Desiccation resistance: effect of cuticular hydrocarbons and water content in *Drosophila melanogaster* adults

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Background. The insect cuticle covers the whole body and all appendages and has bi-directionnal selective permeability: it protects against environmental stress and pathogen infection and also helps to reduce water loss. The adult cuticle is often associated with a superficial layer of fatty acid-derived molecules such as waxes and long chain hydrocarbons that prevent rapid dehydration. The waterproofing properties of cuticular hydrocarbons (CHs) depend on their chain length and desaturation number. *Drosophila* CH biosynthesis involves an enzymatic pathway including several elongase and desaturase enzymes.

Methods. The link between desiccation resistance and CH profile remains unclear, so we tested (1) experimentally selected desiccation-resistant lines, (2) transgenic flies with altered desaturase expression and (3) natural and laboratory-induced CH variants. We also explored the possible relationship between desiccation resistance, relative water content and fecundity in females.

Results. We found that increased desiccation resistance is linked with the increased proportion of desaturated CHs, but not with their total amount. Experimentally-induced desiccation resistance and CH variation both remained stable after many generations without selection. Conversely, flies with a higher water content and a lower proportion of desaturated CHs showed reduced desiccation resistance. This was also the case in flies with defective desaturase expression in the fat body.

Discussion. We conclude that rapidly acquired desiccation resistance, depending on both CH profile and water content, can remain stable without selection in a humid environment. These three phenotypes, which might be expected to show a simple relationship, turn out to have complex physiological and genetic links.

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Abstract

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Results. We found that increased desiccation resistance is linked with the increased proportion of desaturated CHs, but not with their total amount. Experimentally-induced desiccation resistance and CH variation both remained stable after many generations without selection. Conversely, flies with a higher water content and a lower proportion of desaturated CHs showed reduced desiccation resistance. This was also the case in flies with defective desaturase expression in the fat body.

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Introduction

The resistant yet flexible outer layers of the insect exoskeleton, generally known as the cuticle, are involved in many vital functions and possess great structural, mechanical, and chemical complexity (Locke 1966). The cuticle constrains the animal's shape, serves as an attachment point for muscles, and is the basis for a variety of specialized organs such as sensory hairs, respiratory trachea, feeding and copulatory structures (Demerec 1950). It also provides protection against physical and chemical environmental stressors such as desiccation. The cuticle not only limits desiccation (Wigglesworth 1945), it also permits selective exchanges between the organism and the outer world (Moussian 2010).

The soft and unpigmented cuticle of young adult insects undergoes a rapid process of melanization (Andersen 2010) during which stabilized lipids, produced by the oenocytes and fat body, combine with proteins and stiffen the cuticle before sclerotization, which constitutes the final developmental phase (Wigglesworth 1988). Most of our knowledge of the biochemical and genetic parthways involved in these processes comes from *Drosophila*, where they depend on the activity of the neurohormone, bursicon (Honegger et al. 2008), coupled with its rickets receptor (*rk*; (Harwood et al. 2013), as well as on lipid production. A large part of the lipids contained within or secreted onto the epicuticle are long-chain hydrocarbons, generally known as cuticular hydrocarbons (CHs), the production of which depends on the *Cyp4g1* gene (Qiu et al. 2012), and on several genes coding for an elongase (Chertemps et al. 2007; Howard & Blomquist 2005) and for substrate-specific lipid desaturases (*desat1*, *desat2;* Bousquet et al. 2012; Dallerac et al. 2000; Flaven-Pouchon et al. 2016). In *Anopheles gambiae*, a *Cyp4g1* ortholog, associated with insecticide resistance, catalyzes CH production (Balabanidou et al. 2016).

The waxes, lipids and CHs found on the epicuticle appear to have several functions. They act as a barrier against pathogenic microorganisms and insecticides (Balabanidou et al. 2016; da Silva et al. 2015; Gołębiowski et al. 2008), they reduce the heat load by reflecting solar radiation and they deter predators (Eigenbrode & Espelie 2003; Hadley 1994). They also help to limit transpiration (Gibbs 1998; Hadley 1994). Although the adult CH profile shows seasonal and environmental variation (Gibbs & Pomonis 1995; Howard & Blomquist 2005; Savarit & Ferveur 2002; Toolson & Hadley 1979) some of its components serve as sex- and/or species-specific pheromones (Ferveur 2005; Jallon 1984; Savarit et al. 1999). The *desat1* gene affects

78   both the production and the reception of *Drosophila melanogaster* sex pheromones, while it has

79   been suggested that *desat2* is involved in speciation (Fang et al. 2002). *desat1* is also expressed

80   in tissues involved in water balance, in particular the Malpighian tubules (Bousquet & Ferveur

81   2012; Bousquet et al. 2012; Dow 2009).

82   Several studies of *D. melanogaster* have used artificial selection to enhance desiccation

83   resistance by altering water loss, osmotic regulation and CH content. There is a clear link

84   between desiccation resistance and water retention, dry mass and ionic content (Folk & Bradley

85   2004; Gibbs et al. 1997), but the role of CH profile in preventing water loss in such selection

86   experiments remains unclear (Gibbs et al. 1997; Gibbs & Rajpurohit 2010). Selected lines

87   showed no differences in the quantity of CHs, but sex differences in CH chain length were linked

88   to variation in resistance – faced with a desiccation challenge, females survived longer than

89   males (Gibbs et al. 1997; Qiu et al. 2012).

90   To reveal the link between desiccation resistance and CH profile in *D.melanogaster*

91   flies, we carried out a three-part investigation using (*i*) experimentally selected lines, (*ii*) *desat1*

92   transgenics and (*iii*) a *desat2* natural variant and *rk* mutants.

Materials and Methods

Flies

95   *D. melanogaster* were raised on yeast / cornmeal / agar medium and kept at 24±0.5°

96   with 65±5% humidity on a 12 L : 12 D cycle (subjective day=8:00am to 8:00pm). Flies were

97   isolated under light CO_2 anaesthesia either 0–4h (for virgin females) or less than 24h (for all

98   other flies) after eclosion. Male and females were held separately in fresh glass food vials in

99   groups of 10 flies until the day of the experiment (4-5 days old, unless specified). Same-sex flies

100 were then transferred to an empty glass vial to obtain groups of 50±5 individuals.

101 We tested two wild-type stocks, Dijon2000 (Di2) and Zimbabwe30 (Z30), which were

102 collected in France and Zimbabwe respectively (Grillet et al. 2012; Houot et al. 2010). We also

103 used the Di2/*w^{1118}* line (Di2/w), derived from the Di2 strain and introgressed into the genome of

104 the *w^{1118}* strain over five repeated backcross generations.

105   We used several *desat1*-Gal4 transgenic drivers built with putative *desat1* regulatory

106   regions (PRRs) fused with Gal4. PRR(RA)-Gal4 is expressed in the wing margin and in the brain

107   as well as in other tissues; PRR(RC)-Gal4 is exclusively expressed in the fat body; PRR(RE)-

108   Gal4 is expressed in the oenocytes; PRR(RB) is expressed in the Malpighi tubules and midgut;

109   PRR(RD) is expressed in a vaginal moon-shaped structure in the female and in the male

110   ejaculatory bulb; PRR(RDiO)-Gal4 is expressed in neural tissues involved in the discrimination

111   of sex pheromones by male flies (Bousquet et al. 2012). We also used a Gal4 transgene

112   containing the complete *desat1* sequence fused to GAL4 (6908bp; *6908*-Gal4; (Bousquet et al.

113   2016)). Gal4 drivers were used to target the UAS-*desat1*-RNAi transgene (IR; VDRC #33338;

114   This UAS-RNAi line was chosen from three alternative lines for its clear effect on both the

115   hydrocarbon and behavioral phenotypes tested here; Bousquet et al. 2012; Houot et al. 2017);

116   this allowed us to down-regulate *desat1* expression in the tissues where Gal4 is expressed ([IR x

117   driver-Gal4]; (Houot et al. 2017)). We also controlled for the effect of each driver-Gal4 in the

118   Di2 background ([Di2 x driver-Gal4]). To homogenize genetic backgrounds, all UAS and Gal4

119   transgenes were isogenized in the genetic background of the Di2/w^{1118} strain prior to testing.

120   Two *rickets* alleles were used, *rk[1]* and *rk[4]*, which contain different mutations that

121   create premature stop codons, and are considered null alleles (Baker & Truman 2002). We

122   carried out reciprocal crosses between virgin adults carrying the *rk[1]* or the *rk[4]* mutation, both

123   of which were maintained in a heterozygous state by the [SM2, CyO] balancer chromosome.

124   From this cross, we obtained *rk[1]/rk[4]* heteroallelic mutant adults and *rk[1]*/CyO and

125   *rk[4]*/CyO control flies. To control the effect of the SM2, CyO balancer chromosome, we mated

126   Di2/w females with either *rk[1]*/CyO or *rk[4]*/CyO males and collected all CyO flies (i.e. those

127   not carrying the *rk* mutation; CyO/w).

128   **Experimental selection**

129   Groups of same-sex flies (n=50±5) of a given age were kept in glass vials (Fig 1A). Six

130   or seven vials were kept in a transparent plastic box, which was partly filled with silica gel to

131   reduce the humidity to 20%RH and was secured with transparent adhesive tape. We

132   simultaneously tested three or four boxes placed on a hot plate at 25.0±0.2° (StörkTronic,

133   Präzitherm, Düsseldorf, Germany). Every hour or two hours, we counted the number of dead

134   flies in each vial. In each box and in each experiment, we mixed vials containing the different

135   genotypes. For each generation, we performed two or three series of tests that were subsequently

136   pooled.

137   To experimentally select lines for desiccation resistance, the 1-2% flies showing the

138   longest life span during the desiccation challenge were placed in a fresh food vial and allowed to

139   mate, producing the next generation (Fig 1B). This procedure, begun with the wild-type Di2

140   strain, was carried out over six successive generations (F1-F6) and was then sporadically carried

141   out until F57. Of ten F1 lines, flies of the #7 line, which showed high desiccation resistance,

142   were used to create six F2 lines called 77S, which were subject to subsequent selection. Between

143   F2 and F6, we also tested flies resulting from the backcross between 77S females and Di2 males

144   (77S x Di2) and the reciprocal backcross. After F6, no selection was carried out on 77S lines

145   except to create six 77S-Sel lines which were subject to selection; each of these derived from

146   their respective 77S lines (e.g. 77S-Sel1 derived from the $77S_1$ line).

147   **Cuticular hydrocarbons**

148   5-day-old flies were frozen for 5 min at -20°C and their cuticular hydrocarbons then

149   individually extracted for 5 min at room temperature using 30µl of a mixture of hexane and

150   methylene chloride (50/50 by volume). The solution also contained 3.33 ng/µl of C26 (*n*-

151   hexacosane) and 3.33 ng/µl of C30 (*n*-triacontane) as internal standards. Cuticular hydrocarbons

152   were quantified by gas chromatography using a Varian CP3380 gas chromatograph fitted with a

153   flame ionization detector, a CP Sil 5CB column (25 m x 0.25 mm internal diameter; 0.1 µm film

154   thickness; Agilent), and a split–splitless injector (60 ml/min split-flow; valve opening 30 sec

155   after injection) with helium as carrier gas (50 cm/sec at 120°C). The temperature program began

156   at 120°C, ramping at 10°C/min to 140°C, then ramping at 2°C/min to 290°C, and holding for 10

157   min. Individual CH profiles were determined by integration of 46 peak areas in males and

158   females. This corresponded to all the peaks that could be consistently identified in all individuals

159   (Everaerts et al. 2010). The chemical identity of the peaks was checked using gas

160   chromatography – mass spectrometry equipped with a CP Sil 5CB column. The amount

161   (ng/insect) of each component was calculated on the basis of the data obtained from the internal

162   standards. We calculated the absolute amount of each group of CHs (alkene Q, alkane Q), the

163   relative amount of each CH group (alkene %, alkane %) from the overall CH total ($\sum$CH) and

164   their ratio (Desaturated:Linear = D:L). At least 10 flies were tested per condition.

165   **Water content**

166   Groups of 10 live anaesthetized females were weighed on a precision balance (±10μg;

167   Sartorius R160-P) to obtain their fresh weight. Each group of females was then kept for 24 hours

168   in an empty glass vial in a 37° dry incubator to allow complete desiccation. The dead, dry flies

169   were then weighed to obtain the dry weight. The relative level of water in each group was

170   estimated based on the fresh weight : dry weight ratio.

171 **Fecundity**

172   Females and males were kept in groups of ten pairs until they were 4 days old. Females
173 were then isolated (males were discarded) and the total number of male and female adult
174 progeny was noted for 7 days following the emergence of the first offspring. The sex ratio of the
175 progeny was also noted.

176 **Statistics**

177   All statistical analyses were performed using XLSTAT 2012 (Addinsoft 2012). For each
178 desiccation replicate, logistic regression was used to characterize the relationship between
179 mortality and time by estimating the lethal time 50 (LT50) and the regression slope (Robertson
180 & Preisler 1992). Thereafter, for each generation an inter-line comparison for these two
181 parameters was carried out either with a Kruskall-Wallis test with Conover-Iman multiple
182 pairwise comparisons ($p=0.05$, with a Bonferroni correction) or with a Mann-Whitney test, after
183 excluding extreme outliers using Tukey's method (Tukey 1977). The overall amount of CH
184 ($\sum$CH), the Desaturated:Linear ratio (D:L), the fresh weight : dry weight ratio, the total number
185 of adult progeny and the sex ratio were also compared using the same statistical tests.

186 **Results**

187 **Selection for desiccation resistance**

188   5-7 day old flies were placed in groups in a relatively dry environment (20% RH) at
189 25±0.2°C, and two measures of survivorship were taken: LT50 (time at which 50% flies were
190 dead) and lethality slope (the steepness of this curve indicates the proportion of flies dying per
191 hour; Fig 1A). Males were significantly more affected by the dry conditions than females, as
192 shown by a shorter LT50 and a steeper slope ($KW_{(5df)} = 31.74$, $p<10^{-4}$; Fig S1). Because of this
193 sex difference, we subsequently focused on female resistance. No significant differences were
194 found between virgin and mated females (see Materials and Methods). Survivors of this initial
195 desiccation challenge were allowed to mate, and a selection experiment on desiccation resistance
196 was then undertaken, with eight replicate lines. After only one generation, significant resistance
197 appeared in one line (#7; Fig 2); we therefore focused on this line, crossing #7 flies to create six
198 replicate selected lines, known as $77S_{0-5}$. These lines were then used in our experiment on

199   desiccation resistance; data from all six 77S lines were pooled at each generation for statistical

200   analysis.

201   From F3 to F6, 77S females showed significantly increased desiccation resistance as

202   compared to control Di2 flies, as shown by a higher LT50 ($p < 10^{-4} - 10^{-3}$) and a reduced slope (p:

203   0.001-0.037). In order to explore the genetic control of these characters, female and male 77S

204   flies were separately backcrossed to control Di2 flies, and their desiccation resistance was

205   measured. Although both the LT50 and slope of the offspring of the [77S f x Di2 m] backcross

206   were intermediate between control and 77S lines, flies produced by the reciprocal backcross [Di2

207   f x 77S m] were not significantly different from control (p=ns). The effect of selection on male

208   flies was much less, if any (Fig S2). These data suggest that the character(s) that have been

209   selected for in the 77S flies are primarily transmitted through female flies.

210   After F6, systematic selection of the 77S lines was relaxed (Fig 1B). Over 52

211   subsequent generations, desiccation resistance was sporadically tested in the $77S_{0-5}$ lines; we also

212   re-selected females from each 77S line for one or two generations prior to these desiccation

213   resistance tests and tested their progeny (77S-Sel; Fig 3). Compared to control females, 77S

214   females showed a significantly increased LT50 (median value ranges: 14.83-21.67 h and 18.77-

215   24.45 h, respectively) and/or a shallower slope (median value ranges: 9-16 and 5-9 %

216   lethalithy/h, respectively) that showed little consistent variation over time. Reintroduction of

217   selection in the 77S-Sel lines had no effect – these flies were not significantly different from 77S

218   flies that were reared under relaxed selection, indicating that the character(s) isolated in the

219   selection procedure are at fixation in these lines.

220   Effects on associated characters

221   Cuticular hydrocarbons (CH) have regularly been implicated in the evolution of

222   desiccation resistance; we therefore measured CH profiles in F7-F9 $77S_{1-5}$ females and in F8

223   males, and sporadically thereafter between F18 and F59. Beside the absolute CH amount ($\sum$CH),

224   we also determined the absolute (Q) and relative amounts (%) of desaturated CHs (alkenes) and

225   of linear saturated CHs (alkanes) and their ratio (Desaturated:Linear = D:L). Although we

226   observed both interline and intergenerational differences in the $77S_{0-5}$ flies (Fig 4), all F9 females

227   showed increased levels of alkene Q and most showed an increased alkene % ($77S_4$ was an

228   exception). Compared to control Di2 females, most lines showed higher $\sum$CH and D:L (Fig S3 –

229    $77S_0$ was an exception). At F18, following 12 generations of relaxed selection, only $77S_1$ and

230    $77S_2$ females showed increased D:L, while at F19 only $77S_2$ females showed increased D:L (Fig

231   5).

232   At F55, we tested $77S_1$-$77S_5$ flies (the $77S_0$ line was lost between F35 and F55), all of

233   which showed a significantly increased D:L, due to their higher alkene levels (Q and %) and

234   lower alkene Q (Figs 5 & S4). In all 77S lines, $\sum$CH was significantly higher than in Di2

235   females. At F57, 77S females were compared with females that had been re-selected at F55; 77S-

236   Sel – Fig 5). D:L increased in three 77S lines ($77S_1$-$77S_3$), but not in 77S-Sel lines. All 77S lines

237   showed increased $\sum$CH, while only one of the 77S-Sel lines showed such an effect. These

238   differences between 77S and 77S-Sel lines contrast with the results of the desiccation resistance

239   experiments, where there were no significant differences between these sets of lines, and suggest

240   that CH composition and desiccation resistance are not identical.

241   In order to explore the link between CH composition and desiccation resistance, we

242   measured the fresh and dry weight of flies (either freshly killed, or desiccated, respectively) from

243   these lines at F19 and F57/59. Fresh and dry weight can be considered as indirect measures of

244   cuticular surface and their ratio reflects the water retention ability of a particular strain. In F19

245   females, the Fresh : Dry weight ratio was significantly higher in lines $77S_1$-$77S_3$ than in Di2 flies

246   (Fig 6A). However in F57/F59 females, no difference was detected between Di2 and 77S

247   females (Fig 6B).

248   Finally, to confirm that we had not inadvertently selected for changes in sex ratio or

249   number of eggs laid by the 77S females, we counted the total number of adult progeny left by

250   single 77S, 77S-Sel and control females, and calculated their sex-ratio (female : male). There

251   were no overall differences between these groups (Fig S5). Only $77S_3$ females showed a

252   significant variation by producing more progeny than Di2 females (116 and 66, respectively) and

253   more males (64 and 32, respectively), but this did not affect the sex ratio.

Genetic control – *desat1*

255   A major gene involved in CH synthesis is *desat1,* which controls a vital desaturation

256   step in the hydrocarbon biosynthetic pathway. To explore the role of *desat1* in desiccation

257   resistance, we downregulated this gene in subsets of tissues and measured the consequences for

258 desiccation resistance and associated characters. Driver-Gal4 lines, made either with each

259 putative *desat1* regulatory region (PRR-Gal4) or the complete *desat1* regulatory region (6908-

260 Gal4), were used to drive the expression of the UAS-*desat1* RNAi transgenic reporter line (IR).

261 This allowed us to downregulate *desat1* expression either in Gal4-targeted tissues ([PRR-Gal4 x

262 IR]) or in all *desat1*-expressing tissues ([6908-Gal4 x IR]). Under the desiccation conditions used

263 in the selection experiment, [RC-Gal4 x IR] and [6908-Gal4 x IR] females showed reduced

264 desiccation resistance, as shown by a reduced LT50 (Fig 7A), but no difference in the rate at

265 which flies died (as measured by the slope; Fig 7B). This suggests that expression of *desat1*, in

266 particular in the fat body, is required for normal desiccation resistance. Control genotypes were

267 tested simultaneously and showed no effects (Figs 7C, 7D).

268   To confirm that manipulation of *desat1* had altered the CH profiles, the CH levels of [IR

269 x driver-Gal4] females in two generations (F_A and F_B) were compared to simultaneously-raised

270 controls (Di2; Di2/w; [Di2 x IR]; [driver-Gal4 x Di2]; Fig 8). Despite slight quantitative

271 variations between the two generations Figs 8A, 8B), F_A and F_B flies showed similar effects. In

272 particular, [RE-Gal4 x IR], [6908-Gal4 x IR] and to a lesser extent [RC-Gal4 x IR] flies

273 produced lower alkene levels (Q and %; Fig S6). [RE-Gal4 x IR] and [6908-Gal4 x IR] flies also

274 showed increased alkane levels (Q and %) and lower D:L compared to controls. Comparison of

275 Fresh : Dry weight revealed a significant increase in [RC-Gal4 x IR] females compared to

276 control lines (Fig 9), paralleling the effects on CH levels shown by this line. Although [6908-

277 Gal4 x IR] flies also appeared to show an increased ratio, this was not significant compared to

278 [Di2 x IR] controls. No differences in average fecundity were found when F_A and F_B flies were

279 compared with controls. (Fig S7).

Genetic control – natural and lab-induced variants

281   To further explore genetic control of the link between desiccation resistance and CH

282 profile, we studied naturally-occurring and laboratory-induced variants. In Zimbabwe (Z30)

283 flies, the female-specific alkene isomer 5,9-heptacosadiene (5,9HD) largely replaces 7,11HD

284 which is abundant in the other strains studied here (Flaven-Pouchon et al. 2016; Grillet et al.

285 2012). The desiccation resistance shown by these females was not significantly different from

286 Di2 flies. $rk^{1/4}$ mutant females produce high absolute amounts of CH ($\sum$CH, alkene and alkane)

287 but control-like alkene and alkane % and D:L; these mutants showed significantly lower

288   resistance than control females (lower LT50, but no slope variation) (Fig 10). However, *rk*/CyO

289   females showed significantly greater resistance than controls, with a highly increased LT50 and a

290   strongly decreased slope. Increased resistance in *rk*/CyO females was not related to the presence

291   of the CyO balancer –CyO/w females did not show the same effect. Finally, there was a slightly

292   increased resistance (LT50 and slope) shown by Di2/w females compared to Di2 females.

293   **Discussion**

294   Desiccation is a major physiological challenge faced by terrestrial arthropods, and both

295   experimental and theoretical arguments have been deployed to suggest that there is a

296   physiological and evolutionary link between desiccation resistance and the presence of certain

297   cuticular hydrocarbons, which act as a waxy outer layer restricting permeability (Gibbs 1998;

298   Qiu et al. 2012; Toolson & Hadley 1979). Using a range of experimentation approaches, we were

299   able to reveal genetic variation for desiccation resistance in our laboratory strain of *D.*

300   *melanogaster*, as shown by the appearance of a desiccation-resistant strain (#7) after one

301   generation, and then a rapid response to selection for desiccation resistance in multiple replicates

302   of that line which was maintained over dozens of generations despite the subsequent relaxation

303   of selection. Furthermore, we found correlated changes in cuticular hydrocarbon composition in

304   these replicate lines, in particular the overall amount of CH and the D:L ratio, together with

305   water retention ability. We suggest that these were causally linked – altered cuticular

306   hydrocarbon composition enabled the selected lines to retain water and resist desiccation. These

307   findings suggest that genetic variability for desiccation resistance exists even in laboratory lines

308   (the Di2 line was captured in 2000); variability in wild populations is presumably greater, and

309   may help explain the global success of this species.

310   The detection of significant desiccation resistance in line #7 after a single generation

311   enabled us to select for that character over subsequent generations, significantly altering both

312   LT50 and the lethality slope. From the outset, we found that females were significantly more

313   responsive to selection than males, which remained significantly more affected by the

314   desiccation challenge than females. This led us to focus on the female phenotype for the rest of

315   the experiment. Two possible explanations for this sex difference are that the genes we were

316   selecting were sex-limited, expressed only in females, linked to qualitative and quantitative sex

317   differences in CHs (see below), or that the effect was simply due to size – female *Drosophila* are
318   larger than males, with a lower surface area : volume ratio, which in turn would reduce the effect
319   of desiccation.

320   Although selection was relaxed after F6, the resistance character we had selected in the
321   #7 line was stable for over 50 generations, with no significant effect of the reintroduction of
322   selection, indicating that the character(s) involved were at fixation. The fact that these characters
323   were detected after one generation and became fixed after a further five generations of selection
324   suggests that our selection protocol and the available genetic variation in the parent population
325   were well matched and the strongest possible phenotypes were rapidly selected through the
326   available genetic variation.

327   At first glance it could be thought that the main effects we observed – increased
328   desiccation resistance, increased quantities of CH, and increased water content – were all
329   produced by an increase in the size of female flies, which would also increase the quantity of CH
330   by increasing the surface area. Such an increase in size would lead to a decreased surface area :
331   volume ratio, thereby increasing water retention at least at some time points. However, there
332   were no evident size increases in the selected females – certainly nothing approaching the
333   difference in size between males and females, which is visible to the naked eye – and we
334   consider that any potential microscopic changes in size would be unlikely to produce the
335   significant differences in survival we observed in the selected lines.

336   Insight into the link between the effects on CH levels, water content and desiccation
337   resistance can be found by inspecting the genetic mapping of desiccation-related phenotypes in
338   *desat1* transgenic flies. The selective knock-down of *desat1* expression in the female fat body
339   simultaneously induced a decreased desiccation resistance and a markedly increased water
340   content. Knocking down *desat1* expression in all relevant tissues (including the fat body)
341   induced very similar effects: the reduction of both desiccation resistance and the ratio of
342   desaturated : linear alkanes. However, selective targeting of *desat1* expression in oenocytes
343   strongly decreased the ratio of desaturated : linear alkanes, but did not affect desiccation
344   resistance or water content. These data suggest that the relative increase of internal water
345   content, but not the decreased ratio of desaturated : linear alkanes, is involved in reduced
346   desiccation resistance. We also found evidence that a higher amount of internal water may not

347 provide a decisive advantage in resisting desiccation: both *desat1* fat-body targeted genotypes –
348 [RC-Gal4 x IR] and [6908-Gal4 x IR] – showed a lower LT50 but no slope change, suggesting
349 that the earlier mean age of death shown by these transgenic flies may have been due to faster
350 water loss compared to flies with a relatively lower water content.

351   It is possible to interpret these complex data in terms of insect physiology. One of the
352 main functions of the fat body is to store and release energy, whereas oenocytes regulate lipid
353 metabolism (Ferveur 1997; Gutierrez et al. 2007). Energy is stored in adipocyte fat-body cells in
354 the form of fatty acids, glycerol, triglycerides, and glycogen, which is stored in a bulky hydrated
355 form (Arrese & Soulages 2010). The amount of glycogen, which is normally lower than that of
356 fat, can fluctuate depending on locomotor activity and environmental conditions (Lorenz &
357 Anand 2004). For example, if the insect is subject to freezing, low humidity or diapause, the fat
358 body accumulates fat and eliminates water through the activity-dependent regulation of
359 aquaporin water channel genes (Izumi et al. 2007; Liu et al. 2011; Sinclair & Renault 2010). We
360 propose that in our *desat1* fat body-targeted flies the capacity to store lipids was affected. This
361 would explain why, under our desiccation challenge, fat body knock-down transgenic flies lost
362 water more rapidly and died earlier than controls.

363   Further insight into the link between desiccation resistance and CH levels can be found
364 from examining the other mutants studied here. *rk^{1/4}* mutant flies showed a highly increased ∑CH
365 but a control D:L ratio of desaturated : linear alkanes. These flies died earlier than controls,
366 showing that a high CH level, even combined with a control-like D:L ratio, cannot compensate
367 for the underlying genetic defect. We suspect that the defective sclerotization process observed
368 in the *rk* mutant affects the permeability of the adult cuticle: this could enhance both CH
369 trafficking and water loss (Flaven-Pouchon et al. 2016; Gibbs 1998; Moussian 2010).
370 Conversely, *rk*/CyO females with a single *rk* mutant allele and a control-like CH profile showed
371 a lower LT50 and a shallower death slope than controls (this was not due to the CyO marker
372 associated with the SM2 balancer). The reduction in the RK gene product may lead to a slowing
373 down of water loss by changing the ultrastructure of the cuticle or of the spiracles (Chown 2002;
374 Moussian 2010). The clear sex differences in desiccation resistance may also be related to
375 differences in CH levels:" mature females express longer chain CHs than mature males (Antony
376 & Jallon 1982; Gibbs et al. 1997). This may explain why immature flies carrying CHs with
377 longer carbon chains and higher number of double-bonds survived longer than mature flies under

378 dry humidity condition (Fig S1). However, no change in desiccation resistance was induced by

379 the replacement of a alkene (7,11HD) by a closely related isomer (5,9HD) in Z30 mature

380 females, which showed similar desiccation responses to Di2 females (Fig 10).

381 **Conclusion**

382 Our three-pronged approach to desiccation resistance and its underlying genetic and

383 phenotypic components – selection, CH analysis and measurements of water content – provides

384 insight into this fundamental aspect of the ecological physiology of insects. Our data suggest that

385 desiccation resistance is not a simple phenotype: increased and decreased resistance depended on

386 different hierarchies of physiological factors. There was no consistent relationship between

387 increased resistance and various measures of cuticular hydrocarbon composition.

388 Dessication resistance cannot be easily narrowed down to one or two variables, as seen by the

389 different cuticular hydrocarbon composition of the strains studied here. Surprisingly, even the

390 ability to retain a relatively high proportion of water was not related to desiccation resistance –

391 transgenic flies with the highest proportion of water ([RC-Gal4 x IR] and [6908-Gal4 x IR])

392 showed the lowest levels of resistance. These two genotypes also showed reduced D : L (alkenes

393 : alkanes) ratios underlining that a decline in desiccation resistance may occur in flies combining

394 a high water content and low alkene levels. Flies in which these characters were dissociated

395 showed no major change in desiccation resistance. All flies that died rapidly showed a very

396 reduced LT50, but no change in their lethality slope: this suggests that early death equally

397 affected all individuals of a given genotype.

398 Our study has revealed an intricate and non-linear relationship between desiccation

399 resistance, CH profile and internal water content in *D. melanogaster* flies. These three

400 phenotypes, which might be expected to show a simple relationship, turn out to have complex

401 physiological and genetic links. While desiccation resistance and the proportion of desaturated

402 CHs were tightly linked with both measures rapidly increasing after selection and persisting long

403 after selection had been relaxed, a high water content negatively affected resistance, especially in

404 association with a low level of desaturated CHs.

405 **References**

406 Addinsoft. 2012. XLSTAT 2012, Data analysis and statistics with Microsoft Excel. Paris,
407   France.

408 Andersen SO. 2010. Insect cuticular sclerotization: a review. *Insect Biochemistry and Molecular*
409   *Biology* 40:166-178.

410 Antony C, and Jallon JM. 1982. The Chemical Basis For Sex Recognition In Drosophila-
411   Melanogaster. *Journal of Insect Physiology* 28:873-880.

412 Arrese EL, and Soulages JL. 2010. Insect fat body: energy, metabolism, and regulation. *Annual*
413   *Review of Entomology* 55:207-225. doi: 10.1146/annurev-ento-112408-085356

414 Baker JD, and Truman JW. 2002. Mutations in the *Drosophila* glycoprotein hormone receptor,
415   rickets, eliminate neuropeptide-induced tanning and selectively block a stereotyped
416   behavioral program. *Journal of Experimental Biology* 205:2555-2565.

417 Balabanidou V, A. K, M. M, G.J. B, C. T, M.P. J, S.J. M, G. C, A. A, A. L, S. A, J. H, H. R, G.J.
418   L, and J. V. 2016. Cytochrome P450 associated with insecticide resistance catalyzes
419   cuticular hydrocarbon production in *Anopheles gambiae*. *Proc Natl Acad Sci USA*
420   113:9268-9273.

421 Bousquet F, Chauvel I, Flaven-Pouchon J, Farine J-P, and Ferveur J-F. 2016. Dietary rescue of
422   altered metabolism gene reveals unexpected *Drosophila mating* cues. *Journal of Lipid*
423   *Research* 57:1-11. doi:10.1194/jlr.M064683

424 Bousquet F, and Ferveur JF. 2012. desat1: A Swiss army knife for pheromonal communication
425   and reproduction? *Fly* 6:102-107.

426 Bousquet F, Nojima T, Houot B, Chauvel I, Chaudy S, Dupas S, Yamamoto D, and Ferveur JF.
427   2012. Expression of a desaturase gene, desat1, in neural and nonneural tissues separately
428   affects perception and emission of sex pheromones in Drosophila. *Proceedings of the*
429   *National Academy of Sciences of the United States of America* 109:249-254.
430   10.1073/pnas.1109166108

431 Chertemps T, Duportets L, Labeur C, Ueda R, Takahashi K, Saigo K, and Wicker-Thomas C.
432   2007. A female- biased expressed elongase involved in long-chain hydrocarbon
433   biosynthesis and courtship behavior in *Drosophila melanogaster*. *Proc Natl Acad Sci*
434   104:4273-4278.

435 Chown SL. 2002. Respiratory water loss in insects. *Comp Biochem Physiol A Mol Integr Physiol*
436   133:791-804.

437 da Silva SM, Lavander HD, de Santana Luna MM, de Melo Eloi da Silva A, Gálvez AO, and
438   Coimbra MR. 2015. *Artemia franciscana* as a vector for infectious myonecrosis virus
439   (IMNV) to *Litopenaeus vannamei* juvenile. *Journal of Invertebrate Pathology* 126:1-5.

440 Dallerac R, Labeur C, Jallon JM, Knippie DC, Roelofs WL, and Wicker-Thomas C. 2000. A
441   Delta 9 desaturase gene with a different substrate specificity is responsible for the
442   cuticular diene hydrocarbon polymorphism in Drosophila melanogaster. *Proceedings of*
443   *the National Academy of Sciences of the United States of America* 97:9449-9454.

444 Demerec M. 1950. *Biology of Drosophila*. John Wiley: John Wiley.

445 Dow JA. 2009. Insights into the Malpighian tubule from functional genomics. *Journal of*
446   *Experimental Biology* 212:435-445.

447 Eigenbrode SD, and Espelie KE. 2003. Effects of plant epicuticular lpids on insect herbivores.
448   *Annual Review of Entomology* 40:171-194.

449 Everaerts C, Farine JP, Cobb M, and Ferveur JF. 2010. Drosophila cuticular hydrocarbons
450   revisited: mating status alters cuticular profiles. *PLoS One* 5:e9607.
451   doi:10.1371/journal.pone.0009607

452 Fang S, Takahashi A, and Wu CI. 2002. A mutation in the promoter of desaturase 2 is correlated
453   with sexual isolation between drosophila behavioral races. *Genetics* 162:781-784.

454 Ferveur JF. 1997. The pheromonal role of cuticular hydrocarbons in Drosophila melanogaster.
455   *BioEssays* 19:353-358.

456 Ferveur JF. 2005. Cuticular hydrocarbons: Their evolution and roles in Drosophila pheromonal
457   communication. *Behavior Genetics* 35:279-295.

458 Flaven-Pouchon J, Farine JP, Ewer J, and Ferveur JF. 2016. Regulation of cuticular hydrocarbon
459   profile maturation by *Drosophila* tanning hormone, bursicon, and its interaction with
460   desaturase activity. *Insect Biochemistry and Molecular Biology* 78:87-96.

461 Folk DG, and Bradley TJ. 2004. Evolved patterns and rates of water loss and ion regulation in
462   laboratory-selected populations of *Drosophila melanogaster*. *Journal of Experimental*
463   *Biology* 206:2779-2786.

464 Gibbs A, and Pomonis JG. 1995. Physical properties of insect cuticular hydrocarbons: the effects
465   of chain length, methyl-branching and unsaturation. *Comp Biochem Physiol B Biochem*
466   *Mol Biol* 112:243-249.

467 Gibbs AG. 1998. Water-proofing properties of cuticular lipids. *American Zoologist* 38:471-482.

468 Gibbs AG, Chippindale AK, and Rose MR. 1997. Physiological mechanisms of evolved
469   desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology*
470   200:1821-1832.

471 Gibbs AG, and Rajpurohit S. 2010. Cuticular lipids and water balance. In: Blomquist GJ, and
472   Bagnères A-G, eds. *Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology*.
473   Cambridge, UK.: Cambridge University Press, 100-120.

474 Gołębiowski M, Maliński E, Boguś MI, Kumirska J, and Stepnowski P. 2008. The cuticular fatty
475   acids of *Calliphora vicina*, *Dendrolimus pini* and *Galleria mellonella* larvae and their
476   role in resistance to fungal infection. . *Insect Biochemistry and Molecular Biology*
477   38:619-627.

478 Grillet M, Everaerts C, Houot B, Ritchie MG, Cobb M, and Ferveur JF. 2012. Incipient
479   speciation in Drosophila melanogaster involves chemical signals. *Sci Rep* 2:224.
480   10.1038/srep00224

481 Gutierrez E, Wiggins D, Fielding B, and Gould AP. 2007. Specialized hepatocyte-like cells
482   regulate *Drosophila* lipid metabolism. *Nature* 445:275-280.

483 Hadley NF. 1994. Ventilatory patterns and respiratory transpiration in adult terrestrial insectss.
484   *Physiological Zoology* 67:175-189.

485 Harwood BN, Fortin J-P, Gao K, Chen C, Beinborn M, and Kopin AS. 2013. Membrane tethered
486   bursicon constructs as heterodimeric modulators of the Drosophila G protein–coupled
487   receptor Rickets. *Molecular Pharmacology* 83:814-821.

488 Honegger H-W, Dewey EM, and Ewer J. 2008. Bursicon, the tanning hormone of insects: recent
489   advances following the discovery of its molecular identity. *Journal of Comparative*
490   *Physiology A* 194:989-1005.

491 Houot B, Bousquet F, and Ferveur JF. 2010. The consequences of regulation of desat1
492   expression for pheromone emission and detection in Drosophila melanogaster. *Genetics*
493   185:1297-1309. 10.1534/genetics.110.117226

494 Houot B, Cazalé-Debat L, Fraichard S, Everaerts C, Saxena N, Sane SP, and Ferveur J-F. 2017.
495   Gene regulation and species-specific evolution of free-flight odor-tracking in *Drosophila*.
496   *Molecular Biology and Evolution*. doi: 10.1093/molbev/msx241

497 Howard RW, and Blomquist GJ. 2005. Ecological, behavioral, and biochemical aspects of insect
498   hydrocarbons. *Annual Review of Entomology* 50:371-393.

499 Izumi Y, Sonoda S, and Tsumuki H. 2007. Effects of diapause and cold-acclimation on the
500   avoidance of freezing injury in fat body tissue of the rice stem borer, *Chilo suppressalis*
501   Walker. *Journal of Insect Physiology* 53:685-690.

502 Jallon JM. 1984. A Few Chemical Words Exchanged By Drosophila During Courtship And
503   Mating. *Behavior Genetics* 14:441-478.

504 Liu K, Tsujimoto H, Cha S-J, Agre P, and Rasgon JL. 2011. Aquaporin water channel AgAQP1
505   in the malaria vector mosquito *Anopheles gambiae* during blood feeding and humidity
506   adaptation. *Proc Natl Acad Sci USA* 108:6062-6066.

507 Locke M. 1966. The structure and formation of the cuticulin layer in the epicuticle of an insect,
508   *Calpodes ethlius* (Lepidoptera, Hesperiidae). *Journal of Morphology* 118:461-494.
509   10.1002/jmor.1051180403

510 Lorenz MW, and Anand AN. 2004. Changes in the biochemical composition of fat body stores
511   during adult development of female crickets, *Gryllus bimaculatus*. *Archives of Insect*
512   *Biochemistry and Physiology* 56:110-119.

513 Moussian B. 2010. Recent advances in understanding mechanisms of insect cuticle
514   differentiation. *Insect Biochemistry and Molecular Biology* 40:363-375.

515 Qiu Y, Tittiger C, Wicker-Thomas C, Le Goff G, Young S, Wajnberg E, Fricaux T, Taquet N,
516   Blomquist GJ, and Feyereisen R. 2012. An insect-specific P450 oxidative decarbonylase
517   for cuticular hydrocarbon biosynthesis. Proc. Natl. Acad. Sci. U. S. A. 109, . *Proc Natl*
518   *Acad Sci USA* 109:14858–14863.

519 Robertson JL, and Preisler HK. 1992. *Pesticide bioassays with Arthropods*. Boca Raton, FL:
520   CRC Press / Taylor & Francis Group.

521 Savarit F, and Ferveur JF. 2002. Temperature affects the ontogeny of sexually dimorphic
522   cuticular hydrocarbons in Drosophila melanogaster. *Journal of Experimental Biology*
523   205:3241-3249.

524 Savarit F, Sureau G, Cobb M, and Ferveur JF. 1999. Genetic elimination of known pheromones
525   reveals the fundamental chemical bases of mating and isolation in Drosophila.
526   *Proceedings of the National Academy of Sciences of the United States of America*
527   96:9015-9020.

528 Sinclair BJ, and Renault D. 2010. Intracellular ice formation in insects: unresolved after 50
529   years? *Comp Biochem Physiol A Mol Integr Physiol* 155:14-18. doi:
530   10.1016/j.cbpa.2009.10.026.

531 Toolson EC, and Hadley NF. 1979. Seasonal effects on cuticular permeability and epicuticular
532   lipid composition in *Centruroides sculpturatus* (Scorpiones: Buthidae): correlation with
533   thermal effects on cuticular permeability. *Journal of Insect Physiology* 25:271-275.

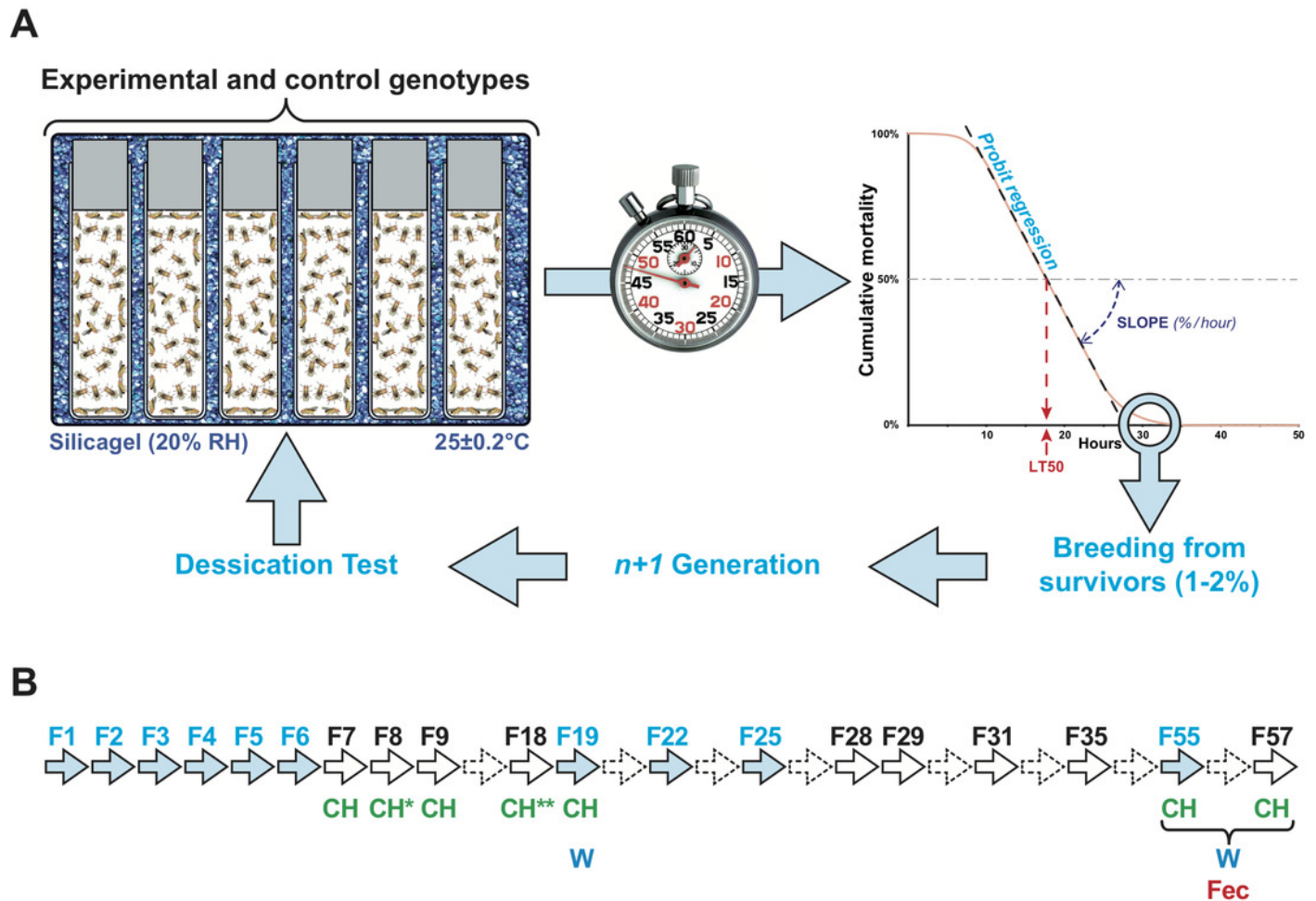
534 Tukey JW. 1977. *Exploratory Data Analysis*.

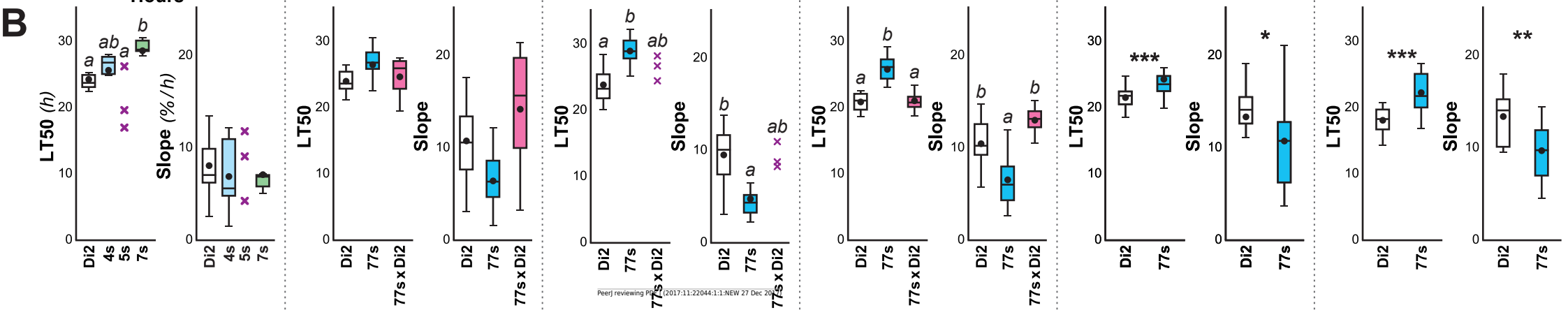
535 Wigglesworth VB. 1945. Transpiration through the cuticle of Insects. *Journal of Experimental*
536   *Biology* 21:97-114.

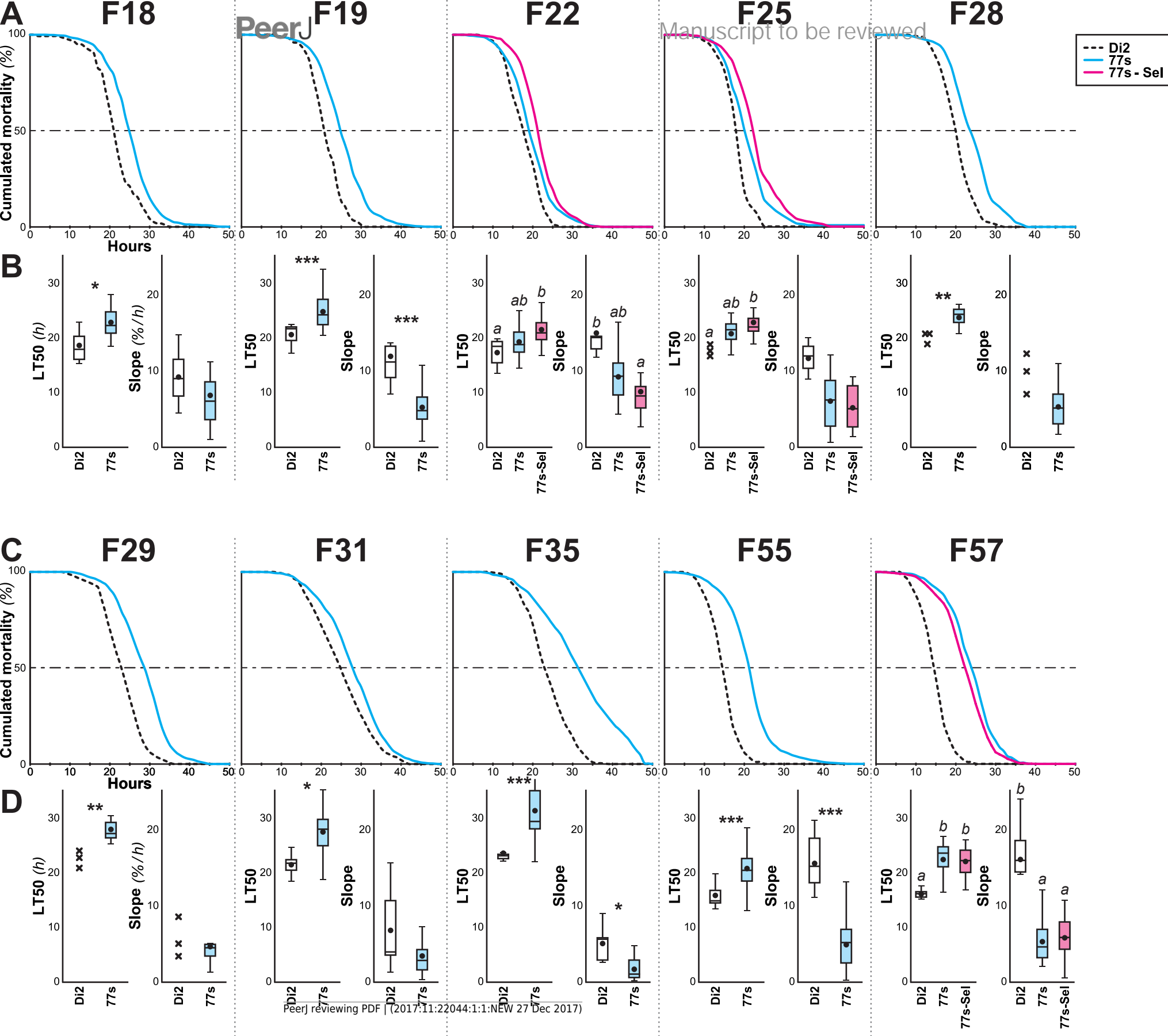
537 Wigglesworth VB. 1988. The control of pattern as seen in the integument of an insect. *BioEssays*
538   9:23-27.

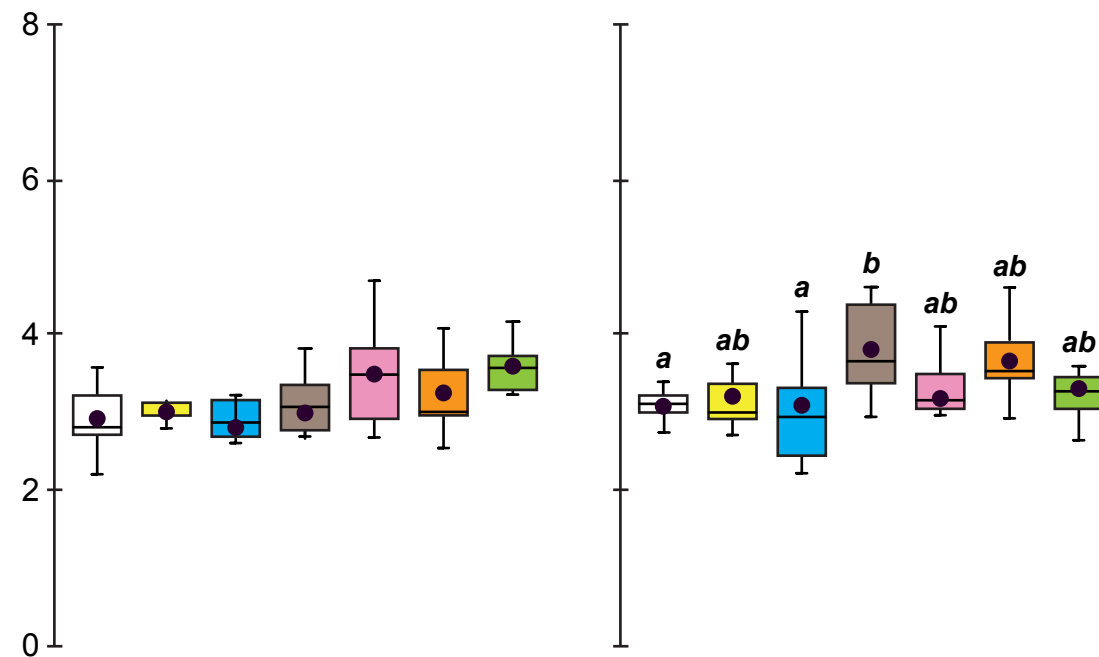
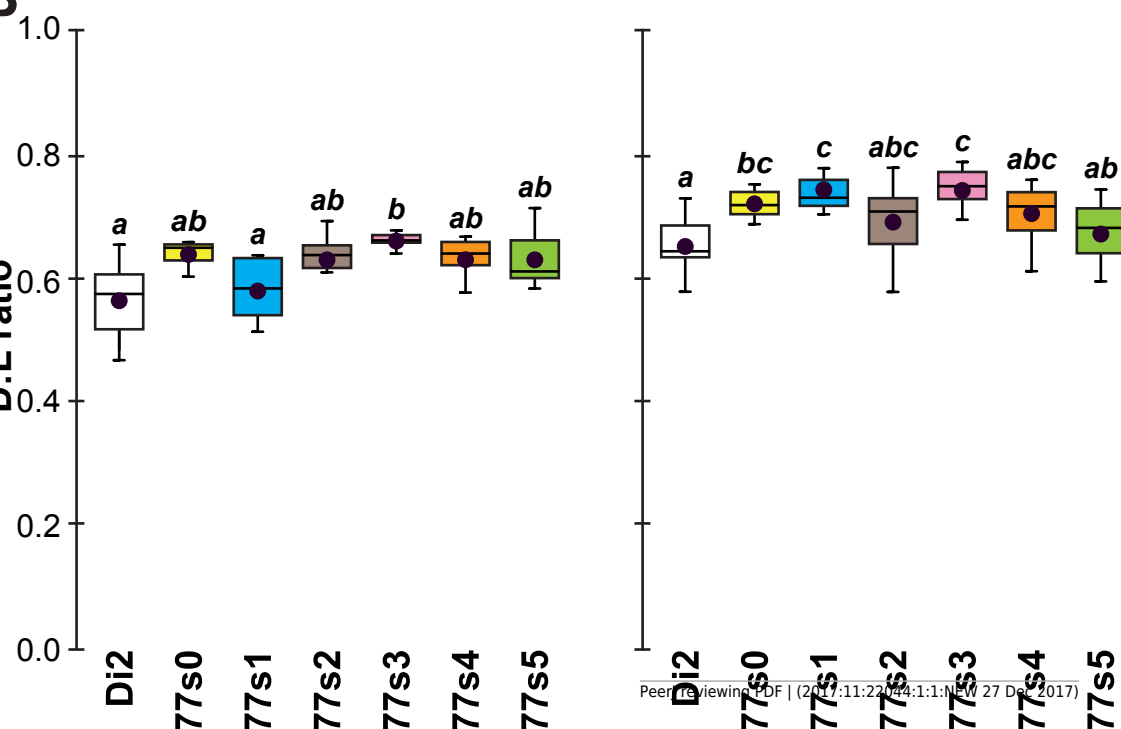
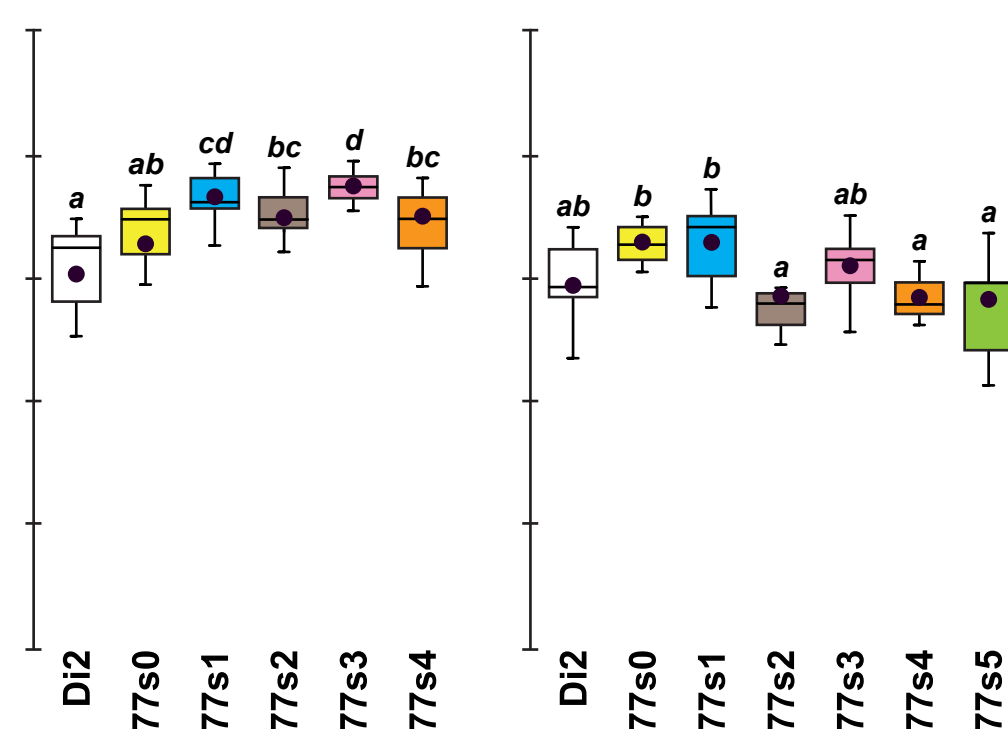
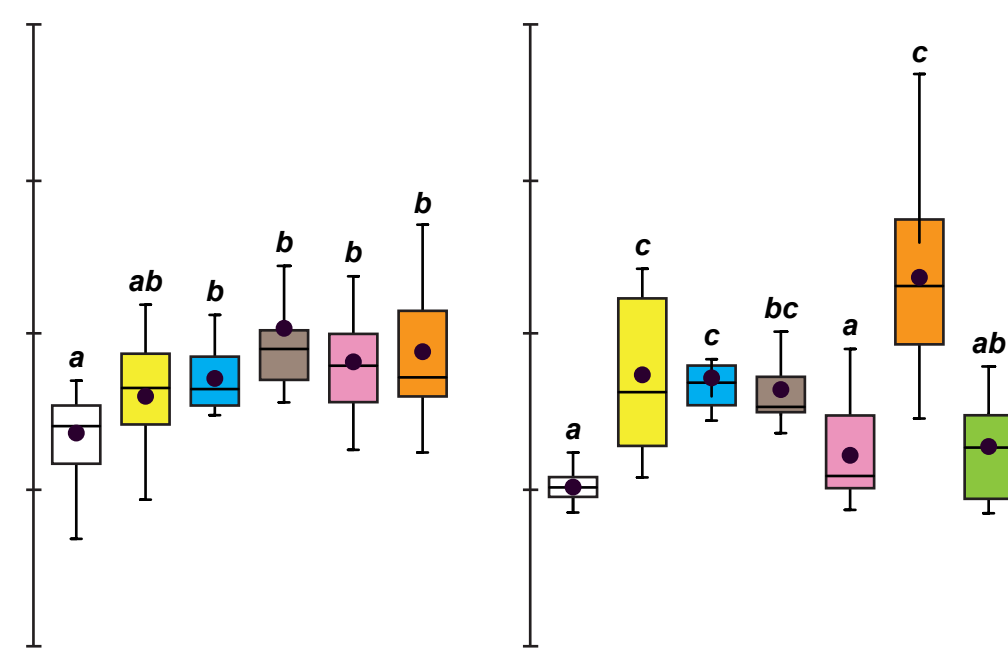
Figure 1

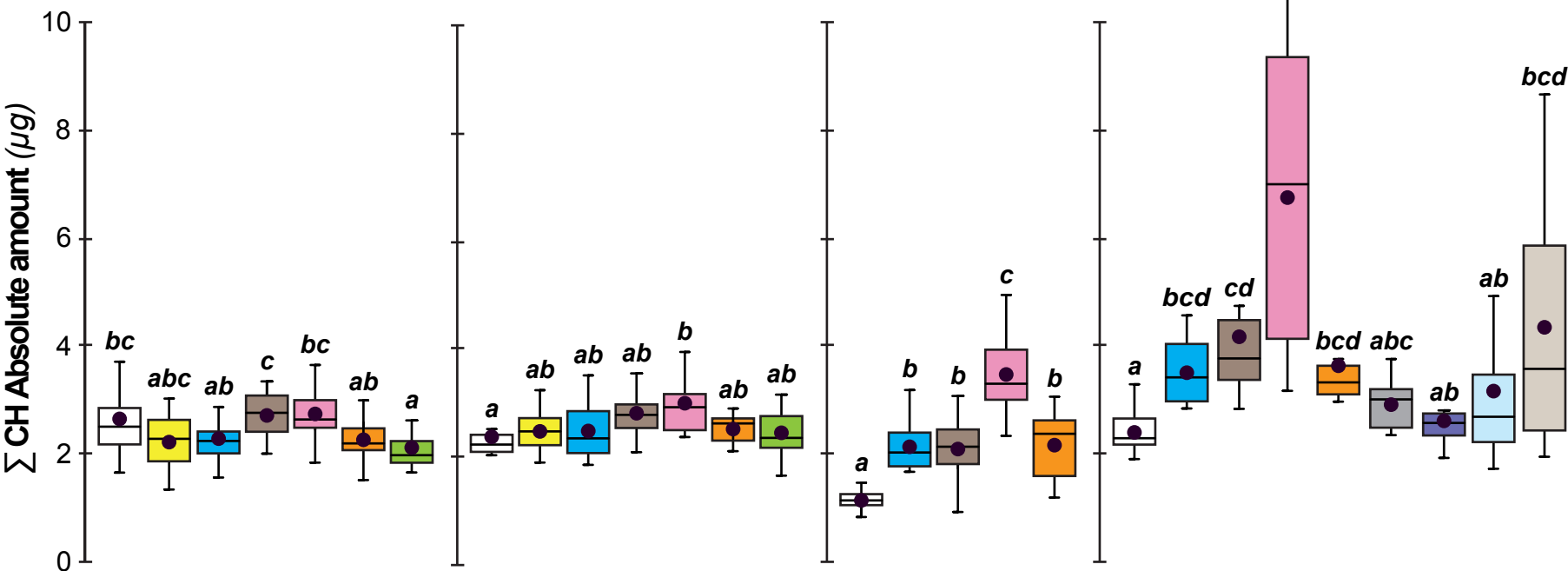
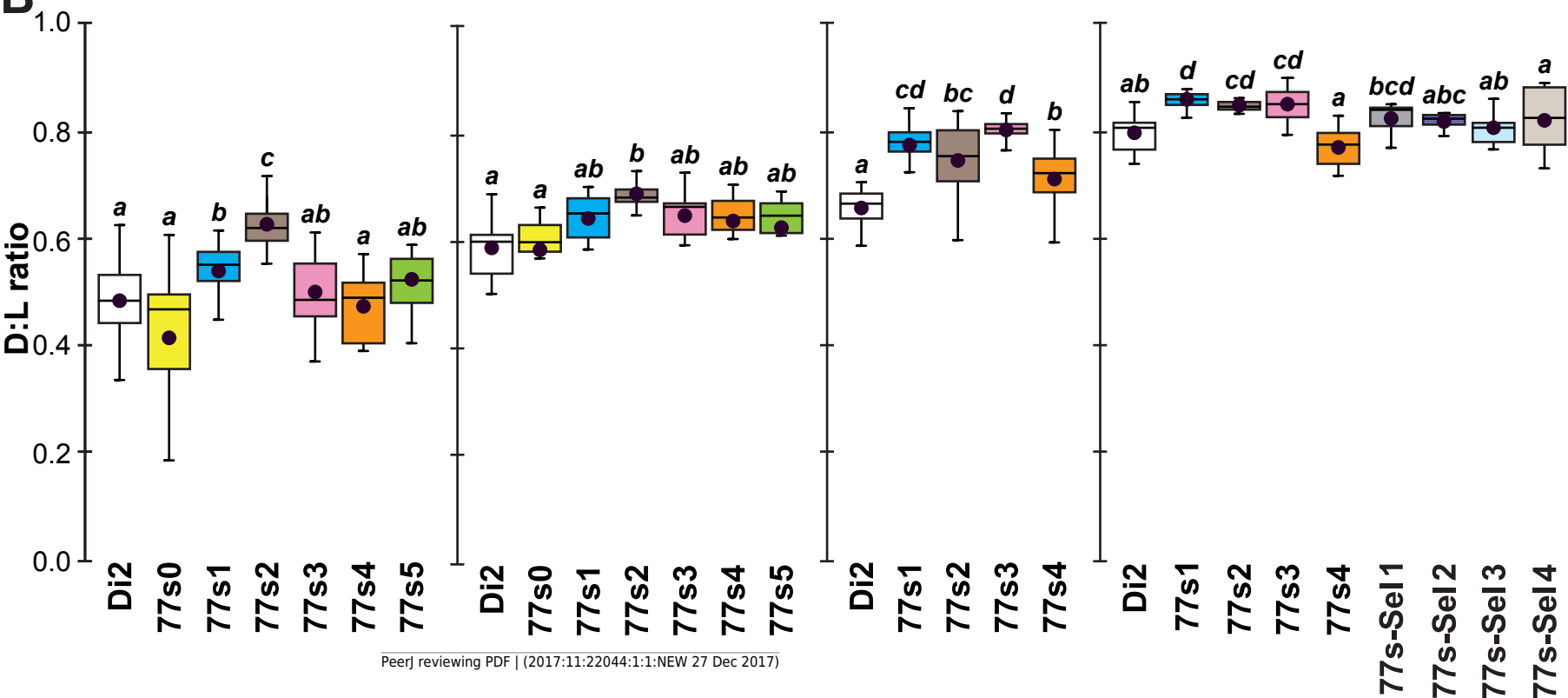
Experimental selection of desiccation resistance line.

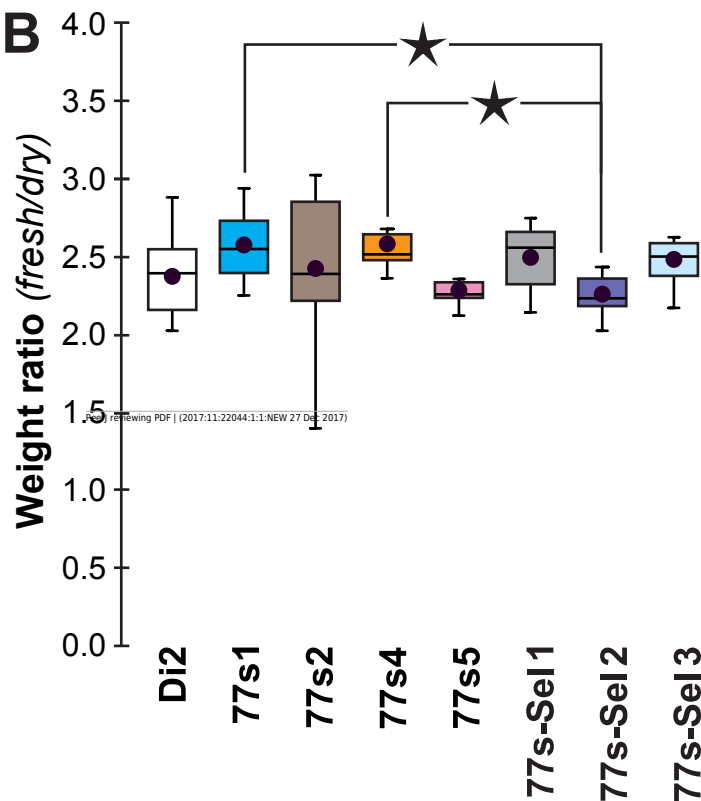
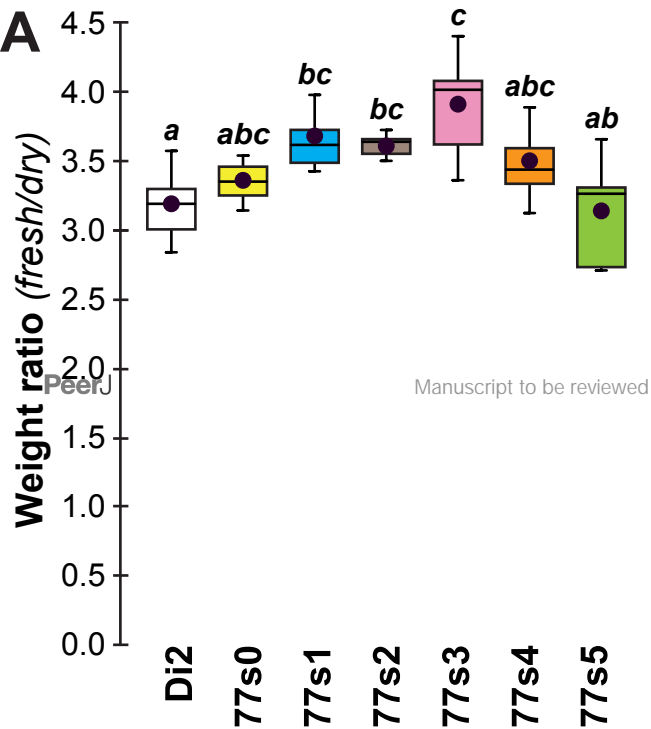
(**A**) Flies were kept in single sex groups of approximately 50 individuals in empty glass vials. 6 or 7 of these glass vials were packed inside an airtight transparent plastic box that was seeded with a layer of silicagel crystals to maintain a low relative humidity (20±1%). The box was placed on a hot plate at 25±0.2°. Four boxes were simultaneously tested. Every two hours, the number of dead flies was counted, providing a measure of survival over time. This allowed us to estimate when 50% flies died (lethality time 50% = LT50), using logistic regression. The slope of the lethality curve was also determined to evaluate the relative lethality per hour. At the end of each experiment, the few surviving flies (maximum 1-2% of all flies) were transferred into fresh food vials and mated with siblings to produce the next generation. (**B**) Arrows indicate the generations of the experiment. Plain arrows indicate the generations during which experimental selection for desiccation (light blue) and/or phenotypic measurements (CH = cuticular hydrocarbons; W = weight; Fec = fecundity) were carried out. Dashed arrows indicate the generations during which no measurement or selection was carried out.

Survival in females selected for desiccation resistance over the first six generations.

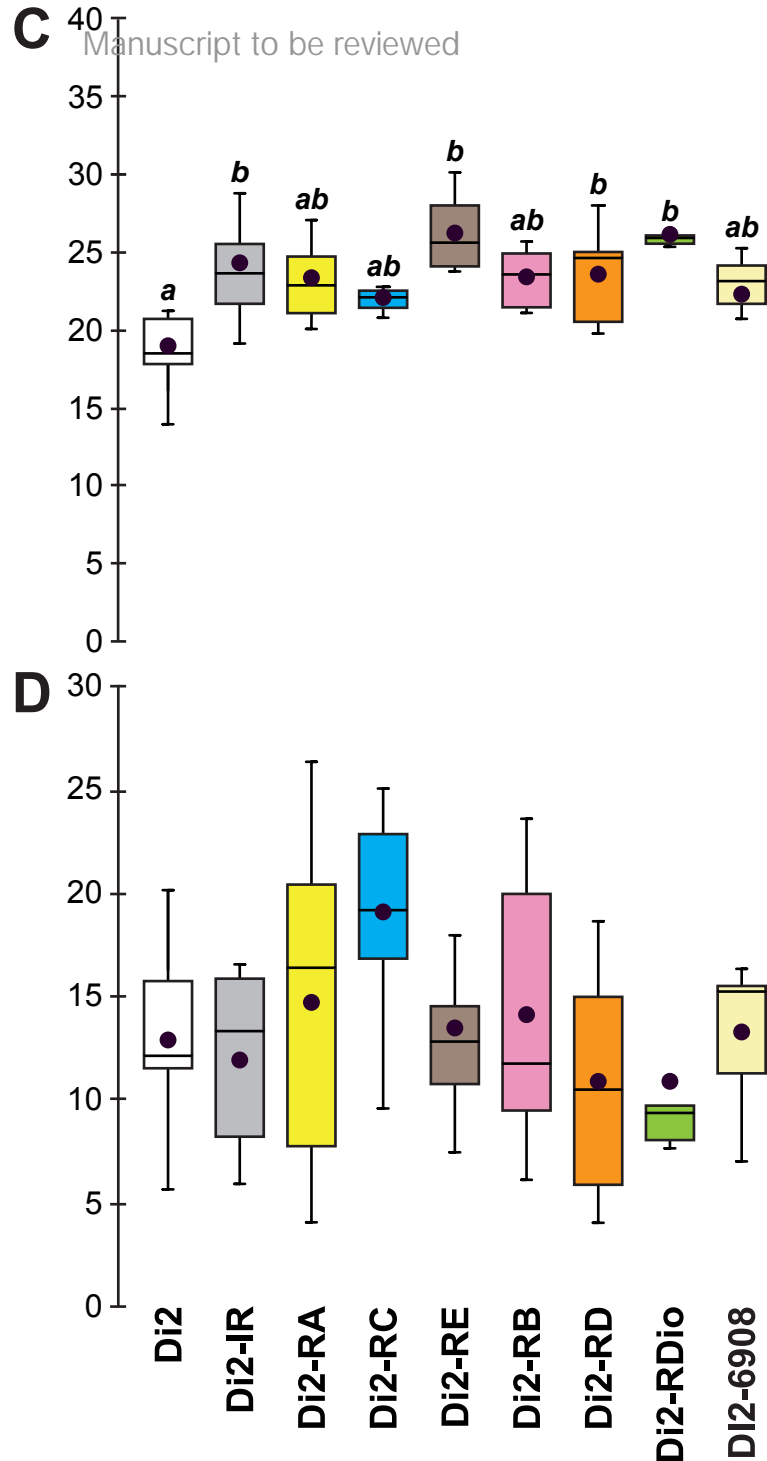
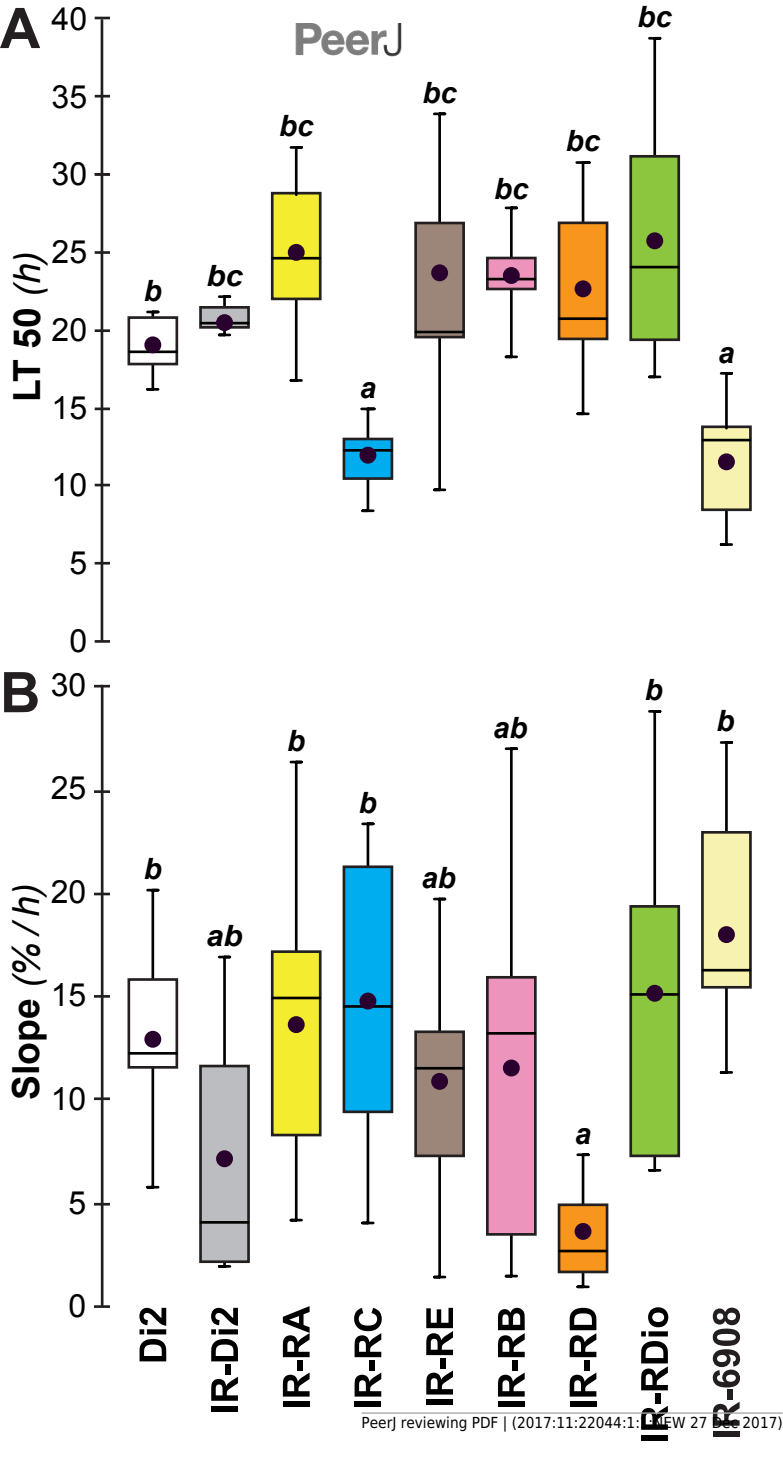
Female flies were selected using the experimental procedure described in Fig. 1. (**A**) For each generation (F1 to F6), the curves represent the survival measured in various genotypes (dashed line = Di2 control line, cyan line = 77S selected lines pooled, magenta line = backcross between 77S females and unselected sibling males; at F1, three selected lines are shown). (**B**) At each generation, the two box-plots represent the LT50 and the lethality slope using colors similar to those of the corresponding genotypes. Data are shown as box plots representing the 50% median data (the small horizontal bar indicates the median value while the plain dot represents the mean). The whiskers shown below and above each box represent the first and third quartiles, respectively. Stars or different letters indicate significant differences. After excluding extreme outliers using Tukey's method, LT50 and slopes were tested using a Kruskall-Wallis test completed by a Conover-Iman multiple pairwise comparisons at level $p=0.05$ (with a Bonferroni correction) or with a Mann-Whitney test. ***: p<0.001; **: p<0.01; *: p<0.05. The absence of a letter or stars indicates that no significant difference was detected. N = 5-17 (except 5S line at F1 and 77sxDi2 line at F3 where N = 3). A similar selection procedure was carried out on males between the F1 and F6 generations (Fig. S2).

Survival in females of selected lines between F18 and F57.

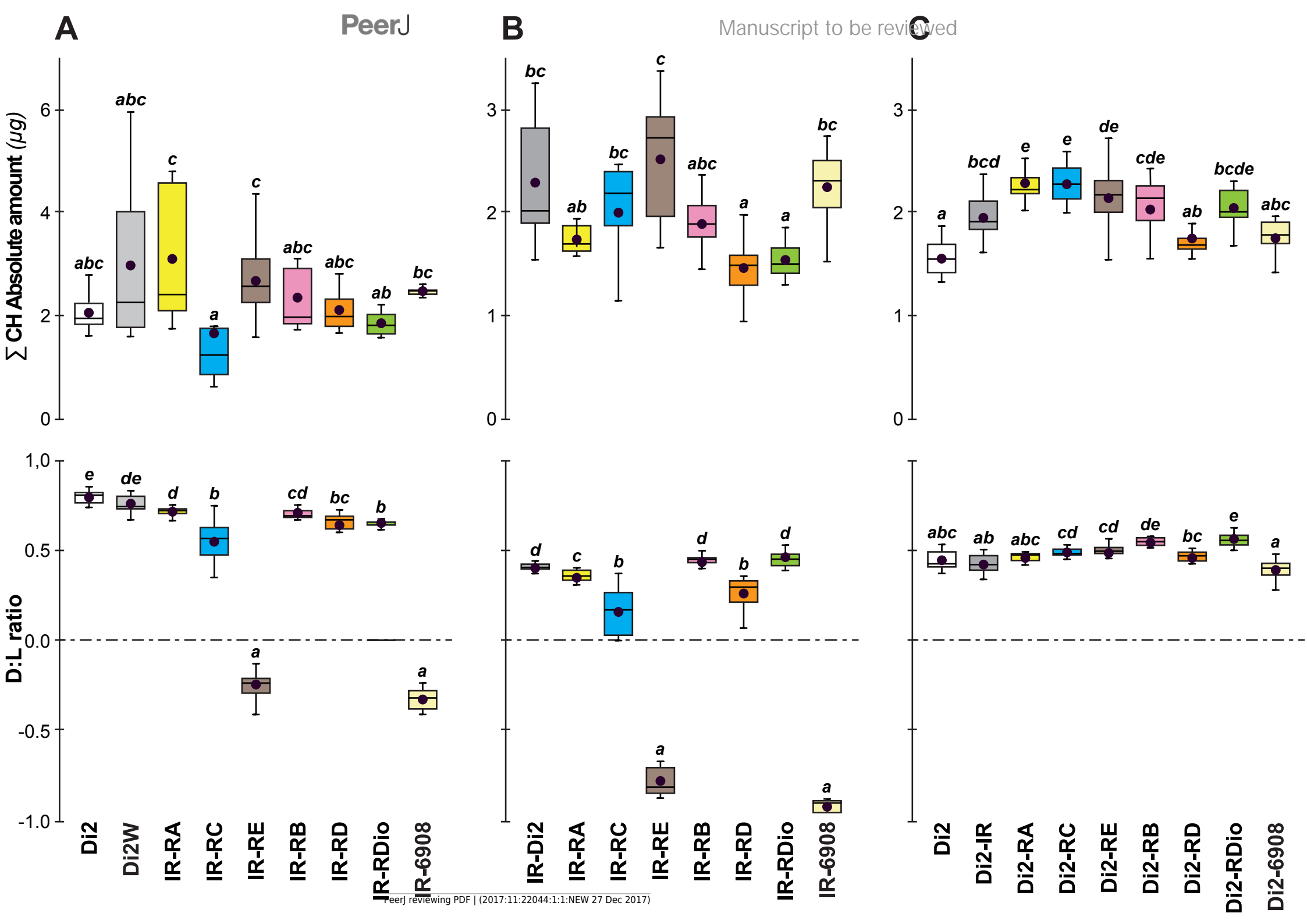
Female flies were selected at the indicated generations using the procedure described in Figs.1 & 2. N = 5-22 (except Di2 line at F18: N =4, and at F25, 28 & 29: N = 3). For parameters and statistics, see Fig. 2 legend.

Principal cuticular hydrocarbons in flies of selected lines following relaxation of selection.

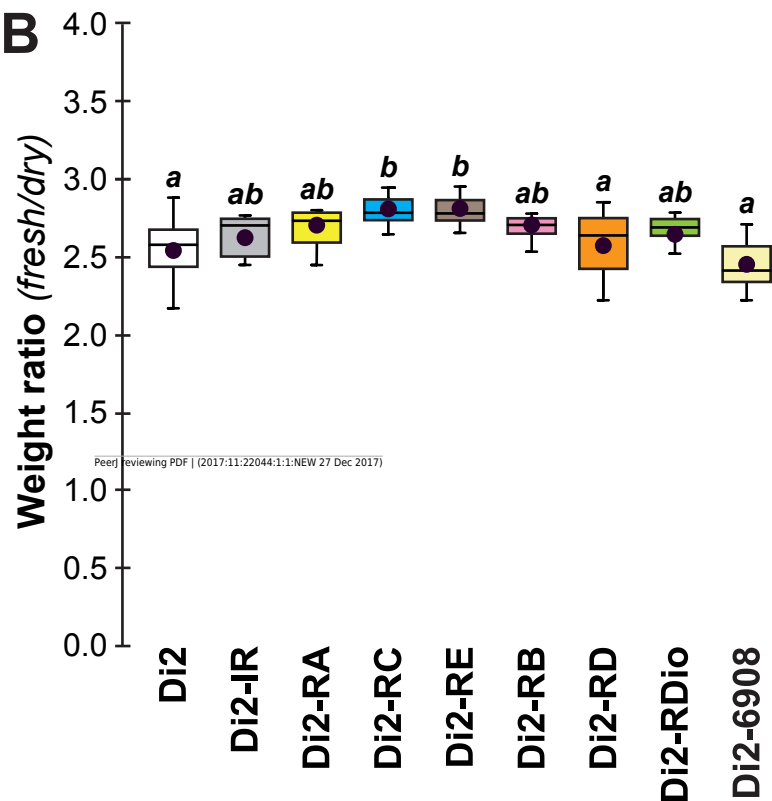
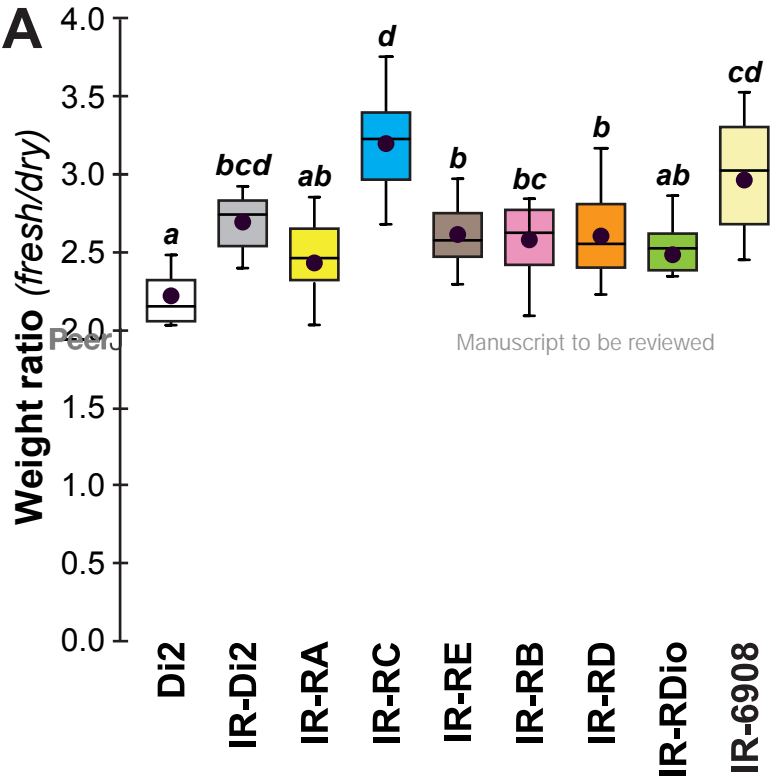
Cuticular hydrocarbon levels (CHs) were measured in F7, F8 and F9 females and in F8 males separately in the six 77S lines ($77S_0$-$77S_5$) experimentally selected for desiccation resistance (F1-F6; see Fig. 2). Here, we show the total absolute amount of CHs ($\sum$CH in µg, **A**) and the ratio of Desaturated : Linear saturated CHs (D : L ratio; **B**) This ratio was calculated using the formula ([D – L] /[D + L]). N = 5-20 for females and N = 9-14 for males. For statistics, see Fig.2 legend. We also determined the absolute (Q) and relative (%) amounts of desaturated CHs (alkenes) and of linear saturated CHs (alkanes) (Fig. S3).

Principal cuticular hydrocarbon levels in females of selected lines between F18 and F57.

$\sum$CH (**A**) and D : L ratio (**B**) were measured in F18, F19, F55 and F57 females. As well as control unselected Di2 females, six 77S lines ($77S_0$-$77S_5$) were tested in F18 and F19; only 4 of these lines ($77S_1$-$77S_4$) survived to F55 and F57. The 77S-Sel lines (77S-Sel1 - 77S-Sel4) that were tested at F57 were the offspring of F55 reselected females from their respective 77S lines (e.g. $77S_1$ females yielded the 77S-Sel1 line). $N = 7\text{-}38$. For more information on parameters, lines and statistics, see legends of Fig.2 and Fig.4. The absolute (Q) and relative (%) amounts of desaturated CHs (alkenes) and of linear saturated CHs (alkanes) determined in these flies are shown in Fig. S4.

Fresh : dry weight ratio in females of selected lines.

Groups of 10 freshly killed females were weighed (fresh weight) and after 24 hours desiccation were weighed again (dry weight). The fresh : dry weight ratio of each group was calculated. Females were weighed at F19 (**A**) and F57 (**B**). N = 6-20. For more information on genotypes and statistics, see legends to Figs. 2, 4 & 5.

Desiccation resistance in various *desat1* transgenic females.

To test the effect of *desat1* knock-down expression in various *desat1*-expressing tissues, we used the female progeny of matings between transgenic females carrying the UAS-*desat1*-IR transgene (IR) and transgenic males either carrying each *desat1* putative regulatory region fused with Gal4 (PRR-Gal4) corresponding to each *desat1* transcript (RA, RC, RE, RB, RD, RDiO), or the complete *desat1* regulatory region (6908bp = 6908). Di2 control females and female progeny resulting of matings between IR females and Di2 males were also tested (Di2; Di2-IR; left box plots). The LT50 (**A**) and the lethality slope (**B**) of all these genotypes were determined. To control for the effect of each *desat1* PRR-Gal4 transgene on the LT50 (**C**) and lethality slope (**D**), we used flies from matings between Di2 females and PRR-Gal4 males, alongside Di2-IR and Di2 control females. *N* = 5-13. For more information on parameters and statistics, see Fig. 2 legend.

Principal cuticular hydrocarbons in various *desat1* transgenic females.

CH levels were measured in all transgenic and control female flies tested for desiccation resistance (see Fig. 7). Transgenic females combining a maternal IR transgene with a paternal PRR-Gal4 or 6908 transgene were tested at both F55 (**A**) and F57 (**B**). Di2, Di2/w and Di2-IR control females were tested (left box-plots) either at F55 (**A**) or at F57 (**B**). Control genotypes carrying a paternal copy of each PRR-Gal4 transgene or of the 6908 transgene combined with a maternal Di2 genome were also tested (**C**). Alongside these control genotypes, we also tested the effect of the IR transgene in the Di2 background (second box-plot from the left. N = 7-16. For more information on CHs, genotypes and statistics, see legends to Figs. 4 & 7.

Fresh : dry weight ratio in various *desat1* transgenic females.

N = 5-15. For more information on genotypes and statistics, see legends to Figs. 2 & 7.

Survival to desiccation in various cuticular hydrocarbon variant females.

The LT50 (**A**) and lethality slope (**B**) of various CH variants was measured. We also assayed the resistance of flies from these lines: control Di2, Di2/w (combining the Di2 genome with the w^{1118} white-eye mutation), Zimbabwe 30 line (Z30), $ricket^{1/4}$ double trans-heterozygote mutant (rk^1/rk^4), rk heterozygotes carrying the SM2 balancer (rk/CyO = rk^1/CyO and rk^4/CyO), and Di2/w flies carrying the SM2 balancer (CyO/w). N = 10-30 (except for CyO/w line: N = 3). For more information on statistics, see legend to Fig. 2.