To beat or not to beat a tick: Comparison of DNA extraction methods from ticks (*Ixodes scapularis*)

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ABSTRACT

Background. Blacklegged ticks (*Ixodes scapularis)* are important disease vectors in the United States, known to transmit a variety of pathogens to humans, including bacteria, protozoa, and viruses. Their importance as a disease vector necessitates reliable and comparable methods for extracting microbial DNA from ticks. Furthermore, to explore the population genetics or genomics of this tick, appropriate DNA extraction techniques are needed for both the vector and its microbes. Although a few studies have investigated different methods of DNA isolation from ticks, they are limited in the number and types of DNA extraction and lack species-specific quantification of DNA yield.

Methods. Here we determined the most efficient and consistent method of DNA extraction from two different developmental stages of *I. scapularis* – nymph and adult - that are the most important for disease transmission. We used various methods of physical disruption of the hard, chitinous exoskeleton, as well as commercial and non-commercial DNA isolation kits. To gauge the effectiveness of these methods we quantified the DNA yield and confirmed the DNA quality via PCR of both tick and microbial genetic material.

Results. DNA extraction using the Thermo GeneJET Genomic DNA Purification kit resulted in the highest DNA yields and the strongest, most consistent PCR amplification. We also found that physical disruption of the tick exoskeleton was most effective using cross-sectional cutting compared to any type of bead-beating matrices used. Storing ticks at -80°C resulted in considerably higher DNA yields than those from ticks stored in ethanol.

Discussion. We contrasted a variety of readily available methods of DNA extraction from single individual blacklegged ticks and presented the results through a quantitative and qualitative assessment.

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INTRODUCTION

Blacklegged ticks (*Ixodes scapularis* Say, 1821) are hard-bodied, hematophagous
arthropod (Arachnida, Ixodida) ectoparasites of vertebrates in North America. During its
two-year life cycle, the tick acquires a bloodmeal at each developmental stage (i.e., larva,
nymph, and adult) prior to molting or egg-laying, in the case of adult females. These ticks
are of great public health importance as disease vectors because they carry and transmit a
variety of human disease agents, such as *Borrelia burdgorferi*, the causative agent of
Lyme disease, *Anaplasma phagocytophilum* which causes granulocytic anaplasmosis, and
Babesia microti, a protozoan responsible for the malaria-like illness, babesiosis
(Speilman, 1976; Steere et al., 1978; Pancholi et al., 1995). Recently, *I. scapularis* has
been found to transmit *Borrelia miyamotoi* (Scoles et al., 2001) and Powassan virus
lineage 2 (a.k.a., Deer Tick Virus) (Telford, 1997). Their importance as human disease
vectors necessitates research that involves successful isolation of genetic material needed
in investigations of both the vector itself and of the wide range of pathogens that they
carry. However, DNA isolation in ticks is challenging due to the hard chitinous
exoskeleton and the small amount of microbial nucleic acids present (Halos et al., 2004).
Furthermore, tick DNA is suseptible to degradation (Hubbard et al., 1995; Hill &
Gutierrez, 2003; Halos et al., 2004) and PCR can be challenged by inhibitors (Halos et
al., 2004). With respect to public health, the unexplored potential for aerosolization of
pathogen DNA may likewise pose a risk (Borst, Box & Fluit, 2004).

Although DNA extraction from ticks for both pathogen isolation and tick genetic
and genomic research is performed routinely by researchers, there is no consensus
regarding the most effective method of DNA isolation from any tick species. A few such
studies have been conducted (Hill & Gutierrez, 2003; Halos 2004; Crowder et al., 2010),
however, they are limited to a handful of extraction techniques, and quantitative data on
DNA concentration is lacking. In this study we aim to identify the optimal DNA isolation
procedure for both tick and microbial DNA from an important tick disease vector, the
blacklegged tick.

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MATERIALS & METHODS

Tick collection

Nymphal and adult female blacklegged ticks are important life stages in the transmission of disease agents compared to larvae, which are rarely infected with human pathogens, and adult males, whose brief feeding bouts minimize the risk of pathogen transmission (Piesman et al., 1986; Falco & Fish, 1988; Falco et al., 1999). Thus, the ability to dependably extract DNA, and in particular microbial DNA, from nymphal and adult female blacklegged ticks is of importance to tick-borne disease research and constitutes the focus of this study.

Unfed, host-seeking nymphal and adult blacklegged ticks were collected by dragging a $1m^2$ flannel cloth along the forest floor or along low vegetation, respectively, during each life stage's peak activity period. Ticks were collected from sites in Westchester County, Putnam County, and Orange County in New York state in the summer and fall of 2009-2011, and were subsequently stored in 70% v/v ethanol until DNA isolation. Additionally, a subset of nymphs and adult females was collected during the summer and fall of 2013-2014 at the Louis Calder Center–Biological Field Station of Fordham University in Armonk, NY and frozen at -80°C until DNA isolation.

DNA isolation

We contrasted the efficiency of extracting DNA from ticks stored in 70% ethanol using five different DNA isolation procedures coupled with either cross-sectional division or bead-based physical disruption of the tick body. These procedures included four commercially available DNA extraction kits: DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA), GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA), Tissue & Insect DNA MicroPrep (Zymo Research, Irvine, CA), PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA); and one noncommercial DNA extraction method using ammonium hydroxide (NH_4OH) and heat, which has been primarily used for DNA

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106   isolation from the European sheep tick, *Ixodes ricinus* (Guy & Stanek, 1991; Rijpkema et

107   al., 1996; Pichon et al., 2003; Humair et al., 2007; Pangrácová et al., 2013). All

108   commercial kits examined here use a silica-based column procedure and have either been

109   used in previous studies for DNA isolation in ticks or are marketed for efficient microbial

110   DNA recovery or insect DNA isolation (Table 1). DNA extraction kits were used

111   according to the manufacturers' recommended protocols with few exceptions (Table 1),

112   including a final elution completed following a 5-min room-temperature incubation in

113   100 µl of deionized, sterilized, distilled water (sdH$_2$O) for consistency. Each tick was air-

114   dried to evaporate the ethanol prior to DNA extraction, as ethanol may inhibit PCR

115   (Hubbard, Cann & Wright 1995; Bessetti et al., 2007; Schrader et al., 2012).

116   The NH$_4$OH method included adding 150 µl of a 0.7-M NH$_4$OH solution to the tick

117   sample in a 1.5-ml snap cap tube, and heating to 100ºC for 15 min. The solution was

118   briefly centrifuged to concentrate fluid at the bottom and then was evaporated to 70-100

119   µl by opening the tubes and heating at 100ºC for an additional 15 min. The solution was

120   then centrifuged for 10 min at 10,000× *g* and the supernatant was collected and respun for

121   2 min at 10,000× *g*. The new supernatent was collected and stored at -20ºC.

122   To determine the most effective method of physical disruption of the hard, chitinous

123   exoskeleton of ticks prior to DNA extraction, we compared cross-sectionally dividing

124   ticks (bisection for nymphs and quadrisection for adult females) and crushing the entire

125   tick in a variety of bead matrices using the BeadBlaster 24 (Benchmark Scientific,

126   Edison, NJ) (Table 1). The MoBio and Zymo kits included their own bead matrices

127   (Table 2). The beads from these two kits were used according to the manufacturers'

128   instructions with either whole or cut ticks. MoBio-processed samples were beaten on a

129   GeneMate vortex mixer (BioExpress, Kaysville, UT) at maximum speed (3200 rpm) for

130   10 min and Zymo-processed samples were beaten on the BeadBlaster 24 for 10 min at 4

131   m/s. For DNA extraction methods that did not include bead matrices (i.e., QIAGEN,

132   Thermo, and NH$_4$OH), we used six different MP Bio Lysing Matrices, which were either

133   marketed for tough samples or, as in the case of Matrices H and I, were marketed

134   specifically for ticks. We beat the ticks with the MP Bio Lysing Matrices for 1.5 and 4

135   min at 4 m/s (Table 2).

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136   A subset of nymphs and adult females stored at -80°C was bead-beaten for 1.5 min at 4

137   m/s with each of the MP Bio matrices and then the DNA was extracted with the QIAGEN

138   kit to assess the effect of storage method on DNA isolation. All DNA extraction and

139   physical disruption methods were conducted on three individual nymphs and three

140   individual adult female blacklegged ticks.

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DNA quantification

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143   The resulting DNA yields were quantified via double-stranded DNA (dsDNA)

144   fluorometric quantitation on a Qubit 2.0 flurometer (Life Technologies, Norwalk, CT)

145   using 10 µl of extracted DNA template in 190 µl of the High Sensitivty (HS) dsDNA

146   assay.

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PCR validation

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149   The isolated DNA was validated using PCR amplification of tick mitochondrial and

150   nuclear loci. We amplified the tick DNA using (1) the cytochrome c oxidase subunit 1

151   (*Cox1*) DNA barcode region (~650bp) located on the mitochondrial genome with the

152   HCO/LCO primers (Folmer et al., 1994), and (2) a dinucleotide $(CA)_n$ microsatellite

153   repeat located on the nuclear genome with the bac7ea/bac7eb primer pair (139-197bp)

154   (Chan, 2012).

155   We targeted the genus *Rickettsia* using PCR to validate the successful extraction of

156   microbial DNA from inside the tick, which is necessary for studies involving PCR

157   detection of human pathogens transmitted by this tick species. Members of this genus are

158   obligate intracellular bacteria and are abundant in blacklegged ticks (Benson et al., 2004;

159   Clay & Fuqua 2010; Moreno et al., 2006; Noda, Munderloh & Kurtti, 1997; Steiner et al.,

160   2008; CP Zolnik, unpublished data). We targeted a 532-bp fragment of the *ompA* gene

161   using *Rickettsia*-specific primers (Vitorino et al., 2007).

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164   All thermal cycling conditions were slightly modified from their published protocols and

165   are detailed below. The thermal cycling conditions for the *Cox1* DNA barcode region

166   began with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for

167   30 s, 50°C for 30 s, and 72°C for 30 s, and then a final extension at 72°C for 7 min

168   (Folmer et al., 1994). The microsatellite region was amplified using a touchdown PCR

169   with an initial denaturation at 95°C for 1 min, then 5 cycles of 95°C for 20 s, 60°C for 20

170   s, and 72°C for 30 s; 30 cycles of 95°C for 20 s, 50°C for 25 s, and 72°C for 30 s; and a

171   final extension for 5 min at 72°C (Chan, 2012). The *Rickettsia ompA* locus was amplified

172   with an initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 52°C for 30 s,

173   and 72°C for 30 s, and a final extension for 7 min at 72°C (Vitorino et al., 2007). All

174   PCR reactions were performed using a Techne Prime Elite Thermal Cycler (Bibby

175   Scientific, Burlington, NJ).

176   Each PCR was performed in a final 25-µl volume with 6.25 µl 2× MyTaq HS Mix

177   (Bioline, Taunton, MA) and 0.2 µM of each forward and reverse primer. The PCRs

178   targeting nuclear and mitochondrial tick DNA were caried out using 16.25 µl sdH$_2$O and

179   1.5 µl DNA template. To account for the lower DNA concentrations of microbial DNA

180   within this tick, and specifically for nymphs, the PCRs targeting the *Rickettsia ompA*

181   fragment were carried out with 15.75 µl sdH$_2$O and 2 µl DNA template from nymphs,

182   and 16.75 µl sdH$_2$O and 1 µl DNA template for adult females. PCRs were confirmed by

183   agarose gel electrophoresis on a 1.5% w/v gel.

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185   **Statistical analysis**

186   Given the substantial differences in size and DNA yield between nymphs and adult

187   females, statistical analyses of the DNA concentration data from the two sets of samples

188   were performed separately. Following a one-way ANOVA, Tukey's HSD test was used

189   for post-hoc analysis of the average DNA quantification values resulting from nymph

190   bisection or female quadrisection across the five DNA isolation methods (QIAGEN,

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191   Thermo, MoBio, Zymo, and NH_4OH). The same procedures were also used to compare

192   the DNA yields resulting from the different MP Bio Lysing Matrices (G, H, I, M, S, and

193   Z) and nymph bisection or female quadrisection methods using data from the two

194   highest-yielding DNA extraction methods. A two-tailed Student's *t*-test was used to

195   compare the average DNA concentrations resulting from bead beating for 1.5 min with

196   those resulting from bead beating for 4 min using the data from the two highest-yielding

197   DNA extraction methods. A *t*-test was also used to compare DNA yields following 1.5

198   min bead beating and DNA extraction with the QIAGEN kit from ticks stored in 70% v/v

199   ethanol with DNA yields from ticks stored at -80°C.

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RESULTS AND DISCUSSION

DNA extraction method comparison

203   The comparison of DNA concentrations resulting from nymph bisection and female

204   quadrisection across the five DNA extraction methods yielded no significant difference

205   between the QIAGEN DNEasy Blood & Tissue Kit and the Thermo GeneJET Genomic

206   DNA Purification Kit (Fig. 1). However, for both nymphs and adult females, the

207   QIAGEN and Thermo methods resulted in significantly higher DNA yields than the

208   Zymo Research Tissue & Insect DNA MicroPrep and the MoBio PowerSoil DNA

209   Isolation Kit ($P<0.01$) (Fig. 1). QIAGEN and Thermo also had significantly higher DNA

210   yields than the NH_4OH method for both nymphs ($P<0.05$) and females ($P<0.05$, $P<0.01$)

211   (Fig. 1). There was no significant difference among the DNA extraction concentrations

212   resulting from the NH_4OH method and Zymo and MoBio kits for both nymphs and

213   females, except for the NH_4OH adult female DNA extraction, which was significantly

214   better than the Zymo method ($P<0.05$) (Fig. 1). Of the five methods, QIAGEN and

215   Thermo generated the highest DNA yields overall.

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Physical disruption methods

218 For the two highest-yielding DNA isolation methods, i.e., QIAGEN and Thermo, in most

219 cases there was no significant difference in the DNA concentration yields among the six

220 different MP Bio Lysing Matrices (G, H, I, M, S, and Z) and bisection for nymphs (Fig.

221 2A). However, both the S matrix and bisection resulted in significantly higher yields than

222 the G and M matrices ($P<0.05$, $P<0.01$) (Fig. 2A). For females in all cases, quadrisection

223 resulted in significantly higher DNA concentrations than any bead beating matrix

224 ($P<0.05$ for H and I; $P<0.01$ for G, M, S, and Z) (Table 2B).

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226 For both nymphs and females that were physically disrupted with the MP Bio Lysing

227 Matrices and underwent a DNA extraction with either the QIAGEN or the Thermo

228 method, there was no significant difference between the DNA yields resulting from bead

229 beating for 1.5 and 4 min (Fig. 3).

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Tick storage method and DNA yield

232 DNA extractions resulting from bead beating for 1.5 min with the MP Bio Lysing

233 Matrices and DNA isolation with the QIAGEN method were significantly better for

234 nymphs and females that were stored at -80°C prior to DNA extraction than for nymphs

235 and females stored in 70% ethanol ($P<0.0001$) (Fig. 4).

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PCR validation

238 Although the QIAGEN and Thermo methods produced similar DNA yields, the Thermo

239 method exhibited the strongest and most consistent gel electrophoresis PCR product

240 bands in the case of both nymphs and females for *Cox1*, microsatellite, and *Rickettsia* sp.

241 *ompA*. Increasing bead beating duration had a positive effect on PCR when DNA was

242 isolated through the QIAGEN method, while the same effect was not evident when using

243 the Thermo method. Among the five DNA isolation methods, Thermo ultimately

244 produced the best-amplifiable genomic DNA.

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246 The DNA extractions resulting from the NH$_4$OH protocol yielded strong and somewhat

247 consistent amplification of the the *Cox1*, microsatellite, and *Rickettsia ompA* loci for

248 adult females. NH$_4$OH yielded significantly lower DNA concentration results than the

249 QIAGEN and Thermo methods when using quadrisected adult female ticks; however, this

250 did not affect PCR success. PCR amplification was consistently poor for nymphs treated

251 with the NH$_4$OH protocol. Only the combination of the NH$_4$OH method with bead

252 beating with the M matrix for 1.5 or 4 min resulted in consistent amplification of the

253 microsatellite target for nymphs, as well as limited success for the *Cox1* and *Rickettsia*

254 *ompA* loci.

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256 The DNA extracted using the Zymo Research Tissue & Insect DNA MicroPrep did not

257 produce nuclear, mitochondrial, or bacterial PCR amplifications for either whole or cut

258 nymphs, or adult females, consistent with the very low DNA yields we measured. DNA

259 extraction from adult female ticks with the MoBio PowerSoil DNA Isolation Kit resulted

260 in the consistent amplification of the three targeted loci, with quadrisection substantially

261 enhancing the amplicon gel band quality in comparison with whole females. However,

262 the very low yields for both bisected and whole nymphs extracted with MoBio

263 corresponded with the limited success of all three PCR protocols with the MoBio method.

264

265 Among the MP Bio Lysing Matrices, the S matrix produced some of the strongest and

266 most consistent PCR results across all targeted loci (nuclear, mitochondrial, and

267 bacterial), DNA extraction methods (QIAGEN, Thermo, and NH$_4$OH), bead beating

268 times (1.5 and 4 min), and life stages (nymphs and females). Although the S matrix did

269 not produce significantly higher DNA concentrations than any other matrix for adult

270 females, yet generated higher DNA yields than the G and M matrices did for nymphs, the

271 PCR results were generally better for the S matrix. The H and I matrices, which the MP

272 Bio website recommends for ticks, also produced comparably good results in terms of

273 strength and consistency for many cases, although the bands resulting from the S matrix

274 were slightly stronger and more consistent following 4 min of bead beating and DNA

275 extraction with the QIAGEN and Thermo kits.

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277 Nymph bisection and female quadrisection produced PCR results that were generally just
278 as strong and consistent as bead beating with the S, H, and I matrices combined with
279 DNA extraction with Thermo and NH_4OH for nuclear and mitochondrial DNA.
280 However, cutting the ticks in combination with the QIAGEN methods produced
281 substantially better results than did bead beating. Additionally, cutting generally
282 produced better results than bead beating for the PCR amplification of rickettsial DNA.
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CONCLUSIONS

285 Successful DNA extraction from tick species is important for both genetic and genomic
286 studies of the tick vector itself, as well as for studies aimed at detecting pathogen
287 presence in these tick vectors. This study was designed to determine the most reliable and
288 efficient method of DNA extraction, including physical disruption of the tick
289 exoskeleton.

290 We determined that the most reliable and consistent DNA extraction method for both
291 nymphal and adult female ticks was the Thermo GeneJET Genomic DNA Purification
292 Kit, which resulted in the highest DNA yields and the strongest and most consistent PCR
293 amplification, demonstrated as PCR bands on electrophoresis agarose gels. The QIAGEN
294 DNeasy Blood & Tissue Kit, though perhaps the most commonly used kit, produced less
295 intense yet consistent results. Cross-sectional cutting was more effective than any type of
296 bead beating matrices for the Thermo and QIAGEN extraction kits.

297 Both the MoBio and Zymo kits were poor choices for extracting DNA from nymphs and
298 adult female ticks, despite the fact that the MoBio kit is marketed for enhanced microbial
299 DNA extraction and the Zymo kit is marketed for DNA extraction from insects (as per
300 the manufacturers' websites). The NH_4OH extraction method is an inexpensive
301 alternative to commercially available kits and produced high-quality PCR products for
302 adult females, although the DNA yield was generally lower than that of commercial kits.
303 However, this method was not useful for DNA extraction from nymphs, resulting in low
304 DNA yield and poor to non-existent PCR amplification, despite the frequent use of this

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305 method in studies on nymphal *Ixodes ricinus* in Europe (Guy & Stanek, 1991; Rijpkema

306 et al., 1996; Pichon et al., 2003; Humair et al., 2007; Pangrácová et al., 2013).

307

308 Although bead beating greatly reduces the time needed for physical disruption of the

309 tick's chitinous exoskeleton and eliminates direct handling of samples, which itself may

310 diminish potential contamination or aerosolization of DNA, we recommend nymphal

311 bisection and female quadrisection for accessing nuclear and mitochondrial tick DNA

312 and internal microbial DNA using the QIAGEN, Thermo and NH_4OH DNA extraction

313 protocols. Cutting the ticks resulted in the strongest and most consistent DNA yields and

314 PCR products. However, if bead beating is used, we recommend using the MP Bio

315 Lysing Matrices S, H, or I with a 4-min beating time. These results confirm MP Bio's

316 recommendation of the H and I matrices for ticks, although the S matrix also results in

317 DNA yields of comparable or slightly better quantity and quality

318 (http://www.mpbio.com/index.php?cPath=2_77_425&country=223).

319

320 Finally, we explored the impact of storing ticks at -80°C in comparison with ethanol-

321 based storage methods (70% ethanol) for a subset of ticks that was extracted using the

322 QIAGEN method following cross-section or bead beating. We found that freezing

323 resulted in signfcantly higher DNA yields than ethanol storage, and we suggest the use

324 of -80°C storage when possible. Our study expands on previous work that determined

325 DNA extraction success from ticks based on PCR amplification alone, without a DNA

326 quantification assessment (Halos et al., 2004). While a recent study quantified DNA

327 yield, the reported values were averaged across multiple tick species and focused only on

328 one developmental stage, adults (Crowder et al., 2010). In order to test the efficiency of

329 the DNA extraction techniques, we kept certain variables constant, such as the time for

330 incubation in lysis buffer, bead beating speed, elution volume, and incubation time prior

331 to elution. Alteration of these variables may result in increased DNA yield and should be

332 considered when DNA concentration is important in downstream applications, such as

333 gene expression, pathogen surveillance, and microbial community profiling.

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447   approach. *Microbiology* 153:160-168.

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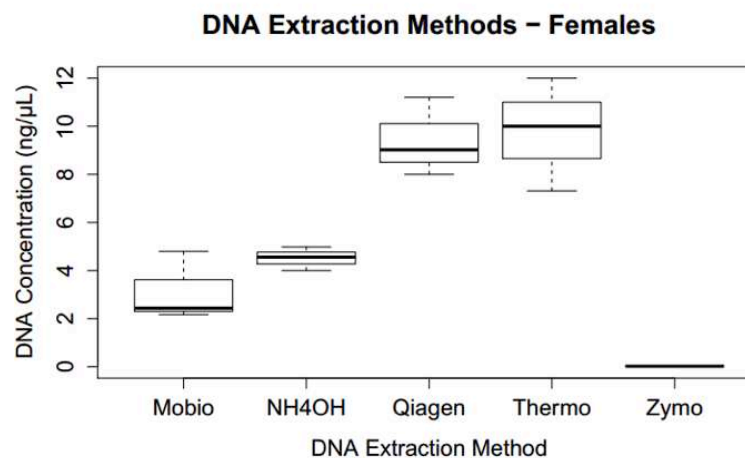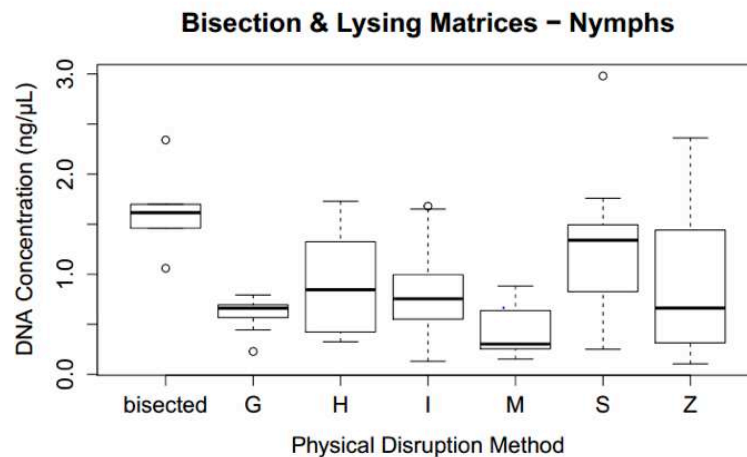449

450

451 **A**

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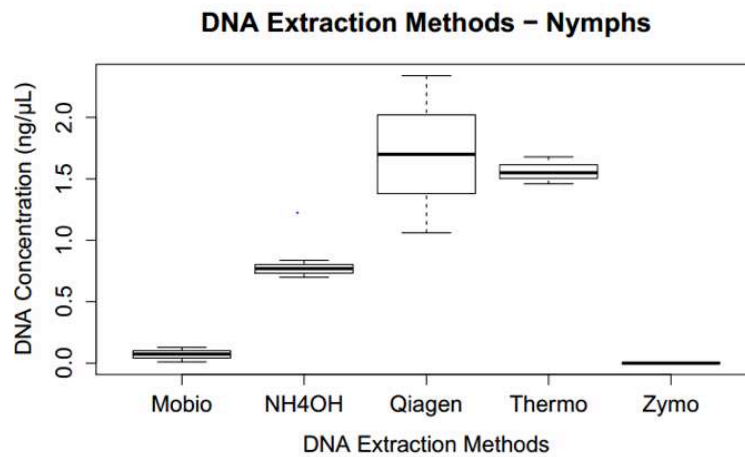
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454 **B**

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457 **Figure 1.** DNA concentrations (ng/μl) resulting from the five DNA extraction methods

458 following nymph bisection and female quadrisection as determined using the Qubit HS

459 dsDNA Assay. Each sample set consisted of three individual tick DNA extractions. The

460 QIAGEN and Thermo methods did not differ significantly. Note the difference in scale

461 between the life stages. **A.** For nymphal ticks, both the QIAGEN and Thermo DNA

462 concentrations were significantly different from those of NH_4OH ($P<0.05$), and Zymo

463 and MoBio ($P<0.01$). **B.** For adult female ticks, the QIAGEN and Thermo concentrations

464 were significantly different from that of NH_4OH ($P<0.05$ and $P<0.01$, respectively). The

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465   QIAGEN and Thermo concentrations were significantly different (P<0.01) from those of

466   MoBio and Zymo yields. The NH_4OH yield differed significantly (P<0.05) from the

467   Zymo yield.

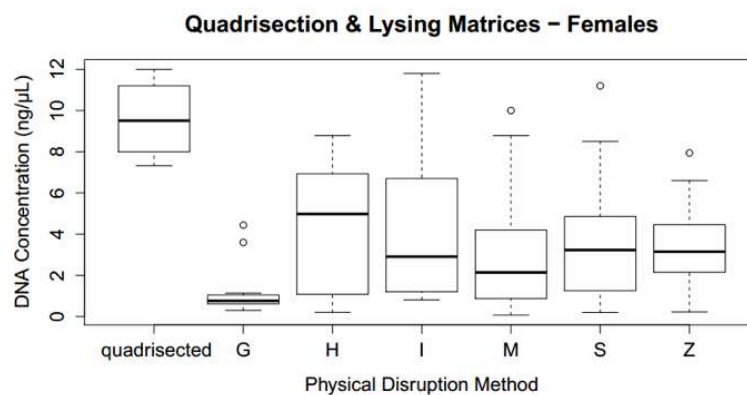468

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470   **A**

Bisection & Lysing Matrices − Nymphs

471

472   **B**

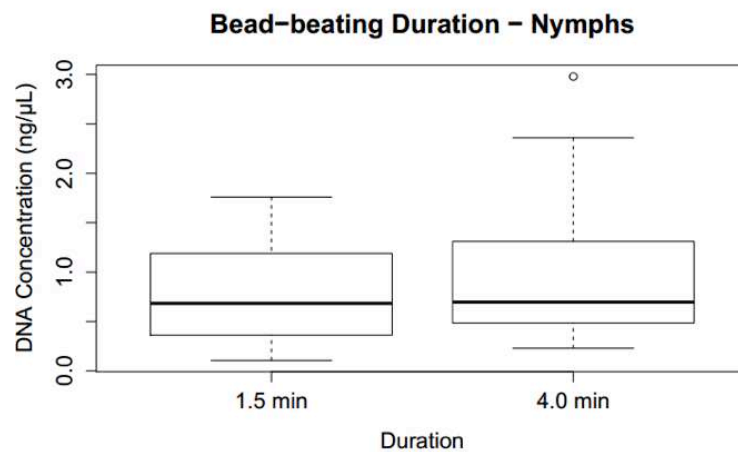Quadrisection & Lysing Matrices − Females

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475   **Figure 2.** DNA concentrations (ng/μl) resulting from the QIAGEN and Thermo DNA

476   extraction methods following the bead beating of whole ticks with each of the MP Bio

477   lysing matrices (G, H, I, M, S, and Z), nymph bisection, and adult female quadrisection,

478   as determined using the Qubit HS dsDNA Assay. Twelve individuals were used for each

479   bead beating treatment. Six individuals were used for each cutting treatment. **A.** For

480   nymphs, bisection was significantly different ($P<0.01$) from the G and M matrices.

481   Matrix S was siginficanlty different from matrices G ($P<0.05$) and M ($P<0.01$). **B.** For

482   adult females, quadrisection produced significantly higher DNA yields than all bead

483   beating matrices ($P<0.05$ against H and I; $P<0.001$ against G, M, S, and Z).

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486   **A**

Bead−beating Duration − Nymphs

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488   **B**

Bead−beating Duration − Females
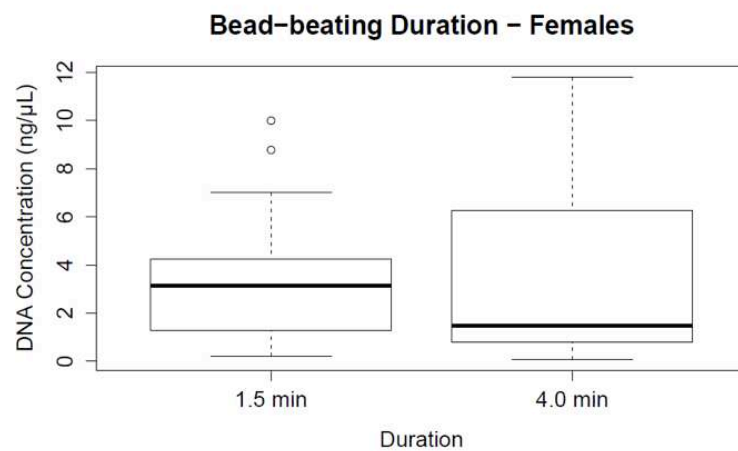
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491   **Figure 3.** DNA concentrations (ng/µl) resulting from the QIAGEN and Thermo DNA

492   extraction methods following the bead beating of whole ticks with each of the MP Bio

493   lysing matrices (G, H, I, M, S, and Z) for 1.5 or 4 min, as determined using the Qubit HS

494   dsDNA Assay. Eighteen individuals were used in each set. The duration treatments were

495   contrasted using a two-tailed Student's *t*-test. **A.** Bisected nymphs (P>0.05). **B.**

496   Quadrisected adult females (P>0.05).

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500   **A**

Storage Methods − Nymphs

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502   **B**

Storage Methods − Females

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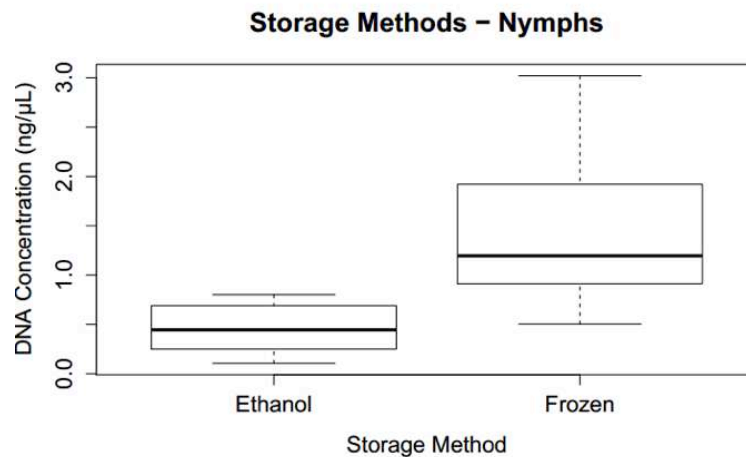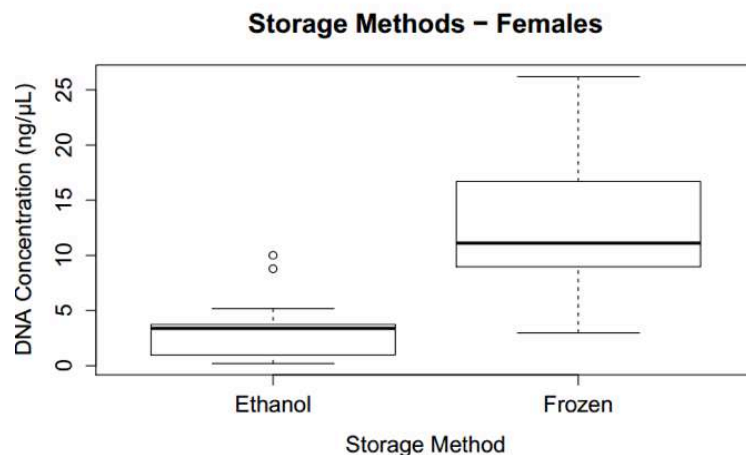
Figure 4. DNA concentrations resulting from DNA extraction from ticks stored at -80°C or in 70% v/v ethanol using the QIAGEN method, following cutting or bead beating of whole ticks with the MP Bio lysing matrices (G, H, I, M, S, and Z) for 1.5 min, as determined using the Qubit HS dsDNA Assay. Eighteen individuals were used in each set. The sample storage treatments were contrasted using a two-tailed Student's *t*-test. **A.** Ethanol-stored vs. frozen nymphs (P<0.0001). **B.** Ethanol-stored vs. frozen, quadrisected adult females (P<0.0001).

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DNA extraction method	Alterations to manufacturer protocols	Physical disruption	Bead beating – speed and duration
QIAGEN DNeasy Blood & Tissue Kit (cat. no. 69506)	Elution in 100 μl dsH$_2$0 with 5 min room temperature incubation	Bisection (Nymphs), Quadrisection (Females)	N/A
		MP Bio Lysing Matrices	4 m/s 1.5 & 4.0 min
Thermo GeneJet Genomic DNA Purification Kit (cat. no. K0722)	Elution in 100 μl dsH$_2$0 with 5 min room temperature incubation	Bisection (Nymphs), Quadrisection (Females)	N/A
		MP Bio Lysing Matrices	4 m/s 1.5 & 4.0 min
Zymo Research Tissue & Insect DNA MicroPrep (cat. no. D6015)	Elution in 100 μl dsH20 with 5 min room temperature incubation	Bisection (Nymphs), Quadrisection (Females) followed by beating with Zymo beads	4 m/s 10 min
		Zymo beads	4 m/s 10 min
MoBio PowerSoil DNA Isolation Kit (cat. no. 12888)	Elution in 100 μl of dsH$_2$0 with 5 min room temperature incubation	Bisection (Nymphs), Quadrisection (Females) followed by beating with MoBio garnet beads	3200 rpm 10 min
	20 μl of Proteinase K added	MoBio provided beads	3200 rpm 10 min
NH$_4$OH (Guy and Stanek, 1991, Pichon et al., 2003)	Initial volume of 150 μl NH$_4$OH Final volume of 70-100 μl dsH20 Second centrifugation for 2 min at 10,000× *g*	Bisection (Nymphs), Quadrisection (Females)	N/A
		MP Bio Lysing Matrices	4 m/s 1.5 & 4.0 min

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517 **Table 1.** Methods of DNA isolation and physical disruption of tick samples. Only
518 samples treated with the MoBio kit were processed on the GeneMate vortex mixer
519 (BioExpress), while all remaining bead beating took place on the BeadBlaster 24
520 (Benchmark Scientific).

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Matrix	Manufacturer	Material	Suggested use
G	MP Bio	1.6 mm silicon carbide particles	Samples with tough, hard, or brittle cell membranes
H	MP Bio	2 mm glass beads & 2 mm zirconium oxide beads	Tough, hard cells and organisms within dense exterior matrices, e.g. whole insects and ticks
I	MP Bio	2 mm zirconium beads & one 4 mm ceramic sphere	Very tough, hard samples including chitin exoskeletons, e.g. whole insects and ticks
M	MP Bio	Two 6.35 mm zirconium oxide-coated ceramic grinding spheres	Tough tissues, seeds, spores
S	MP Bio	3.175 mm stainless steel beads	Tough tissues, seeds, spores
Z	MP Bio	2.0 mm yttria-stabilized zirconium oxide spheres	Tough plant and animal samples
PowerBeads	MoBio	Garnet	Environmental samples
BashingBeads	Zymo	Ceramic	Ticks, mosquitoes, bees, lice, and *Drosophila melanogaster*

528

529 **Table 2.** Bead matrices and their attributes. The compostion, characteristics, and

530 recommended uses for the different bead matrices tested listed are adapted from the

531 manufacturers' websites.

532 (MP Bio: http://www.mpbio.com/index.php?cPath=2_77_425&country=223,

533 MoBio: http://www.mobio.com/soil-dna-isolation/powersoil-dna-isolation-kit.html,

534 Zymo: http://www.zymoresearch.com/dna/genomic-dna/solid-ffpe-tissue-dna/zr-tissue-

535 insect-dna-miniprep)

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Method	Life Stage	Nymphs		Adult females	
		Bisected	Whole	Quadrisected	Whole
QIAGEN	Average	1.70	Table 4	9.41	Table 4
	SD	0.640		1.63	
Thermo	Average	1.68	Table 4	9.77	Table 4
	SD	0.11		2.35	
MoBio	Average	0.070	0.0373	4.51	0.119
	SD	0.0585	0.0457	0.492	0.0133
Zymo	Average	<0.0005	<0.0005	3.14	0.0146
	SD	0.00	0.00	1.44	0.00341
NH4OH	Average	0.677	Table 4	0.0240	Table 4
	SD	0.086		0.0143	

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547 **Table 3.** Average DNA concentration (ng/μl) of whole and cut nymphal and adult

548 female blacklegged ticks. Average and standard deviation of the DNA concentration

549 values determined using the Qubit HS dsDNA Assay. Unless otherwise indicated,

550 samples were stored in 70% v/v ethanol. Three single-tick measurements were included

551 in each treatment. All values listed as < 0.0005 ng/μl indicate a reading of "too low" from

552 the Qubit fluorometer.

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Method	Life Stage	Matrix	Nymphs						Adult females					
			G	H	I	M	S	Z	G	H	I	M	S	Z
QIAGEN 1.5 min	Average		0.725	0.398	0.464	0.364	0.995	0.337	2.995	2.915	1.051	7.147	2.460	2.237
	SD		0.062	0.065	0.336	0.287	0.709	0.260	1.824	2.509	0.302	3.933	1.963	1.116
QIAGEN 4 min	Average		0.634	0.545	0.674	0.290	1.078	0.393	0.889	1.001	2.293	2.589	1.065	4.208
	SD		0.044	0.244	0.153	0.024	0.229	0.180	0.226	0.294	1.903	1.387	0.430	3.864
Thermo 4 min	Average		0.647	1.281	1.493	0.341	1.540	1.195	0.629	6.080	3.220	2.710	3.113	4.300
	SD		0.102	0.426	0.298	0.215	0.195	0.430	0.329	1.148	0.890	1.984	0.904	1.993
Thermo 4min	Average		0.441	1.370	0.707	0.715	1.950	1.847	0.579	7.727	10.773	0.537	8.453	3.147
	SD		0.212	0.085	0.136	0.220	0.896	0.496	0.075	0.981	1.608	0.399	2.770	1.196
NH_4OH 4min	Average		0.256	0.223	0.219	0.527	0.209	0.367	0.841	2.067	1.213	2.760	2.777	1.763
	SD		0.012	0.094	0.072	0.027	0.036	0.104	0.290	1.139	0.154	0.246	0.817	0.161
NH_4OH 4min	Average		0.362	0.309	0.197	0.398	0.252	0.463	0.673	0.423	1.173	2.527	0.771	1.220
	SD		0.034	0.033	0.080	0.023	0.091	0.031	0.234	0.138	0.176	0.794	0.188	0.095

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560 **Table 4.** DNA concentration (ng/µl) of whole nymphal and adult female blacklegged

561 ticks bead-beaten with MP Bio bead matrices. The results of DNA extractions from

562 whole ticks for the QIAGEN, Thermo, and NH_4OH are expanded here to include the

563 individual results from each of the six MP Bio Lysing Matrices.

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Method	Life Stage	Nymphs						Adult females					
	Matrix	G	H	I	M	S	Z	G	H	I	M	S	Z
QIAGEN 1.5 min	Average	0.979	0.729	0.812	1.853	1.853	2.030	10.767	13.267	9.233	5.507	21.900	11.113
	SD	0.374	0.279	0.272	0.462	0.563	1.005	1.274	3.288	1.253	3.691	4.814	7.841

568

569 **Table 5.** DNA concentration (ng/µl) of frozen (-80°C) nymphs and adult females. A two-

570 tailed Student's *t*-test showed that cold storage at -80°C produced significantly greater

571 DNA yields than 70% v/v ethanol storage for both nymphs and females ($P < 0.0001$).

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