



Habitat heterogeneity favors asexual reproduction in natural populations of grasthrips

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Explaining the overwhelming success of sex among eukaryotes is difficult given the obvious costs of sex relative to asexuality. Different studies have shown that sex can provide benefits in spatially heterogeneous environments under specific conditions, but whether spatial heterogeneity commonly contributes to the maintenance of sex in natural populations remains unknown. We experimentally manipulated habitat heterogeneity for sexual and asexual thrips lineages in natural populations and under seminatural mesocosm conditions by varying the number of hostplants available to these herbivorous insects. Asexual lineages rapidly replaced the sexual ones, independently of the level of habitat heterogeneity in mesocosms. In natural populations, the success of sexual thrips decreased with increasing habitat heterogeneity, with sexual thrips apparently only persisting in certain types of hostplant communities. Our results illustrate how genetic diversity-based mechanisms can favor asexuality instead of sex when sexual lineages co-occur with genetically variable asexual lineages.

KEY WORDS: Asexuality, evolution of sex, parthenogenesis, Thysanoptera, Tangled Bank.

Sex: What is it good for? Identifying the factors that drive the maintenance of sexual reproduction in natural populations remains a central goal in evolutionary biology. Sexual reproduction is associated with many direct costs, especially in metazoans, where males often provide little or no resources to their offspring (reviewed by Lehtonen et al. 2012). Since only females can directly produce offspring, sexual females who invest equally in sons and daughters experience a twofold cost relative to asexual females who solely produce daughters (Williams 1975; Maynard Smith 1978). This cost translates into dramatically slower growth rates of the sexual population relative to asexual lines. Even in cases where males do contribute resources to their offspring, sex is typically still costly because it requires the attraction of mates and eventually mating (Bell 1982; Lehtonen et al. 2012). Sex

also generates indirect costs because it breaks up associations between alleles, including beneficial associations that were generated by selection in past generations (reviewed in Agrawal 2006; Otto 2009). These multifaceted costs have motivated a scientific quest for identifying mechanisms and situations that can generate benefits for sex and, therefore, could help explain the overwhelming success of this reproductive mode (e.g., Maynard Smith 1971; Charlesworth 1976; Barton 1995; Peters and Lively 1999; Lenormand and Otto 2000; Gandon and Otto 2007; Agrawal 2009).

Theoretical approaches have identified a number of possible mechanisms that could generate indirect benefits for sex, with support from experimental evolution studies under laboratory conditions for some of them (e.g., Colegrave 2002; Goddard

et al. 2005; Cooper 2007; Morran et al. 2009; Becks and Agrawal 2010; Morran et al. 2011; McDonald et al. 2016). However, it remains unknown whether any of the suggested benefits of sex may be sufficient to outweigh the costs sex generates under natural conditions.

Here, we evaluate whether spatial habitat heterogeneity, a situation that can generate benefits for sex under some conditions, contributes to the maintenance of sex in natural populations. Two classes of theoretical arguments propose that sex can generate benefits under spatial heterogeneity. In the first class, spatial habitat heterogeneity causes spatial variation in selection. Spatial variation in selection can generate locally adapted allele associations with locally maladapted associations introduced by migration (Lenormand 2002). Sex can then provide benefits because it breaks such maladaptive allele associations, provided migration rates are high enough to regularly introduce locally maladapted associations and low enough to not constrain local adaptation via gene flow (Pylkov et al. 1998; Lenormand and Otto 2000; Agrawal 2009). These theoretical predictions were supported by results from an experimental evolution approach in cyclically asexual rotifers. By controlling migration rates between similar and different rearing environments, Becks and Agrawal (2010) showed that higher rates of sex are maintained under migration between different environments. However, theory also showed that spatial variation only favors sex if specific types of epistasis occur in combination with the appropriate migration rates and strengths of selection (Pylkov et al. 1998; Lenormand and Otto 2000), raising the question of how frequently natural populations might fit the theoretical conditions predicted to favor sex under habitat heterogeneity.

The second class of arguments, referred to as “Tangled Bank” models, posit that there is genetically based variation among individuals for exploiting different niches (e.g., Ghiselin 1974; Maynard Smith 1978; Young 1981; Bell 1982; Case and Taper 1986; Doncaster et al. 2000; Song et al. 2011). Genetically identical individuals should, therefore, experience more intense competition than genetically different individuals. Under the assumption that sexual lineages comprise more genetic variation for niche exploitation than asexual ones, sexual lineages can be favored via at least two mechanisms. First, sexual reproduction could reduce competition among siblings because sexually produced offspring display more genetic variation for resource use than asexually produced ones (Maynard Smith 1978; Young 1981). Second, intraspecific competition can negatively impact population growth rates. The negative impact of an asexual lineage on its own growth rate should be more severe than the impact on the growth rate of the sexual lineage (given sexual individuals are genetically variable and different from asexual individuals), which can maintain the coexistence of sexual and asexual lineages (Case and Taper 1986; Doncaster et al. 2000; see also Song et al. 2011).

Empirical tests of Tangled Bank models have used experimental populations consisting either of a single clone (low genetic diversity) or multiple clones (high genetic diversity) of an asexual species and found that genetic diversity can indeed provide benefits (e.g., Antonovics and Ellstrand 1984; Ellstrand and Antonovics 1985; Weeks and Hoffmann 1998, 2008). However, there are currently no experimental tests of whether Tangled Bank mechanisms contribute to the maintenance of competing sexual and asexual lineages in natural populations.

We evaluated the possible contribution of habitat heterogeneity to the maintenance of sex in natural populations by using grasshoppers of the genus *Aptinothrips* (Thysanoptera) as a model system. This genus comprises four species of small (~1 mm) insects, which live and feed on a variety of grasses (Poaceae) (Palmer 1975). Breeding experiments with *Aptinothrips* females from Western Europe revealed reproductive polymorphism in this area. Two *Aptinothrips* species reproduce exclusively asexually (*A. stylifer* and *A. karnyi*), one species (*A. elegans*) reproduces sexually, and the fourth species (*A. rufus*) is characterized by mixed reproduction, with some females reproducing sexually and others asexually (van der Kooij and Schwander 2014). As for most thrips (Hamilton 1967; Lewis 1973), sex ratios for sexual *Aptinothrips* lineages in field populations vary extensively over space and time and are typically highly female-biased (van der Kooij and Schwander 2014). Female-bias is most likely caused by a combination of low investment into males, sex-specific behaviors, and males being very short-lived (Lewis 1973; Mound 1991). Sexual and asexual species of *Aptinothrips* form mixed populations across a large part of their distribution (van der Kooij and Schwander 2014). Several independent transitions to asexuality have been described, and clonal diversity is extremely high in the widely distributed asexual *A. rufus* (van der Kooij and Schwander 2014; Fontcuberta et al. 2016).

We took advantage of the reproductive mode polymorphism among *Aptinothrips* species and lineages to test whether the success of sexual lineages is affected by spatial heterogeneity in selection. Because *Aptinothrips* are dependent on grasses for their entire life cycle, we assumed that different grass species would correspond to different habitat types for the thrips, as host plants often represent different ecological niches for herbivorous insects (Jaenike 1990). We first verified that different grasses do indeed represent habitats of variable quality for *Aptinothrips*, by measuring reproductive success of females on five different grass species. We then used two complementary experimental approaches to test whether grass diversity (i.e., the level of habitat heterogeneity) affected the relative success of competing sexual and asexual lineages. For the first approach, we generated mesocosms comprising either a single grass species (homogeneous habitats) or a mix of three grass species (heterogeneous habitats). Sexual

and asexual grassthrips were then introduced into these habitats and their relative proportions followed over 12 generations. This setup thus mimics a situation where a largely empty habitat is simultaneously colonized by sexual and asexual individuals. In the second approach, we took advantage of experimental hay meadows where grass diversity was manipulated via fertilization and irrigation treatments (Andrey et al. 2014). The original purpose of these treatments was to investigate the effects of fertilization and irrigation on the biodiversity of extensively managed, montane, and subalpine hay meadows, to formulate management recommendations that would simultaneously optimize biodiversity and hay yield. Sexual and asexual thrips co-occur in such extensively managed hay meadows. In contrast to the mesocosm experiments, the meadow treatments thus correspond to a modification of a habitat with established sexual and asexual populations and not to a situation where empty habitat is colonized. We collected grassthrips from these experimental meadows to test whether sexual *Aptinotrips* were favored in treatments generating increased grass diversity.

In summary, our aim was to experimentally manipulate habitat heterogeneity for thrips under the most natural conditions possible and to test whether the level of habitat heterogeneity affects the relative success of sexual and asexual thrips. Our approach, therefore, does not generate artificial conditions that would favor sex specifically via Tangled Bank mechanisms or because it would break up maladaptive gene combinations introduced by migration. Rather, our approach allows us to infer whether habitat heterogeneity contributes to the maintenance of sex under natural conditions independently of the mechanism through which sex would generate benefits. In contrast to hypotheses and experiments under artificial conditions, we show that habitat heterogeneity under natural conditions always favors asexual, rather than sexual, reproduction.

Methods

THRIPS FITNESS ON DIFFERENT GRASS SPECIES

To test whether grass species represent habitats of variable quality for thrips, we measured reproductive success on different grasses for females of the sexual species *A. elegans*, a sexual *A. rufus* lineage, and asexual *A. rufus*. We grew five grass species in 50 ml falcon tubes, with 24–66 replicates per *Aptinotrips* species and grass species: *Molinia caerulea* (moor-grass), *Bromus hordeaceus* (brome), *Festuca rubra* (fescue), *Triticum aestivum* (wheat), and *Secale cereale* (rye). One field-collected, adult female was added to each tube and allowed to reproduce for 24 days at 23°C and 10:14 night/day cycles. Reproductive success of each female was then determined by counting all offspring present in a tube and these offspring numbers were compared between treatments using a GLM with a poisson-distributed error and an empirically estimated scale parameter. Whether thrips species, grass species,

and their interaction explained a significant amount of deviance in the GLM was evaluated using a χ^2 -test.

MESOCOSM EXPERIMENT

Based on the results from the previous experiment, we chose three easy-to-grow grass species that varied in their quality for thrips: wheat, fescue, and rye. With these grasses, we generated four different types of mesocosms: one with a heterogeneous habitat (the three species mixed in equal proportions) and three with homogeneous habitats consisting only of one grass species. Mesocosms were generated in transparent, cylindrical cages (15 cm diameter, 25 cm high), randomly placed on watering tables in a greenhouse under a light regime of L/D = 14:10 superimposed over a natural light regime. These mesocosms were used to test whether habitat heterogeneity affects the relative success of competing sexual and asexual lineages in two different assays, each running over approximately 12 thrips generations (thrips have overlapping generations). In the first assay, we used two *Aptinotrips* lineages that frequently co-occur in natural populations but belong to different species: sexual *A. elegans* and asexual *A. rufus*. Females for this assay were sampled in a hay meadow in Switzerland (N46.657906, W6.533641), where the two species are sympatric with a ratio of 49:51 ($n > 3000$) and that is mainly composed of the grasses *Arrhenatherum elatius*, *Dactylis glomerata*, and *Bromus hordeaceus*. *Aptinotrips elegans* and *A. rufus* females were sorted by species under a dissecting scope at 40× magnification, after CO₂ anesthetization. The two species are very similar but can be distinguished by the shape of antennal segments and position of hairs on the abdomen (Palmer 1975).

In the second competition assay, we used *A. rufus* sexual and asexual strains from laboratory stock populations to account for eventual species differences in the first assay. The stock populations have been maintained in the laboratory for over 3 years on wheat (>30 generations). Under the laboratory conditions used (23°C and 10:14 night/day cycles), the sexual *A. rufus* populations were characterized by an approximately constant proportion of 0.32 males.

For each competition assay, we prepared a total of 160 mesocosms (40 heterogeneous ones and 40 homogeneous ones for each of the three grass species). Six asexual and six mated sexual females were then introduced into each of the 160 mesocosms. This is below the carrying capacity of these mesocosms, which means that competition occurred after a colonisation episode. Furthermore, it is important to note that given the very high clonal diversities in asexual *A. rufus* populations, the six asexual females per mesocosm most likely comprised several different clones. To quantify the frequency of sexual and asexual lineages present, we removed randomly without replacement ten heterogeneous mesocosms and 30 homogeneous ones (10 per grass species) at three successive intervals of 40 ± 2 days (approximately two thrips

generations). Thrips in the remaining ten heterogeneous mesocosms and 30 homogeneous ones were counted after a final interval of 120 days (240 ± 6 days from the start of the experiment). In the sexual *A. elegans* versus asexual *A. rufus* assay, we determined the frequency of sexuals and asexuals by counting the number of adult females of each species. In the sexual versus asexual *A. rufus* assay, we counted the proportion of males among adults because sexual and asexual *A. rufus* females cannot be distinguished morphologically.

Because the mesocosm conditions differed from the conditions in the field (notably because of different grasses), we may have selected for adaptation to new host plants. To test for evidence of adaptation, we collected individuals alive from the 40 mesocosms of the first interval of competing *A. elegans* versus asexual *A. rufus*. Individual *A. elegans* and *A. rufus* females were then transferred to 50 ml tubes with the same habitat as the mesocosm they were extracted from and allowed to reproduce for 24 days as described above. Reproductive output for each thrips species was then compared to the reproductive output under the same conditions prior to the experiment. Thrips from all the remaining mesocosms were counted after extraction into alcohol (adults and larvae) via heat gradients on Berlese funnels.

FIELD EXPERIMENT

To test the effect of habitat heterogeneity on the success of sexual and asexual grassthrips in natural populations, we took advantage of experimental hay meadows in the inner European Alps (Valais, SW Switzerland), where sexual *A. elegans* co-occur with asexual *A. rufus*, *A. stylifer*, and *A. karnyi*. These meadows have been extensively managed for a long time before the start of the experiment. Within each meadow, grass diversity was manipulated via six different irrigation and fertilization treatments (Andrey et al. 2014). These meadows are strongly water and nutrient limited and are occupied by xerophilic species, such that low or intermediate management intensities were expected to increase plant diversity in general and grass diversity in particular (Peter et al. 2009). Precise details on site selection and experimental setups are presented elsewhere (Andrey et al. 2014). Briefly, six circular plots (20 m diameter) were delineated at each of 11 study sites (66 different plots; see Fig. S1) and randomly assigned to the treatments. In the first treatment (“standard”), hay meadows were managed as prior to the start of the experiment with grass cut once per year and no other manipulation. The second treatment consisted in watering the plots once per week, the third treatment in fertilizing them. For the fourth to sixth treatments, plots were simultaneously watered and fertilized in an increasing intensity (see Table S1).

Treatments were initiated in spring 2010 and continued over five years, inducing a gradual transition from the original to new

plant communities (Andrey et al. 2014) over ~40–50 thrips generations. To estimate the final grass diversity in each plot, we used coverage data for grass species from exhaustive plant inventories in rectangles of 2×4 m within each plot, conducted in May–June 2014; thrips were collected in June 2014 (see Supporting Information).

STATISTICAL ANALYSES

All analyses were conducted in R 3.1.1 (R Core Team 2014). We computed Shannon–Wiener α -diversity indices from grass species coverage in experimental plots using the R package *vegan* (Oksanen et al. 2015) to use these diversities as a proxy for habitat heterogeneity. We tested the effects of the treatments on habitat heterogeneity (i.e., grass diversity) using a linear mixed model (LMM). The effect of the treatment and of habitat heterogeneity on the weighted counts of sexual *Aptinothrips* was tested using generalized LMMs (GLMMs) implemented in the R package *lme4* (Bates et al. 2015). Because the residuals in some of our models were overdispersed, we corroborated each GLMM result with an ANOVA using the proportion of sexual *Aptinothrips* rather than their weighted counts (see Supporting Information).

Because our analyses revealed a negative effect of grass diversity on the success of sexual thrips (see results), we investigated whether specific types of grass communities could favor sexual *Aptinothrips*. We first generated a grass community distance dendrogram using Bray–Curtis distances, as implemented in the R package *vegan*. We then used this dendrogram to test whether similar communities were characterized by similar sexual thrips abundances by calculating Pagel’s α with the R package *phytools* (Revell 2012). Pagel’s α is typically used to assess whether phylogenetically related species share similar values of a quantitative trait. In our analyses Pagel’s α thus indicates whether similar grass communities comprise more similar abundances of sexual thrips than expected by chance. For visual evaluation, we further performed a principal component analysis (PCA) as implemented in the R package *vegan*, using a Hellinger transformation for scaling grass cover data (Legendre and Gallagher 2001). Additional grass community analyses are described in the Supporting Information.

Results

Grass species vary significantly in quality for thrips as revealed by the number of progeny females produced on the five species wheat, brome, fescue, rye, and moor-grass (thrips species effect: $df = 2$, $P < 0.0001$; grass species effect: $df = 4$, $P < 0.0001$; interaction: $df = 8$, $P = 0.11$; Fig. 1). Females of the three tested thrips species/lineages were unable to reproduce on moor-grass and we found up to sevenfold differences in reproductive output among the four remaining grasses. Given this broad quality variation among grasses, grass diversity is a suitable proxy for the level of habitat heterogeneity in a thrips population.

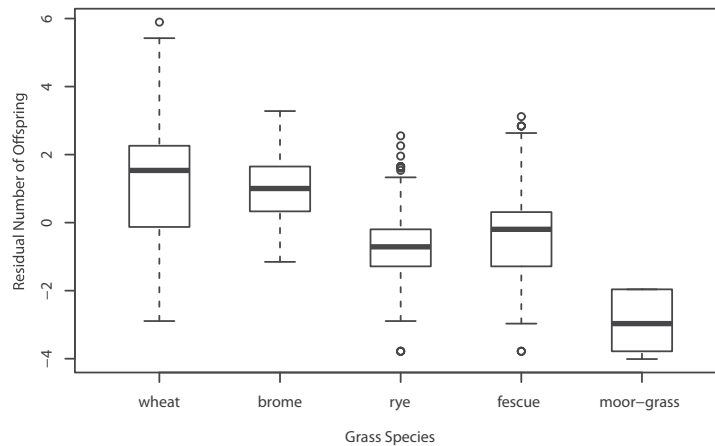


Figure 1. Grassthrips females produce different numbers of offspring depending on the grass species they breed on, indicating that different grasses correspond to habitats of different quality. $N = 24$ –66 females per thrips- and grass species; residual offspring numbers after accounting for variation among thrips species are presented.

We then used three of the five grass species to generate heterogeneous (three species mixed) and homogeneous (one species) mesocosms in which we followed the relative proportion of sexual and asexual thrips over time. Within 80 days of the experiment (approximately four thrips generations), each mesocosm comprised close to 300 individuals, a number that remained approximately constant until the 12th generation (Fig. 2). Independently of the mesocosm type (heterogeneous or different homogeneous ones) and the sexual lineage we used (sexual *A. rufus* or *A. elegans*), sexual females were always completely replaced by asexual ones after 12 generations (Fig. 2). We therefore found no positive effect of habitat heterogeneity on the maintenance of sexual thrips.

In natural populations, the sexual thrips species *A. elegans* frequently co-occurs with asexual *A. rufus*, with individuals used in the mesocosm experiments stemming from a location with approximately equal frequencies of both species. Nevertheless, the rate of population increase of asexual *A. rufus* was much higher than of sexual *A. elegans* in all mesocosms, leading to complete disappearance of *A. elegans* after 4–12 generations. After approximately six generations, *A. elegans* was completely extinct in 10 homogeneous rye mesocosms, and in seven respectively eight of the homogeneous fescue and wheat ones (without a single *A. elegans* individual among over 7000 thrips). *Aptinothrips elegans* females were still present in the 15 remaining mesocosms, but at a very low frequency in 14 of the 15 (one to five individuals among several hundred asexual females). Only one homogeneous wheat mesocosm still comprised 12 *A. elegans* females (among 369 asexual females) after six generations. However, these residual individuals from sexual lineages were completely replaced by asexual females by generation 12, at which point we did not find a single *A. elegans* in any of the 40 mesocosms (combined over 11,000 females). Thus, although the final extinction of sexuals

was somewhat delayed in heterogeneous mesocosms (after six generations: complete extinction in 25 out of 30 homogeneous habitats vs. 0 out of 10 heterogeneous ones; Fisher's exact test $P = 0.01$), sexuals were unable to persist when in competition with asexuals, whatever the conditions.

Part of the success of asexual *A. rufus*, when in competition with *A. elegans* under the used rearing conditions stems from an approximately twofold fecundity advantage of *A. rufus* that is unrelated to reproductive mode but characterizes different species (see below; Fig. 3). However, we also found complete extinction of the sexual lineages after 12 generations for the mesocosms where asexual *A. rufus* were in competition with sexual *A. rufus* lineages. After generation 12, we found not a single male among over 12,000 females (Fig. 2). In contrast to the first competition experiment where sexual lineages were largely extinct by generation six, the proportion of males present at generation six (0–0.11 per mesocosm, Fig. 2) indicated that most mesocosms still comprised sexual lineages. Consequently, we did not find any difference between homogeneous and heterogeneous mesocosms in the residual persistence of sexuals (number of mesocosms without males after six generations: 4 out of 30 homogeneous ones vs. 1 out of 10 heterogeneous ones, $P > 0.99$).

Asexual *A. rufus* and sexual *A. elegans* used in the first mesocosm experiment were collected from a meadow comprising the grass species *Arrhenatherum elatius*, *Dactylis glomerata*, and *Bromus hordeaceus* and without any of the three species used in the mesocosm experiment. We therefore expected to observe some adaptation to the new grass species in the mesocosms. We tested for evidence of adaptation by measuring reproductive output of individual females before the start of the experiment and after two generations in the mesocosms. For asexual *A. rufus* in homogeneous mesocosms, we found, as

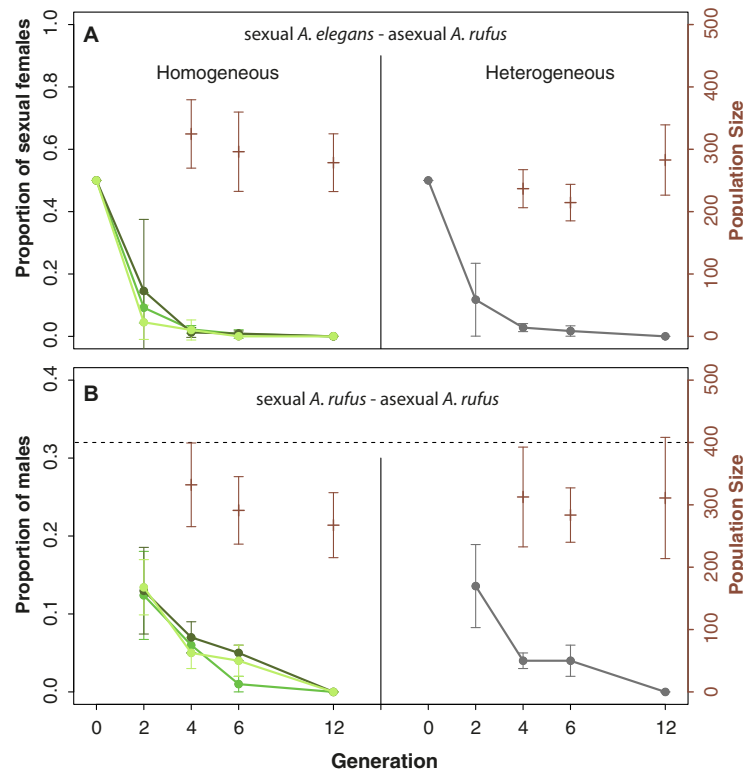


Figure 2. (A) Proportion of sexual *A. elegans* females and (B) proportion of *A. rufus* males in mesocosms across different generations; the dotted line in panel (B) indicates the proportion of males in laboratory populations of sexual *A. rufus* (i.e., the proportion of males corresponding to the fixation of sexual lineages). Generation 0 started with six asexual females and six mated sexual females in all mesocosms, $n = 10$ mesocosms per generation and mesocosm type. The proportions of males, respectively sexual and asexual lineages, were not determined for generations 8–10. Different colours in the homogeneous mesocosms indicate different grass species used in the homogeneous mesocosms (dark green: fescue, intermediate: wheat, light green: rye). Right axis: population size, as measured by the total number of *Aptinotrips* in the mesocosm. Error bars represent standard errors.

expected, that the average number of offspring females produced within 24 days increased after *ca.* two generations of selection (grass species: $df = 2$, $P < 0.0001$; generation: $df = 2$, $P < 0.0001$; Fig. 3A). The reproductive output increase after two generations was accompanied by a significant decrease in variation among females in offspring number on wheat and fescue (one-tailed Ansari–Bradley tests, $AB = 1478.5$, $P = 0.02$ and $AB = 1451$, $P = 0.05$, respectively) as expected if the frequency of genotypes poorly performing on a given plant decreased as a consequence of directional selection. A significant variation decrease was not observed on rye ($AB = 1749$, $P = 0.35$). In contrast to the improved reproductive output after two generations of selection in homogeneous habitats, we found no evidence for adaptation in the heterogeneous mesocosms (generation effect: $df = 2$, $P = 0.98$, Fig. 3A) as expected if adaptation to the new conditions was hampered by the presence of multiple plants (i.e., spatially fluctuating selection).

Sexual reproduction is expected to facilitate adaptation to new biotic and abiotic conditions. However, in contrast to asexual *A. rufus*, we found no evidence for adaptation to mesocosm

conditions in sexual *A. elegans*. The reproductive output of sexual females did not improve significantly after two generations in the mesocosms, neither for homogeneous (plant effect: $df = 2$, $P < 0.0001$; generation effect: $df = 1$, $P = 0.166$) or heterogeneous habitats (generation effect: $df = 1$, $P = 0.293$; Fig. 3B). No measurement of fitness was conducted past the second generation in *A. elegans* because we were unable to collect enough *A. elegans* females from the mesocosms at that point.

In summary, our results from the mesocosm experiments show that sexual lineages (*A. elegans* and *A. rufus*) are unable to persist when in competition with asexual *A. rufus* lineages in homogeneous habitats consisting of a single grass species and in heterogeneous habitats consisting of a mix of three species. However, although a mix of three grasses corresponds to a more complex habitat for thrips than a single grass species, a mix of three grasses may not be representative of natural populations, where typically more than three different grass species co-occur within a field. Therefore, the most relevant insights into the effect of habitat heterogeneity on the success of sexual thrips stem from diversity variation in natural populations.

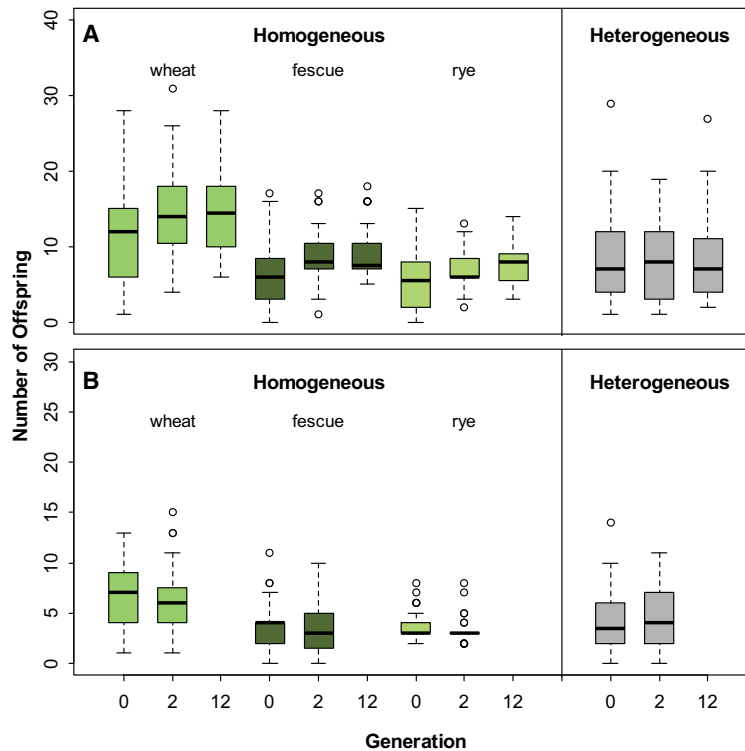


Figure 3. Fitness (number of offspring produced in 24 days) of asexual *A. rufus* (A) and of sexual *A. elegans* females (B) prior to the experiment (Generation 0) and after approximately two and 12 (for *A. rufus*) generations of adaptation to the new conditions.

In the natural setting represented by 66 plots in 11 hay meadows, 29 different grass species were inventoried, with an average of 8.6 (range 2–13) species per plot. The fertilization and irrigation treatments applied to the plots significantly changed grass diversity (LMM with meadow location as random effect: $P = 0.04$; Fig. 4A). The treatments also influenced the proportion of sexual thrips in each plot (GLMM, $\chi^2 = 26.62$, $df = 5$, $P = 0.00007$; LMM: $P = 0.0002$; Fig. 4B). However, contrary to the predictions, the frequency of sexual thrips was negatively affected by treatments increasing grass diversity (i.e., the level of habitat heterogeneity). This effect was most obvious when directly using grass diversity independently of fertilization and irrigation treatments, with a significant negative effect of the grass species diversity on the proportion of sexuals (GLMM $\chi^2 = 4.96$, $df = 1$, $P = 0.026$; LMM: $P = 0.011$, see also Supporting Information). This negative correlation was also observed within standard plots only ($R^2 = 0.46$, $P = 0.02$), indicating it was not solely driven by the shifts in grass communities following the irrigation and fertilization treatments.

Given the unexpected finding that the frequency of sexual thrips was negatively affected by increased grass diversities in the experimental plots, we examined the plots in which a high frequency of sexual thrips was maintained. Specifically, we examined whether plots with a high frequency of sexual thrips comprised specific grass communities, which would indicate

that sexual thrips only occur in a specific type of niche. To this end, we grouped plots by their grass species composition in a dendrogram, using Bray–Curtis distances to quantify the differences between plots. Mapping the proportions of sexual thrips on this dendrogram revealed that plots with similar frequencies of sexual thrips were also characterized by similar grass communities (Pagel’s $\alpha = 0.55$, $P < 0.0001$; Fig. 5A). Similarly, plots with high frequencies of sexual thrips tended to be clustered when the grass communities of the 66 plots were displayed along the first two axes obtained from a PCA (Fig. 5B).

In combination, the grass community analyses support the idea that sexual thrips occur in a specific type of niche. As a first step to characterize this niche, we identified the grass species that were the most strongly correlated with sexual thrips abundances. Only the grass species *Bromus erectus* was consistently associated with sexual *A. elegans* (see Supplementary Methods and Results and Tables S2 and S3 in the Supporting Information), with a strong correlation between the coverage of *B. erectus* in the plots and the proportion of sexual thrips ($R^2 = 0.58$, $P < 0.0001$).

Discussion

Explaining the overwhelming success of sexual reproduction has been a challenge in evolutionary biology for decades. Recent theoretical approaches have identified plausible mechanisms that

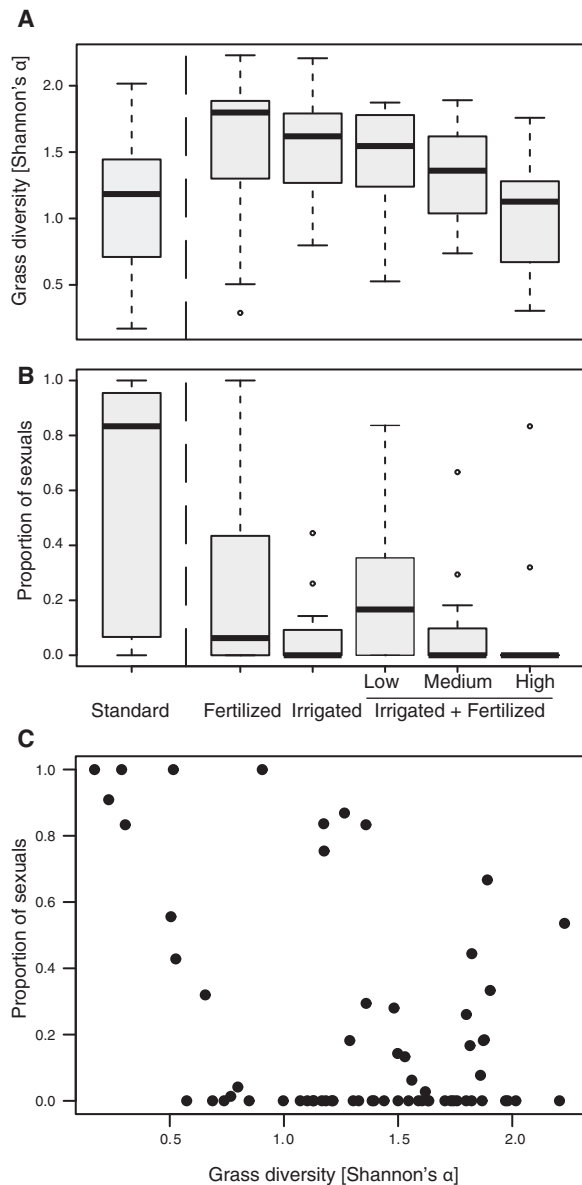


Figure 4. (A) Habitat heterogeneity (measured as grass diversity indices) among plots in experimental hay meadows exposed to fertilization and irrigation treatments. Combined irrigating and fertilizing treatments (irrigated + fertilized) were applied at three different intensity levels (low, intermediate, and high). (B) Proportion of individuals from sexual *Aptinothrips* species with respect to plot treatment. (C) Correlation between grass diversity and the proportion of sexual thrips independently of plot treatments.

could provide benefits to sex and therefore help explain its maintenance. For some of these mechanisms, experimental evolution has provided a “proof of principle” that the predicted mechanisms can work in real organisms given the appropriate conditions (Colegrave 2002; Goddard et al. 2005; Cooper 2007; Morran et al. 2009; Becks and Agrawal 2010; Morran et al. 2011; Becks and Agrawal 2012). However, these conditions may not be realized

in natural populations such that these approaches provide little insights into the maintenance of sex in nature. Indeed, it is impossible to know whether any benefit to sex detected under artificial conditions (that may include controlled migration rates, specific population densities or sizes) would outweigh its immediate costs expressed under natural conditions.

To fill this gap, we tested whether one of the classical conditions predicted to provide benefits to sex, habitat heterogeneity, can contribute to the maintenance of sex under natural conditions. By analyzing the frequency of sexual and asexual grasshoppers in experimental hay meadows, we found that an increase of habitat heterogeneity (measured via grass diversity) results in a decrease of sexual grasshoppers and favors asexual ones. The frequency of sexuals and grass diversity was also negatively correlated in unmanipulated grass communities. In seminatural mesocosm conditions, habitat heterogeneity did not affect the outcome of competition between sexual and asexual grasshoppers; asexual grasshoppers completely replaced their sexual relatives within 4–12 generations. In combination, these experiments show that the potential benefits that sex confers under habitat heterogeneity are negligible compared to its costs when sexual lineages compete with asexuals under natural or seminatural conditions.

Experimental evolution studies have also shown that sex can facilitate adaptation to new environments (Colegrave 2002; Goddard et al. 2005; Cooper 2007; Morran et al. 2009; Becks and Agrawal 2012). Our field and mesocosm experiments both involved a shift of environmental conditions. The treatments applied to the experimental hay meadows were initiated in spring 2010 and induced a gradual transition from the original to new plant communities (Andrey et al. 2014). Unexpectedly, asexual grasshoppers were more successful than sexual ones under these changing conditions. The mesocosm habitats also represented new conditions for the thrips. Yet in both competition assays, the one involving sexual *A. elegans* and asexual *A. rufus* collected from the field, and the one involving sexual and asexual *A. rufus* from laboratory populations, asexuals completely replaced their sexual counterparts. Part of the success of asexuals in the mesocosm experiments could stem from a colonisation advantage at the beginning of the experiment when only 12 individuals were present. However most mesocosms still contained sexuals when the carrying capacity was reached (by generation four), indicating that a colonization advantage of asexuals is unlikely to fully explain the results. For asexual *A. rufus* collected in the field, we further observed an increase of average fitness in two of the three homogeneous mesocosms, and a decrease in fitness variation among females, as expected under adaptation following directional selection. Surprisingly however, we did not find similar patterns for sexual *A. elegans*. A possible explanation for this pattern is that the response to selection in sexuals is slow, perhaps

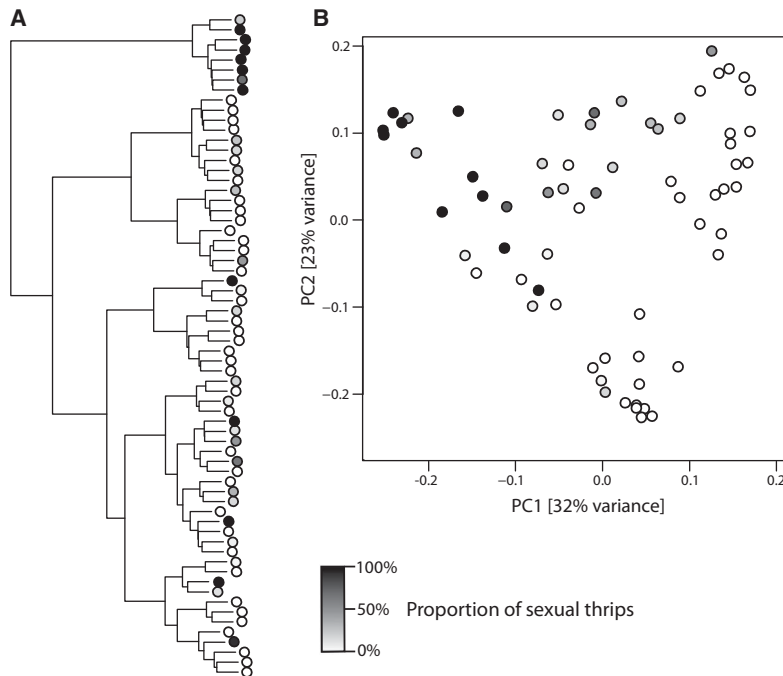


Figure 5. (A) Similarities of grass communities in the experimental plots displayed as a dendrogram. (B) Principal component analysis of the grass communities in the experimental plots. Plot labels are shaded proportionally to the frequency of sexual *Aptinothrips* species.

because of epistasis effects among loci involved in adaptation to different grasses, while genotype sorting in asexuals may happen more rapidly. However, the most likely explanation for these unexpected findings is that natural populations of asexual *Aptinothrips* are characterized by more standing genetic variation for adaptation to different host plants and that the sexual species *A. elegans* is specialized on one or a few grass species.

The same explanation could account for the unexpected success of asexual thrips in experimental plots with high grass diversity. As asexual thrips would be able to efficiently exploit many different species of grasses, and sexual thrips only few of them, the niche space available to asexual thrips would increase as grass diversities increase. Additional studies are required to infer the fundamental niche of the different sexual and asexual grass thrips lineages and their level of specialism vs generalism with respect to host plant use.

The proposition that increased genetic variation improves a lineage’s ability to exploit alternative niches is at the root of Tangled Bank related theories (reviewed in Bell 1982; Song et al. 2011). However, in contrast to our findings, Tangled Bank theories assume that sexual species, rather than asexual ones, would feature extensive genetic variation for exploiting alternative niches. Thus, these theories in fact propose that there is an advantage for genetically based diversity in niche usage; they do not directly show an advantage for sex. In systems where such diversity is larger in asexuals than sexuals, as appears to be the case in grass thrips, Tangled Bank mechanisms predict an advantage for

asexual species. Sexuals would then only persist in specific niches. Such niche-specific persistence might however be transient, pending colonization of that niche by a new set of asexual clones.

What mechanisms could generate genetic variation for niche usage in asexuals? Asexual *A. rufus* populations are distributed throughout the world (van der Kooi and Schwander 2014; Fontcuberta et al. 2016), and the consequently enormous population sizes may be sufficient for mutation, selection, and migration to generate clones adapted to different grass species. Furthermore, asexual *A. rufus* populations consist of a diverse mixture of clones, including clones that independently derived from sexual ancestors (Fontcuberta et al. 2016). Such high levels of clonal diversity may allow different clones to be selected on alternative plant species. More generally, given that populations of asexual “species” are often a mixture of different and independently derived clones (e.g., Neiman et al. 2005; Janko et al. 2008; Ros et al. 2008; Rabeling et al. 2011), the scope for “genetic diversity” based advantages of sex may be more limited in natural populations than typically assumed (e.g., Judson 1997; Ladle et al. 1993; Lively 2010; Neiman and Schwander 2011).

In summary, we maintained competing sexual and asexual thrips in spatially (and temporally) heterogeneous habitat conditions, conditions that have been shown to provide benefits to sex. In spite of this, sexual species decreased in frequency and, in the mesocosm experiments, went extinct. These findings indicate that habitat heterogeneity is unlikely to maintain sex in natural populations given the direct costs sex generates. The question, of

course, remains; whether sex in natural grasshrips populations is on the verge of extinction or whether sexual lineages are maintained by parasite-imposed selection or other factors unrelated to variation in grass composition over space or time. Answering these questions is a task for future studies.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Locations of experimental hay meadows.

Figure S2. Number of thrips of each species in the different treatments.

Figure S3. Distribution of F-values in the bootstrap LMM analyses.

Figure S4. Correlation between grass diversity and the proportion of sexual thrips separately for each hay meadow.

Table S1. Details on the treatments applied to the hay meadows used in the field experiment.

Table S2. Contribution of the different inventoried grass species to the structuring of plant communities.

Table S3. Contribution of the different inventoried grass species to the clustering of plant communities