some public and private collectors. It is perhaps generally felt among researchers, museum curators and collectors that pinned collections maintain genetic information for future molecular studies; however, whilst DNA has been successfully extracted from museum specimens, extraction is often unsuccessful and dry pinning of specimens is not optimal (Junqueira et al., 2002; Chakraborty et al., 2006; Mandrioli et al., 2006). Where only small amounts of DNA or RNA are available from specimens, increasingly expensive or noxious extraction protocols may be required (Fukatsu, 1999; Dean & Ballard, 2001).

In this paper we examine sample storage methods frequently employed by entomologists during collection expeditions and for biodiversity studies. Using bumblebees as a model group, we investigate some of the simplest storage and preservation techniques. We deliberately exclude techniques that require specialised equipment or reagents unavailable to field researchers or low-budget projects. Specifically, we investigated the interaction between DNA extraction and specimen storage techniques by quantifying the rate of DNA decay over two years of storage. We also assessed the quality of the extracted DNA through PCR amplification of the mitochondrial cytochrome b oxidase (Cyt b) gene and a nuclear (28S rRNA) marker. Finally we make recommendations for field researchers to increase the utility of their specimens for a posteriori molecular studies.

MATERIALS AND METHODS

Specimens and preservation methods

Adult workers of *Bombus terrestris* L. were obtained alive from a commercial supplier (Bioline, Syngenta) in 2007. A single hive was placed overnight in a freezer at –20°C to kill the bees. After 24 h, 175 bees of similar size (2.0 to 2.5 cm long) were selected and 25 bees maintained under each of the following conditions: (a) fresh bees analysed immediately after collection (i.e., day 0 of storage); (b) pinned and air-dried in an entomological case at room temperature (~22–25°C); (c) pinned as above but stored at 4°C in a refrigerator; (d) stored at –20°C (freezer) in a plastic vial; (e) stored at –70°C (freezer) in a plastic vial; (f) stored in pure acetone (Fulka, Germany) at room temperature; and (g) stored in 70% ethanol (Sigma, Germany) at room temperature. All samples were stored for 12, 18 and 24 months.

DNA extraction methods

Bumblebee samples were taken from storage and a single hind leg from each bumblebee was removed. The legs were individually washed in distilled water and ground in liquid nitrogen using a micro-pestle. The ground leg tissue was then weighed and DNA was extracted from samples using three commonly employed extraction methods. Each extraction was replicated three times and DNA concentrations were quantified using a UV spectrophotometer (Hitachi U-2001, Japan). The three extraction techniques were as follows:

(a) Commercial Kit: DNA extraction was conducted according to the manufacturer's protocol for the Dneasy Tissue Kit (Qiagen, Germany). This protocol involves enzymatic lysis using proteinase K followed by column purification of DNA using a silica-gel-membrane, without organic extraction (phenol/chloroform/isoamyl alcohol) or ethanol precipitation.

(b) Hexadecyl trimethyl-ammonium bromide (CTAB extraction): The crushed bumblebee tissue was digested with 200 µL of extraction buffer (0.1 M Tris-HCl, 0.2 M EDTA, 1.4 M NaCl, 2.5% ß-mercaptoethanol and 2% CTAB; pH 8.0) and proteinase K (5 µL of 10 mg/mL, Promega) and incubated overnight in a water bath at 37°C. The digested tissue was then centrifuged for 5 min at 13,000 rpm. The supernatant was transferred to a clean centrifuge tube and an equal volume of chloroform : isoamyl alcohol (24 : 1) was added and centrifuged for 5 min at 13,000 rpm; this operation was repeated twice. A 0.5 volume of 5 M NaCl and an equal volume of iced-cold isopropanol were added and the sample was stored overnight at –20°C. The sample was then centrifuged at 13,000 rpm for 10 min, the supernatant was discarded and 500 µL of 70% ethanol was added and centrifuged again for 5 min at 13,000 rpm. The supernatant was discarded and the DNA, set as a pellet, was air-dried. The DNA was then re-suspended in 50 µL of TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.5) (Boyle, 2007).

(c) Adapted phenol method: The hind bumblebee leg was digested with 600 µL of extraction buffer (0.2 M sucrose, 0.1 M Tris-HCl, 2% SDS, 0.5 M EDTA; pH 8.5) and proteinase K (5 µL of 10 mg/mL, Promega) and incubated overnight in a water bath at 37°C. Phenol (500 µL) was added to the digested tissue and carefully agitated for 5 min followed by centrifugation at 13,000 rpm at 4°C for 10 min. The upper phase was transferred

to a clean 1.5 mL centrifuge tube and an equal volume of phenol:chloroform (1 : 1) was added and gently agitated for 10 min followed by centrifugation at 13,000 rpm at 4°C for 10 min. The upper phase was transferred to a clean 1.5 mL centrifuge tube and an equal volume of chloroform : isoamyl alcohol (24 : 1) was added for 10 min followed by a centrifugation at 13,000 rpm at 4°C for 10 min. This step was repeated twice. The upper phase was then transferred to a clean 1.5 mL centrifuge tube and one part of 2 M sodium acetate to nine parts of supernatant solution was added followed by two volumes of 100% pre-cooled ethanol and stored overnight at –20°C. Samples were subsequently centrifuged at 13,000 rpm at 4°C for 10 min and the supernatant was discarded. A 500 mL volume of 75% ethanol was added and the mix was centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was discarded and the DNA, set as a pellet, was air-dried and re-suspended in 50 µL of TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.5) (Boyle, 2007).

Following extraction, all DNA samples were stored at –20°C.

PCR amplification

A partial mitochondrial cytochrome b oxidase gene (580 bp) was amplified using published primers (cyt *b* forward 5'CGWTTAATTCATATAAATGG-3' and cyt *b* reverse 5'TATCATTCWGGTTTAATA-3') by Koulianos et al. (1999). PCR amplifications were carried out in 40 µL volumes containing 2 µL of extracted DNA, 1 X Green buffer (Promega), 2 mM of $MgCl_2$, 0.5 µM of each dNTP (Promega), 1 U *Go-Taq* polymerase (Promega) and 0.2 µM of each primer. The reaction thermocycling profile was as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of initial denaturation at 92°C for 45 s, annealing temperature at 52°C for 1 min, extension at 72°C for 45 s, and a final extension at 72°C for 10 min.

A 200 bp region of the 28S rRNA gene was amplified using D9D10 primers (forward 5'-AGGAGACATGAGAGGTGT AGCA-3 and reverse 5'-GTACCGCCCCAGTCAAACT-3') (Gillespie et al., 2006). PCR amplifications were carried out in 40 µL volumes containing 2 µL of extracted DNA, 1 X Green buffer (Promega), 2 mM of $MgCl_2$, 0.5 µM of each dNTP (Promega), 1 U *Go-Taq* polymerase (Promega) and 0.4 µM of each primer. A touch-down PCR reaction was carried out as follows: 94°C for 4 min followed by 10 cycles of initial denaturation at 94°C for 45 s, an annealing temperature at 65°C for 1 min, decreasing 1°C per cycle, and extension at 72°C for 2 min. This was followed by another 20 cycles of initial denaturation at 94°C for 45 s, an annealing temperature at 55°C for 1 min and extension at 72°C for 2 min, and a final extension at 72°C for 10 min. A negative control (no DNA template) was included in each PCR reaction.

PCR products were electrophoresed at 80 V for 35 min on 0.8% agarose gels in 1 X TAE buffer (0.04 M of Tris acetate; 0.001 M EDTA, pH 8.5) and stained with ethidium bromide (Promega) to a final concentration of 0.5 µg/mL.

Data analyses

DNA concentrations were standardised for bumblebee leg weights. The effect of extraction method on DNA concentration from freshly-killed bees was analysed using ANOVA. The covariate leg-weight was initially included in the model but was removed because it had no significant effect. The effects of sample storage and extraction methods on DNA degradation (fresh DNA concentration – DNA concentration of stored samples) were analysed using repeated measures GLM; the model was as follows: *DNA degradation = time + storage method + extraction method + interactions + error*. Actual DNA concentrations are presented in Fig. 1. Statistical analyses were conducted using SPSS 18.0 (Carver & Nash, 2009). Data were log-transformed and residuals plotted following all analyses to assess normality and homogeneity. Post-hoc Tukey tests were conducted for the main factors (storage methods and extraction techniques) to determine homogenous groups.

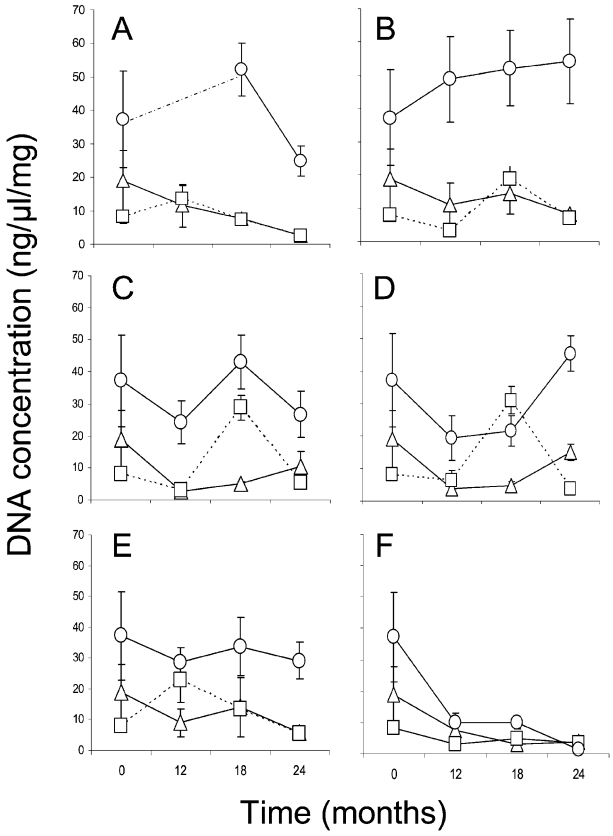

Fig. 1. DNA concentration in ng/µL/mg of tissue extracted from *Bombus terrestris* (hind leg) samples stored as (A) pinned specimens at room temperature; (B) pinned specimens at 4°C; (C) unpinned specimens at –20°C; (D) unpinned specimens at –70°C; (E) specimens in acetone, and (F) specimens in 70% ethanol. DNA was extracted using a phenol method (circles), commercial kit (triangles) and CTAB (squares). DNA concentrations at time = 0 months (i.e., freshly killed bees) are indicated for comparison. Bars indicate standard errors.

RESULTS

Preservation and DNA-extraction methods

DNA was extracted from all samples up to the limits of the study (24 months). DNA could not be reliably determined for a subset of phenol extracted samples contaminated with pollen, in spite of samples being repeatedly washed with distilled water. Therefore, contaminated phenol-extracted samples were excluded from analysis of DNA concentrations. Nevertheless, all storage and extraction methods examined yielded sufficient quality DNA for successful amplification of the target genes using PCR and produced clear bright bands on agarose gels. Furthermore, the sizes of the products were consistent with the sizes of the gene regions.

The concentrations of DNA extracted differed over time and according to storage techniques (Fig. 1). There were significant changes in concentration per mg of sample over the 24 months of storage (Table 1); however,

TABLE 1. Concentration of DNA extracted from hind legs of *Bombus terrestris* using three extraction methods with reductions in DNA concentration following storage under six conditions for 12, 18 and 24 months.

Storage method†		Extraction method†		
	Time (months)	CTAB (B)	Kit (B)	Phenol (A)
		DNA concentration*		
Freshly killed bees	0	85.0 (36.17)	40.0 (8.16)	225.0 (55.60)
		DNA loss ($DNA_{T=0} - DNA_{T=t+n}$)*		
Room temp. (a)	12	–3.62 (7.12)	5.42 (4.01)	37.74 (–)
	18	–11.38 (1.59)	–0.80 (0.86)	14.96 (7.78)
	24	–11.66 (1.73)	–5.69 (0.31)	–12.45 (4.59)
4°C (a)	12	–7.78 (6.37)	–4.68 (0.33)	11.54 (12.82)
	18	–4.28 (6.24)	10.83 (3.60)	14.33 (11.28)
	24	–10.74 (0.56)	–1.05 (1.63)	16.90 (12.61)
–20°C (a)	12	–16.43 (0.31)	–4.98 (0.53)	–13.14 (6.68)
	18	–14.08 (0.85)	20.68 (4.02)	5.90 (8.43)
	24	–8.65 (4.95)	–2.62 (1.51)	–10.58 (7.22)
–70°C (a)	12	–15.18 (0.90)	–4.68 (0.33)	–17.89 (6.84)
	18	–14.28 (1.13)	10.83 (3.60)	–15.81 (4.65)
	24	–4.04 (2.58)	–1.05 (1.63)	8.17 (5.47)
Acetone (a)	12	–10.00 (4.61)	14.68 (7.52)	–8.45 (4.66)
	18	–4.97 (9.67)	5.32 (1.79)	–3.54 (9.53)
	24	–13.34 (0.67)	–2.81 (0.98)	–8.10 (6.07)
Ethanol (b)	12	–11.52 (2.26)	–5.02 (0.92)	–27.18 (3.00)
	18	–15.94 (0.55)	–3.37 (0.88)	–27.48 (1.83)
	24	–15.30 (1.71)	–7.77 (0.09)	–35.96 (0.51)

† – letters in paranthesis (a and b for storage method; A and B for extraction method) indicate homogenous groups (Tukey test); * – standard errors are given in parentheses.

the trends were inconsistent between storage methods and extraction protocols, leading to significant time × storage method × extraction method interactions (Table 2). In some cases there were apparent increases in yield between successive time points. This may be because extractions were conducted at different times and, therefore, under different ambient conditions, and where it occurred, was taken to indicate relatively stable DNA yield (i.e., very low levels of degradation). The phenol-extraction method yielded a higher concentration of DNA from freshly-killed (time = 0) bees compared to the kit method, but was similar to the CTAB method (Table 1 and 2). Accelerated DNA decay in ethanol solution was apparent by significantly lower DNA yields compared to those from all other storage methods (Table 2). The phenol extraction method increased the concentrations of DNA extracted from the samples (Table 2). However, there was a significant storage × extraction method interaction (Table 2) because the phenol method failed to increase DNA yields from ethanol-stored samples (Fig. 1). Significant time × extraction method interactions were largely due to high DNA yields from samples stored for 18 months using the phenol and CTAB methods, but not the kit (Fig. 1, Table 2). Significant time × storage method interactions were due to the lower DNA yields from samples stored for 18 and 24 months in ethanol and as pinned specimens, but similar DNA concentrations from other storage methods and times (Fig. 1, Table 2).

TABLE 2. Results of repeated measure GLM of DNA loss from hind legs of *Bombus terrestris* after storage under six conditions and using three different extraction methods.

Source of variation	Nominator DF	Denominator DF	F-value	P-value
Time (repeated)	2	43	47.42	≤0.001
Extraction method	2	44	71.02	≤0.001
Storage method	5	44	12.042	≤0.001
Time × extraction method	4†	86†	20.05	≤0.001
Time × storage method	10†	86†	8.568	≤0.001
Extraction method × storage method	10	44	2.879	0.008
Time × extraction method × storage method	20†	86†	2.168	0.007

† – DF from multivariate tests.

DISCUSSION

Results from this study indicate that DNA can be successfully extracted from bumblebee samples stored using a variety of cheap and accessible methods. We successfully amplified both mitochondrial and nuclear genes from samples stored over the two years of this study. Nevertheless, two of the most commonly employed storage methods (dry-pinning and ethanol storage) caused declining DNA yields over a relatively short (2-year) period. Consistently high DNA yields could be extracted from pinned specimens using the phenol method, but even this method was largely unsuccessful in extracting DNA from specimens stored for 2 years in 70% ethanol. Inappropriate storage will often require more expensive or noxious DNA extraction methods such as the phenol method used here. Because of increasing restrictions on insect collection, stored specimens will likely be revisited by scientists for increasingly diverse objectives. Our results indicate that specimens should be carefully preserved as valuable sources of genetic information, by storage in acetone, below freezing temperatures or using a combination of preservation techniques (i.e., acetone in cold storage).

Fukatsu (1999), Dean & Ballard (2001) and Mandrioli et al. (2006) have conducted factorial experiments to assess storage techniques for aphids, hymenopterans and moths, respectively. Mandrioli et al. (2006) reported that optimal storage conditions may vary depending on the materials (including the specific insect taxon) in storage. Factors, such as specimen size, the amount of chitin in the samples, and the presence and amounts of water or other contaminants can all affect the efficiency of sample preservation techniques. Museum or archived samples are an important source of specimens that can help not only in resolving taxonomic uncertainty but also in molecular studies (Freeland et al., 2007). Strange et al. (2009) demonstrated the utility of museum pinned *Bombus* spp. in obtaining multilocus microsatellite genotypes to determine trends in genetic diversity in bumblebees. However, our results indicate that DNA decay can occur over a relatively short time (2 years), and that researchers should not rely on dry-pinned archived specimens for posterior molecular work. The quantities of DNA extracted from dried bumblebees declined linearly over the two years of storage at room temperature. Using the phenol extraction method, apparently large quantities of DNA were yielded from dried specimens; however, the extracts were prone to protein contamination which prevented spectrophotometric estimation of DNA concentration for some samples. Quicke et al. (1999) indicated that DNA degradation had occurred in dry ichnuemonid specimens over 35 years. In their study, sequence data for 28S rDNA was unobtainable from up to 40% of pinned samples that were stored for 0 to 5 years (from a total of 64 samples). Furthermore, Dean & Ballard (2001) indicated that pinned specimens that were stored for 2 years had badly sheared DNA and poor PCR success. However, Gilbert et al. (2007), using a method that minimises external morphological damage in museum specimens, successfully extracted DNA from dried specimens stored for 58 years. Based on the present study, cold storage may improve the quality of pinned samples, but sustained cold storage can be expensive (Dessauer et al., 1996). Where samples have been stored for extended periods as pinned specimens or in ethanol solutions, improved DNA yields can be obtained using the phenol method. Phenol was the most toxic of all the extraction reagents used in this study, but this method yielded up to three times more DNA than all other methods tested. Where possible, safer and more convenient DNA extraction protocols should be used, but this will ultimately be dictated by the choice of storage conditions.

Our results indicate that bumblebee specimens stored in 70% ethanol consistently yielded lower DNA concentrations independent of the extraction techniques used. The phenol extraction method, which often yielded up to three times the quantities yielded by the commercial kit and the CTAB methods, extracted significantly less DNA from ethanol-stored specimens than from specimens stored by other methods or those obtained from fresh material. Surprisingly, the samples had been stored for a maximum of only 2 years, and DNA decay had already occurred within the first 12 months. It is possible that the very low preservation success encountered in samples stored in ethanol may have been due to water contamination. Bumblebees are relatively large insects and consequently contain large amounts of water. Therefore, it is possible that they contributed large amounts of water to the solvent, reducing the solvent's effectiveness. Fukatsu (1999) indicates that ethanol is strongly affected by water contamination. DNA yields and quality were generally improved when bumblebee specimens were stored at –20°C and –70°C.

The most consistent storage method in this study was acetone. Although acetone was not significantly better than the other methods, the DNA yields from acetone-stored samples tended to be more stable than those from the other samples, without obvious peaks and troughs. Previous studies have also indicated that acetone is an excellent storage solvent (Fukatsu, 1999; Mandrioli et al., 2006). Acetone, a relatively inexpensive and widely available solvent, appears to penetrate insect tissues more rapidly than ethanol, and is less prone to water contamination. Furthermore, the ability of acetone to preserve DNA does not appear to be taxon dependent (Fukatsu, 1999). In this study we observed that specimens stored in acetone kept their rigid structure when compared to 70% ethanol. These results suggest that specimens stored in acetone are more suitable not only for molecular studies but also for morphological taxonomy. However, in this study, some discoloration of specimens was observed in acetone, indicating that, where colour is a key component of identification, alternative storage solutions should be considered. Although, acetone is an excellent storage method, it is highly flammable and may pose serious transportation problems, in particular when samples are transported in aircraft. We suggest that when acetone cannot be used as a solvent due to transportation restrictions, samples should be soaked in ethanol and drained

prior to transportation. Once in the laboratory the samples should be placed in acetone to aid later molecular analyses.

Concluding remarks

Many bumblebee species are now scarce or have declining numbers. Therefore, sampling from the wild should first avoid killing the bees. Holehouse et al. (2003) suggested that tarsal sampling of either a mid-leg or hind-leg is an effective and acceptable means of sampling DNA from wild bumblebees with no detrimental effect on individual or colony performance. In cases where bumblebees can be collected without concern for their populations, then maximum use should be made of preserved specimens. We recommend freezing or acetone storage of bumblebee samples as optimal for molecular studies, and that weak ethanol solutions (< 75%) should be avoided. Although DNA can be extracted from dried-pinned specimens, very often the DNA is of poor quality leading to unsuccessful PCR amplification in particular for larger fragments. However, dry-pinning is still the best method for morphological and taxonomic studies. Researchers will need to evaluate the cost-effectiveness and practicalities of each storage technique as related to their own research, but will need to evaluate these against the ease or difficulties that are likely to arise during future molecular studies.

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