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

Katrien H. P. Van Petegem [*a], David Renault [b], Robby Stoks [c] and Dries Bonte [a]

[a] Department of Biology, Ghent University, Ghent, Belgium

[b] UMR CNRS 6553 Ecobio, Université de Rennes 1, Rennes Cedex, France

[c] Evolution and Conservation, KU Leuven, Leuven, Belgium

[*] Corresponding author: Katrien.VanPetegem@ugent.be

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

et al. 2009). These studies, however, used plants that were grown from seeds collected directly from the field. Environmental maternal effects can therefore not be excluded. In fact, so far, no study to our knowledge assessed true evolutionary changes in the metabolome of natural populations.

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari, Tetranychidae), a generalist pest species in greenhouses and orchards, expanded its European range from the Mediterranean to at least southern Scandinavia (K. H. P. Van Petegem, personal observation) during the last decades (for more information, see Carbonnelle *et al.* 2007). Previous research with *T. urticae* showed quantitative genetic life-history differentiation along this latitudinal gradient, with daily fecundity, lifetime fecundity and longevity decreasing from range core to edge, and egg survival, dispersal propensity and sex ratio increasing from range core to edge (Van Petegem *et al.* 2015). We expected this life-history differentiation to be associated with an enhancement of different metabolic pathways and thus distinct metabolic phenotypes.

Using a metabolomics approach, the current study aimed to test (i) whether the metabolome of *T. urticae* evolved during its recent range expansion; *i.e.* whether a gradual change in the mite's metabolic phenotype is present from range core to range edge, (ii) whether this metabolic differentiation is associated with the up- or downregulation of certain metabolic pathways and (iii) whether this evolutionary change in the species' metabolic phenotype is 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)

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

most often found in insect samples and that were included in our spectral database(see online supporting information SI.2 for a complete overview of these sixty metabolites). This database consisted, amongst others, out of sixteen amino acids and eleven sugars. The SIM mode ensured a precise annotation of the detected peaks. The calibration curves were set using samples consisting of sixty pure reference compounds at concentrations of 1, 2, 5, 10, 20, 50, 100, 200, 500, 750, 1000, 1500 and 2000 µM. Chromatograms were deconvoluted using XCalibur v2.0.7 software (Thermo Fischer Scientific Inc., Waltham, MA, USA). Finally, metabolite concentrations were quantified according to their calibration curves.

Statistics

A total of forty-three metabolites were identified. In a first step, we examined whether a general pattern in the concentrations of these metabolites exists along the invasion gradient of our study species (*i.e.* whether the metabolic profile is gradually changing from range core to edge). More specifically, we examined whether the metabolite concentrations could be sorted as a function of latitude or as a function of one of the six life-history traits that were previously shown to covary with latitude (daily and lifetime fecundity, egg survival, longevity, dispersal propensity and sex ratio, see Van Petegem *et al.* 2015). The metabolite concentrations were first auto-scaled and cube root- (when looking for covariation with daily fecundity and egg survival) or log- (for latitude) transformed (no transformation was needed for lifetime fecundity, longevity, dispersal propensity and sex ratio). Then, a Partial Least Squares – Discriminant Analysis (PLS-DA) was performed to examine interpopulation variation in the metabolite concentrations. This multivariate analysis was performed using MetaboAnalyst 3.0 (Xia *et al.*

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

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

In a final step, a metabolic pathway analysis was performed in Metaboanalyst 3.0 (Xia *et al.* 2009; Xia *et al.* 2012; Xia *et al.* 2015) with those metabolites that showed significant effects 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

Results

Latitudinal covariation

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

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

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

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

243 are provided in online supporting information SI.6). No associated pathways were found for

244 daily fecundity and sex ratio.

245

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.

Acknowledgements

This project was funded by the Fund for Scientific Research – Flanders (FWO) (project G.0610.11). DB and RS were supported by BelSpo IAP Project "Speedy". We furthermore thank the INEE-CNRS (ENVIROMICS call, project 'ALIENS') for funding DR.

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

References

Ahmed, I. & Khan, M.A. (2006) Dietary branched-chain amino acid valine, isoleucine and leucine requirements of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *British Journal of Nutrition,* **96,** 450-460.

Bundy, J.G., Davey, M.P. & Viant, M.R. (2009) Environmental metabolomics: a critical review and future perspectives. *Metabolomics,* **5,** 3-21.

Carbonnelle, S., Hance, T., Migeon, A., Baret, P., Cros-Arteil, S. & Navajas, M. (2007) Microsatellite markers reveal spatial genetic structure of *Tetranychus urticae* (Acari : Tetranychidae) populations along a latitudinal gradient in Europe. *Experimental and Applied Acarology,* **41,** 225-241.

Chuang, A. & Peterson, C.R. (2015) Expanding population edges: theories, traits, and trade-offs. *Global Change Biology***,** doi: 10.1111/gcb.13107.

Colinet, H., Larvor, V., Laparie, M. & Renault, D. (2012) Exploring the plastic response to cold acclimation through metabolomics. *Functional Ecology,* **26,** 711-722.

Davey, M.P., Burrell, M.M., Woodward, F.I. & Quick, W.P. (2008) Population-specific metabolic phenotypes of *Arabidopsis lyrata* ssp. *petraea*. *New Phytologist,* **177,** 380-388.

Davey, M.P., Woodward, F.I. & Quick, W.P. (2009) Intraspecfic variation in cold-temperature metabolic phenotypes of *Arabidopsis lyrata* ssp. *petraea*. *Metabolomics,* **5,** 138-149.

Fiehn, O. (2002) Metabolomics - the link between genotypes and phenotypes. *Plant Molecular Biology,* **48,** 155-171.

348 Foucreau, N., Renault, D., Hidalgo, K., Lugan, R. & Petillon, J. (2012) Effects of diet and salinity
349 on the survival, egg laying and metabolic fingerprints of the ground-dwelling spider
350 *Arctosa fulvolineata* (Araneae, Lycosidae). *Comparative Biochemistry and Physiology a-*
351 *Molecular & Integrative Physiology,* **163,** 388-395.
352 Frank, T., Noerenberg, S. & Engel, K.-H. (2009) Metabolite Profiling of Two Novel Low Phytic Acid
353 (lpa) Soybean Mutants. *Journal of Agricultural and Food Chemistry,* **57,** 6408-6416.
354 Fronhofer, E.A. & Altermatt, F. (2015) Eco-evolutionary feedbacks during experimental range
355 expansions. *Nature Communications,* **6**.
356 Fuchs, S., Behrends, V., Bundy, J.G., Crisanti, A. & Nolan, T. (2014) Phenylalanine Metabolism
357 Regulates Reproduction and Parasite Melanization in the Malaria Mosquito. *Plos One,* **9**.
358 Fuchs, S., Bundy, J.G., Davies, S.K., Viney, J.M., Swire, J.S. & Leroi, A.M. (2010) A metabolic
359 signature of long life in *Caenorhabditis elegans*. *Bmc Biology,* **8**.
360 Heagle, A.S., Burns, J.C., Fisher, D.S. & Miller, J.E. (2002) Effects of carbon dioxide enrichment on
361 leaf chemistry and reproduction by twospotted spider mites (Acari : Tetranychidae) on
362 white clover. *Environmental Entomology,* **31,** 594-601.
363 Hidalgo, K., Mouline, K., Mamai, W., Foucreau, N., Dabire, K., Bouchereau, A., Simard, F. &
364 Renault, D. (2014) Novel insights into the metabolic and biochemical underpinnings
365 assisting dry-season survival in female malaria mosquitoes of the *Anopheles gambiae*
366 complex. *Journal of Insect Physiology,* **70,** 102-116.
367 Hines, A., Oladiran, G.S., Bignell, J.P., Stentiford, G.D. & Viant, M.R. (2007) Direct sampling of
368 organisms from the field and knowledge of their phenotype: Key recommendations for
369 environmental metabolomics. *Environmental Science & Technology,* **41,** 3375-3381.

370 Khodayari, S., Moharramipour, S., Larvor, V., Hidalgo, K. & Renault, D. (2013) Deciphering the
371 Metabolic Changes Associated with Diapause Syndrome and Cold Acclimation in the
372 Two-Spotted Spider Mite *Tetranychus urticae*. *Plos One,* **8**.
373 Koek, M.M., Jellema, R.H., van der Greef, J., Tas, A.C. & Hankemeier, T. (2011) Quantitative
374 metabolomics based on gas chromatography mass spectrometry: status and
375 perspectives. *Metabolomics,* **7,** 307-328.
376 Kunin, W.E., Vergeer, P., Kenta, T., Davey, M.P., Burke, T., Woodward, F.I., Quick, P., Mannarelli,
377 M.-E., Watson-Haigh, N.S. & Butlin, R. (2009) Variation at range margins across multiple
378 spatial scales: environmental temperature, population genetics and metabolomic
379 phenotype. *Proceedings of the Royal Society B-Biological Sciences,* **276,** 1495-1506.
380 Lankadurai, B.P., Nagato, E.G. & Simpson, M.J. (2013) Environmental metabolomics: an
381 emerging approach to study organism responses to environmental stressors.
382 *Environmental Reviews,* **21,** 180-205.
383 Maity, S., Jannasch, A., Adamec, J., Nalepa, T., Höök, T.O. & Sepúlveda, M.S. (2012) Starvation
384 causes disturbance in amino acid and fatty acid metabolism in *Diporeia*. *Comparative*
385 *Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* **161,** 348-355.
386 Matsuda, F., Okazaki, Y., Oikawa, A., Kusano, M., Nakabayashi, R., Kikuchi, J., Yonemaru, J.-I.,
387 Ebana, K., Yano, M. & Saito, K. (2012) Dissection of genotype-phenotype associations in
388 rice grains using metabolome quantitative trait loci analysis. *Plant Journal,* **70,** 624-636.
389 Mevi-Schutz, J. & Erhardt, A. (2005) Amino acids in nectar enhance butterfly fecundity: A long-
390 awaited link. *American Naturalist,* **165,** 411-419.

391 Miller, M.G. (2007) Environmental metabolomics: A SWOT analysis (strengths, weaknesses,
392 opportunities, and threats). *Journal of Proteome Research,* **6,** 540-545.
393 O'Brien, D.M., Fogel, M.L. & Boggs, C.L. (2002) Renewable and nonrenewable resources: Amino
394 acid turnover and allocation to reproduction in Lepidoptera. *Proceedings of the National*
395 *Academy of Sciences of the United States of America,* **99,** 4413-4418.
396 Oliver, S.G., Winson, M.K., Kell, D.B. & Baganz, F. (1998) Systematic functional analysis of the
397 yeast genome. *Trends in Biotechnology,* **16,** 373-378.
398 Padfield, D., Yvon-Durocher, G., Buckling, A., Jennings, S. & Yvon-Durocher, G. (2016) Rapid
399 evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecology*
400 *Letters,* **19,** 133-142.
401 Phillips, B.L., Brown, G.P. & Shine, R. (2010) Life-history evolution in range-shifting populations.
402 *Ecology,* **91,** 1617-1627.
403 Raina, M. & Ibba, M. (2014) tRNAs as regulators of biological processes. *Frontiers in Genetics,* **5,**
404 171.
405 Ricklefs, R.E. & Wikelski, M. (2002) The physiology/life-history nexus. *Trends in Ecology &*
406 *Evolution,* **17,** 462-468.
407 Robinson, A.R., Ukrainetz, N.K., Kang, K.-Y. & Mansfield, S.D. (2007) Metabolite profiling of
408 Douglas-fir (*Pseudotsuga menziesii*) field trials reveals strong environmental and weak
409 genetic variation. *New Phytologist,* **174,** 762-773.
410 Rodriguez, J.G. & Hampton, R.E. (1966) Essential amino acids determined in 2-spotted spider
411 mite *Tetranychus urticae* KOCH (Acarina, Tetranychidae) with glucose-U-C^{14}. *Journal of*
412 *Insect Physiology,* **12,** 1209-1216.

413 Sacktor, B. (1955) Cell structure and the metabolism of insect flight muscle. *Journal of*
414 *Biophysical and Biochemical Cytology,* **1,** 29-46.
415 Sardans, J., Penuelas, J. & Rivas-Ubach, A. (2011) Ecological metabolomics: overview of current
416 developments and future challenges. *Chemoecology,* **21,** 191-225.
417 Shine, R., Brown, G.P. & Phillips, B.L. (2011) An evolutionary process that assembles phenotypes
418 through space rather than through time. *Proceedings of the National Academy of*
419 *Sciences of the United States of America,* **108,** 5708-5711.
420 Snart, C.J.P., Hardy, I.C.W. & Barrett, D.A. (2015) Entometabolomics: applications of modern
421 analytical techniques to insect studies. *Entomologia Experimentalis Et Applicata,* **155,** 1-
422 17.
423 Tamanna, N. & Mahmood, N. (2014) Emerging roles of branched-chain amino acid
424 supplementation in human diseases. *International Scholarly Research Notices,* **2014**.
425 Tenenhaus, M. (1998) *La régression PLS*. Editions Technip, Paris.
426 Tulisalo, U. (1971) Free and bound amino acids of three host plant species and various fertilizer
427 treatments affecting the fecundity of the two-spotted spider mite, *Tetranychus urticae*
428 Koch (Acarina, Tetranychidae). *Annales Entomologicae Fennicae,* **37,** 155-163.
429 Van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources - Their influence
430 on variation in life-history tactics. *American Naturalist,* **128,** 137-142.
431 Van Petegem, K., Boeye, J., Stoks, R. & Bonte, D. (2015) Spatial selection and local adaptation
432 jointly shape life-history evolution during range expansion. *bioRxiv*.
433 Viant, M.R. (2008) Recent developments in environmental metabolomics. *Molecular Biosystems,*
434 **4,** 980-986.

435 Xia, J., Mandal, R., Sinelnikov, I.V., Broadhurst, D. & Wishart, D.S. (2012) MetaboAnalyst 2.0—a
436 comprehensive server for metabolomic data analysis. *Nucleic Acids Research*.
437 Xia, J., Psychogios, N., Young, N. & Wishart, D.S. (2009) MetaboAnalyst: a web server for
438 metabolomic data analysis and interpretation. *Nucleic Acids Research,* **37,** W652-W660.
439 Xia, J., Sinelnikov, I.V., Han, B. & Wishart, D.S. (2015) MetaboAnalyst 3.0—making metabolomics
440 more meaningful. *Nucleic Acids Research*.
441 Zera, A.J. & Harshman, L.G. (2001) The physiology of life history trade-offs in animals. *Annual*
442 *Review of Ecology and Systematics,* **32,** 95-126.
443
444

Figure legends

Figure 1

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

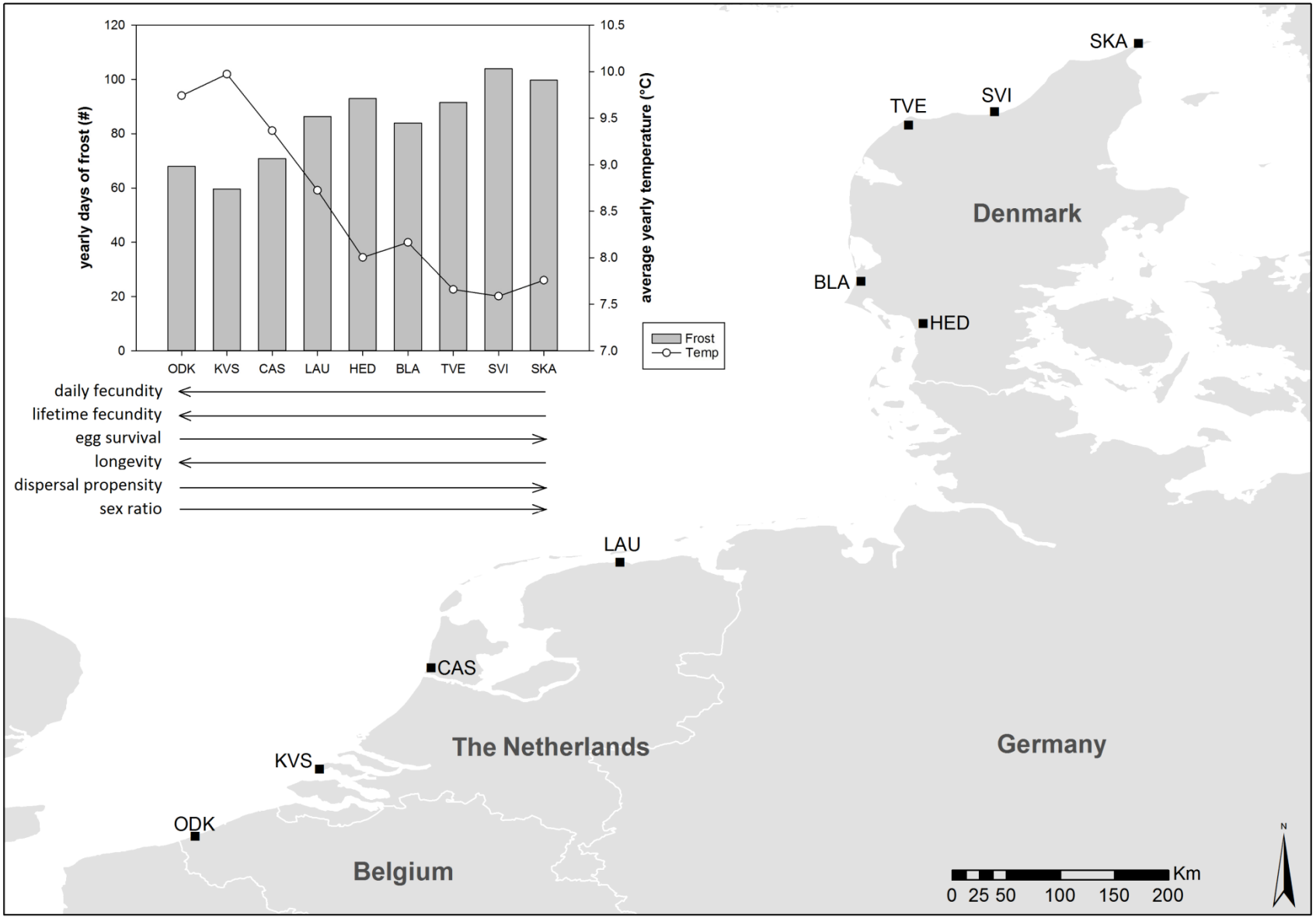
Figure 2

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

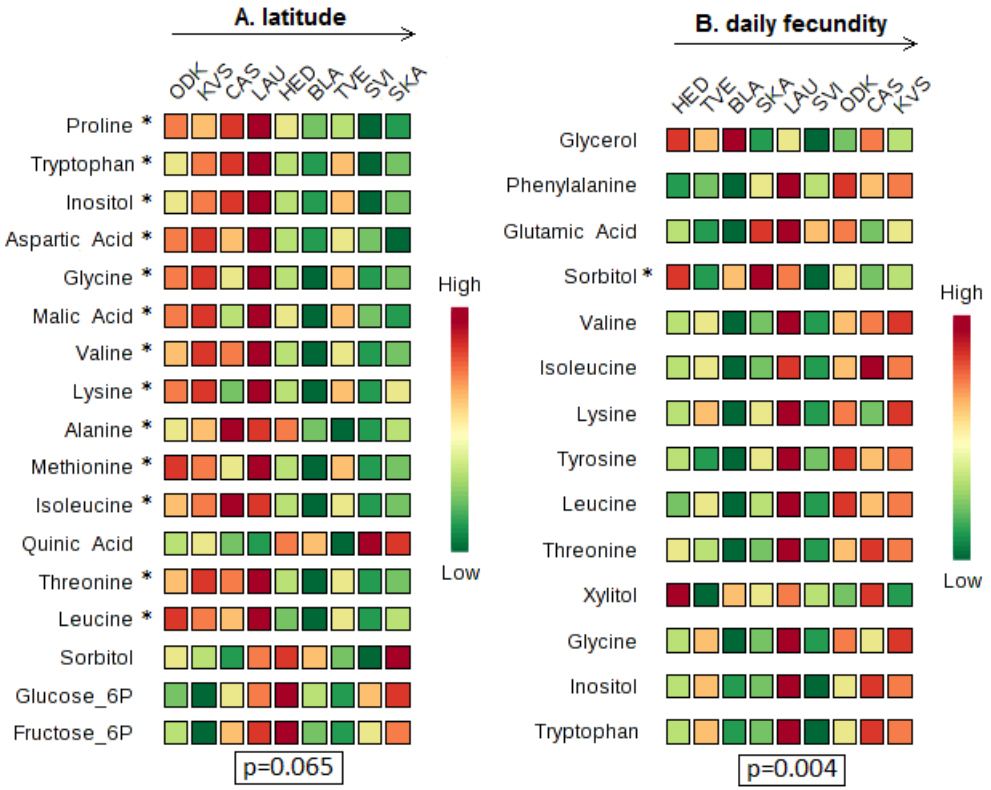
Figures

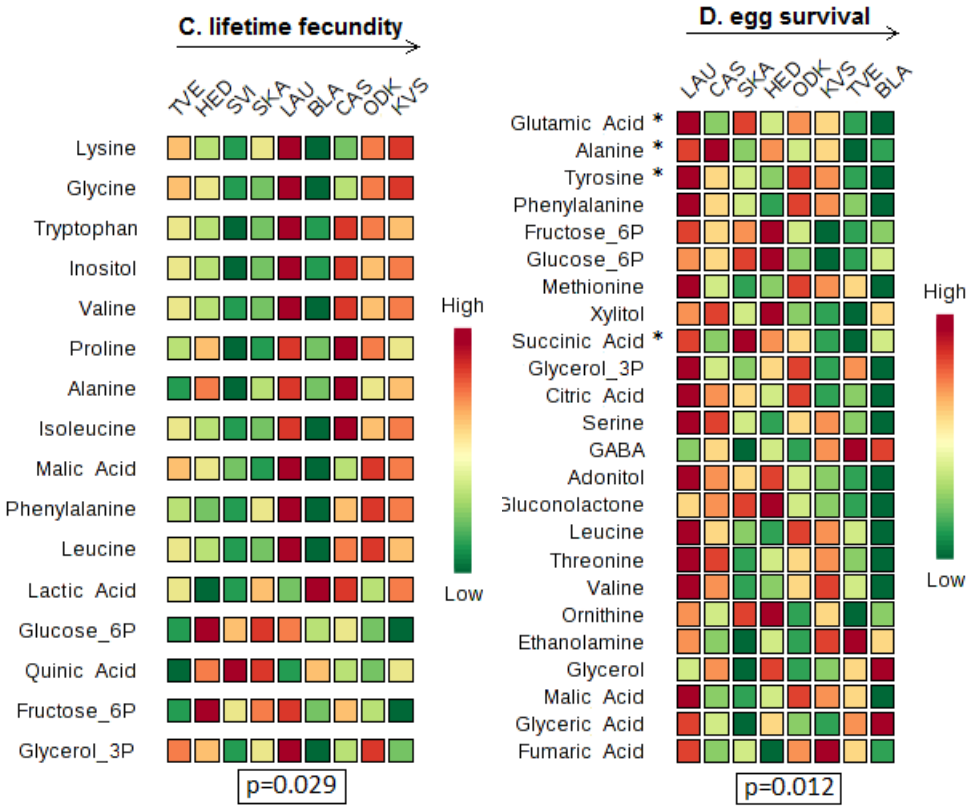
Figure 1


yearly days of frost (#)
average yearly temperature (°C)
120
100
80
60
40
20
0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
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
N
Km
0 25 50 100 150 200

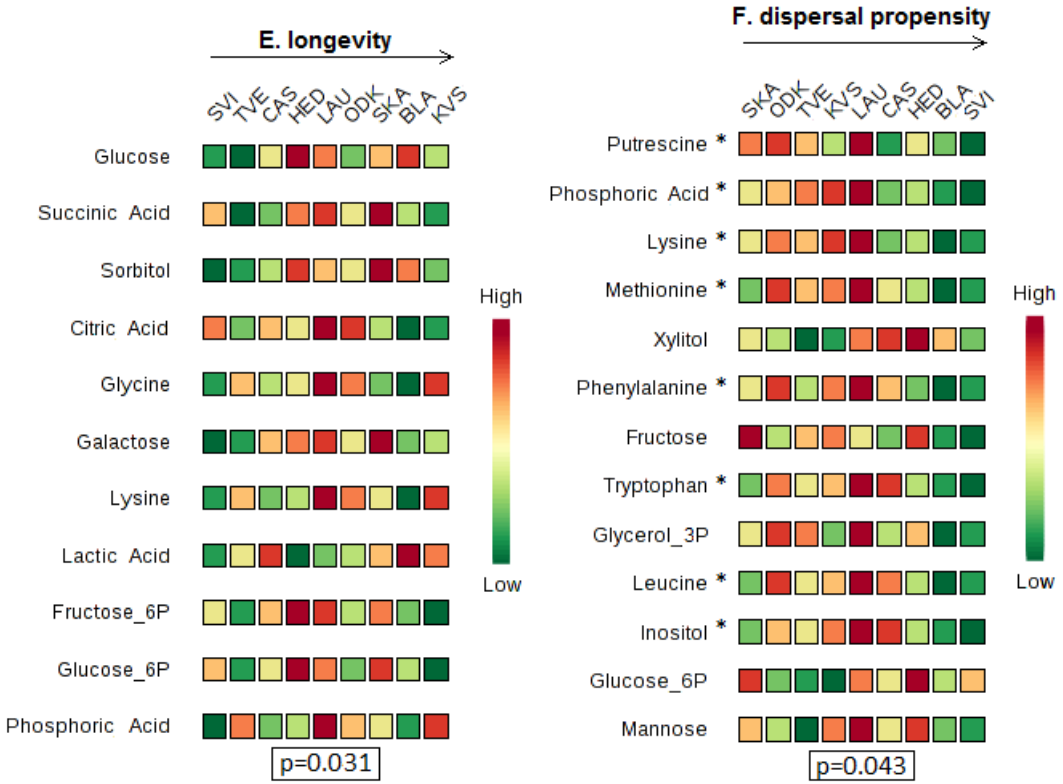
478

Figure 2

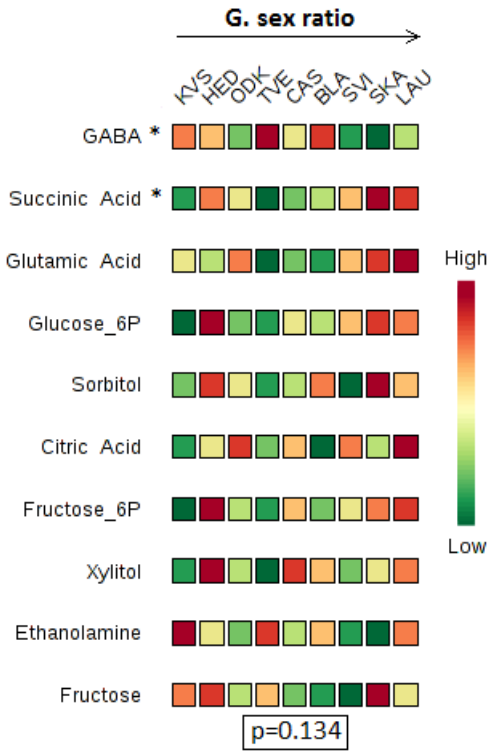

A. latitude
ODK KVS CAS LAU HED BLA TVE SVI SKA
Proline *
Tryptophan *
Inositol *
Aspartic Acid *
Glycine *
Malic Acid *
Valine *
Lysine *
Alanine *
Methionine *
Isoleucine *
Quinic Acid
Threonine *
Leucine *
Sorbitol
Glucose_6P
Fructose_6P
High
Low
p=0.065
B. daily fecundity
HED TVE BLA SKA LAU SVI 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
High
Low
p=0.031
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
High
Low
p=0.043

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