Metabolic adaptations underlie life-history evolution in a range expanding arthropod

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Running headline: Metabolomics of a range expanding arthropod

Summary

1. During range expansions or range shifts, species' life histories evolve due to changing selection pressures or spatial sorting. Despite an increasing number of studies documenting such life-history evolution, we lack a mechanistic understanding of the underlying physiological processes.
2. We used a powerful metabolomics approach to study physiological changes associated with the recent range expansion of a model arthropod, the two-spotted spider mite *Tetranychus urticae.*
3. Mite populations were sampled in the field along a latitudinal gradient from range core to range edge, and reared under common garden conditions for two generations. Thereafter, we obtained metabolic population profiles using Gas Chromatography-Mass Spectrophotometry (GC-MS).
4. We found gradual metabolic differentiation along the latitudinal gradient, indicating rapid evolution of the metabolome in association with range expansion. In addition, we observed that some of this differentiation covaried with the life-history evolution previously found to be associated with the mite's range expansion.
5. Particularly, more northern populations, which evolved a higher dispersal tendency, showed lowered concentrations of several essential and non-essential amino acids, suggesting a downregulation of certain metabolic pathways and a potential dispersal-foraging trade-off.

6. This study is the first to demonstrate how metabolic adaptations might underlie life-history evolution during range expansion.

Keywords: amino acids, common garden, GC-MS metabolomics, global change, latitudinal gradient, *Tetranychus urticae*

Introduction

During range expansions or range shifts, species' life histories can evolve at contemporary timescales (Phillips, Brown & Shine 2010). Changing environmental conditions force species to locally adapt and spatial assortment of dispersive phenotypes leads to increased dispersiveness at the expanding/shifting range edge (Shine, Brown & Phillips 2011). These evolutionary processes of local adaptation and spatial selection affect key life-history traits like fecundity, development and dispersal (reviewed in Chuang & Peterson 2015). We therefore expect range edge populations to exhibit physiological adaptations that underlie these observed trait evolutions, more particularly at the metabolism level. Indeed, any elevation of the performance of one life-history trait augments its energetic and metabolite demands at the expense of other traits (*cfr.* the "Y" model of resource allocation, Van Noordwijk & de Jong 1986; Zera & Harshman 2001), thus modifying the global metabolic network operation. As a result, life-history differentiation is expected to be associated with changes in the metabolome (*i.e.* the set of circulating metabolites within an organism, Oliver *et al.* 1998), as was for example found for ageing in *Caenorhabditis elegans* (Fuchs *et al.* 2010) and reproduction in the Malaria Mosquito (Fuchs *et al.* 2014).

Metabolomics is a powerful technique which can be used as a candidate approach to explore an organism's response to environmental variations and forthcoming environmental change (Hines *et al.* 2007; Miller 2007; Viant 2008; Bundy, Davey & Viant 2009; Lankadurai, Nagato & Simpson 2013; Hidalgo *et al.* 2014). It provides information on the interaction

between an organism's physiology and its natural environment by identifying metabolites of low to moderate molecular mass within the whole body, cells, tissues or biofluids. Compared to other –omics technologies like genomics and transcriptomics, metabolomics has the significant advantage to focus on 'downstream' cellular functions (Snart, Hardy & Barrett 2015), providing a more direct picture of the functional links between causes and consequences of environmental variation (Foucreau *et al.* 2012). Essentially, metabolomics provides the link between genotypes and phenotypes (Fiehn 2002). When applied on individuals originating from different localities from range core to edge, but reared for several generations under common garden conditions, it can provide insights on the physiological adaptations that underlie life-history evolution during range expansion.

Though a consideration of the whole-organism physiology allows a better understanding of how life-history evolution in natural populations might occur and why this evolution is sometimes constrained (Zera *et al.* 2001; Ricklefs & Wikelski 2002), few studies documented metabolic variation in wild populations along natural gradients (Sardans, Penuelas & Rivas-Ubach 2011). Instead, most studies assess plastic or evolutionary responses to environmental stressors by manipulating abiotic variables in controlled environments (Sardans *et al.* 2011; Colinet *et al.* 2012; Padfield *et al.* 2016). Notable exceptions are the studies on *Arabidopsis lyrata* that demonstrated distinct metabolic phenotypes along the species' latitudinal distribution, with a typical cold-induced metabolome in the north, indicating adaptation to the local climate (Davey *et al.* 2008; Davey, Woodward & Quick 2009), but no difference in metabolic fingerprint between large connected *versus* marginal fragmented populations (Kunin

83 *et al.* 2009). These studies, however, used plants that were grown from seeds collected directly
84 from the field. Environmental maternal effects can therefore not be excluded. In fact, so far, no
85 study to our knowledge assessed true evolutionary changes in the metabolome of natural
86 populations.
87
88 The two-spotted spider mite, *Tetranychus urticae* Koch (Acari, Tetranychidae), a
89 generalist pest species in greenhouses and orchards, expanded its European range from the
90 Mediterranean to at least southern Scandinavia (K. H. P. Van Petegem, personal observation)
91 during the last decades (for more information, see Carbonnelle *et al.* 2007). Previous research
92 with *T. urticae* showed quantitative genetic life-history differentiation along this latitudinal
93 gradient, with daily fecundity, lifetime fecundity and longevity decreasing from range core to
94 edge, and egg survival, dispersal propensity and sex ratio increasing from range core to edge
95 (Van Petegem *et al.* 2015). We expected this life-history differentiation to be associated with an
96 enhancement of different metabolic pathways and thus distinct metabolic phenotypes.
97
98 Using a metabolomics approach, the current study aimed to test (i) whether the
99 metabolome of *T. urticae* evolved during its recent range expansion; *i.e.* whether a gradual
100 change in the mite's metabolic phenotype is present from range core to range edge, (ii) whether
101 this metabolic differentiation is associated with the up- or downregulation of certain metabolic
102 pathways and (iii) whether this evolutionary change in the species' metabolic phenotype is
103 associated with the life-history differentiation that has occurred during its range expansion.

Materials and methods

Field sampling and common garden

In August 2012, we hand-sampled mites from nine localities (one population per locality) along an 800 km latitudinal gradient from north-western Belgium to northern Denmark (Fig. 1). Mites were found on infested leaves of *Lonicera periclymenum* (European honeysuckle) at high latitudes and on *Euonymus europaeus* (European spindle), *Humulus lupulus* (common hop) or *Sambucus nigra* (European black elderberry) at lower latitudes. (More information is provided in online supporting information SI.1.) In the laboratory, fifty to several hundreds of mites per population were put on separate whole bean plants (*Phaseolus vulgaris*, variety Prélude) and kept under controlled conditions at room temperature with a light-regime of 16:8 LD. After one generation, ten adult female mites per population were taken from their bean plant and put on a piece of bean leaf on wet cotton in a Petri dish. Two such Petri dishes were prepared for each population. The Petri dishes were then used to create a pool of synchronised two-day adult female mites for each population. (Two-day adult females were preferred, since these are significantly bigger than fresh adults.) For this purpose, all females were allowed to lay eggs during 24 hours in a climate room at 27 °C, with a light-regime of 16:8 LD. The resulting same-aged eggs were subsequently left to develop until they were two-day adult mites, of which only females (which are easily recognized) were selected. As mites were kept in common garden for two generations, all direct environmental effects were excluded.

Metabolomic profiling using Gas Chromatography-Mass Spectrophotometry (GC-MS)

126 As we wanted to scan metabolites from different metabolite families (because of their
127 various but connected roles in general organismal physiology), we used GC-MS metabolomics
128 (Koek *et al.* 2011; Khodayari *et al.* 2013). For each population, we constructed the metabolomic
129 profile of five replicated pooled sets of fifty two-day-adult female mites. Each set was placed in
130 a microtube and snap-frozen at -80 °C. To be able to measure true quantities of the metabolites,
131 it is important to standardise the initial masses of each extract. However, even when pooling
132 fifty individuals, the masses of the replicates were too low to be accurately measured. Yet,
133 previous research showed that female adult size does not differ among the nine sampled
134 populations (Van Petegem *et al.* 2015). We could thus confidently use and interpret metabolite
135 concentrations in nmol/sample. The samples were first homogenized in ice-cold (-20 °C)
136 methanol-chloroform (2:1), using a tungsten-bead beating equipment (RetschTM MM301,
137 Retsch GmbH, Haan, Germany) at 25 Hz. After addition of ice-cold ultrapure water, the samples
138 were centrifuged at 4,000 g for 5 min at 4 °C. The upper aqueous phase was then transferred to
139 new chromatographic glass vials, dried-out and resuspended in 30 µl of 20 mg L-1
140 methoxyamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in pyridine and incubated
141 under automatic orbital shaking at 40 °C for 60 min. Subsequently, 30 µl of N-methyl-N-
142 (trimethylsilyl) trifluoroacetamide (MSTFA; Sigma, #394866) was added and the derivatisation
143 was conducted at 40 °C for 60 min under agitation. The samples were then analysed in a GC-MS
144 system (Thermo Fischer Scientific Inc., Waltham, MA, USA), using the same settings as in
145 Khodayari *et al.* (2013). For this purpose, one microliter of each sample was injected in the GC-
146 MS system using the split mode (split ratio: 25:1). After that, the selective ion monitoring (SIM)
147 mode (electron energy: -70 eV) was used to search for the sixty primary metabolites that are

148 most often found in insect samples and that were included in our spectral database(see online
149 supporting information SI.2 for a complete overview of these sixty metabolites). This database
150 consisted, amongst others, out of sixteen amino acids and eleven sugars. The SIM mode
151 ensured a precise annotation of the detected peaks. The calibration curves were set using
152 samples consisting of sixty pure reference compounds at concentrations of 1, 2, 5, 10, 20, 50,
153 100, 200, 500, 750, 1000, 1500 and 2000 µM. Chromatograms were deconvoluted using
154 XCalibur v2.0.7 software (Thermo Fischer Scientific Inc., Waltham, MA, USA). Finally, metabolite
155 concentrations were quantified according to their calibration curves.
156
157 **Statistics**
158 A total of forty-three metabolites were identified. In a first step, we examined whether a
159 general pattern in the concentrations of these metabolites exists along the invasion gradient of
160 our study species (*i.e.* whether the metabolic profile is gradually changing from range core to
161 edge). More specifically, we examined whether the metabolite concentrations could be sorted
162 as a function of latitude or as a function of one of the six life-history traits that were previously
163 shown to covary with latitude (daily and lifetime fecundity, egg survival, longevity, dispersal
164 propensity and sex ratio, see Van Petegem *et al.* 2015). The metabolite concentrations were
165 first auto-scaled and cube root- (when looking for covariation with daily fecundity and egg
166 survival) or log- (for latitude) transformed (no transformation was needed for lifetime fecundity,
167 longevity, dispersal propensity and sex ratio). Then, a Partial Least Squares – Discriminant
168 Analysis (PLS-DA) was performed to examine interpopulation variation in the metabolite
169 concentrations. This multivariate analysis was performed using MetaboAnalyst 3.0 (Xia *et al.*

170 2009; Xia *et al.* 2012; Xia *et al.* 2015). By ordering the populations according to their latitude or
171 according to one of the six life-history traits covarying with latitude, it was possible to check for
172 trends in the metabolite concentrations. To check the significance of this interpopulation
173 variation, permutation tests (2,000 permutations) were run using separation distance (B/W) test
174 statistics. The PLS-DA provided Variable Importance in Projection (VIP) scores, which were
175 subsequently used to select the most important metabolites in explaining the variation among
176 populations (low VIP-scores depict a weak, and high scores a strong global pattern). Using a
177 step-wise procedure, only those metabolites with a VIP score of at least 1.2 (1.0 for egg survival
178 because removing the metabolites with a score between 1.0 and 1.2 resulted in a decreased
179 percentage of variation explained) for the first and/or second component were retained for
180 further analysis (compared to 0.8 in Tenenhaus 1998).

181 In a second step, univariate analyses were performed to test, metabolite by metabolite,
182 whether the global patterns obtained in the previous step could be confirmed. Using SAS 9.4
183 (SAS Institute Inc. 2013), linear regressions were run for all those metabolites that were
184 retained during the above described multivariate analysis. As our study is explorative (though
185 on highly standardised samples regarding environmental conditions during development), we
186 wanted to avoid false negatives (with the chance of making a Type II error). We therefore did
187 not correct for multiple comparisons (*e.g.* Bonferroni correction). Given the large number of
188 statistical tests, such a correction would have greatly diminished our statistical power.

189 In a final step, a metabolic pathway analysis was performed in Metaboanalyst 3.0 (Xia *et*
190 *al.* 2009; Xia *et al.* 2012; Xia *et al.* 2015) with those metabolites that showed significant effects
191 in the univariate analyses. These pathway analyses were performed with a Fisher's exact test

192 algorithm, which we ran using the metabolic pathways of *Drosophila melanogaster* (*i.e.* the
193 closest relative of *T. urticae* available in the program). The algorithm calculates the match
194 (number of hits) between the metabolites in a dataset and the totality of metabolites present in
195 a specific pathway. Furthermore, it uses a pathway topology analysis to compute a value for the
196 impact of these metabolites on the pathway. As multiple comparisons are made, corrected
197 Holm p-values are provided. This final step linked the selected individual metabolites with one
198 or more metabolic pathways, thus identifying those pathways that were potentially up- or
199 downregulated during the range expansion of *T. urticae*.
200

201 **Results**

202

203 ***Latitudinal covariation***

204 The PLS-DA showed a separation between the nine populations, which was visible on 3-D
205 score plots (see online supporting information SI.3). Of the forty-three identified metabolites,
206 seventeen had VIP scores of at least 1.2 and were thus retained in the analysis (Fig. 2A). They
207 showed a clear general trend from high values in southern to low values in northern populations
208 (Fig. 2A).

209 In the subsequent linear regressions, thirteen of these seventeen metabolite
210 concentrations decreased significantly with increasing latitude (Fig. 2A). Among these thirteen
211 metabolites, six essential amino acids, five non-essential amino acids (see Rodriguez & Hampton
212 (1966) for an overview of all essential amino acids in *T. urticae* –we defined tryptophan, which is
213 not included in this overview, as essential) and one intermediate of the citric acid cycle can be
214 mentioned.

215 Pathway analysis indicated that of these thirteen metabolites, eleven play a significant
216 role in the aminoacyl-tRNA biosynthesis (total: 67, hits: 11, impact=0, Holm p=2.6066E-10) and
217 four in the valine, leucine and isoleucine biosynthesis (total: 13, hits: 4, impact=0.9999, Holm
218 p=8.5705E-4) (both pathway maps are provided in online supporting information SI.6).

219

220 ***Life history covariation***

221 The PLS-DA showed a separation between the nine (eight for egg survival, for which no
222 data were available for population SVI) populations, which is visible on 3-D score plots (see
223 online supporting information SI.3). Of the forty-three identified metabolites, only those which
224 explained most of the interpopulation variation for a certain life-history trait (high VIP score, see
225 above) were retained for further analysis. Fourteen were retained for daily and sixteen for
226 lifetime fecundity, twenty were retained for egg survival, eleven for longevity, thirteen for
227 dispersal propensity and ten for sex ratio (Fig. 2B-G). Figure 2 (B-G) shows clear indications of a
228 positive correlation between lifetime fecundity and its sixteen selected metabolites. In contrast,
229 figure 2 (B-G) suggests a negative correlation between the twenty and thirteen metabolites
230 selected for, respectively, egg survival and dispersal propensity. For daily fecundity, longevity
231 and sex ratio, no clear trends were visible.

232 In the subsequent linear regressions, performed with the selected metabolites, one
233 significant correlation was found for daily fecundity (a polyol), four for egg survival (one
234 essential amino acid, two non-essential amino acids and one intermediate of the citric acid
235 cycle), eight for dispersal propensity (including five essential amino acids) and two for sex ratio
236 (including one intermediate of the citric acid cycle). No significant results were found for
237 lifetime fecundity or longevity (Fig. 2B-G).

238 Pathway analysis indicated that three of the four metabolites that were negatively
239 correlated with egg survival play a significant role in the alanine, aspartate and glutamate
240 metabolism (total: 23, hits: 3, impact=0.2770, Holm p=0.0032) and that five of the eight
241 metabolites which negatively correlated with dispersal propensity play a significant role in the
242 aminoacyl-tRNA biosynthesis (total: 67, hits: 5, impact=0, Holm p=0.0041) (both pathway maps

are provided in online supporting information SI.6). No associated pathways were found for daily fecundity and sex ratio.

Discussion

Of the forty-three metabolites identified in the GC-MS analysis, twenty-one correlated with latitude and/or one or more life-history traits. More specifically, thirteen showed a significantly reduced concentration with increasing latitude, eight showed a negative correlation with dispersal propensity, four showed a negative correlation with egg survival, two showed a correlation (one positive, one negative) with sex ratio and one showed a negative correlation with daily fecundity. Of the twenty-one different metabolites, thirteen amino acids and one intermediate of the citric acid cycle could be shown to play an important role in the aminoacyl-tRNA biosynthesis, the valine, leucine and isoleucine biosynthesis and/or the alanine, aspartate and glutamate metabolism (see pathway maps provided in online supporting information SI.6).

Our results indicate that the life-history evolution which occurred during the recent range expansion of *T. urticae* (Van Petegem *et al.* 2015) was not associated with shifts in the mites' energetic metabolism, but rather with shifts in its anabolism. While our spectral database contained eleven sugars, not a single sugar accounted for the separation among populations. This suggests that the genes involved in encoding the mite's energetic metabolism (*i.e.* glycolysis, citric acid cycle, which typically involve sugars) have not been affected during the range expansion of *T. urticae*. Instead, the observed differentiation in the mites' metabolome probably involved evolutionary changes in the mites' anabolism, where amino acids play a central role in the metabolic turnover of proteins. In more northern and more dispersive populations, the aminoacyl-tRNA biosynthesis was downregulated. In this pathway, aminoacyl-

268 tRNA is formed by charging tRNA with an amino acid. The aminoacyl-tRNA then serves as a
269 substrate in protein synthesis or plays one of its many other roles in, for example, cell wall
270 formation or antibiotic biosynthesis (Raina & Ibba 2014). In accordance, the valine, leucine and
271 isoleucine biosynthesis, important for protein synthesis as well (Ahmed & Khan 2006; Tamanna
272 & Mahmood 2014), was downregulated in more northern populations. We did nonetheless find
273 one possible connection with the energetic metabolism. In populations with a high egg survival,
274 the alanine, aspartate and glutamate metabolic pathway, which acts directly in fuelling
275 energetic metabolism (Sacktor 1955; Maity *et al.* 2012), was downregulated.
276
277 The affected amino acids showed decreased concentrations toward higher latitudes and
278 showed a negative correlation with the dispersal propensity and egg survival of *T. urticae*, both
279 of which increase towards the north. While, in general, amino acids are considered fundamental
280 for egg production and thus fecundity (Tulisalo 1971; O'Brien, Fogel & Boggs 2002; Mevi-Schutz
281 & Erhardt 2005; Fuchs *et al.* 2014, but see Heagle *et al.* 2002), not a single correlation was found
282 for fecundity, despite a clear positive trend in the PLS-DA. Of the fourteen affected amino acids,
283 eight were essential and six non-essential. While the non-essential amino acids could have been
284 synthesized *de novo* from glucose, the essential amino acids could only have been supplied
285 through the mite's diet (Rodriguez *et al.* 1966). Though all mites were kept in common garden,
286 mites from northern, more dispersive populations were found to contain lower essential amino
287 acid concentrations. In line with the recent finding of Fronhofer & Altermatt (2015) that a
288 dispersal-foraging trade-off leads to a reduced exploitation of resources at range margins, our
289 results could indicate that northern, dispersive mites evolved lower essential amino acid

290 concentrations because they consume less of their food source. We should, however, keep in
291 mind that metabolites were measured only at one point in time and from whole organism
292 samples. We are therefore missing the temporal fluctuations of the metabolome over a day,
293 and our data therefore represent only a snapshot of the existing balance in terms of metabolite
294 demand among metabolic pathways.
295
296 An important challenge for metabolomics is understanding the relative contribution of
297 environmental and genetic factors in shaping an organism's metabolic phenotype (Bundy *et al.*
298 2009). In the current study, mites were kept in common garden for two generations, during
299 which they were reared under optimal conditions. As such, only genetic factors were retained.
300 Though genetic factors are considered less determining than environmental factors (Robinson
301 *et al.* 2007; Frank, Noerenberg & Engel 2009; Matsuda *et al.* 2012), our results demonstrate a
302 clear genetic signal of metabolic differentiation. With our common garden setup, we could
303 show that the metabolome of *T. urticae* underwent persistent changes through local adaptation
304 and/or spatial selection along the species' invasion gradient. Interestingly, as metabolic changes
305 were mainly linked with latitude and dispersal propensity, the metabolic differentiation is
306 anticipated to be mostly caused by spatial selection and not local adaptation.
307
308 This explorative study is the first that specifically examined whether range expansion
309 results in evolutionary changes in an organism's metabolism. Despite non-stressful common
310 garden conditions, approximately forty per cent of the identified metabolites showed rapid
311 evolutionary changes. Though effects were small, our results clearly indicate that the

312 metabolome of *T. urticae* underwent genetic changes during the species' recent range
313 expansion. By linking this metabolic differentiation with the life-history evolution observed in a
314 previous study, we could furthermore show that for dispersal propensity and egg survival, some
315 of the trait variation was associated with changes in the mite's metabolism.

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Data accessibility

- Sample locations and life-history trait values: uploaded as online supporting information

- Results of GC-MS analysis: will be deposited in the Dryad repository (and added to the reference list)

- Outcomes of statistical analyses: uploaded as online supporting information

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328

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443
444

445 ## Figure legends

446

447 **Figure 1**

448 The map shows the nine field collection sites, which are situated in Belgium, The Netherlands
449 and Denmark. The graph shows the yearly number of frost days and the average yearly
450 temperature for each collection site along the latitudinal gradient. These climatic data were
451 obtained from FetchClimate (Microsoft Research, Cambridge) and were averaged over a period
452 of 35 years (1980 to 2015). Below the graph, arrows for each of six life-history traits depict their
453 trend along the latitudinal gradient (increase, decrease). (For more detailed information, see
454 online supporting information SI.1 and Van Petegem *et al.* 2015.)

455

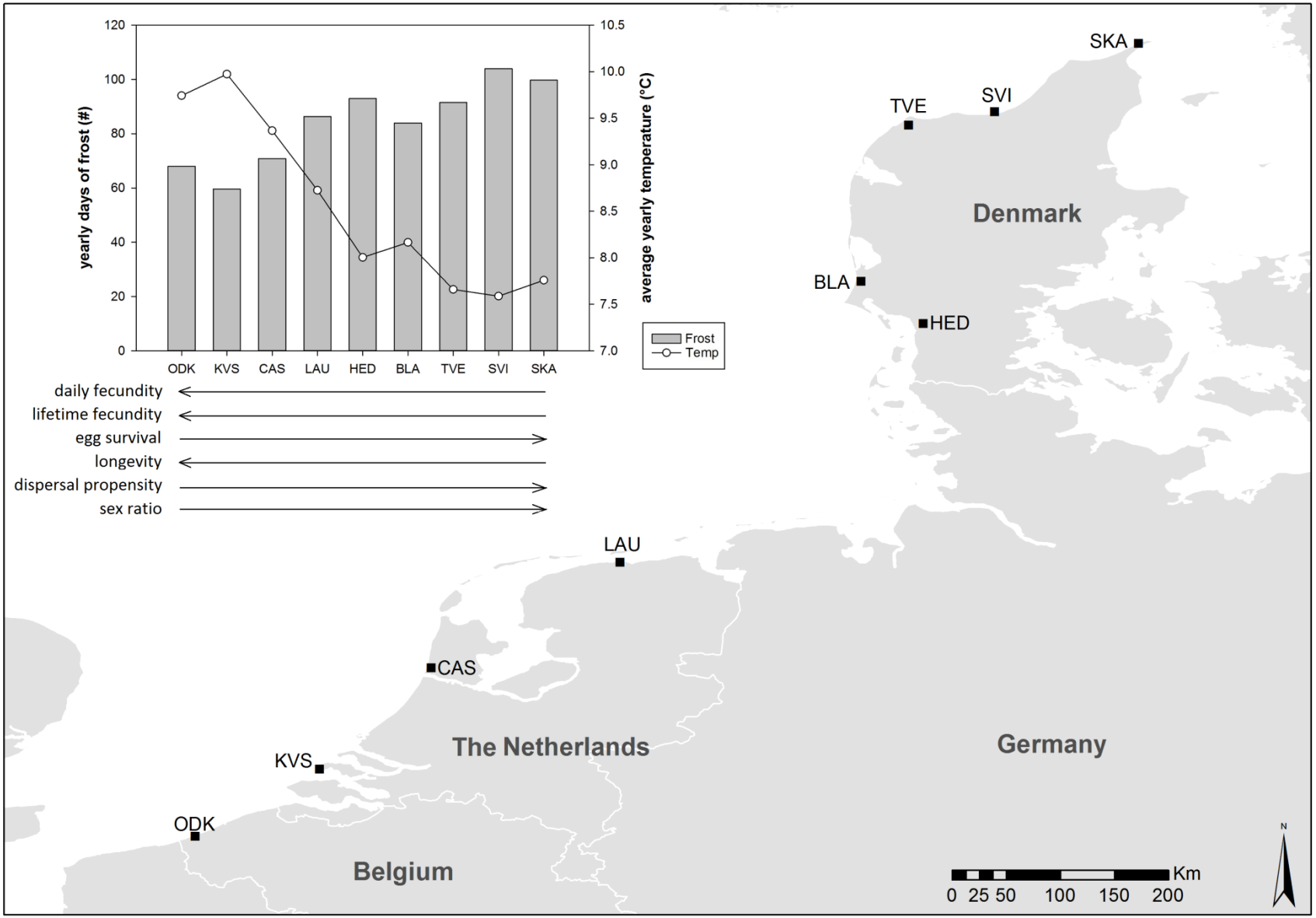
456 **Figure 2**

457 Variable importance plots resulting from the multivariate analyses (PLS-DA) on the metabolomic
458 data. These plots list those metabolites that, based on their VIP score, contribute the most to
459 explaining the variation among the nine populations in our dataset (ODK, KVS, CAS, LAU, HED,
460 BLA, TVE; SVI, SKA). The metabolites are ordered from high to low VIP scores for component 1
461 (an overview off all scores for component 1 and 2 is provided in online supporting information
462 SI.4). The colour codes indicate the relative concentration of a given metabolic compound for
463 each population (green=low concentration, to red= high concentration). The populations
464 themselves are ordered according to their latitude (A), or from low values at the left to high
465 values at the right for a given life-history trait (daily (B) or lifetime (C) fecundity, egg survival (D),
466 longevity (E), dispersal propensity (F) or sex ratio (G)). For example, in Fig. 3A, LAU is the

467 population with the highest concentration of proline and ODK is the southernmost population
468 (lowest latitude). At the bottom of each plot, the p-value resulting from the permutation test is
469 given. At the left side of each plot, an asterisk next to a metabolite name indicates a significant
470 correlation between this metabolite and latitude or the denoted life-history trait. For example,
471 in Fig. 3A, proline shows a significant negative correlation with latitude. (A detailed overview,
472 including p- and F-values, of all significant linear regressions is found in online supporting
473 information SI.5.)
474

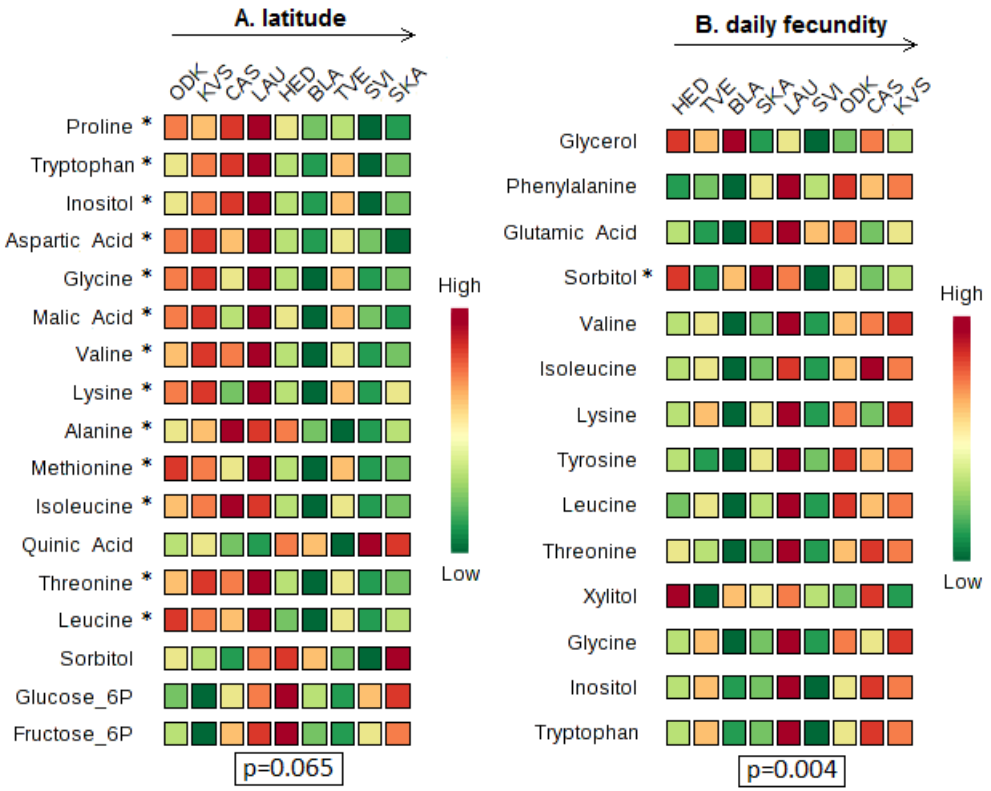
Figures

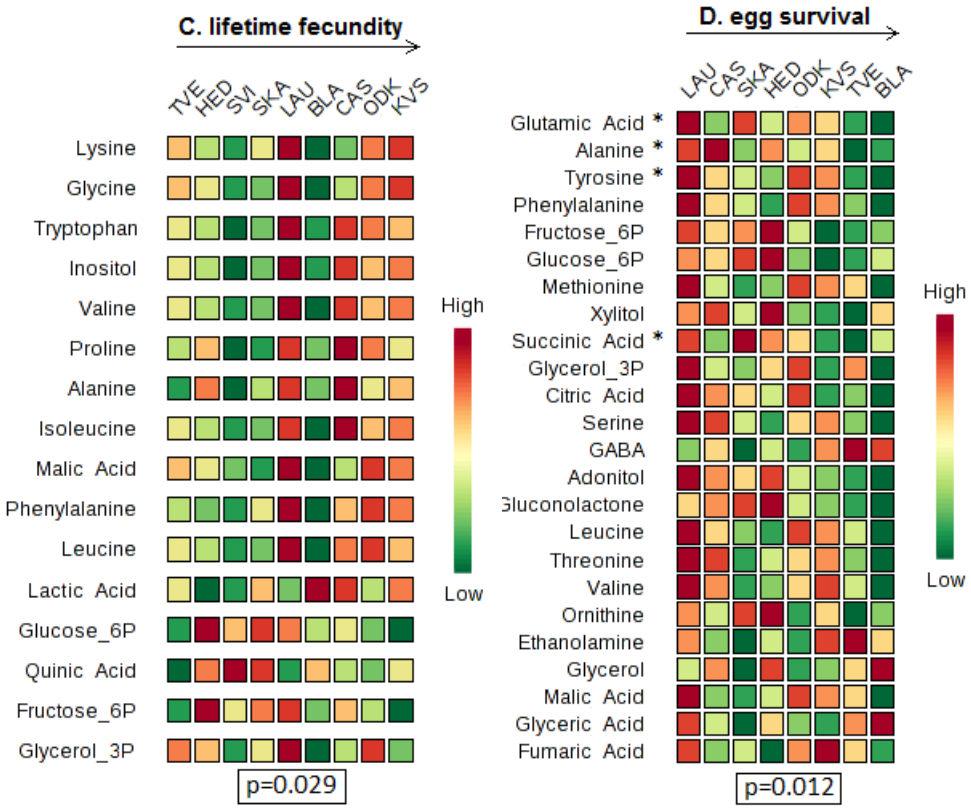
Figure 1


yearly days of frost (#)
average yearly temperature (°C)
ODK KVS CAS LAU HED BLA TVE SVI SKA
Frost
Temp
daily fecundity
lifetime fecundity
egg survival
longevity
dispersal propensity
sex ratio
SKA
TVE
SVI
Denmark
BLA
HED
LAU
CAS
The Netherlands
Germany
KVS
ODK
Belgium
Km
0 25 50 100 150 200
N

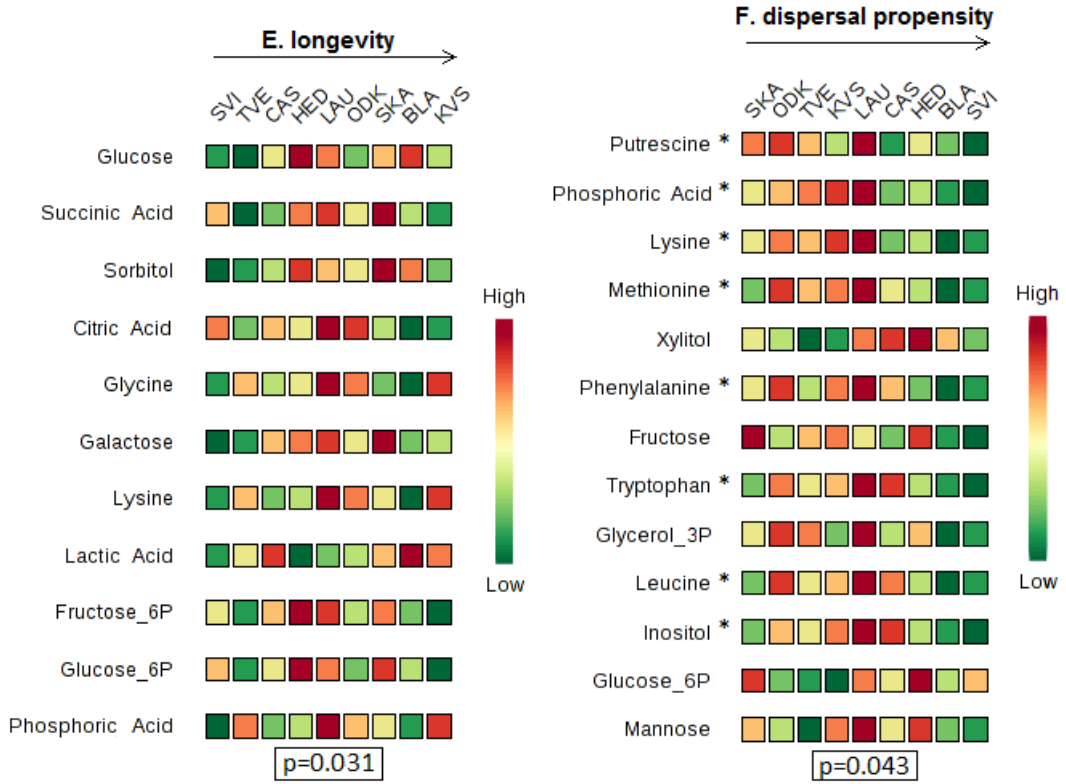
478

Figure 2

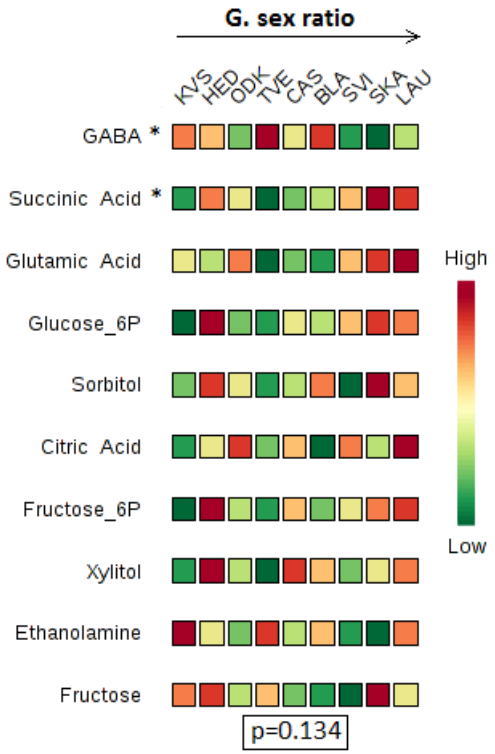

A. latitude
ODK
KVS
CAS
LAU
HED
BLA
TVE
SVI
SKA
Proline *
Tryptophan *
Inositol *
Aspartic Acid *
Glycine *
Malic Acid *
Valine *
Lysine *
Alanine *
Methionine *
Isoleucine *
Quinic Acid
Threonine *
Leucine *
Sorbitol
Glucose_6P
Fructose_6P
High
Low
p=0.065
B. daily fecundity
HED
TVE
BLA
SKA
LAU
SVI
ODK
CAS
KVS
Glycerol
Phenylalanine
Glutamic Acid
Sorbitol *
Valine
Isoleucine
Lysine
Tyrosine
Leucine
Threonine
Xylitol
Glycine
Inositol
Tryptophan
High
Low
p=0.004


C. lifetime fecundity
TVE
HED
SVI
SKA
LAU
BLA
CAS
ODK
KVS
Lysine
Glycine
Tryptophan
Inositol
Valine
Proline
Alanine
Isoleucine
Malic Acid
Phenylalanine
Leucine
Lactic Acid
Glucose_6P
Quinic Acid
Fructose_6P
Glycerol_3P
High
Low
p=0.029
D. egg survival
LAU
CAS
SKA
HED
ODK
KVS
TVE
BLA
Glutamic Acid *
Alanine *
Tyrosine *
Phenylalanine
Fructose_6P
Glucose_6P
Methionine
Xylitol
Succinic Acid *
Glycerol_3P
Citric Acid
Serine
GABA
Adonitol
Gluconolactone
Leucine
Threonine
Valine
Ornithine
Ethanolamine
Glycerol
Malic Acid
Glyceric Acid
Fumaric Acid
High
Low
p=0.012

481


E. longevity
SVI
TVE
CAS
HED
LAU
ODK
SKA
BLA
KVS
Glucose
Succinic Acid
Sorbitol
Citric Acid
Glycine
Galactose
Lysine
Lactic Acid
Fructose_6P
Glucose_6P
Phosphoric Acid
p=0.031
High
Low
F. dispersal propensity
SKA
ODK
TVE
KVS
LAU
CAS
HED
BLA
SVI
Putrescine *
Phosphoric Acid *
Lysine *
Methionine *
Xylitol
Phenylalanine *
Fructose
Tryptophan *
Glycerol_3P
Leucine *
Inositol *
Glucose_6P
Mannose
p=0.043
High
Low

482


G. sex ratio
KVS
HED
ODK
TVE
CAS
BLA
SVI
SKA
LAU
GABA *
Succinic Acid *
Glutamic Acid
Glucose_6P
Sorbitol
Citric Acid
Fructose_6P
Xylitol
Ethanolamine
Fructose
High
Low
p=0.134

483

484