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Gauging DNA degradation among common insect trap preservatives

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Abstract

Genetic methods for species identification are becoming increasingly popular and can accelerate insect monitoring. However, obtaining good DNA quality and quantity from insect traps remains a challenge for field studies. Ethylene glycol, propylene glycol, and Renner solution have been previously suggested as suitable preservatives for the collection of genetic material, but a systematic overview of their performance under compromising field conditions is lacking. Here we experimentally test whether and how different preservatives affect DNA quality under different conditions and evaluate how choice of preservative may affect metabarcoding and more demanding downstream applications (e.g., RADseq). For this, we used the house cricket, *Acheta domesticus* (L.) (Orthoptera: Gryllidae), and tested propylene glycol, ethylene glycol, and Renner solution for their ability to preserve DNA over 27 days in various dilutions and temperatures. DNA quality was measured as DNA fragmentation and success rates in PCR amplifying a COI fragment of 658, 313, or 157 bp. Undiluted propylene glycol and ethylene glycol always retained high molecular weight DNA at room temperature. No high molecular weight DNA was preserved at 37 °C or in any dilution. Nevertheless, the COI sequence could be amplified from samples at every condition. Renner solution did not preserve high molecular weight DNA and fragmentation increased over time at 37 °C until amplification was impossible. The results suggest that propylene glycol and ethylene glycol are suitable preservatives for collecting both genetic and morphological material, but dilution or high temperatures compromise their ability to preserve high molecular weight DNA. For genomic approaches requiring high DNA quality, additional preservatives may need to be tested.

KEYWORDS
Acheta domesticus, barcoding, DNA fragmentation, DNA quality, ethylene glycol, Gryllidae, metabarcoding, monitoring, Orthoptera, propylene glycol, Renner solution, species identification

INTRODUCTION

More than two-thirds of all terrestrial species are arthropods (Purvis & Hector, 2000) and among these, insects are the most abundant and diverse group (Stork, 2018). Arthropods play central roles in a variety of ecosystem functions including pollination (Ollerton et al., 2011), pest control (Redhead et al., 2020), and decomposition (Wu et al., 2015) and are central in food webs (Nyffeler et al., 2018). A decline in the number of insects has been shown in several studies in the last years, with a potential for strong negative consequences on ecosystems and human health (Seibold et al., 2019; Forister et al., 2021). To understand the drivers of insect decline and to monitor the success of conservation efforts, quick and reliable detection methods are necessary; however, these are often limited by the significant taxonomical expertise needed to identify species morphologically (Staab et al., 2015; Dopheide et al., 2019). To facilitate rapid identification of species, genetic methods such as metabarcoding have

become increasingly popular (Uhler et al., 2021; Leroy et al., 2022), but they add DNA preservation as an important new requirement for insect trap fluids, besides the preservation of morphological features.

Commonly used sampling methods such as flight-interception traps (Micó et al., 2015; Knuff et al., 2019), Malaise traps (Karlsson et al., 2020; Skvarla et al., 2021), or pitfall traps (Lange et al., 2014) often need to be in the field for extended periods of time (several weeks) to sample a representative fraction of the local community. Furthermore, when sampling in isolated locations, collection intervals for traps may be even longer due to difficulties in accessing the area (Aristophanous, 2010). During the sampling period, the DNA of the collected specimens is susceptible to degeneration by exonucleases (Barnes et al., 2014) and chemical influences, e.g., acid hydrolysis induced by changes in pH (Strickler et al., 2015; Tsuji et al., 2017). Degradation can be introduced and increased by adverse weather effects such as rainfall, which is a problem especially for pitfall traps (Costa-Silva et al., 2019). Dilution with rain water may compromise the preservability of the trapping liquid for both morphology (Costa-Silva et al., 2019) and DNA by lowering the preservatives' concentration and pH. Furthermore, temperature can relate to preservation. DNA itself is stable, also under high temperatures (Bartlett & Stirling, 2003; Karni et al., 2013) but enzymatic activity, e.g., by exonucleases, increases already at moderately high temperatures and this can significantly speed up DNA degradation during the summer (Barnes et al., 2014; Strickler et al., 2015; Kasai et al., 2020).

A variety of preservatives retaining the morphological features of specimens under field conditions are established, e.g., sodium chloride or glycerine (Stoeckle et al., 2010). However, most of these do not preserve DNA sufficiently for genetic analyses (Stoeckle et al., 2010). Ethanol is thus being frequently used for collection (Zhang et al., 2016; Uhler et al., 2021); however, at the commonly used concentration of 70%, DNA preservation is not optimal and at 100% concentration specimens become brittle (Marquina et al., 2021) and the preservative evaporates quickly (Nagy, 2010). Therefore, tests with other preservatives have been conducted in the last few years, which identified ethylene glycol (Stoeckle et al., 2010; Gossner et al., 2016), Renner solution (Stoeckle et al., 2010; Gossner et al., 2016), and propylene glycol (Vink et al., 2005; Nakamura et al., 2020; Martoni et al., 2021; Robinson et al., 2021) as promising options for preservation of both morphology and genetic material. These recommendations are based on the amplification or sequencing success of DNA barcode fragments (Nakamura et al., 2020; Martoni et al., 2021; Robinson et al., 2021) with the exception of Gossner et al. (2016) and Vink et al. (2005), who additionally provide DNA fragment lengths as a direct measure of DNA integrity. So far, data on the possibility of recovering high molecular weight DNA from insect trap specimens is not available, whereas it is a necessary prerequisite to recommend these preservatives for genetic applications that require genomic fragments longer than the standard 658 base pair *cytochrome c oxidase subunit I* (COI) barcode.

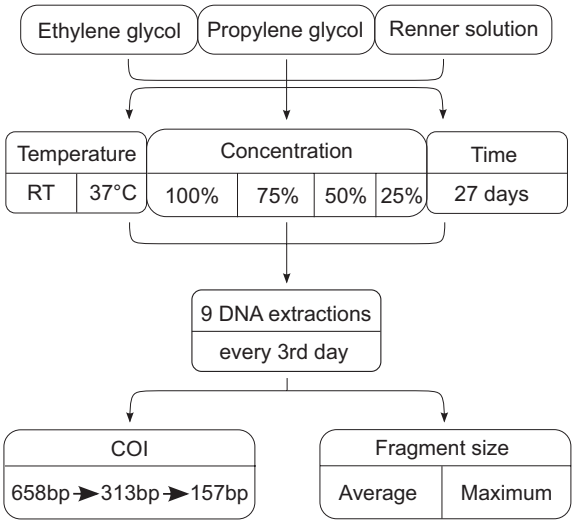

FIGURE 1 Overview of the experimental design. We tested three preservatives at room temperature (RT) and 37 °C, and four concentrations over 27 days with three biological replicates for each combination. At 3-day intervals, we analysed DNA quality by testing COI amplification and measuring average and maximum DNA fragment size to analyse the presence of high molecular weight DNA and overall DNA fragmentation.

In an experimental setting, using the house cricket, *Acheta domesticus* (L.) (Orthoptera: Gryllidae), we tested the influence of temperature, dilution, and time on DNA fragment length when samples are stored in either ethylene glycol, propylene glycol, or Renner solution. For all preservatives, we expected to measure an increase of DNA fragmentation over time, with increasing dilution, and temperature and consequently a decreased chance for recovering high molecular weight DNA.

MATERIAL AND METHODS

Experimental design

We performed a systematic experiment using the house cricket *A. domesticus* as the test organism. Three replicates were made for each combination of three preservatives, four concentrations, and two temperatures. The setup was repeated to have nine consecutive extractions (every 3rd day) summing up to a total timeframe of 27 days with 686 samples in total (Figure 1).

Sample set up and extraction

For this study, we used 694 commercially available live *A. domesticus* 'Micro' specimens, of homogenous size and age, from a zoo shop. The immature stage 'Micro' was chosen because the specimens easily fitted in standard 1.5-ml tubes without damaging body parts, which

ensured that the baseline from which degradation processes started was the same for all specimens, and because at this size the weight of available specimens was most homogenous.

Propylene glycol (CAS nr. 57–55-6), ethylene glycol (CAS nr. 107–21-1), and Renner solution (ethanol, glycerine, acetic acid, and water at 40:20:10:30 ratio) were prepared in concentrations of 100, 75, 50, and 25% by dilution with distilled water. Of each dilution, 1 ml was pipetted into sterile 1.5-ml tubes and three, live, approximately 0.5-cm-large cricket specimens were added to each, to serve as biological replicates per condition. The crickets were immobilized at –20 °C prior to handling, to ensure that they stayed fully submerged and consequently drowned within a similar time (a few minutes). This setup was duplicated to be stored at room temperature (20–24 °C, from now on referred to as RT) and in an incubator at ca. 37 °C and subsequently replicated 9× to be available for nine extractions. Temperatures were chosen based on the highest temperatures recorded in 2018 in the Southern Black Forest (Storch et al., 2020), which is representative for current maximum summer temperatures in several parts of central Europe. Additionally, on every extraction date for both temperatures, a tube with only one specimen in distilled water was prepared as a no-preservative control (from now on referred to as NC). Because the specimens in the NC were not subjected to experimental treatments and were homogenous in size and age, we did not expect to see a strong variation in the degradation progress of replicates under controlled laboratory conditions. We thus decided, as the NC would not be used quantitatively in the statistical analysis but only for qualitative comparison, that one specimen per tube would be sufficient, in order to keep the number of specimens used in this study at a minimum. The tubes were all stored in lightproof carton boxes to avoid any additional effects of light and UV radiation. At the beginning of the experiment (day 1), four specimens were extracted as a positive control (from now on referred to as PC) and all preservatives and dilutions were tested for potential contamination with DNA.

Over a course of 27 days, DNA was extracted every 3rd day from all conditions and the corresponding NC. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) with a standardized protocol following the manufactures recommendations (Supplementary data 1). After extraction, the DNA was eluted in 100 µl DEPC-treated water and stored at –20 °C until further processing. DNA concentrations were measured on a Qubit 4 Fluorometer with the corresponding Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

Fragmentation analysis

Polymerase chain reaction (PCR) was performed using HotStarTaq Plus DNA Polymerase (Qiagen) with 2 ng of DNA per sample or the maximum amount possible (PCR protocol in Table S1). For every sample, we first tested whether amplification of the whole length COI (658 bp) region is possible using the primer pair dgLCO1490 (5′ GGT CAA CAA ATC ATA AAG AYA TYG G 3′) and dgHCO2198 (5'TAA ACT TCA GGG TGA CCA AAR AAY CA 3′; Meyer, 2003). If all three biological replicates of a condition did not show a product, the reverse dgHCO2198 primer was combined with the forward mlCOIintF primer (5′ GGW ACW GGW TGA ACW GTW TAY CCY CC 3′; Leray et al., 2013) to target a shorter 313 bp region. Finally, if there was no product for all replicates, a 157 bp region was targeted with the ZBJ-ArtF1c (5′ AGA TAT TGG AAC WTT ATA TTT TAT TTT TGG 3′) and ZBJ-ArtR2c (5′ WAC TAA TCA ATT WCC AAA TCC TCC 3′; Zeale et al., 2011) primer pair (primer overview in Table S2). We chose to only test a triplet of replicates with the next primer pair if all three replicates failed, to avoid technical failure influencing the results, as we did not do technical replicates for the PCR. The success of the PCR was checked on 2% agarose gels stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific). For every extraction point, one positive PCR product was randomly chosen from each preservative for Sanger sequencing with one randomly chosen PC and NC for comparison. Sequencing was performed by Microsynth (Balgach, Switzerland). All samples, including PC and NC, were measured on a 5200 Fragment Analyser System (Agilent Technologies, Santa Clara, CA, USA) with the HS Genomic DNA 50-kb Kit (Agilent Technologies) and their average and maximum fragment size (independent of concentration) extracted with the PROSize v.3.0 Software (Agilent Technologies). In the fragment Analyser System output, fragment lengths above or equal to the high molecular weight minimum (>50000 bp) are not further differentiated. As in the PCR, we did not do technical replicates for the fragment measurements. Samples which did not return any result were cross checked with the Qubit data if concentration was already known to be very low or outside the measurable range.

Data were analysed with R v.4.1.1 (RStudio, Boston, MA, USA), using the packages *ggplot2* (Wickham, 2016), *tidyverse* (Wickham et al., 2019), *psych* (Revelle, 2013), *lmtest* (Zeileis & Hothorn, 2002), and *viridis* (Garnier et al., 2021). Average fragment size and maximum fragment size of every preservative, grouped by temperature, were checked for collinearity with Kendall correlation (Figure S1). Because values were highly correlated in every case, suggesting the presence of high molecular weight DNA (Figure S1), only maximum fragment size instead of average fragment size was subsequently tested. For all three preservatives, a linear two-way ANOVA was used to test the influence of preservative concentration, extraction date, and temperature, on maximum fragment size as response variable. Temperature and concentration were also tested as an interaction, because both enhance reaction rates by increasing molecule collision and can

therefore be expected to potentiate or otherwise influence each other.

RESULTS

COI amplification with different primer pairs

The amplification success for COI differed between preservatives and concentrations. For propylene glycol at RT, 100 of 108 samples resulted in a successful amplification of the 658 bp fragment, and at 37 °C, 102 of 108 samples had successful amplification (Figure 2). The ethylene glycol samples kept at RT also had 101 of 108 positive PCR results, and at 37 °C, 80 of 108 samples had successful amplification of the 658 bp fragment. For the samples with 100% concentration of days 24 and 27, all three replicates failed for the full-length product. For the sample from day 24, the 313 bp product could be successfully amplified in one replicate (Figure 2).

Renner solution performed as well as the other preservatives at RT, with 100 of 108 samples resulting in successful amplification of the full-length product (Figure 2). At 37 °C, however, only 20 of the 108 samples had amplification of the full-length product; of the failed replicate triplets 15 of 78 samples had amplification of the 313 bp product, and of the recurrently unsuccessful replicate triplets only 1 of 60 samples resulted in amplification of the 157 bp product. The decrease in size of the amplifiable product started on day 6 until no product was generated anymore from day 15 on. The higher the concentration of the preservative, the sooner the decrease started (Figure 2). All samples from the PC and also all the NCs (crickets in water) could be used to successfully amplify the 658 bp PCR product. For every preservative, Sanger sequencing confirmed the expected sequence of eight from nine selected products each. For the three cases in which the product could not be confirmed, sequencing failed completely.

Fragment size

Maximum fragment size for samples kept in propylene glycol increased with concentration ($F_{1,211} = 93.709$, $P < 0.001$) but was not related to time. Temperature affected maximum fragment size ($F_{1,211} = 13.349$, $P = 0.003$) and there was also an interaction between temperature and concentration ($F_{1,211} = 34.956$, $P < 0.001$) (Table S5). Fragmentation

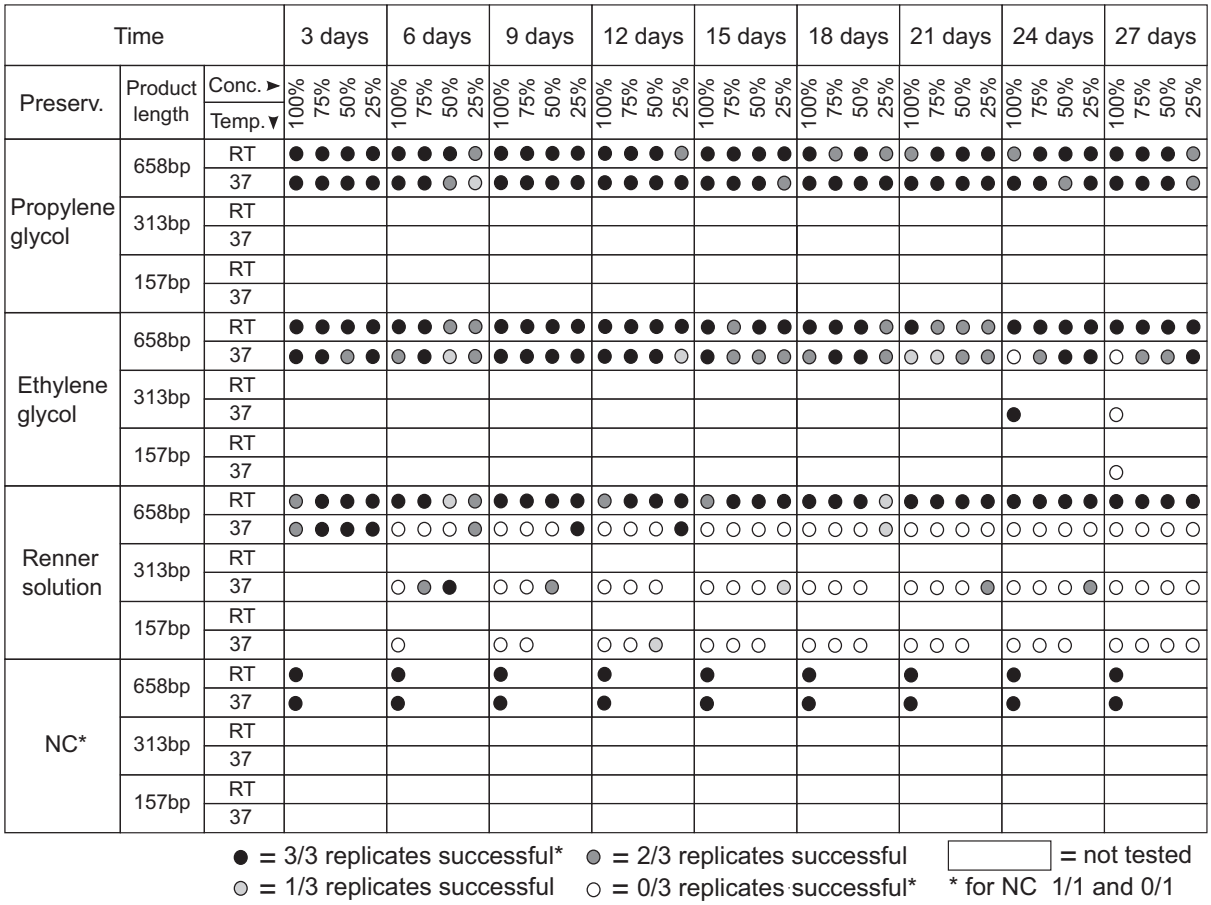

FIGURE 2 Overview of PCR success for propylene glycol, ethylene glycol, and Renner solution at 100, 75, 50, and 25% concentration, at room temperature (RT) and 37 °C for the nine extraction dates, as well as the no-preservative control (NC). All samples were first tested for the 658 bp amplicon, if all three replicates failed the 313 bp amplicon was tested, and finally if all three replicates failed the 157 bp amplicon.

pattern was overall higher at 37 °C. At RT, the 100% concentration samples contained high molecular weight DNA at all time points and maximum fragment size was of equal value as the PC (exception on day 15). On days 3, 21, and 27 single replicates of the 75% concentration samples contained high molecular weight DNA (Figure 3). At 37 °C, on days 3, 6, 12, and 18 high molecular weight DNA was contained in the 100% concentration samples, on days 6 and 9 in 50% concentration samples (Figure 3).

For ethylene glycol samples, maximum fragment size increased with concentration ($F_{1,211}$ = 118.501, P < 0.001) and decreased with extraction date ($F_{1,211}$ = 8.877, P = 0.003). Maximum fragment size also decreased with temperature on its own ($F_{1,211}$ = 66.777, P < 0.001) and again there was also an interaction between temperature and concentration ($F_{1,211}$ = 85.749, P < 0.001) (Table S5). The 100% samples at RT contained high molecular weight DNA on all time points and were of equal value as the PC. The 75% samples contained high molecular weight DNA on days 3 and 9 in single replicates (Figure 3). At 37 °C, only one of the 100% concentration replicates of the first extraction point contained high molecular weight DNA (Figure 3).

The results for Renner solution were very different than those for propylene glycol and ethylene glycol. Maximum fragment size was not affected by concentration ($F_{1,211}$ = 0.008, P = 0.93) or extraction date ($F_{1,211}$ = 0.525, P = 0.47). Maximum fragment size decreased with temperature ($F_{1,211}$ = 12.027, P < 0.001) but there was no interaction between concentration and temperature ($F_{1,211}$ = 0.012, P = 0.91). Of all samples, only one replicate of 75% concentration at RT on day 9, and one replicate of 25% concentration at RT on day 24 contained high molecular weight DNA (Figure 3). Overall, the average fragment size ranged around only 250 bp for most samples at RT (Figure S2). No sample contained high molecular weight DNA at 37 °C and average fragment length ranged around 150 bp (Figure S2).

DISCUSSION

Genetic applications such as metabarcoding are becoming increasingly popular in ecology to accelerate species identification (Dopheide et al., 2020; Uhler et al., 2021; Leroy et al., 2022) and investigate population dynamics (Keller et al., 2012; Lozier et al., 2016; Bateman et al., 2018; Wittische et al., 2019). A large variety of insect trap preservatives optimised to preserve morphological features of specimens is established but the list of preservatives with DNA retaining characteristics is still short in comparison (Vink et al., 2005; Stoeckle et al., 2010; Gossner et al., 2016; Martoni et al., 2021). Propylene glycol, ethylene glycol, and Renner solution have all been successfully used to barcode or metabarcode arthropod specimens (Gossner et al., 2016; Robinson et al., 2021), but so far no study compared all three options and their limitations, as well as measured actual DNA fragmentation on top of PCR success.

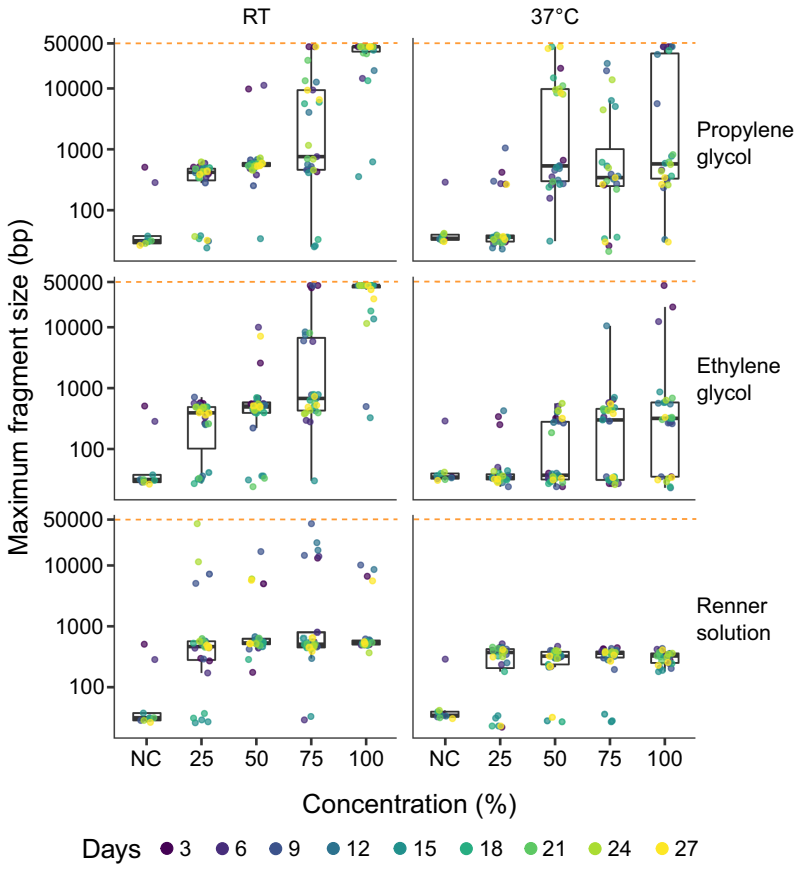

FIGURE 3 Maximum fragment size of samples kept in 100, 75, 50, and 25% concentration of propylene glycol, ethylene glycol, or Renner solution on nine extraction dates (days 3–27), on every 3rd day, as well as the no-preservative control (NC). The preservatives were tested at room temperature (RT) and 37 °C. The dashed lines indicate the maximum fragment size of the positive control. Fragment lengths ≥50000 bp are condensed in the Fragment Analyser system output. Note that the y-axis is displayed on a log-scale. Statistical details are reported in Figure S1.

Effects of dilution, temperature, and time on PCR success and fragmentation

We tested propylene glycol, ethylene glycol, and Renner solution for their performance under room and elevated temperature (37 °C), at various concentrations (100, 75, 50, and 25%) over a course of 27 days, with extractions on every 3rd day. COI amplification success was robust for propylene glycol and ethylene glycol independent of dilution and largely independent of temperature. Renner solution performed just as well at room temperature (RT) but caused unexpected problems at 37 °C, which may be related to the low pH of this preservative. Possibly the higher kinetic energy at 37 °C increased reactivity of the 20% acetic acid content of Renner solution and thus catalysed degradation of DNA by acid hydrolysis (Pollmann & Schramm, 1961; Lindahl, 1993; Liu et al., 2014). This would consequently explain why the higher concentrations started failing in PCR first. In contrast, the fragmentation measurements for propylene and ethylene glycol showed better DNA preservation with increasing concentration. At 100% and RT, high weight genomic DNA could be extracted reliably, but it was severely impaired at 37 °C. The negative influences of dilution and temperature are expected results. By reducing the concentration, dilution by water not only decreases the preservatives effect on the contained DNA but also opens reaction space for biochemical DNA degradation for example by exonucleases (Evans et al., 2002; Barnes et al., 2014; Peng et al., 2018). Our results indicate that this effect is further enhanced by temperature and for ethylene glycol also over time. For Renner solution, the effect by the various conditions on fragmentation was less explicit, as fragmentation was always high, independent of dilution temperature and time.

Consequences for arthropod sampling based on downstream applications

For metabarcoding studies of insects ethanol is often the sampling preservative of choice (Zhang et al., 2016; Uhler et al., 2021). However, at high concentration ethanol evaporates quickly (Nagy, 2010; Marquina et al., 2021), which makes this preservative unsuited when traps have to operate in the field for longer times, or when sampling is conducted in hot climates. Furthermore, because of the dehydrating properties of ethanol, the integrity of the specimens is impaired at high concentration, which can complicate morphological identification (Marquina et al., 2021). Renner solution, ethylene glycol, and propylene glycol have thus been proposed to preserve both morphological features and DNA suitable for PCR amplification (Gossner et al., 2016; Martoni et al., 2021). We found that PCR amplification is indeed robust with ethylene and propylene glycol, but only at 100% concentration and moderate temperature high molecular weight DNA is preserved reliably. Interestingly, we could also successfully amplify the longest commonly used COI fragment (Meyer, 2003) from our no-preservative control (crickets in water), indicating that even in water without any preservative added some fragments long enough for PCR amplification remain, at least under clean and controlled laboratory conditions. However, as we have tested only one species, we disclaim that DNA of different insect species may show different degradation patterns, as is indicated by the species-specific barcoding success in Gossner et al. (2016). Nonetheless, in comparison to museum specimens, which commonly have to be barcoded with mini barcodes (Cárdenas & Moore, 2019; Velasco-Cuervo et al., 2019), individual barcoding for the whole COI fragment should be unproblematic with specimens collected even at low preservative concentrations.

Analysing DNA from a single species, we could also not directly test metabarcoding in this study. However, considering the extent of DNA fragmentation in our experiment and the unknow factor of taxon-specific barcoding amenability, we recommend to use propylene or ethylene glycol at concentrations of at least 50–75% in the field, if the aim is to sequence specimens in a bulk sample of different size and taxonomy. With the expected higher amount of exonucleases and risk of further dilution under field conditions, a too-low starting concentration could otherwise lead to biased results due to uneven degradation states. At concentrations of 75% or higher, a surface-breaking detergent may be necessary to ensure that specimens sink into the solution quickly (Martoni et al., 2021). When higher temperatures are expected, propylene glycol is more suitable than ethylene glycol, as the preservability seems to be more resistant to heat, and evaporation is slower due to the larger molecule size. From our fragmentation measurements we not only infer that just at 100% concentration of propylene and ethylene glycol high molecular weight DNA is preserved reliably, but also that the fragmentation significantly increases at 75% concentration already. Independent of PCR success a sample with a maximum fragment size of 1000 bp has to be considered severely degraded in comparison to the maximum fragment size of 50000 bp in the positive control and 100% concentration samples. Several downstream applications that target larger parts of the genome, such as hybridization RAD (Suchan et al., 2016; Schmid et al., 2017), are specifically designed for highly fragmented DNA but are expensive in comparison to the common RAD or double digest RAD approaches. These basic versions of RAD will suffer a quality loss if the fragmentation of the input DNA is too severe (Graham et al., 2015; Guo et al., 2018).

Whole genome sequencing is not as dependent on DNA fragment length (Oosting et al., 2020), but the quality of the assembly directly depends on the quality of the input DNA (Dominguez Del Angel et al., 2018; Oosting et al., 2020). Similarly, long-read sequencing options like the Oxford Nanopore technologies can generate results with fragmented DNA, but as the length of a DNA fragment will directly be translated to the length of the obtained reads (Oosting et al., 2020; Volarić et al., 2021), high molecular

weight DNA is necessary for the best output. Generally, for downstream applications that target the whole or larger parts of the genome we recommend that specimens should be collected with propylene or ethylene glycol at 100% starting concentration with surface breaking detergent added. The specimens should be collected in as short intervals as logistically possible. Alternatively, specimens could be transferred into cooling directly, e.g., after canopy fogging (Leroy et al., 2022). Considering the severe fragmentation of DNA from Renner solution at both temperatures and the PCR failure at 37 °C we do not recommend Renner solution as a DNA preservative in insect traps. In line with Gossner et al. (2016), who suspected pH as a problem of copper sulphate as well, we would generally recommend to avoid acid-based preservatives including diluted acetic acid, Renner solution, or FAACC (formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%), or preservatives with low pH such as copper sulphate.

CONCLUSION

Genetic approaches have the potential to accelerate and standardise identification and understanding of insects and their population dynamics. The necessary sequencing procedures have different demands to DNA quality, which are not always addressable with the same collection approach. To further increase the knowledge on DNA preservation options, future studies could expand the taxonomic coverage for a better understanding of differences in preservability and conduct systematic comparisons using metabarcoding approaches. We here use DNA fragmentation analysis as a direct measure of DNA quality, in addition to PCR, as an application-based indicator, to provide a systematic overview on the influence of concentration, temperature, and time on preservability. With propylene and ethylene glycol we find two preservatives which can preserve specimen morphology and high molecular weight DNA under the right conditions and show where they reach their limits. With Renner solution, we also remove one candidate from the list of potential DNA preservatives and demonstrate the need for exploration of preservative performance under worst-case conditions.

AUTHOR CONTRIBUTIONS

Laura-Sophia Ruppert: Conceptualization (lead); formal analysis (lead); methodology (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Gernot Segelbacher:** Conceptualization (supporting); formal analysis (supporting); funding acquisition (lead); methodology (supporting); resources (lead); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting). **Michael Staab:** Formal analysis (supporting); supervision (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Nathalie Winiger:** Conceptualization (equal); methodology (supporting); writing – original draft (supporting).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. PCR protocol for COI amplification with insect DNA

Table S2. List of primers used for PCR amplification of a 658 bp, 313 bp, and 157 bp COI amplicon

Table S3. ANOVA results for the relationship between maximum fragment size (response variable) and concentration, extraction date, and temperature for specimens kept in either propylene glycol, ethylene glycol, or Renner solution

Figure S1. Kendall correlation graph for the maximum (Max) and average (Avg) fragment size of propylene glycol (PG), ethylene glycol (EG), and Renner solution (RE) at room temperature (RT) and 37 °C (37). For all preservatives at both temperatures average and maximum fragment size are strongly correlated.

Figure S2. Average fragment size of samples kept in 100, 75, 50, and 25% concentration of propylene glycol, ethylene glycol, or Renner solution on nine extraction dates on every third day with a total experiment time of 27 days and the negative control (NC). The average fragment size of the positive control is indicated by the dashed line. Fragment lengths ≥50000 bp are condensed in the Fragment Analyser system output. The y-axis is transformed logarithmical for better visualisation.

Supplementary data 1: DNA extraction protocol for QIAGEN DNeasy Blood & Tissue Kit.

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