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Opportunistic crimes: Evaluation of DNA from regularly-used knives after a brief use by a different person

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Key words

Forensic DNA analysis; trace DNA; DNA transfer; DNA persistence; regular use; multiple users; DNA-TPPR

Abstract

When evaluating trace DNA recovered from evidential items in forensic casework, it is crucial to consider how the DNA got there, and such evaluative interpretations should ideally be informed by published experimental data. A key activity-level question is whether the DNA obtained comes from the regular user, the last user (ostensibly the user at the time of the crime) or from indirect transfer events. The aim of this experiment was to provide data to contribute to answering this question, particularly when considering opportunistic crimes, in which an offender might grab the nearest item at hand required for their purpose, e.g. a weapon or tool, and therefore only handle it very briefly. Volunteers ('regular users') used knives in a prescribed manner to simulate regular use (one user per knife); DNA recovery by mini-tapes from these knives gave ~1-10 ng DNA, with <16% non-donor DNA from indirect transfer events. Different volunteers ('second users') then stabbed replicate sets of regularly-used knives into a foam block for either 2, 30 or 60 sec (on different occasions), with each timeframe in triplicate, and DNA was recovered from the knife handles using mini-tapes. For knives regularly-used by three of the four volunteers, the ratios of regular user to second user DNA were approximately 4:1, 2:1 and 1:1 for durations of use by the second user of 2, 30 and 60 sec, respectively. Analysis of the respective quantities of DNA showed that this trend resulted from a

35 decrease in regular user DNA via transfer to the second user's hands, rather than an
36 increase in DNA deposition from the second user. However, for knives regularly-used by
37 the fourth volunteer, DNA from the regular user remained at significantly higher quantities
38 than DNA from the second user and unknown sources, irrespective of duration of use by
39 the second user. Furthermore, one volunteer deposited a similar amount of DNA through
40 regular use as the amount of indirectly-transferred unknown DNA deposited by another
41 volunteer's hands. These observations indicate that caution should be taken when
42 relying solely on absolute quantities of DNA to inform evaluative interpretations, and other
43 parameters, such as profile quality and relative contributions to mixed profiles, should
44 also be taken into account. To better assist activity level assessments, more extensive
45 studies of this manner should be conducted to obtain probability distributions of different
46 types of profiles resulting from this kind of activity.

47

48 **1. Introduction**

49 When evaluating trace DNA recovered from evidential items in forensic casework, it is
50 now widely accepted that considerations at the activity level, that is how the DNA got
51 there, are crucial. It is recommended that such activity level evaluations be informed by
52 empirical data with that data coming from published (peer-reviewed) structured
53 experiments where possible [1-4]. A key activity-level question when examining items in
54 forensic casework for so-called "touch DNA" (i.e. DNA assumed to have been deposited
55 during an action of touching), or trace DNA, is whether the DNA obtained comes from the
56 regular user, the last user (ostensibly the user at the time of the crime) or from indirect
57 transfer events. The aim of the experiment presented here is to produce data to
58 contribute to answering this question.

59

60 There are a number of published studies that have started to address the general issue
61 of interpreting DNA findings when there are multiple users of an item [5-16]. These
62 studies fall into three categories: the touching or handling of clean surfaces by multiple
63 individuals [5, 7, 8, 15], the regular use of fabric items (e.g. clothing, bedding etc.) that
64 are then used by a different individual [6, 9-13], and the regular use of items made of
65 hard non-porous substrates (e.g. pens, keyboards, screwdrivers etc.) that are then used
66 by a different individual [9, 12, 14, 16]. It is this latter category that we are focusing on

67 here, as we consider the specific issue regarding the use of hand-held items (e.g. those
68 that are comparable to items used as weapons or burglary tools) that have been regularly
69 used by one individual and then used by a different 'one-off' user. To our knowledge,
70 there are only three published studies that address this specific issue [9, 12, 16], which
71 emphasises the need for research into this topic. Research is required to investigate the
72 factors (e.g. shedder status and manner, frequency and duration of handling by the
73 regular or second user) that may impact the DNA results obtained to understand how
74 these factors can be accommodated in casework evaluation, where the specific details
75 of the incident are likely to be unknown. Research is also required to provide the data to
76 generate probability distributions of different types of profiles resulting from this kind of
77 activity, and, with so few studies available addressing this specific topic, more extensive
78 studies are needed before consistent results characterised by the majority of studies can
79 be elucidated.

80
81 Pens have been used as hard, non-porous, plastic surfaces to represent surfaces
82 encountered as tools or weapons in casework [9, 12]. In one study, the 'regular user'
83 vigorously rubbed plastic pens for 30-60 sec on four consecutive days to give a total of
84 3.5 min of use, and then second users wrote with the pens for various durations [12].
85 Approximately equal proportions of DNA from the regular and second users were
86 observed when the second users wrote with the pens for 1-30 min, with a greater
87 contribution of DNA from the second users being observed when duration of use
88 increased to more than 30 min [12]. In another study, pens were handled by the regular
89 user for a minimum of 20 min per day for 10 consecutive days to give an average total of
90 240 min of use, and then the second users handled the pens for 5, 30 or 120 min [9].
91 Similar findings were observed in which approximately equal proportions of regular and
92 second user DNA were recorded at 30 min of use by the second user, with the second
93 user DNA tending to a major profile at 120 min of use [9]. Whilst this study reported that
94 DNA from the regular user tended to be the major profile at 5 min of use by the second
95 user [9], which differed from the findings by van Oorschot *et al.* [12], this is presumably
96 due to the difference in total duration of initial use by the regular user (i.e. 240 min versus
97 3.5 min).

98

99 However, in an opportunistic crime, an offender might grab the nearest item at hand, e.g.
100 a weapon or tool, required for their purpose in that moment, and therefore handle it for a
101 shorter timeframe of seconds rather than minutes. Pfeifer & Wiegand [16] start to address
102 this by considering the effect of a second user handling burglary tools (screwdrivers,
103 hammers and crowbars) for 30 sec after initial use by the 'owner'. They also investigated
104 the impact of different types of handling by the second user, by considering how a burglar
105 might intensely handle the tool to break into a property versus how the tool would normally
106 be used. They found that DNA tended to be recovered from the second user rather than
107 the owner when the tool was intensely used by the second user, although the owner only
108 used the tools for 30 sec prior to use by the second user [16]. Here, we created 'regularly-
109 used' knives in the same manner as Meakin *et al.* [17], such that the regular user handled
110 knives for a total of 4 min across two days prior to use by a second individual, which is
111 comparable to the study with multiple users by van Oorschot *et al.* [12]. The knives were
112 then stabbed for 2, 30 or 60 sec by a different individual (second user) to assess the
113 impact of shorter timeframes of second use on the DNA results obtained. In addition, we
114 assessed the DNA data to consider whether changes in respective proportions of DNA
115 from the regular and second users, with increased duration of use by the second user,
116 are due to an increase in second user DNA deposition or a reduction in the persistence
117 of regular user DNA.

118
119

120 **2. Materials and Methods**

121 *2.1 Materials and volunteers*

122 Plastic-handled steak knives and stabbing apparatus (consisting of a plastic box
123 containing a foam block covered in foil) were prepared and cleaned of DNA as described
124 by Meakin *et al.* [17]. Four participants, denoted A, B, C and D, were selected from those
125 who volunteered on the basis of availability to attend the laboratory at the times required.
126 Each volunteer gave informed consent to participate, and provided a buccal swab from
127 which a reference DNA profile was generated.

128

129 *2.2 Experimental set-up*

130 Before participating in the study, the four volunteers were instructed not to have contact
131 with each other and to avoid touching any shared items for the duration of the study. The
132 participants were also directed not to use anti-bacterial gels and not to wash their hands
133 in the hour immediately prior to laboratory visits.

134
135 Simulated 'regularly-used' knives were set up as described by Meakin *et al.* [17], such
136 that each knife was handled by a single volunteer (referred to as the 'regular user') for a
137 total of 4 min across two days. For each volunteer, three regularly-used knives were
138 mini-taped to provide DNA samples as positive controls and three regularly-used knives
139 were prepared each week for three consecutive weeks to give a total of 36 knives used
140 in the following handling experiments.

141
142 Each participant attended the laboratory individually for the three consecutive days after
143 the preparation of regularly-used knives to give ~24 h between each visit. During
144 attendance, the participant (referred to as the 'second user') selected a knife that had
145 been regularly used by a different volunteer and then stabbed it into the stabbing
146 apparatus at a rate of 1 stab per 2 s [17]. During each week, participants stabbed for 2,
147 30 or 60 s, with a different knife used each day to give triplicate results for each duration
148 of stabbing. DNA was recovered from each knife handle using a mini-tape within an hour
149 of each stabbing event. The volunteers were paired at their convenience, such that when
150 volunteers A, B, C and D were the regular users of the knives, volunteers B, A, D and C
151 were the second users, respectively. The pairings remained the same throughout this
152 study.

153

154 *2.3 Processing of DNA samples*

155 Extraction, quantification and profiling of DNA from the mini-tapes, and extraction and
156 profiling of DNA from the buccal swabs, were performed as described by Meakin *et al.*
157 [17]. In brief, the QIAamp® DNA Investigator Kit (QIAGEN, Germany), the Quantifiler®
158 Human DNA Quantification Kit (Applied Biosystems, USA), and the AmpF/STR® NGM
159 SElect™ PCR Amplification Kit (Applied Biosystems, USA) using 10 µl DNA extracts
160 were used for the mini-tape samples. DNA extracts from each individual knife handle

161 were quantified in duplicate to enhance accuracy; averages of these duplicate
162 quantifications are used in Fig. 1a and 3. The buccal swabs were processed using the
163 SwabSolution™ Kit (Promega, USA) with 2 µl extracts profiled using NGM SElect™. The
164 30 cycle protocol was used for all samples and PCR products were then separated using
165 the DNA Analyzer 3730x1 (Applied Biosystems, USA). DNA profiles were generated
166 using GeneMapper® 4.0 software with a 100 rfu peak height threshold, as per the
167 laboratory's internal validation study.

168

169 *2.4 Data analyses*

170 Relative contributions of DNA from the regular user, second user and any non-donor
171 sources (referred to as 'unknown' DNA) to the profiles obtained from the knife handles
172 were determined by comparison to the reference DNA profiles of the regular and second
173 users. These calculations used the relative peak height contributions from the unique
174 alleles that could be attributed to each of the respective reference profiles at each locus,
175 and averaged across the STR loci and across the three replicates per sample [17]. To
176 determine the amount of DNA deposited by each user, the relative contributions were
177 multiplied by the total amount of DNA recovered for each sample. Where the minimum
178 numbers of contributors are stated, these were determined with consideration of both
179 number of alleles and respective peak heights. SPSS® Version 22 (IBM) was used to
180 examine any trends in or differences between datasets. Datasets per individual volunteer
181 were normally distributed according to the Shapiro-Wilk test ($p > 0.05$), enabling
182 parametric statistics to be used for comparisons between volunteers and for investigating
183 correlations with duration of stabbing. When data from different volunteers were
184 combined (for example, to compare amounts of DNA detected from the regular user
185 versus from unknown sources), these datasets were not normally distributed ($p < 0.05$),
186 such that the Mann-Whitney U test was used.

187

188 **3. Results**

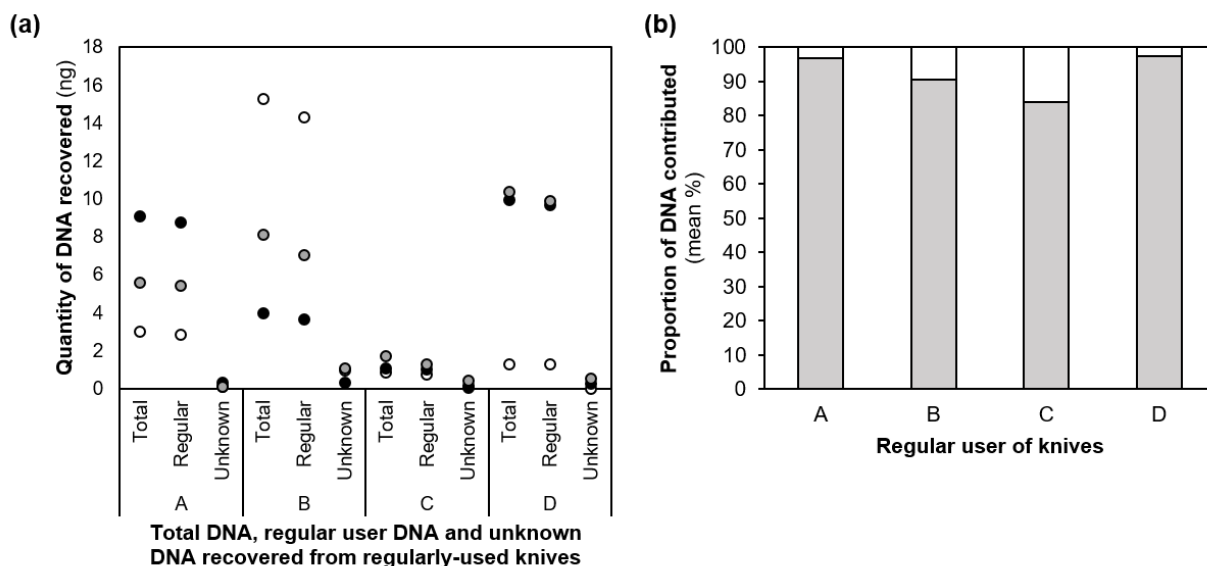
189 *3.1 DNA recovered from knife handles after simulated regular use*

190 Prior to the handling experiments, knives that had been handled in a manner to simulate
191 regular use were examined for DNA. The mean quantities of total DNA retrieved from

192 these knives used only by volunteers A, B, C and D were 5.9, 9.1, 1.2 and 7.2 ng,
193 respectively; the quantities recovered for each of the three replicate knife handles are
194 shown in Fig. 1a. These total DNA quantities varied both across replicates for the same
195 volunteer and among samples obtained from different volunteers (Fig. 1a; ANOVA
196 $F = 4.712$, $p < 0.05$). Pairwise tests using the Student's t -test revealed DNA samples
197 from knives handled by volunteers A, B and D were not significantly different, as also
198 indicated by the range of quantities shown in Fig. 1a. However, samples recovered from
199 knives handled by volunteer C contained significantly less DNA than those from the other
200 volunteers ($p < 0.05$ for the three comparisons; Fig. 1a).

201
202 To calculate the quantities of DNA deposited through direct handling of the knives versus
203 via indirect transfer events, the proportions of the DNA profiles obtained that could be
204 attributed to the regular user versus unknown sources of DNA were first determined.
205 These showed that, of the DNA profiles from the knives handled by volunteers A, B and
206 D, 91-97% came from the regular user with 3-9% coming from unknown sources
207 (Fig. 1b). The minimum number of contributors to the unknown component of these
208 profiles ranged from 1 to 2 with a mean of 1.0. A higher proportion of unknown DNA at
209 16% was observed for knives handled by volunteer C (Fig. 1b), which was attributed to
210 DNA from their romantic partner, as previously observed when volunteer C participated
211 in a previous study (as volunteer X in [17]). When the total DNA quantities recovered
212 were multiplied by these proportions, the mean quantities of DNA attributed to the regular
213 user were 5.7, 8.3, 1.0 and 7.0 ng, and those attributed to indirectly-transferred DNA were
214 0.19, 0.86, 0.20 and 0.19 ng, for the knives handled by volunteers A, B, C and D,
215 respectively. The range of quantities are shown as individual data points for each
216 replicate knife handle in Fig. 1a. Comparison of the DNA quantities attributed to the
217 regular user with those from unknown sources, across all four volunteers' samples
218 combined, showed that significantly more DNA was deposited from the regular user than
219 from unknown sources (Mann Whitney $U = 12.0$, $p < 0.001$). For the DNA attributed to
220 the regular user, full profiles were observed for volunteers A, B and D, but some allele
221 drop-out was observed for volunteer C to give partial profiles of 83-93%.

222



223

224

225

Fig. 1. Quantities of DNA (a) and respective proportions of DNA (b) contributing to the DNA mixtures recovered from the handles of simulated regularly-used knives. In (a), quantities of DNA are from the three replicate knives (white, grey and black dots) corresponding to the total DNA recovered, the DNA attributed to the regular user, and the DNA attributed to unknown sources. In (b), proportions of DNA are means of three replicate knives contributed from the regular user (light grey bars) and other unknown sources (white bars).

231

232

233

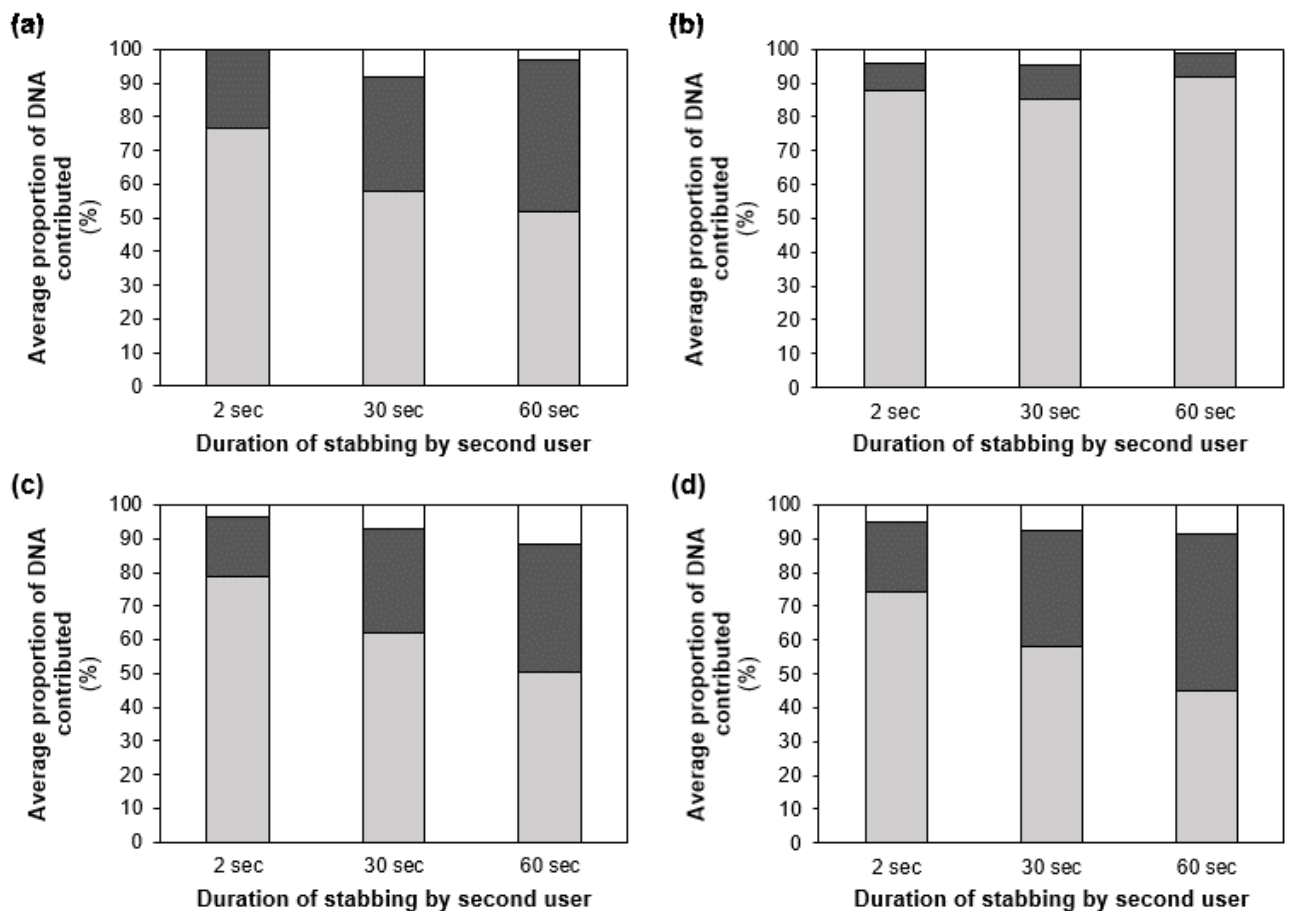
3.2 Respective contributions from both users to mixed DNA profiles obtained

234

Each 'regularly-used' knife was then stabbed into the stabbing apparatus for a set period of time by a different user, and DNA recovered from the knife handles. For the knives that had been regularly used by volunteers A, C and D, when the second user stabbed the knife for 2 sec, the proportion of regular user to second user DNA was approximately 4:1 (Fig. 2a, c & d). This changed to approximately 2:1 when the second user stabbed for 30 sec, and to approximately 1:1 when the second user stabbed the knife for 60 sec (Fig. 2a, c & d). These observations showed a significant correlation for the decrease in proportion of regular user DNA with increasing duration of stabbing by the second user (Pearson $r = -0.56, -0.49, -0.49$ for knives initially handled by volunteers A, C and D, respectively; $p < 0.001$). A corresponding significant correlation for the increase in proportion of second user DNA with increasing stabbing duration was also observed (Pearson $r = -0.52, -0.38, -0.45$ for knives initially handled by volunteers A, C and D, respectively; $p < 0.001$). However, these correlations were not observed for knives

246

247 regularly handled by volunteer B and subsequently stabbed into the apparatus by
 248 volunteer A. For these knives, the proportion of regular user DNA remained high at 85-
 249 92%, irrespective of the duration of stabbing by the second user (Fig. 2b).



250
 251 **Fig. 2.** Proportions of DNA contributed from the regular user (light grey bars), second user (dark grey bars)
 252 and other unknown sources (white bars) to the mixed DNA profiles recovered from knife handles that had
 253 been regularly-used by volunteers A (a), B (b), C (c) and D (d) and then stabbed in the foam block by a
 254 second user for 2, 30 or 60 sec.

255
 256 **3.3 DNA quantities deposited from the users and unknown sources**

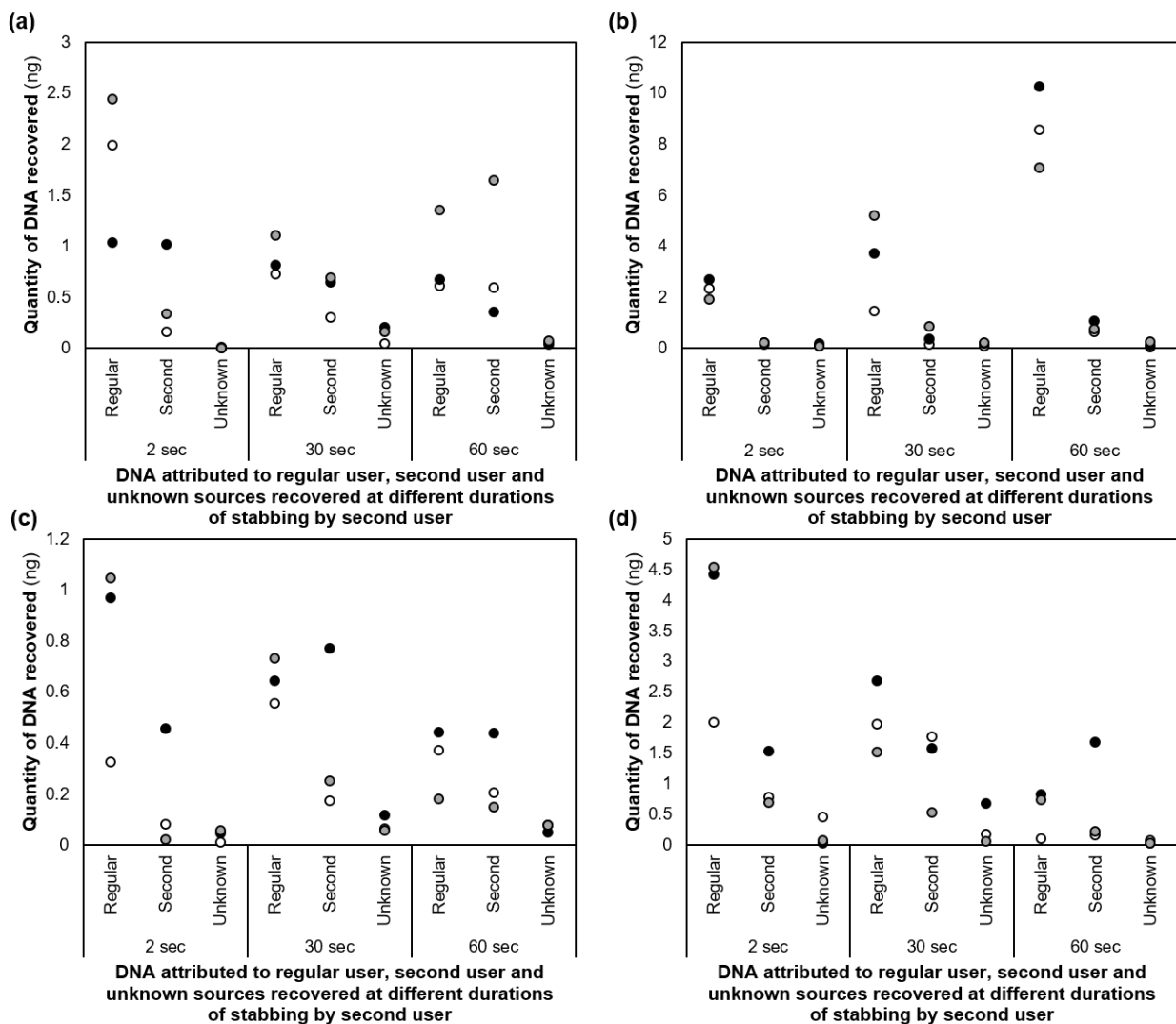
257 To determine the quantities of DNA deposited by the two users and to interrogate the
 258 observed trends further, the respective proportions of DNA in Fig. 2 were multiplied by
 259 the total quantities of DNA recovered for each knife handle. The quantities of DNA
 260 attributed to the regular user, second user, and unknown non-donor sources are
 261 presented individually for each replicate knife handle in Fig. 3. For the knives initially

262 handled by volunteers A, C and D, although the quantities of DNA recovered from the
263 regular user are quite varied, they appear to decrease with increasing duration of
264 stabbing by the second user (Fig. 3a, c & d). However, although also varied, the
265 quantities of DNA from the second user appear to stay at similar levels, irrespective of
266 the duration of stabbing (Fig. 3a, c & d). A Pearson's correlation was used to examine
267 whether the quantities of DNA were significantly related to the duration of stabbing. The
268 observed decrease in quantity of regular user DNA was significantly correlated with
269 increasing duration of stabbing by the second user for knives initially handled by
270 volunteers A ($r = -0.55$, $p = 0.01$), C ($r = -0.61$, $p < 0.01$) and D ($r = -0.80$, $p < 0.001$). No
271 significant correlations were observed between duration of stabbing and quantities of
272 DNA from the second user ($p > 0.3$ for all three volunteers' knives). Fig. 3b shows that
273 no such decrease in regular user DNA was observed for volunteer B's knives;
274 alternatively, the regular user DNA rather unexpectedly increased, particularly on the
275 knives that were handled by the second user for 60 sec.

276

277 To consider further the amounts of DNA deposited by the two users and those coming
278 from unknown non-donor sources, the results from the sets of knives initially handled by
279 volunteers A, C and D were combined and examined using the Mann-Whitney U test.
280 For these three volunteers, significantly more DNA was recovered from the regular user
281 than the second user, when duration of stabbing by the second user lasted 2 sec
282 ($U = 42.0$, $p < 0.001$) and 30 sec ($U = 100.5$, $p = 0.05$). However, when the stabbing
283 lasted 60 sec, there was no significant difference between the quantities of DNA
284 recovered from the two users ($U = 132.0$, $p = 0.34$), as per the observation of
285 approximately 1:1 ratios in proportions of DNA from the two users (Fig. 2a, c and d). Also
286 for these knives, the quantities of unknown DNA recovered from the knife handles were
287 significantly lower than the DNA quantities attributed to either user ($p < 0.001$). For the
288 knives regularly used by volunteer B, DNA from the regular user was recovered at
289 significantly greater quantities than those from the second user and those from unknown
290 sources for all durations of stabbing ($p < 0.01$; Fig. 2b & 3b). However, although the
291 quantities of DNA from the second user were significantly higher than those from
292 unknown sources for 2 and 60 sec stabbing durations ($t = 2.9$, $p = 0.015$ for 2 sec; $t = 7.6$,
293 $p < 0.001$ for 60 sec), there was no significant difference in the quantities of DNA

294 recovered from the second user and unknown sources for 30 sec duration of stabbing
 295 ($t = 2.1$, $p = 0.088$). For all the knives handled by two users, the minimum number of
 296 contributors to the unknown component of the DNA profiles obtained ranged from 1 to 2
 297 with a mean of 1.3. This is essentially the same as the minimum number of contributors
 298 to the unknown component of the DNA profiles obtained from the knives handled only by
 299 the regular user (Section 3.1). This was surprising, as a higher number of contributors
 300 might be expected in the unknown component of the second set of knives given that they
 301 were handled by two individuals, each presumably contributing DNA from separate
 302 unknown origins.



303
 304 **Fig. 3.** Quantities of DNA attributed to the regular user, second user and other unknown sources recovered
 305 from knife handles that had been regularly-used by volunteers A (a), B (b), C (c) and D (d) and then stabbed
 306 in the foam block by a second user for 2, 30 or 60 sec. DNA quantities are presented individually from
 307 three replicate knives (white, grey and black dots).

308 4. Discussion

309 4.1 DNA deposition during regular use

310 During simulated regular use of the knives, volunteers deposited quantities of DNA in the
311 1-10 ng range, consistent with findings by previous studies for comparable non-porous
312 items that were either regularly used in a simulated manner or actually regularly used [9,
313 12, 13, 17, 18]. Volunteer C deposited significantly less DNA than the other volunteers,
314 also as previously observed when this volunteer participated as volunteer X in a prior
315 study [17]. These observations provide further support for the concept of 'shedder
316 status', which was first proposed by Lowe *et al* in 2002 [19] and is gaining wider
317 acceptance in recent years [9, 17, 20-23]. DNA from unknown sources was also
318 recovered, which had been indirectly-transferred to the knife handles via the hands of the
319 participants. In general, this contributed to <16% of the mixed profiles obtained, as has
320 been observed previously by numerous studies (e.g. [9, 12, 15, 17, 21]), with the slightly
321 higher level being observed for volunteer C due to the transfer of DNA from their romantic
322 partner.

323
324 DNA quantities are being increasingly relied upon to assist in distinguishing between
325 different activity scenarios when evaluating trace DNA evidence [24]. Here, when the
326 total quantities of DNA were multiplied by the relative proportions of DNA attributed to the
327 regular user and unknown sources, the quantities of DNA deposited by the regular user
328 were significantly greater than those deposited from indirect transfer events. This
329 suggests that it might be possible to use such data to help distinguish between DNA
330 deposited directly via regular use and DNA indirectly deposited via the hands of the
331 regular users. However, it is also important to note that the amount of regular user DNA
332 recovered from volunteer C's knives (1.0 ± 0.4 ng) is similar to the amount of unknown
333 DNA from volunteer B's knives (0.86 ± 0.48 ng). This could be due to volunteer C being
334 a 'poor shedder', and suggests that caution should be taken when considering whether
335 the quantity of DNA can be used to distinguish modes of transfer, particularly when the
336 shedder statuses of the respective DNA contributors are not known. In such situations,
337 it might therefore be more appropriate to consider the respective proportions of the DNA
338 observed in mixed profiles obtained, rather than the absolute DNA quantities.

339

340 4.2 Effect of increased use by second user on DNA recovered

341 For knives regularly-handled by three of the four volunteers (A, C and D), an apparent
342 trend was observed showing a significant decrease in proportion of regular user DNA
343 with a corresponding significant increase in proportion of second user DNA, as duration
344 of stabbing by the second user increased. Whilst a similar trend has been observed by
345 previous studies when durations of handling by the second user were minutes to hours
346 [9, 12], these data are the first to show that this trend can also occur when handling by
347 the second user is for just two seconds to a minute. At 30 sec of use by the second user,
348 DNA from the regular user was still observed at a higher proportion to that from the
349 second user, which is in contrast to the findings by Pfeifer and Wiegand [16]. This is
350 presumably due to differences in experimental design; firstly, the manner of handling by
351 second users varied, and secondly, second users in that study handled tools that were
352 either genuinely regularly-used, but had not been handled for at least two weeks prior to
353 the study, or bought new and handled once for 30 sec by the designated 'owner'. Here,
354 regular use was simulated through the handling of clean knives for a total of 4 min across
355 the two days immediately prior to the second user handling the knives.

356
357 Analysis of the DNA quantities attributed to the respective users revealed that the
358 quantities of second user DNA remained similar across the knives, irrespective of the
359 duration of use, which is consistent with the concept that increasing the duration of a
360 single contact does not necessarily increase DNA deposition [18]. The increased
361 proportion of DNA from the second user by 60 sec of use is therefore not due to an
362 increase in deposition, but instead due to a decrease in the quantity of DNA from the
363 regular user persisting on the knife handles, giving similar amounts of DNA from the two
364 users and resulting in the approximately 1:1 ratio observed here and by other studies [9,
365 12]. This decrease in quantity of regular user DNA, as duration of use by the second
366 user increases, supports a previously proposed explanation by van Oorschot *et al.* [12],
367 that there is simultaneous transfer of DNA from the knife handle to hand, such that the
368 hand of the second user takes increasing amounts of DNA away from the knife handles
369 with increasing duration of use. Differences in the nature of the two substrates coming
370 into contact, i.e. hand versus plastic knife handle, might also contribute to this finding.

371

372 Interestingly, the above change in respective proportions of DNA from the two users was
373 not observed for the knives that were regularly handled by volunteer B and then used by
374 volunteer A. Fig. 1a shows that the average quantity of DNA recovered from the knives
375 that were only handled by volunteer B appeared to be higher than the other volunteers,
376 although the t-test showed this was not statistically significant. Rather unexpectedly, Fig.
377 3b shows that the quantities of DNA deposited by volunteer B were higher in the samples
378 where the knives were handled for longer durations by volunteer A. It is not clear why
379 the quantities of DNA deposited by volunteer B varied in this manner; it may be an artefact
380 of the intra-person variation in DNA deposits observed by Goray *et al.* [21], even though
381 the volunteers handled the knives at similar times of day and were directed to handle the
382 knives in the same manner each time. However, these higher quantities of DNA from
383 volunteer B may be the reason why increasing the duration of use by volunteer A did not
384 result in the decrease in regular user DNA observed in the other pairings of volunteers.
385 Comparison of Fig.s 1a and 3 also shows that volunteer A deposited less DNA during the
386 second handling experiment, than during the positive control sampling for regular use,
387 which may also contribute to this finding. Correspondingly, the quantities of regular user
388 DNA for volunteer B remained significantly greater than those from the second user
389 (volunteer A) and those from unknown sources for all durations of use.

390

391 *4.3 Further consideration of DNA quantities from direct and indirect transfer*

392 For the knives that were regularly handled by volunteers A, C and D and then used by a
393 second user, indirectly-transferred unknown DNA was observed at significantly lower
394 quantities than DNA from both users. This supports the observations that, when
395 examining regularly-used items, DNA that has been transferred indirectly is detected at
396 lower levels than that which has been directly transferred [9, 12], even during an
397 experiment purposefully designed to investigate indirectly-transferred DNA [17].
398 However, for the knives that were regularly handled by volunteer B, although DNA from
399 the regular user was deposited at significantly greater amounts than indirectly-transferred
400 unknown DNA, second user DNA was only recovered at significantly greater quantities
401 than indirectly-transferred unknown DNA at 2 and 60 sec stabbing durations. For this
402 pairing of volunteers, at 30 sec duration of use by the second user, similar quantities of
403 DNA from the second user and unknown sources were recovered. This further

404 demonstrates that there are occasions when caution should be taken when relying solely
405 on DNA quantity to distinguish between modes of transfer.

406

407 *4.4 Concluding remarks*

408 DNA recovery from knives that were regularly-used in a simulated manner support prior
409 observations that regular use of non-porous items deposits ~1-10 ng DNA, with variation
410 depending on the donor's 'shedder status', and includes <16% non-donor DNA from
411 indirect transfer events. The use of knives, initially regularly-used by three of four
412 participants, for just 2-60 sec by a second user resulted in a decrease in contribution from
413 regular user DNA with a simultaneous increase in contribution from second user DNA.
414 Analysis of the quantities of DNA contributed revealed that this trend is due to a decrease
415 in regular user DNA via transfer to the second user's hands, rather than an increase in
416 DNA deposition from the second user. This should be investigated further using a larger
417 sample size of participants and 'real-life' regularly-used knives and other non-porous
418 items. In particular, participants that vary in their shedder status should be used, given
419 that when knives were initially regularly-used by a volunteer who could be considered a
420 'good shedder', subsequent use by a second user did not reduce the DNA present from
421 the regular user, such that the proportion remained high and the quantity of DNA from
422 the regular user remained significantly greater than that from the second user.

423

424 The data reported herein contribute to the data available for use in the determination of
425 likelihood ratios addressing activity-level evaluations of DNA evidence in forensic
426 casework, such as in cases of opportunistic crimes, when an offender might only briefly
427 handle the nearest item at hand, e.g. a weapon or tool, required for their purpose in that
428 moment. However, whilst the general trend described above was identified, it is important
429 to acknowledge that, even with just four participants in this experiment, a deviation from
430 that general trend was observed with knives regularly handled by one of the four
431 volunteers. Furthermore, one volunteer deposited a similar amount of DNA through
432 regular use as the amount of indirectly-transferred unknown DNA deposited by another
433 volunteer's hands. These observations indicate that caution should be taken when
434 relying solely on absolute quantities of DNA to inform evaluative interpretations. As has

435 been discussed previously [17], other parameters, such as profile quality and relative
436 contributions to mixed profiles, should also be taken into account.

437
438 Complex and variable scenarios are frequently encountered in forensic science and being
439 able to offer reproducible and transparent inferences is important for robust forensic
440 reconstructions [2]. The data presented in this study illustrate the broad value of
441 developing casework informed empirical studies that can provide data to underpin
442 evaluative interpretations of activity level propositions [4, 25]. To better assist activity
443 level assessments, more extensive studies of this manner should be conducted to obtain
444 probability distributions of different types of profiles resulting from this kind of activity. This
445 will also enable an elucidation of consistent results characterised by the majority of
446 studies for use in casework.

447

448 **Conflicts of interest**

449 None.

450

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456

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