**Effect of temperature on the survival and development of
three forensically relevant Dermestes species (Coleoptera:
Dermestidae)**

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11 **Effect of temperature on the survival and development of three forensically**
12 **relevant *Dermestes* species (Coleoptera: Dermestidae)**

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Effect of temperature on the survival and development of three forensically relevant *Dermestes* species (Coleoptera: Dermestidae)

Abstract

Most *Dermestes* species (Coleoptera: Dermestidae) are scavengers during both larval and adult stages, with a preference for dry organic matter. Because of this, *Dermestes* beetles are potentially useful indicators in forensic investigations concerning skeletonized and/or mummified human remains. However, there is a paucity of reference developmental data on most forensically relevant *Dermestes* species. This study analyses the effect of five constant temperatures (15, 20, 25, 30 and 35 °C) on the survival and developmental rates of three of the forensically most relevant dermestids: *Dermestes frischii* [Kugelan](#), *Dermestes maculatus* [De Geer](#) and *Dermestes undulatus* [Brahm](#). Pig skin was used as rearing substrate, in order to use a substrate as similar as possible to that exploited in nature. Overall, the temperature had a significant effect on the survival and the duration of development, with optimal values at intermediate temperatures. Both *D. frischii* and *D. maculatus* showed similar developmental rates and the shortest developmental times at 30 °C, whereas *D. undulatus* developed faster at lower temperatures. At 15 °C, both *D. frischii* and *D. undulatus* did not oviposit, whereas no *D. maculatus* individuals survived beyond the pupal stage. An inconsistent number of larval instars per individual were observed across different constant temperatures in the three species. The present study aims to provide baseline developmental data for further advances in the potential use of *Dermestes* beetles as forensic tools in long *post-mortem* interval cases.

Resumen

La mayoría de las especies del género *Dermestes* (Coleoptera: Dermestidae) son carroñeras durante la fase larvaria y la fase adulta, con preferencia por la materia orgánica seca. Debido a esto, los derméstidos son indicadores potencialmente útiles en las investigaciones forenses que impliquen restos humanos momificados y/o esqueléticos. Lamentablemente, los datos de referencia sobre el desarrollo de la mayoría de las especies de *Dermestes* de relevancia forense son escasos. Este estudio analiza el efecto de cinco temperaturas constantes (15, 20, 25, 30 y 35 °C) sobre la supervivencia y la tasa de desarrollo de tres de los derméstidos de mayor relevancia forense: *Dermestes frischii* Kugelan, *Dermestes maculatus* De Geer y *Dermestes undulatus* Brahm. Como sustrato de cría se utilizó piel de cerdo, con el objetivo de utilizar un sustrato lo más parecido posible al explotado en condiciones naturales. En general, la temperatura tuvo un efecto significativo sobre la supervivencia y la duración del desarrollo, con valores óptimos bajo las temperaturas intermedias. *D. frischii* y *D. maculatus* mostraron tasas de desarrollo similares y tiempos de desarrollo más cortos a 30 °C, mientras que *D. undulatus* se desarrolló más rápido a temperaturas inferiores. A 15 °C, ni *D. frischii* ni *D. undulatus* pusieron huevos, mientras que ningún individuo de *D. maculatus* sobrevivió más allá de la fase pupa. Se observó un número inconsistente de estadios larvarios por individuo en las diferentes temperaturas constantes y en las tres especies. Este estudio tiene como objetivo proporcionar datos de referencia sobre el desarrollo para futuros avances en el uso de los derméstidos como herramientas forenses en casos de intervalos *post mortem* largos.

KEY WORDS

Dry remains, forensic entomology, post-mortem interval, skeletonization, stored products

67 Introduction

68 Both adult and larval stages of most species of the genus *Dermestes* Linnaeus
69 (Coleoptera: Dermestidae) are scavengers with a preference for dry, protein-rich organic
70 matter (Hinton 1945, Plata Negrache 1972, Braack 1987, Zhantiev 2009). Because of
71 this, *Dermestes* beetles are of high economic importance as they can represent major
72 pests of stored animal products, including food, silkworm cocoons, leather and other
73 textile items, and museum collections (Hinton 1945, Linnie 1999, Rajendran and Hajira
74 Parveen 2005, Fontenot et al. 2015). Moreover, some species may occasionally act as
75 intermediate hosts of parasites or as vectors of disease organisms (Hinton 1945), as well
76 as cause allergic reactions (Rustin and Munro 1984, Cuesta-Herranz et al. 1997).
77 Paradoxically, although *Dermestes* beetles can be a serious pest in natural history
78 collections, they are also a helpful tool for curators as they are traditionally used for
79 cleaning bones (Hall and Russell 1933). *Dermestes* beetles are certainly able to greatly
80 accelerate the skeletonization process of animal carcasses under optimal environmental
81 conditions (Hall and Russell 1933) and, of course, this is also applicable to human
82 corpses (Schroeder et al. 2002, Charabidze et al. 2014). Consequently, *Dermestes*
83 beetles are potentially useful indicators in both medicolegal and archaeological
84 investigations concerning skeletonized and/or mummified human remains (Charabidze
85 et al. 2013, Huchet et al. 2013, Magni et al. 2015). However, the most relevant
86 application of insects in forensic investigations, the estimation of a minimum post-
87 mortem interval ($_{\min}$ PMI), is hampered in the case of *Dermestes* beetles due to the lack
88 of data on developmental rates for certain species and under certain environmental
89 variables (Magni et al. 2015).

90 Understanding how different abiotic variables can affect the life cycle of
91 *Dermestes* species is essential for a proper management of stored products and for using

92 these insects in forensic investigations. Environmental temperature is arguably the most
93 important variable, as it highly influences the developmental rates of insects because of
94 their poikilothermia. Accordingly, there are a number of published studies dealing with
95 the influence of temperature, as well as other factors like humidity, on the development
96 of *Dermestes* beetles (see Magni et al. 2015, for a review). Those studies come mainly
97 from the pest research literature, with the cosmopolitan *Dermestes maculatus* De Geer
98 as the main target species (e.g. Hinton 1945, Scoggin and Tauber 1949, Pisfil and
99 Korytkowski 1974, Raspi and Antonelli 1995, Fontenot et al. 2015). Although *D.*
100 *maculatus* is perhaps the most important species economically in the genus (Hinton
101 1945), other *Dermestes* species like the cosmopolitan *Dermestes frischii* Kugelan can be
102 serious pests as well, and therefore they have also received attention from the pest
103 management perspective (e.g. Andres 1925, Hinton 1945, Howe 1953, Amos 1968). On
104 the other hand, the studies on the life cycle of *Dermestes* species from a forensic
105 perspective are very scarce and strictly focused on *D. maculatus* (Richardson and Goff
106 2001, Zanetti et al. 2016b). *Dermestes maculatus* is indeed a forensically relevant
107 species as it is frequently cited in association with both animal carcasses and human
108 corpses in every biogeographic region (see Magni et al. 2015, for a review). However, a
109 recent review of forensic case studies performed in France during two decades
110 (Charabidze et al. 2013) showed that two other *Dermestes* species, namely *D. frischii*
111 and *D. undulatus* Brahm, were clearly the most frequent ones found on human corpses.
112 This is in line with the results from several succession studies using pig carcasses in
113 different regions of the Iberian Peninsula (e.g. Castillo-Miralbés 2001, Prado e Castro et
114 al. 2013), where both *D. frischii* and *D. undulatus* were certainly the dominant
115 dermestid species in the carrion beetle assemblages across different habitats (Martín-
116 Vega and Baz 2012). In contrast to *D. maculatus* and *D. frischii*, *D. undulatus* shows a

117 more limited geographical distribution (it is apparently restricted to central Asia, Europe
118 and North America) and it is only occasionally found infesting stored products (Hinton
119 1945). Nonetheless, there are no available studies on the development of *D. undulatus*
120 and, as mentioned, the life cycle of *D. frischii* has received only limited attention from a
121 pest management perspective and not at all from a forensic point of view. Moreover, no
122 study has compared the developmental rates of more than one forensically relevant
123 *Dermestes* species under the same conditions.

124 Furthermore, it must be taken into account that the studies on the developmental
125 rates of *Dermestes* species for pest management purposes typically use protein-rich
126 products from the food industry as rearing substrate. For example, cheese, bacon,
127 fishmeal or lamb-and-rice-based dogfood have been used as experimental larval diets
128 (e.g. Howe 1953, Amos 1968, Pisfil and Korytkowski 1974, Raspi and Antonelli 1995,
129 Fontenot et al. 2015), but even forensically oriented studies have also used commercial
130 food products like dried fish (Richardson and Goff 2001) or beef (Zanetti et al. 2016b).
131 On a cadaver, under natural conditions, *Dermestes* larvae are usually the main fauna
132 responsible for the complete consumption of skin whereas other insect taxa typically
133 consume the soft tissues (Braack 1987). The potential forensic value of *Dermestes*
134 beetles comes, indeed, from their occurrence on dry, skeletonized and/or mummified
135 human remains (Charabidze et al. 2013, Magni et al. 2015). Therefore, from a
136 medicolegal perspective, it would be interesting to study the developmental rates of
137 *Dermestes* beetles on a substrate as similar as possible to that exploited in nature,
138 because different biological performance has been reported using different food
139 products as experimental diet (Osuji 1978, Woodcock et al. 2013).

140 The present paper analyses the effect of temperature on the survival and
141 developmental rates of three forensically relevant *Dermestes* species (*D. frischii*, *D.*

142 *maculatus* and *D. undulatus*), using pig skin as rearing substrate. This study aims to
143 provide baseline developmental data in order to improve our knowledge of the
144 usefulness of *Dermestes* beetles as forensic indicators in long PMI cases (Charabidze et
145 al. 2013, Magni et al. 2015).

146 **Materials and Methods**

147 Laboratory colonies of *D. frischii*, *D. maculatus* and *D. undulatus* were established
148 using adults collected on pig and sheep carcasses in supplementary feeding stations for
149 scavenging bird populations in central Spain and on pork-rib baits placed in a periurban
150 plot belonging to the University of Alcalá (Madrid, Spain) during late spring 2014.
151 Details on the supplementary feeding stations and their management can be found in
152 Martín-Vega and Baz (2011). Details on the locality sampled with pork-rib baits can be
153 found in Baz et al. (2015). The adult beetles were placed in plastic containers (40 x 30 x
154 30 cm) containing sterilized soil 2–3 cm deep and water *ad libitum*. Food (pork ribs)
155 was regularly provided. The three main colonies (one of each species) were maintained
156 at room temperature (25 ± 3 °C) and under natural light.

157 For each species, 15–25 adult pairs were transferred to another plastic container
158 (25 x 15 x 15 cm) with sterilized soil 1–2 cm deep, water *ad libitum* and pork ribs
159 regularly provided. The process was replicated five times and each sub-colony was
160 placed into an incubator under a constant temperature (15, 20, 25, 30 and 35 ± 1 °C),
161 60–80 % RH and a photoperiod of 12:12 (light:dark) during 3 days for acclimatization.
162 Several studies have shown that *Dermestes* species require relative humidities above 50
163 % to develop successfully (see Magni et al. 2015, for a review). Then, the adults from
164 each sub-colony were transferred to a new, identical plastic container into the same

165 incubator, in order to set a time zero for oviposition and ensuring that all the eggs were
166 laid under the same conditions.

167 After the transfer to new containers, laid eggs were collected on the soil using a
168 fine brush every 24 hours. A group of 10 eggs was placed into a small plastic container
169 (8 x 8 x 7 cm) containing a piece of pig skin (c. 5 x 5 cm) previously slightly dried in a
170 heater, and tissue paper as a refuge for pupation (Fontenot et al. 2015). The plastic
171 container was placed in an incubator under the correspondent experimental temperature
172 (15, 20, 25, 30 and 35 ± 1 °C). Pig skins were obtained from fresh carcasses in the
173 supplementary feeding stations for scavenging birds (see above). The skin sections
174 contained the epidermis, dermis and subcutaneous tissue (i.e. the lowermost layer of
175 connective tissue and elastin which attaches to underlying bones and muscles).
176 Dermestid species typically show relatively high mortality rates under certain
177 temperatures (e. g. Amos 1968, Zanetti et al. 2016b). Hence, for each species and
178 experimental temperature, the process was replicated a minimum of 20 and a maximum
179 of 70 times, in order to obtain a minimum of 100 individuals reaching adulthood (Table
180 1; note that for *D. frischii* only 95 individuals could be reared to adulthood at 35 °C).
181 Two different incubators were used for each experimental temperature.

182 The plastic containers were examined at 24-hour intervals, replacing the pig skin
183 with a fresh piece when needed and recording the developmental stage (egg, larval
184 instar, pupa or adult) shown by each individual. The observed developmental stages
185 were delimited by the following developmental milestones: (i) egg hatching (i.e. when
186 the embryonic development is complete and the first instar larva hatches from the egg);
187 (ii) a variable number of larval ecdyses (i.e. the moulting of the old cuticle, each
188 ecdysis—except the last one, see below—resulting in a new larval instar); (iii)
189 postfeeding larval phase (i.e. when the last larval instar ceases feeding, searches for a

place for pupation and becomes quiescent); (iv) pupation (i.e. formation of the pupa, which takes place within the cuticle of the last larval instar; the cuticle is then moulted and the pupa remains inactive and undergoes metamorphosis); and (v) adult emergence (the adult emerging by shedding the pupal cuticle). Larval ecdyses were recorded on the basis of the reduced sclerotization shown by larvae after moulting the cuticle and the presence of exuviae (i.e. the old cuticle), which were collected and preserved in 70 % ethanol. Daily examinations continued until all specimens had reached adulthood or died. Newly emerged adults were transferred to the main colony of the correspondent species to be used for further studies.

Survival under different constant temperatures was analysed using modified Kaplan-Meier survival curves and Mantel-Cox log-rank tests were performed to test the differences between survival distributions (Kleinbaum and Klein 2011). Kaplan-Meier survival curves are a series of declining horizontal steps, with the length of the vertical lines illustrating the extent of the decrease in survival within consecutive sampled events. In this case, the x-axis plotted the three immature life stages (egg, larval and pupal stage), so each life stage showed two values corresponding to the survival rate at the beginning and the end of the stage, respectively. Comparisons of the duration for each developmental stage between temperatures were performed using one-way analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD) post hoc test. Statistical analyses were performed using Statgraphics Centurion (Statistical Graphics Corp. 1994–2000). Differences were considered to be significant at the < 0.05 level in every analysis. Isomorphen diagrams were built plotting the mean time to reach each developmental milestone (see above) against temperature using Microsoft Excel v. 14.0 (Microsoft 2010–2016).

Results

215 *Survival*

216 Table 1 shows the cumulative survival rates of the three species for each immature stage
 217 and constant temperature. No oviposition occurred at 15 °C for *D. frischii*, and neither
 218 at 15 °C nor at 35 °C for *D. undulatus*. In general, the larval stage showed a more rapid
 219 drop in survival than the pupal stage in every species and at every constant temperature
 220 (Fig. 1). Cannibalism was determined when the remains of a clearly devoured larva or
 221 pupa were observed. Cannibalism was observed in the three species and at every
 222 constant temperature, but only representing a relatively low percentage of the total
 223 number of deaths at both larval (*D. maculatus*: 9.41 %, *D. frischii*: 12.28 %, *D.*
 224 *undulatus*: 4.3 %) and pupal (*D. maculatus*: 0.89 %, *D. frischii*: 1.37 %, *D. undulatus*:
 225 5.88 %) stages.

226 For both *D. maculatus* and *D. frischii*, the highest survival rates were observed
 227 at 25 °C, i.e. the intermediate temperature within the range considered in this study (Fig.
 228 1A–B; Table 1). In those two species, survival rates at 25 °C were higher for every
 229 immature stage with the exception of the egg stage in *D. maculatus*, which showed the
 230 highest survival rate at 15 °C, i.e. the lowest constant temperature within the considered
 231 range. However, interestingly, the survival rate at 15 °C dropped drastically during the
 232 subsequent larval stage with only ~1 % of the individuals reaching pupation, and no
 233 individuals surviving to the pupal stage (Fig. 1A–B; Table 1). On the other hand, the
 234 survival rates were low in every immature stage at 35 °C, i.e. the highest constant
 235 temperature within the considered range, although in this case 25 % and 20 % of the
 236 individuals survived until the adulthood in *D. maculatus* and *D. frischii*, respectively
 237 (Fig. 1A–B; Table 1). Finally, the survival rates at both 20 °C and 30 °C were overall
 238 very similar in those two species, but nonetheless lower than the rates observed at 25 °C
 239 (Fig. 1A–B; Table 1). Indeed, log-rank tests showed significant differences ($p < 0.05$) in

the survival distributions between every constant temperature with the exception of the comparison between 20 °C and 30 °C (*D. maculatus*: $\chi^2 = 1.6$, $p = 0.21$; *D. frischii*: $\chi^2 = 0.03$, $p = 0.81$).

Unlike *D. maculatus* and *D. frischii*, *D. undulatus* showed the highest survival rates in every immature stage at 20 °C and the lowest at 30 °C (Fig. 1C; Table 1). At 25 °C, the survival rates showed intermediate values although closer to those of 20 °C. Nevertheless, log-rank tests showed significant differences ($p < 0.05$) in the survival distributions between every constant temperature.

Duration of development

For the three species, the relative duration of each immature developmental stage (egg, larva and pupa) remained fairly constant among temperatures (Table 2). The egg stage was the shortest stage, lasting less than 10 % of the total duration of development, followed by the pupal stage, which lasted about 15–25 % of the total duration of the development (Table 1). The larval stage was the longest developmental stage lasting about 65–75 % of the total duration of the development, considering both the feeding and postfeeding larval phases (Table 2).

As expected, the duration of development was significantly influenced by temperature (Tables 3–5). In general, the duration of the development decreased with an increasing temperature (Fig. 2A–C); this was certainly the case for the egg stage in the three species (Fig. 2A). However, for both larval and pupal stages, the total duration time slightly increased at 35 °C in comparison to 30 °C in *D. maculatus* and *D. frischii*, and at 30 °C in comparison to 25 °C in *D. undulatus* (no oviposition occurred at 35 °C in this species; Fig. 2B–C). The same pattern was consequently observed in the total development duration time (i.e. the time from oviposition to adult emergence), ranging

from 78.82 days at 20 °C to 26.8 days at 30 °C but increasing to 30.7 days at 35 °C in *D. maculatus* (Table 3), from 77.1 days at 20 °C to 31.4 days at 30 °C but increasing to 38.6 days in *D. frischii* (Table 4), and from 60.1 days at 20 °C to 34.7 days at 25 °C but increasing to 35.4 ~~days~~^{°C} in *D. undulatus* (Table 5). With a few exceptions between close temperatures, significant differences were found within the three species in the developmental times between constant temperatures, for each of the different developmental milestones (Table 3–5): egg hatching, larval ecdyses, postfeeding larval phase, pupation and adult emergence.

It is remarkable that an inconsistent number of larval instars per individual were observed during this study across different constant temperatures in the three species (Fig. 3A–C), with the exception of *D. frischii* at 25 °C, where 100 % of the individuals showed five larval instars (Fig. 3B). In the remaining cases, the three species showed a variable number of larval instars per individual: from a minimum of five up to a maximum of ten instars in *D. maculatus* (Fig. 3A); from a minimum of five up to a maximum of nine instars in *D. frischii* (Fig. 3B); and from a minimum of four up to a maximum seven instars in *D. undulatus* (Fig. 3C). Although the largest variability in the number of instars per individual was observed at the highest constant temperature allowing the completion of the development in each species (35 °C in *D. maculatus* and *D. frischii*; 30 °C in *D. undulatus*; Fig. 3), no clear relationship between temperature and number of instars could be determined. Thus, the highest number of larval instars per individual was observed at 35 °C in *D. frischii* (up to nine instars; Fig. 3B) and at 30 °C in *D. undulatus* (up to seven instars; Fig. 3C), but at 15 °C in *D. maculatus* (up to ten instars; Fig. 2A). On the other hand, the additional ninth larval instar observed in *D. maculatus* and *D. frischii* at 35 °C, and the seventh larval instar observed in *D. undulatus* at 30 °C, were shown by only the 1.4 %, 1.96 % and 5.1 % of the individuals,

289 respectively (Fig. 3). Nevertheless, notwithstanding the final number of larval instars,
290 the relative duration of both feeding and postfeeding larval stages remained fairly
291 constant among different temperatures within each species (Table 2).

292 Isomorphen diagrams (Fig. 4A–C) show how the average time to reach each
293 developmental milestone gradually decreases (and, consequently, the gap between
294 curves gradually narrows) with increasing temperature, but slightly increases again
295 under the maximum constant temperature, as mentioned above. It must be borne in
296 mind the aforementioned variability in the number of larval instars per individual in the
297 three species (Fig. 3A–C), which was translated into an almost overlap between the
298 mean times of the last additional larval ecdysis and the onset of the postfeeding larval
299 phase in some cases (Fig. 4A–C). For example, it explains the fact that the observed
300 mean time for the ninth ecdysis in *D. maculatus* at 15 °C was slightly higher than the
301 mean time for the onset of the postfeeding larval phase, as the latter was calculated
302 considering individuals showing a final number of either nine or ten larval instars (Fig.
303 3A).

304 Discussion

305 *Survival*

306 Richardson and Goff (2001) determined a positive correlation between survival and
307 temperature in *D. maculatus*, with significantly higher percentages of individuals
308 reaching adulthood at 30 °C and 35 °C within the same temperature range as the one
309 used in our study. However, other studies on *D. maculatus* observed the highest survival
310 at intermediate temperatures (20–30 °C) over similar temperature ranges (Raspi and
311 Antonelli 1996, Zanetti et al. 2016b). Similar results were reported by Amos (1968) on
312 *D. frischii*. Our results agree with the latter studies, suggesting that the optimal

temperatures for both *D. maculatus* and *D. frischii* are among the intermediate temperatures (20–30 °C) over the range considered (Fig. 1A–B). Moreover, the survival rates from egg to adult observed in the present study (Table 1) also agree with the rates observed by Fontenot et al. (2015) using different protein-rich commercial diets. The upper temperature threshold for both *D. maculatus* and *D. frischii* might be not much above 35 °C, taking into account the rapid drop in survival in these two species at 35 °C (Fig. 1A–B). Indeed, Raspi and Antonelli (1996) observed a mortality of 100 % at both 38 °C and 40 °C in *D. maculatus*. Similarly, Howe (1953) and Amos (1968) observed a mortality of 100 % at 40 °C in *D. frischii* — Amos (1968) observed some eggs hatching at this constant temperature but all the individuals died during the first or second larval instar. On the other hand, *D. undulatus* appears to have a more restricted range of optimal temperatures, with low survival rates at 30 °C (Fig. 1C), whereas its upper temperature threshold must be below 35 °C, at least for eggs development in adults, as no oviposition occurred at this temperature in the present study (Table 1).

Regarding the minimum temperature threshold, our results suggest that it might be between 15 °C and 20 °C-degrees for the three species, as *D. maculatus* did not complete its development at 15 °C and the other two species did not even oviposit under this temperature (Table 1). Raspi and Antonelli (1996) managed to rear *D. maculatus* individuals from egg to adult at 18 °C although with a high mortality (89 %), whereas at 15 °C all their individuals died during the larval stage. Similar results were obtained by Richardson and Goff (2001) and Amos (1968) in *D. maculatus* and *D. frischii*, respectively, with egg hatching occurring at 15 °C but larvae failing to reach pupation. Interestingly, Amos (1968) recorded high survival rates at the egg stage in *D. frischii* at 15 °C, similarly to our experiments with *D. maculatus*, where the survival rate was clearly high at 15 °C but dropped drastically during the larval stage, with a low

percentage reaching pupation and no individuals completing their development (Fig. 1A). By contrast, also in *D. maculatus* at 15 °C, Zanetti et al. (2016b) observed a percentage of individuals surviving from egg to adult of only 55 %. It would be interesting to explore if that different minimum temperature threshold is due to variations in rearing protocols or to potential geographic differences between Neotropical (Zanetti et al. 2016b) and Holarctic (Raspi and Antonelli 1996, Richardson and Goff 2001; also this study) populations of *D. maculatus*.

High larval densities and the absence of refuge sites for pupation can favour the occurrence of cannibalism by larvae on both larvae and pupae (Howe 1953, Archer and Elgar 1998). Although it is beyond the scope of the current study to discuss the occurrence of cannibalism, it seems that providing a refuge for pupation certainly minimizes the cannibalism by larvae on pupae in laboratory cultures (Fontenot et al. 2015). From a forensic perspective, this emphasizes the need for studies on the pupation behaviour of dermestids, which could result in tunnelling and damage on human bones as they seek refuge sites (Huchet et al. 2013).

Duration of development

Overall, our results are in accordance with the developmental times reported in previous studies for both *D. maculatus* and *D. frischii*, with very similar times at the same constant temperatures (Hinton 1945, Howe 1953, Raspi and Antonelli 1996, Zanetti et al. 2016b). However, the times from egg to adult provided for *D. maculatus* by Richardson and Goff (2001) are slightly longer, ~6–10 days more for every constant temperature. Interestingly, Richardson and Goff (2001) reported that, although no larvae were able to reach pupation at 15 °C, some individuals remained alive at this temperature up to nine months (~270 days). This contrasts with our results, where some

362 *D. maculatus* larvae reached pupation at ~190 days and no larvae survived beyond that
363 time (Table 3), whereas both results contrast with the study of Zanetti et al. (2016b),
364 where *D. maculatus* completed its development (i.e. from egg to adult) in ~114 days at
365 15 °C. Previous studies have suggested that different diets may result in different larval
366 growth rates (Woodcock et al. 2013), but a complete study comparing the development
367 on different tissues (including skin) is still pending. Humidity has also been identified
368 as factor influencing larval development (Magni et al. 2015), but Richardson and Goff
369 (2001), Zanetti et al. (2016b) and the present study used rearing protocols with similar
370 relative humidity levels. Nonetheless, once again, although the potential effect of
371 different rearing protocols should not be discarded, it would be interesting to explore
372 potential differences among distinct geographical populations. On the other hand,
373 Richardson and Goff (2001) noted that the number of accumulated degree days (ADD)
374 required for development decreased with increasing temperature but at 35 °C the
375 number of ADD began to increase, which they suggested could be related to a stress
376 effect of high temperatures. This is in accordance with the slight increase in
377 developmental times observed at 35 °C in comparison to 30 °C in both *D. maculatus* and
378 *D. frischii* (Fig. 2A–B; Table 3–4), and with previous studies including 35 °C in their
379 experimental temperature range (e.g. Howe 1953, Raspi and Antonelli 1996).
380 Considering this, Richardson and Goff (2001) suggested that the optimal temperature
381 for *D. maculatus* development would be around 30 °C. Nevertheless, it must be
382 emphasized that, although developmental times are shorter at 30 °C for every stage in *D.*
383 *maculatus* and *D. frischii* (Fig. 4A–B; Table 3–4), survival was found to be significantly
384 higher at 25 °C (Fig. 1A–B). A different optimal temperature for survival and for shorter
385 developmental times had been previously reported not only in *D. maculatus* and *D.*
386 *frischii* (e.g. Howe 1953, Amos 1968, Raspi and Antonelli 1996, Zanetti et al. 2016b),

387 but also in necrophagous species of other Coleoptera families (e.g. Midgley and Villet
 388 2009). On the other hand, optimal environmental temperature conditions can favour the
 389 occurrence of high larval densities feeding on a cadaver (Schroeder et al. 2002). In those
 390 cases, larval-mass heating effects and their potential impact on larval growth rates
 391 (Charabidze et al. 2011, Johnson and Wallman 2014) cannot be discarded. Richardson
 392 and Goff (2001) suggested that the total developmental time from egg to adult may
 393 decrease with increasing larval density; however, a parallel drop in survival was also
 394 observed. Further studies should delve into the potential larval mass effect on survival
 395 and developmental rates and its forensic implications.

396 Regarding the third species considered in this study, *D. undulatus* showed
 397 interesting differences with *D. maculatus* and *D. frischii*. *D. undulatus* appears to
 398 develop within a more restricted temperature range as it did not develop at 35 °C.
 399 Martín-Vega and Baz (2012) found both *D. frischii* and *D. undulatus* across different
 400 types of wild habitats in central Spain, although *D. frischii* showed higher abundances
 401 in those localities with higher average temperatures. In the most arid environments of
 402 southeastern Spain, Sánchez Piñero (1997) reported high abundances of *D. frischii* and
 403 also the occurrence of *D. maculatus*, but not *D. undulatus*. ~~Nevertheless, i~~Interestingly,
 404 *D. undulatus* showed shorter developmental times at 25 °C (Fig. 4C; Table 5) but higher
 405 survival at ~~32~~20 °C (Fig. 1C) in the present study, ~~whereas just the opposite to~~ both *D.*
 406 *maculatus* and *D. frischii* showed shorter developmental times at 30 °C (Fig. 4C; Table
 407 5) but higher survival at 25 °C (Figs. 1A–B, 4A–B; Table 3–4). Perhaps even more
 408 interestingly, the current study reveals that *D. undulatus* is able to pupate after four
 409 larval instars, in contrast to the minimum of five larval instars apparently required by *D.*
 410 *maculatus* and *D. frischii* (Fig. 3A–C). The completion of a minimum of four larval
 411 instars before pupation had been previously reported in the species *Dermestes lardarius*

412 Linnaeus (Magni et al. 2015). Regrettably, there are no previous studies on the
413 development of *D. undulatus* to compare with our data.

414 The variability in the number of larval instars had been previously reported in
415 several species of dermestids, including both *D. maculatus* and *D. frischii* (e.g.
416 Keyenberg 1928, Gay 1938, Howe 1953, Hinton 1945, Fleming and Jacob 1986, Esperk
417 et al. 2007, Zanetti et al. 2016a, 2016b). The intraspecific variability in the number of
418 larval instars occurs widely across insect taxa, in both hemimetabolous and
419 holometabolous insects (Esperk et al. 2007). Several factors influencing the number of
420 larval instars in different insect groups have been identified: temperature, photoperiod,
421 food quality and quantity, humidity, population density, inheritance and sex, among
422 others (Esperk et al. 2007). However, several studies have revealed that under similar
423 conditions *Dermestes* larvae can show an inconsistent number of instars and that,
424 specifically, the variation is not correlated to temperature, humidity, sex or diet (Gay
425 1938, Fleming and Jacob 1986, Zanetti et al. 2016a, 2016b). This is in accordance with
426 our results, where the three species showed variability in the number of instars in every
427 temperature, with the sole exception of *D. frischii* at 25 °C (Fig. 3A–C). Zanetti et al.
428 (2016a) suggested that inherited genetic factors might affect the larval instar number, as
429 it has been reported in other insect species (Esperk et al. 2007). Nonetheless, further
430 research on this issue is needed to fully understand this phenomenon and its
431 implications in forensic research.

432 The intraspecific variability in the number of larval instars and its inconstancy
433 under similar conditions raises some questions about the actual applicability of
434 dermestids in forensic investigations. Future studies will explore in detail the variability
435 in the number of larval instars and its effect in the duration of the development by
436 monitoring specimens individually during development. Nevertheless, the optimal use

437 of isomorphen diagrams (Fig. 4A–C) would require a reliable instar classification if the
438 entomological evidence consists of dermestid larvae. Recent studies (e.g. Frątczak and
439 Matuszewski 2016) have developed methods to reliably classify carrion beetle larvae
440 according to instar in forensic practice on the basis of morphometric characters. It
441 would be interesting to test if similar classifiers can also be developed for reliable instar
442 classifications in the case of dermestids regardless of the intraspecific variability in the
443 number of instars. In future studies, morphometric measurements during the larval stage
444 and the use of more constant temperatures will allow the development of isomegalen
445 diagrams and thermal summation models, in order to achieve statistically robust
446 estimations of minPMI using dermestid beetles. In general, necrophagous beetles show
447 significantly longer developmental times than flies (Midgley and Villet 2009) and,
448 among them, dermestids show longer developmental times than other forensically
449 relevant families (e.g. Midgley and Villet 2009, Wang et al. 2016), making them
450 potentially very useful in long PMI case investigations. Moreover, the different optimal
451 temperatures and developmental rates observed in *D. undulatus* in comparison to both
452 *D. frischii* and *D. maculatus*, suggest that these species may supply complementary
453 information as the coexistence of both *D. frischii* and *D. undulatus* on human cadavers
454 is not infrequent (Charabidze et al. 2013). The present data thus provide a baseline for
455 further advances in the potential use of these beetles as invaluable tools in long PMI
456 cases. In those situations, several generations may develop on a cadaver, so future
457 studies should also investigate the fecundity and the time required by females to mature
458 and produce eggs.

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580 **Table legends**

581 **Table 1.** Cumulative survival rates of *Dermestes maculatus*, *Dermestes frischii* and
582 *Dermestes undulatus* reared at different constant temperatures

583 **Table 2.** Duration of each developmental stage as a percentage of the total time from
584 oviposition to adult emergence in *Dermestes maculatus*, *Dermestes frischii* and
585 *Dermestes undulatus* reared at different constant temperatures

586 **Table 3.** Mean ($\pm$ SD) development duration (days) of *Dermestes maculatus* at five
587 constant temperatures

588 **Table 4.** Mean ($\pm$ SD) development duration (days) of *Dermestes frischii* at four
589 constant temperatures

590 **Table 5.** Mean ($\pm$ SD) development duration (days) of *Dermestes undulatus* at three
591 constant temperatures.

592

593

594 **Figure legends**

595 **Fig. 1.** Modified Kaplan-Meier survival curves at different constant temperatures. **(A)**
596 *Dermestes maculatus*. **(B)** *Dermestes frischii*. **(C)** *Dermestes undulatus*.

597 **Fig. 2.** Mean $\pm$ SD time (days) to complete each immature stage at different constant
598 temperatures in *Dermestes maculatus*, *Dermestes frischii* and *Dermestes undulatus*. **(A)**
599 Egg stage. **(B)** Larval stage. **(C)** Pupal stage.

600 **Fig. 3.** Variability in the final number of larval instars at different constant
601 temperatures, as the percentage of individuals reaching the postfeeding larval stage. **(A)**
602 *Dermestes maculatus*. **(B)** *Dermestes frischii*. **(C)** *Dermestes undulatus*.

603 **Fig. 4.** Isomorphen diagrams plotting the duration (mean $\pm$ SD) of each developmental
604 milestone against temperature. **(A)** *Dermestes maculatus*. **(B)** *Dermestes frischii*. **(C)**
605 *Dermestes undulatus*.

Table 1. Cumulative survival rates of *Dermestes maculatus*, *Dermestes frischii* and *Dermestes undulatus* reared at different constant temperatures

Species	Temperature	Initial number of eggs	Survival rate of egg stage	Accumulated survival rate at larval stage	Accumulated survival rate at pupal stage	Number of individuals surviving to adult stage
<i>D. maculatus</i>	15 °C	360	0.94	0.01	0	0
	20 °C	330	0.68	0.56	0.52	171
	25 °C	240	0.79	0.72	0.72	172
	30 °C	240	0.68	0.59	0.58	139
	35 °C	460	0.46	0.27	0.25	115
<i>D. frischii</i>	20 °C	200	0.80	0.55	0.52	104
	25 °C	240	0.83	0.72	0.71	171
	30 °C	220	0.68	0.55	0.54	119
	35 °C	480	0.57	0.23	0.20	95
<i>D. undulatus</i>	20 °C	300	0.78	0.58	0.49	147
	25 °C	430	0.65	0.48	0.44	190
	30 °C	700	0.29	0.17	0.16	110

No oviposition occurred at 15 °C for *D. frischii*, and at 15 °C and 35 °C for *D. undulatus*.

Table 2. Duration of each developmental stage as a percentage of the total time from oviposition to adult emergence in *Dermestes maculatus*, *Dermestes frischii* and *Dermestes undulatus* reared at different constant temperatures

	Developmental stage	20 °C	25 °C	30 °C	35 °C
<i>D. maculatus</i>	Egg	7.73	6.79	7.46	5.35
	Feeding larva	65.54	60.33	60.12	69.53
	Postfeeding larva	10.18	12.89	12.01	9.43
	Pupa	16.55	19.99	20.41	15.69
<i>D. frischii</i>	Egg	6.48	7.49	6.40	6.37
	Feeding larva	63.23	55.50	61.49	66.26
	Postfeeding larva	11.67	14.62	14.53	13.22
	Pupa	18.62	22.39	17.58	14.15
<i>D. undulatus</i>	Egg	8.89	9.39	6.41	-
	Feeding larva	55.45	52.06	58.67	-
	Postfeeding larva	13.00	11.70	16.64	-
	Pupa	22.66	23.86	18.28	-

No oviposition occurred at 35 °C for *D. undulatus*. Oviposition at 15 °C only occurred in *D. maculatus*, but no individual reached pupation

Table 3. Mean ($\pm$ SD) development duration (days) of *Dermestes maculatus* at five constant temperatures

Developmental milestone	15 °C (mean $\pm$ SD)	20 °C (mean $\pm$ SD)	25 °C (mean $\pm$ SD)	30 °C (mean $\pm$ SD)	35 °C (mean $\pm$ SD)	F	p
Egg hatching	13 $\pm$ 0.1	6.1 $\pm$ 0.5	2.9 $\pm$ 0.3	2 $\pm$ 0.01	1.6 $\pm$ 0.5	52073.98	< 0.001
	N = 338	N = 226	N = 190	N = 164	N = 214		
1st ecdysis	32.2 $\pm$ 3.9	12.3 $\pm$ 1.2	6.2 $\pm$ 0.5	4 $\pm$ 0.5 *	4.1 $\pm$ 0.9 *	7109.23	< 0.001
	N = 173	N = 211	N = 185	N = 159	N = 169		
2nd ecdysis	50.2 $\pm$ 6	18.2 $\pm$ 1.4	9.2 $\pm$ 0.6	6 $\pm$ 0.6	6.7 $\pm$ 1.2	7291.39	< 0.001
	N = 145	N = 201	N = 183	N = 157	N = 164		
3rd ecdysis	67.9 $\pm$ 8.1	23.9 $\pm$ 1.5	12.2 $\pm$ 0.6	7.8 $\pm$ 0.7	9.2 $\pm$ 1.5	7584.06	< 0.001
	N = 135	N = 196	N = 182	N = 155	N = 162		
4th ecdysis	84 $\pm$ 8.7	29.4 $\pm$ 1.6	15.2 $\pm$ 0.7	10 $\pm$ 0.7	11.5 $\pm$ 1.6	9998.18	< 0.001
	N = 130	N = 195	N = 182	N = 154	N = 155		
5th ecdysis	99.3 $\pm$ 9.3	34.9 $\pm$ 1.6	18.7 $\pm$ 0.9	12.6 $\pm$ 0.8	13.7 $\pm$ 1.5	10124.89	< 0.001
	N = 126	N = 192	N = 142	N = 98	N = 148		
6th ecdysis	116.3 $\pm$ 9.2	41.1 $\pm$ 1.8	-	15.8 $\pm$ 1.6 *	16.2 $\pm$ 1.6 *	7702.11	< 0.001
	N = 118	N = 95		N = 9	N = 140		
7th ecdysis	133.3 $\pm$ 9.1	-	-	-	19.9 $\pm$ 1.5	-	-
	N = 113	-			N = 73		
8th ecdysis	151.8 $\pm$ 9.9	-	-	-	23.5 $\pm$ 3.5	-	-
	N = 105				N = 2		
9th ecdysis	170.77 $\pm$ 11.36	-	-	-	-	-	-
	N = 47						
Postfeeding larva	169 $\pm$ 33.7	57.7 $\pm$ 5.8	28.6 $\pm$ 3	18.1 $\pm$ 2.4	23 $\pm$ 3.3	2776.74	< 0.001
	N = 26	N = 189	N = 180	N = 153	N = 142		
Pupation	190.2 $\pm$ 11.69	65.7 $\pm$ 6.4	34.1 $\pm$ 2.9	21.3 $\pm$ 2.5	25.9 $\pm$ 3.3	3716.23	< 0.001
	N = 5	N = 185	N = 173	N = 142	N = 124		
Adult emergence	-	78.8 $\pm$ 6.4	42.6 $\pm$ 2.9	26.8 $\pm$ 2.6	30.7 $\pm$ 3.1	5123.42	< 0.001
		N = 171	N = 172	N = 139	N = 115		

Values within the same row having an asterisk (*) do not differ significantly from each other based on a one-way ANOVA + LSD test at $p < 0.05$.

Table 4. Mean ($\pm$ SD) development duration (days) of *Dermestes frischii* at four constant temperatures

Developmental milestone	20 °C (mean $\pm$ SD)	25 °C (mean $\pm$ SD)	30 °C (mean $\pm$ SD)	35 °C (mean $\pm$ SD)	F	<i>p</i>
Egg hatching	5 $\pm$ 0.5	2.7 $\pm$ 0.5	2 $\pm$ 0.1	1.5 $\pm$ 0.5	2217.24	< 0.001
	N = 160	N = 200	N = 153	N = 273		
1st ecdysis	10.1 $\pm$ 1	5.9 $\pm$ 0.5	4.2 $\pm$ 0.8	3.7 $\pm$ 0.9	2181.68	< 0.001
	N = 149	N = 197	N = 141	N = 254		
2nd ecdysis	15.3 $\pm$ 1.5	8.9 $\pm$ 0.5	6.9 $\pm$ 1	7.3 $\pm$ 1.4	1719.76	< 0.001
	N = 147	N = 195	N = 137	N = 219		
3rd ecdysis	20.3 $\pm$ 1.7	11.7 $\pm$ 0.6	9.1 $\pm$ 1.3	9.8 $\pm$ 1.7	2006.05	< 0.001
	N = 141	N = 190	N = 132	N = 213		
4th ecdysis	25.6 $\pm$ 2	15 $\pm$ 0.6	11.4 $\pm$ 1.4	12 $\pm$ 1.7	2890.4	< 0.001
	N = 139	N = 186	N = 128	N = 212		
5th ecdysis	32 $\pm$ 1.6	-	14.6 $\pm$ 1.7	14.1 $\pm$ 1.8	4162.34	< 0.001
	N = 109		N = 96	N = 209		
6th ecdysis	40.1 $\pm$ 1.8	-	18.4 $\pm$ 2.6	17 $\pm$ 1.9	555.53	< 0.001
	N = 8		N = 21	N = 191		
7th ecdysis	-	-	-	20.5 $\pm$ 2.1	-	-
				N = 65		
8th ecdysis	-	-	-	21.25 $\pm$ 2.5	-	-
				N = 4		
Postfeeding larva	53.8 $\pm$ 5.6	22.7 $\pm$ 1.3	21.3 $\pm$ 3.0	28 $\pm$ 4	2307.89	< 0.001
	N = 135	N = 182	N = 126	N = 199		
Pupation	62.6 $\pm$ 5.9	27.9 $\pm$ 1.8	25.9 $\pm$ 3.7	33.1 $\pm$ 5.1	1967.77	< 0.001
	N = 110	N = 173	N = 124	N = 109		
Adult emergence	77.1 $\pm$ 5.8	36.0 $\pm$ 1.9	31.4 $\pm$ 3.5	38.6 $\pm$ 4.9	3011.91	< 0.001
	N = 104	N = 171	N = 119	N = 95		

Values within the same row having an asterisk (*) do not differ significantly from each other based on a one-way ANOVA + LSD test at $p < 0.05$. No oviposition occurred at 15 °C

Table 5. Mean ($\pm$ SD) development duration (days) of *Dermestes undulatus* at three constant temperatures.

Developmental milestone	20 °C (mean $\pm$ SD)	25 °C (mean $\pm$ SD)	30 °C (mean $\pm$ SD)	F	<i>p</i>
Egg hatching	5.3 $\pm$ 0.62	3.3 $\pm$ 0.56	2.3 $\pm$ 0.44	182,021	< 0.001
	N = 234	N = 281	N = 203		
1st ecdysis	9.8 $\pm$ 1.1	6.1 $\pm$ 0.6	4.5 $\pm$ 0.9	2108.19	< 0.001
	N = 213	N = 264	N = 188		
2nd ecdysis	14.3 $\pm$ 1.3	8.8 $\pm$ 0.8	7.3 $\pm$ 1.3	2080.67	< 0.001
	N = 204	N = 261	N = 177		
3rd ecdysis	19.3 $\pm$ 1.5	12 $\pm$ 0.8	10.4 $\pm$ 1.9	2229.94	< 0.001
	N = 201	N = 260	N = 176		
4th ecdysis	24.9 $\pm$ 1.5	15.4 $\pm$ 0.9	13.9 $\pm$ 2.3	1323.01	< 0.001
	N = 97	N = 81	N = 159		
5th ecdysis	34.1 $\pm$ 1.1	-	18.7 $\pm$ 2.2	299.36	< 0.001
	N = 8		N = 64		
6th ecdysis	-	-	23.3 $\pm$ 1.19	-	-
			N = 8		
Postfeeding larva	38.6 $\pm$ 4.5	22.3 $\pm$ 1.6	23 $\pm$ 4.1	1511.49	< 0.001
	N = 184	N = 256	N = 169		
Pupation	46.5 $\pm$ 5.8	26.4 $\pm$ 1.6	28.9 $\pm$ 3.9	1270.22	< 0.001
	N = 176	N = 206	N = 122		
Adult emergence	60.1 $\pm$ 5.4	34.7 $\pm$ 1.6*	35.4 $\pm$ 3.8*	2215.11	< 0.001
	N = 147	N = 190	N = 110		

Values within the same row having an asterisk (*) do not differ significantly from each other based on a one-way ANOVA + LSD test at $p < 0.05$. Only those milestones with more than 10 observations ($N > 10$) were subjected to analyses. No oviposition occurred at 15 °C and 35 °C

A

B

C