Insect. Soc. 51 (2004) 197–202
0020-1812/04/020197-06
DOI 10.1007/s00040-003-0709-x

Insectes Sociaux

Technical note

Recommendations on the use of alcohols for preservation of ant specimens (Hymenoptera, Formicidae)

J.R. King[1,2] and S.D. Porter[2]

[1] *University of Florida, Department of Entomology & Nematology, PO Box 110620, Gainesville, FL 32611, USA, e-mail: jking@gainesville.usda.ufl.edu*
[2] *Center for Medical, Agricultural & Veterinary Entomology, USDA – ARS, PO Box 14565, 1600 SW 23rd Dr., Gainesville, FL 32604, USA*

Received 8 July 2003; revised 23 October 2003; accepted 27 October 2003.

Summary. Collectors use a variety of concentrations and types of alcohols to preserve ant specimens. We evaluated existing literature, experimental evidence, and expert myrmecological advice to determine what kind and concentration of alcohol will result in the best preserved specimens for card-point mounting and DNA extraction. For our experimental evaluation, we killed and stored *Solenopsis invicta*, *Camponotus floridanus*, and *Dorymyrmex bureni* workers in isopropanol and ethanol at four concentrations (70, 85, 95, 100%) over three time periods (24 h, 1 month, 6 months). We then compared specimen condition and amenability to manipulation for mounting on card points. Specimens stored in either 95% isopropanol or 95% ethanol for time periods longer than 24 h produced the best specimens for mounting. A literature review revealed that DNA is best preserved in 95–100% ethanol due to the ability of ethanol to more rapidly penetrate cellular membranes and deactivate DNase activity than other primary alcohols. We recommend that general collections of adult ant specimens should be killed and stored in 95% ethanol. Following this recommendation will result in ant specimens that are easier to mount for museum collections and better preserved for molecular studies. A variety of other killing and preservation techniques relevant to the study of ants are also discussed.

Key words: Formicidae, ethanol, isopropanol, specimen preservation, DNA.

Introduction

Ant collectors have a number of choices available for killing, preserving, and storing specimens. Immersion in some type of alcohol has long been the most commonly used method because of its greater safety and availability, low cost, and effectiveness in producing well-preserved, usable specimens relative to other killing and preserving agents (Wheeler, 1910; Borror et al., 1989; Upton, 1994). Although alcohol use has become nearly universal, it is by no means standardized; there is a great deal of variability in the type and concentration of alcohol used by collectors. The absence of comprehensive information detailing the effect of alcohol storage on insect specimens has created the potential for collectors to unknowingly preserve specimens in such a way that they may be unusable for certain types of study. In particular, some molecular techniques, which have increasingly become an integral component of systematic and ecological work on ants, may be impracticable if specimens are improperly killed and preserved (Post et al., 1993; Dillon et al., 1996). The purpose of this article is to provide recommendations on the use of alcohols as killing and preserving agents for ants. We provide an overview of how the features of ants respond, qualitatively, to different types and concentrations of alcohol. Furthermore, we outline how alcohols preserve specimens and discuss why some alcohols are better than others for preserving ants. We present this information in the hope of establishing a standard protocol for alcohol use by ant collectors which will improve both the curation of specimens and the preservation of DNA.

The ongoing biodiversity crisis encumbers insect collectors with a responsibility to preserve biological information for current and future study. With few exceptions, following the recommendations of this review will ensure that adequate external morphological and molecular information is retained in collected ant specimens and available for future study. Additionally, workers intending to use ant specimens that have already been killed and preserved in alcohol or another killing agent may gain some insight into whether those specimens will be viable for certain types of study. We organized our recommendations by whether myrmecologists are seeking to mount or preserve specimens for examination of external morphology or to extract DNA. In addition we make some comments on how alcohols may affect internal

tissue and organs. Although our recommendations are specific to the preservation of adult ant specimens, they are also generally relevant to many other adult insects with small bodies and heavily sclerotized exoskeleton.

Materials and methods

Perhaps the most common reason for collecting ants for study is to observe their external features for identification. To ameliorate the process of identifying most ant species, representative, adult worker specimens must be mounted properly. Lattke (2000) provides a comprehensive, step-by-step review of ant specimen processing and curating for identification purposes and we favor his methodology with the caveat that collectors should employ an alcohol type and concentration consistent with our recommendations. When mounted, specimens are glued on points of paper inserted on the right side of the body between the middle and hind coxae to provide support across the ventral body surface. Regardless of the mounting technique used, there is usually some manner of manipulation of a specimen's body or body parts that must be done prior to mounting to assure that the point can be properly placed and that none of the features of the ant necessary for identification are obscured or hidden by their position. The type and concentration of alcohol used in the process of sorting and storing specimens affects their rigidity and can greatly facilitate the process of mounting specimens. Ideally, specimens should have some rigidity so that appendages will remain in position without being too rigid, brittle, or conversely, flexible to the point that limbs will not remain in the position they are placed.

To assess how alcohol may influence the process of mounting adult ants, we tested the effect of different types and concentrations of alcohol on three species of ants from three different subfamilies in a four-way fixed-level factorial design. We tested both isopropyl (isopropanol) and ethyl (ethanol) alcohol at 4 concentrations: 70%, 85%, 95%, and 100%, over three time periods: 24 h, 1 month, and 6 months. The different concentrations were made by diluting 100% isopropanol and ethanol with deionized water. *Solenopsis invicta* Buren, *Dorymyrmex bureni* (Trager), and *Camponotus floridanus* (Buckley), were used for the experiment. We selected these species because they represent a wide range in body sizes (<1 mm to >10 mm in total body length), commonly collected subfamilies (Myrmicinae, Formicinae, and Dolichoderinae) and genera. Seventy-two 2-dram (8 ml) glass, screw-top vials were used. Each vial received three workers (representative sizes were selected from the polymorphic species, *S. invicta* and *C. floridanus*), for a total of 216 specimens. At the end of each time period specimens were removed from alcohol and briefly dried by placing them on paper towel. To reduce bias, manipulations and observations were then performed by a different person without knowledge of the source of the specimen. To qualitatively evaluate the outcome of the effects of alcohol type and concentration on the condition of specimens intended for mounting, the second and third legs on the right side of each specimen were manipulated into a position appropriate for mounting (i.e. they were typically spread apart and arranged below the midline of the body). If the legs remained as placed, they were scored "yes", if not, they were scored "no." Condition (e.g. brittleness, flexibility, damage) of each specimen was also noted.

A logit analysis of a five-way contingency table was used to assess the relationship of species, alcohol type, time in alcohol, and alcohol concentration with specimen condition. For the analysis, a generalized linear model was fit to data using a logistic link function in *proc genmod* in SAS (Allison, 1991; SAS Institute, 2000; Stokes et al., 2000). Interactions were not found to be significant and were excluded from the final model (Allison, 1991). In addition, it should be noted that an examination of the parameter coefficients in *proc genmod* is, by default, a contrast between the highest value of a classification variable and all other values (Allison, 1991).

Results

Alcohol concentration (X^2 = 97.5, 3 d.f., $p < 0.0001$) and time in alcohol (X^2 = 12.73, 2 d.f., $p = 0.0017$) were the only variables that affected the outcome response, while species and alcohol type had no affect on the adjustability of specimen legs. An analysis of the parameter estimates showed that the outcome responses at alcohol concentrations 70% (X^2 = 28.69, 1 d.f., $p < 0.0001$) and 85% (X^2 = 30.11, 1 d.f., $p < 0.0001$) were significantly different from outcome responses at 100% (Table 1). The outcome response at 95% was not significantly different from 100%. Additionally, specimens exposed to alcohol for only 24 h were significantly different (X^2 = 7.74, 1 d.f., $p < 0.0054$), from those exposed for 6 months, while those immersed for 1 month were not significantly different from specimens immersed for 6 months. These results indicate that regardless of species or type of alcohol, specimens exposed to higher concentrations of alcohol (95% or higher) for periods of time greater than 24 h will be more amenable to manipulation for mounting (but see Table 1, and comments below on specimen condition).

Qualitatively, among alcohol concentrations there were distinct differences among specimens. Specimens killed and stored in both 70% and 85% ethanol and isopropanol were generally flexible and rubbery. Legs, antennae, and body position could all be easily manipulated without damage; however, they tended to return to their original position and could rarely be coaxed to hold a position that facilitated mounting. Additionally, some specimens stored in 70% alcohol for the six months were noticeably swollen, typically with distended pleural and intersegmental membranes on the abdomen. In contrast, specimens stored in 100% alcohol tended to produce extremely stiff and brittle specimens that often lost tarsi and antennae – even when handled with extreme care. Similarly, species with relatively softer bodies (*D. bureni* and *C. floridanus*) tended to have shriveled abdomens (and in a few cases, head capsules) when stored for 1–6 months in 100% alcohol. Overall, regardless of species, alcohol type, or time of storage, killing and preserv-

Table 1. Frequencies of positive and negative ant specimen scores for mounting grouped by the length of time immersed in alcohol and the concentration of alcohol used. Specimens were scored "yes" if they were amenable to mounting (i.e. if legs and body remained in position after manipulation). Generally, specimens stored in absolute ethanol and isopropanol were extremely brittle and could not be manipulated without breaking appendages. In contrast, specimens stored in 95% alcohols were not brittle and were easily manipulated.

Concentration	Time					
	24 hours		1 month		6 months	
	yes	no	yes	no	yes	no
70%	6	12	4	14	6	12
85%	3	15	8	10	4	14
95%	9	9	18	0	18	0
100%	15	3	18	0	18	0

ing ants in 95% alcohol produced specimens best suited for manipulation and mounting, with minimal shrinkage or swelling of body parts, and without brittleness.

Discussion

Proper curation and preservation of biological specimens is a crucial component of both the ecological and evolutionary study of ants and insects. For collectors to attain the best-preserved specimens for mounting and DNA extraction, the processes of tissue degradation and DNA decay must be severely limited or stopped while damage to external features and molecular structures is minimized. Therefore, to be most effective a preservative (or preservative process) should remove water and oxygen and sterilize tissues to prevent damage from autolytic processes (e.g. DNase activity), hydrolytic processes (e.g. hydrolysis of phosphodiester or carbon-nitrogen bonds), and micro-organisms (Baker, 2000; Prendini et al., 2002). Furthermore, to prevent denaturation and degradation of proteins and nucleic acids over time, oxidative processes must also be slowed or stopped by lowering temperatures and excluding light (Prendini et al., 2002).

Primary (monohydric) alcohols such as ethanol and isopropanol are effective preservatives because they coagulate proteins and disrupt cellular membrane function (halting DNase activity and killing micro-organisms) without unduly damaging external features. Additionally, primary alcohols enter specimen tissues in proportion to their water content, effectively precipitating DNA from solution with water (preventing hydrolysis of phosphodiester and carbon-nitrogen bonds). This process occurs as, in the presence of ethanol and isopropanol at high concentrations (e.g. greater than 50% aqueous solution), the interhelical interactions become so strong that the tertiary structure of DNA becomes stabilized due to an almost complete absence of hydrogen bonding and base-stacking; i.e. the DNA becomes insoluble, aggregated, and thermally stable (Piškur and Rupprecht, 1995; Sergeyev et al., 1999).

Primary alcohols are effective preservatives for insects because of their small size and chitinous integument. Soft, membranous cuticle, typically found in areas of joint flexion and intersegmental regions, is more hydrophilic than hard cuticle and is likely where most diffusion through external integument occurs for polar, primary alcohols. When specimens are immersed in alcohols their tissues become saturated. Both ethanol and isopropanol first enter ant specimens primarily through the alimentary canal and the respiratory system and then penetrate internal tissues by passively diffusing through cellular membranes. However, diffusion may occur slowly or be hindered if specimens close oral, anal, or respiratory tracts upon immersion or if the specimens are very large. Additionally, the type and concentration of alcohol will determine the rate of diffusion and the final water to alcohol ratio within specimen tissues.

Mounting specimens

The concentration of primary alcohol (ethanol or isopropanol) used to kill and store ants clearly impacts the quality of specimens intended for mounting. Based on our results, storage of specimens in 95% ethanol and isopropanol produces specimens most amenable to mounting without the excessive flexibility or brittleness seen in specimens stored at lower and higher concentrations, respectively. In addition, we have also observed that ant specimens killed and (temporarily) stored in alternative killing agents commonly employed in field collecting techniques (e.g. pitfall traps, malaise traps, kill jars, etc.) such as ethylene glycol, propylene glycol, glycerin, and ethyl acetate are typically not adversely affected for mounting purposes if they are cleaned and eventually stored in alcohol prior to mounting.

There is evidence that long-term storage of ant specimens in isopropanol may discolor specimens more than ethanol (S. Cover, J. Lattke, pers. comm.). This is likely a result of the greater propensity of isopropanol (and other longer-chain alcohols) to extract lipids and proteins (including pigments) from cellular membranes over time at high concentrations (Goates and Knutson, 1994). However, damage to external features resulting from any long-term (e.g. decades or longer) storage of specimens in alcohol may be prevented or reduced if they are stored near or below the freezing point (0 °C) in a dark environment (Masner, 1994). The volume ratio of alcohol to specimens should be kept very high (30 or more times specimen volume) to avoid dilution of the preservative (Martin, 1977). This is likely only to be a problem with ants if large worker or colony series are collected or if small vials are used to store very large specimens. Large volumes of high concentrations (> 70%) of alcohols kept for long periods of time should be maintained in fireproof conditions. Glass vials should be used for long-term storage as plastic is less fire resistant and may also eventually be degraded by high concentrations of ethanol (Prendini et al., 2002). Highly concentrated primary alcohols are flammable liquids and may require special packaging and labeling requirements according to the transportation laws of the countries of origin and destination. Shipping companies (e.g. Federal Express) or government agencies (e.g. United States Department of Transportation) in origin and destination countries should be consulted prior to the shipment of specimens in alcohol to assure proper handling and delivery. Martin (1977) and Upton (1991) provide further comprehensive, general entomological literature resources for collection, curation, preservation, and storage techniques not covered here.

Although live or freshly killed specimens may be preferable, a wide variety of methods of fluid preservation for insects intended for internal morphological study can be used for adult and immature life-stages of ants. Among those choices, the use of alcohol as a killing agent and preservative for specimens should be carefully considered prior to collection as some histological and slide-mounting methods may be confounded by the use of alcohol (Wheeler and Wheeler, 1960; Barbosa, 1974; Martin, 1977). Conversely, many established fluid preservation techniques (particularly those

that require the use of formalin or acetic acid) may damage external features or prevent DNA extraction. For both the immature and adult life stages of many insects, the use of alcohol (particularly concentrations higher than 85%) is contraindicated as a killing agent for many histological techniques and internal morphological studies as it may make internal tissues brittle, shrink organs, or discolor organs and tissue; although it may be recommended for storage after fixation in other agents such as acetic alcohols, Carnoy's solution, boiling water, or others (Wheeler and Wheeler, 1960; Barbosa, 1974; Martin, 1977; Borror et al., 1989; Upton, 1991, 1994).

Wheeler and Wheeler (1960) have written an excellent paper on techniques for the study of ant larvae and it should be used as a guideline for the study of internal and external morphology of immature ant forms. Further reading on the broad subject of histological and internal morphological study techniques for insects, including formulae of commonly used fixatives and clearing, staining, and relaxing media applicable to adult ants can be found in Barbosa (1974), Martin (1977), Borror et al. (1989), and Upton (1991, 1994).

Extraction of DNA

Molecular techniques, including DNA amplification methods (i.e. Polymerase Chain Reaction, or PCR), require at least some intact DNA. Because it is often extremely difficult to obtain or transport and maintain live specimens of the majority of ant species, preservation techniques that conserve intact DNA are crucial for molecular studies. Accordingly, effective preservation protocols have been developed for many taxa (including many insect groups), but none have been published specifically for ants. Although there are differences among insect orders and families in the effectiveness of various preservation techniques (Post et al., 1993; Reiss et al., 1995; Dillon et al., 1996; Austin and Dillon, 1997; Koch et al., 1998; Prendini et al., 2002), it is generally accepted that the most consistently effective method of DNA preservation amenable to field collecting techniques is immersion in absolute ethanol (Baker, 2000; Prendini et al., 2002).

Ants, like wasps (Dillon et al., 1996; Austin and Dillon, 1997), may be robust to different methods of alcohol preservation as intact (amplifiable) DNA can be extracted from specimens stored in lower concentrations (e.g. 70–90%) of ethanol and isopropanol stored at room temperature (S. Valles, pers. comm.). However, for the most consistent results both adult (workers and sexuals) and immature ant specimens intended for molecular study should be killed and preserved in 95–100% ethanol and stored under cold (e.g. <4 °C), dark conditions (e.g. in a freezer) (Prendini et al., 2002; P. Ward, pers. comm.). Specimens stored in 95–100% ethanol kept at room temperature for longer than two months may yield diminishing quantities of DNA (Reiss et al., 1995). For long-term fluid preservation (e.g. for many months or years) specimens should be stored in absolute ethanol and frozen at –20 °C or colder (and kept in the dark) (Dean and Ballard, 2001). Genetic voucher specimens should be prepared using cryo-fixation methods and deposited in permanent, liquid-nitrogen cryo-preservation facilities (e.g. American Museum of Natural History's Ambrose Monell Cryo Collection) that maintain specimens near –150 °C to ensure DNA stability for decades or longer (Hanner and Webster, 2002).

Generally, fixatives other than ethanol (e.g. isopropanol, methanol, formalin, Carnoy's solution) should be avoided for storage of specimens intended for molecular work as it has been shown that they do not preserve insect DNA as well (Post et al., 1993; Reiss et al., 1995; Dillon et al., 1996). Additionally, lower concentrations of ethanol (<95%) should be avoided for storing specimens, particularly if dilution caused by a large specimen to alcohol volume ratio cannot be avoided. To minimize the effects of dilution, alcohol should be replaced promptly after initial immersion (at 24 h), and periodically (once per week for several weeks) thereafter for very large samples (Prendini et al., 2002).

Why does ethanol seem to preserve DNA in specimens better than other primary alcohols? The answer to this question is likely dependent on a combination of how quickly the alcohols penetrate cellular membranes and how effectively they precipitate DNA and halt DNase activity intracellularly. The most comprehensive data on the diffusion of primary alcohols across cellular membranes comes from the field of investigative dermatology using the stratum corneum, the lipid-enriched keratin layer of human epidermis, as a chemically similar analogue for all cellular membranes (Scheuplein, 1965). The rate of diffusion of primary alcohols has been shown be a product of (and is proportional to) the diffusion coefficient of the molecules across membranes: at the same concentration gradient, small polar molecules will diffuse more rapidly across a phospholipid bilayer than large polar molecules (Scheuplein, 1965; Scheuplein and Blank, 1973). Accordingly the rate of diffusion for primary alcohols through epidermis follows the order methanol < ethanol < isopropanol. Additionally, based on research on the preservation of DNA in a variety of plant tissues using alcohol, Flournoy et al. (1996) have proposed that ethanol may more effectively denature DNases than methanol. In combination, this evidence supports the hypothesis that ethanol is the best primary alcohol for use as a preservative of specimens intended for molecular work as it will penetrate cell membranes more rapidly than isopropanol and more effectively precipitate DNA and coagulate DNases than methanol (Adams et al., 1999; R.P. Adams, pers. comm.).

A variety of killing agents, including ethanol, isopropanol, ethyl acetate, ethylene glycol, and various fixatives have been evaluated for their impact on the extractability of DNA from various insect groups (including Coleoptera and Hymenoptera) (Post et al., 1993; Reiss et al., 1995; Dillon et al., 1996; Austin and Dillon, 1997; Koch et al., 1998; Prendini et al., 2002). Generally, none of these agents performed as well as absolute ethanol for preserving DNA, although wasps killed and stored in ethylene glycol reliably produced amplifiable DNA (Dillon et al., 1996). Additionally, specimens killed in agents other than 95–100% ethanol, even if

eventually stored in 95–100% ethanol, may not yield extractable DNA (Reiss et al., 1995; Dillon et al., 1996; Austin and Dillon, 1997). Other molecular methods, such as cuticular hydrocarbon analysis, may be confounded by the use of alcohols (D. Carlson, pers. comm.). Accordingly, specimens intended for cuticular hydrocarbon analysis or multiple molecular technique analyses should be live or temporarily preserved in hexane (Narang and Seawright, 1990; Narang et al., 1993). Further research on the effects of various killing agents on extracting DNA from ants is warranted, particularly for non-evaporating agents such as ethylene glycol ("toxic" antifreeze) or propylene glycol ("non-toxic" antifreeze) which are often used in field techniques (e.g. pitfall traps).

Neither reagent grade isopropanol nor ethanol can be obtained commercially as true absolute; the actual percentage is likely to be between 99.5–99.9% with water and possibly trace amounts of other materials such as benzene which may remain if used in the manufacturing process (which may affect DNA extraction – see Ito, 1992) (Sigma-Aldrich Co., customer service, pers. comm.). Lower concentrations of reagent grade alcohol (e.g. 95%) typically contain water and may contain other fluids such as methanol. Denatured alcohol (ethanol to which toxic substances have been added) may contain ingredients such as methanol, isopropanol, ethyl acetate, methyl ethyl ketone, methyl isobutyl ketone, and various hydrocarbons (Sigma-Aldrich Co., customer service, pers. comm.). Denatured alcohol, while often less expensive and more widely available, may affect specimen condition (e.g. discoloration) if used for long-term storage (S. Cover, J. Lattke, pers. comm.) and should be avoided for molecular work (Post et al., 1993; Dillon et al., 1996). Baker (2000) and DeSalle et al. (2002) provide further, detailed information on molecular techniques, preservation methods, and DNA resources.

Conclusions

Specimens intended for study of external morphology or DNA extraction (or both) should be killed and stored in 95% reagent grade ethanol whenever possible to ameliorate the process of curation (mounting) while increasing the likelihood of DNA preservation. If molecular extraction is the primary goal for a study of ants, the collectors should consider using absolute ethanol and cryo-preserving specimens for longer-term storage to minimize DNA degradation. Specimens stored in lower concentrations of alcohol (< 95%) or in other liquids can be placed in higher concentrations of ethanol or isopropanol to make them more amenable for mounting. Similarly, specimens stored in absolute methanol, ethanol, or isopropanol can be made less brittle by storing them in lower concentrations of ethanol or isopropanol, or by placing them in water for a short time (< 24 h). Dried specimens can also be rehydrated in this manner although other methods of specimen relaxation may be preferred (Barbosa, 1974; Borror et al., 1989; Upton, 1991). Storing soft bodied insect specimens intended for morphological study, such as ant larvae, in alcohols at concentrations higher than 85% is not recommended (Wheeler and Wheeler, 1960).

Acknowledgements

This paper was inspired by J.R. King's discussion of the use of alcohol for preservation of ants with instructors at the 2001 Ant Course at the Southwest Research Station in Portal, Arizona. We sincerely thank Stefan Cover, John Lattke, Phil Ward, Steven Valles, David Carlson, and Robert Adams for contributing their expert opinions and advice. We thank Lloyd Davis for collecting *S. invicta* queens and aiding in specimen processing. Steven Valles, Lloyd Morrison, and Lloyd Davis provided comments on an earlier version of this manuscript. J.R. King was supported by a University of Florida Alumni Fellowship.

References

Adams, R.P., M. Zhong and Y. Fei, 1999. Preservation of DNA in plant specimens: inactivation and reactivation of DNases in field specimens. *Mol. Ecol. 8*: 681–684.

Allison, P.D., 1991. *Logistic Regression Using the SAS System: Theory and Application*. SAS Institute, Inc., Cary, North Carolina. 288 pp.

Austin, A.D. and N. Dillon, 1997. Extraction and PCR of DNA from parasitoid wasps that have been chemically dried. *Aus. J. Entomol. 36*: 241–244.

Baker, A.J. (Ed.), 2000. *Molecular Methods in Ecology*. Blackwell Science, Oxford. 337 pp.

Barbosa, P., 1974. *Manual of Basic Techniques in Insect Histology*. Autumn Publishers, Amherst, Massachusetts. 253 pp.

Borror, D.J., C.A. Triplehorn and N.F. Johnson, 1989. *An Introduction to the Study of Insects, Sixth edition*. Saunders College Publishing, Fort Worth, Texas. 875 pp.

Dean, M.D. and J.W.O. Ballard, 2001. Factors affecting mitochondrial DNA quality from museum preserved *Drosophila simulans*. *Entomol. Exp. Applic. 98*: 279–283.

DeSalle, R., G. Giribet and W. Wheeler (Eds.), 2002. *Techniques in Molecular Systematics and Evolution*. Birkhäuser Verlag, Basel, Switzerland. 407 pp.

Dillon, N., A.D. Austin and E. Bartowsky, 1996. Comparison of preservation techniques for DNA extraction from hymenopterous insects. *Ins. Mol. Bio. 5*: 21–24.

Flournoy, L.E., R.P. Adams and R.N. Pandy, 1996. Interim and archival preservation of plant specimens in alcohols for DNA studies. *Biotechniques 20*: 657–660.

Goates, C.Y. and K. Knutson, 1994. Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohols. *Biochim. Biophys. Acta 1195*: 169–179.

Hanner, R. and B. Webster, 2002. Cryopreservation of whole flies, a technique for the preparation of genetic voucher specimens. *Dros. Inf. Serv. 84*: 185–186.

Ito, K., 1992. Nearly complete loss of nucleic acids by commercially available highly purified ethanol. *Biotechniques 12*: 69–70.

Koch, D.A., G.A. Duncan, T.J. Parsons, K.P. Pruess and T.O. Powers, 1998. Effects of preservation methods, parasites, and gut contents of black flies (Diptera: Simuliidae) on polymerase chain reaction products. *J. Med. Entomol. 35*: 314–318.

Lattke, J.E., 2000. Specimen processing: building and curating an ant collection. In: *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. (D. Agosti, J.D. Majer, L.E. Alonso and T.R. Schultz, Eds.), Smithsonian Institution Press, Washington, D.C. pp. 155–171.

Martin, J.E.H., 1977. *The Insects and Arachnids of Canada Part 1: Collecting, Preparing, and Preserving Insects, Mites, and Spiders*.

Publication 1643, Research Branch, Canada Department of Agriculture, Hull, Québec, Canada. 182 pp.
Masner, L., 1994. Effect of low temperature on preservation and quality of insect specimens stored in alcohol. *Ins. Coll. News 9*: 14–15.
Narang, S.K. and J.A. Seawright, 1990. Hexane preserves biological activity of isozymes and DNA. *J. Am. Mosq. Control. Assoc. 6*: 533–534.
Narang, S.K., J.A. Seawright, S.E. Mitchell, P.E. Kaiser and D.A. Carlson, 1993. Multiple-technique identification of sibling species of the *Anopheles quadrimaculatus* complex. *J. Am. Mosq. Control. Assoc. 9*: 463–464.
Piškur, J. and A. Rupprecht, 1995. Aggregated DNA in ethanol solution. *FEBS Letters 375*: 174–178.
Post, R.J., P.K. Flook and A.L. Millest, 1993. Methods for the preservation of insects for DNA. *Biochem. Syst. Ecol. 21*: 85–92.
Prendini, L., R. Hanner and R. DeSalle, 2002. Obtaining, storing and archiving specimens and tissue samples for use in molecular studies. In: *Techniques in Molecular Systematics and Evolution.* (R. DeSalle, G. Giribet and W. Wheeler, Eds.), Birkhäuser Verlag, Basel, Switzerland. pp. 176–248.
Reiss, R.A., D.P. Schwert and A.C. Ashworth, 1995. Field preservation of Coleoptera for molecular genetic analyses. *Environ. Entomol. 24*: 716–719.
SAS Institute, 2000. *SAS/STAT User's Guide, Release 8.02*. SAS Institute, Inc., Cary, North Carolina. 3884 pp.
Scheuplein, R.J., 1965. Mechanism of percutaneous adsorption. *J. Invest. Derm. 45*: 334–345.
Scheuplein, R.J. and I.H. Blank, 1973. Mechanism of percutaneous absorption. IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. *J. Invest. Derm. 60*: 286–296.
Sergeyev, V.G., S.V. Mikhailenko, O.A. Pyshkina, I.V. Yaminsky and K. Yoshikawa, 1999. How does alcohol dissolve the complex of DNA with cationic surfactant? *J. Am. Chem. Soc. 121*: 1780–1785.
Stokes, M.E., C.S. Davis and G.C. Koch, 2000. *Categorical Data Analysis Using the SAS System*. SAS Institute, Inc., Cary, North Carolina. 626 pp.
Upton, M.S., 1991. Methods for collecting, preserving, and studying insects and allied forms. *Aus. Entomol. Soc. Misc. Pub. 3*: 1–86.
Upton, M.S., 1994. Collection and preservation of specimens. In: *Systematic and Applied Entomology: An Introduction*. (I.D. Naumann, Ed.), Melbourne University Press, Carlton, Victoria, Australia. pp. 204–222.
Wheeler, G.C. and J. Wheeler, 1960. Techniques for the study of ant larvae. *Psyche 67*: 87–94.
Wheeler, W.M., 1910. *Ants: Their Structure, Development, and Behavior*. Columbia University Press, New York. 666 pp.