1   # Life history evolution in response to changes in metapopulation

2   # structure in an arthropod herbivore

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Abstract

1. *The persistence and dynamics of populations largely depends on the way they are configured and integrated into space and the ensuing eco-evolutionary dynamics.*

2. *We manipulated spatial and temporal variation in patch size in replicated experimental metapopulations of the herbivore mite* Tetranychus urticae *and* followed *evolutionary dynamics over approximately 30 generations.*

3. *A significant divergence in life history traits, physiological endpoints and gene expression was recorded in the spatially and spatiotemporally variable metapopulation, but also a remarkable convergence relative to the stable reference metapopulation in traits related to size and fecundity and in its transcriptional regulation.*

4. *The observed evolutionary dynamics are tightly linked to demographic changes, more specifically frequent episodes of resource shortage* that *increased the reproductive performance of mites on tomato, a challenging host plant. This points towards a general, adaptive stress response in stable spatial variable and spatiotemporal variable metapopulations that pre-adapts a herbivore arthropod to novel environmental stressors.*

Introduction

49 Changes in land-use levy a strong pressure on our natural habitat and leads to habitat loss

50 and isolation, which are both a major thread for biodiversity (Dirzo & Raven 2003). Species

51 conservation therefore relies largely on optimal reserve planning which in turn is rooted

52 within the principles of metapopulations (Levins 1969; Hanski 1998). This concept defines

53 population-extinction dynamics and eventually extinction thresholds within in networks of

54 interconnected habitat patches. Most spatially structured populations can be classified as

55 *patchy* or *mainland-island* metapopulations (Harrison & Taylor 1997), and the omnipresence

56 of classical Levin's metapopulations has been recently questioned (Fronhofer *et al.* 2012).

57 This spatial variation in habitat availability not only affects patch occupancy dynamics, it also

58 impacts the local- and metapopulation-level demography. Some of us demonstrated

59 experimentally that spatial variation in habitat availability decreases variance in

60 metapopulation size at the metapopulation level (De Roissart, Wang & Bonte 2015).

61 Conversely, spatiotemporal variation in habitat availability increases patch extinction rates,

62 but decreases local population and metapopulation sizes. These demographic changes

63 minimised metapopulation-level variability in mainland-island metapopulations, relative to

64 classical and patchy ones.

65

66 Because of these changes in population dynamics, selection pressures in metapopulations

67 are expected to act on more than one level of population structure (Olivieri, Couvet &

68 Gouyon 1990). For instance, in metapopulations where local population extinctions occur

69 regularly, increased dispersal rates are selected relative to metapopulations where

70 extinctions are rare or where patch sizes or heterogeneous, since long-term survival is only

71 possible if genotypes are able to re-colonise patches from where they have become locally

72 extinct. With increasing asymmetry in patch size, however, dispersal will evolve to lower

73 rates because benefits of dispersal are only prevalent for a minority of the individuals (Travis

74 & Dytham 1999). Additional and/or alternative adaptive strategies might also evolve through

75 the adjustment of sex-ratio (Macke *et al.* 2011), age-at-death (Dytham & Travis 2006) and

76 density-dependency (Bierbaum, Mueller & Ayala 1989) according to changes in spatial

77 structure and associated variation in the prevalence and strength of local resource

78 competition (Clark 1978; Cameron *et al.* 2013, 2014) and other stressors (Margulis & Sagan

79 2000; Parsons 2005).

80

81　While evolutionary theory to date is centred on single trait dynamics, multivariate selection

82　in life history and physiology is anticipated in response to changes in spatial habitat

83　configuration. These evolutionary responses then simultaneously feedback on the ecological

84　dynamics, rendering both ecology and evolution heavily intertwined. We now begin to

85　understand such eco-evolutionary dynamics in either natural or experimental

86　metapopulations (Bell & Gonzalez 2011). The importance of eco-evolutionary dynamics is

87　most obvious in metapopulations where dispersal determines the genetic composition and

88　demography of different populations (Kokko & Lopez-Sepulcre 2007). Seminal examples

89　include the Glanville fritillary (Hanski and Mononen 2011) or stick insect metapopulations

90　(Farkas *et al.* 2013). Often, these eco-evolutionary dynamics lead to evolutionary rescue, the

91　process where adaptive evolution allows a population (Gomulkiewicz & Holt 1995),

92　metapopulation (Bell & Gonzalez 2011; Travis *et al.* 2013) or an expanding population

93　(Boeye *et al.* 2013) to recover from negative growth as a result from environmental change

94　(Gomulkiewicz & Holt 1995). Evolutionary rescue is known to be strongly determined by

95　demographic and genetic factors of local populations, but also by entire metapopulation

96　changes (Carlson, Cunningham & Westley 2014).

97

98　The genetic basis of life history differentiation can now be disentangled by the development

99　of several –omic approaches. Transcriptomic analyses may uncover genes that significantly

100　alter their transcript levels as a response to the implemented selection pressure and provide

101　detailed insights on the pleiotropic effects underlying phenotypic. For instance, in *Drosophila*

102　*melanogaster,* many genes that are up- or downregulated in response to stress are equally

103　associated with mobility and aggression (Wheat 2012). In the spider mite *Tetranychus*

104　*urticae*, transcriptomic analysis of populations that developed pesticide resistance or that

105　were exposed to challenging host plants reveals the presence of common adaptive

106　responses and identified key candidates genes for xenobiotic adaptation in this polyphagous

107　mite (Dermauw *et al.* 2013)

108

109　Experimental evolution in artificial metapopulations provides a unique formal test to

110　understand to which degree spatial variation in habitat availability affects life history

111　divergence (Kawecki *et al.* 2012). We installed three types of experimental metapopulation

4

112   inhabited by spider mites (*T. urticae;* Fig. 1): a stable metapopulation consisting of patches of

113   similar size (the patchy metapopulation; HOM), a metapopulation where habitat quality

114   varies in space and time (the classical metapopulation; TEM) and a metapopulation where

115   patches are of different size (the mainland-island metapopulation; SPA). We earlier

116   documented how this variation in spatial structure affected demographic changes (De

117   Roissart *et al.* 2015) and now report on the evolutionary phenomic changes as measured at

118   the end of that experiment. Following the observed changes in demography and theoretical

119   expectations outlined above, we predicted that:

120

121   1. Asymmetry in patch and population size in mainland-island metapopulations selects

122   for lower dispersal rates, while in classical metapopulations, the spatiotemporal

123   variation in population size leads to higher evolved dispersal rates

124   2. Increased local extinction rates and local population variability in classical

125   metapopulation selects for faster life histories, i.e. increased fecundity, decreased

126   longevity and/or reduced developmental time

127   3. Low local and metapopulation variability in mainland-island metapopulations

128   increase local competition and therefore select for a more male-biased sex ratio

129   and/or increased developmental time (age-at-maturity).

130 Materials and methods

131 Experimental setup of the artificial metapopulations

132 Metapopulation dynamics of *Tetranychus urticae* were studied using experimental

133 microcosms. We used as a base population the "LS-VL" *T. urticae* strain, because it is known

134 to be highly evolvable due to its genetic variability (Van Leeuwen *et al.* 2008; Fronhofer,

135 Stelz & Lutz 2014). Artificial metapopulations consisted of a transparent plastic box with 9

136 patches arranged in a 3 x 3 lattice. We constructed three types of artificial metapopulations

137 with an equal metapopulation-level carrying capacity but varying spatial configuration of the

138 patches. Patches were detached bean (*Phaseolus vulgaris L.*) leaves placed on a Tanglefood

139 layer in closed boxes. This hostile matrix prevents mites from leaving the patches. Bean

140 leaves were renewed on a weekly basis to avoid starvation of the mites. The size of the bean

141 leaves introduced to each patch was dependent upon the treatment. Two times a week, for

142 8 hours a wind current (1.5m/s) was induced by a fan and allowed aerial dispersal. Three

143 metapopulation types were installed each of which was replicated three times. With

144 exception of the HOM metapopulation, all replicates are similar regarding the specific

145 attributes but different in exact spatial configuration. The three metapopulation types are:

146

147   i)   a patchy metapopulation consisting of nine patches weekly refreshed with leaves

148   of 20 cm² (spatially homogenous distribution of resources; further referred to as

149   HOM)

150   ii)   a mainland-island metapopulation consisting of three patches of standard leaf

151   size (20 cm²) and three of double size; another three patches of these

152   metapopulations remained constantly empty (spatial heterogeneous distribution

153   of resources; further referred to as SPA)

154   iii)   a spatiotemporal heterogeneous metapopulation (further referred to as TEM) in

155   which we assigned nine single-patch resources (standard leaf) randomly to one of

156   the nine patches. Due to this algorithm, the distributions of the resources (and

157   thus local carrying capacity or island size) changed weekly among the nine

158   patches and varied between zero (no resource renewal and local extinction) and

159   double or exceptionally triple island size. In consequence, patch sizes and thus

160   local carrying capacities fluctuated over time and space, but we ensured again a

161   constant metapopulation carrying capacity (9 x 20 cm²) over time.

162

163   At the beginning of the experiment, 20 randomly collected adult female mites, from the base

164   population, were assigned to each patch within each metapopulation type and allowed to

165   establish the triplicated populations. All metapopulations were kept under controlled

166   conditions (23°C, 16:8 LD photoperiod, 85% humidity).

167

168   **Quantification of mite life-history**

169   Spider mite life-history traits were measured at the initiation of the experiment and after 10

170   months, corresponding to approximately 30 mite generations. All traits were measured on

171   F2 mites (raised for two generations in common garden on detached leaf discs) to minimise

172   maternal and environmental effects caused for instance by local conditions of crowding.

173   Young inseminated females of each experimental metapopulation were individually allowed

174   to oviposit on bean leaf discs. Leaf discs were placed with the abaxial part upwards on

175   moistened filter paper to prevent mites from escaping and to maintain leaf turgor. Different

176   life history parameters of the descendants were recorded daily: juvenile survival,

177   developmental time (time from egg until the adult stage), fecundity (daily number of eggs),

178   adult longevity and sex-ratio. Since spider mites deposit the majority of their eggs during the

179   first seven days after maturity, we monitored fecundity only during that period. Dispersal

180   propensity of the mites was assessed by transferring mated females to test arenas for trials

181   of aerial dispersal (after two whole generations under common garden to avoid confounding

182   maternal effects). The experimental setup for aerial dispersal assessment was identical to

183   the one applied in (Li & Margolies 1994) (details in Appendix S1 in supporting information).

184

185   **Mite performance**

186   Mite performance was followed by quantifying rate of intrinsic growth as a proxy of fitness

187   (Cameron *et al.* 2013). To detect possible differences in individual performance between

188   treatments, an integrated individual-level fitness measure, the rate of intrinsic growth (r_m),

189   was calculated by combining the estimated parameter distributions of the different life

190   history parameters according (see statistical analyses) to the equation $\Sigma\, e^{-(r_m)x}\, l_x f_x = 1$ (with l_x

191   survival till maturity x and f_x the number of female offspring at age x) which represents the

192   contribution of each female to the number of females in the subsequent generation. We

193   performed 10000 simulations and reported the mean value and standard deviation while

194   testing its significance in comparing whether 2.5% tails of the distribution overlap. We

195   additionally measured a set of physiological endpoints (mass, glucose, trehalose and

196   triglycerid levels) after the common garden treatment at the start of the experiment and

197   after 30 generations of selection in a metapopulation context as indicators for mite

198   performance. All physiological parameters were measured following (Laparie *et al.* 2012) on

199   F2 mites (Appendix S1 in supporting material).

200

201   **Differential gene expression after experimental evolution**

202   To examine the effects of metapopulation structure on the mite transcriptome, Agilent dual

203   colour gene expression micro-array analysis was performed on female mites raised for two

204   generations in a common garden of every selection regime. The microarray data have been

205   deposited in the Gene Expression Omnibus (GEO) (accession number: GSE55623). For the

206   hierarchical clustering, data of previous *T. urticae* studies were incorporated (Bryon *et al.*

207   2013; Zhurov *et al.* 2014). Final statistical processing and analysis was conducted in limma

208   (Smyth 2005). Gene Ontology (GO) annotation was executed using Blast2GO software

209   (Conesa *et al.* 2005). Using the Blast2GO generated annotation and the statistical output of

210   limma as input, Gene Set Analysis (GSA) was performed with the Bioconductor package

211   piano (Parametric Analysis of Gene set Enrichment, PAGE)(Väremo, Nielsen & Nookaew

212   2013). More details of the gene expression and GO-term analysis are provided in Appendix 1

213   from Supporting Material.

214

215   **Performance on a challenging new host**

216   Our LS-VL base population has been maintained on bean for more than 10 years. We

217   assessed performance on a novel suboptimal host by quantifying isofemale growth rate on

218   tomato (*Solanum lycopersicum;* variety Moneymaker) grown under controlled laboratory

219   conditions (23°C, 16:8 L:D photoperiod). Experimental arenas were constructed with leaves

220   from 4-week old tomato plants. Moist tissue paper was used to cover 10 cm² leaf edges that

221   prevented mites from escaping. Twenty fertilized F2 females (raised for two generations in

222   common garden to reduce maternal and environmental effects) from each artificial

223   metapopulation were placed on a leaf-arena and allowed to establish a population. All leaf-

224   arenas were kept under controlled conditions (23°C, 16:8 L:D photoperiod). Population

8

225   growth was assessed weekly for 3 weeks by counting the number of eggs, juveniles, adult

226   males and females.

227

228   **Statistical analysis**

229   We first tested for overall multivariate divergence in life history after experimental evolution

230   and subsequently used GLMM to test the univariately according to the imposed treatments .

231   The measured traits follow different statistical distributions and therefore applied a

232   Permutational Multivariate Analysis of Variance (PERMANOVA). Because our measurements

233   were taken with different units on different scales, the correctly estimated replicate-level

234   averages of the life history and physiological endpoints (see GLMM further) were scaled

235   prior to PERMANOVA analysis based on Euclidean distances among replicates belonging to

236   one of the three metapopulation treatments (PERMANOVA; with ADONIS function in R;

237   (Anderson 2005). To visualise metapopulation divergence based on life history, Nonmetric

238   Multidimensional Scaling (NMDS) analyses were performed on the scaled distance matrix (all

239   life history and physiological traits) using the METAMDS function (vegan library, R.2.15.1;).

240   The significantly diverging traits were subsequently identified by a Multivariate Analysis of

241   Variance (MANOVA) on the scaled averaged data per replicate.

242

243   We examined how metapopulation type affected the different life history traits and

244   physiological endpoints using generalized linear mixed models (GLMM). The model included

245   metapopulation type (HOM, SPA, TEM) as fixed factor and each individual metapopulation as

246   a random effect to control for dependency among the three replicates from each

247   metapopulation treatment. Depending on the dependent variable, a Gaussian (all

248   physiological endpoints), Poisson (fecundity, developmental time, longevity and population

249   size on the novel host) or binomial error (sex ratio, juvenile mortality) structure was

250   modelled with appropriate link functions. Non-significant contributions (P>0.05) were

251   removed by backwards procedure. Effective degrees of freedom were estimated using

252   Kenward-Rogers procedure. All analyses were conducted with SAS 9.3 (SAS Institute Inc

253   2006) by using the GLIMMIX procedure.

254

255 **Results**

256 **Population-level divergence in life history traits**

257 Experimental evolution caused significant divergence in life history traits between the

258 treatments (PERMANOVA F_2= 2.75; p=0.03). MANOVA analyses showed sex-ratio (F_2=7.77;

259 p=0.02) and fecundity (F_2=10.35; p=0.01) as the two main life history endpoints underlying

260 this divergence. A detailed analysis on the individual trait distribution after experimental

261 evolution confirmed divergence in fecundity and sex ratio, but also in longevity (Table 1; Fig

262 2). An analysis of the trait variation at the start of the experiment is provided in Appendix S2

263 from the supporting material.

264

265 The average proportion of male offspring was higher in clutches originating from the SPA

266 metapopulations (0.34 ± 0.02SE) relative to the HOM (0.26 ± 0.02SE). Mites from the SPA

267 and TEM treatment evolved a high daily fecundity (t=-3.79; p=0.01) than those from the

268 HOM treatment (respectively 5.21± 0.28SE and 5.59± 0.28SE versus 4.31± 0.25SE). Similarly,

269 mites that evolved in the SPA (35.20± 2.01SE) and TEM treatment (36.91± 2.1SE) had a

270 significantly higher fecundity (t=-3.53, p=0.0014) than those from the reference population

271 HOM (27.61± 2.01SE).

272

273 Mites that evolved in the TEM metapopulations died earlier after reaching maturity (9.65 ±

274 0.42SE days) than mites from homogeneous (HOM) metapopulations (after 11.24 ± 0.45SE

275 days) and spatial variable (SPA) metapopulations (after 11.62 ± 0.47SE days) (Fig. 2C). Under

276 the prevailing lab conditions, males developed in 7.99 days on average while the female

277 reached maturity after 8.40 days (t=-3.28; p=0.0010). Mites from homogeneous

278 metapopulations reached maturity earlier (7.97 ± 0.12) than mites from the spatially

279 heterogeneous (SPA) metapopulation (8.43 ± 0.11) (t=-2.85; p=0.012) (Fig. 2D). The

280 interaction between sex and treatment was not significant ($F_{2,2228}$=0.25; p=0.78). No

281 significant differences in juvenile survival of mites among treatments were observed

282 ($F_{2,7.201}$=0.25; p=0.79), and no differences were detected in aerial dispersal propensity

283 ($F_{2,5.185}$=0.02; p=0.98).

284

285 The simulated growth rate at the start of the experiment was 3.56 (SD=0.19). After

286 experimental evolution, growth rates were slightly lower in the homogeneous

287   metapopulation treatment relative to the other two, but this difference was not significant

288   based on the inferred 95% confidence intervals (r_{Hom}=3.38, SD=0.21; r_{TEMP}=3.52; SD=0.20;

289   r_{SPA}=3.55, SD=0.19).

290

291   **Divergence in physiological endpoints**

292   Although not significant ($F_{2,32}$= 3.08; p=0.06), a trend towards a lower mass per 50 mites was

293   observed for mites from homogeneous metapopulations (424 ± 25SE μg) compared to mites

294   from metapopulations with spatial (510 ± 25SE μg) or spatiotemporal variation (441 ± 31SE

295   μg). Glucose levels were significantly different among the metapopulation treatments

296   ($F_{2,67}$=3.52; p=0.03; Fig. 3), with the lowest levels for HOM (1.39 ± 0.25SE) relative to those

297   from SPA (2.33 ± 0.25SE) (t=-2.64; p=0.027). No significant differences in trehalose

298   ($F_{2,60}$=0.43; p=0.51) or triglyceride level were observed among treatments ($F_{2,56}$=2.07;

299   p=0.14).

300

301   **Divergence in gene expression**

302   Based on genome-wide gene-expression data of adult female mites raised under common

303   garden for two generations, SPA and TEM treatments diverged from the control HOM

304   regime, in a parallel direction. We found 152 and 181 differentially expressed genes in SPA

305   and TEM lines, respectively, using the HOM treatment as reference (FDR-corrected *p*-value

306   <0.05 and log_2-converted fold change (FC)>0.585) (Fig. 4). Fig. S1 depicts the expression

307   patterns of the triplicated lines within each treatment separately. Of these differentially

308   expressed genes, 81.6% and 70.7% exhibited down-regulation in SPA and TEM, relative to

309   HOM, respectively (Fig. 4, Fig. S1 in supporting material). Pearson correlation indicated that

310   the altered transcript levels in SPA and TEM were significantly correlated (ρ=0.80, df=260,

311   p<0.0001).

312

313   Using Blast2GO (Conesa *et al.* 2005), a total of 164 Biological Process GO-sets were assigned

314   to the differentially expressed genes that were associated with evolution in the TEM and SPA

315   regimes. Approximately half of these GO-terms (n=84) were present in both treatments.

316   Twenty gene sets were significantly up- and down-regulated in either the SPA or TEM

317   regimes, relative to HOM (Fig. 5). The associated labels of the GO-IDs are listed in Table S1 in

318   supporting Material. Using this GSA, we observed a convergence in the down-regulation of

319　genes involved in methionine biosynthesis (Fig. S3 in supporting material). In contrast, the

320　transcriptional responses to TEM and SPA selection pressures diverged in the gene sets of

321　which members were associated with gluconeogenesis and interconnected pathways and in

322　gene sets of which members code for glycoside hydrolases (Fig. 5 and Fig. S2 in supporting

323　material).

324

325　**Population performance on novel hosts**

326　After one week of challenging the novel host, mite survival did not differ according to the

327　spatial setting to which they evolved ($F_{2,122}$=2.19; p=0.12). However, significant differences in

328　fecundity were observed ($F_{2,122}$=66.81; p<0.0001), with a lower number of deposited eggs in

329　mites that evolved in the homogeneous populations (49.33 ± 1.07SE eggs) relative to SPA

330　(68.29 ± 1.29SE eggs) and TEM (55.83 ± 1.17 eggs). All pairwise differences were significant

331　(Fig.6A). After three weeks, the first cohort of offspring matured which differed in

332　population size among treatments ($F_{2,5.635}$=5.83; p=0.04; Fig. 6B). Again, population sizes

333　were lowest in mites originating from HOM (5± 0.90SE) relative to SPA (10.26 ± 1.71SE) and

334　TEM (10.62 ± 1.77SE).

335

336

337 **Discussion**

338 While there is an increasing awareness that changes in spatial structure affect population

339 dynamics, and that these ecological dynamics interact with evolutionary trajectories, there is

340 a limited understanding of how these reciprocal eco-evolutionary interactions are governed

341 by metapopulation-level selection pressures. We followed a phenomic approach in which we

342 contrasted life history traits, physiological endpoints and transcriptomes from mites that

343 evolved in classical and mainland-island metapopulations with those that evolved in stable

344 patchy metapopulations. Using stable patchy metapopulations (HOM) as a reference, mites

345 from the spatially (mainland-island, SPA) and spatiotemporally (classical, TEM) variable

346 metapopulations showed evolutionary convergence in traits related to size and fecundity

347 and in its transcriptional regulation of methionine biosynthesis. However, these mites

348 equally evolutionary diverged in longevity, sex ratio, glucose content and in the expression

349 of genes involved in gluconeogenesis and that code for glycoside hydrolases (Fig. S2 in

350 supporting information). Despite this divergence in the separate life traits, overall

351 population growth rate on the ancestral host did not differ among the treatments because

352 of different population-level trade-offs.

353

354 Relative to HOM, spatiotemporal variation in habitat availability in the classical

355 metapopulations (TEM) generated high levels of local variation in population density and on

356 average lower metapopulation sizes due to frequent patch extinctions and lagged

357 colonisation dynamics (De Roissart *et al.* 2015). Mites evolving in these metapopulations

358 showed a significant down-regulation of transcription of genes associated with

359 gluconeogenesis and ATP production (Fig. S2). Genes of the gluconeogenesis pathway affect

360 various metabolic fluxes and energy production and are known to influence life history traits

361 (e.g. dispersal, life span and basal metabolic rate), which determine survival in a

362 metapopulation structure. Here, mite adaptation to spatiotemporal dynamics led to an

363 increased fecundity and reduced adult longevity, which may have been mediated by the

364 transcriptional changes in their gluconeogenesis pathway (Fig S2 in supporting information).

365 These altered traits and resulting trade-off reflect a change in resource allocation between

366 survival and reproduction (Magalhães *et al.* 2007), leading to the evolution of more *r*-

13

367 strategic traits (Ronce, Perret & Olivieri 2000; Wheat & Hill 2014). In general, the evidence

368 for the existence of such trade-offs is poorly documented.

369

370 Mainland-island metapopulations (SPA) are characterised by both a low local and

371 metapopulation-level variability in population size, large metapopulation sizes and low

372 metapopulation-level dispersal (De Roissart *et al.* 2015). We detected no such pattern at the

373 individual level. This contrasts with theoretical predictions (Ronce *et al.* 2000) and can be

374 explained by the densities in the trials at which differences are not expressed, the

375 behavioural lability which hides potential minor average differnces, or the overruling impact

376 of kin competition in our system that hides potential minor differences in this behaviour.

377 More importantly, however, an evolution towards more male biased, fecund and slowed

378 aging strategies relative to mites from patchy metapopulations (HOM) was detected. Sex

379 ratio changes are known to evolve in response to local resource (Clark 1978) or mate

380 competition (Macke *et al.* 2011). While mites from stable metapopulations with low

381 dispersal and stable local population sizes are expected to evolve more female biased sex-

382 ratios due to elevated kin-competition, our results point in the direction of resource

383 competition. Spider mites show scramble competition, with fast resource depletion when

384 population sizes are high (Krips *et al.* 1998). As such, slowing down population growth by

385 extending age-at-maturity (Cameron *et al.* 2014; Monro & Marshall 2014) and more male-

386 biased sex ratios (Johnson 1988; Zhurov *et al.* 2014) can be considered as an adaptive

387 strategy under stable conditions and elevated resource competition. The significantly higher

388 transcription in gene sets that code for glycoside hydrolases, enzymes which are crucial for

389 the digestion of complex carbohydrates in an arthropod herbivore's diet and consequently a

390 higher feeding efficiency (Terra & Ferreira 1994), can be regarded as a consequence of mite

391 adaptation to the stable conditions and the resulting elevated resource competition.

392 Interestingly, two of the differentially expressed *GH* genes (*tetur29g01280* and

393 *tetur29g01230*) code for glycoside hydrolases of the GH32 family and seem to have been

394 incorporated in the mite's genome by a horizontal gene transfer event from a bacterial

395 donor species (Grbić *et al.* 2011). The mite genome harbours a remarkable number of

396 horizontally transferred genes and the observed evolutionary response in the transcription

397 of these *GH* genes to metapopulation selection seems to support the hypothesis that

14

398 horizontal gene transfer is a driving force in the adaptive evolution of spider mites (Grbic et
399 al. 2011).

400

401 Despite the evolved divergent phenotypic profiles reported above, life history traits and
402 genome-wide gene-expression showed an overall convergence in the spatial and
403 spatiotemporal variable metapopulation configurations, relative to the stable patchy one.
404 The convergence is apparent in (a) similar evolution of higher fecundity rates and to a lesser
405 degree sex-ratio, age and mass-at-maturity, (b) and increased glucose content and (c) the
406 identical direction of the differential expression of 71 genes (including genes of the
407 methionine anabolic pathway; see Fig. S3 in supporting information). Increased fecundity
408 shows trade-offs with longevity and sex ratio in respectively the TEM and SPA
409 metapopulation. Therefore, mites did not evolve an increased per capita growth rate and on
410 average reached maturity at later age. A slower growth, is typically associated with
411 competitive environments (Kawecki 1993). For the SPA treatment, such increased
412 competitive interactions have been discussed above, but densities were on average lower in
413 the TEM treatments (De Roissart *et al.* 2015), rendering high densities as a common
414 competitive environment a poor explanation for the detected convergence. Systematic high
415 densities in the SPA treatments and resource limitation combined with unoccupied patches
416 in the TEM treatment (De Roissart et al. 2015) must have led to more frequent episodes of
417 *per capita* resource shortage, relative to the HOM metapopulations. Elevated glucose levels
418 have been associated with responses to cope with increased starvation resistance in a
419 ground beetle (Laparie *et al.* 2012) and are typically associated with low metabolic rates
420 under food limitation(Packard & Boardman 1999; Božič & Woodring 2015). A slower
421 development and higher glucose levels of mites from the SPA and TEM are thus in
422 concordance with evolutionary trajectories towards stress resistance (Sulmon *et al.* 2015).

423

424 Our theory of a general stress response was further supported by the transcriptional
425 responses. First, identical expression profiles of a GO-term related to methionine synthesis
426 indicates a common response that could interfere with methylation processes. Such
427 methylation is often induced by general, oxidative stresses, for instance –and in line with our
428 findings- due to compensatory growth after food restriction (De Block & Stoks 2008).
429 Moreover, the more frequent resource shortage experienced by TEM and SPA populations

15

430 may have restricted their access to sulfur-rich proteins containing methionine from *P.*

431 *vulgaris* (George *et al.* 1993). During the common garden experiment, the increased access

432 to the beans for SPA and TEM may have reduced the need for methionine synthesis, an

433 amino acid that has been proven a key player in driving fecundity in *Drosophila*

434 *melanogaster* (Zajitschek *et al.* 2013).

435 Significantly up-and down-regulated gene sets upon adaptation to (spatio)temporal stress

436 included sets that are associated with basal metabolic pathways. Genetic changes in these

437 pathways are a common response to environmental stressors, with enzymes of the

438 gluconeogenesis/glycolysis and citric acid pathways as one of the prime targets (Marden

439 2013). It is interesting to notice that glucose 6-phosphatase shares a substrate with PGI (see

440 Figure S2 in supporting material), a protein previously connected to performance in specific

441 metapopulation structures in butterfly species (Wheat & Hill 2014). It may therefore

442 represent an important link between stress resistance and dynamics in metapopulations.

443

444

445 Adaptive metabolic changes are known to lead to the development of cross-tolerance in

446 organisms, enabling organisms to cope with unfamiliar stressors. We demonstrated that

447 evolutionary dynamics resulting from changes of the metapopulation spatial structure, pre-

448 adapt mites to cope with a challenging novel host. This is an important finding which

449 definitively needs more study in other (model) organisms. If general, such an evolutionary

450 response is expected to have a strong impact on community- and food web dynamics under

451 natural conditions (Farkas *et al.* 2013). We show that altered population dynamics due to

452 changes in metapopulation spatial structure may induce general stress resistance responses.

453 Since multiple stressors are jointly operational under global change, evolutionary responses

454 towards changes in spatial structure and the coupled spatiotemporal variation in

455 demography may offset the need for adaptation to other environmental stressors by

456 maintaining a general stress response and lead to evolutionary rescue (Carlson *et al.* 2014).

457

458 **Acknowledgements**

464

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609

Author contributions

611   ADR, DB & TVL designed the study, ADR & NW performed the research, ADR wrote the first

612   draft of the manuscript and analyzed the data, and all authors contributed substantially to

613   revisions.

614

Supporting information

616   Additional supporting information may be found in the online version of this article.

617   Appendix S1: additional information methodologies

618   Appendix S2: trait variation at the start of the experiment

619   Table S1: GO labels

620   Fig S1: expression heatmap

621   Fig S2: citric acid cycle, glycolysis and gluconeogenesis

622   Fig S3: methionine synthesis pathway

623

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625

626 **Figure legends**

627

628 **Figure 1:** Spider mites as a model for experimental evolution (mature female, on bean).

629 Photo credit by Gilles San Martin

630

631

632 **Figure 2: Effects of variation in metapopulation structure on life history parameters (mean**

633 **values ± SE) of mites.** A: longevity, B: total fecundity, C: sex ratio (males/total clutch size), D:

634 developmental time; E: Daily fecundity. Dotted lines represent parameter values before 30

635 generations of selection. Equal notations indicate non-significant contrast for the respective

636 measurements. Error bars represent standard errors.

637

638

639 **Figure 3: Effects of variation in metapopulation structure on glucose level (nmol) per 50**

640 **mites (mean values ± SE).**Equal notations indicate non-significant contrast for the respective

641 measurements. Error bars represent standard errors.

642

643 **Figure 4: Scatterplot showing the log$_2$-scaled fold change of the differentially expressed**

644 **genes in SPA and TEM, using HOM as reference.** The inset represents a Venn-diagram

645 depicting the number of differentially expressed genes in the TEM and SPA lines, relative to

646 HOM. The labels on the log$_2$-scaled x- and y-axis represent the non-transformed fold change

647 (FC) values.

648

649 **Figure 5: Gene set analysis of biological processes for differentially expressed genes in**

650 **spider mites after adaptation to spatial and spatiotemporal variability**. Nodes and edges

651 represent gene sets and overlap of members between interconnected sets, respectively.

652 Using PAGE, red, blue and grey indicate whether a gene set was significantly up-regulated,

653 down-regulated or not differentially expressed, respectively (Varemo et al., 2013). Gene sets

654 are labelled with the GO-ID (corresponding GO-labels are listed in Table S1 in the supporting

655   material). Green, orange and purple halos surrounding nodes show which gene sets code for

656   genes involved in methionine biosynthesis, gluconeogenesis and interconnected pathways,

657   and genes coding for glycoside hydrolases, respectively.

658

659   **Figure 6: Effects of long-term evolution in the different metapopulation contexts on**

660   **population growth on a novel host (mean values ± SE).** A: number of eggs after one week,

661   B: number of female offspring reaching adulthood after 21 days. Equal notations indicate

662   non-significant contrast for the respective measurements. Error bars represent standard

663   errors.

664

665 **Tables**

666 **Table 1:** Results for fixed effects from mixed linear models with fecundity, developmental

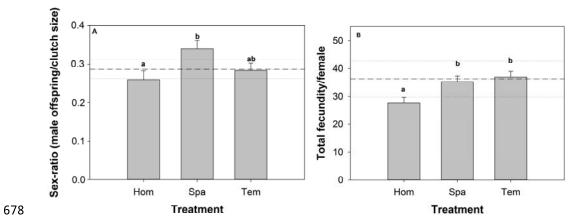667 time, sex-ratio, longevity and juvenile survival as response variable.

668

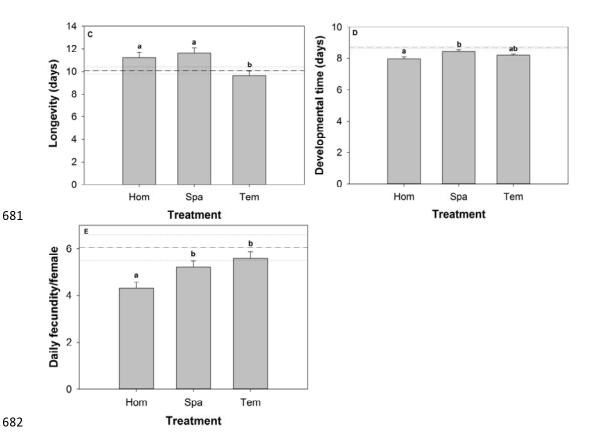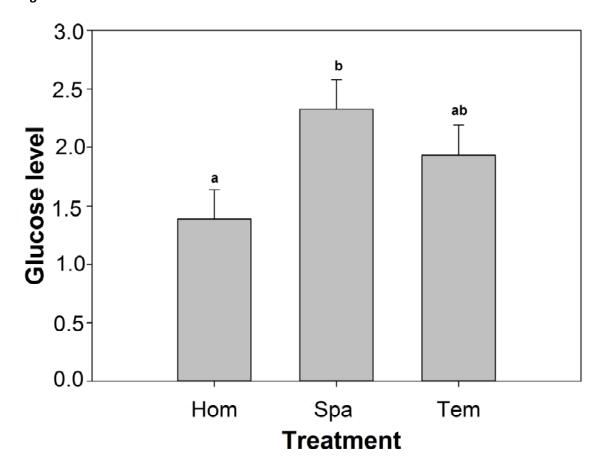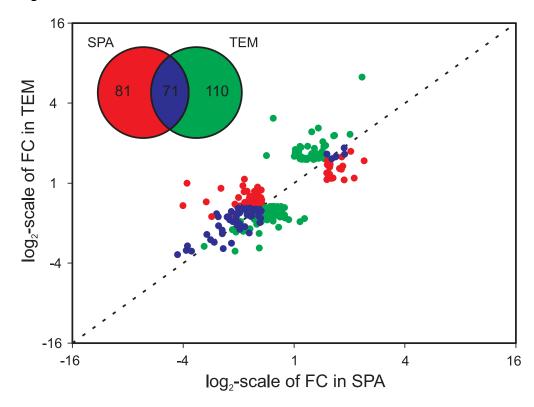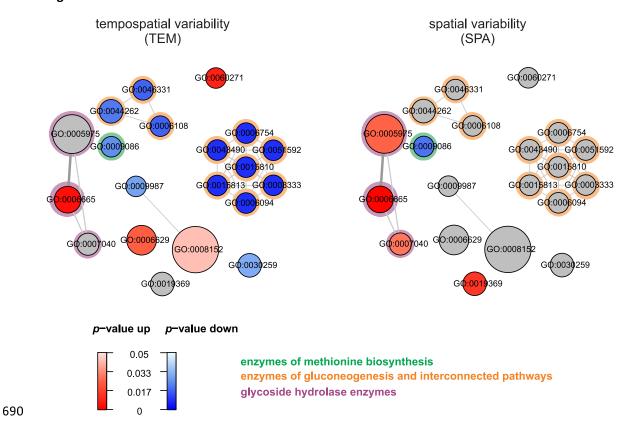| Factor	Num df	Den df	F	p
Daily fecundity				
Treatment	2	8.732	9.34	0.0068
Day	1	1891	693.77	<.0001
Day*Treatment	2	1891	2.99	0.0507
Total fecundity				
Treatment	2	106.2	6.79	0.0017
Sex-Ratio				
Treatment	2	189.8	3.55	0.0308
Developmental time				
Treatment	2	2228	4.09	0.0168
Sex	1	228	10.78	0.0010
Treatment*Sex	2	2228	0.25	0.7782
Longevity				
Treatment	2	158	5.43	0.0053
Juvenile survival				
Treatment	2	7.201	0.25	0.7882

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671   **Figures**

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673   **Figure 1**

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676   **Figure 2**

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685   **Figure 3**

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687   **Figure 4**

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689   **Figure 5**

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691 **Figure 6**

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