1 # General environmental heterogeneity as the

2 # explanation of sexuality? Comparative

3 # study shows that ancient asexual taxa are

4 # associated with both biotically and

5 # abiotically homogeneous environments

6

7 Authors

8 Jan Toman*, Jaroslav Flegr

9 J. Toman, J. Flegr: Laboratory of Evolutionary Biology, Department of Philosophy and History

10 of Sciences, Faculty of Science, Charles University in Prague, Vinicna 7, 128 00 Prague 2,

11 Czech Republic

12 J. Toman: Tel.: +(420)221951821. Email: tomanj@natur.cuni.cz

13 J. Flegr: Tel.: +(420)221951821. Email: flegr@cesnet.cz

14 * Corresponding author

Abstract

Ecological theories of sexual reproduction assume that sexuality is advantageous in certain conditions, for example, in biotically or abiotically more heterogeneous environments. Such theories thus could be tested by comparative studies. However, the published results of these studies are rather unconvincing. Here we present the results of a new comparative study based solely on the ancient asexual clades. The association with biotically or abiotically homogeneous environments in these asexual clades was compared with the same association in their sister, or closely related, sexual clades. Using the conservative definition of ancient asexuals (i.e. age > 1 million years), we found six pairs for which relevant ecological data are available. The difference between the homogeneity type of environment associated with the sexual and asexual species was then compared in an exact binomial test. Based on available literature, the results showed that the vast majority of ancient asexual clades tend to be associated with biotically or abiotically, biotically, and abiotically more homogeneous environments than their sexual controls. In the exploratory part of the study, we found that the ancient asexuals often have durable resting stages, enabling life in subjectively homogeneous environments, live in the absence of intense biotic interactions, and are very often sedentary, inhabiting benthos and soil. The consequences of these findings for the ecological theories of sexual reproduction are discussed.

KEYWORDS: ancient asexuals – asexual reproduction – Frozen evolution theory – habitat heterogeneity – sexual reproduction

2

Introduction

Paradox of sexual reproduction

The term "sexual" may be assigned to a broad array of processes (see e.g. Redfield, 2001; Birky, 2009; Gorelick & Carpinone, 2009; Schön et al., 2009a). On the other hand, sex in its strict sense (i.e. amphimixis) is defined as the alternation of meiosis and syngamy (Butlin et al., 1998a).

Sexual reproduction (*sensu* amphimixis) is one of the most enigmatic phenomena in evolutionary biology, especially if its overwhelming predominance in eukaryotes is taken into account (see e.g. Williams, 1975; Maynard Smith, 1978; Bell, 1982; Meirmans & Strand, 2010). The reason is that it brings many obvious disadvantages in comparison to asexual reproduction—the well-known two-fold cost of sex being only the first and most obvious one (see e.g. Lehtonen et al., 2012). Thus, an asexual lineage invading the sexual population would be expected to quickly drive its sexual conspecifics extinct (Maynard Smith, 1978). None of these disadvantages apply to all sexual species because of the highly variable nature of their reproduction. But, under many circumstances, the disadvantages apply profoundly (Lehtonen et al., 2012). Thus, sexual reproduction remains an enigma that calls for explanation.

Theories of sexual reproduction

Many main concepts and their countless variants were proposed to explain the paradox of sexual reproduction (reviewed e.g. in Williams, 1975; Maynard Smith, 1978; Bell, 1982, 1985; Kondrashov, 1993; Meirmans & Strand, 2010). The genetic advantages of sexual reproduction as the explanation for the maintenance of sexual reproduction are highlighted by theories like the Weismann's idea of sex generating variability, later delimited as the concept of Vicar of Bray (Bell, 1982), Fisher-Muller's accelerated evolution of sexual species (Muller, 1932; Fisher, 2003), breaking free of neighbouring deleterious mutations (Crow, 1970), reduction of the spread of genomic parasites (Sterrer, 2002), advantage of diploidy (Lewis & Wolpert, 1979), repair of DNA (Bernstein & Bernstein, 2013), restoration of epigenetic signals (Gorelick & Carpinone, 2009), eventually stochastic and deterministic variants of Muller's ratchet hypothesis (Muller, 1964; Kondrashov, 1982).

Ecological theories of sexual reproduction, on the other hand, stress the assumption that sex provides some ecological advantage to sexual species. Certain trends can be clearly found in

3

65   the geographic distribution of sexual reproduction, as was recently summarized by Hörandl

66   (2006, 2009) or Vrijenhoek & Parker (2009). As most advantages of sexual reproduction

67   postulated by genetic theories could be achieved by automixis (Gorelick & Carpinone, 2009;

68   Neiman & Schwander, 2011), the correct answer to the "greatest paradox of evolutionary

69   biology" probably lies in the group of ecological theories of sexual reproduction, or at least in

70   some form of theoretical synthesis that includes them (West et al., 1999).

Ecological theories of sexual reproduction and their predictions

72   Ecological theories such as Red Queen theory (Hamilton et al., 1990), evolutionary arm-

73   races hypothesis (Dawkins & Krebs, 1979), and the fast-sexual-response hypothesis of Maynard

74   Smith (1993) emphasize the sexually reproducing organisms' advantage when interacting with

75   other organisms, which are able to dynamically react in a co-evolutionary manner. According to

76   these "biotic heterogeneity advantage" theories, sexual species should prosper in spatially and

77   temporally biotically heterogeneous environments, i.e. environments with many biotic

78   interactions from competitors, predators, and parasites – e.g. tropical rainforests, low-latitude

79   coral reefs, ancient lakes, climax communities, or generally species-rich ecosystems In such

80   environments, selective pressures affecting the offspring differ profoundly from those that

81   affected the parents because of the coadaptation (or rather counter-adaptation) between

82   interacting organisms. In the presence of such intensive biotic interactions, sexual species would

83   be especially favoured because they maintain high genetic polymorphism and could quickly react

84   to the countermoves of their evolutionary opponents by a simple change of allele frequencies in

85   the population. The speed, not the depth, of adaptation is essential in these environments

86   (Maynard Smith, 1993).

87   Another group of ecological theories of sexual reproduction sees the main advantage of

88   sexual reproduction in the higher fitness sexual individuals or species have in abiotically

89   heterogeneous environments. This group of "abiotic heterogeneity advantage" theories

90   comprises, for example, the lottery and Sisyphean genotypes hypothesis (Williams, 1975), elbow

91   room hypothesis (Maynard Smith, 1978), tangled bank hypothesis (Bell, 1982), hypothesis of

92   fluctuating selection (Smith, 1980), and hypothesis of reduced response to fluctuating selection

93   (Roughgarden, 1991). Such environments are abiotically very variable in space and/or time, i.e.

94   diverse, unpredictable, and with unequally distributed resources – e.g. temporary or ephemeral

95   habitats, exposed habitats, dynamically changing freshwater environments, coastal habitats or

4

96   more generally biomes of high latitudes and/or altitudes. The offspring usually inhabit an

97   environment different from that of their parents due to the dispersal in time and/or space. An

98   abiotic environment does not co-evolutionarily react to the evolutionary moves of its inhabitants,

99   allowing them to perfectly adapt to it under certain circumstances, e.g. under conditions of slow,

100   long-term changes. Under these circumstances, the asexual species might have an advantage

101   because, for example, they do not suffer from segregation and recombination loads (Crow,

102   1970). However, if the environment is very heterogeneous and unpredictable in time and/or

103   space, the sexual species have the same advantage as in the biotically heterogeneous

104   environment.

105   The heterogeneity of the environment, both biotic and abiotic, can be comprehended as

106   the sum of heterogeneity in space (in the sense of variability) and time (in the sense of instability,

107   especially when the change is unpredictable). The temporal and spatial aspects of heterogeneity,

108   even though differing substantially at first sight, could act remarkably similarly in terms of

109   favouring sexual species (Kondrashov, 1993; Otto & Lenormand, 2002; Neiman & Schwander,

110   2011). In principle, the most important factor is always whether the environment inhabited by the

111   offspring differs in its character (i.e. selective pressures) from the environment inhabited by their

112   parents. There are two conditions under which such a situation is fulfilled; the environment

113   either changes dynamically in time, or is very patchy and the offspring often live in different

114   microhabitats.

115   Both spatial and temporal heterogeneity could be the consequences of both biotic and

116   abiotic factors (Li & Reynolds, 1995). Thus, according to the major source of the environmental

117   heterogeneity, it is essentially possible to differentiate between "biotic" and "abiotic" ecological

118   theories of sexual reproduction, despite the fact that the biotic and abiotic parts of the

119   environmental heterogeneity are usually interconnected and that they influence and complement

120   each other in their effects on the advantage of sexual or asexual reproduction (Glesener &

121   Tilman, 1978). Abiotically homogeneous environments can (in the case of plentiful resources

122   and mild conditions), but do not have to (e.g. in the case of extreme environments) cause an

123   increase of the biotic aspect of environmental heterogeneity (e.g. by increasing the complexity of

124   the ecosystem) (Tokeshi, 1999). Abiotic heterogeneity could act both ways too. It might cause an

125   increase of biotic heterogeneity of the environment, or at least of the species diversity. On the

126   other hand, abiotically heterogeneous environments with conditions widely fluctuating to

127   extremes (e.g. the desiccating ponds) tend to be biotically homogeneous (Tokeshi, 1999).

128   The "biotic" and "abiotic" theories of sexual reproduction mentioned above have

129   different predictions regarding the character of the environment that will be advantageous for

130   sexual and asexual species. However, their predictions are not absolutely disparate—one could

131   easily devise examples of environments suitable for asexual species according to both groups of

132   theories, e.g. stable extreme environments. Similarly, the individual theories of sexual

133   reproduction are far from being disparate; they are usually interconnected in their basic

134   principles, they intermingle and complement each other (Meirmans & Strand, 2010). Many, if

135   not all "abiotic" theories can be reinterpreted "biotically". It is therefore possible that the

136   differentiation of "biotic" and "abiotic" ecological theories is important in theory, but not

137   important in the real world, and that sexual organisms do have an advantage in environments that

138   are both biotically and abiotically relatively heterogeneous (i.e. overall heterogeneous

139   environments).

140   It was suggested by Williams (1975, pp. 145–146, 149–154, 169) and explicitly discussed

141   by Flegr (2013) that the lower ability of sexual species to fully adapt to transient environmental

142   changes brings them, paradoxically, a major advantage in randomly fluctuating environments,

143   i.e. in environments expressing large (biotic or abiotic) heterogeneity in time. Evolutionary

144   "plastic" (*sensu* Flegr) asexual species, sooner or later, adapt to transiently changed

145   environmental conditions and become extinct when the conditions return to normal. Sexual

146   species are not able to fully adapt to temporal environmental changes; they always retain some

147   genetic polymorphism that helps them escape extinction when the conditions quickly return to

148   normal. This hypothesis was supported by the results of certain experimental studies, e.g. long-

149   term patterns of fitness and genetic variability (Renaut et al., 2006) or dynamics of adaptation

150   (Colegrave et al., 2002; Kaltz & Bell, 2002) in sexually and asexually reproducing

151   *Chlamydomonas*. According to this (meta-)hypothesis, asexuals would prevail in stable or

152   predictively slowly changing, possibly extreme, environments of low temporal heterogeneity

153   (Flegr, 2013). Given the similarities between the effect of temporal and spatial heterogeneity

154   mentioned above, this notion can be readily extended to encompass both temporal and spatial

155   heterogeneity. Similar remarks were made, e.g. by Williams (1975, p. 153) and Roughgarden

156   (1991), while a combination of several aspects of heterogeneity was implicitly also proposed as

6

157 the explanation of the presence of sexual reproduction by Glesener & Tilman (1978) and some

158 interpreters of the Red Queen theory (e.g. Butlin et al., 1999) or the tangled bank hypothesis (e.g.

159 Bell, 1982).

Comparing the ecology of sexual and asexual groups

161 Most of the organisms that live on Earth, Archaea and Bacteria, are primarily asexual,

162 while most of the known species, eukaryotes, are primarily sexual (Speijer et al., 2015).

163 However, some eukaryotic lineages switched to secondary asexual reproduction (de Meeus,

164 Prugnolle & Agnew, 2007; Van Dijk, 2009; Speijer et al., 2015). It is therefore possible to

165 compare the environmental biotic and abiotic heterogeneity of such secondary asexual clades

166 with that of their sexual relatives.

167 Various studies aimed at testing and discriminating between individual ecological

168 theories of sexual reproduction on the basis of their predictions about the environmental

169 correlates of sexual and asexual lineages show largely inconclusive results. Often their aim was

170 to test particular theoretical concepts: lottery hypothesis and Sisyphean genotypes hypothesis

171 (Williams, 1975; Hörandl, 2009), elbow room hypothesis (Garcia & Toro, 1992; Koella, 1993),

172 Red Queen theory (Burt & Bell, 1987; Neiman & Koskella, 2009), fast-sexual-response

173 hypothesis (Becerra et al., 1999), hypothesis of optimal responsibility to fluctuating selection

174 (Griffiths & Butlin, 1995; Schön & Martens, 2004), hypothesis of prevention of loss of genetic

175 variability under fluctuating selection (Maynard Smith, 1993; Hörandl, 2009; Vrijenhoek &

176 Parker, 2009) or tangled bank hypothesis (Vrijenhoek, 1984; Burt & Bell, 1987; Griffiths &

177 Butlin, 1995; Domes et al., 2007; Maraun et al., 2012); or at least they were later interpreted as

178 such. The most extensive comparison not focused on testing one particular theoretical concept

179 was performed by Bell (1982) on multicellular animals (Metazoa). In his comprehensive study,

180 he concluded that the results mostly supported the tangled bank hypothesis. However, his study

181 included both old and young asexual taxa and might not fully appreciate the influence of

182 different life strategies on the character of the environment the species subjectively experience—

183 see the Discussion. Experiments aimed at discriminating the selective pressures of biotically (see

184 e.g. Fischer & Schmid-Hempel, 2005) or abiotically (see e.g. Becks & Agrawal, 2010)

185 heterogeneous and homogeneous environments were also performed, mostly pointing to the

186 conclusion that heterogeneous environments select higher rates of recombination or sexual

187 reproduction. However, particular mechanisms that favour higher levels of sex are hard to

188 determine in these cases that are, moreover, often based on facultatively sexual organisms.

Short-term and long-term asexual groups

190   The main problem of the comparative studies mentioned above may be the inclusion of

191 both old and young asexual taxa. Most secondary asexual groups probably are not evolutionarily

192 viable in the long term, as could be deduced from the distribution of asexual lineages on the "tree

193 of life." With the exception of several ancient asexuals (AAs), they form only the terminal

194 twigs—species and genera (Butlin, 2002). This pattern could be the consequence of the

195 disadvantage that secondary asexual species have in species selection (Nunney, 1989). In the

196 large majority of cases, young asexuals do not speciate, and when the conditions suitable for

197 their opportunistic transition to asexuality change, they become extinct. Another suggested

198 reason for this macroevolutionary failure of asexual species is their failure in the interspecific

199 sorting in fluctuating environments—asexual species are able to adapt to temporarily changed

200 conditions more easily, but they fail to readapt quickly enough because of the lack of genetic

201 polymorphism, and therefore become extinct when the conditions return back to normal

202 (Williams, 1975; Flegr, 2013). At least some young asexual lineages could, in fact, consist of

203 short-lived clones continuously cleaved from maternal sexual population (Janko et al., 2008;

204 Vrijenhoek & Parker, 2009). Alternatively, they could be sustained by an occasional

205 hybridisation with related sexual lineages (Turgeon & Hebert, 1994; van Raay & Crease, 1995;

206 Butlin et al., 1998b) or an infrequent transfer of genetic material from "host species" in

207 hybridogenetic and gynogenetic lineages (Mantovani et al., 2001; Bogart et al., 2007). In sum,

208 young asexuals do not have to exhibit the properties that would allow them to survive in the long

209 term and, contrary to the mainstream view, they could in fact bring a significant noise into the

210 studies of long-term maintenance of sexual (and secondary asexual) reproduction. In other

211 words, the existence of most asexual lineages might be a consequence of certain ecological

212 opportunism and the reasons for their survival might be, in contrast to the AA lineages, different

213 from case to case. We speculate that this may be the main reason for the inconclusiveness of

214 most comparative studies mentioned above—they focused only on short-term asexual clones or

215 did not take into account the age of different clones.

216   The phenomenon of asexual "terminal twigs contra ancient asexuals" is still somewhat

217 controversial and its real existence is being discussed (see e.g. Schön et al., 1996; Neiman et al.,

8

218   2009; Schwander & Crespi, 2009; Janko et al., 2011). It was even suggested that the cumulative

219   frequency distribution of ages of asexual lineages is "fairly regular" and nearly linear on the

220   logarithmically transformed scale, i.e. it shows no obvious break between the young and old

221   asexuals (Neiman et al., 2009). As a consequence, AAs need not be substantially different from

222   other asexuals and their delimitation could be more or less arbitrary. On the other hand, Janko et

223   al. (2011) strongly questions the methodology of Neiman et al. (2009), especially the choice of

224   linear regression on logarithmic scale and its interpretation. They argue that the data distribution

225   presented by Neiman et al. (2009) is unlikely to provide any strong conclusions regarding the

226   universality of processes affecting clonal life spans—in fact, it fits even better to other

227   distributions, including a multidiffonential distribution, which is characteristic of distributions

228   generated by a mix of different underlying processes. Moreover, using their analysis of clonal

229   genetic variability, Janko et al. (2011) identified a growing selective pressure against asexual

230   lineages based on their age, corroborating the hypothesis of the long-term disadvantage of

231   asexuality. Regardless of these discussions, it is obvious that out of all the secondary asexual

232   clades only the AAs have been able to survive or even diversify in an asexual state for millions

233   of years (Judson & Normark, 1996; Normark et al., 2003; Neiman et al., 2009; Schurko et al.,

234   2009; Schwander & Crespi, 2009). This is the main reason that our study is based exclusively on

235   AAs as they already proved to be evolutionarily viable in the long term. Contrastingly, young

236   opportunistic clonal lineages still face a serious extinction risk during the first pronounced

237   change of conditions.

238   However, it is worth mentioning that the focus on AAs puts forward another serious

239   difficulty: these clades were separated from their sister sexual lineages a long time ago (at least 1

240   million years ago, see Materials and Methods) and both sexual and asexual lineages thus

241   underwent considerable time periods of independent evolution. Therefore, both lineages

242   independently acquired numerous adaptations that distinguished them but need not be related to

243   the mode of their reproduction. Singular case studies comparing AAs and their sexual sister

244   lineage thus are not expected to have a strong predictive value in the long-term maintenance of

245   asexual reproduction. On the other hand, an extensive comparative study would enable the

246   comparison of several such pairs of AA and sexual control and reveal possible common

247   adaptations of AAs related to their long-term survival in an asexual state.

248 **Aims of the study**

249   The main aim of the current study is to test whether AAs more often inhabit 1) generally

250 less heterogeneous environments, 2) less biotically heterogeneous environments, or 3) less

251 abiotically heterogeneous environments. To this end, we used paired exact tests to compare the

252 ecological demands of sexual species and AA species within all six unrelated clades of

253 eukaryotic organisms in which the presence of differences in associated environmental

254 heterogeneity can be recognised on the basis of available published data. Namely, we tested the

255 hypothesis that, within such pairs, the asexual species inhabit more homogenous environment

256 more often than the sexual species. In the exploratory part of the study, we searched for

257 particular environmental properties and organismal adaptations that are common among the AA

258 members of the pairs.

259 # Materials and Methods

260 **Identification of ancient asexuals and their sexual controls**

261 **Ancient asexual groups**

262   The definition of the "ancient asexual group" is rather vague. Some researchers consider

263 a lineage to be AA if it reproduces obligately asexually for at least 50 000 generations or 0.5

264 million years (Law & Crespi, 2002); some prefer one million generations (Schwander et al.,

265 2011), yet others just speak about "millions of years" (Judson & Normark, 1996; Normark et al.,

266 2003). It was even suggested that AAs are not substantially different from other asexuals and

267 their delimitation is more or less arbitrary (Neiman et al., 2009). It is not the aim of this study to

268 argue for the substantial difference of AAs from other asexuals or against it. We focus only on

269 groups that were proven to survive in an asexual state for a considerable amount of time. Thus,

270 regardless of the discussion on the fundamental distinction of young and old asexual taxa, which

271 was briefly presented in the Introduction, in the current study we defined AAs conservatively as

272 those secondary asexual eukaryotic lineages that reproduce obligately asexually with a great deal

273 of certainty for at least one million years.

274   The condition of obligate asexuality is at least equally as important as the age of the

275 studied asexual groups in the context of this study, as many seemingly asexual lineages might

276 experience rare sexual events or other forms of genetic exchange. These options constitute a

277　greater problem in short-term asexuals than in AAs, which are spatially and temporally isolated

278　from their sexual sister lineages. Nevertheless, their obligate asexuality was discussed, studied,

279　and verified thoroughly (see Table S1).

280　　　At the beginning, we identified well supported AA groups with the help of literary

281　sources. We started with published secondary literature such as Judson & Normark (1996),

282　Normark et al. (2003), Neiman et al. (2009), Schurko et al. (2009), Schwander & Crespi (2009)

283　and Speijer et al. (2015), investigated cited primary literature and other novel primary literal

284　sources concerning putative AA groups. We also investigated other possible AAs proposed in the

285　primary literature and some lineages traditionally believed to be long term asexual. The evidence

286　for confirmation or rejection of putative AAs included organismal, life-history, palaeontological,

287　biogeographical, molecular, individual genetic, and population genetic data and also other

288　indices of ancient asexuality proposed in the AA literature listed above. The list of supported and

289　contested AA candidates, as well as reasons for our decision, are summarized in Table S1. The

290　confirmed AA groups were eight: bdelloid rotifers (Bdelloidea), darwinulid ostracods

291　(Darwinulidae), several lineages of oribatid mites (Oribatidae), several lineages of mites from the

292　suborder Endeostigmata and order Trombidiformes, shoestring fern *Vittaria appalachiana*

293　(Farrar & Mickel), three species of stick insects from the genus *Timema*, and several lineages of

294　bivalve genus *Lasaea.* Only these groups were included in our comparative study.

Sexual controls

296　　　In the next step, we identified ecologically comparable sexual sister lineages for the eight

297　AA groups by using literary sources. In those individual cases in which the phylogenetic

298　relations between the sexual and asexual lineages were not entirely clear, we used the closest

299　possible comparable clades—clades proven to be closely related and comparable in terms of

300　their ecology (see Table S2). Three of the AA groups were monophyletic (Bdelloidea,

301　Darwinulidae, and *Vittaria*). The remaining AA groups were polyphyletic, i.e. they included

302　several related monophyletic asexual sub-lineages with interstitial sexual lineages. In each case

303　of a polyphyletic AA taxon, these closely related AA lineages were taken together and treated as

304　single unit in the analysis. In the opposite case, i.e. if the monophyletic lineages of these

305　polyphyletic taxa were incorporated into the analysis as single units, the risk would arise that the

306　observations would not be independent and the results of statistical analysis could be biased

307　because of the effect of pseudoreplications. Thus, in these cases, we compared every individual

11

308   AA lineage with its sister sexual lineage in the monophyletic sub-taxa of the polyphyletic AA

309   groups and based our conclusions on the prevailing trend (i.e. over 50 % of the cases; however,

310   all actual trends were much more convincing, see Table S3) in the polyphyletic groups.

311   Unfortunately, with the exception of *Timema*, the internal phylogenetic relationships of the

312   studied polyphyletic AA groups were more or less unclear. Where possible, we proceeded using

313   the most probable relationships (Bdelloidea, Darwinulidae, Oribatidae, Nematalycidae and

314   Proteonematalycidae, Grandjeanicidae and Oehserchestidae, see Table S2). In the cases with

315   several equally probable alternative phylogenetic relationships of AA and sexual lineages (both

316   in monophyletic /*Vittaria*/, and polyphyletic /*Alicorhagia* and *Stigmalychus*, Pomerantziidae,

317   *Vittaria*, *Lasaea*/ AA taxa), we compared AA lineages with alternative sexual controls to

318   determine the consistency of the trend in the association of AA lineages or sexual controls with

319   biotically and/or abiotically more heterogeneous environments (all trends were consistent over

320   all alternative sexual controls, see Table S3).

321   It could be argued that it is legitimate to consider particular asexual monophyletic

322   lineages in the polyphyletic AA group as independent observations when we inquire into the

323   reasons for maintaining sexual reproduction, not into the reasons for the transition to sexuality

324   (Thornhill & Fincher, 2013). However, we chose the more conservative approach because, as

325   noted above, the phylogenetic relationships of the individual AA and sexual lineages were often

326   not clear enough to form sisterly pairs of sexual and asexual lineages in the polyphyletic AA

327   groups with necessary certainty.

328   **Determination of environmental heterogeneity**

329   After the identification of the AA groups and their sexual controls, we focused on the

330   comparison of the character of their environments. Using relevant literary resources, we

331   collected and analysed data on the (biotically or abiotically more heterogeneous or

332   homogeneous) character of environments inhabited by the studied groups (see Table S3). Biotic

333   and abiotic environmental heterogeneity clearly have a non-trivial relationship to each other (see

334   the Discussion), but it is essentially possible to distinguish them. Environmental heterogeneity,

335   both biotic and abiotic, is an emergent property stemming from different factors and different

336   adaptations in various AAs and thus could not be quantified and rated on a single universal scale.

337   On the other hand, there is no obstacle of comparing AAs and their sexual controls based on this

338   general property of their environment.

Biotic heterogeneity

We define biotically highly heterogeneous environments as those in which selective pressures affecting the offspring differ profoundly from those that previously affected their parents because of the coadaptation (or rather counter-adaptation) of interacting organisms. Thus, the most biotically heterogeneous environments are the habitats with a high degree of competition, predation, and parasitism. Biotic heterogeneity has both temporal (the coadaptation of interacting organisms) and spatial (e.g. the migration of the offspring to the areas with new competitors, predators, and parasites) dimensions. Changes in the biotic heterogeneity are essentially unpredictable, with the exception of some ecological cycles (e. g. host-predator or host-parasite cycles). In the latter case, environments with unpredictable changes were considered more biotically heterogeneous.

For example, the environment of organisms that live in tight association with other organisms is biotically very heterogeneous. This applies especially to predators and parasites that are forced to respond to the evolutionary counter-moves of their prey and hosts (Dawkins & Krebs, 1979). The more specific relationship with prey and hosts they have, the stronger the selective pressures of counter-adapting prey and hosts affect them (Dawkins & Krebs, 1979). Therefore, it is expected that organisms that use non-specific predatory strategies, e.g., filtering (especially if they filter both living organisms and dead organic matter), are under relatively weak selective pressure from their prey. Their environment is consequently biotically relatively homogenenous in this regard. On the other hand, the environment of organisms that are themselves under strong selective pressure of predators and parasites is highly biotically heterogeneous (Dawkins & Krebs, 1979). The environemnt of organisms that are not under strong selective pressures of predators and parasites for various reasons is biotically more homognenous in this regard (e.g. the environment of Darwinulidae, see Schön et al., 2009b or Bruvo et al., 2011). Another important component of environmental biotic heterogeneity is competition. The environments with complex ecosystems that are characterized by high a degree of competition, predation and parasitism among their inhabitants (e.g. ancient lakes, see Martens, 1998; Martens & Schön, 2000; Schön & Martens, 2004) are highly biotically heterogeneous for them. On the other hand, the environments with a low degree of competition, predation and parasitism (especially extreme habitats, e.g. the environments of extremely high temperatures, see Tobler, 2007, or, for photosynthetic organisms, poorly lit environments, see Farrar, 1978,

13

370   1998), are biotically very homogeneous for their inhabitants. The vast majority of environments

371   on Earth are thus somewhat biotically heterogeneous. In spite of that, we can find important

372   exceptions. This factor of environmental heterogeneity is considerably weakened especially in

373   extreme, ephemeral and marginal habitats that cannot sustain complex ecosystems because of

374   their extreme conditions, rapid unpredictable changes, low carrying capacity and/or insufficient

375   energy sources (see e.g. Bell, 1982).

376   An important factor that affects the biotic heterogeneity of environment the organisms

377   experience is the way of life practiced by aquatic organisms. On the average, lesser biotic

378   heterogeneity is experienced by benthic (or sedentary) organisms in comparison with planktonic

379   aquatic organisms. The reason is that the latter are subject to fast and effective transmission of

380   parasites and pathogens, especially viruses (see e.g. Suttle et al., 1990; Bratbak et al., 1993;

381   Fuhrman, 1999; Wommack & Colwell, 2000; Suttle, 2005, 2007), because of the character of

382   their environment—mixing of water masses lead to frequent encounters of various individuals

383   (Emiliani, 1993a, b). Crucial difference of the resulting risks for benthic and planktonic

384   organisms was pointed out by Emiliani (1982; 1993a). Emiliani (1993a) documented that benthic

385   representatives of Foraminifera have lower risk of extinction in comparison with planktonic

386   ones. The average length of existence of their benthic species was 20 million years, whereas

387   planktonic species lasted only about 7 million years. The most probable explanation of this

388   pattern is higher susceptibility of planktonic organisms to extinction caused by lethal parasitic,

389   especially viral, infections. This finding gave rise to the viral theory of background extinctions

390   (Emiliani 1993a, b). Evidence for the lower risks arising for benthic organisms from parasites

391   and pathogens was also supported by other ecological studies. For example, Filippini et al.

392   (2006) observed lower prevalence of individuals infected by viruses and consequent mortality

393   among benthic bacteria (~0,03 %) in comparison with bacteria from water column (~6 %).

394   Moreover, this pattern held despite much larger abundance of viruses in the sediment of studied

395   temperate lake. Fisher et al. (2003) and Bettarel et al. (2006) came to very similar conclusions on

396   the basis of studies of temperate oxbow lake and freshwater habitats in tropical Africa,

397   respectively (but see Danovaro et al., 2008 for somewhat contrasting results from marine benthic

398   sediment). Putting aside the limitations of parasite and pathogen transmission among benthic

399   organisms, the protection of benthic organisms against viruses might be further enhanced by the

400   adsorption of viruses into organic and inorganic particles of sediment and the aggregation of

401 benthic organisms (Filippini et al. 2006; Fisher et al., 2003). Whereas the extinction of whole

402 species due to viral infection (eventually infection by another parasite or pathogen, e.g. fungus)

403 is possible only under very limited conditions (see e.g. Buckwold, 1994, or de Castro & Bolker,

404 2005), local extinctions caused by pathogen or parasitic infections are probably quite common

405 (Emiliani, 1982, 1993a; de Castro & Bolker 2005). The planktonic way of life thus probably

406 considerably increases the selective pressures of parasites and pathogens and consequently the

407 biotic heterogeneity of the environment.

408   The argument for lower biotic heterogeneity of benthic (or sedentary) organisms is

409 possible to extend also to organisms that inhabit soil. From the viewpoint of the viral theory of

410 background extinctions (Emiliani, 1993a, b), soil represents an environment that considerably

411 impedes the spread of pathogens and parasites. Interactions with parasites and pathogens are very

412 limited both in intensity and frequency due to the tortuous, i.e., multidimensional, character of

413 soil matrix—it is best described as semi-discontinuous network of pores filled with air and/or

414 water, or water films surrounding solid particles (Lavelle & Spain, 2003; for more information

415 about the character of soil environment see also Wallwork, 1970; Coleman et al., 2004, or Paul,

416 2007). These features of soil matrix limit both the passive spread of parasites and pathogens and

417 the frequency of encounters among their transmitters and organisms in general. The tortuous

418 character of soil environments was stressed as a factor that limits the passive spread of viruses in

419 benthic sediment by Fisher et al. (2003), whereas Murphy & Tate (1996) emphasized its

420 limitations on the spread of bacteria. These observations are in agreement with Drake et al.

421 (1998), who observed a negative correlation between the concentration of viral particles and

422 sediment grain size. The sieving effect of the soil for organisms of various sizes is also

423 commented by Paul (2007). Moreover, the tortuous character of soil impedes also active

424 dispersion of organisms, e.g. when searching for prey (Elliott et al., 1980). Only a few larger

425 organisms are able to effectively move larger distances within soil or even create their own

426 habitats; movements of most organisms are locally constrained (Lavelle & Spain, 2003). Direct

427 encounters between organisms, even organisms of the same species, are thus relatively

428 infrequent. This leads, together with the limited spread of pheromones (Karasawa & Hijii, 2008),

429 for example, to frequent transitions to indirect fertilisation with the help of deposited

430 spermatophores (see e.g. Wallwork, 1970, or Lavelle & Spain, 2003). The pattern of spatial

431 autocorrelation of genetic lineages in soil communities, for example in rotifers (Robeson et al.,

432   2011), further supports the limited dispersal abilities of soil organisms. The genetic diversity of

433   various Bdelloidea lineages in soil is correlated only on small spatial scales (up to 54-133 m).

434   Operational taxonomic units identified by Robeson et al. (2011) almost did not overlap above

435   this distance. Habitats that distanced only tens to hundreds of meters were thus inhabited

436   overwhelmingly by separate genetic lineages. Moreover, rotifer communities differed to a certain

437   degree even in the smallest investigated distance of 16 cm (Robeson et al., 2011)[1].

438   Taken together, the tortuous character of the soil affects all soil organisms at various

439   scales not only in terms of the reduced spread of parasites and pathogens, but also lower

440   frequencies of encounters with predators and competitors. This leads to an overall reduction of

441   biotic pressures in soil, which is further supported by the striking evolutionary stasis of many

442   lineages of soil inhabiting organisms (Pilato, 1979). Moreover, species richness and population

443   sizes, including parasites, predators and competitors, markedly decreases with the depth of soil

444   horizon (Lavelle & Spain, 2003; Paul, 2007). Deep soil horizons are therefore even more

445   abiotically homogeneous. The specific character of soil environment does not imply its general

446   spatial homogeneity. On the contrary, soil is often spatially heterogeneous, especially on a larger

447   scale (see e.g. Lavelle & Spain, 2003; Coleman, Crossley & Hendrix, 2004, or Paul, 2007). It is

448   the tortuous and multidimensional character of soil matrix that reduces biotic pressures affecting

449   its inhabitants and makes this environment biotically very homogeneous.

450   The biotic heterogeneity of the environment the organisms experience might be reduced

451   by the presence of durable resting stages. Organisms may get rid of parasites and pathogens,

452   survive unfavourable environmental conditions, or colonize new habitats with naïve parasites,

453   predators, pathogens and competitors in these stages (as do, for example, Bdelloidea—see

454   Wilson, 2011). The geographical trend of decreasing biotic heterogeneity with increasing latitude

455   might be expected on a global scale. Species diversity and ecosystem complexity decrease with

456   distance from equator (Tokeshi, 1999). These events are coupled with a decreasing intensity of

457   parasitization, abundance, prevalence and a relative diversity of parasites (Rohde, 1986; Rohde

458   & Heap, 1998). An analogous trend of decreasing biotic heterogeneity with increasing depth

---

[1] High genetic diversity of Bdelloidea in gene cox1 is not very surprising in the light of severe DNA breaks that originate during anhydrobiosis, following repairs of these breaks and consequent intensive horizontal gene transfer (see e.g. Gladyshev et al., 2008).

459   might be expected in deeper parts of the water column for the same reasons (see e.g. Etter et al.,
460   2005).

Abiotic heterogeneity

462   Abiotically highly heterogeneous environments are defined as those that are highly
463   variable regarding changes of abiotic factors. They are diverse, unstable, and have unequally
464   distributed resources. Again, the abiotic heterogeneity of the environment has both spatial (in the
465   sense of variability) and temporal (in the sense of instability) dimensions. The offspring thus
466   usually inhabit an environment different from that of their parents due to their dispersal in time
467   and/or space. Changes in the abiotic environment could be predictable (e.g. cyclical) or
468   unpredictable, and their intensity and frequency vary on different timescales. We are interested
469   in ecological timescales in this study so we consider short-term unpredictably changing
470   environments as the most abiotically heterogeneous.

471   Temporally and spatially highly changeable ephemeral and marginal habitats are
472   especially abiotically heterogeneous environments (see e.g. Pejler, 1995). However, most of the
473   surface terrestrial habitats are considerably abiotically heterogeneous. On the contrary, sheltered
474   habitats such as caves, ground water reservoirs or soil environment are greatly abiotically
475   homogenenous. Such environments protect their inhabitants from solar radiation and buffer
476   short-term fluctuations in outer environment (e.g. changes of temperature and humidity),
477   protecting their inhabitants from the direct impacts of such changes (Wallwork, 1970; Farrar,
478   1978, 1990, 1998; Krivolutsky & Druk, 1986; Lavelle & Spain, 2003; Devetter & Scholl, 2014).
479   Most changes in soil matrix are much slower in comparison with surface habitats (Lavelle &
480   Spain, 2003). The abiotic homogeneity of soil environment further increases with the depth of
481   the soil horizon. For example, there is a specific depth of soil horizon in each geographical
482   region under which the temperature is perienally stable, depending on its latitude, altitude and
483   other climatic factors (Wallwork, 1970; Lavelle & Spain, 2003; Coleman et al., 2004; Paul,
484   2007). The buffering effect of soil on moisture fluctuations also increases with depth (see e.g.
485   Quesada et al., 2004). Moreover, soils of certain biomes (especially forest soils) are temporally
486   abiotically more homogeneous than soils of other biomes (see e.g. Siepel, 1994, 1996).

487   Regarding aquatic environments, freshwater habitats and coastal areas are the most
488   abiotically heterogeneous (Sheldon, 1996). The decrease of abiotic heterogeneity with increasing
489   depth is also expected—water masses buffer surface environmental changes in a similar way to

17

490   soil (see e.g. Etter et al., 2005). Certain extreme environments that are temporally stable (e.g. hot

491   springs or subsurface cavities) are also very abiotically homogeneous (Bell, 1982), but this does

492   not apply to all environments referred to as extreme.

493   In a similar way to the biotic heterogeneity of the environment, also the abiotic one might

494   be reduced in the populations of organisms producing durable resting stages. Such an adaptation

495   enables them to survive unfavourable fluctuations of an abiotic environment and promotes

496   colonization of new habitats (see e.g. Wilson, 2011). On the other hand, mobility probably does

497   not strongly affect the abiotic environmental heterogeneity that the organisms experience.

498   Mobile organisms might hypothetically experience more different abiotic conditions in their life,

499   but they can also easily stick with those most suitable for them. The geographical trend of

500   increasing abiotic heterogeneity with increasing latitude and altitude might be expected to occur

501   on global scale (Hörandl, 2006, 2009; Vrijenhoek & Parker, 2009). However, it is noteworthy

502   that such a trend might be countered by an opposite trend of the decreasing biotic heterogeneity

503   mentioned above in its effects on sexual and asexual species (and *vice versa*). All of the

504   expectations mentioned above need not apply absolutely, but may serve as useful leads in

505   judging the environmental heterogeneity of various organisms if their peculiarities are taken into

506   account.

507   In two AA groups (*Lasaea*, *Timema*) we were unable to identify any consistent

508   differences in the heterogeneity of the environments inhabited by their sexual and asexual

509   lineages. The probability that any two groups experience absolutely identical habitat

510   heterogeneity is negligible and the absence of difference must be just a result of lack of empirical

511   data. *Lasaea* and *Timema* were thus not included in the statistical analysis because the binomial

512   test can analyse only binary variables (see e.g. McDonald, 2014) and our current knowledge on

513   these groups' ecology does not allow us to decide whether asexual or sexual species live in more

514   heterogeneous environments. The same applies for the abiotic heterogeneity of the environment

515   of Darwinulidae. Thus, the analysis was based on only six pairs of AAs and their sexual controls,

516   out of which six differed in the abiotic heterogeneity of their environment and five in the biotic

517   heterogeneity.

Statistics

519   Collected data were analysed using the R v. 3.1.2 software environment (R_Core_Team,

520   2014). We chose to evaluate data by an exact test, specifically a one-tailed binomial test, due to

18

521 the low number of compared sexual-asexual pairs (six pairs in total, six for biotic heterogeneity

522 and five for abiotic heterogeneity), the character of the data (pair, binary), and the type of the

523 tested hypothesis. By this means, we tested three hypotheses, namely that AA groups inhabit

524 predominantly (1) biotically or abiotically, (2) biotically, and (3) abiotically more homogeneous

525 environments than their sister or closely related ecologically comparable sexual groups.

Results

Ancient asexuals

528 Reviewing relevant literary resources, including several recent studies, we concluded that

529 at least eight of the putative AA groups do fulfil our strict criteria of ancient asexuality: bdelloid

530 rotifers (Bdelloidea), darwinulid ostracods (Darwinulidae), some mite lineages of the Oribatidae,

531 Endeostigmata, and Trombidiformes, shoestring fern *Vittaria appalachiana*, some lineages of

532 stick insects from the genus *Timema*, and some lineages of the bivalve genus *Lasaea*; see

533 Supplemental table S1. Their sister or closely related ecologically comparable sexual groups

534 were identified consequently with the help of relevant literature; see Table S2.

Comparative analysis of the habitat homogeneity of ancient asexuals and their sexual controls

537 The comparison of the character of environments inhabited by the AAs and their sexual

538 controls showed that AAs inhabit biotically or abiotically (6 of 6, p = 0.016), biotically (6 of 6, p

539 = 0.016), and abiotically (5 of 5, p = 0.031) more homogeneous environments. All these results

540 are statistically significant. Details of the results are summarized in the Supplemental review of

541 AA ecology and Table S3.

Exploratory part of the study

543 The exploratory part of this study is based on data gathered in the course of the main

544 analytic study. We identified several properties and adaptations that are common to a

545 considerable number of studied AAs. The most notable are durable resting stages, life in benthos

546 and soil, and life in the absence of intense biotic interactions. These findings are discussed

547 below.

Discussion

In contrast with other comparative studies in the field, the presented one is (1) based exclusively on the AA taxa. Moreover, (2) biotic and abiotic environmental heterogeneity has been strictly distinguished. We conclude that all 6 of the 6 analysed AA groups inhabit biotically more homogeneous environments and all 5 of the 5 ones inhabit abiotically more homogeneous environments when compared with their sexual controls. Some of the studied AA groups are found in environments of a predominantly low biotic homogeneity (mainly Darwinulidae) or a predominantly low abiotic homogeneity (mainly Oribatidae), but most of them are associated with environments substantially more homogeneous both biotically and abiotically (Bdelloidea, non-Oribatidae mites, *Vittaria*). No AA group lives in an environment abiotically or biotically more heterogeneous than its sexual control. In the cases excluded from the analysis (abiotic heterogeneity in Darwinulidae and both biotic and abiotic heterogeneity in *Timema* and *Lasaea*), it was not possible to distinguish whether the heterogeneity is lower in the AA group or in the sexual control. More specifically, enough information could not be found to decide either way.

The associations with biotically and abiotically more homogeneous environments overlap almost perfectly. Thus, the results of the comparative analysis clearly indicate that either the AA groups tend to be associated with overall (both biotically and abiotically) homogeneous environments, or that these two types of heterogeneity are so strongly correlated that it is impossible to decide in favour of theories of sexual reproduction that stress the key role of biotic or abiotic heterogeneity. In general, our results obtained on AAs support, but of course do not prove, the hypotheses that consider both biotic and abiotic heterogeneities acting as one factor in their effect on organisms (Williams, 1975, pp. 145–146, 149–154, 169; Roughgarden, 1991; Flegr, 2010, 2013).

Despite the widespread apprehension that the long independent evolution of AAs and their sexual controls would hamper any ecological comparative analysis of the type presented here (leading to the preference of young asexual lineages, see Introduction), we found that both groups usually inhabit quite similar and considerably homogeneous environments. Contrary to the initial expectation, this can, in fact, complicate analyses aimed at evaluating the differences of the environments of AAs and their sexual controls in the opposite way (as was the case of *Timema* and *Lasaea*, see Table S3). On the other hand, their common ancestor's association with the homogeneous environments could have been a preadaptation to the successful and long-term

20

579   transfer to asexual reproduction in the AAs. This tendency is obvious especially in

580   Darwinuloidea-Cypridoidea, but it can also be seen in Bdelloidea-Monogononta, Oribatidae, and

581   Endeostigmata (see Table S3).

582   It is interesting in this regard that a lot of contested AAs (see Table S1) also inhabit

583   considerably homogeneous environments—e.g. arbuscular mycorrhizal fungi of the order

584   Glomales (Croll & Sanders, 2009), tardigrades (Pilato, 1979; Mobjerg et al., 2011), nematode

585   genus *Meloidogyne* (Castagnonesereno et al., 1993), ostracods *Heterocypris incongruens*

586   (Ramdohr) and *Eucypris virens* (Jurine) (Butlin et al., 1998b; Martens, 1998), bristle fern

587   *Trichomanes intricatum* (Farrar) (Farrar, 1992), basidiomycete fungal families Lepiotaceae and

588   Tricholomataceae (Currie et al., 1999a, b), ambrosia fungi Ophiostomatales (Farrell et al., 2001),

589   or brine shrimp „*Artemia parthenogenetica*" (Bowen & Sterling) (Vanhaecke et al., 1984)—and

590   their adaptations are similar to those of the AAs included in this study (see below).

What environmental properties and organismal adaptations are associated with AA taxa?

593   The exploratory part of this study is based on data gathered in the course of the analytic

594   part of the study. Besides the tendency to inhabit biotically and abiotically homogeneous

595   environments, we discovered several properties and adaptations that are common to a

596   considerable number of studied AAs, do occur in AAs strikingly more often and could be the

597   particular adaptations enabling their long-term survival in the environments mentioned above.

598   Universally distributed adaptations potentially connected to the mode of reproduction were not

599   expected to be found in our sample because of markedly different ecological strategies of the

600   studied AAs. In spite of that, we found characteristics that have broad distribution among studied

601   taxa:

Alternative exchange of genetic information

603   Alternative ways of exchange of genetic information could theoretically substitute sexual

604   reproduction and thus were repeatedly proposed as the key adaptation to asexuality (Butlin et al.,

605   1998a; Gladyshev et al. 2008; Boschetti et al. 2011; Debortoli et al., 2016; Schwander, 2016).

606   However, we identified this factor only once in the AAs included in our study (i.e. in 1/8 cases),

607   namely in Bdelloidea that experience intensive horizontal gene transfer (Gladyshev et al. 2008;

608   Boschetti et al. 2011; Debortoli et al., 2016). Another mechanism of genetic exchange,

21

609 parasexuality (*sensu* Pontecorvo 1954), was proposed in some contested ancient asexuals—

610 Glomales (Croll & Sanders, 2009), Tricholomataceae and Lepiotaceae (Mikheyev et al., 2006)

611 and certain protists (Birky, 2009). However, considering only the well supported AAs, these

612 mechanisms have limited distribution.

Durable resting stages and subjectively homogeneous environment

614 There is one particular, mostly unnoticed adaptation that could be essentially important

615 for the understanding of the links between the heterogeneity of environment and the mode of

616 reproduction. It is based on the insight that the character of the environment is "read" much

617 differently by its inhabitants than by the human observer. In case that a particular organism

618 reacts to the adverse change of environmental conditions by entrenching itself in the resting or

619 durable persistent stages (e.g. anabiosis) then, as a result, it *de facto* does not experience the

620 unfavourable conditions at all. Its objectively heterogeneous environment becomes subjectively

621 much more homogeneous.

622 This subjectivity addresses especially abiotic factors of environment, e.g. desiccation,

623 which is survived in the anabiotic stages by Bdelloidea (Pilato, 1979; Ricci, 2001), or freeze and

624 desiccation, which is survived in a state of torpor by Darwinulidae and some of their sexual

625 relatives (Carbonel et al., 1988). Similar durable stages could also be found in some contested

626 AAs, namely „*Artemia parthenogenetica* "(Vanhaecke et al., 1984) and tardigrades (Mobjerg et

627 al., 2011). Moreover, AA *Lasaea* is able to become mostly inactive and rests during the adverse

628 conditions for some time as well (Morton et al., 1957). On the other hand, at least in Bdelloidea,

629 the anhydrobiosis serves as the escape from biotic stresses too—especially parasites, both

630 directly (the individual gets rid of parasites during desiccation) and indirectly (by enabling the

631 escape from parasites in space and time) (Wilson, 2011). The distribution of durable resting

632 stages among well supported AAs looks rather scarce (3/8 cases). However, these 2-3 groups

633 comprise all studied AAs associated with significantly objectively abiotically heterogeneous

634 habitats.

635 Despite the undeniable importance of this phenomenon and the fact that it was repeatedly

636 highlighted, both historically (von Uexküll, 1982) and recently (Pilato, 1979), there is no general

637 awareness of the idea of difference between the subjective environment experienced by the

638 organism and the objective environment perceived by the human observer. This might be another

639 reason why most researchers did not come to unambiguous conclusions in their comparative

640   analyses of the ecology of sexual and asexual organisms. For example, a lot of "extreme"

641   environments are not necessarily abiotically very homogeneous, whereas some environments

642   that appear very abiotically heterogeneous on first sight (periodical ponds, dendrotelms etc.)

643   could be very subjectively homogeneous for local inhabitants (e.g. anhydrobiotic Bdelloidea).

644   After all, the "extremeness" of the environment itself depends on the adaptation of the observer,

645   including the presence or absence of the durable stages, and thus is highly subjective.

646   For a species, the ability to survive adverse conditions entrenched in durable persistent

647   stages could have crucial evolutionary consequences. It can be at least partially responsible for

648   the phenomenon of evolutionary stasis, great morphological uniformity, and conservatism of

649   numerous AA groups (see below) and indirectly responsible for the ability of successful and

650   long-term transition to asexuality. Such organisms are shielded against the selective pressures of

651   the environment. Their evolution is slow, but they are perfectly adapted to the conditions of their

652   habitat (Pilato, 1979).

653   **Sedentary life and life in benthos**

654   At least three well-supported AA groups (Bdelloidea, Darwinulidae, and *Lasaea*) are

655   exclusively benthic or sessile in contrast to their sexual relatives (Dole-Olivier et al., 2000; Ricci

656   & Balsamo, 2000; Ó Foighil, 1989). Some species of rotifer group Monogononta (sexual control

657   for Bdelloidea) (Pejler, 1995) and ostracod group Cypridoidea (sexual control for Darwinulidae)

658   (Martens et al., 2008) are planktonic; one of the two sexual lineages in genus *Lasaea* has

659   planktonic larvae (Ó Foighil, 1988). Life in benthos could theoretically hamper and reduce the

660   spread of parasites. Sessile organisms can resist hypothetical lethal epidemics postulated by the

661   viral theory of background extinction more efficiently (Emiliani, 1993a, b).

662   On the other hand, paleontological studies show that species with planktonic larvae have

663   decreased extinction rates in background extinction, particularly that species without planktonic

664   larvae go extinct more rapidly (Jablonski, 1986). In *Lasaea*, it seems that lineages with direct

665   development have a lower risk of extinction. These representatives also have a high chance of

666   speciation after migration into the new area (Ó Foighil & Eernisse, 1988), but the main reason is

667   probably that the direct developing lineages are surprisingly much better colonizers of distant

668   areas thanks to the rafting on vegetation (Ó Foighil, 1989).

669   In a similar way to resting stages, the distribution of benthic or sedentary lifestyle among

670   well supported AAs looks rather scarce on the first sight (3/8 cases). However, these 3 groups

23

671   comprise all studied AAs that are (at least partially) associated with aquatic habitats. It is

672   interesting that numerous contested aquatic AAs are also exclusively benthic: flatworm

673   *Schmidtea polychroa* (Schmidt) (Pongratz et al., 2003), New Zealand mudsnail *Potamopyrgus*

674   *antipodarum* (Gray) (Neiman et al., 2005), and ostracods *Heterocypris incongruens* (Ramdohr)

675   and *Eucypris virens* (Jurine) (Butlin et al., 1998b; Martens, 1998).

Life in the soil

677   Another adaptation widely distributed among AA groups is the inhabitancy of soil,

678   especially deeper parts of the soil horizon. This tendency can be seen mainly in the AA mites

679   from groups Oribatidae, Endeostigmata, and Trombidiformes (Karasawa & Hijii, 2008; Maraun

680   et al., 2009; Walter, 2009). Bdelloidea and Darwinuloidea tend to be associated with semi-

681   terrestrial habitats (Schön et al., 2009b). Moreover, AA Bdelloidea dominate among the soil

682   rotifers (Pejler, 1995). Most representatives of Darwinulidae inhabit soil (respectively interstitial)

683   too (Schön et al., 2009b). Taken together, 5/8 studied AA groups have numerous soil-inhabiting

684   representatives and a tendency to inhabit soil. Moreover, Bdelloidea dominate there over its

685   sexual control and AA mites have a stronger tendency to inhabit soil (Oribatidae) or its deep

686   horizons (Endeostigmata, Trombidiformes) in comparison with their sexual controls.

687   Living in soil could, in a similar way to life in benthos, reduce the capacity of parasites to

688   spread (*sensu* Emiliani, 1993a, b) Moreover, the tortuous character of the soil reduces any

689   interactions of soil organisms and thus negatively affect not only parasitization but also predation

690   and competition (Elliott et al., 1980; Murphy & Tate, 1996; Drake et al., 1998; Fisher et al.,

691   2003; Lavelle & Spain, 2003; Paul, 2007). Besides, soil is an abiotically very stable environment

692   shielding its inhabitants from fluctuations in temperature and humidity, as well as from UV

693   radiation, and could be very favourable for asexuals also for this reason (Pilato, 1979;

694   Krivolutsky & Druk, 1986; Siepel, 1994). However, other explanations have also been proposed

695   for the asexuals' association with soil habitats. Oribatidae could suffer less intense selective

696   pressures in the soil than in the arboreal environment where they have to respond to the co-

697   evolving lichens, their main food source (Maraun et al., 2009). Asexuality can be also more

698   advantageous in soil because of the difficulties with seeking out sexual partners, less effective

699   pheromone dispersal etc. (Karasawa & Hijii, 2008). Numerous contested AAs are soil

700   inhabitants too: Glomales (Croll & Sanders, 2009), tardigrades (Pilato, 1979; Jorgensen,

701   Mobjerg & Kristensen, 2007), and *Meloidogyne* (Castagnonesereno et al., 1993).

702   Thus, for the reasons mentioned above, life in soil habitats probably erases most of the

703   evolutionary advantages of sexuality. Selection pressures of biotic and abiotic environments are

704   weak in soil: The abiotic environment is relatively more stable and homogeneous; the

705   interactions with competitors, parasites, and predators are very limited both in intensity and

706   frequency due to the tortuous and multidimensional character of the environment (the shortest

707   way from point A to point B in soil is only rarely a straight line). The evolutionary consequences

708   of living in the soil are similar to those of having the capacity to form durable persistent stages

709   (see above). This is in accordance with the presence of noticeable evolutionary stasis of soil-

710   inhabiting groups described by Pilato (1979). It can also be seen in Bdelloidea (Ricci, 1987;

711   Poinar & Ricci, 1992) and Darwinulidae (Martens et al., 1998; Schön et al., 1998, 2009b). On

712   the other hand, the main factor causing evolutionary stasis in these two groups may be the

713   subjective homogeneity of the environment caused by the presence of dormant stages. However,

714   evolutionary stasis can be also found in other soil-inhabiting AAs: Oribatidae (Krivolutsky &

715   Druk, 1986; Norton, 1994; Heethoff et al., 2007) and other AA mites (Norton et al., 1993;

716   Walter, 2009; Walter et al., 2009); globally in 5/8 studied AA groups. Taking into account the

717   contested AA groups, it can be found in Glomales (Remy et al., 1994; Redecker, Kodner &

718   Graham, 2000) and tardigrades (Pilato, 1979; Jorgensen et al., 2007). All of these groups are

719   very uniform and have undergone almost no change in tens to hundreds of millions of years

720   (Pilato, 1979).

Absence of life strategies with intensive biotic interactions

722   It is noticeable that there are practically no typical predators and parasites among the AAs

723   we studied—this property is characteristic for all 8 studied groups. Remarkably often they feed

724   on dead organic matter or are autotrophic; parasites are almost absent, and in the case of a

725   predatory lifestyle, they are phytophagous or filtering (see Table S3). One possible explanation is

726   that they are unable to keep up in the co-evolutionary race with their sexual hosts or prey. Thus,

727   they can be successful in the long term, especially in the case of a predatory lifestyle, only if they

728   adopt (or are preadapted to) such nonspecific ecological strategies.

25

Succumbing to domestication and delegation of concern for its own benefit to another biological entity

The tendency for asexual reproduction is particularly interesting in the contested AA fungi domesticated by ants (Formicidae) and bark beetles (Scolytinae). The ant symbionts are from the basidiomycete groups Tricholomataceae and Lepiotaceae (Mueller et al., 1998), whereas bark beetles domesticate the ambrosia fungi of the ascomycete group Ophiostomatales (Farrell et al., 2001). The association is particularly close in the ants. They care for the fungi intensively, remove fungal predators and parasites, and the founding queen always carries filamentous bacteria, which synthetize an antidote against the main fungal pathogen—ascomycete *Escovopsis* (Currie et al., 1999a, b) —not to mention the stable temperature and humidity in the nest. By doing so, they provide a very favourable, biotically and abiotically stable environment. Moreover, there is some evidence that they prevent fungi from their already minimal attempts at sexual reproduction. On the other hand, the situation may be more complicated because some of these fungi create sexual structures predominantly in the presence of ants (Mueller, 2002). This phenomenon provides an alternative view on some aspects of human agriculture. A lot of crops raised by humans are asexual or self-pollinating too, which probably facilitates their breeding but increases their susceptibility to parasites and pathogens (Flegr, 2002). Life in association with another organism that takes care of the symbiont can also be found in the contested AA group Glomales (Croll & Sanders, 2009) and various prokaryotic and eukaryotic endosymbionts, see e.g. Douglas (2010). However, it has not been found in any of the 8 well supported AA groups we studied, and its effect on the long-term maintenance of asexual reproduction thus remains only speculative.

Conclusions

The analytical part of this study, i.e. the comparative analysis of the environment of AAs and their sexual relatives, supported the hypothesis that AA groups are associated with overall (biotically and abiotically) more homogeneous environments in comparison with their sister or closely related ecologically comparable clades. It consequently supported the theoretical concepts that postulate the essential advantage of sexual species in heterogeneous environments and consider the (biotic and abiotic, temporal and spatial) heterogeneity of the environment affecting the organisms to be one factor that can exhibit itself in many ways (Williams, 1975, pp.

759 145–146, 149–154, 169; Roughgarden, 1991; Flegr, 2010, 2013). Particular ecological

760 adaptations, from which durable resting stages, life in the absence of intense biotic interactions,

761 and the association with soil and benthic habitats are most notable, could be considered special

762 cases of the general AAs' association with overall homogeneous environments.

763   Therefore, the general notion that proposed theories of sexual reproduction (see

764 Introduction) need not exclude each other, that the effects proposed by some or all of them might

765 intertwine and affect individuals and evolutionary lineages simultaneously, or that they even

766 may, ultimately, represent only different aspects of one more general explanation, seems to be

767 well supported. Moreover, overall environmental heterogeneity, regardless of its complicated

768 conceptualization and study, seems to be a suitable candidate for this hypothetical general

769 explanation.

770   Most putative AA lineages are still critically understudied. One way of elaborating the

771 foundations laid out by this study would be comparing the heterogeneity of environments in a

772 broader spectrum of AA lineages as soon as more lineages are discovered or confirmed (e.g. the

773 protist lineages proposed by Speijer et al., 2015). It would also be very desirable to investigate

774 the ecology of *Lasaea*, *Timema*, and Darwinulidae in greater detail. Additionally, it would be

775 appropriate to focus on the interaction of biotic and abiotic environmental heterogeneities and

776 their effect on organisms. According to Flegr (2008, 2010, 2013), sexual groups should exhibit

777 more pronounced evolutionary conservation of niches in comparison with asexuals—on the

778 whole, they are expected to stick closely around the phenotype of their common ancestor. This

779 hypothesis could be tested by comparing the variance of properties of individual species within

780 an AA and its related sexual clade. It would be also possible to test whether the sexual species

781 are able to survive under a wider range of conditions of the heterogeneous environment due to

782 their high genetic variability and hypothetical "elastic" reaction on selection, as was suggested

783 by Flegr (2008, 2010, 2013).

784 ## Acknowledgments

789 particular asexual taxa and useful insights into the biology of these species. We would also like

790 to thank Karel Kotrly, Vlasta Pachtova and Vojtech Zarsky for their help with acquiring some

791 hardly accessible literary sources and Eva Priplatova, Lenka Priplatova, Jinka Bousova, Julie

792 Nokola Novakova and Charlie Lotterman for the final revisions of our text.

793 **Ethics approval and consent to participate**

794 Not applicable

795 **Consent for publication**

796 Not applicable

797 **Availability of data and materials**

798 All data generated or analysed during this study are included in this published article and its

799 supporting information files.

800 **Competing interests**

801 The authors have no conflict of interests to declare.

804 Authors' contributions

805 JT gathered the data, prepared the figures and tables and was the greatest contributor in writing

806 the manuscript. JF contributed the analysis tools. Both JT and JF conceived and designed the

807 study, analysed the data and reviewed the drafts of the paper. All authors read and approved the

808 final manuscript.

809 Additional files

810 **Additional file 1:** E&E supporting information.pdf (TITLE: 3 Supplemental tables and

811 Supplemental review of AA ecology. DESRCRIPTION: Three Supplemental tables mentioned

812 in the manuscript and Supplemental review that corroborates the claims in Supplemental table 3

813 and explains them in more detail).

References

814

815 Becerra M, Brichette I, Garcia C. 1999. Short-term evolution of competition between genetically

816   homogeneous and heterogeneous populations of Drosophila melanogaster. Evolutionary

817   Ecology Research 1(5): 567-579.

818 Becks L, Agrawal AF. 2010. Higher rates of sex evolve in spatially heterogeneous environments.

819   Nature 468(7320): 89-93.

820 Bell G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. London, UK:

821   Croom Helm.

822 Bell G. 1985. Two theories of sex and variation. *Experientia* 41(10): 1235-1245.

823 Bernstein H, Bernstein C. 2013. Evolutionary origin and adaptive function of meiosis. In:

824   Bernstein H, Bernstein C, eds. Meiosis. InTech, 41-75. *Available at:*

825   *http://www.intechopen.com/books/meiosis/evolutionary-origin-and-adaptive-function-of-*

826   *meiosis* (accessed 23 may 2017). DOI: 10.5772/56557

827 Bettarel Y, Bouvy M, Dumont C, Sime-Ngando T. 2006. Virus-bacterium interactions in water

828   and sediment of West African inland aquatic systems. Applied and Environmental

829   Microbiology 72(8): 5274-5282.

830 Birky CJ. 2009. Sex and evolution in eukaryotes. Encyclopedia of Life Support Systems

831   (EOLSS). Oxford, UK: Eolss Publishers. *Available at: http://www.eolss.net/sample-*

832   *chapters/c03/e6-183-14-00.pdf* (accessed 23 may 2017).

833 Bogart JP, Bi K, Fu JZ, Noble DWA, Niedzwiecki J. 2007. Unisexual salamanders (genus

834   Ambystoma) present a new reproductive mode for eukaryotes. Genome 50(2): 119-136.

835 Boschetti C, Pouchkina-Stantcheva N, Hoffmann P, Tunnacliffe A. 2011. Foreign genes and

836   novel hydrophilic protein genes participate in the desiccation response of the bdelloid

837   rotifer *Adineta ricciae*. *Journal of Experimental Biology* 214(1): 59-68.

838 Bratbak G, Egge JK, Heldal M. 1993. Viral mortality of the marine alga *Emiliania huxleyi*

839   (Haptophyceae) and termination of algal blooms. Marine Ecology Progress Series 93: 39-

840   48.

841 Bruvo R, Adolfsson S, Symonova R, Lamatsch D, Schön I, Jokela J, Butlin R, Muller S. 2011.

842   Few parasites, and no evidence for *Wolbachia* infections, in a freshwater ostracod

843   inhabiting temporary ponds. Biological Journal of the Linnean Society 102(1): 208-216.

844 Buckwold VE. 1994. Viral-induced extinctions unlikely. Nature 368(6471): 562.

845   Burt A, Bell G. 1987. Mammalian chiasma frequencies as a test of two theories of
846   recombination. Nature 326(6115): 803-805.

847   Butlin R. 2002. The costs and benefits of sex: new insights from old asexual lineages. Nature
848   Reviews Genetics 3(4): 311-317.

849   Butlin R, Schön I, Griffiths H. 1998a. Introduction to reproductive modes. In: Martens K, ed.
850   Sex and Parthenogenesis: Evolutionary Ecology of Reproductive Modes in Non-Marine
851   Ostracods. Leiden: Backhuys, 1-24.

852   Butlin R, Schön I, Martens K. 1998b. Asexual reproduction in nonmarine ostracods. *Heredity*
853   81(5): 473-480.

854   Butlin R, Schön I, Martens K. 1999. Origin, age and diversity of clones. *Journal of Evolutionary*
855   *Biology* 12(6): 1020-1022.

856   Carbonel P, Colin J, Danielopol D, Loffler H, Neustrueva I. 1988. Paleoecology of limnic
857   ostracodes: a review of some major topics. *Palaeogeography Palaeoclimatology*
858   *Palaeoecology* 62(1-4): 413-461.

859   Castagnonesereno P, Piotte C, Uijthof J, Abad P, Wajnberg E, Vanlerberghemasutti F,
860   Bongiovanni M, Dalmasso A. 1993. Phylogenetic relationships between amphimictic and
861   parthenogenetic nematodes of the genus *Meloidogyne* as inferred from repetitive DNA
862   analysis. *Heredity* 70(2): 195-204.

863   Colegrave N, Kaltz O, Bell G. 2002. The ecology and genetics of fitness in *Chlamydomonas*.
864   VIII. The dynamics of adaptation to novel environments after a single episode of sex.
865   *Evolution* 56(1): 14-21.

866   Coleman DA, Crossley DA, Hendrix PF. 2004. *Fundamentals of Soil Ecology, Second edition*.
867   USA: Elsevier Academic Press.

868   Croll D, Sanders I. 2009. Recombination in *Glomus intraradices*, a supposed ancient asexual
869   arbuscular mycorrhizal fungus. *Bmc Evolutionary Biology* 9(1).

870   Crow J. 1970. Genetic loads and the cost of natural selection. In: Kojima K, ed. *Biomathematics.*
871   *Volume 1. Mathematical topics in population genetics*. Berlin: Springer-Verlag, 128-177.

872   Currie C, Mueller U, Malloch D. 1999a. The agricultural pathology of ant fungus gardens.
873   *Proceedings of the National Academy of Sciences of the United States of America* 96(14):
874   7998-8002.

875   Currie C, Scott J, Summerbell R, Malloch D. 1999b. Fungus-growing ants use antibiotic-
876   producing bacteria to control garden parasites. *Nature* 398(6729): 701-704.

877   Danovaro R, Dell'Anno A, Corinaldesi C, Magagnini M, Noble R, Tamburini C, Weinbauer M.
878   2008. Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature*
879   454(7208): 1084-1087.

880   Dawkins R, Krebs JR. 1979. Arms races between and within species. Proc*eedings of the Royal*
881   *Society B-Biological Sciences* 205(1161): 489-511.

882   Debortoli N, Li X, Eyres I, Fontaneto D, Hespeels B, Tang CQ, Flot J, Van Doninck K. 2016.
883   Genetic exchange among bdelloid rotifers is more likely due to horizontal gene transfer
884   than to meiotic sex. *Current Biology* 26(6): 723-732.

885   de Castro F, Bolker B. 2005. Mechanisms of disease-induced extinction. *Ecology Letters* 8(1):
886   117-126.

887   de Meeus T, Prugnolle F, Agnew P. 2007. Asexual reproduction: genetics and evolutionary
888   aspects. *Cellular and Molecular Life Sciences* 64(11): 1355-1372.

889   Devetter M, Scholl K. 2014. Hydrobiont animals in floodplain soil: are they positively or
890   negatively affected by flooding? *Soil Biology & Biochemistry* 69: 393-397.

891   Dole-Olivier M, Galassi D, Marmonier P, Des Chatelliers M. 2000. The biology and ecology of
892   lotic microcrustaceans. *Freshwater Biology* 44(1): 63-91.

893   Domes K, Scheu S, Maraun M. 2007. Resources and sex: soil re-colonization by sexual and
894   parthenogenetic oribatid mites. *Pedobiologia* 51(1): 1-11.

895   Douglas A. 2010. *The Symbiotic Habit*. Princeton, NJ: Princeton University Press.

896   Drake LA, Choi KH, Haskell AE, Dobbs FC. 1998. Vertical profiles of virus-like particles and
897   bacteria in the water column and sediments of Chesapeake Bay, USA. *Aquatic Microbial*
898   *Ecology* 16(1): 17-25.

899   Elliott ET, Anderson RV, Coleman DC, Cole CV. 1980. Habitable pore space and microbial
900   trophic interactions. *Oikos* 35(3): 327-335.

901   Emiliani C. 1982. Extinctive evolution: extinctive and competitive evolution combine into a
902   unified model of evolution. *Journal of Teoretical Biology* 97(1): 13-33.

903   Emiliani C. 1993a. Extinction and viruses. *Biosystems* 31(2-3): 155-159.

904   Emiliani C. 1993b. Viral extinctions in deep-sea species. *Nature* 366(6452): 217-218.

905   Etter RJ, Rex MA, Chase MR, Quattro JM. 2005. Population differentiation decreases with depth
906   in deep-sea bivalves. *Evolution* 59(7): 1479-1491.

907   Farrar D. 1978. Problems in the identity and origin of the Appalachian *Vittaria* gametophyte, a
908   sporophyteless fern of the eastern United States. *American Journal of Botany* 65(1): 1-12.

909   Farrar D. 1990. Species and evolution in asexually reproducing independent fern gametophytes.
910   *Systematic Botany* 15(1): 98-111.

911   Farrar D. 1992. *Trichomanes intricatum*: the independent *Trichomanes* gametophyte in the
912   eastern United States. *American Fern Journal* 82(2): 68-74.

913   Farrar D. 1998. The tropical flora of rockhouse cliff formations in the eastern United States.
914   *Journal of the Torrey Botanical Society* 125(2): 91-108.

915   Farrell BD, Sequeira AS, O'Meara BC, Normark BB, Chung JH, Jordal BH. 2001. The evolution
916   of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution* 55(10):
917   2011-2027.

918   Filippini M, Buesing N, Bettarel Y, Sime-Ngando T, Gessner MO. 2006. Infection paradox: high
919   abundance but low impact of freshwater benthic viruses. *Applied and Environmental*
920   *Microbiology* 72(7): 4893-4898.

921   Fischer O & Schmid-Hempel P. 2005. Selection by parasites may increase host recombination
922   frequency. *Biology Letters* 1(2): 193–195.

923   Fisher R. 2003. *The Genetical Theory of Natural Selection: A Complete Variorum Edition*. New
924   York, NY: Oxford University Press.

925   Fisher UR, Wieltschnig C, Kirschner AKT, Velimirov B. 2003. Does virus-induced lysis
926   contribute significantly to bacterial mortality in the oxygenated sediment layer of shallow
927   oxbow lakes? *Applied and Environmental Microbiology* 69(9): 5281-5289.

928   Flegr J. 2002. Was Lysenko (partly) right? Michurinist biology in the view of modern plant
929   physiology and genetics. *Rivista Di Biologia-Biology Forum* 95: 259-271.

930   Flegr J. 2008. *Frozen evolution: Or, that's not the way it is, mr. Darwin - Farewell to selfish*
931   *gene*. USA: Createspace Independent Pub.

932   Flegr J. 2010. Elastic, not plastic species: frozen plasticity theory and the origin of adaptive
933   evolution in sexually reproducing organisms. *Biology Direct* 5(1).

934   Flegr J. 2013. Microevolutionary, macroevolutionary, ecological and taxonomical implications
935   of punctuational theories of adaptive evolution. *Biology Direct* 8(1).

936   Fuhrman JA. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature*
937   399(6736): 541-548

938   Garcia C, Toro M. 1992. Sib competition in *Tribolium*: a test of the elbow-room model. *Heredity*
939   68(6): 529-536.

940   Gladyshev E, Meselson M. 2008. Extreme resistance of bdelloid rotifers to ionizing radiation.
941   *Proceedings of the National Academy of Sciences of the United States of America*
942   105(13): 5139-5144.

943   Glesener R, Tilman D. 1978. Sexuality and the components of environmental uncertainty: clues
944   from geographic parthenogenesis in terrestrial animals. *The American Naturalist*
945   112(986): 659-673.

946   Gorelick R, Carpinone J. 2009. Origin and maintenance of sex: the evolutionary joys of self sex.
947   *Biological Journal of the Linnean Society* 98(4): 707-728.

948   Griffiths H, Butlin R. 1995. A timescale for sex versus parthenogenesis: evidence from subfossil
949   ostracods. *Proceedings of the Royal Society of London Series B-Biological Sciences*
950   260(1357): 65-71.

951   Hamilton W, Axelrod R, Tanese R. 1990. Sexual reproduction as an adaptation to resist parasites
952   (a review). *Proceedings of the National Academy of Sciences of the United States of*
953   *America* 87(9): 3566-3573.

954   Heethoff M, Domes K, Laumann M, Maraun M, Norton RA, Scheu S. 2007. High genetic
955   divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite
956   *Platynothrus peltifer* (Acari, Oribatida). *Journal of Evolutionary Biology* 20(1): 392-402.

957   Hörandl E. 2006. The complex causality of geographical parthenogenesis. *New Phytologist*
958   171(3): 525-538.

959   Hörandl E. 2009. Geographical parthenogenesis: opportunities for asexuality. In: Schön I,
960   Martens K, van Dijk P, eds. *Lost Sex: The Evolutionary Biology of Parthenogenesis*.
961   Dordrecht: Springer Science+Business Media B.V., 161-186.

962   Jablonski D. 1986. Background and mass extinctions: the alternation of macroevolutionary
963   regimes. *Science* 231(4734): 129-133.

964   Janko K, Drozd P, Eisner J. 2011. Do clones degenerate over time? Explaining the genetic
965   variability of asexuals through population genetic models. *Biology Direct* 6(1).

966   Janko K, Drozd P, Flegr J, Pannell J. 2008. Clonal turnover versus clonal decay: a null model for
967   observed patterns of asexual longevity, diversity and distribution. *Evolution* 62(5): 1264-
968   1270.

969   Jorgensen A, Mobjerg N, Kristensen R. 2007. Molecular study of the tardigrade *Echiniscus*
970   *testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical
971   area. *Journal of Limnology* 66(1s): 77-83.

972   Judson OP, Normark BB. 1996. Ancient asexual scandals. *Trends in Ecology & Evolution* 11(2):
973   41-46.

974   Kaltz O, Bell G. 2002. The ecology and genetics of fitness in *Chlamydomonas*. XII. Repeated
975   sexual episodes increase rates of adaptation to novel environments. *Evolution* 56(9):
976   1743-1753.

977   Karasawa S, Hijii N. 2008. Vertical stratification of oribatid (Acari: Oribatida) communities in
978   relation to their morphological and life-history traits and tree structures in a subtropical
979   forest in Japan. *Ecological Research* 23(1): 57-69.

980   Koella J. 1993. Ecological correlates of chiasma frequency and recombination index of plants.
981   *Biological Journal of the Linnean Society* 48(3): 227-238.

982   Kondrashov A. 1982. Selection against harmful mutations in large sexual and asexual
983   populations. *Genetical Research* 40(03): 325-332.

984   Kondrashov A. 1993. Classification of hypotheses on the advantage of amphimixis. *Journal of*
985   *Heredity* 84(5): 372-387.

986   Krivolutsky D, Druk A. 1986. Fossil oribatid mites. *Annual Review of Entomology* 31(1): 533-
987   545.

988   Lavelle P, Spain AV. 2003. *Soil Ecology*. New York, USA: Kluwer Academic Publishers.

989   Law J, Crespi B. 2002. Recent and ancient asexuality in *Timema* walkingsticks. *Evolution* 56(8):
990   1711-1717.

991   Lehtonen J, Jennions M, Kokko H. 2012. The many costs of sex. *Trends in Ecology & Evolution*
992   27(3): 172-178.

993   Lewis J, Wolpert L. 1979. Diploidy, evolution and sex. *Journal of Theoretical Biology* 78(3):
994   425-438.

995   Li H, Reynolds J. 1995. On definition and quantification of heterogeneity. *Oikos* 73(2): 280-284.

996   Mantovani B, Passamonti M, Scali V. 2001. The mitochondrial cytochrome oxidase II gene in
997   *Bacillus* stick insects: ancestry of hybrids, androgenesis, and phylogenetic relationships.
998   *Molecular Phylogenetics and Evolution* 19(1): 157-163.

999   Maraun M, Erdmann G, Schulz G, Norton RA, Scheu S, Domes K. 2009. Multiple convergent
1000   evolution of arboreal life in oribatid mites indicates the primacy of ecology. *Proceedings*
1001   *of the Royal Society B-Biological Sciences* 276(1671): 3219-3227.

1002 Maraun M, Norton RA, Ehnes RB, Scheu S, Erdmann G. 2012. Positive correlation between
1003   density and parthenogenetic reproduction in oribatid mites (Acari) supports the structured
1004   resource theory of sexual reproduction. *Evolutionary Ecology Research* 14(3): 311-323.

1005 Martens K. 1998. Sex and ostracods: a new synthesis. In: Martens K, ed. *Sex and*
1006   *Parthenogenesis: Evolutionary Ecology of Reproductive Modes in Non-Marine Ostracds.*
1007   Leiden: Backhuys, 295-321.

1008 Martens K, Horne D, Griffiths H. 1998. Age and diversity of non-marine ostracods. In: Martens
1009   K, ed. *Sex and Parthenogenesis: Evolutionary Ecology of Reproductive Modes in Non-*
1010   *Marine Ostracds.* Leiden: Backhuys, 37-55.

1011 Martens K, Schön I. 2000. The importance of habitat stability for the prevalence of sexual
1012   reproduction. In: Minoura K, ed. *Lake Baikal: a Mirror in Time and Space for*
1013   *Understanding Global Change Processes. Yokohama Symposium 1998*. Amsterdam:
1014   Elsevier, 324-330.

1015 Martens K, Schön I, Meisch C, Horne D. 2008. Global diversity of ostracods (Ostracoda,
1016   Crustacea) in freshwater. *Hydrobiologia* 595(1): 185-193.

1017 Maynard Smith J. 1978. *The evolution of sex.* Cambridge, UK: Cambridge University Press.

1018 Maynard Smith J. 1993. *The theory of evolution.* Cambridge, UK: Cambridge University Press.

1019 McDonald JH. 2014. *Handbook of Biological Statistics (3rd ed.).* Baltimore, USA: Sparky
1020   House Publishing.

1021 Meirmans S, Strand R. 2010. Why are there so many theories for sex, and what do we do with
1022   them? *Journal of Heredity* 101(1s): S3-S12.

1023 Mikheyev AS, Mueller UG, Abbot P. 2006. Cryptic sex and many-to-one colevolution in the
1024   fungus-growing ant symbiosis. *Proceedings of the National Academy of Sciences of the*
1025   *United States of America* 103(28): 10702-10706.

1026   Mobjerg N, Halberg K, Jorgensen A, Persson D, Bjorn M, Ramlov H, Kristensen R. 2011.
1027   Survival in extreme environments – on the current knowledge of adaptations in
1028   tardigrades. *Acta Physiologica* 202(3): 409-420.

1029   Morton JE, Boney AD, Corner EDS. 1957. The adaptations of *Lasaea rubra* (Montagu), a small
1030   intertidal lamellibranch. *Journal of the Marine Biological Association of the United*
1031   *Kingdom* 36(02): 383-405.

1032   Mueller UG. 2002. Ant versus fungus versus mutualism: ant–cultivar conflict and the
1033   deconstruction of the attine ant–fungus symbiosis. *American Naturalist* 160(4s): S67-
1034   S98.

1035   Mueller UG, Rehner SA, Schultz TR. 1998. The evolution of agriculture in ants. *Science*
1036   281(5385): 2034-2038.

1037   Muller H. 1932. Some genetic aspects of sex. *The American Naturalist* 66(703): 118-138.

1038   Muller H. 1964. The relation of recombination to mutational advance. *Mutation Research* 1(1):
1039   2-9.

1040   Murphy SL, Tate RL. 1996. Bacterial movement through soil. In: Stozsky G & Bollag JM, ed.
1041   *Soil Biochemistry Vol. 9.* New York: Marcel Dekker, 253-286.

1042   Neiman M, Jokela J, Lively C. 2005. Variation in asexual lineage age in *Potamopyrgus*
1043   *antipodarum*, a New Zealand snail. *Evolution* 59(9): 1945-1952.

1044   Neiman M, Koskella B. 2009. Sex and the Red queen. In: Schön I, Martens K, van Dijk P, eds.
1045   *Lost sex: the Evolutionary Biology of Parthenogenesis*. Dordrecht: Springer
1046   Science+Business Media B.V., 133-159.

1047   Neiman M, Meirmans S, Meirmans P, Schlichting C, Mousseau T. 2009. What can asexual
1048   lineage age tell us about the maintenance of sex? *Annals of the New York Academy of*
1049   *Sciences* 1168(1): 185-200.

1050   Neiman M, Schwander T. 2011. Using parthenogenetic lineages to identify advantages of sex.
1051   *Evolutionary Biology* 38(2): 115-123.

1052   Normark B, Judson O, Moran N. 2003. Genomic signatures of ancient asexual lineages.
1053   *Biological Journal of the Linnean Society* 79(1): 69-84.

1054   Norton R. 1994. Evolutionary aspects of oribatid mite life histories and consequences for the
1055   origin of the Astigmata. In: Houck M, ed. *Mites. Ecological and Evolutionary Analyses*
1056   *of Life-History Patterns*. New York: Chapman & Hall, 99-135.

1057 Norton R, Kethley J, Johnston D, O'Connor B. 1993. Phylogenetic perspectives on genetic
1058   systems and reproductive modes of mites. In: Wrensch D, Ebbert M, eds. *Evolution and*
1059   *diversity of sex ratio in insects and mites*. New York: Chapman and Hall, 8-99.

1060 Nunney L. 1989. The maintenance of sex by group selection. *Evolution* 43(2): 245-257.

1061 Otto S, Lenormand T. 2002. Resolving the paradox of sex and recombination. *Nature Reviews*
1062   *Genetics* 3(4): 252-261.

1063 Ó Foighil D. 1988. Random mating and planktotrophic larval development in the brooding
1064   hermaphroditic clam *Lasaea australis* (Lamarck, 1818). *Veliger* 31(3-4): 214-221.

1065 Ó Foighil D. 1989. Planktotrophic larval development is associated with a restricted geographic
1066   range in *Lasaea*, a genus of brooding, hermaphroditic bivalves. *Marine Biology* 103(3):
1067   349-358.

1068 Ó Foighil D, Eernisse D. 1988. Geographically widespread, non-hybridizing, sympatric strains of
1069   the hermaphroditic, brooding clam *Lasaea* in the northeastern Pacific Ocean. *Biological*
1070   *Bulletin* 175(2): 218-229.

1071 Paul EA. 2007. *Soil Microbiology, Ecology and Biochemistry*. USA: Academic Press.

1072 Pejler B. 1995. Relation to habitat in rotifers. *Hydrobiologia* 313(1): 267-278.

1073 Pilato G. 1979. Correlations between cryptobiosis and other biological characteristics in some
1074   soil animals. *Italian Journal of Zoology* 46(4): 319-332.

1075 Poinar GO, Ricci C. 1992. Bdelloid rotifers in Dominican amber: evidence for parthenogenetic
1076   continuity. *Experientia* 48(4): 408-410.

1077 Pongratz N, Storhas M, Carranza S, Michiels N. 2003. Phylogeography of competing sexual and
1078   parthenogenetic forms of a freshwater flatworm: patterns and explanations. *Bmc*
1079   *Evolutionary Biology* 3(1).

1080 Pontecorvo D. 1954. Mitotic recombination in the genetic systems of filamentous fungi.
1081   *Caryologia* 6: 192-200.

1082 Quesada CA, Miranda AC, Hodnett MG, Santos AJB, Miranda HS, Breyer LM. 2004. Seasonal
1083   and depth variation of soil moisture in a burned open savanna (campo sujo) in central
1084   Brazil. *Ecological Applications* 14(4s): 33-41.

1085 Redecker D, Kodner R, Graham L. 2000. Glomalean fungi from the Ordovician. *Science*
1086   289(5486): 1920-1921.

1087 Redfield R. 2001. Do bacteria have sex? *Nature Reviews Genetics* 2(8): 634-639.

1088 Remy W, Taylor T, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular
1089   mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of*
1090   *America* 91(25): 11841-11843.

1091 Renaut S, Replansky T, Heppleston A, Bell G. 2006. The ecology and genetics of fitness in
1092   *Chlamydomonas*. XIII. The fitness of long-term sexual and asexual populations in benign
1093   environments. *Evolution* 60(11): 2272-2279.

1094 Ricci C. 2001. Dormancy patterns in rotifers. *Hydrobiologia* 446(1): 1-11.

1095 Ricci C, Balsamo M. 2000. The biology and ecology of lotic rotifers and gastrotrichs.
1096   *Freshwater Biology* 44(1): 15-28.

1097 Ricci CN. 1987. Ecology of bdelloids: how to be successful. *Hydrobiologia* 147(1): 117-127.

1098 Robeson MS, King AJ, Freeman KR, Birky CW, Martin AP, Schmidt SK. 2011. Soil rotifer
1099   communities are extremely diverse globally but spatially autocorrelated locally.
1100   *Proceedings of the National Academy of Sciences of the United States of America*
1101   108(11): 4406-4410.

1102 Rohde K. 1986. Differences in species diversity of monogenea between the pacific and atlantic
1103   oceans. *Hydrobiologia* 137(1): 21-28.

1104 Rohde K, Heap M. 1998. Latitudinal differences in species and community richness and in
1105   community structure of metazoan endo- and ectoparasites of marine teleost fish.
1106   *International Journal For Parasitology* 28(3): 461-474.

1107 Roughgarden J. 1991. The evolution of sex. *American Naturalist* 138(4): 934-953.

1108 R_Core_Team. 2014. *R: A language and environment for statistical computing, version 3.1.2.*
1109   Vienna, Austria: R Foundation for Statistical Computing. *Available at: https://www.r-*
1110   *project.org* (accessed 23 may 2016).

1111 Schurko A, Neiman M, Logsdon J. 2009. Signs of sex: what we know and how we know it.
1112   *Trends in Ecology & Evolution* 24(4): 208-217.

1113 Schwander T, Crespi BJ. 2009. Twigs on the tree of life? Neutral and selective models for
1114   integrating macroevolutionary patterns with microevolutionary processes in the analysis
1115   of asexuality. *Molecular Ecology* 18(1): 28-42.

1116 Schwander T, Henry L, Crespi B. 2011. Molecular evidence for ancient asexuality in *Timema*
1117   stick insects. *Current Biology* 21(13): 1129-1134.

1118   Sheldon P. 1996. Plus ça change—a model for stasis and evolution in different environments.

1119   *Palaeogeography, Palaeoclimatology, Palaeoecology* 127(1-4): 209–227.

1120   Schön I, Butlin R, Griffiths H, Martens K. 1998. Slow molecular evolution in an ancient asexual

1121   ostracod. *Proceedings of the Royal Society of London Series B-Biological Sciences*

1122   265(1392): 235-242.

1123   Schön I, Martens K. 2004. Adaptive, pre-adaptive and non-adaptive components of radiations in

1124   ancient lakes: a review. *Organisms Diversity & Evolution* 4(3): 137-156.

1125   Schön I, Martens K, Rossi V. 1996. Ancient asexuals: scandal or artifact? *Trends in Ecology and*

1126   *Evolution* 11(7): 296-297.

1127   Schön I, Martens K, van Dijk P. 2009a. *Lost Sex: The Evolutionary Biology of Parthenogenesis*.

1128   Dordrecht: Springer Science+Business Media B.V.

1129   Schön I, Rossetti G, Martens K. 2009b. Darwinulid ostracods: ancient asexual scandals or

1130   scandalous gossip? In: Schon I, Martens K, van Dijk P, eds. *Lost Sex: The Evolutionary*

1131   *Biology of Parthenogenesis*: Dordrecht: Springer Science+Business Media B.V., 217-

1132   240.

1133   Schwander T. 2016. Evolution: The end of an ancient asexual scandal. *Current Biology* 26(6):

1134   R233–R235.

1135   Siepel H. 1994. Life-history tactics of soil microarthropods. *Biology and Fertility of Soils* 18(4):

1136   263-278.

1137   Siepel H. 1996. Biodiversity of soil microarthropods: the filtering of species. *Biodiversity &*

1138   *Conservation* 5(2): 251-260.

1139   Smith J. 1980. Selection for recombination in a polygenic model. *Genetical Research* 35(03):

1140   269-277.

1141   Speijer D, Lukes J, Elias M. 2015. Sex is a ubiquitous, ancient, and inherent attribute of

1142   eukaryotic life. *Proceedings of the National Academy of Sciences of the United States of*

1143   *America* 112(29): 8827-8834.

1144   Sterrer W. 2002. On the origin of sex as vaccination. *Journal of Theoretical Biology* 216(4): 387-

1145   396.

1146   Suttle CS. 2005. Viruses in the sea. *Nature* 437(7057): 356-361.

1147   Suttle CS. 2007. Marine viruses—major players in the global ecosystem. *Nature Reviews*

1148   *Microbiology* 5(10): 801-812.

1149 Suttle CS, Chan AM, Cottrell MT. 1990. Infection of phytoplankton by viruses and reduction of
1150   primary productivity. *Nature* 347(6292): 467-469.

1151 Thornhill R, Fincher C. 2013. The comparative method in cross-cultural and cross-species
1152   research. *Evolutionary Biology* 40(4): 480-493.

1153 Tobler M, Schlupp I, de Leon F, Glaubrecht M, Plath M. 2007. Extreme habitats as refuge from
1154   parasite infections? Evidence from an extremophile fish. *Acta Oecologica* 31(3): 270-
1155   275.

1156 Tokeshi M. 1999. *Species Coexistence: Ecological and Evolutionary Perspectives*. Oxford, UK:
1157   Blackwell Science Ltd.

1158 Turgeon J, Hebert P. 1994. Evolutionary interactions between sexual and all-female taxa of
1159   *Cyprinotus* (Ostracoda: Cyprididae). *Evolution* 48(6): 1855-1865.

1160 Van Dijk P. 2009. Apomixis: basics for non-botanists. In: Schön I, Martens K, van Dijk P, eds.
1161   *Lost Sex: The Evolutionary Biology of Parthenogenesis*. Dordrecht: Springer
1162   Science+Business Media B.V., 47-62.

1163 van Raay T, Crease T. 1995. Mitochondrial DNA diversity in an apomictic *Daphnia complex*
1164   from the Canadian High Arctic. *Molecular Ecology* 4(2): 149-161.

1165 Vanhaecke P, Siddall S, Sorgeloos P. 1984. International study on *Artemia*. XXXII. Combined
1166   effects of temperature and salinity on the survival of *Artemia* of various geographical
1167   origin. *Journal of Experimental Marine Biology and Ecology* 80(3): 259-275.

1168 von Uexküll J. 1982. The theory of Meaning. *Semiotica* 42(1): 25-82.

1169 Vrijenhoek R. 1984. The evolution of clonal diversity in *Poeciliopsis*. In: Turner B, ed.
1170   *Evolutionary Genetics of Fishes*. New York: Plenum Press, 399-429.

1171 Vrijenhoek R, Parker E. 2009. Geographical parthenogenesis: general purpose genotypes and
1172   frozen niche variation. In: Schön I, Martens K, van Dijk P, eds. *Lost Sex: The*
1173   *Evolutionary Biology of Parthenogenesis*: Dordrecht: Springer Science+Business Media
1174   B.V., 99-131.

1175 Walter D. 2009. Suborder Endeostigmata. In: Krantz G, Walter D, eds. *A manual of acarology*.
1176   Lubbock: Texas Tech University Press, 421-429.

1177 Walter D, Lindquist E, Smith I, Cook D, Krantz G. 2009. Order Trombidiformes. In: Krantz G,
1178   Walter D, eds. *A manual of acarology*. Lubbock: Texas Tech University Press, 233-420.

1179   Wallwork, JA. 1970. *Ecology of soil animals*. London, UK: McGRAW-HILL Publishing
1180   Company Limited.

1181   West S, Lively C, Read A. 1999. A pluralist approach to sex and recombination. *Journal of*
1182   *Evolutionary Biology* 12(6): 1003-1012.

1183   Williams GC. 1975. *Sex and evolution*. Princeton, NJ: Princeton University Press.

1184   Wilson CG. 2011. Desiccation-tolerance in bdelloid rotifers facilitates spatiotemporal escape
1185   from multiple species of parasitic fungi. *Biological Journal of the Linnean Society*
1186   104(3): 564-574

1187   Wommack KE, Colwell RR. 2000. Virioplankton: Viruses in Aquatic Ecosystems. *Microbiology*
1188   *and Molecular Biology Reviews* 64(1): 69-114.