2 **Loser-effect duration evolves independently of fighting ability.**

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**Abstract**

Winning or losing contests can impact subsequent competitive behaviour and the

duration of these effects can be prolonged. While it is clear effects depend on social

and developmental environments, the extent to which they are heritable, and

hence evolvable, is less clear and remains untested. Furthermore, theory predicts

that winner and loser effects should evolve independently of actual fighting ability,

but again tests of this prediction are limited. Here we used artificial selection on

replicated beetle populations to show that the duration of loser effects can evolve,

with a realized heritability of about 17%. We also find that naïve fighting ability

does not co-evolve with reductions in the duration of the loser effect. We discuss

the implications of these findings and how they corroborate theoretical

predictions.

**Key words:** Winner effect, loser effect, fighting, contest, realised heritability

**Introduction**

In many animals aggressive contests occur for limited resources like territories, food and mates (1). Additionally, individuals frequently engage in repeated contests, and previous fighting experience often influences current contest outcomes (2). Thus prior winning often increases the probability of winning subsequent contests, and prior losing decreases the probability, phenomena known as winner and loser effects respectively. While the precise underlying causes of winner/loser effects are often unclear, they are assumed to have some genetic underpinnings (reviewed in 3). However, despite the widespread occurrence of winner/loser effects (2, 3), this claim is rarely tested – in fact we could find no examples where explicit tests of a genetic basis to winner/loser effects had been undertaken. Additionally, although we expect genetic variation to underpin behavioural phenotypes (4), this might not always be the case (5).

One explanation for winner and loser effects is that prior experience shapes future contests by providing contestants information about their relative resource-holding potential (RHP) or fighting ability (6), and two non-mutually exclusive hypotheses for the effects have been proposed (3). Individuals either

51    gain information on their own RHP (a self-assessment mechanism) or winning and

52    losing produces status-related cues that affect the assessment of subsequent

53    opponents (a social-cue mechanism). With a social-cue mechanism, individuals are

54    predicted to detect previous winning or losing by their opponents from visible or

55    chemical 'cues' emitted by them, and should adjust self-behaviors based on

56    opponent's previous experience (3). Social-cues include signs of exhaustion or

57    injuries (7), and odors (8). Both hypotheses require there to be variation in

58    fighting ability in the population so that there is value in working out who to fight

59    and who not to fight (9). A typical example of self-assessment is learning through

60    prior fighting (10, 11). Here, individuals adjust their behavior based on their

61    previous experience (e.g., 12-14), and there is abundant evidence that individuals

62    vary in their behavioural adjustments, including in contest duration (2) and the

63    type of adjustments employed (14-16). Additionally, variation in behavioral

64    adjustment may be underpinned by differences in perception (10) and learning

65    ability (11), and this variation can be related to behavioral syndromes or

66    personality (17). Finally, although the evolution of winner and loser effects can be

67    inferred from such among-individual differences (3, 11), direct evidence for

68    genetic variation and responses to selection of winner-loser effects appears to be

69    lacking. This may be because these effects arise from experience, effectively the

70    environment. But of course the environment is responsible for all manner of gene

71    expression variation that generates physiological changes in an individual, and any

72    genetic variation in gene expression (e.g. 18) will mean genetic variation for

73    winner/loser effects. Thus genetics will also be important (2).

74

75    Here we only focused on loser effects and their duration. This is primarily because

76    theory suggests loser effects can evolve without corresponding winner effects,

77    while the reverse is not true (3, 19). This loser only evolution should occur when

78    the costs of fighting (C: the rate of increase in costs of over-estimating RHP in

79    terms of heightened risk of getting into and losing escalated fights) are moderate

80    and the fitness benefits of dominance (V: relative fitness of dominant individuals)

81    are substantial (e.g. V > C > 0), a pattern reported for several taxa (e.g., 20-22).

82    Furthermore, although experience effects are generally short-lived, as noted above,

83    variable durations are found within and across taxa (reviewed in 3). For example,

84    effects can persist from 10 minutes to 10 days (e.g. 23, 24), and although there is

85    limited evidence for the intra-specific variation in loser effects (2), variation has

86    been found in the cricket *Gryllus bimaculatus* (23, 25-27). Variation in the duration

5

87    of effects is thought to be influenced by the frequency of social interactions and

88    population density (22, 28), as well as the costs and benefits of fighting (2), which

89    all implies that these effects can evolve. Interestingly, effects may be due to

90    perception only.   That is, absolute fighting ability need not reflect the duration of

91    loser effects and *vice versa*.   So loser effects could potentially evolve without

92    affecting fighting ability, although this remains to be demonstrated experimentally.

93

94    Broad-horned flour beetles (*Gnatocerus cornutus*) are increasingly well studied,

95    especially with respect to their fighting behaviour and its consequences (e.g. 14,

96    29-36). Males freely engage in combat for access to females (14) and experience a

97    loser effect when they are beaten in these fights. The loser effect lasts for about

98    four days, during which time fewer than 25% of losers will engage in combat (75%

99    of losers will not fight), and there is no apparent decay of the effect during that

100   four-day period (14). Rather than fighting, losing males tend to disperse to new

101   territories (which may or may not contain other males) and increase their

102   investment in sperm production (14, 35). It should be emphasized that there is no

103   modulation of male behavior due to winning (i.e. winners are not different from

104   naïve males), which is consistent with theoretical predictions that loser effects can

105    evolve alone (3, 19). Here we investigated whether the duration of the loser effect

106    could evolve through artificial selection in experimental populations of *G. cornutus*.

107    Any response to selection would then facilitate estimating the heritability of the

108    response duration and enable testing for correlated evolution of male fighting

109    behaviour. Furthermore, demonstrating such evolutionary responsiveness would

110    establish the broad-horned flour beetle system as a model for explicit testing of

111    theoretical predictions about the conditions under which pure loser effects are

112    expected to evolve (19).

113

114    **Materials and Methods**

115    The *G. cornutus* beetle culture originated from adults collected in Miyazaki City

116    (31° 54'N, 131°25'E), Japan, and has been maintained in the laboratory of the

117    National Food Research Institute, Japan, for ~50 years on whole meal enriched

118    with yeast. The stock contains 1500–2000 beetles per generation. This beetle is a

119    stored product pest, and thus, the laboratory conditions very closely mimic what

120    have become natural conditions over the last 4500 years (37). All rearing and

121    subsequent experimentation was conducted in a chamber maintained at 25°C, 60%

122    relative humidity and with a photoperiod cycle of 14:10h light/dark.

123 To obtain virgin adults for experiments, one final instar larva was placed in each

124 well of a 24-well tissue culture plate with 1g of food (Cellstar; Greiner Bio-One,

125 Frickenhausen, Germany) (14, 32). Individuals were placed in the wells

126 immediately after eclosion, and did not interact with conspecifics until the start of

127 the experiments. Thus, we ensured that animals were virgin and had no previous

128 fighting experience. Adults 15-20 days old (after final eclosion) were used for the

129 experiments (For a more detailed description of the stock culture see references

130 14, 32). The body size (prothorax width: 14, 32, 38) of each experimental

131 individual was measured (±0.01 mm), using a dissecting microscopic monitoring

132 system (VM-60; Olympus, Tokyo, Japan)(see 32 for landmarks).

133

134 ***Identifying losers***

135 Following established protocols (14), adult males with no fighting experience were

136 collected from the stock culture (collected as final instar larvae and housed alone

137 until adulthood). To control for the effect of body size on fighting success, males

138 were paired so that the difference in body size between contestants was less than

139 0.01 mm thus competitors differed by in size by less than 2% (14). Pairs were

140 placed on filter-paper (17 mm diameter) in a plastic container (17 mm diameter,

8

141 20 mm high) and allowed to interact (and fight) for one hour - previous work has

142 shown that male fights occur in almost all trials when staged in this manner (14).

143 Males that pushed opponents and chased them were denoted the winner (14).

144 Losers (L-males) were those that retreated from the winner. For a more detailed

145 description of the methods, see (14). Subsequently, each L-male was placed in one

146 well of a 24-well tissue culture plate with food (1 g), as described above, until

147 testing for the selection and control populations. These focal males were marked

148 with white or pink spots [Mitsubishi Paint-Marker] on their elytra; in half of the

149 trials, focal L males were white, and in half of the trials, focal L males were pink.

150

151 ***Selection protocol***

152 As shown previously (14), loser effects last about 4 days, with no apparent decay

153 in the proportion of males affected during that period (and again note there is no

154 modulation of behavior due to winning fights). Here we selected for a reduced

155 duration of the loser effect after losing fights. We first collected males from the

156 stock culture to manipulate the loser effect, as described above, to establish three

157 selection and three control populations (initially with ca. 75 males/population). To

158 investigate whether the loser effect influenced the outcome of a subsequent fight,

159    each loser male (males that lost initial fights) was matched with an opponent male

160    collected from the stock culture (tester male), at 4 days after first fight losses. The

161    tester males had no fighting experience in these or other experiments. Contestants

162    were matched for body size (as above) and outcomes were assessed as above. We

163    then selected the 12 losers that won these second fights (i.e., males that had not

164    modulated their behavior until day 4 due to their previous losing experience) as

165    sires of the reduced loser-effect-duration populations (RLE Populations). To

166    propagate control populations (C Population) 12 random (with respect to their

167    fighting behaviour in these second bouts) (previous) losers where selected to act

168    as sire. That is, control males had also lost initial fights, but we did not take their

169    subsequent win/lose status into account when choosing them as sires.   The 12

170    males/population were randomly divided into four groups (three males in each),

171    and each group was placed in a plastic cup (7 cm diameter, 2.5 cm height) with 20

172    g of medium and three females collected from the stock culture. Groups were

173    maintained this way for 2 months with males able to mate with females and

174    females were allowed to lay eggs in each group, until final instar larvae were

175    obtained (38). Final instar larvae were collected (as above) to obtain the adults for

176    subsequent generations. When the adults reached 10–15 days old, 144

177    males/population were randomly collected and 72 male pairs/population were

178    matched within each population and tested again as above. We then took losers

179    from these fights and selected the 12 losers that won second fights against tester

180    males (4 days later) to propagate RLE Populations, and randomly selected 12

181    previous losers regardless of their winner or loser status in second fights to

182    propagate controls (C Populations). Females were randomly chosen as dams from

183    within each experimental population. This regime continued for 10 generations.

184    We randomly collected 50 males from each experimental population at generation

185    5 and 10 and examined whether the duration of the loser effect had decayed at day

186    4; we examined whether males were attacked first by or lost fights to tester males,

187    again noting that usually almost all losers will not fight so not initiating attacks is a

188    measure of loser effect duration (14). Winning or losing a fight provides an

189    estimate of fighting ability.

190

191    To compare population rates of attacking first and losing fights, we applied a

192    generalized linear model (GLM) with a binomial distribution, a logit-link function,

193    and overdispersion test. Replicate (population) was nested within selection regime

194    [RLE = reduced loser effect duration and C = control (no artificial selection on loser

195     effect duration)]. Losing (losing=1, winning=0) and attacks (attacked=1,

196     attacking=0) were the response variables. All model assumptions were met. All

197     statistical analyses were carried out using JMP 7 (39). The realized heritabilities

198     were calculated according to the liability model (40) as cumulative response to

199     selection divided by cumulative selection differential (also see 41).

200

201     In addition to testing for an impact of selection on loser effect at day 4, we also

202     tested effects of losing on males at days 1-5 after they lost their initial fights (using

203     the methods previously described, with different males used for each day – each

204     male only fought twice). On each day after initial losses, 20 losers (per day) were

205     observed per population ($n$ = 600 in total), in which the experimental losers

206     competed against tester males from the stock culture. Population rates of being

207     attacked first or losing to by tester males were compared using GLMs with a

208     binomial distribution, a logit-link function, and overdispersion test. Replicate was

209     again nested within selection regime (RLE and C), and this and test time (day after

210     initial loss 1-5) were used as the explanatory variables. Losing (losing=1,

211     winning=0) and attacks (attacked=1, attacking=0) were the response variables.

212     When significant interaction terms (selection regime × day) were observed, as a

213    post-hoc test, we compared population rate at each day using GLMs with a

214    binomial distribution, a logit-link function, and overdispersion test. Replicate was

215    nested within selection regime (RLE and C), and this was used as the explanatory

216    variable. We note here that when we used model selection (Table S1) inferences

217    were unchanged.

218

219    ***Correlated responses in fighting success***

220    At generation 10 adults were also collected to assess the fighting success of naïve

221    males as a correlated response to selection on loser effect duration. Males from

222    each of the six experimental populations (RLE and C) were used to assess fighting

223    success when they had experienced no previous fighting – their first fights when

224    they were naïve. We observed 30 contests per population ($n$ = 180 in total) in

225    which focal experimental males competed against a tester male (as described

226    above). Trials were then continuously observed until fight outcomes could be

227    scored. Population rates of being attacked by and losing to tester males were

228    compared using GLM with a binomial distribution, a logit-link function, and

229    overdispersion test. Replicate was nested within selection regime (RLE and C) and

230    this was used as the explanatory variable. Losing (losing=1, winning=0) and

231    attacks (attracted=1, attack=0) were the response variables.

232

233    ***Correlated response in body size***

234    Thirty males were also randomly chosen from each of the six experimental

235    populations (RLE and C) at generation 10 and were used to assess body size

236    (prothorax width). Body size was compared using GLM with a normal distribution

237    and an identity-link function. Replicate was nested within selection regime (RLE

238    and C), which was the primary explanatory variable.

239

240    **Results**

241    The proportion of previous losing males that lost focal fights to tester males at 4

242    days after initial losses showed a clear direct response to selection (figure 1), with

243    a steady divergence between selection and control populations (Generation 5:

244    selection, d. f. = 1, $\chi^2$ = 21.51, $p$ < 0.001; replicate (within selection), d. f. = 4, $\chi^2$ =

245    4.27, $p$ = 0.31. Generation 10: selection, d. f. = 1, $\chi^2$ = 47.87, $p$ < 0.001; replicate

246    (within selection), d. f. = 4, $\chi^2$ = 1.11, $p$ = 0.89; figures 1 and 2ac). After 10

247    generations of selection, the proportion of males losing their second fights 4 days

248    after initial losses in the RLE populations had fallen to around 50%, whereas it was

249    always higher than 80% in control populations. Similar results were observed in

250    the proportion of previous losing males that were first attacked by tester males at

251    4 days after initial losses (Generation 5: selection, d. f. = 1, $\chi^2$ = 15.52, $p$ < 0.001;

252    replicate (within selection), d. f. = 4, $\chi^2$ = 2.12, $p$ = 0.71. Generation 10: selection, d.

253    f. = 1, $\chi^2$ = 27.69, $p$ < 0.001; replicate (within selection), d. f. = 4, $\chi^2$ = 1.18, $p$ = 0.88;

254    figure 2bd). However, males of the RLE populations had not become completely

255    immune to losing fights. In the first three days after initial fight loss, the RLE males

256    behaved much the same as control males losing second contests about 85% of the

257    time (figure 3a), while the control males did not fall to the day 4 levels of selection

258    male success (50% of fights won) until 5 days after initial losses (figure 3a).

259    Similar results were found when we compared which males attacked first, with

260    RLE males tending to become more aggressive only at day 4 and control males not

261    achieving this level of aggression until day 5 after initial losses (figure 3b). This all

262    indicates that loser effect decayed after 3 days in the RLE populations, and after 4

263    days in control populations. Thus, we found a significant difference in the duration

264    of loser effects between experimental treatments.

265

266    This rapid response to the selection indicated heritable variation in the effects

267   losing has on males. Realized heritabilities were significantly different from zero

268   for all RLE Populations [$h^2$ (± SE) - RLE I, 0.188 (0.015): RLE II, 0.179 (0.021); RLE

269   III: 0.161 (0.024). All $p < 0.001$], with 16-19% of the variation in the duration of the

270   loser effect estimated to be due to additive gene action.

271

272   Selection on the duration of the loser effect did not affect male fighting success and

273   likelihood of initiating attacks on rivals when males had no previous fighting

274   experience. Naïve males from the selection populations attacked as much and

275   won/lost as much in their initial fights as males from control populations (Initiate

276   Attacks – RLE, 0.53, 0.50, 0.57: C, 0.47, 0.43, 0.53: selection, d. f. = 1, $\chi^2$ = 0.56, $p$ =

277   0.46; replicate (within selection), d. f. = 4, $\chi^2$ = 0.89, $p$ = 0.93. Fights Lost – RLE,

278   0.43, 0.53, 0.53: C, 0.50, 0.57, 0.47: selection, d. f. = 1, $\chi^2$ = 0.02, $p$ = 0.88, replicate

279   (within selection), d. f. = 4, $\chi^2$ = 1.43, $p$ = 0.84). Furthermore body size did not

280   evolve as a correlated response to selection on loser effect duration (Body size

281   (mm± SE): RLE, 1.214 (0.006), 1.222 (0.006), 1.214 (0.005): C, 1.217 (0.007), 1.207

282   (0.005), 1.208 (0.007): selection, d. f. = 1, $\chi^2$ = 1.42, $p$ = 0.23, replicate (within

283   selection), d. f. = 4, $\chi^2$ = 2.84, $p$ = 0.58).

284

**Discussion**

Our major findings here were that the duration of loser effects can evolve, with narrow sense heritabilities of about 17%, and furthermore, the evolved, reduced duration of the loser effect was not simply due to a general loss of the effect. Additionally there appeared to be no general change in fighting ability (as measured by fighting success in first fights) or body size that evolved as correlated responses to selection on loser effect duration. We discuss these findings further below.

Perhaps the most interesting finding was that in the populations that evolved shorter loser-effect durations, fighting success in contests between naïve animals did not evolve – there was no difference in success rates between control and experimental populations. This suggests that actual fighting ability in these dyadic contests had not evolved in response to our selection, but clearly there was a reduction in the effects losing had on subsequent behaviours in the experimental populations. This contrasts somewhat with crickets were winning is associated with a broader range of fighting tactics (42), but the fact that beetle populations evolving reduced impacts of losing had not changed their fighting success (% naïve

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303  wins) only serves to highlight the differences between fighting ability and the

304  impacts of losing. Indeed, the fact that loser effects can evolve independently of

305  fighting ability establishes broad-horned flour beetle as an ideal system to test

306  formal theoretical predictions about when loser effects are expected to evolve by

307  themselves (19). That is, in testing how fighting costs and dominance benefits

308  affect the disconnect between loser and winner effects, and for example, testing

309  whether increasing variation in fighting ability within populations selects for

310  stronger loser effects as predicted by theory (9, 19). Future work could therefore

311  manipulate key parameters in different populations and quantify any concomitant

312  evolutionary change in loser effects.

313

314  Body size also did not evolve as a correlated response to selection, which given the

315  lack of change in fighting ability is arguably not surprising. Size frequently

316  determines RHP, and RHP should correlate with an individual's absolute

317  probability of winning fights (6). However, fighting ability is also associated to

318  other factors like fighting skills and physical performance (43-45). Indeed, recent

319  work has shown that fighting ability can be linked to measureable functional traits

320  such as bite force (reviewed in 43), and the loser effect is associated with a

321  decrease in bite force in the cricket *Acheta domesticus* (44). Further studies are

322  required to investigate precisely what determines fighting ability in *G. cornutus*,

323  but our results suggest that the loser effect and fighting ability of naïve (with

324  respect to fighting) males are not closely genetically linked in this species - males

325  from populations selected for reduced duration of the loser effect did not win more

326  initial fights than control males (nor where they larger), so it appears functional

327  traits linked to absolute ability did not coevolve with reduced loser effects. This

328  finding corroborates assumptions in the theoretical literature, which posit that

329  loser (and winner) effects reflect changes in subjective estimates of the

330  distribution of fighting abilities in the population but not changes in individual

331  fighting abilities *per se* (9).

332

333  While experience effects are often short-lived, they vary in their durations and

334  duration can be affected by costs and benefits of fighting and social interaction

335  frequency (2, 22). These general inferences are mirrored in a theoretical study of *G.*

336  *cornutus* fighting behaviour, which predicted that the optimal duration of the loser

337  effect would depend on the frequency of social interactions, the mating success

338  derived from fighting (benefit) and the decrease in longevity resulting from

339    fighting (cost) (28). Again, these findings all suggest effects can evolve, as we have

340    shown here. Interestingly, the heritability of the loser effect we report is on the low

341    side for a behaviour (46) and this probably reflects the fact that there are many

342    links in the causal pathway generating the effect. That is, we may have selected on

343    memory retention or metabolic rate for example, but have not directly estimated

344    the heritability of memory or metabolism. Additionally, a number of studies have

345    implicated biogenic amines such as octopamine or dopamine as neurochemical

346    mechanisms of winner/loser effects (e.g. 23, 47; reviewed in 48). Thus by selecting

347    on the duration of the loser effect we may well have altered the time course of

348    octopamine effects, or those of an octopamine agonist. We did not test for these

349    possible changes, and there are of course mechanisms other than these that could

350    be involved in generating the evolutionary change we document, including a raft of

351    other physiological and neurological processes (49, 50) that could have been

352    altered by the artificial selection we applied. We finally note that realized

353    heritabilites are only approximations of base-population heritabilities (40), and

354    that there was no evolution of effects in the control lines.

355

356    The relatively low heritability also implies that, as expected, much of the variation

357    in the loser effect is environmental. Outcomes of direct physical fights will

358    obviously depend on opponents and will provide reliable information enabling

359    self-assessment of ones own fighting ability relative to others in the population.

360    Thus the social environment and an individuals' developmental environment must

361    influence winner/loser effects to a large degree and thus contribute much to

362    phenotypic variation in these effects (e.g. 51, 52).

363

364    Behavioural modulations resulting from winning fights have not been recorded in

365    *G. cornutus* (14) even though the loser effect has a relatively long duration. This

366    matches a general pattern of effect decay, with loser effects generally lasting longer

367    than winner effects (2). For example, losing fights impacts sticklebacks for around

368    6 hours, but the winner effect has largely disappeared after about 3 hours (53).

369    From a proximate perspective, it has been suggested that this asymmetry is a

370    consequence of fundamental learning processes: losers may have more control

371    over situation outcomes (i.e. they can retreat but individuals cannot determine

372    whether a fight will occur or not as that depends on opponent behaviour) and

373    hence links (activity-outcome) are easier to establish and remember (19, 54).

374

375 Given the methodological impacts on winner/loser effect assessment, it is

376 important to note that individuals in our investigation were self-selecting (*sensu* 2)

377 (i.e. we did not randomly allocate subjects to winner/loser treatments), were

378 isolated for much of their lives and had very few encounters with competitors.

379 Each of these factors can potentially affect individual experience (2). In our beetles,

380 individuals are normally likely to encounter multiple rivals throughout their lives,

381 these multiple encounters will probably result in more complex effects, with each

382 individual experience potentially contributing to cumulative effects on future

383 contest outcomes (e.g. 55, 56).

384

385 Fighting experience effects can also impact multiple behaviours and ecological

386 processes that we did not assess here (e.g. 57, 58). Indeed, the loser effect can

387 impact various reproductive and dispersal strategy in *G. cornutus* beyond the

388 fighting outcome itself (14, 35). Similarly, theory predicts that many factors can

389 influence the strength of loser effects, including age and experience (e.g. 59,

390 reviewed in 9), and many of these are untested in flour beetles. Furthermore, the

391 effects of male experience could impact female reproductive behaviours in this

392 beetle. There are direct fitness costs imposed on females by aggressive,

393　competitively superior males (60). These males are highly aggressive towards rival

394　males (38) but also attack females (60).　Thus female fitness-costs are probably

395　side-effects of misdirected male aggression, as suggested for the dung fly, *Sepsis*

396　*cynipsea* (61). Many studies have now demonstrated that highly competitive males

397　can be harmful to females (e.g. 62-66) and in *G. cornutus,* show female

398　mate-preference may be constrained, because although females prefer males that

399　fight less, they frequently do not get to mate with them (33, 60).

400

401　To conclude, we used artificial selection to cause micro-evolution of the duration of

402　the loser effect. We also found that the reduction in response duration was not

403　associated with a change in fighting ability (as measured by the likelihood of

404　fighting success), which supports theoretical predictions. Further investigations of

405　these effects and on the precise neural/physiological mechanism underpinning the

406　outcomes of our artificial selection are warranted. Our work also suggests

407　broad-horned flour-beetles are an excellent model to explicitly test theoretical

408　predictions about the conditions under which pure loser effects are expected to

409　evolve.

410

**Competing interests**

We have no conflicts of interest.


**Authors' contributions**

KO collected data, carried out the statistical analyses, participated in the design of

the study and drafted the manuscript; YO, SD and DH conceived of the study,

coordinated the design of the study and drafted the manuscript. All authors gave

final approval for publication.

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*Figure legends*

617 **Figure 1.** Responses to selection on loser-effect duration. The proportion of males

618 with losing experience (L males) that lost subsequent fights to a tester male at 4

619 days after losing initial fights (our measure of loser effect duration). White circles,

620 are the populations where we selected for reduced duration of the loser effect

621 (Reduced Loser Effect duration: RLE). Black circles are the Control Populations

622 (C) that were not subjected to selection on the duration of the loser effect.

623

624 **Figure 2.** The proportion of males with losing experience (L males) that lost

625 subsequent fights to a tester male at 4 days after losing fight (our measure of

626 loser effect duration) and L males that were attacked first by a tester male (i.e.

627 focal males that did not initiate attacks) at 4 days after losing fight at generation 5

628 (a, b) and 10 (c, d). RLE populations are those where we selected for reduced

629 duration of the loser effect (Reduced Loser Effect duration). The Control

630 Populations (C) were not subjected to selection on the duration of the loser effect.

631

632 **Figure 3**. Loser effects at each day after losing initial fights in focal experimental

633 males – White circles, are the populations where we selected for reduced

33

634    duration of the loser effect (Reduced Loser Effect duration: RLE). Black circles are

635    the Control Populations (C) that were not subjected to selection on the duration

636    of the loser effect. (a) is the proportion of focal males that lost subsequent fights,

637    and (b) is the proportion of focal males attacked first by tester males (i.e. focal

638    males that did not initiate attacks). There was neither an effect of selection

639    regime nor replication, but there was a significant interaction between selection

640    regime and day (Selection × Day, d. f. = 4, $\chi^2$ = 30.26, $p$ < 0.001).

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642