

1 **Evolution in spatial and spatiotemporal variable metapopulations**
2 **changes a herbivore's host plant range**

3

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30

31 **Abstract**

32 *The persistence and dynamics of populations largely depends on the way they are configured*
33 *and integrated into space and the ensuing eco-evolutionary dynamics. We manipulated*
34 *spatial and temporal variation in patch size in replicated experimental metapopulations of*
35 *the herbivore mite Tetranychus urticae. Evolution over approximately 30 generations in the*
36 *spatially and spatiotemporally variable metapopulations induced a significant divergence in*
37 *life history traits, physiological endpoints and gene expression, but also a remarkable*
38 *convergence relative to the stable reference patchy metapopulation in traits related to size*
39 *and fecundity and in its transcriptional regulation. The observed evolutionary dynamics are*
40 *tightly linked to demographic changes, more specifically frequent episodes of resource*
41 *shortage, and increased the reproductive performance of mites on tomato, a challenging*
42 *host plant. This points towards a general, adaptive stress response in stable spatial variable*
43 *and spatiotemporal variable metapopulations that pre-adapts a herbivore arthropod to*
44 *novel environmental stressors.*

45 Changes in land-use levy a strong pressure on our natural and pristine habitat and leads to
46 habitat loss and isolation, which are both a major threat for biodiversity¹. Species
47 conservation therefore relies largely on optimal reserve planning which in turn is rooted
48 within the principles of metapopulations^{2,3}. This concept defines population-extinction
49 dynamics and eventually extinction thresholds within in networks of interconnected habitat
50 patches. Most spatially structured populations can be classified as *patchy* or *mainland-*
51 *island* metapopulations⁴, and the omnipresence of classical Levin's metapopulations has
52 been recently questioned⁵. This spatial variation in habitat availability not only affects patch
53 occupancy dynamics, it also impacts the local- and metapopulation-level demography. Some
54 of us⁶ demonstrated experimentally that spatial variation in habitat availability decreases
55 variance in metapopulation size at the metapopulation level. Conversely, spatiotemporal
56 variation in habitat availability increases patch extinction rates, but decreases local
57 population and metapopulation sizes. These demographic changes minimised
58 metapopulation-level variability in mainland-island metapopulations, relative to classical and
59 patchy ones.

60

61 Because of these changes in population dynamics, selection pressures in metapopulations
62 are expected to act on more than one level of population structure⁷. For instance, in classical
63 metapopulations where local population extinctions occur regularly, increased dispersal
64 rates are selected since long-term survival is only possible if genotypes are able to re-
65 colonise patches from where they have become locally extinct. With increasing asymmetry
66 in patch size, however, dispersal will evolve to lower rates because benefits of dispersal are
67 only prevalent for a minority of the individuals⁸. Additional and/or alternative adaptive
68 strategies might also evolve through the adjustment of sex-ratio⁹, age-at-death¹⁰ and
69 density-dependency¹¹ according to changes in spatial structure and associated variation in
70 the prevalence and strength of local resource competition¹²⁻¹⁴ and other stressors^{15,16}.

71

72 While evolutionary theory to date is centred on single trait dynamics, multivariate selection
73 in life history and physiology is anticipated in response to changes in spatial habitat
74 configuration. These evolutionary responses then simultaneously feedback on the ecological
75 dynamics, rendering both ecology and evolution heavily intertwined. We now begin to
76 understand such eco-evolutionary dynamics in either natural or experimental

77 metapopulations¹⁷. The importance of eco-evolutionary dynamics is most obvious in
78 metapopulations where dispersal determines the genetic composition of different
79 populations. Seminal examples include the Glanville fritillary¹⁸ or stick insect
80 metapopulations¹⁹. Often, these eco-evolutionary dynamics lead to evolutionary rescue, the
81 process where adaptive evolution allows a population²⁰, metapopulation^{17,21} or an
82 expanding population²² to recover from negative growth as a result from environmental
83 change²⁰. Evolutionary rescue is known to be strongly determined by demographic and
84 genetic factors of local populations, but also by entire metapopulation changes²³.

85

86 The genetic basis of life history differentiation can now be disentangled by the development
87 of several -omic approaches. Transcriptomic analyses may uncover genes that significantly
88 alter their transcript levels as a response to the implemented selection pressure and provide
89 detailed insights on the pleiotropic effects underlying phenotypic. For instance, in *Drosophila*
90 *melanogaster*, many genes that are up- or downregulated in response to stress are equally
91 associated with mobility and aggression²⁴. In the spider mite *Tetranychus urticae*,
92 transcriptomic analysis of populations that developed pesticide resistance or that were
93 exposed to challenging host plants reveals the presence of common adaptive responses and
94 identified key gene candidates for xenobiotic adaptation in this polyphagous mite²⁵

95

96 Experimental evolution in artificial metapopulations provides a unique formal test to
97 understand to which degree spatial variation in habitat availability affects life history
98 divergence²⁶. Using this approach, we studied phenomic divergence of replicated spider mite
99 populations *T. urticae* that evolved in three types of artificial metapopulations. Following
100 theoretical expectations outlined above, and the earlier documented demographic changes⁶
101 we derived the following predictions on life history evolution for mainland-island and
102 classical metapopulations, relative to patchy metapopulations where habitat is constantly
103 available in time and space:

104

- 105 1. Asymmetry in patch and population size in mainland-island metapopulations selects
106 for lower dispersal rates, while in classical metapopulations, the spatiotemporal
107 variation in population size leads to higher evolved dispersal rates

- 108 2. Increased local extinction rates and local population variability in classical
109 metapopulation selects for faster life histories, i.e. increased fecundity, decreased
110 longevity and/or reduced developmental time
- 111 3. Low local and metapopulation variability in mainland-island metapopulations
112 increase local competition and therefore select for a more male-biased sex ratio
113 and/or increased developmental time (age-at-maturity).

114 **Results**

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

116 Experimental evolution caused significant divergence in life history traits of the variable
117 treatments with the homogeneous treatment (PERMANOVA $F_2=2.75$; $p=0.03$) (Fig. 1A).
118 MANOVA analyses showed sex-ratio ($F_2=7.77$; $p=0.02$) and fecundity ($F_2=10.35$; $p=0.01$) as
119 the two main life history endpoints underlying this divergence. A detailed analysis on the
120 individual trait distribution after experimental evolution confirmed divergence in fecundity
121 and sex ratio, but also in longevity (Table 1; Fig 2). An analysis of the trait variation at the
122 start of the experiment is provided in supplementary material S2.

123

124 The average proportion of male offspring was higher in clutches originating from the SPA
125 metapopulations ($0.34 \pm 0.02SE$) relative to the HOM ($0.26 \pm 0.02SE$). Mites from the SPA
126 and TEM treatment evolved a high daily fecundity ($t=-3.79$; $p=0.01$) than those from the
127 HOM treatment (respectively $5.21 \pm 0.28SE$ and $5.59 \pm 0.28SE$ versus $4.31 \pm 0.25SE$). Similarly,
128 mites that evolved in the SPA ($35.20 \pm 2.01SE$) and TEM treatment ($36.91 \pm 2.1SE$) had a
129 significantly higher fecundity ($t=-3.53$, $p=0.0014$) than those from the reference population
130 HOM ($27.61 \pm 2.01SE$).

131

132 Mites that evolved in the TEM metapopulations died earlier (after $9.65 \pm 0.42SE$ days) than
133 mites from homogeneous (HOM) metapopulations (after $11.24 \pm 0.45SE$ days) and spatial
134 variable (SPA) metapopulations (after $11.62 \pm 0.47SE$ days) (Fig. 2C). Under the prevailing lab
135 conditions, males developed in 7.99 days on average while the female reached maturity
136 after 8.40 days ($t=-3.28$; $p=0.0010$). Mites from homogeneous metapopulations reached
137 maturity earlier (7.97 ± 0.12) than mites from the spatially heterogeneous (SPA)
138 metapopulation (8.43 ± 0.11) ($t=-2.85$; $p=0.012$) (Fig. 2D). The interaction between sex and
139 treatment was not significant ($F_{2,2228}=0.25$; $p=0.78$). No significant differences in juvenile
140 survival of mites among treatments were observed ($F_{2,7,201}=0.25$; $p=0.79$), and no differences
141 were detected in aerial dispersal propensity ($F_{2,5,185}=0.02$; $p=0.98$).

142

143 The simulated growth rate at the start of the experiment was 3.56 (SD=0.19). After
144 experimental evolution, growth rates were slightly lower in the homogeneous
145 metapopulation treatment relative to the other two, but this difference was not significant

146 based on the inferred 95% confidence intervals ($r_{\text{Hom}}=3.38$, $SD=0.21$; $r_{\text{TEMP}}=3.52$; $SD=0.20$;
147 $r_{\text{SPA}}=3.55$, $SD=0.19$).

148

149 **Divergence in physiological endpoints**

150 Although not significant ($F_{2,32}=3.08$; $p=0.06$), a trend towards a lower mass per 50 mites was
151 observed for mites from homogeneous metapopulations ($424 \pm 25\text{SE } \mu\text{g}$) compared to mites
152 from metapopulations with spatial ($510 \pm 25\text{SE } \mu\text{g}$) or spatiotemporal variation ($441 \pm 31\text{SE}$
153 μg). Glucose levels were significantly different among the metapopulation treatments
154 ($F_{2,67}=3.52$; $p=0.03$; Fig. 3), with the lowest levels for HOM ($1.39 \pm 0.25\text{SE}$) relative to those
155 from SPA ($2.33 \pm 0.25\text{SE}$) ($t=-2.64$; $p=0.027$). No significant differences in trehalose
156 ($F_{2,60}=0.43$; $p=0.51$) or triglyceride level were observed among treatments ($F_{2,56}=2.07$;
157 $p=0.14$).

158

159 **Divergence in gene expression**

160 Based on genome-wide gene-expression data of adult female mites raised under common
161 garden for two generations, SPA and TEM treatments diverged from the control HOM
162 regime, in a parallel direction (Fig. 1B). We found 152 and 181 differentially expressed genes
163 in SPA and TEM lines, respectively, using the HOM treatment as reference (FDR-corrected p -
164 value <0.05 and \log_2 -converted $FC>0.585$) (Fig. 4). Fig. S1 depicts the expression patterns of
165 the triplicated lines within each treatment separately. Of these differentially expressed
166 genes, 81.6% and 70.7% exhibited down-regulation in SPA and TEM, relative to HOM,
167 respectively (Fig. 4, Fig. S2). Pearson correlation indicated that the altered transcript levels in
168 SPA and TEM were significantly correlated ($\rho=0.80$, $df=260$, $p<0.0001$).

169

170 Using Blast2GO²⁷, a total of 164 Biological Process GO-sets were assigned to the
171 differentially expressed genes that were associated with evolution in the TEM and SPA
172 regimes. Approximately half of these GO-terms ($n=84$) were present in both treatments.
173 Twenty gene sets were significantly up- and down-regulated in either the SPA or TEM
174 regimes, relative to HOM (Fig. 5). The associated labels of the GO-IDs are listed in Table S1.
175 Using this GSA, we observed a convergence in the down-regulation of genes involved in
176 methionine biosynthesis (Fig. S3). In contrast, the transcriptional responses to TEM and SPA
177 selection pressures diverged in the gene sets of which members were associated with

178 gluconeogenesis and interconnected pathways and in gene sets of which members code for
179 glycoside hydrolases (Fig. 5 and Fig. S2).

180

181 **Population performance on novel hosts**

182 After one week of challenging the novel host, mite survival did not differ according to the
183 spatial setting to which they evolved ($F_{2,122}=2.19$; $p=0.12$). However, significant differences in
184 fecundity were observed ($F_{2,122}=66.81$; $p<0.0001$), with a lower number of deposited eggs in
185 mites that evolved in the homogeneous populations ($49.33 \pm 1.07SE$ eggs) relative to SPA
186 ($68.29 \pm 1.29SE$ eggs) and TEM (55.83 ± 1.17 eggs). All pairwise differences were significant
187 (Fig.6A). After three weeks, the first cohort of offspring matured which differed in
188 population size among treatments ($F_{2,5.635}=5.83$; $p=0.04$; Fig. 6B). Again, population sizes
189 were lowest in mites originating from HOM ($5 \pm 0.90SE$) relative to SPA ($10.26 \pm 1.71SE$) and
190 TEM ($10.62 \pm 1.77SE$).

191

192

193 Discussion

194 While there is an increasing awareness that changes in spatial structure affect population
195 dynamics, and that these ecological dynamics interact with evolutionary trajectories, there is
196 a limited understanding of how these reciprocal eco-evolutionary interactions are governed
197 by metapopulation-level selection pressures. We followed a phenomic approach in which we
198 contrasted life history traits, physiological endpoints and transcriptomes from mites that
199 evolved in classical and mainland-island metapopulations with those that evolved in stable
200 patchy metapopulations. Using stable patchy metapopulations (HOM) as a reference, mites
201 from the spatially (mainland-island, SPA) and spatiotemporally (classical, TEM) variable
202 metapopulations showed evolutionary convergence in traits related to size and fecundity
203 and in its transcriptional regulation of methionine biosynthesis. However, these mites
204 equally evolutionary diverged in longevity, sex ratio, glucose content and in the expression
205 of genes involved in gluconeogenesis and that code for glycoside hydrolases (Fig. S2).

206

207 Relative to HOM, spatiotemporal variation in habitat availability in the classical
208 metapopulations (TEM) generated high levels of local variation in population density and on
209 average lower metapopulation sizes due to frequent patch extinctions and lagged
210 colonisation dynamics⁶. Mites evolving in these metapopulations showed a significant
211 down-regulation of transcription of genes associated with gluconeogenesis and ATP
212 production (Fig. S2). Genes of the gluconeogenesis pathway affect various metabolic fluxes
213 and energy production and are known to influence life history traits (e.g. dispersal, life span
214 and basal metabolic rate), which determine survival in a metapopulation structure. Here,
215 mite adaptation to spatiotemporal dynamics led to an increased fecundity and reduced
216 longevity, which may have been mediated by the transcriptional changes in their
217 gluconeogenesis pathway (Fig S2). These altered traits reflect a change in resource allocation
218 between survival and reproduction²⁸, leading to the evolution of more *r*-strategic traits^{29,30}.

219

220 Mainland-island metapopulations (SPA) are characterised by both a low local and
221 metapopulation-level variability in population size, large metapopulation sizes and low
222 metapopulation-level dispersal⁶. We detected a similar trend of reduced dispersal at the
223 individual level -in line with theoretical predictions²⁹, but more importantly an evolution

224 towards more male biased, fecund and slowed aging strategies relative to mites from patchy
225 metapopulations (HOM). Sex ratio changes are known to evolve in response to local
226 resource¹² or mate competition⁹. While mites from stable metapopulations with low
227 dispersal and stable local population sizes are expected to evolve more female biased sex-
228 ratios due to elevated kin-competition, our results point in the direction of resource
229 competition. Spider mites show scramble competition, with fast resource depletion when
230 population sizes are high³¹. As such, slowing down population growth by extending age-at-
231 maturity^{14,32} and more male-biased sex ratios^{33,34} can be considered as an adaptive strategy
232 under stable conditions and elevated resource competition. The significantly higher
233 transcription in gene sets that code for glycoside hydrolases, enzymes which are crucial for
234 the digestion of complex carbohydrates in an arthropod herbivore's diet³⁵, can be regarded
235 as a consequence of mite adaptation to the stable conditions. Interestingly, two of the
236 differentially expressed *GH* genes (*tetur29g01280* and *tetur29g01230*) were horizontally
237 transferred and code for glycoside hydrolases of the GH32 family³⁶.

238

239 Despite the evolved divergent phenotypic profiles reported above, life history traits and
240 genome-wide gene-expression showed an overall convergence in the spatial and
241 spatiotemporal variable metapopulation configurations, relative to the stable patchy one.
242 This was unexpected since the HOM (meta-)population dynamics are intermediately situated
243 between the SPA and TEM regimes. The convergence is apparent in (a) similar evolution of
244 higher fecundity rates and to a lesser degree sex-ratio, age and mass-at-maturity, (b) and
245 increased glucose content and (c) the identical direction of the differential expression of 71
246 genes (including genes of the methionine anabolic pathway; see Fig. S3). Increased fecundity
247 shows trade-offs with longevity and sex ratio in respectively the TEM and SPA
248 metapopulation. Therefore, mites did not evolve an increased per capita growth rate and on
249 average reached maturity at later age. A slower growth, larger size at maturity are typically
250 traits associated with competitive environments³⁷. For the SPA treatment, such increased
251 competitive interactions have been discussed higher, but densities were on average lower in
252 the TEM treatments⁶, rendering high densities as a common competitive environment a
253 poor explanation for the detected convergence. In relative terms, however, high densities in
254 the SPA treatments and resource limitation combined with unoccupied patches in the TEM
255 treatment must have led to more frequent episodes of *per capita* resource shortage, relative

256 to the stable HOM metapopulations. Elevated glucose levels have been associated with
257 responses to cope with increased starvation resistance in a ground beetle³⁸ and are typically
258 associated with low metabolic rates under food limitation^{39,40}. The decreased developmental
259 rates, the higher glucose levels of mites from the SPA and TEM are thus in concordance with
260 evolutionary trajectories towards stress resistance⁴¹.

261

262 Our theory of a general stress response was further supported by the transcriptional
263 responses. First, identical expression profiles of a GO-term related to methionine synthesis
264 indicates a common response that could interfere with methylation processes. Such
265 methylation is often induced by general, oxidative stresses, for instance –and in line with our
266 findings- due to compensatory growth after food restriction⁴². Significantly up-and down-
267 regulated gene sets upon adaptation to (spatio)temporal stress included sets that are
268 associated with basal metabolic pathways. Genetic changes in these pathways are a
269 common response to environmental stressors, with enzymes of the
270 gluconeogenesis/glycolysis and citric acid pathways as one of the prime targets⁴³.

271

272 Adaptive metabolic changes are known to lead to the development of cross-tolerance in
273 organisms, enabling organisms to cope with unfamiliar stressors. We demonstrated that
274 evolutionary dynamics resulting from changes of the metapopulation spatial structure, pre-
275 adapt mites to cope with a challenging novel host. This is an important finding which
276 definitively needs more study in other (model) organisms. If general, such an evolutionary
277 response is expected to have a strong impact on community- and food web dynamics under
278 natural conditions¹⁹. We show that altered population dynamics due to changes in
279 metapopulation spatial structure may induce general stress resistance responses. Since
280 multiple stressors are jointly operational under global change, evolutionary responses
281 towards changes in spatial structure can thus enable organisms of some species to cope with
282 additional stressors like changes in habitat quality and pollution (i.e., evolutionary rescue;
283 ²³).

284

285 **Materials and methods**

286 **Experimental setup of the artificial metapopulations**

287 Metapopulation dynamics of *Tetranychus urticae* were studied using experimental
288 microcosms. We used as a base population the “LS-VL” *T. urticae* strain, because it is known
289 to be highly evolvable due to its genetic variability^{44,45}. Artificial metapopulations consisted
290 of a transparent plastic box with 9 patches arranged in a 3 x 3 lattice. We constructed three
291 types of artificial metapopulations with an equal metapopulation-level carrying capacity but
292 varying spatial configuration of the patches. Patches were detached bean (*Phaseolus vulgaris*
293 *L.*) leaves placed on a Tanglefood layer in closed boxes. This hostile matrix prevents mites
294 from leaving the patches. Bean leaves were renewed on a weekly basis to avoid starvation of
295 the mites. The size of the bean leaves introduced to each patch was dependent upon the
296 treatment. Two times a week, for 8 hours a wind current (1.5m/s) was induced by a fan and
297 allowed aerial dispersal. Three metapopulation types were installed each of which was
298 replicated three times:

299

- 300 i) a patchy metapopulation consisting of nine patches weekly refreshed with leaves
301 of 20 cm² (spatially homogenous distribution of resources; further referred to as
302 HOM)
- 303 ii) a mainland-island metapopulation consisting of three patches of standard leaf
304 size (20 cm²) and three of double size; another three patches of these
305 metapopulations remained constantly empty (spatial heterogeneous distribution
306 of resources; further referred to as SPA)
- 307 iii) a spatiotemporal heterogeneous metapopulation (further referred to as TEM) in
308 which we assigned nine single-patch resources (standard leaf) randomly to one of
309 the nine patches. Due to this algorithm, the distributions of the resources (and
310 thus local carrying capacity or island size) changed weekly among the nine
311 patches and varied between zero (no resource renewal and local extinction) and
312 double or exceptionally triple island size. In consequence, patch sizes and thus
313 local carrying capacities fluctuated over time and space, but we ensured again a
314 constant metapopulation carrying capacity (9 x 20 cm²) over time.

315

316 At the beginning of the experiment, 20 randomly collected adult female mites, from the base
317 population, were assigned to each patch within each metapopulation type and allowed to
318 establish the triplicated populations. All metapopulations were kept under controlled
319 conditions (23°C, 16:8 LD photoperiod, 85% humidity).

320

321 **Quantification of mite life-history**

322 Spider mite life-history traits were measured at the initiation of the experiment and after 10
323 months, corresponding to approximately 30 mite generations. All traits were measured on
324 F2 mites (raised for two generations in common garden on detached leaf discs) to minimise
325 maternal and environmental effects caused for instance by local conditions of crowding.
326 Young inseminated females of each experimental metapopulation were individually allowed
327 to oviposit on bean leaf discs. Leaf discs were placed with the abaxial part upwards on
328 moistened filter paper to prevent mites from escaping and to maintain leaf turgor. Different
329 life history parameters of the descendants were recorded daily: juvenile survival,
330 developmental time (time from egg until the adult stage), fecundity (daily number of eggs),
331 longevity and sex-ratio. Since spider mites deposit the majority of their eggs during the first
332 seven days after maturity, we monitored fecundity only during that period. Dispersal
333 propensity of the mites was assessed by transferring mated females to test arenas for trials
334 of aerial dispersal (after two whole generations under common garden to avoid confounding
335 maternal effects). The experimental setup for aerial dispersal assessment was identical to
336 the one applied in⁴⁶ (details in supplementary material S1).

337

338 **Mite performance**

339 Mite performance was followed by quantifying rate of intrinsic growth as a proxy of
340 fitness¹³. To detect possible differences in individual performance between treatments, an
341 integrated individual-level fitness measure, the rate of intrinsic growth (r_m), was calculated
342 by combining the estimated parameter distributions of the different life history parameters
343 according (see statistical analyses) to the equation $\sum e^{-(r_m)x} l_x f_x = 1$ (with l_x survival till maturity
344 x and f_x the number of female offspring at age x) which represents the contribution of each
345 female to the number of females in the subsequent generation. We performed 10000
346 simulations and reported the mean value and standard deviation while testing its

347 significance in comparing whether 2.5% tails of the distribution overlap. We additionally
348 measured a set of physiological endpoints (mass, glucose, trehalose and triglycerid levels)
349 after the common garden treatment at the start of the experiment and after 30 generations
350 of selection in a metapopulation context as indicators for mite performance. All physiological
351 parameters were measured following³⁸ on F2 mites (see suppl. material S1).

352

353 **Differential gene expression after experimental evolution**

354 To examine the effects of metapopulation structure on the mite transcriptome, Agilent dual
355 colour gene expression micro-array analysis was performed on female mites raised for two
356 generations in a common garden of every selection regime. The microarray data have been
357 deposited in the Gene Expression Omnibus (GEO) (accession number: GSE55623). For the
358 hierarchical clustering, data of previous *T. urticae* studies were incorporated^{33,47}. Final
359 statistical processing and analysis was conducted in limma (Smyth 2005). Gene Ontology
360 (GO) annotation was executed using Blast2GO software²⁷. Using the Blast2GO generated
361 annotation and the statistical output of limma as input, Gene Set Analysis (GSA) was
362 performed with the Bioconductor package piano (Parametric Analysis of Gene set
363 Enrichment, PAGE)⁴⁸. More details of the gene expression and GO-term analysis are
364 provided in supplementary material S1.

365

366 **Performance on a challenging new host**

367 Our LS-VL base population has been maintained on bean for more than 10 years. We
368 assessed performance on a novel suboptimal host by quantifying isofemale growth rate on
369 tomato (*Solanum lycopersicum*; variety Moneymaker) grown under controlled laboratory
370 conditions (23°C, 16:8 L:D photoperiod). Experimental arenas were constructed with leaves
371 from 4-week old tomato plants. Moist tissue paper was used to cover 10 cm² leaf edges that
372 prevented mites from escaping. Twenty fertilized F2 females (raised for two generations in
373 common garden to reduce maternal and environmental effects) from each artificial
374 metapopulation were placed on a leaf-arena and allowed to establish a population. All leaf-
375 arenas were kept under controlled conditions (23°C, 16:8 L:D photoperiod). Population
376 growth was assessed weekly for 3 weeks by counting the number of eggs, juveniles, adult
377 males and females.

378

379 **Statistical analysis**

380 Because the measured traits follow different statistical distributions, we first tested for
381 multivariate differences in the measured traits after experimental evolution by applying a
382 Permutational Multivariate Analysis of Variance (PERMANOVA). Because our measurements
383 were taken with different units on different scales, the correctly estimated replicate-level
384 averages of the life history and physiological endpoints (see GLMM further) were scaled
385 prior to PERMANOVA analysis based on Euclidean distances among replicates belonging to
386 one of the three metapopulation treatments (PERMANOVA; with ADONIS function in R;⁴⁹. To
387 visualise metapopulation divergence based on life history, Nonmetric Multidimensional
388 Scaling (NMDS) analyses were performed on the scaled distance matrix (all life history and
389 physiological traits) using the METAMDS function (vegan library, R.2.15.1;). The significantly
390 diverging traits were subsequently identified by a Multivariate Analysis of Variance
391 (MANOVA) on the scaled averaged data per replicate.

392

393 We examined how metapopulation type affected the different life history traits and
394 physiological endpoints using generalized linear mixed models (GLMM). The model included
395 metapopulation type (HOM, SPA, TEM) as fixed factor and each individual metapopulation as
396 a random effect to control for dependency among replicates from each metapopulation
397 treatment. Depending on the dependent variable, a Gaussian (all physiological endpoints),
398 Poisson (fecundity, developmental time, longevity and population size on the novel host) or
399 binomial error (sex ratio, juvenile mortality) structure was modelled with appropriate link
400 functions. Non-significant contributions ($P > 0.05$) were removed by backwards procedure.
401 Effective degrees of freedom were estimated using Kenward-Rogers procedure. All analyses
402 were conducted with SAS 9.3 (SAS Institute Inc 2006) by using the GLIMMIX procedure.

403

404

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411

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527

528 **Author contributions**

529 ADR, DB & TVL designed the study, ADR & NW performed the research, ADR wrote the first
530 draft of the manuscript and analyzed the data, and all authors contributed substantially to
531 revisions.

532

533 The authors declare no competing financial interests.

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536

537 **Figure legends**

538

539 **Figure 1: Visualization of the life history data.** A: nonmetric multidimensional scaling of the
540 metapopulations showing dissimilarities between metapopulations based on 'Euclidean
541 distance', B: Hierarchical clustering of the transcriptomic profiles of the three
542 metapopulation types and the ancestral mite population living in cold conditions (17°C),
543 relative to a reference strain (London).

544

545 **Figure 2: Effects of variation in metapopulation structure on life history parameters (mean
546 values \pm SE) of mites.** A: longevity, B: total fecundity, C: sex ratio (males/total clutch size), D:
547 developmental time; E: Daily fecundity. Dotted lines represent parameter values before 30
548 generations of selection. Equal notations indicate non-significant contrast for the respective
549 measurements. Error bars represent standard errors.

550

551

552 **Figure 3: Effects of variation in metapopulation structure on glucose level (nmol) per 50
553 mites (mean values \pm SE).** Equal notations indicate non-significant contrast for the respective
554 measurements. Error bars represent standard errors.

555

556 **Figure 4: Scatterplot showing the log₂-converted FC of the differentially expressed genes in
557 SPA and TEM, using HOM as reference.** The inset represents a Venn-diagram depicting the
558 number of differentially expressed genes in the TEM and SPA lines, relative to HOM.

559

560 **Figure 5: Gene set analysis of biological processes for differentially expressed genes in
561 spider mites after adaptation to spatial and spatiotemporal variability.** Nodes and edges
562 represent gene sets and overlap of members between interconnected sets, respectively.
563 Using PAGE, red, blue and grey indicate whether a gene set was significantly up-regulated,
564 down-regulated or not differentially expressed, respectively. Gene sets are labelled with the
565 GO-ID (corresponding GO-labels are listed in Table S1). Green, orange and purple halos
566 surrounding nodes show which gene sets code for genes involved in methionine
567 biosynthesis, gluconeogenesis and interconnected pathways, and genes coding for glycoside
568 hydrolases, respectively.

569

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

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

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

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

574 errors.

575

576 **Tables**

577 **Table 1:** Results for fixed effects from mixed linear models with fecundity, developmental
 578 time, sex-ratio, longevity and juvenile survival as response variable.

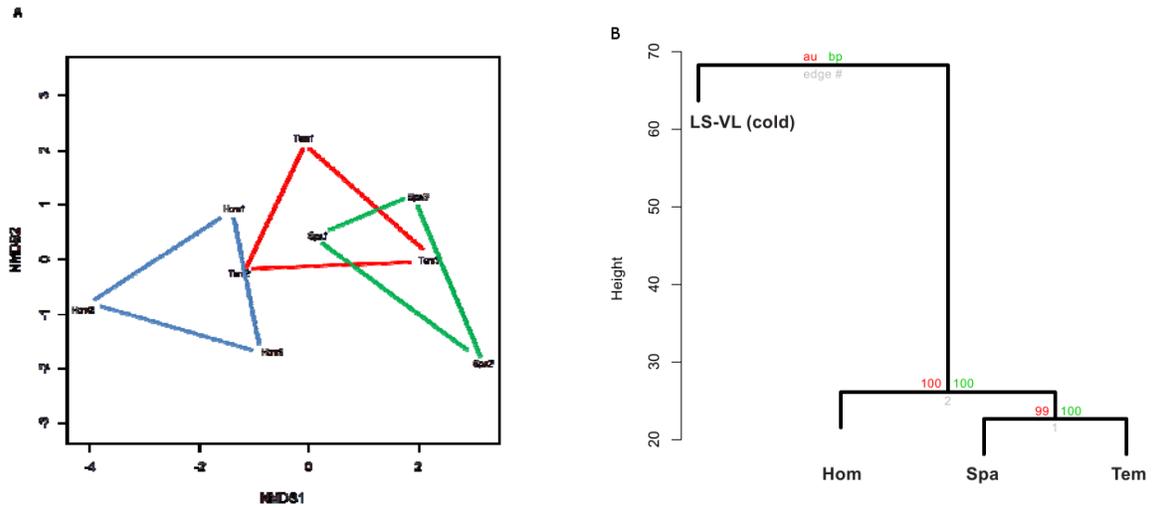
Factor	Num df	Den df	F	p
579				
Daily fecundity				580
Treatment	2	8.732	9.34	0.0068
Day	1	1891	693.77	<.0001
Day*Treatment	2	1891	2.99	0.0507
581				
Total fecundity				
Treatment	2	106.2	6.79	0.0017
Sex-Ratio				
Treatment	2	189.8	3.55	0.0308
582				
Developmental time				
Treatment	2	2228	4.09	0.0168
Sex	1	228	10.78	0.0010
Treatment*Sex	2	2228	0.25	0.7782
583				
Longevity				
Treatment	2	158	5.43	0.0053
584				
Juvenile survival				
Treatment	2	7.201	0.25	0.7882

582 **Figures**

583

584 **Figure 1**

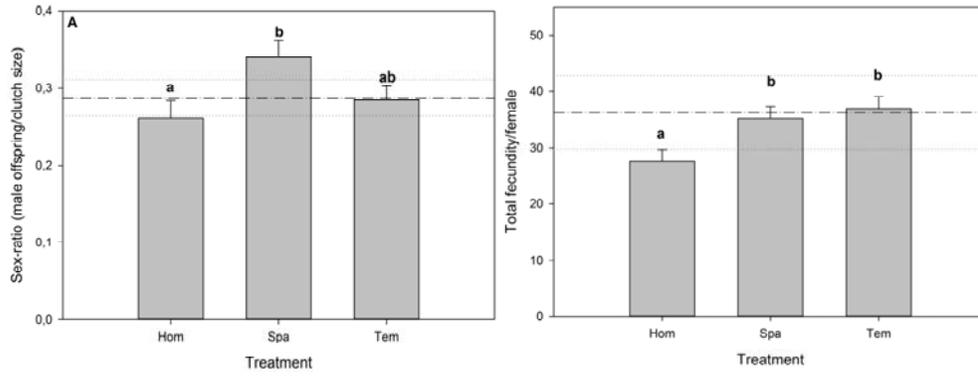
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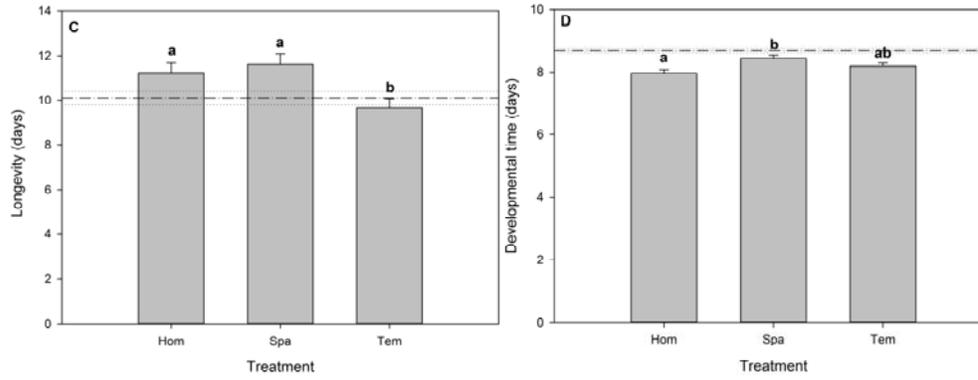
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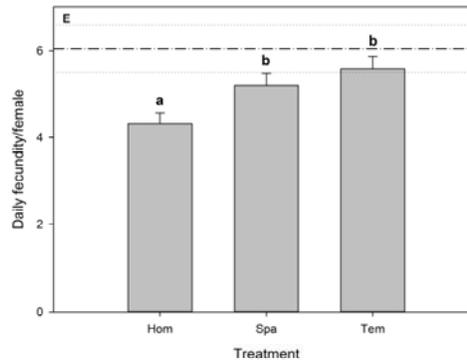
588 **Figure 2**



589



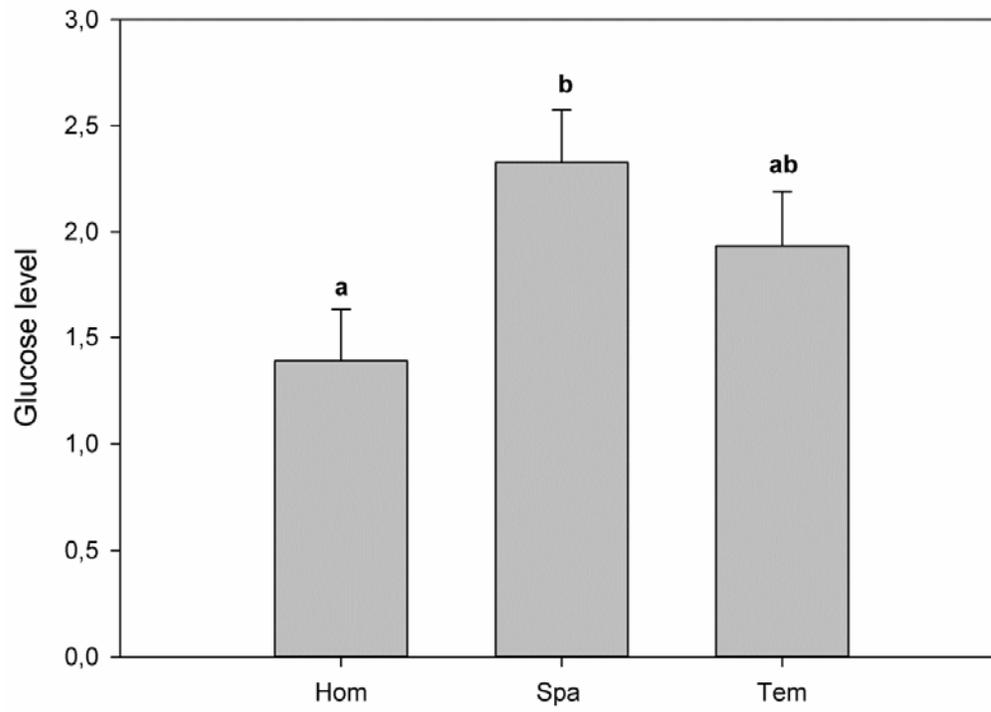
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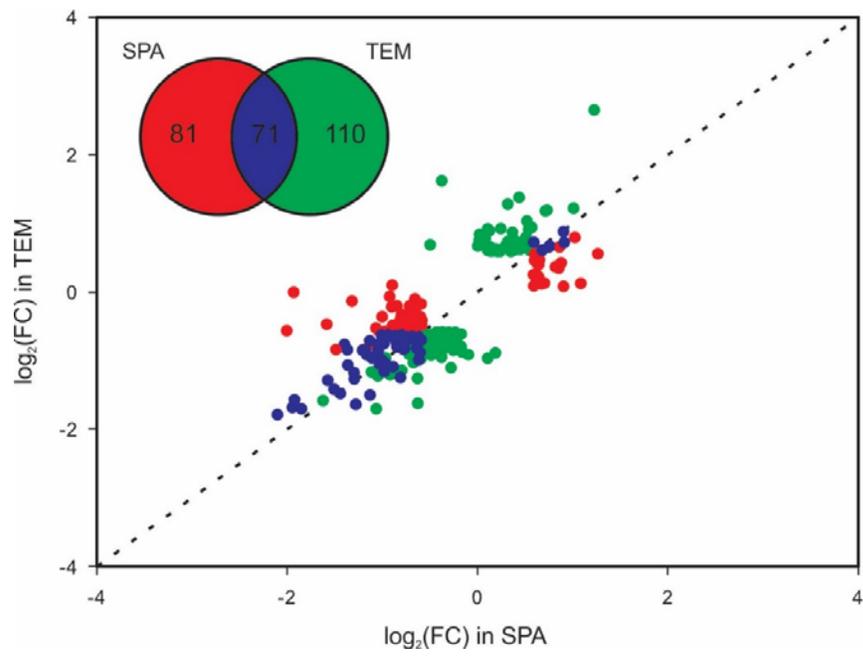
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593 **Figure 3**



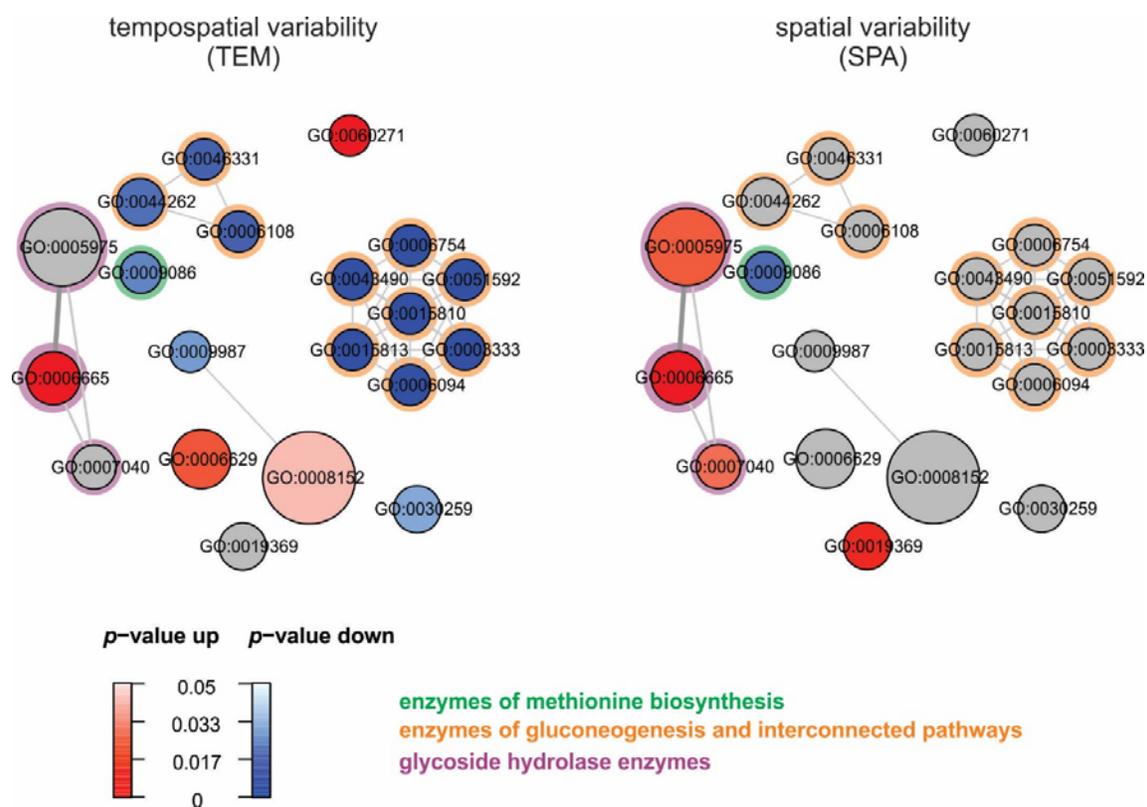
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595 **Figure 4**



596

597 **Figure 5**



598

599 **Figure 6**

