Individual differences in developmental trajectory leave a male polyphenic signature in bulb mite populations

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Abstract

Developmental plasticity alters phenotypes and can in that way change the response to selection and affect, and be affected by, population size-structure when alternative phenotypes show different life history trajectories, in an eco-evolutionary interaction. Developmental plasticity often functions to anticipate future conditions but it can also mitigate current stress conditions, raising the question if these two kinds of developmental plasticity underlie different eco-evolutionary population dynamics. Here, we tested in a long-term population experiment using the male polyphenic bulb mite (*Rhizoglyphus robini*), if the selective harvesting of juveniles on different developmental trajectories elicits eco-evolutionary population responses that are expected if the male polyphenism is assumed to be anticipatory or mitigating. We found that the frequency of adult males that expressed costly (fighter) morphology was lowest under the most severe juvenile harvesting conditions. This response cannot be explained if we assume that adult male phenotype expression is to anticipate adult (mating) conditions because, in that case, only the manipulation of adult performance would have an effect. This response can, however, be explained by juveniles mitigating their increased mortality risk by expediating ontogeny to forego the development of costly morphology and mature quicker but as a defenceless scrambler. If, like in mammals and birds where early-life stress effects are extensively studied, we account for such pre-adult viability selection in coldblooded species, it would allow us to better characterise natural selection on trait development like male polyphenisms, how it can affect the response to other selections in adulthood, and how such trait dynamics influence, and are influenced by, population dynamics.

Keywords: alternative male phenotypes, dispersal, eco-evolutionary dynamics, male morph coexistence, polyphenism

Introduction

Across the animal kingdom, developmental plasticity comprises a switch to alternative developmental pathways that depends on an individual's condition – the resource budget available for the production and maintenance of adaptive traits (Hill 2011; Casasa et al. 2020; Nijhout & McKenna 2018) – and which leads to the expression of alternative phenotypes in adulthood (polyphenism) (West-Eberhard 2003; Nijhout & McKenna 2018). For example, many intraspecific, arthropod male dimorphisms comprise large majors that have weapons for male-male competition, and smaller, weaponless minors that sneak matings (Moczek 2003; Smallegange 2011; Buzatto & Machado 2014). Importantly, these condition-dependent alternative phenotypes typically have different survival, growth and reproduction rates (West-Eberhard 2003; Smallegange 2011; Weir et al. 2016). Differences in individual development will thus affect a population's structure, size and growth. Such population changes can in turn influence individual development through processes like density-dependent competition over resources (food) and density-dependent selection (Travis et al. 2013), creating eco-evolutionary feedbacks at the population level (Smallegange & Deere 2014; Croll et al. 2019; Smallegange 2022). However, accurately predicting the population dynamical responses to perturbation of developmental trajectories is still difficult (Smallegange et al. 2019; Smallegange 2022).

The way in which a perturbation to individual development can impact the eco-evolutionary dynamics of populations depends on what selection pressures are assumed to act on developmental plasticity (Smallegange 2022). In case of the minor and major males described above, whether a male develops into an armed major male or an unarmed minor male depends on whether it reaches a critical resource budget or body size threshold during development (Plaistow et al. 2005; Sasson et al. 2016; Cotton et al. 2004). If developmental plasticity is anticipatory, this condition-cued threshold mechanism is the evolutionary result of disruptive sexual selection on alternative mating tactics (West-Eberhard 2003; Smallegange et al. 2019): good-condition juvenile males maximise fitness by becoming majors, anticipating competitive mating success; bad-condition juveniles anticipate failure

at competitive success and salvage fitness by becoming minors (Shuster & Wade 2003). On the other hand, the condition-cued threshold mechanism could be part of a heritable developmental system that adaptively mitigates stressful environmental conditions (Badyaev 2005, Del Giudice et al. 2018; Ellis & Del Giudice 2019). Such mitigating developmental plasticity would be the result of natural selection that favours developmental systems that tend to construct phenotypes that are successful, relative to other variant systems, at surviving and reproducing (Griffiths & Gray 1994). This means that juvenile males can mitigate stress during maturation by refraining from developing costly morphology to maintain fitness and mature and survive as an adult minor, as opposed to risk dying during maturation when investing in costly major morphology (Smallegange et al. 2019). To summarise, in both kinds of plasticity, the fitness functions of the alternative male phenotypes cross over a condition gradient, but anticipatory developmental plasticity assumes that the threshold for alternative male phenotype expression at the cross-section of the fitness functions is evolutionarily regulated by how adult males perform, whereas mitigating developmental plasticity assumes that it is regulated by the dynamics of population density, food competition and individual resource budgets. Together with the fact that the condition distribution itself informs on population size (Smallegange & Coulson 2013), we have the ingredients to create predictions on how anticipatory or mitigating developmental plasticity fuel eco-evolutionary population change in response to perturbations to individual development.

We conducted a long-term population experiment to test hypotheses on how anticipatory and mitigating developmental plasticity impact eco-evolutionary population responses to the selective removal of juveniles of different condition in populations of the male polyphenic bulb mite *Rhizoglyphus robini*. Adult fighter males of the bulb mite possess a proportionally thickened third leg pair with dagger-like claws that are used in fights to kill rival males (Radwan et al. 2000). Whereas fighter males metamorphose from good-condition juveniles that have a large resource budget, nutrition-deprived juveniles that grow slow or are of worse condition when metamorphosing into an adult instead express the scrambler phenotype that does not have such leg modifications

(Rhebergen et al. 2022). Further, in response to unfavourable environmental conditions, *R. robini* expresses a facultative dispersal morph during development, called the deutonymph, which does not feed and is adapted to phoretic dispersal (Díaz et al. 2000). Deutonymph expression occurs mid-development, prior to the final instar stage from which juveniles metamorphose into adults (Díaz et al. 2000), and, thus, prior to when adult male phenotype is determined (which is immediately before metamorphosis [Rhebergen et al. 2022]). Because deutonymph expression carries developmental costs, including reduced size at metamorphosis in both sexes and reduced egg production in females (Deere et al. 2015), our working assumption is that only individuals that are of sufficiently good condition can develop into one if cued by poor environmental conditions. This assumption is supported by the fact that in two of our previous studies (Smallegange & Coulson 2011; Deere et al. 2015), male deutonymphs always matured as a fighter, from which we surmise that males always were in sufficiently good condition to develop costly fighter legs.

In our experiment, we selectively removed individuals of different conditions by removing deutonymphs, or other juveniles. Assuming that the male polyphenism is anticipatory, we predict that removing deutonymphs from the population will lower the righthand side of the distribution of individual conditions (because individuals of relatively good condition are removed), and thus population size (compare blue dotted distribution size [before harvesting] with red dashed distribution size [after harvesting] in Fig. 1c). However, we expect that male fitness is unaffected by juvenile performance and thus that the mean threshold for fighter expression will evolutionarily track the latter change in the condition distribution because relative, and not absolute condition is important in determining alternative phenotype fitness (Tomkins et al. 2011) (compare the proportions of the differently shaded areas under the condition distribution in Fig. 1a [before harvesting] with Fig. 1c [after harvesting]). Likewise, we predict that the selective removal of juveniles (bar deutonymphs) of random condition has no effect on fighter expression (compare the proportions of the differently shaded areas under the condition distribution in Fig. 1a [before harvesting] with Fig. 1e [after harvesting]), because fitness functions remain unaltered. The

reduction in population size because of the harvesting of juveniles, however, will be reflected in an overall reduced size of the condition distribution (compare blue dotted distribution size [before harvesting] with red dashed distribution size [after harvesting] in Fig. 1e).

In contrast, assuming that the male polyphenism is mitigating, the stress mechanism of the developmental system can fuel a mitigating response to increased deutonymph mortality because males can mature early as a minor to escape the juvenile stage quickly (Ernande et al. 2004), fuelling the evolution of scrambler expression. Mean fighter expression is then predicted to decrease in response to deutonymph harvesting (compare the proportions of the differently shaded areas under the condition distribution in Fig. 1b [before harvesting] with Fig. 1d [after harvesting]) and in response to the selective harvesting of other juveniles (compare the proportions of the differently shaded areas under the condition distribution in Fig. 1b [before harvesting] with Fig. 1f [after harvesting]). Further, because scramblers mature earlier in life (Smallegange 2011), live longer than fighters (Radwan & Bogacz 2000) and sire more offspring than fighters (van den Beuken et al. 2019), an increase in scrambler expression can increase population size (Smallegange & Deere 2014), and, thus, increase the size of the condition distribution ((compare blue dotted distribution size [before harvesting] with red dashed distribution size [after harvesting] in Fig. 1d and 1f). But note that the removal of good-condition juveniles when removing deutonymphs will result in the right-hand side of the distribution to be at lower condition values (compare blue dotted distribution size [before harvesting] with red dashed distribution size [after harvesting] in Fig. 1d).

121 **Methods**

122

123 *Study system*

124 The bulb mite has six life stages: egg, larva, protonymph, deutonymph, tritonymph an adult. The
125 deutonymph stage is a facultative, non-feeding dispersal stage and mites only develop into this stage
126 during unfavourable conditions (i.e. low food quality and quantity) (Díaz et al 2000). When growing
127 from one stage to the next, mites moult; this stage is known as the quiescent stage which is
128 immobile and non-feeding. Source populations were established from mites collected in 2010 from
129 flower bulbs in storage rooms in North Holland. Mites were kept on an oats diet and maintained as
130 described in Smallegange (2011). Experimental treatments were established from these source
131 populations.

132

133 *Experimental procedure*

134 The experiment ran between June 2016 and April 2017 (302 days) and comprised five treatments: (i)
135 harvesting of 100% of all deutonymphs (D100), (ii) harvesting of 50% of deutonymphs (D50), (iii)
136 harvesting of other juveniles (excluding eggs and deutonymphs) at the same percentage at which
137 deutonymphs where harvested from all juveniles in D100 (J-D100) to keep the demographic impact
138 of harvesting comparable, (iv) harvesting of other juveniles (excluding eggs and deutonymphs) at the
139 same percentage at which deutonymphs where harvested from all juveniles in D50 (J-D50), again, to
140 keep the demographic impact of harvesting comparable, and (v) no harvesting (C; general control
141 treatment). Two levels of harvesting deutonymphs (D100, D50) and juveniles (J-D100 and J-D50)
142 were chosen to be able to compare harvesting impacts. Treatments were replicated three times
143 resulting in fifteen populations. The juvenile harvesting populations were started 8 weeks after the
144 other treatments, but before harvesting commenced (Fig. 2), for logistical reasons. Populations were
145 kept in 20 mm diameter, flat-bottomed glass tubes with a plaster of Paris and powdered charcoal
146 base, which was kept moist to avoid desiccation of the mites. Tubes were sealed by a circle of very

147 fine mesh (allowing gaseous diffusion), which was held in place by the tubes' standard plastic caps
148 with ventilation holes cut into them. Populations were kept in an unlit climate chamber at 25°C and
149 >70% relative humidity.

150 Each population was established with 50 randomly selected mites from the source
151 population with founder mites, which we removed after 15 days to reduce founder effects (founder
152 mites can be easily distinguished because they are much bigger than the first generation emerging
153 after 15 days in the experimental population). Each population was fed approximately 1mg of oats
154 per day (the weight was never less than 0.9g and never exceeded 1.1g). After population
155 initialization, on each Thursday, mites of each life stage in all population were counted using a hand
156 counter at 15 X magnification. For three weeks at the start of each time period (day 85-161, day 162-
157 238 and day 239-302: see statistical analyses below), photos were taken of up to 5 individual
158 deutonymphs, fighters, scramblers, and females as proxies to assess any shifts in mean condition
159 across the different life stages in response to experimental conditions. The body length of mites on
160 each photo was measured to the nearest 0.1 µm using ZEN software blue edition (ZEISS microscopy,
161 Carl Zeiss, Germany). We measured body length for adult mites as the ventral distance of the
162 idiosoma (the white bulbous posterior part of the body; from the mouthparts to the furthest part of
163 the genitalia); for deutonymphs as the dorsal anterior-posterior length (without mouthparts); and
164 for quiescent tritonymphs as the lateral body length (without mouthparts). Harvesting treatments
165 started 85 days after populations were initialized, which is a sufficiently long time for dynamics to
166 stabilise (which typically happens at 40 days after initialisation [Cameron and Benton 2004;
167 Smallegange and Deere 2014]) (see also Fig. 1). Harvesting was done once a week (on Thursday),
168 after counting and before feeding. The harvesting treatments were applied for a total of 217 days,
169 which in a similar experiment was sufficient to observe an evolutionary shift in fighter expression
170 (Smallegange & Deere 2014). However, even if this period of time is too short to observe an
171 evolutionary response to harvesting, we can still distinguish between our anticipatory and mitigating
172 hypotheses. Specifically, under juvenile harvesting (J-100 and J-D50), fighter expression is predicted

173 to remain unaltered if it is anticipatory (Fig. 2e). However, fighter expression is predicted to decrease
174 if it is mitigating (Fig. 2f) because juvenile males can respond quickly (within a generation) to
175 increased juvenile mortality by expediating ontogeny and mature as a scrambler. This plastic
176 response, in turn, will further fuel the evolution towards developmental systems that produce
177 scramblers in response to the juvenile harvesting selection pressure.
178
179 *Life-history assays*
180 Following the end of the long-term population experiment we conducted a common garden life
181 history assay to assess if any differences in body size and fighter expression between treatments
182 were plastic or genetic. For each assay we removed ten adult females from each population,
183 individually isolated them and gave them ad lib access to oats and allowed them to lay eggs. Three of
184 the ten females (isolating more individuals was logistically not possible) were then randomly
185 selected and their offspring individually isolated, given ad lib access to oats, and followed until they
186 reached maturity. Deutonymphs, quiescent tritonymphs and adults (2-5 days after maturation) were
187 size-measured as above and their sex and morph scored (we measured a total of 420 individuals).
188 The effects of maternal nutritional conditions are negligible in this species (the effect size of
189 offspring environment is 15 times larger than that of maternal environment: Smallegange 2011a),
190 therefore, rearing mites in a common garden environment condition for one generation is sufficient
191 to eliminate maternal effects. Females and their offspring were kept in 10 mm diameter tubes with a
192 plaster of Paris and powdered charcoal base, which were kept in an unlit climate chamber at 25°C
193 and >70% relative humidity.
194
195 *Statistical analyses*
196 To analyse the results from the population experiment, we only used data from day 85 onwards
197 (when harvesting commenced) and excluded two population tubes that had fallen over during the
198 population experiment (one tube in treatment D100 and one in treatment J-D50). We then divided

199 the experimental period into three periods, day 85-161, day 162-238 and day 239-302 to assess
200 long-term (instead of transient) temporal changes in the response variables within each treatment
201 group. To analyse the results of the population experiment, we used a generalised linear mixed
202 model (GLMM) with Gaussian errors to analyse the effect of the fixed factors harvesting treatment
203 (C, D100, D50, J-D100, J-D50) and time period (period 1, 2, and 3) and their interaction, including
204 'population tube' as a random effect (to account for the repeated measures within each
205 experimental population) on: fighter expression (the number of fighters with the number of adult
206 males set as an offset so that the model prediction will be proportion of fighter males), and the
207 mean size of deutonymphs, adult fighters, scramblers and females. The model assumptions of
208 Gaussian errors and homoscedacity were confirmed by inspecting the probability plots and error
209 structures. We used a GLMM with Poisson errors to analyse the effects of the fixed factors
210 harvesting treatment and time period, their interaction, with 'population tube' as a random effect,
211 on the number of deutonymphs and on total population size.
212 To analyse the results of the life history assay conducted at the end of the population
213 experiment, we used a GLMM with Gaussian errors to analyse the effect of the fixed factor
214 harvesting treatment (C, D100, D50, C-D100, C-D50), including 'population tube' and 'maternal
215 identity' as random effects on the mean size of adult fighters, scramblers and females. The model
216 assumptions of Gaussian errors and homoscedacity were again confirmed by inspecting the
217 probability plots and error structures. We used a GLMM with binomial errors to analyse the effect of
218 the harvesting treatment on fighter expression ('0' if a male developed into a scrambler, and '1' if in
219 a fighter), including 'population tube' and 'maternal identity' as random effects.
220 In each GLMM, the significance of the treatment effects was tested using a model
221 simplification procedure, whereby the full model was fitted after the least significant term had been
222 removed (never deleting random terms from the model), if the deletion caused an insignificant
223 increase in deviance as assessed by a maximum likelihood ratio test (LRT). Pairwise comparisons
224 among the treatment levels were conducted by creating a reduced model within which two levels

225 were combined. If the reduced model caused an insignificant increase in deviance as assessed by a
226 maximum likelihood ratio test (α = 0.05), then the two levels were considered to be not significantly
227 different. All analyses were conducted in R and for all GLMMs we used the *lme4* package (www-r-
228 project.org).
229
230 **Results**
231
232 *Population size, fighter expression and number of deutonymphs*
233 Mean total population size had stabilised across all treatments at the start of harvesting (Fig. 2) but
234 was significantly affected by harvesting (χ^2_4 = 15.58, p=0.004) and differed between time periods (χ^2_2
235 = 262.07, p<0.001) (non-significant interaction: χ^2_8 = 7.30, p=0.063). Population size was highest
236 when 100% of deutonymphs were harvested (D100), lower when 50% of deutonymphs were
237 harvested (D50), and lowest in all the other treatments (C, J-D50 and J-D100) (treatments C and J-
238 D50 did not differ significantly (χ^2_1 = 0.35, p=0.553), and neither did C and J-D50 differ significantly
239 from J-D100 (χ^2_1 = 0.47, p=0.494)) (Fig. 3A). Irrespective of harvesting treatment, total population
240 size significantly increased over time (Fig. 3B).
241 The harvesting treatment significantly affected the proportion of fighters but differently at
242 different time periods (significant interaction harvesting $\times$ time period: Σ^2_8 = 29.92, p<0.001). Within
243 the interaction, the harvesting treatments C and J-D50 did not significantly differ in their effect on
244 proportion of fighters over time (χ^2_3 = 4.22, p=0.239) and were combined into one level. The next
245 two similar responses to the harvesting treatments J-D100 and D50 did, however, differ significantly
246 (χ^2_3 = 11.80, p=0.008) (Fig. 3C). In summary, the proportion of fighters was always low when 100% of
247 deutonymphs were harvested (D100 treatment) (Fig. 3C), but when other juveniles were harvested
248 at the same percentage as in D100, (J-D100), and when harvesting 50% of deutonymphs (D50), the
249 proportion of fighters significantly decreased towards the end of the experiment (Fig. 3C). In

250 contrast, under the combined harvesting treatments of C and J-D50, the proportion of fighters was
251 always high over the whole course of the experiment (Fig. 3C).
252 The harvesting treatment also significantly affected the number of deutonymphs, but, again,
253 differently at different time periods (significant interaction harvesting × time period: χ^2_8 = 49.70,
254 p<0.001). Within the interaction, the harvesting treatment levels J-D100 and C did not significantly
255 differ (χ^2_3 = 0.40, p=0.940) and were combined into one level. Likewise, the latter combined level did
256 not significantly differ from the J-D50 harvesting treatment (χ^2_3 = 3.08, p=0.380) and all three control
257 levels were combined into one level. These three levels, combined, significantly differed from the
258 D50 harvesting level (χ^2_3 = 12.42, p=0.006) and from the D100 harvesting level (χ^2_3 = 67.71, p<0.001).
259 We therefore find that, counterintuitively, the number of deutonymphs significantly increased over
260 time when all deutonymphs were harvested (D100), and, to a lesser extent, when 50% of
261 deutonymphs were harvested (D50) (Fig. 3D). In contrast, in the other treatments combined, the
262 number of deutonymphs slightly decreased over the course of the experiment (Fig. 3D).
263 Furthermore, at any given time period, the number of deutonymphs was always the highest in the
264 treatment where weekly harvesting removed all deutonymphs (D100) (Fig. 3D).
265
266 *Deutonymph, fighter, scrambler and adult female mean size*
267 Mean deutonymph size was not significantly affected by harvesting (χ^2_4 = 4.81, p=0.307) but
268 significantly decreased over the course of the experiment (significant effect of time period: χ^2_2 =
269 106.31, p<0.001) (Fig. 4A) (non-significant interaction: χ^2_8 = 7.22, p=0.513). Mean fighter size also
270 decreased over time but size differed significantly between the different harvesting treatments
271 (significant interaction harvesting × time period: Σ^2_8 = 19.44, p=0.013) (Fig. 4B). Within the
272 interaction, fighter size in the harvesting treatment levels J-D50 and J-D100 did not significantly
273 differ (χ^2_3 = 2.33, p=0.507) and were combined into one level (Fig. 4B). Similarly, fighter size in the
274 harvesting treatment levels C and D50 did not significantly differ (χ^2_3 = 1.83, p=0.609) and were
275 combined into one level (Fig. 4B). The overall result was that mean fighter size in the combined

levels J-D50 and J-D100 and in the combined levels C and D50 decreased over time, whereas mean fighter size in the D100 treatment did not decrease until the last time period (Fig. 4B). Mean scrambler size was not significantly affected by harvesting (χ^2_4 = 1.17, p=0.883) but significantly decreased over the course of the experiment (significant effect of time period: χ^2_2 = 96.44, p<0.001) (Fig. 4C) (non-significant interaction: χ^2_8 = 9.48, p=0.303). Finally, mean adult female size was also not significantly affected by harvesting (χ^2_4 = 0.62, p=0.961) but significantly decreased over the course of the experiment (significant effect of time period: χ^2_2 = 67.65, p<0.001) (Fig. 4D) (non-significant interaction: χ^2_8 = 14.55, p=0.068).

Life history assay

Neither fighter size (Σ^2_4 = 6.58, p=0.160, mean size: 439.76 μm ± 2.39 SE, n = 190), scrambler size (Σ^2_4 = 2.77, p=0.597, mean size: 401.07 μm ± 7.33 SE, n = 14) or female adult size (Σ^2_4 = 3.75, p=0.440, mean size: 334.66 μm ± 1.65 SE, n = 206) were affected by the harvesting treatments in the common garden life history assay. Fighter expression was also not significantly affected by harvesting treatment (Σ^2_4 = 0.51, p=0.973). However, only 14 scramblers emerged in the life history assay (as opposed to 190 fighters), prohibiting any statistical inference. Nevertheless, it is noteworthy that mean scrambler size was lowest (Fig. 5A) and mean fighter expression was highest (Fig. 5B) for those treatments where fighter expression in the long-term population experiment was also highest (control treatment C and juvenile harvesting treatment J-D50) (Fig. 3C). Similarly, mean scrambler size was highest (Fig. 5A) and mean fighter expression lowest (Fig. 5B) for those treatments where fighter expression in the long-term population experiment was also relatively the lowest (deutonymph harvesting treatments D100 and D50 and juvenile harvesting treatment J-D100) (Fig. 3C).

299 **Discussion**

300 We tested two hypotheses on how developmental plasticity impacts the eco-evolutionary response
301 of male polyphenic bulb mite populations to the selective removal of individuals of different
302 developmental stages. If male alternative male phenotype expression is taken to be anticipatory
303 (anticipating future mating success), we predicted that the selective harvesting of deutonymphs and
304 other juveniles would not impact fighter expression. In contrast, if alternative male phenotype
305 expression is taken to be mitigating, we predicted that the selective harvesting of deutonymphs and
306 other juveniles would decrease fighter expression both plastically, as male juveniles can expediate
307 development and escape the juvenile stage quicker by maturing as a scrambler, and evolutionarily,
308 as the selective removal of juveniles fuels the evolution of developmental systems that produce
309 scramblers. In line with the mitigating hypothesis, we found that fighter expression was overall
310 lowest when 100% of all deutonymphs were harvested (D100), and, at the end of the experiments,
311 had reached similarly low levels in the treatments where 50% of deutonymphs were harvested (D50)
312 and where the highest number of other juveniles were harvested (J-D100). In contrast, fighter
313 expression remained high throughout the experiment in the control treatment and in the treatment
314 where the lowest number of juveniles were harvested (J-D50), perhaps because, there, the
315 harvesting impact was the lowest. Our life history assay at the end of the population experiment did
316 not have sufficient statistical power to tell if these shifts in fighter expression were (in part)
317 evolutionary. However, it is striking that the harvesting treatments where we observed the lowest
318 levels of fighter expression in the population experiment are the same as where we observed the
319 lowest fighter expression levels in the life history assay. But even if this pattern was coincidental and
320 not indicative of an evolutionary change in fighter expression, our findings still point to a role for
321 mitigating developmental plasticity. That is, we expected no shift in fighter expression at all in
322 response to the harvesting of juveniles (bar deutonymphs) under anticipatory developmental
323 plasticity (Fig. 1e), but a reduction in fighter expression under mitigating developmental plasticity
324 (Fig. 1f) because individuals mature earlier as a scrambler to escape the risky juvenile stage. The

325 latter reduction is indeed what we observed towards the end of the experiment in the treatment
326 where we harvested the highest number of juveniles other than deutonymphs (J-D100). Our results
327 therefore suggest that nutrition-deprived male juveniles do not 'make the best of a bad job' by
328 anticipating a non-fighting mating tactic (Dawkins 1980; Eberhard 1982), but instead develop into
329 that male adult phenotype because they can adaptively mitigate their early-life stress (Rhebergen
330 2022). Such adaptive early-life stress responses that have negative effects in adulthood are common
331 in (warm-blooded) mammals and birds (Lindström 1999; Fisher et al. 2006; Wells 2011; Vickers
332 2014), likely because their growth during development is physiologically constrained. However, if we
333 account for such pre-adult viability selection in coldblooded species that typically show flexible
334 growth, it would allow us to better characterise natural selection on trait development like male
335 polyphenisms in these species, how it can affect the response to other selections in adulthood (e.g.,
336 sexual selection: Mojica and Kelly 2010; Lürig & Matthews 2021; Rhebergen 2022), and how such
337 trait dynamics influence, and are influenced by, population dynamics (Smallegange 2022).
338 One unexpected finding in our experiment was that the number of deutonymphs was
339 highest when we harvested all of them, each week. Further, despite being harvested, deutonymph
340 number increased as the experiment progressed. We can only speculate why this happened.
341 Perhaps this overcompensatory response was because the per capita food availability increased as
342 we removed individuals (Verhulst 1838, Schaefer 1954, Hilborn and Walters 1992), although we still
343 only maximally removed a small percentage of the total population in the deutonymph harvesting
344 treatments. Furthermore, initiation of the deutonymph stage in this species is due to poorer
345 resource availability and so our findings are unlikely due to an increase in per capita food availability.
346 The higher number of deutonymphs we found could also reflect eco-evolutionary dynamics in which
347 another selection pressure, for example imposed by strong-density dependence (population size was
348 highest in the 100% deutonymph harvesting treatment), opposed the effects of direct harvesting
349 (Edeline & Loeuille 2021). Failure to account for either of these eco-evolutionary processes might
350 fundamentally hamper our ability to understand the expression of dispersal phenotypes. Another

351 explanation is that it was the actual lack of deutonymphs and their olfactory cues that triggered
352 deutonymph development. Olfactory cues are common in acarine mites. All acarines have a sensory
353 site on their forelegs known as the "foretarsal sensory organ" and olfaction is one of this site's
354 functions (Carr & Roe 2016). If the harvesting of deutonymphs reduced the concentration of
355 deutonymph olfactory cues, this could trigger more juveniles to develop into a deutonymph
356 because, with fewer deutonymphs around, competition for attachment sites on insects is lower
357 (deutonymphs disperse via phoresy on insects [Díaz et al 2000]). We realise, however, that this is
358 speculation and olfactory cues produced by deutonymphs have yet to be tested in *R. robini*. Finally,
359 in our previous studies (Smallegange & Coulson 2011; Deere et al. 2015), male deutonymphs always
360 matured as a fighter, but here, increases in deutonymph numbers did not coincide with increases in
361 fighter frequency. Perhaps this was because twice as many females emerge from deutonymphs than
362 males (fighters) (Deere et al. 2015; Stewart & Smallegange in prep) so that our statistical analyses
363 were unable to pick up significant differences in the few more fighters that emerged. It does
364 highlight, however, that we still have some way to go to unravel the intricate links between
365 deutonymph expression, alternative male phenotype expression and sex ratio and how they
366 together drive population dynamics.

367 Because a change in the distribution of a trait will almost always also change the size of the
368 distribution of that trait, it follows that trait change is accompanied by a change in population size. If
369 a trait is heritable, this constitutes eco-evolutionary change at the population level (Smallegange &
370 Coulson 2013). This is especially so in the case of alternative phenotypes where we expect a shift in
371 phenotype expression to be accompanied by a change in population size because the different
372 phenotypes often have different demographic rates. For example, in the case of bulb mite males,
373 scramblers mature earlier in life (Smallegange 2011), live longer than fighters (Radwan & Bogacz
374 2000) and sire more offspring than fighters (van den Beuken et al. 2019) so that an increase (or
375 decrease) in scrambler (or fighter) expression will increase population size. Indeed, we found the
376 highest population size in the harvesting treatment with the lowest level of fighter expression (D100:

100% deutonymph harvesting). We suspect that the increase in population size that we observed over the course of the experiment is associated with the observed decrease in mean body size over time in response to the strong, density-dependent food conditions, because smaller organisms typically reproduce faster and can reach higher carrying capacities than larger ones (e.g., Huryn & Benke 2007). Indeed, Gilbert et al. (2022) recently showed in an experiment with the protist *Tetrahymena pyriformis* that mean body size decreased in response to increased density-dependence and that this rapid change in mean body size had a larger effect on population density than the reverse. Reductions in mean body size have been observed across a range of taxa in response to adverse environmental change (Gardner et al. 2011; Sheridan & Bickford 2011). Often, these reductions are associated with increased mortality rates (e.g., in red knots *Calidris canutus* [van Gils et al., 2016]). What is more, when populations not only suffer from directional environmental change but also from selective harvesting, the removal of good condition individuals (of large size) can lead to population extinction (Knell & Martínez-Ruiz 2017). Thus, unravelling the intertwined ecological and evolutionary dynamics of population size, body size, condition and condition-dependent (alternative) phenotypes is integral to understanding eco-evolutionary population responses to change (Edeline & Loeuille 2021).

Data availability statement

The raw data and the R scripts that we used to analyse the data can be found on Figshare (Deere & Smallegange 2023).

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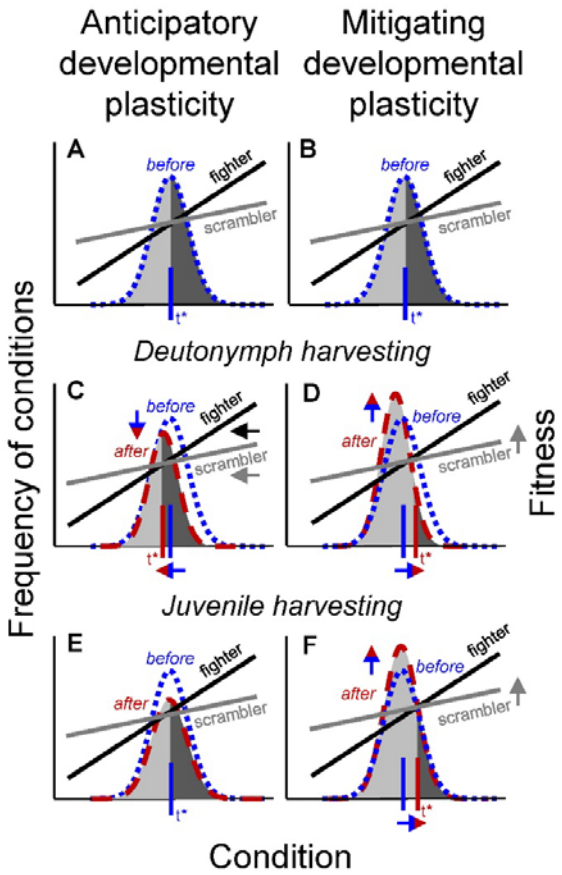


Figure 1. Eco-evolutionary changes due to the selective harvesting of deutonymphs and other juveniles. Fitness functions of fighters (black lines) and scramblers (grey lines) cross over a condition gradient; the area under each condition distribution curve represents population size. The proportion of individuals adopting alternative phenotypes (contrastingly shaded areas under the curves: light grey for scramblers and dark grey for fighters) is determined by the threshold (t^*) condition value at the intersection of the fitness functions (indicated by a vertical line). Blue dotted lines and red dashed lines represent condition distributions before (**a,b**) and after harvesting (**c-f**), respectively. **(c)** Although the selective harvesting of deutonymphs will reduce the size of the condition distribution, anticipatory developmental plasticity predicts that the fitness functions, and, thus, the threshold (t^*) for fighter expression, will track the change in the condition distribution and remain in the same, position relative to the condition distribution. In this example, the threshold tracks the mean of the condition distribution. (**e**) Assuming that the harvesting of juveniles reduces population size but does not change mean condition, the threshold (t^*) for fighter expression remains unaffected in case of anticipatory developmental plasticity. (**d,f**) In contrast, the stress mechanism of a developmental system can facilitate the mitigating response to increased deutonymph or juvenile mortality, because males can mature early as a minor to escape the juvenile stage quickly. This will increase scrambler fitness, fuelling the evolution of scrambler expression to a higher threshold (t^*) for fighter expression. Additionally, because scramblers mature earlier in life (Smallegange 2011), live longer than fighters (Radwan & Bogacz 2000) and sire more offspring than fighters (van den Beuken et al. 2019), an increase in scrambler expression can increase population size (Smallegange & Deere 2014), and thus increase the size of the condition distribution (**d,f**).

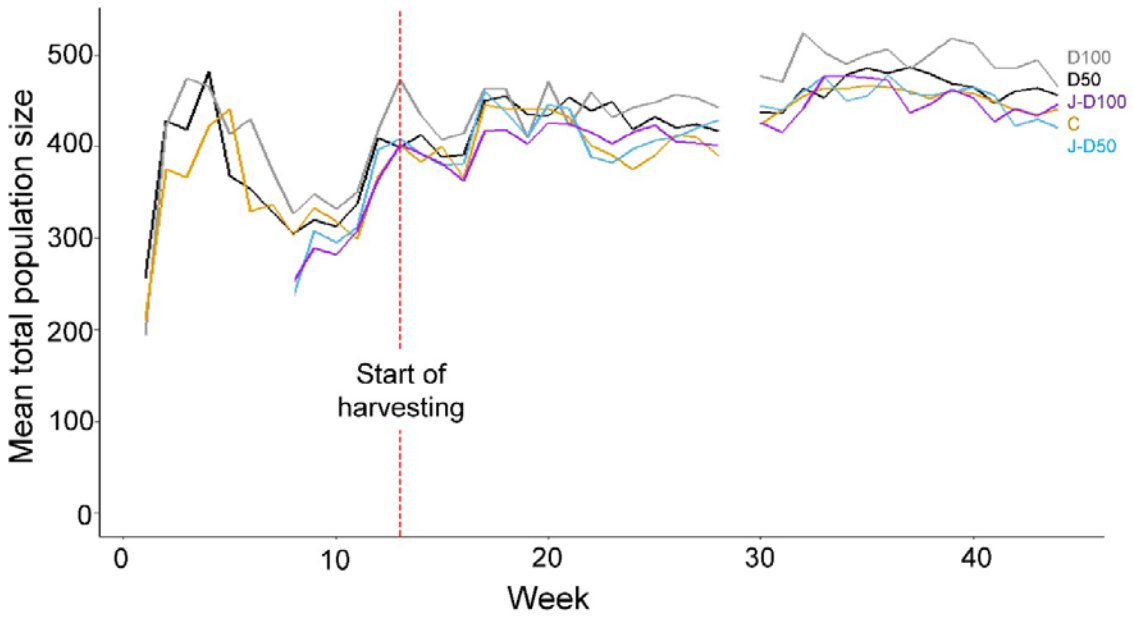


Figure 2. Time series of mean total population size, averaged per harvesting treatment. The treatments were: harvesting of 100% of all deutonymphs (D100: grey line), harvesting of 50% of deutonymphs (D50: black line), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which deutonymphs where harvested from all juveniles in D100 (J-D100: purple line), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which deutonymphs where harvested from all juveniles in D50 (J-D50: indigo line), and no harvesting (C; control treatment: orange line).The harvesting treatments began in week 13 of the experiment. No data were collected in week 29 of the experiment due to illness.

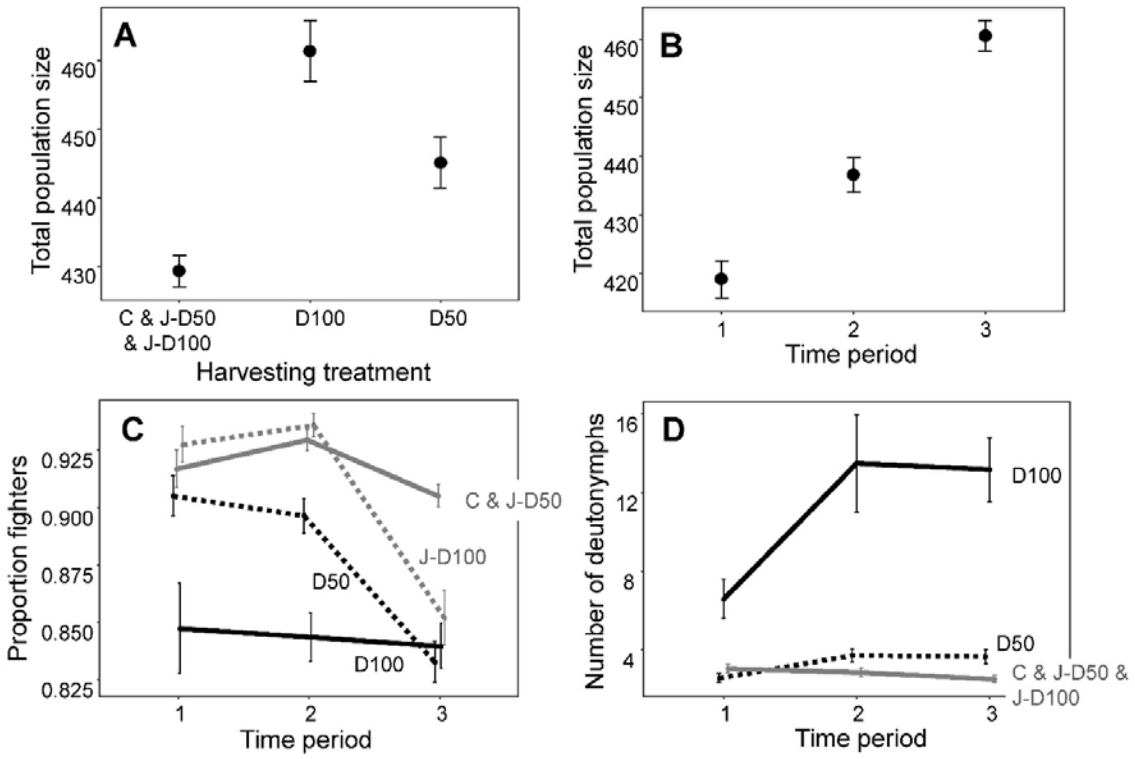
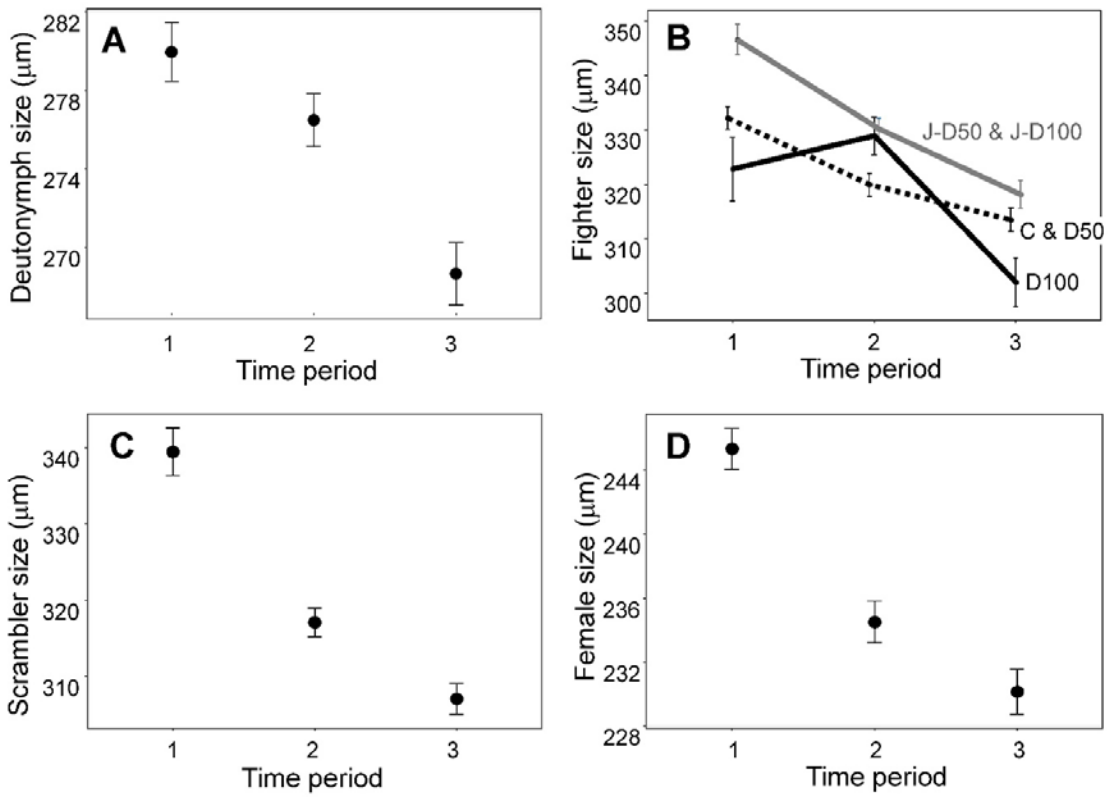


Figure 3. Population size and structure of the long-term population experiment. Mean total population size differed between different harvesting treatments **(a)** and between the different time periods **(b)**. Fighter expression varied over time in most but not all harvesting treatments **(c)**. The number of deutonymphs also varied over time in some but not all harvesting treatments **(d)**. Errors in all panels are standard errors. Harvesting treatments were: harvesting of 100% of all deutonymphs (D100: solid black lines), harvesting of 50% of deutonymphs (D50: dashed black lines), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which deutonymphs where harvested from all juveniles in D100 (J-D100: grey dashed line (c) and solid grey line (d)), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which deutonymphs where harvested from all juveniles in D50 (J-D50: solid grey lines), and no harvesting (C; control treatment: solid grey lines).



552

553 **Figure 4. Mean body sizes observed in the long-term population experiment.** Mean deutonymph
554 size (µm) **(a)**, mean fighter size (µm) **(b)**, mean scrambler size (µm) **(c)** and mean female size (µm) **(d)**
555 all significantly decreased over the course of the experiment, but for fighters, this decrease differed
556 between harvesting treatments (d). Errors in all panels are standard errors. Harvesting treatments
557 were: harvesting of 100% of all deutonymphs (D100: solid black line), harvesting of 50% of
558 deutonymphs (D50: dashed black line), harvesting of other juveniles (excluding deutonymphs) at the
559 same percentage at which deutonymphs where harvested from all juveniles in D100 (J-D100: grey
560 solid line), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which
561 deutonymphs where harvested from all juveniles in D50 (J-D50: grey solid line), and no harvesting (C;
562 control treatment: dashed black line).

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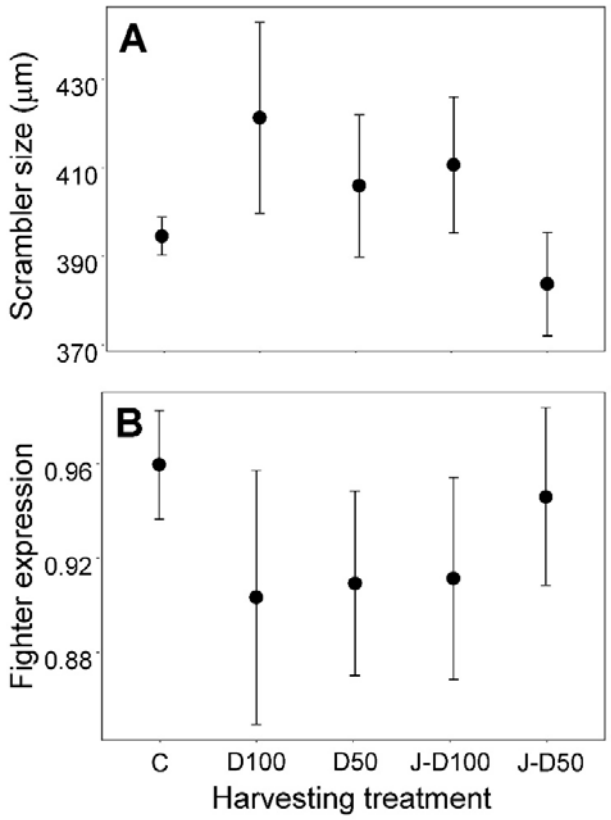

Figure 5. Life history assay results. Mean scrambler size (µm) **(a)** and mean fighter expression **(b)** for each harvesting treatment. Errors in all panels are standard errors. Harvesting treatments were: harvesting of 100% of all deutonymphs (D100), harvesting of 50% of deutonymphs (D50), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which deutonymphs where harvested from all juveniles in D100 (J-D100), harvesting of other juveniles (excluding deutonymphs) at the same percentage at which deutonymphs where harvested from all juveniles in D50 (J-D50), and no harvesting (C; control treatment).