



The evolution of sex : theoretical study based on quantitative genetics models ad experimental approach using the facultatively sexual rotifer *Brachionus plicatilis*

Eloïse Vanhoenacker

► To cite this version:

Eloïse Vanhoenacker. The evolution of sex : theoretical study based on quantitative genetics models ad experimental approach using the facultatively sexual rotifer *Brachionus plicatilis*. Reproductive Biology. Sorbonne Université, 2018. English. NNT : 2018SORUS347 . tel-02865511

HAL Id: tel-02865511

<https://theses.hal.science/tel-02865511>

Submitted on 11 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Thèse de doctorat de Sorbonne Université

Ecole doctorale 227 :

Sciences de la Nature et de l'Homme : Evolution et Ecologie

MNHN - Sorbonne Université

En vue de l'obtention du grade de

DOCTEUR DE SORBONNE UNIVERSITÉ

The evolution of sex: theoretical study based on quantitative genetics models and experimental approach using the facultatively sexual rotifer *Brachionus plicatilis*

Présentée et soutenue publiquement par

Eloïse Vanhoenacker

Le 13 Décembre 2018

MEMBRES DU JURY

Christine DILLMANN	Professeur, Université Paris Saclay	Rapporteur
Christoph HAAG	Directeur de recherche, CNRS Montpellier	Rapporteur
Emmanuelle PORCHER	Professeur, MNHN Paris	Examinatrice
Henrique TEOTÓNIO	Maître de conférence, ENS Paris	Examineur
Denis ROZE	Chargé de recherche, CNRS Roscoff	Directeur de thèse

Remerciements¹

Tout d'abord, je souhaite remercier Christine Dillmann et Christoph Haag d'avoir accepté d'être les rapporteurs de ce manuscrit, ainsi qu'Emmanuelle Porcher et Henrique Teotónio d'avoir accepté de faire partie de mon jury de thèse. Je souhaite également remercier María José Carmona et Eduardo García-Roger, à Valence, qui nous ont fait découvrir leur travail sur les rotifères, et qui (le plus important) ont accepté de nous envoyer des échantillons de sédiment pour initier nos populations de rotifères. Un grand merci à l'équipe BEDIM de m'avoir accueillie en son sein, et tout particulièrement à Denis, mon encadrant de thèse, qui a fait le pari de me faire confiance pendant trois ans, et qui a su m'accompagner au cours de ces trois années.

Bon, moins sérieusement, c'est quand même la fin de trois années de travail, le bébé est né, c'est le moment de se lâcher ! Denis, je suis fière d'avoir pu travailler avec toi pendant trois ans, tu es toujours calme, toujours bienveillant, toujours impliqué (que tu sois en week-end ou en vacances), et tu m'as permis de réaliser cette thèse dans les meilleures conditions possibles. J'ai appris énormément de choses, tu m'as initiée aux joies de la modélisation, à l'horreur qu'est d'essayer de faire un joli graphique sous Mathematica, et aux théories sur le sexe, avec de longues discussions qui te font sentir intelligent pendant les 5 minutes qui suivent (ça retombe vite en général...). Merci de m'avoir fait découvrir ces sales bêtes que sont les rotifères (au fond, on les aime bien, même si comme Elisa l'a si bien formulé, ils nous apprennent à repousser les limites de notre patience). En parlant de patience, merci à Jérôme de m'avoir tant de fois aidée à bosser avec ces petites bêtes, ce qui a considérablement réduit les heures passées à les transférer et les compter; et merci à Stéphane d'avoir génotypé nos individus, d'avoir passé des heures à relire, corriger, repasser nos plaques qui ne faisaient que s'empiler, malgré ta quantité de travail. Je voudrais également remercier Elisa et Océane, sans qui une grosse partie de ce travail n'aurait pas pu être réalisée.

Merci à Barbara et à toute l'équipe du pôle RH qui m'ont souvent grandement facilité la tâche dans toutes mes démarches administratives. Merci à la plateforme ABiMS qui s'occupe du Cluster, malgré quelques plantages, je l'ai tout de même

¹Cette thèse est garantie 100% sans caféine.

grandement sollicité au cours de ces trois années. Merci au service de laverie scientifique et à tous les services techniques (que l'on oublie que trop) sans qui notre travail serait bien moins facile.

Le plus difficile reste à venir, c'est là qu'on se rend compte à quel point c'est difficile de trouver les bons mots pour remercier les personnes auxquelles on tient². Une grosse pensée pour toi Laure, au moment où j'écris ces mots, tu es probablement dans le même état d'épuisement que moi (courage !). Ça a beau avoir commencé dans une tente, tu es l'une des personnes qui m'a le plus marquée ici. On a toutes les deux des forts caractères, et peu (pas) de filtre, mais c'est probablement parce que je t'aime le plus que c'est aussi le plus "explosif" (qui aime bien chatie bien il paraît). Merci de m'avoir écoutée, conseillée, d'avoir été là, dans tous les moments, les bons comme les moins bons, même quand je n'étais pas forcément très réceptive. Un énorme merci pour Alexis, avec qui j'aurais passé le plus de pauses clopes sans fumer. Tu as toujours été là pour me changer les idées, pour discuter de tout, sans tabous, sans jugements, avec qui j'ai eu d'énormes fous rires, tu as toujours réussi à nous motiver pour des soirées improvisées improbables (qui bizarrement finissent toujours de la même façon), merci d'être comme tu es. J'en profite aussi pour remercier Thibaut, même si tu es arrivé dans la famille plus tard, c'est comme si tu avais toujours été là. Obligée de remercier le Camtors' Café pour tous les bons moments qu'on y passe ! Après le départ d'Alexis j'ai gardé les mêmes habitudes, sauf que les pauses clopes sans clopes se sont transformées en pauses WTF dans votre bureau. Merci Camille d'avoir toujours été de bon conseil, de m'avoir écoutée râler tant de fois sans broncher, d'être toujours de bonne humeur et de faire passer les autres avant toi (Hufflepuff power!). Victor, aussi insupportable qu'adorable, malgré les apparences d'ours mal luné à peu près 87% du temps (on va pas se mentir), tu débordes de gentillesse et tu es toujours prêt à aider. J'en profite pour remercier Jean pour toutes ces discussions plus ou moins sérieuses, plus ou moins philosophiques, ainsi que Thierry et Christophe, vous savez rendre les pauses café toujours plus animées. Je voudrais remercier Marine et Ambre, qui ont partagé mon bureau pendant deux ans, et qui m'ont intégrée et fait rencontrer tout le monde; merci à Louise et Aurélien (aka Louis) qui ont pris leur suite. Bien que c'était sympa d'avoir un énorme bureau pour

² En aucun cas rangés par ordre d'importance !

moi toute seule, c'est toujours plus agréable quand on a des gens avec qui râler – euh, discuter. D'ailleurs, Louise, merci d'être toujours aussi gentille, pleine de générosité et d'enthousiasme, tu réussis à partager ta bonne humeur autour de toi et tu as su me redonner des élans de motivation quand elle était au plus bas. En parlant de motivation, merci à toi Eric, tu m'as motivée à me remettre au sport et m'a fait découvrir le volley (je suis presque accro maintenant). Tu es complètement taré (tu collectionnes les chaussettes et les chats quand même), mais c'est pour ça qu'on passe les meilleurs moments avec toi. Je n'oublie pas bien sûr tous les autres, la "team Ty Pierre" comme on pourrait simplement les appeler, merci à Ulysse, Damien (ouais mamène), Jeremy, Mathilde, Peche pour votre humour plutôt pas toujours très subtil, votre enthousiasme, votre joie de vivre. Merci à ceux qui sont partis, et à ceux qui restent. Merci aux équipes du Ty Pierre d'ailleurs, d'être toujours de bonne humeur, et d'accueillir tous les mercredi soirs des énormes tables de zinzins, joyeux certes, mais pas toujours très sortables.

Enfin, un énorme merci à ma famille qui me soutient tous les jours, qui est venue me voir aussi souvent que possible, malgré la distance, et qui croit toujours en moi dans n'importe quelle situation. Merci à Daphnée, d'avoir toujours été là pour moi, de jour comme de nuit, à m'écouter parler pendant des heures, à me donner des conseils, parce que tu me connais mieux que moi-même depuis 22 ans. Merci à toi Nico (pardon, Dr. Burghgraeve), de m'avoir accompagnée et soutenue au début de cette thèse, et toujours maintenant, ne change jamais ce que tu es. Et finalement, merci à Toi, de m'avoir supportée pendant la pire période dans la vie d'un thésard, de m'avoir remonté le moral, encouragée, changé les idées, parfois forcée à travailler, pour que je puisse enfin terminer ce travail et en être fière.

Contents

I	Introduction	5
II	Theoretical study based on quantitative genetics models	19
1	Stabilizing selection, mutational bias and the evolution of sex	21
2	Evolution of sex in temporally changing environments	41
2.1	Introduction	41
2.2	Simulation results	45
2.2.1	Linear change in optimum	45
2.2.2	Cyclical change in optimum	49
2.2.3	Stochastic change in optimum	50
2.3	Discussion	53
III	Experimental approach using the facultatively sexual rotifer <i>Brachionus plicatilis</i>	57
3	Preliminary experiments on the <i>Brachionus plicatilis</i> system	59
3.1	Monogonont rotifers as an experimental system to explore the benefits of sex	59
3.1.1	Morphology, life-cycle and reproduction	59
3.1.2	Variability in the propensity for sex and mixis initiation . .	64
3.1.3	Variability in dormancy and hatching cues	67

3.1.4	Monogonont rotifers as a model system to explore the benefits of sex	68
3.2	Preliminary experiments	70
3.2.1	Standard culture conditions	70
3.2.2	Isolation of strains	71
3.2.3	Tests on hatching rates	74
3.2.4	Genetic characterization of strains and reproductive system .	78
3.2.5	Effect of different forms of abiotic stress and of reproductive mode on fitness	84
4	Variability in the propensity for sex	91
4.1	Preliminary experiments on the propensity for sex in experimental rotifer populations	91
4.1.1	Setup of the chemostat system to maintain rotifer populations	91
4.1.2	Propensity for sex	94
4.1.3	Evolution of the genetic composition of experimental populations	99
4.2	Regulation of investment in sex in <i>Brachionus plicatilis</i>	102
4.2.1	Introduction	102
4.2.2	Material and methods	106
4.2.3	Results	111
4.2.4	Discussion	118
5	Adaptation of rotifers to stressful environments and the benefits of sex	123
5.1	Introduction	123
5.2	Material and Methods	127
5.2.1	Study system	127
5.2.2	Experimental populations	128
5.2.3	Fitness measures and microsatellite genotyping	132
5.2.4	Fitness of sexually produced individuals after adaptation . .	133
5.2.5	Statistical analysis	134
5.3	Results	135

5.4 Discussion	148
IV General discussion	153
LITERATURE CITED	165
Appendices	185
S1 Supplementary files from Vanhoenacker et al. (2018)	187
S2 Mean fitness and rate of sex at equilibrium when the optimum changes steadily over time ($a = 0.1$)	235
S3 Mean rate of sex at equilibrium when the optimum changes cycli- cally over time ($P < 10$ and $a = 1$)	241
S4 Composition of f/2 culture medium	245
S5 Protocol for species discrimination	249
S6 Composition of PCR mixes and PCR cycles for 11 microsatellite markers	253
S7 Multilocus genotypes of the clones for 11 microsatellite loci	257
S8 Proportion of new genotypes in experimental populations G and D over time	267
S9 Setup of the chemostats in the adaptation experiment	270
S10 Effect of treatment and reproductive origin on the variance in fitness of experimental populations	275
S11 Density of rotifers over time in the experimental populations.	279

Part I

Introduction

1 Theoretical hypotheses on the evolution of sex

The question of the evolution and maintenance of sexual reproduction has troubled evolutionary biologists for decades. Indeed, while sex is widespread in the eukaryotic kingdom, it comes with many costs: the cost of finding and attracting a mate, the risk of catching a sexually transmitted disease, an increased risk of predation while mating, etc. (see Lehtonen et al., 2012 for review), which impact the individuals directly. Moreover, in species with differentially sized gametes (anisogamous), a sexual female typically uses 50% of its resources to produce males, that generally do not provide any resource to their offspring, while an asexual female only produces females that transmit resources to their offspring. This generates a twofold cost for sexual reproduction (Maynard Smith, 1971). At the genetic level, sex and recombination can break apart possibly advantageous genotypes. By itself, sex is thus an evolutionary paradox.

From the end of the 19th century, it was argued that sex can be beneficial because it generates the variation necessary for natural selection to act (Weismann, 1889). However, sex does not always generate higher genetic variation, and when it does, it is often accompanied by a reduction in fitness (Otto, 2009).

Many theoretical studies tried to understand the possible benefits of sexual reproduction. Some of the first models explored the evolution of recombination (Kimura, 1956; Nei, 1967) or sex (Dolgin and Otto, 2003) modifier genes, but

found that genetic mixing never evolved. These models were considering infinite populations at equilibrium under selection alone (no mutation, no drift) in constant environments, and in such situations, sex and recombination are never favored (“reduction principle”, Feldman et al., 1997). Different models considered situations in which the environment changed, or with a continuous input of new mutations (mutation-selection balance). These models showed that a negative curvature of the fitness function (generating negative genetic associations between loci) in populations facing directional selection (Charlesworth, 1993; Barton, 1995) or populations at mutation-selection equilibrium (Feldman et al., 1980; Kondrashov, 1984; Charlesworth, 1990; Barton, 1995; Otto and Feldman, 1997) could favor the spread of modifiers increasing recombination or sex rates (Otto, 2003), because they break apart negative associations, and enhance the response to selection. Such a negative curvature of the fitness function can be caused by negative epistasis between loci, or dominance interactions between alleles (in diploids) (see Otto, 2009). Yet, in these models, the parameter space in which high rates of sex or recombination can evolve is rather restricted, due to the fact that the short-term effect of breaking genetic associations is to reduce the mean fitness of offspring (recombination or segregation load; Figure 1). Furthermore, they are often based on strong assumptions (*e.g.*, infinite populations, fixed selection), which are not necessarily realistic.

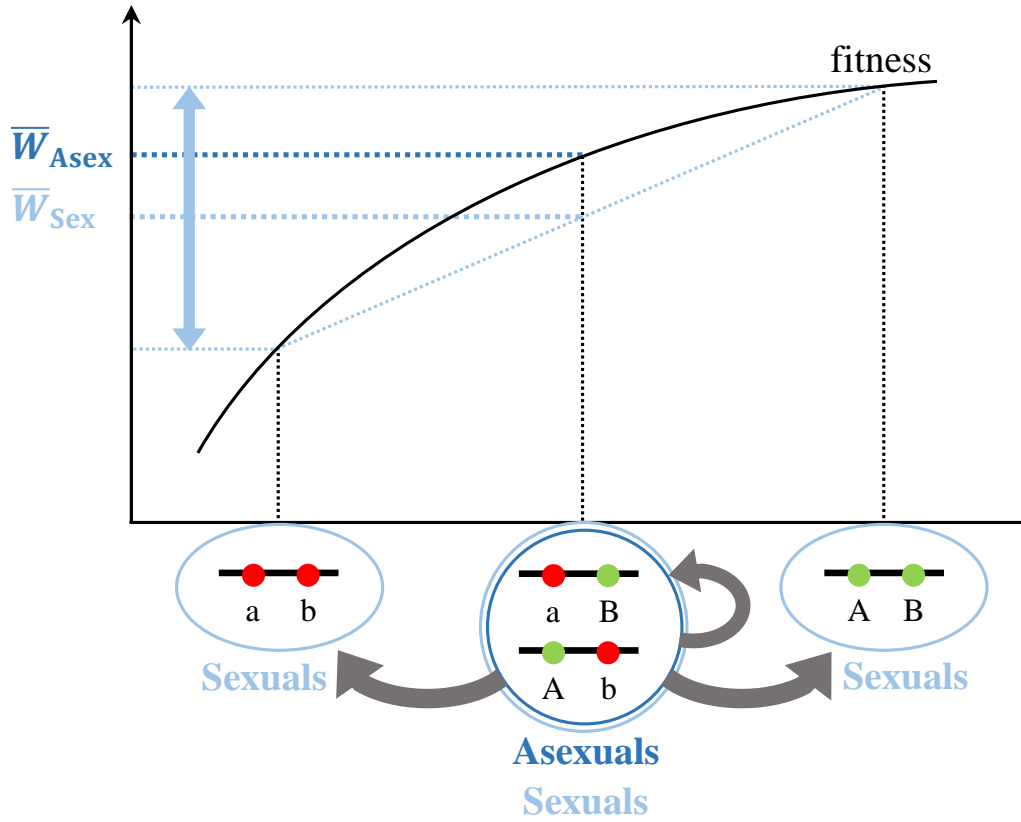


Figure 1: Short-term fitness effect of sexual reproduction. Haploid genotypes are represented by horizontal bars, where red and green dots represent deleterious and beneficial alleles, respectively. The initial population consists of aB and Ab genotypes. Asexual reproduction generates strictly identical genotypes, while sexual reproduction (grey arrows) will also generate ab and AB genotypes by recombination. The initial population harbors negative genetic associations (each beneficial allele is associated with the deleterious allele at the other locus), so sexual reproduction increases the variance in fitness among offspring. Although the average fitness of sexually produced individuals is lower on average ($\bar{W}_{SEX} < \bar{W}_{ASEX}$) due to the negative curvature of the fitness function (negative epistasis), the fittest genotype (AB genotype) is sexually produced. This genotype will increase in frequency, carrying along alleles that increase the rate of sex. Adapted from Roze (2012).

Finite population models have shown that stochastic effects may also play an important role in the evolution of sex or recombination. Indeed, finite population size tends to generate negative linkage disequilibrium between selected loci, that is to say that beneficial alleles are more often associated with deleterious alleles at other loci (Hill-Robertson effect, Hill and Robertson, 1966), slowing down the action of selection. Sex and recombination are beneficial because they break those negative combinations, restoring genetic variance and increasing the efficiency of selection. Simulation and analytical models showed that this effect favors modifiers increasing rates of sex or recombination (Felsenstein and Yokoyama, 1976; Otto and Feldman, 1997; Barton and Otto, 2005; Roze and Barton, 2006; Martin and Lenormand, 2006; Roze, 2014). Interestingly, individual-based simulation models have shown that sex and recombination may evolve over a broader range of epistatic interactions in finite population (epistasis needs not be necessarily negative; Otto and Barton, 2001; Keightley and Otto, 2006).

Another set of models have shown that sex can be favored when selection varies over time or space. Some authors have highlighted the importance of inter-specific interactions in the evolution of sex. The “Red Queen” hypothesis (Bell, 1982) stipulates that host and parasites are in a continuous arms race, and in this case, sexual reproduction is advantageous because it accelerates the evolution of one or the other species. In itself, this theory is not necessarily different from the

previous ones: in host-parasite coevolution, sex can accelerate adaptation due to the generation of negative linkage disequilibrium by negative epistasis or by Hill-Robertson effect. However, it has been shown that a different mechanism can favor recombination when selection fluctuates sufficiently fast, so that genotypes advantageous in a given generation are disadvantageous just a few generations later (fluctuating epistasis), in which case, recombination can increase the mean fitness in offspring. Various models have shown that this type of fluctuations can be generated by host-parasite coevolution (Peters and Lively, 1999; Gandon and Otto, 2007). Different models have explored the effect of spatial variation in selection, and showed that migration between locally adapted patches can also create genetic associations that favor sex and recombination (Pylkov et al., 1998; Lenormand and Otto, 2000; Agrawal, 2009).

Other authors have evoked the possibility that sex and recombination may be maintained among most eukaryotes because there might be some constraints to losing sexuality (*e.g.*, Charlesworth, 1989; Nunney, 1989). For instance, in daphnia, aphids or rotifers, sex leads to the production of resistant or dormant stages, which enable individuals to survive to detrimental environmental conditions. According to the “species selection argument”, only the species in which asexual mutants cannot occur due to some constraints (for example, due to the fact that it is not possible to produce a resting stage asexually) persist over evolutionary time, the

other species becoming asexual and eventually extinct. While such constraints may be an important element for the maintenance of sex in many groups, this hypothesis is rather difficult to test, and constraints are not always very well identified. For example, some populations of *Daphnia pulex* are able to produce resting eggs asexually (*e.g.*, Innes and Hebert, 1988).

Overall, theoretical studies have shown that sex and recombination may be favored under a number of possible realistic scenarios. However, to what extent these selective forces (either acting alone or in combination) can favor high rates of sex despite the strong costs mentioned above remains unclear. Multilocus simulations showed that selection for low rates of sex may be strong in fully asexual populations, while the evolution of high rates of costly sex seems more difficult to explain (Keightley and Otto, 2006; Roze, 2014). These simulations make strong assumptions on the genetic architecture of fitness, however (all mutations have the same effect, no epistasis or fixed epistasis between all pairs of loci), and it would be important to obtain results using more realistic models.

2 Experimental tests of the possible benefits of sexual reproduction

Experimental tests of the theories mentioned above remain scarce. An important component of these theories is the effect of sexual reproduction on the mean

and variance in fitness among offspring. While these effects are usually difficult to measure in natural populations, several authors obtained data from laboratory populations of various organisms.

Comparisons between recombinant and non-recombinant populations of *Drosophila melanogaster* showed that recombination enhanced the efficiency of selection, indicating that recombination increases the variance in fitness, but also had the immediate effect of reducing mean fitness (Charlesworth and Charlesworth, 1975; Rice and Chippindale, 2001). A similar effect of increased variance but decreased mean fitness was found in sexual populations of *Chlamydomonas reinhardtii* compared to asexual populations (Da Silva and Bell, 1996). However, as discussed above, sex needs not always increase the genetic variance in fitness. Indeed, several studies measured a decreased variance in fitness among sexually produced offspring (see Lynch and Deng, 1994; Allen and Lynch, 2008; Becks and Agrawal, 2011), reflecting the existence of positive genetic associations in these populations.

Evolution experiments have explored the effect of sexual reproduction on the speed of adaptation, either by examining the effect of one (Colegrave et al., 2002) or several episodes of sexual reproduction (Colegrave, 2002; Kaltz and Bell, 2002) during the adaptive phase, or by comparing the adaptation of populations regularly engaging in sex to that of purely asexual populations (*e.g.*, Goddard et al., 2005; Renaut et al., 2006). In the latter case, asexual populations usually contain muta-

tions preventing meiosis to occur, so that they can be placed in the sex-inducing environment like the sexual populations (ensuring that both types of populations always evolve in the same environmental conditions). Overall, these experiments showed that one episode of sex had the immediate effect of increasing genetic variance, and thus increased the rate of adaptation to new environments (Colegrave et al., 2002). Adaptation is also faster in populations with repeated episodes of sex than in asexual populations (Colegrave, 2002; Kaltz and Bell, 2002; Goddard et al., 2005; Renaut et al., 2006). Recently, McDonald et al. (2016) obtained genomic data from adapting sexual and asexual yeast populations, and showed that sexual reproduction decouples beneficial mutations from linked deleterious ones.

Although these experiments clearly show that adaptation tends to proceed faster in sexual populations, whether genotypes coding for higher rates of sex or recombination may be favored during adaptation remains unclear. To address this question, facultatively sexual organisms with genetically variable rates of sex are of particular interest. Becks and Agrawal (2010) investigated the evolution of the propensity for sex in experimental populations of monogonont rotifers (planctonic animals that reproduce both sexually and asexually), and found that higher rates of sex may evolve in populations living in a heterogeneous environment, compared to a homogeneous environment. In a more recent study, the authors observed an increase of the propensity for sex in populations adapting to a new environ-

ment, while the rate of sex decreases once the populations are adapted (Becks and Agrawal, 2012); the authors also showed that sex increases the variance in fitness among offspring during adaptation, while decreasing the mean fitness of offspring. Using similar experiments, Luijckx et al. (2017) showed that higher rates of sex evolve in populations facing more complex environmental changes. These different results show that indirect benefits (due to the production of new genotypes) may potentially compensate for the costs of sex in adapting populations; however, it is not always fully clear whether the observed increase in the rate of sex corresponds to a genetic rather than a plastic response.

3 Objectives of the present thesis

The aim of this thesis was to explore the evolutionary benefits of sex, using both a theoretical and an experimental approach. The theoretical part consisted in using quantitative trait models, in order to introduce more realistic genetic architectures involving distributions of epistatic effects among loci and possible compensatory effects between mutations (Part I, chapters 1 and 2). The experimental approach consisted in developing the facultatively sexual rotifer *Brachionus plicatilis* as a biological system to test for the possible benefits of sexual reproduction using experimental evolution approaches (Part II, chapters 3, 4 and 5). The thesis is structured as follows:

1. The first chapter presents a model of quantitative traits under stabilizing selection, used to explore the effect of mutational bias on the mean fitness of individuals and on the evolution of the rate of sex in a constant environment.
2. Using a simplified version of the model (presented in chapter 2), we combined stabilizing and directional selection on a single quantitative trait, to explore the effect of different forms of environmental change (linear, cyclical or stochastic) on the evolution of the rate of sex.
3. The third chapter presents the results of preliminary experiments performed on the *Brachionus plicatilis* system, exploring in particular the hatchability of resting eggs, characterizing the mode of asexual reproduction and measuring fitness in different environments.
4. The fourth chapter shows the results of a study exploring genetic variation for investment in sex between our strains of *B. plicatilis*, and demonstrating the existence of transgenerational maternal effects affecting the rate of sex of individuals.
5. Finally, the last chapter presents the results of an evolution experiment aimed at exploring the potential benefits of sex during adaptation to a new environment.

Part II

Theoretical study based on
quantitative genetics models

Chapter 1

Stabilizing selection, mutational bias and the evolution of sex

Stabilizing selection, mutational bias, and the evolution of sex*

Eloïse Vanhoenacker,^{1,2} Linnéa Sandell,³ and Denis Roze^{1,2,4}

¹CNRS, UMI 3614 Evolutionary Biology and Ecology of Algae, 29688 Roscoff, France

²Sorbonne Université, 29688 Roscoff, France

³Department of Zoology, University of British Columbia, Vancouver, BC V6T1Z4, Canada

⁴E-mail: roze@sb-roscoff.fr

Received April 19, 2018

Accepted June 20, 2018

Stabilizing selection around a fixed phenotypic optimum is expected to disfavor sexual reproduction, since asexually reproducing organisms can maintain a higher fitness at equilibrium, while sex disrupts combinations of compensatory mutations. This conclusion rests on the assumption that mutational effects on phenotypic traits are unbiased, that is, mutation does not tend to push phenotypes in any particular direction. In this article, we consider a model of stabilizing selection acting on an arbitrary number of polygenic traits coded by biallelic loci, and show that mutational bias may greatly reduce the mean fitness of asexual populations compared with sexual ones in regimes where mutations have weak to moderate fitness effects. Indeed, mutation and drift tend to push the population mean phenotype away from the optimum, this effect being enhanced by the low effective population size of asexual populations. In a second part, we present results from individual-based simulations showing that positive rates of sex are favored when mutational bias is present, while the population evolves toward complete asexuality in the absence of bias. We also present analytical (QLE) approximations for the selective forces acting on sex in terms of the effect of sex on the mean and variance in fitness among offspring.

KEY WORDS: Adaptive landscape, epistasis, evolutionary quantitative genetics, multilocus population genetics, reproductive systems.

Various possible evolutionary benefits of sexual reproduction have been proposed in order to explain the widespread occurrence of this reproductive mode among eukaryotes (e.g., Agrawal 2006; Otto 2009; Hartfield and Keightley 2012). These broadly fall into two categories: direct selective advantages of meiotic recombination, in particular in terms of DNA repair (e.g., Bernstein et al. 1985, 1988), or indirect benefits stemming from the disruption of linkage disequilibria and other forms of genetic associations through recombination and segregation. Breaking genetic associations affects the mean fitness of offspring when the fitness effect of alleles depends on the genetic background (dominance, epistasis); it may also affect the variance in fitness among offspring, and thus the response to selection. In the absence of dominance or epistasis and under random mating, stochastic events occurring in finite populations tend to generate negative genetic associations—

negative linkage disequilibrium between selected loci (Hill and Robertson 1966; Felsenstein 1974) and excess heterozygosity in diploids (e.g., Balloux et al. 2003). Breaking these negative associations increases the variance in fitness among offspring and the efficiency of natural selection, favoring higher rates of sex or recombination (Otto and Barton 1997, 2001; Barton and Otto 2005; Roze and Barton 2006; Martin et al. 2006; Roze and Michod 2010). Multilocus simulation programs showed that selection for recombination generated by such stochastic effects may be strong when sex is rare, but decreases rapidly as the baseline rate of sex in the population increases (Keightley and Otto 2006; Hartfield et al. 2010; Roze 2014).

Genetic associations may also be produced by deterministic forces: in particular, dominance and epistatic interactions between alleles affecting fitness are known to be widespread (e.g., de Visser and Elena 2007; Martin et al. 2007; Halligan and Keightley 2009; Manna et al. 2012), and represent another source of linkage disequilibria or deviations from Hardy–Weinberg

*This article corresponds to Clo, J. 2018. Digest: How mutational bias could explain the maintenance of sex. *Evolution*. <https://doi.org/10.1111/evo.13578>.

equilibrium. In randomly mating populations living in a constant environment, breaking associations generated by dominance or epistasis decreases the mean fitness of offspring (segregation or recombination load), generating a short-term cost for sex and recombination (Barton 1995; Charlesworth and Barton 1996; Otto 2003)—this short-term cost may turn into a short-term benefit when mating is nonrandom or when the environment changes in space or time (Lenormand and Otto 2000; Otto 2003; Roze and Lenormand 2005; Gandon and Otto 2007; Agrawal 2009). In a longer term, sex is generally beneficial when interactions cause a negative curvature of the fitness function (e.g., negative epistasis, partially recessive deleterious alleles), generating negative genetic associations that limit the efficiency of selection (Barton 1995; Otto 2003). Multilocus simulations including fixed epistasis between loci have suggested that epistatic interactions may only play a secondary role in the evolution of recombination, however, stochastic (Hill-Robertson) effects being often stronger (Otto and Barton 2001; Keightley and Otto 2006). Nevertheless, epistatic interactions are known to vary across pairs of loci (e.g., Phillips et al. 2000; de Visser and Elena 2007; Martin et al. 2007), and this variation (which should generally disfavor recombination, Otto and Feldman 1997) has not been considered in recent multilocus simulation studies on the evolution of sex and recombination.

Models of stabilizing selection acting on quantitative phenotypic traits represent a simple way of introducing distributions on epistatic interactions (on fitness), including possible compensatory effects between mutations (indeed, a mutation displacing a phenotypic trait away from the optimum can be compensated by another mutation having the opposite effect on the trait). Interestingly, the predicted distribution of fitness effects and epistatic interactions among mutations obtained from classical models such as Fisher's geometric model of adaptation with a Gaussian shaped fitness function have been shown to accurately describe empirical distributions of epistasis in bacteria and viruses (Martin et al. 2007), justifying the use of such models to explore the effects of the variance in epistasis. Selection for recombination under stabilizing, directional, or fluctuating selection acting on one or several polygenic traits has been explored by previous simulation models (Maynard Smith 1980, 1988; Kondrashov and Yampolsky 1996). They showed that while recombination is disfavored under stabilizing selection around a fixed optimum, environmental change may favor recombination. A mathematical analysis based on the infinitesimal model was proposed by Charlesworth (1993) (see also Appendix 2 in Barton 1995), showing that recombination increases the phenotypic variance by breaking negative genetic associations generated by epistatic interactions among loci, thereby increasing the speed of adaptation.

As in most evolutionary quantitative genetics models, the studies just mentioned assume unbiased mutational effects on

phenotypic traits: mutations are always as likely to increase as to decrease the value of a given trait. Several authors explored the effect of mutational bias on quantitative traits (e.g., Waxman and Peck 2003; Zhang and Hill 2008; Charlesworth 2013a, 2013b), and showed that such a bias may significantly reduce the mean fitness of populations in regimes where drift has substantial effects at loci coding for the traits, by displacing mean phenotypes away from their optimal values (thereby introducing a component of directional selection). Although the effect of mutational bias has only been explored in sexual populations, it should in principle be stronger in asexual populations, due to their reduced effective population size caused by interference effects between loci. This may generate selection for sex and recombination in the absence of environmental change.

In this article, we explore the effect of mutational bias in a simple, isotropic model of stabilizing selection acting on an arbitrary number of phenotypic traits, in a haploid, facultatively sexual population. We first assume a fixed rate of sex in the population, and show that mutational bias may strongly reduce the mean fitness of populations in which sex is rare or absent, provided that mutations affecting phenotypic traits have weak to moderate fitness effects. We then introduce genetic variation for the rate of sex, and show that the equilibrium rate of sex is an increasing function of the degree of mutational bias. Finally, we use the methods of Barton (1995) and Charlesworth and Barton (1996) to express different components of selection for sex in terms of the effect of sex on the mean fitness and additive variance in fitness among offspring, and show that these expressions provide correct predictions when selection is sufficiently weak.

Methods

LIFE CYCLE

The different parameters and variables of the model are summarized in Table 1. We consider a population of N haploid organisms with discrete generations. Each individual may generate a fraction of its offspring asexually (by mitosis), the remaining fraction being produced sexually. In the last case, gametes are produced by mitosis and fuse at random in the population to form zygotes, which immediately undergo meiosis to produce haploid juveniles. We will first consider that all individuals invest equally into sexual reproduction, the parameter σ representing the rate of sex in the population (proportion of sexually produced offspring): $\sigma = 0$ corresponds to obligate asexual reproduction, and $\sigma = 1$ to obligate sex. In a second step (described below), we will introduce genetic variation for the rate of sex. We assume that individuals are hermaphroditic (generating both male and female gametes) and produce very large (effectively infinite) numbers of juveniles, among which N are sampled randomly to form the next adult

Table 1. Parameters and variables of the model.

N	Population size
n	Number of selected traits
m	Degree of pleiotropy of mutations
$\rho = m/n$	Scaled pleiotropy
V_e	Environmental variance (on selected traits)
ω^2	Strength of stabilizing selection on phenotypic traits
$V_s = \omega^2 + V_e$	Strength of stabilizing selection on breeding values g_α
$W_{g,\max} = (\omega^2/V_s)^{n/2}$	Mean fitness of an optimal genotype
ℓ	Number of loci affecting selected traits
u	Mutation rate per locus per generation
$U = u\ell$	Overall mutation rate on loci affecting selected traits
R	Genome map length
a^2	Variance of mutational effects on selected traits
b	Mutational bias on selected traits
$\theta = b^2/(a^2 + b^2)$	Scaled mutational bias
$\langle X \rangle$	Expected value of X at mutation-selection-drift equilibrium
\bar{s}_d	Average deleterious effect of mutations on log fitness (in an optimal genotype)
z_α	Value of phenotypic trait α (in a given individual)
g_α, e_α	Genetic and environmental components of trait α
$g_{\alpha j}$	Effect of the allele present at locus j on trait α
\bar{z}_α	Average value of trait α (in the population)
$V_{g,\alpha}$	Genetic variance for trait α (variance of g_α)
$C_{g,\alpha\beta}$	Genetic covariance between traits α and β
$\mathcal{D}_{\alpha\alpha}, \mathcal{D}_{\alpha\beta}$	Effect of linkage disequilibria on $V_{g,\alpha}$ and $C_{g,\alpha\beta}$
$r_{\alpha j}$	Effect of allele 1 at locus j on trait α
p_j, q_j	Frequencies of allele 1 and allele 0 at locus j
s	Investment into sexual reproduction
c	Cost of sex
$\sigma = \frac{s}{c(1-s)+s}$	Rate of sex (proportion of sexually produced offspring among maternally produced offspring)
$\bar{\sigma}$	Mean rate of sex in the population
$V_{g,\sigma}$	Genetic variance for the rate of sex σ
ℓ_s	Number of loci affecting s
U_s	Mutation rate per generation on loci affecting s
a_s^2	Variance of mutational effects on s
$V_{e,s}$	Environmental variance on s

generation (note that hermaphroditic haploid individuals occur in some species of mosses, ferns, and algae).

Throughout the article, fitness W denotes the overall fecundity of an individual and depends on the values of n quantitative phenotypic traits under stabilizing selection, represented by the vector $\mathbf{z} = (z_1, z_2, \dots, z_n)$. In the following, we use greek letters $\alpha, \beta, \gamma, \dots$ to denote phenotypic traits, while latin letters i, j, k, \dots will denote loci. We assume that each phenotypic trait can be decomposed into a genetic and an environmental component:

$$z_\alpha = g_\alpha + e_\alpha \quad (1)$$

where g_α is the individual's genetic contribution to trait α ("breeding value"), and where the environmental effect e_α is independent of the genotype of the individual and is sampled from a Gaussian distribution with mean 0 and variance V_e (the same for all traits).

Average phenotypes and breeding values in the population are denoted \bar{z}_α and \bar{g}_α (with $\bar{z}_\alpha \approx \bar{g}_\alpha$ when the population is sufficiently large). As we assume no genotype \times environment interaction, the variance of trait α is given by:

$$V_\alpha = V_{g,\alpha} + V_e \quad (2)$$

where $V_{g,\alpha}$ is the genetic variance for trait α (variance of g_α). The genetic covariance between traits α and β (covariance between g_α and g_β) will be denoted $C_{g,\alpha\beta}$. Finally, $\langle X \rangle$ will denote the expected value of the quantity X at mutation-selection-drift equilibrium: for example, $\langle V_{g,\alpha} \rangle$ is the average genetic variance for trait α at equilibrium.

As we will see, some of our analytical results on the selective forces acting on the rate of sex do not depend on the specific shape of the fitness function. However, our simulation programs

and some of our approximations assume an isotropic, Gaussian-shaped fitness function around the phenotypic optimum, located at $\mathbf{z} = (0, 0, \dots, 0)$:

$$W = \exp\left[-\frac{\sum_{\alpha=1}^n z_{\alpha}^2}{2\omega^2}\right], \quad (3)$$

where ω^2 represents the strength of selection. The mean fitness associated with a given genotype (obtained by averaging over the distribution of environmental effects e_{α}) is given by:

$$W_g = W_{g,\max} \exp\left[-\frac{\sum_{\alpha=1}^n g_{\alpha}^2}{2V_s}\right] \quad (4)$$

where $V_s = \omega^2 + V_e$, and where $W_{g,\max} = (\omega^2/V_s)^{n/2}$ is the mean fitness of an optimal genotype (e.g., Lande 1976a).

GENETIC ARCHITECTURE OF TRAITS AND MUTATIONAL BIAS

We assume that selected traits are coded by ℓ loci with additive effects, so that

$$g_{\alpha} = \sum_{j=1}^{\ell} g_{\alpha j} \quad (5)$$

where $g_{\alpha j}$ is the contribution of the allele at locus j on trait α . Loci are assumed biallelic (although some of our results on the selective forces acting on sex are valid under more general architectures), the alleles at each locus being denoted 0 and 1. Assuming that an individual carrying allele 0 at all loci is at the phenotypic optimum, the contribution of locus j on trait α can be written as:

$$g_{\alpha j} = r_{\alpha j} X_j, \quad (6)$$

where X_j is an indicator variable equal to 1 if the individual carries allele 1 at locus j (while $X_j = 0$ otherwise), and $r_{\alpha j}$ is the effect of allele 1 at locus j on trait α (note that $r_{\alpha j}$ may be negative). The frequency of allele 1 at locus j is denoted p_j , while $q_j = 1 - p_j$. At each locus, we assume that mutation occurs at the same rate u in both directions (from 0 to 1 and from 1 to 0), while $U = u\ell$ denotes the mutation rate on the whole set of loci affecting selected traits. As in previous works (Chevin et al. 2010; Lourenço et al. 2011; Roze and Blanckaert 2014), we introduce a parameter m measuring the degree of pleiotropy of mutations: each locus only affects a subset m (sampled randomly and independently for each locus) of the n traits under selection. We assume that the distribution of $r_{\alpha j}$ over all loci affecting trait α has average b and standard deviation a — the same for all traits — without any covariance between mutational effects on the different traits. From equation (4), the average deleterious

effect of mutations on $\log W_g$ (in an optimal genotype) is given by:

$$\bar{s}_d = \frac{1}{\ell} \sum_{j=1}^{\ell} \sum_{\alpha=1}^n \frac{r_{\alpha j}^2}{2V_s} = \frac{m(a^2 + b^2)}{2V_s}. \quad (7)$$

The parameter b represents the degree of mutational bias, since mutation tends to displace mean phenotypes away from the optimum when $b \neq 0$. In the following, mutational bias will be measured using a scaled parameter θ , defined as $\theta = b^2/(a^2 + b^2)$ and varying between 0 and 1. For a given value of \bar{s}_d (mean fitness effect of mutations), θ will thus allow us to explore a continuum between two extreme situations corresponding to two classical models: $\theta = 0$ corresponds to Fisher's geometrical model without mutational bias, with a variance a^2 of mutational effects and possible compensatory effects among different mutations, while $\theta = 1$ corresponds to a situation where all mutations have the same fitness effect ($a^2 = 0$) and selection thus becomes directional (alleles 1 are disfavored), without any possible compensatory effect among mutations. Note that a^2 and b^2 are simply expressed in terms of \bar{s}_d and θ , as $a^2 = 2V_s(1 - \theta)\bar{s}_d/m$ and $b^2 = 2V_s\theta\bar{s}_d/m$. Furthermore, equation (4) indicates that the parameters a , b , ω^2 , and V_e should only affect changes in genotype frequencies through the scaled parameters $\tilde{a} = a/\sqrt{2V_s}$ and $\tilde{b} = b/\sqrt{2V_s}$, since genotypic fitnesses become independent of V_s when expressed in terms of the scaled phenotypic traits $\tilde{g}_{\alpha} = g_{\alpha}/\sqrt{2V_s}$. For a given choice of \bar{s}_d and θ , the results should thus not depend on ω^2 and V_e .

Using the parameters \bar{s}_d and θ (instead of \tilde{a} and \tilde{b}) will allow us to change the degree of mutational bias θ (between 0 and 1) while keeping the average fitness effect of mutations \bar{s}_d constant. This is equivalent to the approach used by Zhang and Hill (2008), in which the variance of mutational effects decreases as the degree of mutational bias increases in order to maintain a constant mutational variance V_M , defined as the per generation increase in phenotypic variance due to mutation (in our model, $V_M = \frac{m}{n} U(a^2 + b^2) = 2V_s \bar{s}_d U/n$). Finally, we can note that while the average coefficient of epistasis (on fitness) between mutations is zero in the absence of bias (e.g., Martin et al. 2007), it becomes negative when $\theta > 0$. Indeed, defining epistasis e as a deviation from additivity of mutational effects on $\log W_g$, we have (assuming that the number of loci ℓ is large):

$$\bar{e} = -\frac{2}{\ell(\ell-1)} \sum_{j \neq k} \sum_{\alpha=1}^n \frac{r_{\alpha j} r_{\alpha k}}{2V_s} = -2\rho\theta\bar{s}_d \quad (8)$$

with $\rho = m/n$. In the extreme case when $\theta = 1$ and $\rho = 1$ (all mutations have exactly the same phenotypic effect), epistasis becomes constant for all pairs of mutations and equals $-2\bar{s}_d$.

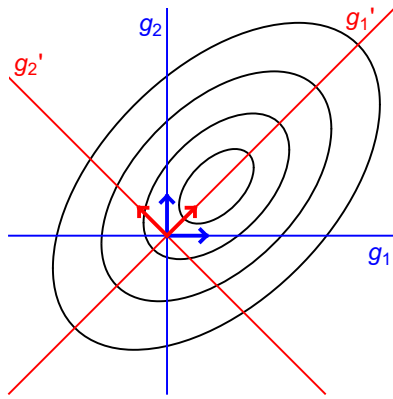


Figure 1. Effect of mutational bias, illustrated for $n = 2$ (the fitness optimum corresponds to the axes' origin). The black curves show the shape of the frequency distribution of individuals with different values of traits g_1 and g_2 (blue axes). Due to the symmetry of our model, mutational bias tends to displace \bar{g}_1 and \bar{g}_2 from their optimal values by the same amount, and generates a positive covariance among traits. Traits g_1' and g_2' are defined by rotating the phenotypic basis (eqs. 9 and 10, red axes) so that the covariance between g_1' and g_2' is zero, while mutational bias displaces phenotypes along the g_1' axis.

CHANGE IN PHENOTYPIC BASIS

Due to the symmetry of our model, average trait values and genetic variances at equilibrium should be the same for all traits, while mutational bias ($\theta > 0$) will tend to displace the mean phenotype of the population in the direction of the $(1, 1, \dots, 1)$ vector. Although the effects of mutations on the different traits are not correlated, mutation generates genetic covariances $C_{g, \alpha\beta}$ between traits in the population (of the same magnitude for all pairs of traits), since individuals carrying more 1 alleles in their genomes tend to lie further in the direction of the $(1, 1, \dots, 1)$ vector. For analytical derivations, it is useful to define a new phenotypic basis in which the average mutational bias lies along the first axis and in which the genetic variance-covariance matrix is diagonal, thus eliminating covariances between traits (see Fig. 1). This can be done by defining new breeding values g_{α}' as:

$$g_1' = \frac{1}{\sqrt{n}} \sum_{\beta=1}^n g_{\beta} \quad (9)$$

$$g_{\alpha}' = \frac{1}{\sqrt{(\alpha-1)\alpha}} \left[(\alpha-1)g_{\alpha} - \sum_{\beta=1}^{\alpha-1} g_{\beta} \right], \quad \alpha > 1 \quad (10)$$

(e.g., p. 380 in Anton 2005). The fitnesses of genotypes in the new basis are still given by equation (4), replacing g_{α} by g_{α}' . The average effect of mutations on $\tilde{g}_1' = g_1'/\sqrt{2V_s}$ is given by:

$$\tilde{b}_1' = \frac{1}{\ell} \sum_{j=1}^{\ell} \tilde{r}_{1j}' = \frac{1}{\ell} \sum_{j=1}^{\ell} \frac{1}{\sqrt{n}} \sum_{\beta=1}^n \frac{r_{\beta j}}{\sqrt{2V_s}}, \quad (11)$$

yielding:

$$\tilde{b}_1' = \sqrt{\rho \theta \bar{s}_d} \quad (12)$$

where again $\rho = m/n$ (note that eq. 8 may thus be written as $\bar{e} = -2\tilde{b}_1'^2$). Due to the mutational bias, \tilde{z}_1' will tend to be positive, while the genetic variance along the first axis ($V_{g,1}'$) will be larger than along the other axes (see Fig. 1).

GENETIC CONTROL OF THE RATE OF SEX

In order to explore the selective forces acting on reproductive mode, we will assume that a given individual may invest proportions s and $1-s$ of its resources in sexual and asexual reproduction (respectively), and that genetic variation for s exists in the population. As in previous articles (Roze and Michod 2010; Roze and Otto 2012; Roze 2014), we introduce a direct cost of sex c by assuming that the probabilities that an individual is the maternal parent of a juvenile through asexual and sexual reproduction are proportional to $1-s$ and s/c , respectively ($c = 1$ in the absence of cost, while $c = 2$ corresponds to a twofold cost of sex). This cost may be caused by anisogamy (cost of males): for example $c = 2$ when half of the resources invested in sex are used to produce male gametes, assuming that the same amount of resources is needed to produce a female gamete and an asexual spore. Alternatively, the cost may result from the failure of gametes to find a partner (assuming that a proportion $1-1/c$ of gametes are lost), or to extra energetic costs associated with gamete production compared with asexual spore production. The rate of sex σ of an individual is defined as the proportion of sexually produced individuals among its maternally produced offspring, given by:

$$\sigma = \frac{s}{c(1-s) + s} \quad (13)$$

($\sigma = s$ in the absence of cost). We assume that, like the other traits, investment in sex can be decomposed into an additive genetic and an environmental component:

$$s = \bar{s} + g_s + e_s \quad (14)$$

where \bar{s} is the average investment in sex in the population, $g_s = \sum_i g_{si}$ (g_{si} being the effect of the allele present at locus i on investment in sex) while e_s is sampled from a centered Gaussian distribution with variance $V_{e,s}$. These equations assume that the distribution of values of s in the population is not too close to 0 or 1 (otherwise the assumption of additivity may not hold, as s cannot be lower than 0 or higher than 1). As above, the variance in s in the population is given by $V_{g,s} + V_{e,s}$ (where $V_{g,s}$ is the variance in g_s). Throughout the article, we will assume that loci affecting investment in sex do not affect the traits under stabilizing selection.

Assuming that the variance in s in the population is sufficiently small, the rate of sex σ may also be decomposed into an additive genetic and an environmental component:

$$\sigma = \bar{\sigma} + g_{\sigma} + e_{\sigma} \quad (15)$$

where $\bar{\sigma}$ is the mean rate of sex. From equations (13) and (14) (and assuming that g_s and e_s are small, of order ϵ), we have:

$$\bar{\sigma} \approx \frac{\bar{s}}{c(1 - \bar{s}) + \bar{s}}, \quad V_{g, \sigma} \approx \frac{c^2}{[c(1 - \bar{s}) + \bar{s}]^4} V_{g, s} \quad (16)$$

(to leading order in ϵ) where $V_{g, \sigma}$ is the genetic variance for the rate of sex (variance of g_{σ}).

SIMULATION PROGRAMS

Our individual-based simulation programs (written in C++) are available from Dryad, and described in Supplementary File S1. The genome of each individual consists in a single linear chromosome with map length R (average number of cross-overs at meiosis). The ℓ biallelic loci affecting the n traits under stabilizing selection are equally spaced along the chromosome, each of these loci affecting a subset of m randomly sampled traits as described above. Investment in sexual reproduction s is coded by ℓ_s multiallelic loci (with an infinite number of possible alleles per locus), which are also equally spaced along the chromosome (see Fig. 2); assuming multiallelic loci ensures that all possible rates of sex between 0 and 1 may be achieved even when the number of loci affecting investment in sex is low. Mutational effects at these loci are sampled from a centered Gaussian distribution with variance a_s^2 (the mutational effect being added to the value coded by the allele before mutation). Investment in sex s is obtained by summing allelic effects at all these ℓ_s loci, and adding an environmental component drawn from a centered Gaussian distribution with variance $V_{e, s}$ (if the value obtained is lower than 0 or higher than 1, it is then set to 0 or 1). In a different version of the program the ℓ_s multiallelic loci do not affect investment in sex (which is fixed), but correspond to neutral loci that are used to estimate the effective population size N_e . For this, diversity at each of these neutral loci is computed as $D = 1 - \sum_i p_i^2$ (where p_i is the frequency of allele i), and the effective population size is estimated by $N_e \approx \bar{D}/[2\mu(1 - \bar{D})]$, where \bar{D} is the average diversity over neutral loci and generations, and μ the mutation rate at each neutral locus (generally fixed to 10^{-3}). Simulations with a fixed rate of sex generally lasted 10^5 generations, while simulations in which investment in sex was free to evolve lasted 2×10^6 generations (however the rate of sex generally reached an equilibrium within the first 5×10^5 generations).

Effect of Mutational Bias on Mean Fitness

In this section, we assume that the rate of sex σ is fixed, and explore the effect of σ and of mutational bias on mean fitness. The mutation load L measures the reduction in mean fitness of the population due to the presence of deleterious alleles, and is defined as:

$$L = 1 - \frac{\bar{W}}{W_{g, \max}}, \quad (17)$$

where \bar{W} is mean fitness and $W_{g, \max}$ the fitness of an optimal genotype. Throughout this section, we assume an isotropic, Gaussian-shaped fitness function (eq. 3). Assuming that the variance in log-fitness in the population is small and that population size is large, we have (see Supplementary File S2):

$$\langle L \rangle \approx 1 - \exp \left[-\frac{1}{2V_s} \sum_{\alpha=1}^n (\langle V_{g, \alpha} \rangle + \langle \bar{g}_{\alpha}^2 \rangle) \right]. \quad (18)$$

In the absence of mutational bias, the effect of deviations of mean phenotypes from their optimal values (the term in $\langle \bar{g}_{\alpha}^2 \rangle$ in eq. 18) is proportional to $1/N_e$, and should thus remain small when N_e is sufficiently large (Lande 1976b; Charlesworth 2013b). However, in the presence of mutational bias, drift may cause substantial deviations of mean phenotypes away from the optimum (Zhang and Hill 2008). Simple approximations for the load can be obtained in the regime where selection is negligible relative to drift at all loci. Assuming that the variance of \bar{g}_{α} due to drift is small, we have $\langle \bar{g}_{\alpha}^2 \rangle \approx \langle \bar{g}_{\alpha} \rangle^2$, while $\langle \bar{g}_{\alpha} \rangle = \sum_{j=1}^{\ell} r_{\alpha j} \langle p_j \rangle$ in our biallelic model. Using equation (12), and the fact that $\langle p_j \rangle = 1/2$ under symmetric mutation when the effect of selection at locus j is neglected, one obtains:

$$\frac{1}{2V_s} \sum_{\alpha=1}^n \langle \bar{g}_{\alpha}^2 \rangle \approx \frac{1}{4} (\ell \tilde{b}_1')^2 \quad (19)$$

where $\tilde{b}_1' = \sqrt{\rho \bar{\theta} \bar{s}_d}$ is the (scaled) magnitude of mutational bias (along the z_1' axis). Furthermore, linkage disequilibria between loci should be close to zero on average when selection is sufficiently weak, in which case the genetic variance for trait α is given by:

$$\langle V_{g, \alpha} \rangle \approx \sum_{j=1}^{\ell} r_{\alpha j}^2 \langle p_j q_j \rangle \quad (20)$$

(e.g., Lynch and Walsh 1998). Given that $\langle p_j q_j \rangle \approx Nu/(1 + 4Nu)$ at mutation-drift balance, one obtains from equation (20):

$$\frac{1}{2V_s} \sum_{\alpha=1}^n \langle V_{g, \alpha} \rangle \approx \bar{s}_d \frac{NU}{1 + 4Nu}, \quad (21)$$

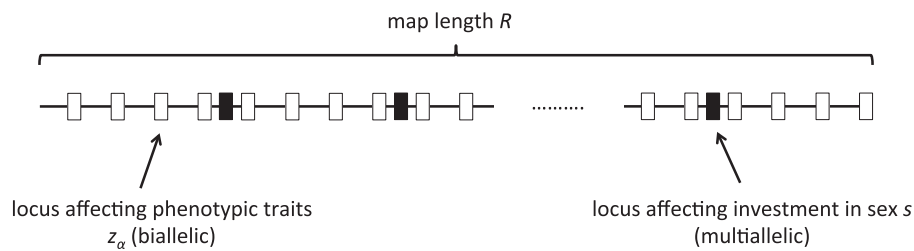


Figure 2. Simulated genetic architecture. Traits affecting fecundity are coded by ℓ biallelic loci uniformly distributed along a chromosome with map length R Morgans. Investment in sex is coded by ℓ_s multiallelic loci, which are also regularly spaced along the chromosome. When $\ell_s = 1$, the locus affecting investment in sex is located at the mid-point of the chromosome.

finally giving:

$$\langle L \rangle \approx 1 - \exp \left[-\bar{s}_d \frac{NU}{1 + 4Nu} - \frac{1}{4} (\ell \tilde{b}_1')^2 \right] \quad (22)$$

Equation (22) is equivalent to equation (8) in Roze and Blanckaert (2014) in the absence of mutational bias ($\tilde{b}_1' = 0$). It is expected to hold only when selection (measured by \bar{s}_d) is so weak that its effect on the distribution of trait values in the population is negligible. As \bar{s}_d increases, $\langle \bar{g}_\alpha \rangle$ and $\langle V_{g,\alpha} \rangle$ depart more and more from the expressions given above; however, simulations indicate that equation (21) stays valid over a wider range of values of \bar{s}_d than equation (19), in agreement with previous observations that selection may have significant effects on mean trait values even when $\langle p_i q_i \rangle$ at each locus is mainly controlled by mutation and drift (Robertson 1960; Campbell 1984; Barton 1989; Charlesworth 2013a). Based on this, it is possible to derive a better approximation for low \bar{s}_d by taking the effect of selection on $\langle \bar{g}_\alpha \rangle$ into account, while still neglecting the effect of selection on genetic variance (and neglecting linkage disequilibria). This yields (see Supplementary File S2 for derivation):

$$\langle L \rangle \approx 1 - \exp \left[-\bar{s}_d \frac{NU}{1 + 4Nu} - \frac{(\ell \tilde{b}_1')^2}{4 \left[1 + \frac{\bar{s}_d}{n} [1 + \theta(m-1)] \frac{N\ell}{1+4Nu} \right]^2} \right]. \quad (23)$$

Approximations for the regime where genetic variances are significantly affected by mutation, selection, and drift are more difficult to obtain. Under very strong selection against mutant alleles (so that the contribution to future generations of individuals deviating from the optimum can be neglected), the mutation load becomes (for both sexual and asexual populations):

$$L \approx 1 - e^{-U} \quad (24)$$

(e.g., Kimura and Maruyama 1966). Under sexual reproduction, equation (24) also holds under weaker selection in the absence of mutational bias ($\theta = 0$), as long as drift and linkage disequilibria may be neglected (e.g., Bürger 1998, Supplementary File S2). In the case of an asexual population, an expression for the load at mutation-selection balance (still in the absence of mutational

bias, and neglecting drift) can be obtained assuming a Gaussian distribution of trait values in the population:

$$L \approx 1 - \exp \left[-\sqrt{\frac{n}{2}} U \bar{s}_d \right] \quad (25)$$

(Lande 1980a; Roze and Blanckaert 2014). Generalizing these expressions to introduce mutational bias is not straightforward in the context of our biallelic model, as the degree of mutational bias changes depending on the position of mean phenotypes; however, previous studies have shown that the effect of mutational bias is generally small in regimes where drift is negligible (Waxman and Peck 2003; Zhang and Hill 2008). In Supplementary File S2, we show that a deterministic approximation for the load in a sexual population under the maximum level of bias ($\theta = 1$) is given by:

$$L \approx 1 - \exp \left[-\frac{4\rho U - \bar{s}_d + \sqrt{\bar{s}_d(8\rho U + \bar{s}_d)}}{8\rho} \right] \quad (26)$$

(see Supplementary File S2 for the same expression in terms of \tilde{b}_1' , \bar{s}_d , and U).

Figure 3 shows the equilibrium mutation load as a function of \bar{s}_d , for different rates of sex and levels of mutational bias. In the absence of mutational bias ($\theta = 0$), the load is generally higher in sexual ($\sigma = 1$) than in asexual ($\sigma = 0$) populations, due to the fact that recombination breaks combinations of alleles with compensatory effects (recombination load). This pattern reverses for high values of \bar{s}_d ($\bar{s}_d = 0.1$ in Fig. 3), as the frequency of deleterious alleles is increased by Hill-Robertson effects in asexual populations. While the effect of mutational bias (with $\theta = 0.1$) on the mean fitness of sexual populations stays modest, it greatly increases the load of asexual populations for small values of \bar{s}_d (between 10^{-5} and 10^{-3}) — see Figure S1 for results under stronger bias ($\theta = 0.5$). Figure S2 shows that this increase in L is caused by deviations of mean phenotypes from the optimum, due to the combined effects of mutational bias and drift. Indeed, Figure 3 shows that the effective population size of asexual populations (estimated from the average diversity at neutral loci, see Methods) is greatly reduced by background selection effects.

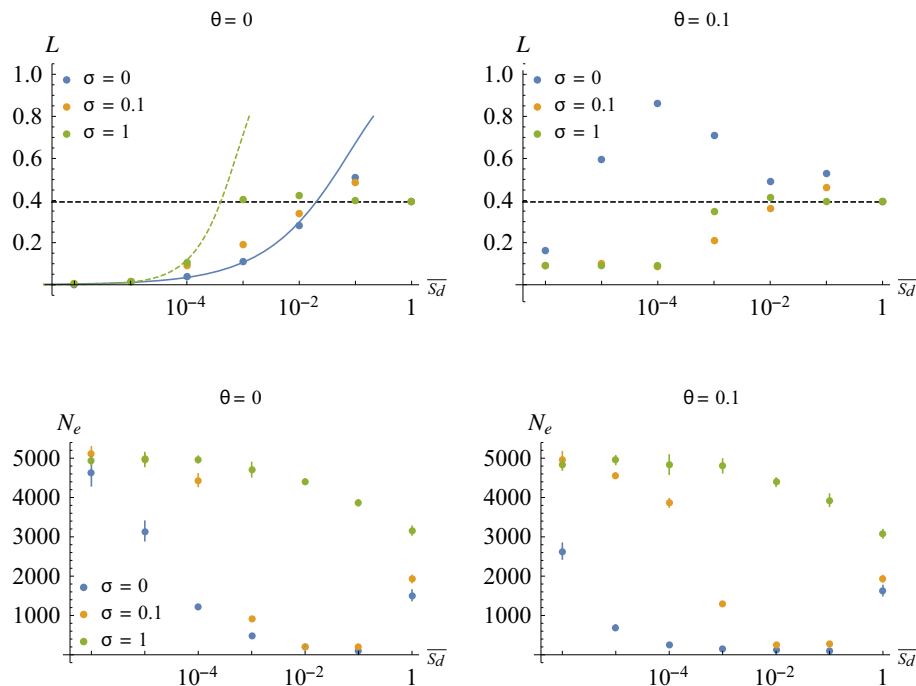


Figure 3. Top: average mutation load as a function of the mean fitness effect of mutations \bar{s}_d , for different rates of sex σ and different degrees of mutational bias θ . Dots: simulation results (note that all points are superposed for $\bar{s}_d = 1$). In this and the following figures, error bars (computed by splitting the last generations of the simulation into six batches of 10^4 generations and calculating the standard error over batches) are smaller than the size of symbols in most cases. The horizontal dashed line correspond to equation (24) ($1 - e^{-U}$), the green dashed curve to equation (22) and the solid blue curve to equation (25). Bottom: estimated effective population size N_e (see Methods) for the same parameter values. Parameter values are $N = 5000$, $U = 0.5$, $\ell = 10^4$, $n = 50$, $m = 5$, $R = 10$.

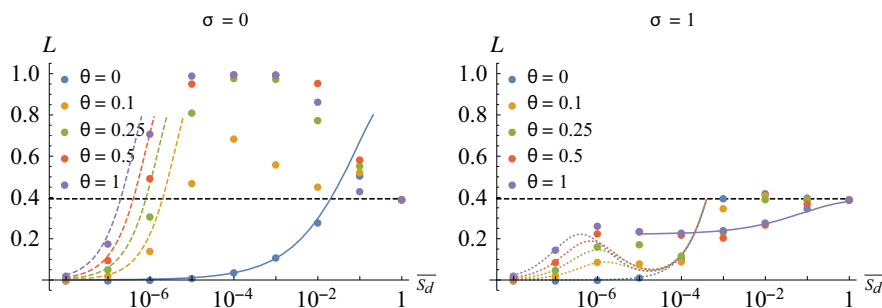


Figure 4. Average mutation load in asexual (left) and sexual (right) populations as a function of the mean fitness effect of mutations \bar{s}_d , for different degrees of mutational bias θ . The horizontal dashed lines correspond to equation 24 ($1 - e^{-U}$). Left: the colored dashed curves correspond to equation (22), and the solid blue curve to equation (25). Right: the dotted curves correspond to equation (23), and the solid curve to equation (26). Parameter values are as in Figure 3.

As shown by Figure 4, equation (22) correctly predicts the increase in load caused by mutational bias at very low values of \bar{s}_d , but rapidly overestimates L as \bar{s}_d increases, as it neglects the effects of selection (see Fig. S3 for the relative effects of genetic variance and of deviations of mean phenotypes from the optimum). In the case of sexual populations, equation (23) provides better predictions (dotted curves in Fig. 4) but still fails when \bar{s}_d is not very small, as it neglects the effect of selection on genetic variances. In agreement with previous results (Waxman and Peck 2003; Zhang and Hill 2008), we find that in sexual populations,

the effect of mutational bias stays rather small in the deterministic regime ($N\bar{s}_d \gg 1$). Very strong levels of bias ($\theta = 0.5, 1$) decrease the load in this regime, this effect being correctly predicted by our deterministic approximation for $\theta = 1$ (eq. 26): this is due to the fact that mutational bias generates negative epistasis (on average) between deleterious alleles (eq. 8), reducing the mutation load of sexual populations (e.g., Kimura and Maruyama 1966; Kondrashov and Crow 1988). Figure 5 shows that the effect of mutational bias increases as the number of loci ℓ increases (allowing stronger deviations from the fitness optimum) and as

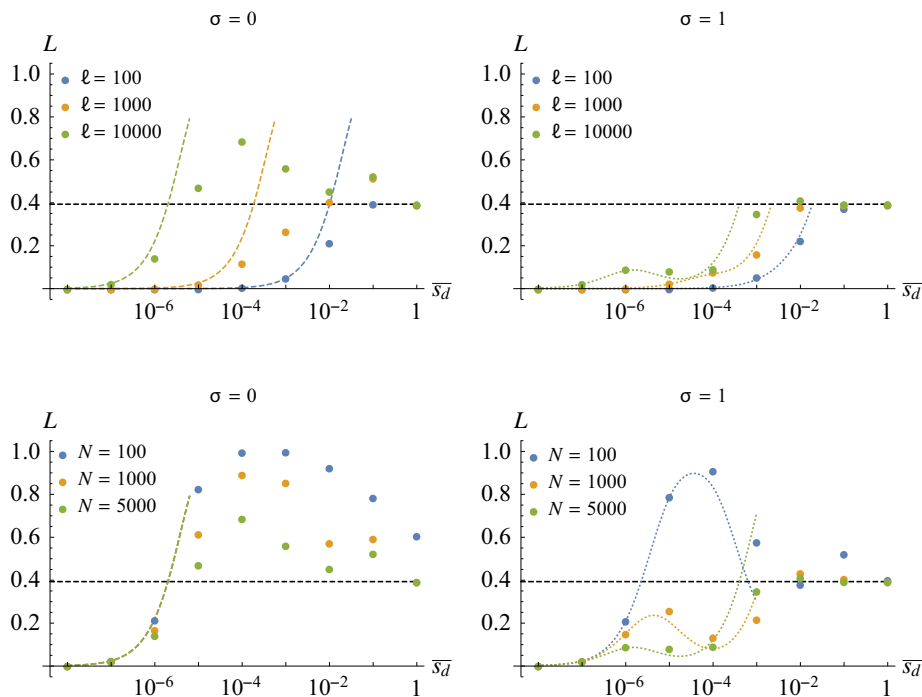


Figure 5. Average mutation load in asexual (left) and sexual (right) populations as a function of the mean fitness effect of mutations \bar{s}_d , for different numbers of loci ℓ affecting selected traits (top) and different values of population size N (bottom). The horizontal dashed lines correspond to equation (24) ($1 - e^{-U}$), the dashed curves to equation (22), and the dotted curves to equation (23). Parameter values are as in Figure 3, with $\theta = 0.1$.

population size decreases (see Figs. S4-S6 for results under stronger bias and for the relative effects of genetic variance and of deviations of mean phenotypes from the optimum on the load). The effects of the degree of pleiotropy of mutations m and of the total number of selected traits n are shown on Figure 6. The mutation load increases with the degree of pleiotropy (Fig. 6, top panels): indeed, the magnitude of mutational bias \tilde{b}_1' increases with $\rho\theta$ (with $\rho = m/n$, eq. 12). Increasing m while keeping $\rho\theta$ constant has only little effect on the load (Fig. 6, middle panels), confirming that m mostly affects the load through its effect on \tilde{b}_1' . Finally, Figure 6 shows that increasing n while keeping m/n (and thus \tilde{b}_1') constant has little effect on the load in sexual populations, while it increases the load of asexual populations due to stronger deviations of mean phenotypes from the optimum (see Figs. S7). Indeed, increasing the dimensionality n of the fitness landscape reduces the chances that a deleterious allele can be compensated by mutations at other loci, and thus enhances the effect of mutational bias in asexuals.

Overall, these results show that the combined action of mutational bias and genetic drift may greatly reduce the mean fitness of asexual populations when the average fitness effect of mutations is small to moderate, this increase in load being maximized for intermediate strengths of selection against deleterious alleles \bar{s}_d , higher values of pleiotropy m/n , number of selected traits n and number of loci ℓ , and for lower values of population size N . In

the next section, we will see how this translates into selection on modifier genes affecting the rate of sex of individuals.

Evolution of Sex

ANALYTICAL APPROXIMATIONS

Expressions for the effect of selection on the rate of sex are derived in Supplementary File S3, assuming weak selection, a Gaussian distribution of traits affecting fecundity (the z_α 's) and a low variance for the rate of sex in the population. Under these assumptions, the change in the mean rate of sex over one generation ($\Delta\bar{\sigma}$) can be decomposed into two terms, representing the effect of the cost of sex (direct selection), and indirect selection caused by the effect of sex on genetic associations between loci affecting fecundity:

$$\Delta\bar{\sigma} = \Delta_{\text{cost}} \bar{g}_\sigma + \Delta_{\text{ind}} \bar{g}_\sigma. \quad (27)$$

As shown in Supplementary File S3, $\Delta_{\text{cost}} \bar{g}_\sigma \approx \beta_{\text{cost}} V_{g,\sigma}$, where

$$\beta_{\text{cost}} \approx -\frac{c-1}{1+(c-1)\bar{\sigma}} \quad (28)$$

represents the direct selection gradient (selecting against sex when $c > 1$). Indirect selection in turn decomposes into two terms,

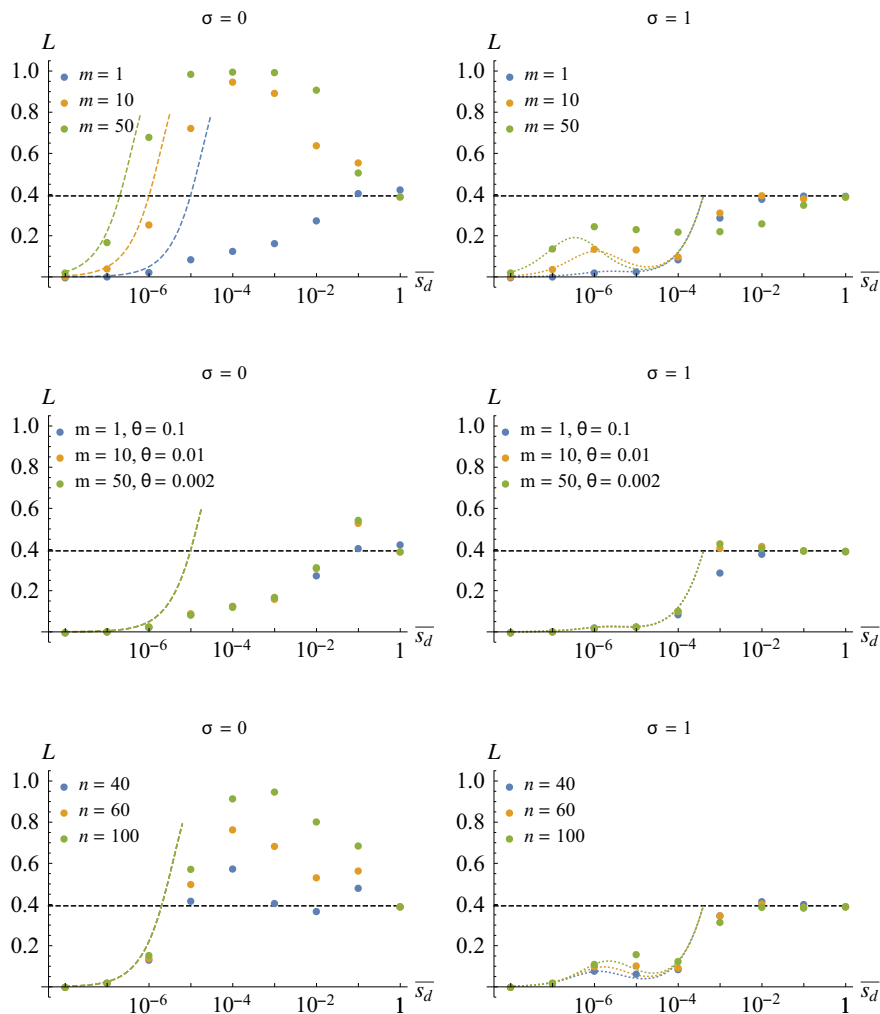


Figure 6. Average mutation load in asexual (left) and sexual (right) populations as a function of the mean fitness effect of mutations \bar{s}_d , for different degrees of pleiotropy of mutations m and numbers of selected traits n . In the middle panels, $m\theta$ is kept constant by decreasing θ as m increases, while m/n is kept constant in the bottom panels by increasing m as n increases (*i.e.*, $m = 4, 6$ and 10 when $n = 40, 60$, and 100 , respectively). The horizontal dashed lines correspond to equation (24) $(1 - e^{-U})$, the dashed curves to equation (22), and the dotted curves to equation (23). Parameter values are as in Figure 3 with $\theta = 0.1$ and $n = 50$ unless specified otherwise.

sometimes called the “short-term” and “long-term” effect of breaking genetic associations (e.g., Agrawal 2006):

$$\Delta_{\text{ind}} \bar{g}_\sigma = \Delta_{\text{short}} \bar{g}_\sigma + \Delta_{\text{long}} \bar{g}_\sigma. \quad (29)$$

The short-term effect is due to the fact that, in the presence of epistatic interactions, breaking genetic associations between loci affects the mean fitness of offspring. Under our isotropic fitness function (eq. 3), and assuming that phenotypes are measured in a basis that eliminates covariances between traits (the basis defined by eqs. 9 and 10), $\Delta_{\text{short}} \bar{g}_\sigma$ is given by:

$$\Delta_{\text{short}} \bar{g}_\sigma \approx \sum_{\alpha=1}^n \frac{\partial \ln \bar{W}}{\partial V_{g,\alpha}} M_{g,\sigma\alpha\alpha} \quad (30)$$

where $M_{g,\sigma\alpha\alpha}$ is the third moment $E[(g_\sigma - \bar{g}_\sigma)(g_\alpha - \bar{g}_\alpha)^2]$ (where E stands for the average over all individuals). A more general ex-

pression for arbitrary fitness function is given in the Appendix (see Supplementary File S3 for derivation). Under stabilizing selection, $\partial \ln \bar{W} / \partial V_{g,\alpha}$ is negative (mean fitness decreases as the genetic variance for selected traits increases). Furthermore, selection tends to generate associations (linkage disequilibria) between alleles at different loci with compensatory effects on selected traits, thereby reducing $V_{g,\alpha}$. By breaking these associations, sex increases the genetic variance among offspring: therefore, the genetic variance tends to be higher among individuals that engage more in sex (*i.e.*, with higher values of g_σ) than among individuals that engage less in sex, translating into a positive value of $M_{g,\sigma\alpha\alpha}$. The term representing the short-term effect ($\Delta_{\text{short}} \bar{g}_\sigma$) is thus negative, corresponding to the short-term cost of breaking genetic associations that have been generated by selection — one can show that this term is equivalent to the term in

δV_g in Charlesworth's (1993) recombination modifier model, see also Appendix 2 of Barton (1995).

The long-term effect stems from the fact that increasing the genetic variance among offspring allows a better response to directional selection, and can be written as:

$$\Delta_{\text{long}} \bar{g}_\sigma \approx \sum_{\alpha=1}^n \frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} C_{g,\sigma\alpha} \quad (31)$$

where $C_{g,\sigma\alpha} = E[(g_\sigma - \bar{g}_\sigma)(g_\alpha - \bar{g}_\alpha)]$ is the genetic covariance between the rate of sex σ and trait α . Equation (31) corresponds to the classical expression describing the effect of selection on correlated characters (Lande 1979): if selection favors higher values of trait α ($\partial \ln \bar{W} / \partial \bar{z}_\alpha > 0$), a positive genetic covariance between traits α and σ will lead to the evolution of higher values of σ . In our model, directional selection is caused by mutational bias displacing mean phenotypes from the optimum, and thus occurs along the first phenotypic axis of the basis defined by equations (9) and (10) ($\partial \ln \bar{W} / \partial \bar{z}_\alpha = 0$ along all other axes). Because sex increases the response to directional selection by increasing the genetic variance among offspring, trait values tend to be closer to the optimum in individuals that engage more in sex: $C_{g,\sigma\alpha}$ has the same sign as $\partial \ln \bar{W} / \partial \bar{z}_\alpha$, and $\Delta_{\text{long}} \bar{g}_\sigma$ is thus positive — this term is equivalent to the term in $\delta \bar{z}$ in Charlesworth (1993).

Charlesworth (1993) and Barton (1995) showed how the short-term and long-term effect can be expressed in terms of mean trait values and genetic variances for selected traits in a recombination modifier model, neglecting the effects of genetic drift on genetic associations and using a quasi linkage equilibrium (QLE) approximation. An equivalent derivation for the case of the present model is given in Supplementary File S3, the main results being summarized in the Appendix. For this, we assume an infinite population size, large number of loci affecting fecundity, weak selection and low variance for the rate of sex in the population; we also assume that the rate of sex is not too low (for the QLE approximation to hold). One obtains that $\Delta_{\text{short}} \bar{g}_\sigma \approx \beta_{\text{short}} V_{g,\sigma}$, $\Delta_{\text{long}} \bar{g}_\sigma \approx \beta_{\text{long}} V_{g,\sigma}$, where the short and long-term selection gradients are given by:

$$\beta_{\text{short}} \approx -\frac{1}{2V_s^2 r_{h,1} \bar{\sigma}^2} \left(\sum_{\alpha=1}^n V_{g,\alpha}^2 - \frac{\bar{z}_1^2 V_{g,1}^2}{V_s} \right), \quad (32)$$

$$\beta_{\text{long}} \approx \left(\frac{1}{r_{h,2} \bar{\sigma}} - \frac{1}{r_{h,1}} \right) \frac{1}{\bar{\sigma}^2} \frac{\bar{z}_1^2 V_{g,1}^2}{V_s^3}. \quad (33)$$

Equations (32) and (33) assume that traits are measured in the phenotypic basis given by equations (9) and (10), so that only the first phenotypic trait (with average \bar{z}_1 and genetic variance $V_{g,1}^2$) is under directional selection. The terms $r_{h,1}$ and $r_{h,2}$ that appear in the denominators of β_{short} and β_{long} correspond to harmonic mean recombination rates among loci. Defining r_{ijk} as the probability

that at least one recombination event occurs at meiosis between a locus i affecting investment in sex and loci j and k affecting selected traits, $r_{h,1}$ is the harmonic average of r_{ijk} over all possible triplets of loci i , j , and k , while $r_{h,2}$ is the harmonic average of $r_{ij} r_{ijk}$, where r_{ij} is the recombination rate between loci i and j . The maximum possible values of $r_{h,1}$ and $r_{h,2}$ (obtained for the case of freely recombining loci) are thus 3/4 and 3/8, respectively.

Equations (32) and (33) indicate that both the short-term and long-term selection gradients increase as the mean rate of sex in the population $\bar{\sigma}$ decreases, β_{long} increasing more rapidly (due to the term in $1/\bar{\sigma}^3$). However, both expressions diverge as $\bar{\sigma}$ tends to zero, due to the QLE approximation. Equation (33) also shows that the long-term effect vanishes in the absence of mutational bias ($\bar{z}_1 = 0$). The genetic architecture of investment in sex affects β_{long} and β_{short} through $r_{h,1}$ and $r_{h,2}$. Provided that the number of loci affecting fecundity is large and that their distribution over the genome is relatively uniform, the harmonic averages of r_{ijk} and $r_{ij} r_{ijk}$ over all j and k should be similar for all loci i affecting investment in sex, and the indirect selection gradient should thus be little affected by the number of loci coding for the rate of sex.

As we have seen in the previous section, it is difficult to obtain general analytical expressions for mean trait values (\bar{z}_1) and genetic variances ($V_{g,\alpha}$) at mutation-selection-drift equilibrium under mutational bias, for arbitrary values of \bar{s}_d and $\bar{\sigma}$, and we were thus not able to express the mean rate of sex in the population at equilibrium in terms of the different parameters of the model. One can note, however, that the approximations above for the short and long-term selection gradients can be expressed in terms of the effect of sex on the average and variance in fitness among offspring, that could (at least in principle) be measured from an experimental population (Barton 1995; Charlesworth and Barton 1996). Indeed, denoting \bar{W}_{sex} and \bar{W}_{asex} the mean fitness of sexually and asexually produced offspring (respectively), and $\text{Var}_{A,\text{sex}}(\ln W)$, $\text{Var}_{A,\text{asex}}(\ln W)$ the additive variance in log fitness among sexually and asexually produced offspring, we have (see Supplementary File S3):

$$\beta_{\text{short}} \approx \frac{\Delta_1}{r_{h,1} \bar{\sigma}}, \quad \beta_{\text{long}} \approx \left(\frac{1}{r_{h,2} \bar{\sigma}} - \frac{1}{r_{h,1}} \right) \frac{\Delta_2}{\bar{\sigma}} \quad (34)$$

with:

$$\Delta_1 = \ln \bar{W}_{\text{sex}} - \ln \bar{W}_{\text{asex}}, \quad (35)$$

$$\Delta_2 = \text{Var}_{A,\text{sex}}(\ln W) - \text{Var}_{A,\text{asex}}(\ln W). \quad (36)$$

Equations (34) – (36) are valid in principle for any shape of the fitness function (not necessarily Gaussian), as long as selection is sufficiently weak and the number of selected loci is sufficiently large. However, as the previous results, they assume that genetic

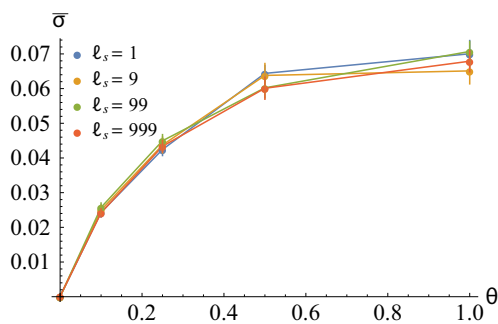


Figure 7. Mean rate of sex in the population at equilibrium as a function of the degree of mutational bias θ , for different values of the number of loci ℓ_s affecting investment in sex. Parameter values: $N = 5000$, $\bar{s}_d = 10^{-3}$, $n = 50$, $m = 5$, $\ell = 10^4$, $U = 0.5$, $R = 10$, $c = 1.2$, $U_s = 10^{-3}$, $a_s^2 = V_{e,s} = 5 \times 10^{-5}$, initial investment in sex: $s_{\text{init}} = 0.05$. In this and the following figures, error bars were computed by splitting the last generations of the simulation into 15 batches of 10^5 generations and calculating the standard error over batches.

associations remain small (QLE approximation), causing them to diverge as the mean rate of sex in the population tends to zero.

SIMULATION RESULTS

Figure 7 shows that, in agreement with the discussion above, the number of loci affecting investment in sex has very little effect on the mean rate of sex in the population ($\bar{\sigma}$) at equilibrium (the numbers 9, 99, and 999 were chosen so that the number of loci affecting fecundity between two loci affecting sex is 1000, 100, and 10, respectively — see Fig. 2). As shown by Figures 7–9, the population evolves toward asexuality in the absence of mutational bias ($\theta = 0$), while increasing the magnitude of mutational bias \tilde{b}_1' (by increasing either θ or m/n , see eq. 12) increases the equilibrium rate of sex. Higher rates of sex evolve under higher values of the mutation rate U , larger numbers of selected loci ℓ and lower values of population size N , due to stronger effects of mutational bias (Figs. 8 and 9). Similarly, increasing the dimensionality of the fitness landscape n while keeping m/n constant (so that \tilde{b}_1' stays constant) enhances the effect of mutational bias in asexuals (Figs. 6, S7), favoring higher rates of sex (Fig. 8). The mean fitness effect of deleterious alleles has a nonmonotonic effect on selection for sex, the equilibrium rate of sex being maximized for intermediate values of \bar{s}_d (Fig. 9). The genome map length R also has a nonmonotonic effect on the equilibrium rate of sex (Fig. 9): up to a certain point, increasing linkage favors sex since the long-term benefit of sex increases faster than the short-term cost as linkage becomes tighter (as can be seen from eqs. 32 and 33, and the fact that $r_{h,2}$ decreases faster than $r_{h,1}$ as recombination rates decrease). However, indirect selection vanishes when R tends to zero (since sex becomes genetically equivalent to asexual reproduction), in which case the rate of sex evolves toward zero

when sex is costly — Figure 9 shows that low rates of sex may be maintained in the population, probably due to hitchhiking effects between loci affecting investment into sex and loci affecting selected traits. Finally, Figure 8 shows that higher rates of sex are maintained in the absence of a direct cost of sex ($c = 1$), although the rate of sex still evolves toward zero when mutational bias is absent ($\theta = 0$).

Our simulation program was modified in order to test the validity of the QLE approximations shown above (eqs. 32–34) for different values of $\bar{\sigma}$. In this modified version, we introduce genetic variation for investment in sex but constrain $\bar{\sigma}$ to stay in a given range by sampling the value of alleles at loci affecting sex after mutation from a uniform distribution with variance a_s^2 , without adding the value of the allele before mutation. The short and long-term selection gradients were estimated from equations (30) and (31) (divided by $V_{g,\sigma}$), using equations (A2) and (A3) and measuring the moments \bar{z}_α , $V_{g,\alpha}$, $M_{g,\sigma\alpha}$, and $C_{g,\sigma\alpha}$ for all traits α . For this, the value of g_σ was estimated for each individual from the average rate of sex σ of 100 clonally produced offspring (all with different environmental components of investment in sex e_s), given by equation (13). The terms Δ_1 and Δ_2 of equation (34) were also measured every 100 generations by producing a pool of offspring by sexual reproduction and another pool by asexual reproduction, and measuring the mean fitness and additive variance in log fitness within each pool of offspring. The additive variance in log fitness was estimated from the covariance in log fitness $\text{Cov}(\ln W)$ between sexually (or asexually) produced offspring and their own sexually produced offspring, using $\text{Var}_A(\ln W) = 4\text{Cov}(\ln W) - \text{Var}(\ln W)$ (Lynch and Walsh 1998, Supplementary File S3). Figure 10 shows that the QLE approximation provides correct predictions of the indirect selection gradients when selection is sufficiently weak ($\bar{s}_d = 10^{-4}$, for the parameter values used in Fig. 10) and for intermediate rates of sex (while the QLE expressions diverge as $\bar{\sigma}$ approaches zero). Discrepancies appear for $\bar{s}_d = 10^{-3}$, however, and become more important for $\bar{s}_d = 10^{-2}$. These discrepancies are probably due to a breakdown of the different assumptions used to derive equations (32)–(36) (e.g., weak genetic associations, negligible effect of associations involving more than 2 or 3 loci, distribution of breeding values close to a Gaussian distribution), and possibly also to the effect of drift on genetic associations (through the Hill-Robertson effect), which is not taken into account in our analysis.

Discussion

Epistasis and drift are the two major sources of genetic associations that have been considered in theoretical studies on the benefits of sex and recombination. Epistasis may favor recombination when it is negative on average, that is, when the fitness effect of a deleterious allele is increased by the presence

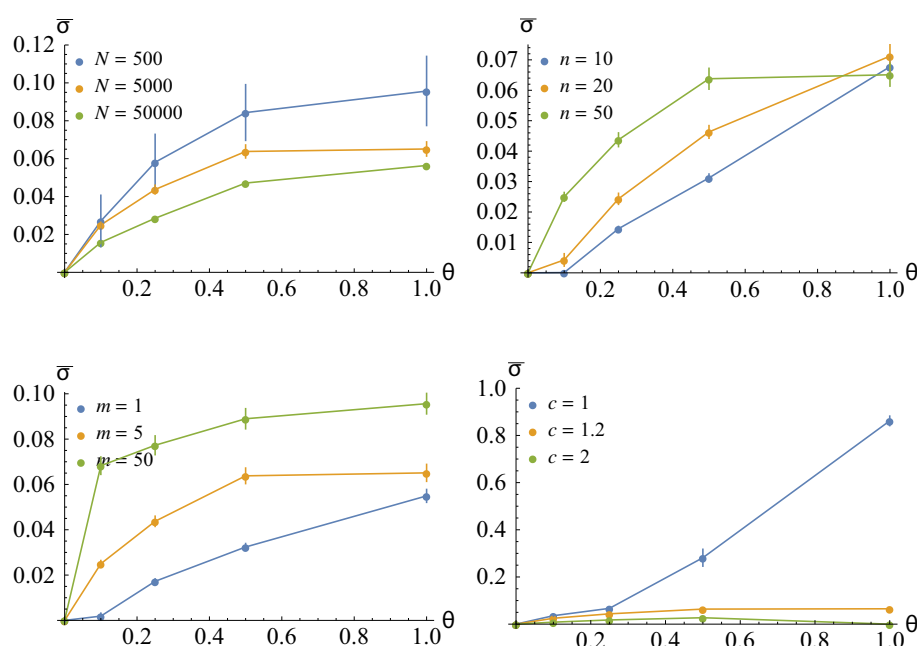


Figure 8. Mean rate of sex at equilibrium as a function of the degree of mutational bias θ , for different values of population size N , number of selected traits n , degree of pleiotropy m and cost of sex c . Parameter values are as in Figure 7 with $\ell_s = 9$ unless specified otherwise. The m/n ratio is kept constant (and equal to 0.1) in the panel showing results for different values of n (top right panel), i.e., $m = 1, 2$ and 5 for $n = 10, 20$ and 50 , respectively.

of other deleterious alleles at other loci, or conversely when the fitness effect of a beneficial allele is decreased by the presence of other beneficial alleles in the genome. However, epistatic interactions also generate a short-term cost for recombination (since recombinant offspring tend to have a lower mean fitness than their parents in a constant environment), so that high rates of recombination can only be favored when epistasis is weak relative to the strength of selection, and not too variable across loci (Barton 1995; Otto and Feldman 1997). Epistatic interactions (on fitness) arise naturally in models of selection acting on quantitative phenotypic traits. In agreement with the results mentioned above, Gaussian (or quadratic) stabilizing selection around a fixed optimum in an infinite population is expected to disfavor recombination in the absence of mutational bias (Charlesworth 1993). Indeed, at equilibrium the mean phenotype of the population is centered on the optimum, in which case epistasis between deleterious alleles is zero on average, with a given variance (Martin et al. 2007)—epistasis between two alleles displacing the phenotype in the same direction is negative (due to the negative curvature of the fitness function), while epistasis between alleles having opposite (compensatory) effects on the phenotype is positive. Away from the optimum, epistasis between deleterious alleles is negative on average (while epistasis between beneficial alleles is also negative, e.g., Martin et al. 2007), generating a deterministic advantage for recombination (Charlesworth 1993).

Our simulation results confirm that, in the absence of mutational bias on phenotypic traits, populations evolve toward obligate asexuality when the phenotypic optimum remains constant over time, even when population size is finite. This stands in contrast with previous simulation results assuming fixed epistasis across loci (always negative or always positive), that found only minor effects of epistasis compared with the stochastic (Hill-Robertson) effects that favor recombination in initially asexual (or nonrecombining) populations (Keightley and Otto 2006). When mutational bias is included in the model, however, positive rates of sex are maintained in the population at equilibrium. Indeed, mutational bias tends to displace mean phenotypes away from the optimum (thereby increasing the mutation load), this effect being stronger in asexual populations in which the variance in fitness may be greatly lowered by negative associations between loci, reducing their ability to respond to directional selection. Extending Barton's (1995) QLE analysis to our model, we obtained deterministic approximations for the short- and long-term indirect selection gradients acting on sex in terms of mean trait values and genetic variances, and showed that these approximations provide reasonable predictions when selection acting at the different loci is sufficiently weak and when the rate of sex is not too low (Fig. 10). This implies that, in this parameter range, selection for sex is mainly driven by negative linkage disequilibria caused by epistasis (although drift may play a significant role by increasing the distance between the mean phenotype and the

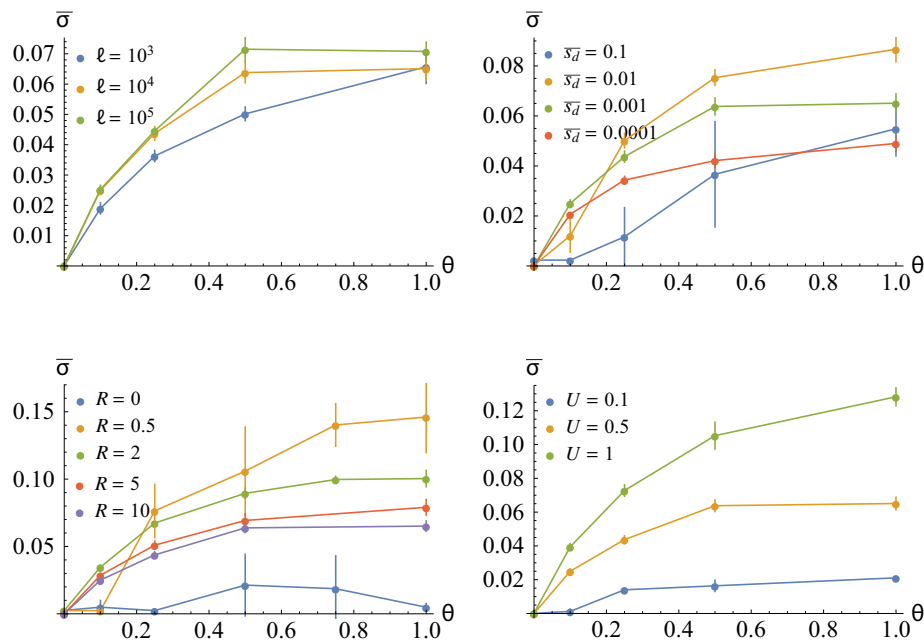


Figure 9. Mean rate of sex at equilibrium as a function of the degree of mutational bias θ , for different values of the number of selected loci ℓ , average deleterious effect of mutations \bar{s}_d , genome map length R and overall mutation rate on selected traits U . Parameter values are as in Figure 7 with $\ell_s = 9$ unless specified otherwise.

optimum, and therefore the magnitude of directional selection). The Hill-Robertson effect may become more important in parameter ranges where the QLE approximation fails (strong selection and/or low rate of sex); however, the lack of suitable analytical method to cover such regimes makes it difficult to assess its relative effect.

Recent experimental evolution studies showed that higher rates of sex or outcrossing may evolve in populations adapting to a new environment or coevolving with a pathogen, possibly through the generation of advantageous genotypes by recombination and segregation (Becks and Agrawal 2010, 2012; Morran et al. 2011; Luijckx et al. 2017). In adapting populations of monogonont rotifers, Becks and Agrawal (2012) showed that sexually produced offspring tend to have a lower mean fitness and a higher variance in fitness than asexually produced offspring, in agreement with predictions from models with concave fitness functions such as the one used in this article. However, how to relate the effect of sex on the mean and variance in fitness of offspring with the strength of indirect selection for sex is not immediately obvious. Transposing Barton's (1995) and Charlesworth and Barton's (1996) analysis of recombination modifier models to our sex modifier model, we showed that simple relations exist between the short- and long-term selection gradients for sex and the effect of sex on the fitness of offspring (eqs. 34–36). However, several important caveats must be noted: (i) these relations only hold in the QLE regime, and thus break down when the rate of sex in the population is low; (ii) they depend on average recom-

bination rates between loci affecting fitness and loci affecting the rate of sex (through $r_{h,1}$ and $r_{h,2}$ in equation 34), which are generally unknown (although lower bounds for selection gradients can be obtained by replacing these terms by their values under free recombination, that is $r_{h,1} = 3/4$ and $r_{h,2} = 3/8$); (iii) the long-term selection gradient is expressed in terms of the effect of sex on the *additive* variance in fitness among offspring, which will generally be more difficult to measure than the variance in fitness. Nevertheless, estimations of the effect of sex on the mean and variance in fitness among offspring still convey important information on the existence and sign of short- and long-term selection gradients on sex (e.g., Peters and Otto 2003; Sharp and Otto 2016).

For a given genomic mutation rate U , our model predicts that increasing the dimensionality of the fitness landscape n increases selection for sex (Fig. 8). Indeed, the variance of epistasis between mutations decreases as n increases (Martin et al. 2007), epistasis vanishing as n tends to infinity, since mutations become orthogonal in this limit (without any possible compensatory effect). In other words, strong epistatic interactions (in particular, compensatory effects between deleterious alleles) are more likely to occur when the dimensionality of the fitness landscape is low, and these strong interactions tend to favor asexual reproduction (that can maintain coadapted multilocus genotypes). However, we can note that our model assumes that all loci have the same probability of affecting any trait: under a more modular genetic architecture where different sets of loci affect different sets

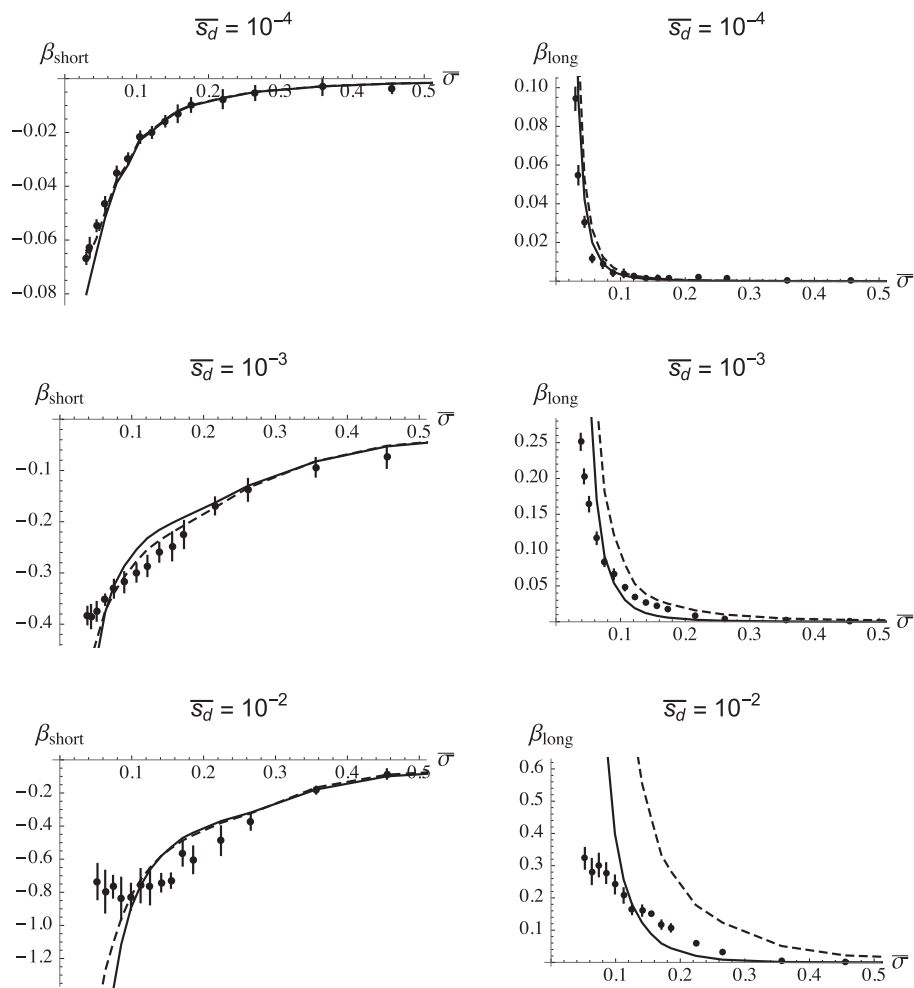


Figure 10. Short- and long-term selection gradients for sex as a function of the mean rate of sex in the population, for different values of $\overline{s_d}$. The dots show β_{short} and β_{long} estimated using equations (30) and (31) (divided by $V_{g,\sigma}$) and equations (A2) – (A4). Solid curves correspond to equations (32) and (33) (using the values of $\overline{\sigma}$, $\overline{\alpha}$, and $V_{g,\alpha}$ measured in the simulations), and dashed curves to equations (34) – (36) (where Δ_1 and Δ_2 are measured in the simulations as explained in the main text). Parameter values are as in Figure 7 with $\ell_s = 9$ and $\theta = 0.1$, leading to $r_{h,1} \approx 0.66$ and $r_{h,2} \approx 0.13$.

of traits (modular pleiotropy, e.g., Welch and Waxman 2003; Chevin et al. 2010; Chebib and Guillaume 2017), the magnitude of epistatic interactions may be more dependent on the average number of traits coded by a given module than on the total number of selected traits, which may lead to different results. In general, the range of realistic values for the dimensionality of fitness landscapes remains difficult to assess: while a large number of traits in an organism may be under selection, many of those traits are probably correlated, reducing the effective dimensionality of the landscape (Martin and Lenormand 2006). In VSV and ϕ X174 viruses, the effective number of selected traits was estimated to be around 10 and 45 (respectively) based on predictions from Fisher's geometric model on the relation between N_e and population mean fitness (Tenaillon et al. 2007; Lourenço et al. 2011), but this number may be much higher in multicellular eukaryotes.

As we have seen, mutational bias is required for sex to be favored in a constant environment. Some evidence for mutational bias on quantitative traits has been obtained from *Drosophila* and *Caenorhabditis elegans* (e.g., Santiago et al. 1992; Lyman et al. 1996; Keightley and Ohnishi 1998; Ostrow et al. 1997; García-Dorado et al. 1999); however, how to relate these data with the parameter θ measuring bias in our model is not immediately obvious. In particular, a downward mutational bias is often observed on traits that may be seen as fitness components, but such a bias is expected in our model at the optimum even when $\theta = 0$ (since fitness can only decrease at an optimum). Traits that have a less direct relation with fitness sometimes show mutational bias (e.g., metabolite pool size, Davies et al. 2016), sometimes not (e.g., mitotic spindle traits, Farhadifar et al. 2016) but it is again difficult to relate such measures to θ , since the relation between these traits and fitness is generally poorly known. Information on θ

may rather be obtained from the distribution of fitness effects of mutations. Indeed, bias causes mutation to push phenotypic traits in a given direction away from the optimum, so that the proportion of beneficial mutations should always stay below 0.5, even for small-effect mutations occurring in a nonoptimal genotype. By contrast, in the absence of bias the proportion of beneficial mutations tends to 0.5 as one moves away from the optimum, the convergence to 0.5 being faster for smaller effect mutations. As a consequence of this high rate of compensatory mutations, drift load generally stays mild in the absence of bias unless population size is very small (the load being roughly proportional to n/N , e.g., Lande 1980b; Hartl and Taubes 1998; Poon and Otto 2000), while it may reach much higher values when mutational bias is present, as shown in the present article. Compensatory mutations has been best studied in model organisms such as bacteriophages, bacteria, nematodes, and yeasts where they were shown to be common (e.g., Levin et al. 2000; Poon and Chao 2005; Estes et al. 2011; Szamecz et al. 2014). However, more work is needed to better understand how the rate of compensatory mutations changes with the degree of maladaptation of individuals, in order to gain more insights on realistic levels of mutational bias (as modeled here).

Finally, we can note that the equilibrium rate of sex in the population generally stays small when the cost of sex is moderate to strong (Figs. 8 and 9), the highest rates of sex being always achieved under complete bias ($\theta = 1$), that is, when compensatory mutations are not possible. Similarly, low levels of costly sex are also maintained in most cases in models on the evolution of sex due to deleterious mutations without epistasis (Roze and Michod 2010; Roze and Otto 2012; Roze 2014). Exploring to what extent higher levels of sex may be maintained in models including environmental change would thus be of interest, and will be the subject of future work.

Appendix : General QLE Results

Assuming that the distribution of phenotypic traits affecting fecundity in the population is approximately Gaussian and that selection is weak, a general expression for indirect selection on the rate of sex is given by (see Supplementary File S3 for derivation):

$$\Delta_{\text{ind}} \bar{g}_{\sigma} \approx \sum_{\alpha=1}^n \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\alpha}} C_{g,\sigma\alpha} + \sum_{\alpha \leq \beta} \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} M_{g,\sigma\alpha\beta} \quad (\text{A1})$$

where the second sum is over all possible pairs of selected traits, including $\alpha = \beta$. Equation (A1) is equivalent to Charlesworth's (1993) decomposition of the selection gradient for a recombination modifier allele into two terms (eq. A10 in Charlesworth 1993, see also Appendix 2 of Barton 1995). The first term of equation A1 (equivalent to the term in $\delta \bar{z}$ in Charlesworth 1993) represents

indirect selection caused by the effect of sex on mean phenotypes. With our Gaussian, isotropic fitness function (eq. 3), we have:

$$\frac{\partial \ln \bar{W}}{\partial \bar{z}_{\alpha}} = -\frac{\bar{z}_{\alpha}}{V_{g,\alpha} + V_s}, \quad (\text{A2})$$

which is approximately $-\bar{z}_{\alpha}/V_s$ when selection is weak ($V_{g,\alpha} \ll V_s$). In our model, directional selection occurs along the axis corresponding to the direction of the mutational bias, and therefore only the first term of the sum (for $\alpha = 1$) will contribute when phenotypes are measured in the basis defined by equations (9) and (10). The second term of equation (A1) (equivalent to the term in δV_g in Charlesworth 1993) represents indirect selection caused by the effect of sex on the genetic variance-covariance matrix: $\partial \ln \bar{W} / \partial C_{g,\alpha\beta}$ describes how mean fitness is affected by the genetic covariance between traits α and β , while the third moment $M_{g,\sigma\alpha\beta} = E[(g_{\sigma} - \bar{g}_{\sigma})(g_{\alpha} - \bar{g}_{\alpha})(g_{\beta} - \bar{g}_{\beta})]$ (where E stands for the average over all individuals) describes to what extent the genetic covariance between traits α and β differs between subsets of the populations with different rates of sex. As shown in Supplementary File S3, under an isotropic, Gaussian fitness function, and measuring phenotypes in the basis defined by equations (9) and (10), only the terms with $\alpha = \beta$ differ from zero, and the second term of equation (A1) thus becomes $\sum_{\alpha=1}^n (\partial \ln \bar{W} / \partial V_{g,\alpha}) M_{g,\sigma\alpha\alpha}$. The selection gradient $\partial \ln \bar{W} / \partial V_{g,\alpha}$ measures the strength of stabilizing selection on trait α , and is given by:

$$\begin{aligned} \partial \ln \bar{W} / \partial V_{g,\alpha} &= -\frac{1}{2(V_{g,\alpha} + V_s)} + \frac{1}{2} \left(\frac{\partial \ln \bar{W}}{\partial \bar{z}_{\alpha}} \right)^2 \\ &\approx -\frac{1}{2V_s} \left(1 - \frac{\bar{z}_{\alpha}^2}{V_s} \right) \end{aligned} \quad (\text{A3})$$

where again the term \bar{z}_{α}^2 will differ from zero only for the first phenotypic trait in the basis defined by equations (9) and (10). Note that the second term of equation (A1) does not appear in classic expressions describing the effect of selection on correlated characters (Lande 1979), as these assume a multivariate Gaussian distribution of phenotypic traits. Here, we cannot assume that the joint distribution of the rate of sex σ and of the traits affecting fecundity is multivariate Gaussian: in particular, sex tends to increase $V_{g,\alpha}$ by breaking negative genetic associations (linkage disequilibria between alleles with compensatory effects on trait α), generating a positive third moment $M_{g,\sigma\alpha\alpha}$.

Following Charlesworth (1993) and Barton (1995), the moments $C_{g,\sigma\alpha}$ and $M_{g,\sigma\alpha\beta}$ that appear in equation (A1) may be expressed in terms of the genetic variance for the rate of sex $V_{g,\sigma}$ and genetic variances (and covariances) for selected traits using a QLE argument. The derivation (shown in Supplementary File S3) supposes that selection is weak relative to effective recombination rates between loci (and thus that the rate of sex is not too low), so that linkage disequilibria remain small. Furthermore, it neglects

the effects of genetic associations involving more than three loci. Under these assumptions, one obtains for $M_{g,\sigma\alpha\beta}$:

$$M_{g,\sigma\alpha\beta} \approx -\frac{\Delta_{\text{sel}}\mathcal{D}_{\alpha\beta}}{r_{h,1}\bar{\sigma}^2} V_{g,\sigma} \quad (\text{A4})$$

where $r_{h,1}$ is defined in the main text. The term $\Delta_{\text{sel}}\mathcal{D}_{\alpha\beta}$ in the numerator of equation (A5) measures the change in $\mathcal{D}_{\alpha\beta}$ (per generation) due to selection, where $\mathcal{D}_{\alpha\beta}$ is the contribution of linkage disequilibria to the genetic covariance between traits α and β . As shown in Supplementary File S3, when phenotypes are measured in a basis that eliminates covariances between traits, we have (assuming that the number of loci affecting selected traits is large):

$$\Delta_{\text{sel}}\mathcal{D}_{\alpha\beta} \approx \left[(1 + I_{\alpha\beta}) \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} - \frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} \frac{\partial \ln \bar{W}}{\partial \bar{z}_\beta} \right] V_{g,\alpha} V_{g,\beta} \quad (\text{A5})$$

where $I_{\alpha\beta}$ equals 1 if $\alpha = \beta$, and 0 otherwise. Under an isotropic, Gaussian fitness function, it is possible to show that the term between brackets in equation (A6) equals 0 when $\alpha \neq \beta$, while it is approximately $-1/V_s$ when $\alpha = \beta$ (Supplementary File S3, eq. (A3)). In this case, equations (A3) – (A5) yield equation (32) in the main text.

The QLE expression for the genetic covariance $C_{g,\sigma\alpha}$ that appears in the first term of equation (A1) writes (see Supplementary File S3 for derivation):

$$C_{g,\sigma\alpha} \approx -\left(\frac{1}{r_{h,2}\bar{\sigma}} - \frac{1}{r_{h,1}} \right) \frac{1}{\bar{\sigma}^2} \sum_{\beta=1}^n \frac{\partial \ln \bar{W}}{\partial \bar{z}_\beta} (\Delta_{\text{sel}}\mathcal{D}_{\alpha\beta}) V_{g,\sigma} \quad (\text{A6})$$

where $r_{h,2}$ is defined in the main text. Under an isotropic, Gaussian fitness function, equations (A2) – (A5) yield equation (33) in the main text.

AUTHOR CONTRIBUTIONS

E.V., L.S., and D.R. analyzed the model, E.V. and D.R. wrote the article.

ACKNOWLEDGMENTS

We thank Brian Charlesworth for suggesting considering the effect of mutational bias, two anonymous reviewers for helpful comments, and the bioinformatics and computing service of Roscoff's Biological Station (Abimis platform) for computing time. This work was supported by the French Agence Nationale de la Recherche (project SexChange, ANR-14-CE02-0001).

DATA ARCHIVING

The DOI for our data is <http://10.5061/dryad.50q65s0>.

LITERATURE CITED

Agrawal, A. F. 2006. Evolution of sex: why do organisms shuffle their genotypes? *Curr. Biol.* 16:R696–R704.
 ———. 2009. Spatial heterogeneity and the evolution of sex in diploids. *Am. Nat.* 174:S54–S70.

Anton, H. 2005. Elementary linear algebra, 9th ed. John Wiley & Sons, Hoboken, NJ.
 Balloux, F., L. Lehmann, and T. de Meuwis. 2003. The population genetics of clonal and partially clonal diploids. *Genetics* 164:1635–1644.
 Barton, N. H. 1989. The divergence of a polygenic system subject to stabilizing selection, mutation and drift. *Genet. Res.* 54:59–77.
 ———. 1995. A general model for the evolution of recombination. *Genet. Res.* 65:123–144.
 Barton, N. H., and S. P. Otto. 2005. Evolution of recombination due to random drift. *Genetics* 169:2353–2370.
 Becks, L., and A. F. Agrawal. 2010. Higher rates of sex evolve in spatially heterogeneous environments. *Nature* 468:89–93.
 ———. 2012. The evolution of sex is favoured during adaptation to new environments. *PLoS Biol.* 5:e1001317.
 Bernstein, H., H. C. Byerly, F. A. Hopf, and R. E. Michod. 1985. Genetic damage, mutation, and the evolution of sex. *Science* 229:1277–1281.
 Bernstein, H., F. A. Hopf, and R. E. Michod. 1988. Is meiotic recombination an adaptation for repairing DNA, producing genetic variation, or both? Pp. 139–160 in R. E. Michod and B. R. Levin, eds. *The evolution of sex. An examination of current ideas*. Sinauer, Sunderland.
 Bürger, R. 1998. Mathematical properties of mutation-selection models. *Genetica* 102/103:279–298.
 Campbell, R. B. 1984. The manifestation of phenotypic selection at constituent loci. I. Stabilizing selection. *Evolution* 38:1033–1038.
 Charlesworth, B. 1993. Directional selection and the evolution of sex and recombination. *Genet. Res.* 61:205–224.
 ———. 2013a. Stabilizing selection, purifying selection, and mutational bias in finite populations. *Genetics* 194:955–971.
 ———. 2013b. Why we are not dead one hundred times over. *Evolution* 67:3354–3361.
 Charlesworth, B., and N. H. Barton. 1996. Recombination load associated with selection for increased recombination. *Genet. Res.* 67:27–41.
 Chebib, J., and F. Guillaume. 2017. What affects the predictability of evolutionary constraints using a G-matrix? The relative effects of modular pleiotropy and mutational correlation. *Evolution* 71:2298–2312.
 Chevin, L.-M., G. Martin, and T. Lenormand. 2010. Fisher's model and the genomics of adaptation: restricted pleiotropy, heterogeneous mutation, and parallel evolution. *Evolution* 64:3213–3221.
 Davies, S. K., A. Leroi, A. Burt, G. J. Bundy, and C. F. Baer. 2016. The mutational structure of metabolism in *Caenorhabditis elegans*. *Evolution* 70:2239–2246.
 de Visser, J. A. G. M., and S. F. Elena. 2007. The evolution of sex: empirical insights into the roles of epistasis and drift. *Nat. Rev. Genet.* 8:139–149.
 Estes, S., P. C. Phillips, and D. R. Denver. 2011. Fitness recovery and compensatory evolution in natural mutant lines of *C. elegans*. *Evolution* 65:2335–2344.
 Farhadifar, R., J. Miguel Ponciano, E. C. Andersen, D. J. Needleman, and C. F. Baer. 2016. Mutation is a sufficient and robust predictor of genetic variation for mitotic spindle traits in *Caenorhabditis elegans*. *Genetics* 203:1859–1870.
 Felsenstein, J. 1974. The evolutionary advantage of recombination. *Genetics* 78:737–756.
 Gandon, S., and S. P. Otto. 2007. The evolution of sex and recombination in response to abiotic or coevolutionary fluctuations in epistasis. *Genetics* 175:1835–1863.
 García-Dorado, A., C. López-Fanjul, and A. Caballero. 1999. Properties of spontaneous mutations affecting quantitative traits. *Genet. Res.* 74:341–350.

- Halligan, D. L., and P. D. Keightley. 2009. Spontaneous mutation accumulation studies in evolutionary genetics. *Ann. Rev. Ecol. Evol. Syst.* 40:151–172.
- Hartfield, M., and P. Keightley. 2012. Current hypotheses regarding the evolution of sex and recombination. *Integr. Zool.* 7:192–209.
- Hartfield, M., S. P. Otto, and P. D. Keightley. 2010. The role of advantageous mutations in enhancing the evolution of a recombination modifier. *Genetics* 184:1153–1164.
- Hartl, D. L., and C. H. Taubes. 1998. Towards a theory of evolutionary adaptation. *Genetica* 103:525–33.
- Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. *Genet. Res.* 8:269–294.
- Keightley, P. D., and O. Ohnishi. 1998. EMS-induced polygenic mutation rates for nine quantitative characters in *Drosophila melanogaster*. *Genetics* 148:753–766.
- Keightley, P. D., and S. P. Otto. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* 443:89–92.
- Kimura, M., and T. Maruyama. 1966. The mutational load with epistatic gene interactions in fitness. *Genetics* 54:1337–1351.
- Kondrashov, A. S., and J. F. Crow. 1988. King's formula for the mutation load with epistasis. *Genetics* 120:853–856.
- Kondrashov, A. S., and L. Y. Yampolsky. 1996. Evolution of amphimixis and recombination under fluctuating selection in one and many traits. *Genet. Res.* 68:165–173.
- Lande, R. 1976a. The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genet. Res.* 26:221–235.
- . 1976b. Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30:314–334.
- . 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution* 33:402–416.
- . 1980a. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* 94:203–215.
- . 1980b. Genetic variation and phenotypic evolution during allopatric speciation. *Am. Nat.* 116:463–479.
- Lenormand, T., and S. P. Otto. 2000. The evolution of recombination in a heterogeneous environment. *Genetics* 156:423–438.
- Levin, B. R., V. Perrot, and N. Walker. 2000. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* 154:959–997.
- Loureço, J., N. Galtier, and S. Glémin. 2011. Complexity, pleiotropy and the fitness effect of mutations. *Evolution* 65:1559–1571.
- Luijckx, P., E. K. H. Ho, M. Gasim, S. Chen, A. Stanic, C. Yanchus, Y. S. Kim, and A. F. Agrawal. 2017. Higher rates of sex evolve during adaptation to more complex environments. *Proc. Natl. Acad. Sci. USA* 114:534–539.
- Lyman, R. F., F. Lawrence, S. V. Nuzhdin, and T. F. C. Mackay. 1996. Effects of single *P*-element insertions on bristle number and viability in *Drosophila melanogaster*. *Genetics* 143:277–292.
- Lynch, M., and J. B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, MA.
- Manna, F., R. Gallet, G. Martin, and T. Lenormand. 2012. The high-throughput yeast deletion fitness data and the theories of dominance. *J. Evol. Biol.* 25:892–903.
- Martin, G., S. F. Elena, and T. Lenormand. 2007. Distributions of epistasis in microbes fit predictions from a fitness landscape model. *Nat. Genet.* 39:555–560.
- Martin, G., and T. Lenormand. 2006. A general multivariate extension of Fisher's geometrical model and the distribution of mutation fitness effects across species. *Evolution* 60:893–907.
- Martin, G., S. P. Otto, and T. Lenormand. 2006. Selection for recombination in structured populations. *Genetics* 172:593–609.
- Maynard Smith, J. 1980. Selection for recombination in a polygenic model. *Genet. Res.* 35:269–277.
- . 1988. Selection for recombination in a polygenic model—the mechanism. *Genet. Res.* 51:59–63.
- Morran, L. T., O. G. Schmidt, I. A. Gelarden, R. C. I. Parrish, and C. M. Lively. 2011. Running with the Red Queen: host-parasite coevolution selects for biparental sex. *Science* 333:216–218.
- Ostrow, D., N. Phillips, A. Avalos, D. Blanton, A. Boggs, T. Keller, L. Levy, J. Rosenbloom, and C. F. Baer. 1997. Mutational bias for body size in rhabditid nematodes. *Genetics* 176:1653–1661.
- Otto, S. P. 2003. The advantages of segregation and the evolution of sex. *Genetics* 164:1099–1118.
- . 2009. The evolutionary enigma of sex. *Am. Nat.* 174:S1–S14.
- Otto, S. P., and N. H. Barton. 1997. The evolution of recombination: removing the limits to natural selection. *Genetics* 147:879–906.
- . 2001. Selection for recombination in small populations. *Evolution* 55:1921–1931.
- Otto, S. P., and M. W. Feldman. 1997. Deleterious mutations, variable epistatic interactions, and the evolution of recombination. *Theor. Popul. Biol.* 51:134–47.
- Peters, A. D., and S. P. Otto. 2003. Liberating genetic variance through sex. *BioEssays* 25:533–537.
- Phillips, P. C., S. P. Otto, and M. C. Whitlock. 2000. Beyond the average: the evolutionary importance of gene interactions and variability of epistatic effects. Pp. 20–38 in J. B. Wolf, E. D. Brodie, and M. J. Wade, eds. *Epistasis and the evolutionary process*. Oxford Univ. Press, New York.
- Poon, A., and L. Chao. 2005. The rate of compensatory mutation in the DNA bacteriophage ϕ X174. *Genetics* 170:898–899.
- Poon, A., and S. P. Otto. 2000. Compensating for our load of mutations: freezing the meltdown of small populations. *Evolution* 54:1467–1479.
- Robertson, A. 1960. A theory of limits in artificial selection. *Proc. Roy. Soc. (Lond.) B* 153:234–249.
- Roze, D. 2014. Selection for sex in finite populations. *J. Evol. Biol.* 27:1304–1322.
- Roze, D., and N. H. Barton. 2006. The Hill-Robertson effect and the evolution of recombination. *Genetics* 173:1793–1811.
- Roze, D., and A. Blanckaert. 2014. Epistasis, pleiotropy and the mutation load in sexual and asexual populations. *Evolution* 68:137–149.
- Roze, D., and T. Lenormand. 2005. Self-fertilization and the evolution of recombination. *Genetics* 170:841–857.
- Roze, D., and R. E. Michod. 2010. Deleterious mutations and selection for sex in finite, diploid populations. *Genetics* 184:1095–1112.
- Roze, D., and S. P. Otto. 2012. Differential selection between the sexes and selection for sex. *Evolution* 66:558–574.
- Santiago, E., J. Albornoz, A. Domínguez, M. A. Toro, and C. López-Fanjul. 1992. The distribution of spontaneous mutations on quantitative traits and fitness in *Drosophila melanogaster*. *Genetics* 132:771–781.
- Sharp, N. P., and S. P. Otto. 2016. Evolution of sex: using experimental genomics to select among competing theories. *BioEssays* 38:751–757.
- Szamecz, B., G. Boross, D. Kalapis, K. Kovács, G. Fekete, Z. Farkas, V. Lázár, M. Hrtan, P. Kemmeren, M. J. A. Groot Koerkamp, et al. The genomic landscape of compensatory evolution. *PLoS Biol.* 12:e1001935.

Tenaillon, O., O. K. Silander, J.-P. Uzan, and L. Chao. 2007. Quantifying organismal complexity using a population genetic approach. *PLoS One* 2:e217.

Waxman, D., and J. R. Peck. 2003. The anomalous effects of biased mutation. *Genetics* 164:1615–1626.

Welch, J. J., and D. Waxman. 2003. Modularity and the cost of complexity. *Evolution* 57:1723–1734.

Zhang, X.-S., and W. G. Hill. 2008. The anomalous effects of biased mutation revisited: mean-optimum deviation and apparent directional selection under stabilizing selection. *Genetics* 179:1135–1141.

Associate Editor: J. Moorad
Handling Editor: M. Servedio

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1: Description of simulation programs.

File S2: Approximations for the effect of mutational bias on mean fitness.

File S3: QLE model for the evolution of sex Supplementary Figures.

Chapter 2

Evolution of sex in temporally changing environments

2.1 Introduction

In the article presented in Chapter 1, we observed that when the environment is stable (fixed phenotypic optimum), positive rates of sex can evolve in the presence of mutational bias. However, in most cases, equilibrium sex rates remained quite low when the cost of sex is not null. The aim of this chapter is to investigate to what extent higher rates of sex can be favored when the environment is changing.

Models taking into account environmental changes have been explored by simulations, for example, some models investigated the fate of a modifier affecting recombination between loci coding for a quantitative trait, in the case of a chang-

ing environment. Maynard Smith (1980, 1988) observed that there is a conflict between stabilizing selection (selecting against recombination) and directional selection (selecting for increased rates of recombination). However, the model only involved a few loci. Kondrashov and Yampolsky (1996) used a simulation model representing the evolution of one or several quantitative traits under various modes of selection. They observed that alleles causing an increase in recombination were favored when the fluctuations of the environment were moderate. Bürger (1999) and Waxman and Peck (1999) used other simulation models involving quantitative traits to compare the rate of adaptation of sexual and asexual populations (without letting sex or recombination evolve), and observed a strong advantage of sexual recombining populations – over asexual or sexual non-recombining populations – when the environment changes steadily or fluctuates over time, in terms of increased genetic variance (causing an increase in mean fitness) or survival rate.

Analytical approximations based on the infinitesimal model (quantitative trait coded by a very large number of loci, each with very weak effect) on selection at a recombination modifier locus have been obtained by Charlesworth (1993), considering different forms of temporal change in the phenotypic optimum (corresponding to a steady, cyclical or random change). In particular, he obtained predictions (detailed below) for the effect of the parameters describing environmental change (*i.e.* speed of environmental change, length of the environmental

2.1. Introduction

cycle) on the strength of selection for recombination.

In this chapter, we simplified the model used in the previous chapter, to represent a single selected phenotypic trait coded by a large number of loci. This time, loci are multiallelic with an infinite number of possible alleles per locus (continuum-of-alleles model), so that the evolution of the population was not limited by the amount of genetic diversity. The value of the phenotypic trait can be decomposed into a genetic and an environmental component:

$$z = g + e, \tag{2.1}$$

where the genetic contribution g is the sum of allelic effects on all loci, and the environmental effect e is sampled from a centered gaussian distribution with variance V_e (which was set to 1). We assumed no mutational bias, so that the phenotypic effect of mutations at loci affecting the trait are sampled from a gaussian distribution with mean 0 and variance a^2 . However, directional selection results from changes in the optimal phenotype over time. The gaussian shaped fitness function is given by:

$$W = \exp \left[-\frac{(z - o)^2}{2\omega^2} \right], \tag{2.2}$$

where z is the value of the phenotypic trait, o the value of the phenotypic optimum and ω^2 represents the strength of selection on the trait (fixed to 10). With only

Chapter 2. Evolution of sex in temporally changing environments

one selected trait, the mean fitness effect of mutations is given by $\bar{s} = a^2/(2V_s)$, where $V_s = \omega^2 + V_e$ (see equation (7) in the article). We explored the effect of the same forms of environmental change as in Charlesworth (1993) (*i.e.* steady change, cyclical change or stochastic change of optimum over time) on the mean fitness and rate of sex at equilibrium. We explored various ranges of parameters to investigate which conditions may possibly favor higher rates of sex. The details of the simulations program are fairly similar to the previous model (see Appendix S1). Simulations were run for 10^6 generations, including 10^5 preliminary generations during which the investment in sexual reproduction was not allowed to evolve (enough to reach a fitness equilibrium). The list of parameters of the model is detailed in Table 2.1.

Table 2.1: Parameters of the model

N	Population size
ℓ	Number of selected loci
U	Overall mutation rate on loci affecting the selected trait
a^2	Variance of the mutational effects on the selected trait
R	Genome map length
c	Cost of sex
ℓ_s	Number of loci affecting the rate of sex
U_s	Mutation rate per generation on loci affecting the rate of sex
a_s^2	Variance of mutational effects on loci affecting the rate of sex
$\omega^2 = 10$	Strength of stabilizing selection on the phenotypic trait

2.2 Simulation results

2.2.1 Linear change in optimum

In the case of a steady change of the environment, the phenotypic optimum changes at a constant rate over time, so that: $o(t) = \alpha t$, with α corresponding to the speed of environmental change.

As observed in Chapter 1, when the environment stays constant ($\alpha = 0$), the population evolves towards pure asexuality. However, the rate of sex at equilibrium increases with the speed of environmental change, in all sets of parameters tested, until the population becomes extinct (fitness reached 0 within the first 10^5 generations) for really high values of α , when the change in optimum becomes too fast for the population to track it – the figures only show results up to α_{max} , which corresponds to the maximal value of α for which the population is able to adapt.

For the same speed of environmental change (α), a lower population size favors higher rates of sex; this may be due to stronger Hill-Robertson effects between selected loci, and also to the fact that genetic variation is more limiting in smaller populations (increasing the benefits of recombination). However, population extinction occurs at lower values of α in smaller populations ($\alpha_{max} = 2$ for $N = 500$; Figure 2.1). High rates of sex may evolve for high rates of environmental change, even in the presence of a substantial cost of sex ($c = 1.2$; Figure 2.1 and 2.2). As

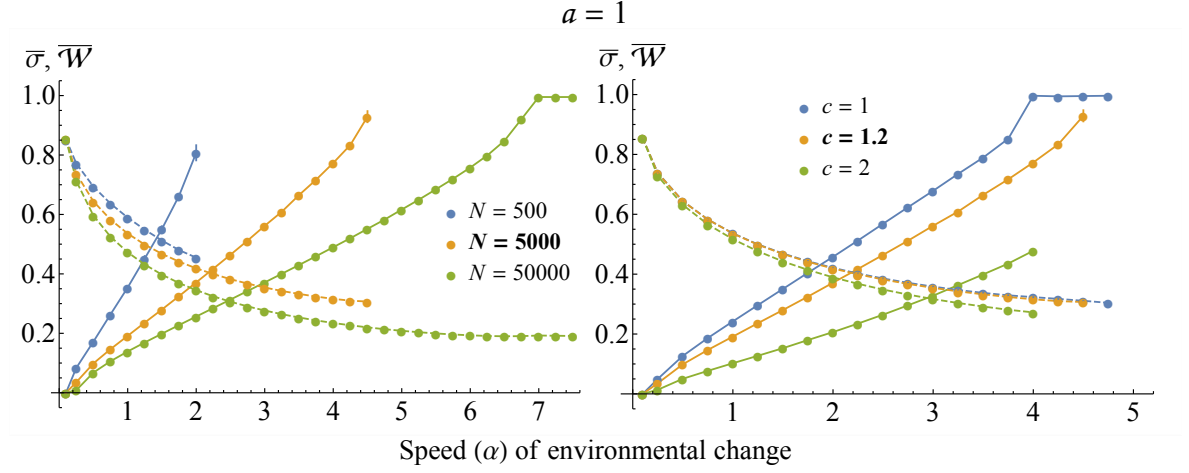


Figure 2.1: Mean fitness (decreasing curves, dashed) and mean rate of sex (increasing curves, solid) at equilibrium as a function of the rate of environmental change α , for different values of population size N and cost of sex c , for $a = 0.1$. Parameter values: $N = 5000$, $\ell = 10^4$, $U = 0.1$, $R = 10$, $c = 1.2$, $\ell_s = 10$, $U_s = 0.1$ and $a_s = 0.01 = V_{e,s}$, for $a = 1$ (corresponding to $\bar{s} \approx 0.045$). Initial investment in sex is 0.5. Error bars were computed by splitting the last generations of the simulation into 8 batches of 10^5 generations and calculating the standard error over batches.

expected, with a strong cost of sex ($c = 2$, twofold cost of sex), equilibrium sex rates are lower but still reach 50% (see Figure 2.1). Figure 2.2 shows that the number of loci ℓ affecting the quantitative trait does not have much effect on the mean fitness or mean rate of sex at equilibrium. Similarly, the genetic architecture of the rate of sex (number of loci coding for the rate of sex, ℓ_s) has no effect on the evolution of investment in sex. As in the previous chapter, the genome map length R has a non-monotonic effect on the equilibrium rate of sex: when R decreases, the benefit of sex (due to the increase of variance in the offspring) increases faster than the cost due to the reduction in mean fitness among offspring, favoring higher

2.2. Simulation results

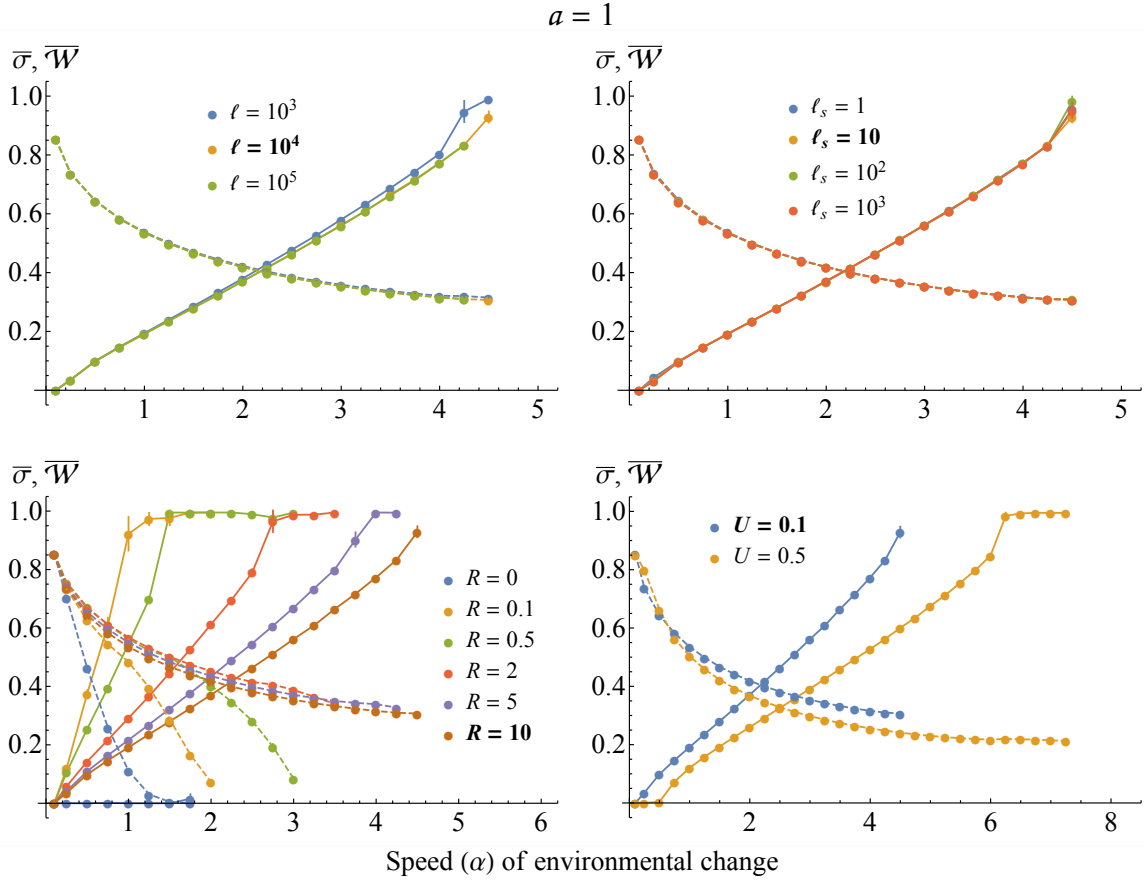


Figure 2.2: Mean fitness (decreasing curves, dashed) and mean rate of sex (increasing curves, solid) at equilibrium as a function of the rate of environmental change α , for different values of the number of selected loci ℓ , genome map length R and overall mutation rate on selected traits U , and for $a = 1$ (corresponding to $\bar{s} \approx 0.045$). Parameter values are as in Figure 2.1.

rates of sex. However, sex cannot be favored when $R = 0$, and the population becomes asexual due to the cost of sex. Finally, an increased mutation rate U on selected loci leads to a lower investment in sex for the same rate of environmental change, probably due to the fact that recombination is less critical for the production of new beneficial genotypes when the mutation rate is higher. However, with a higher mutation rate, the population can survive to faster environmental change, with $\alpha_{max} = 7.25$ when $U = 0.5$ compared to 4.5 when $U = 0.1$ (Figure 2.2). The mutation rate U_s on loci affecting sex rates only enables the population to survive to faster environmental change, but does not affect the equilibrium rate of sex (see Figure S2.3, when $a = 0.1$).

Charlesworth (1993) predicted that recombination should be favored only when the rate of change of the environment is strong enough, so that the increased variance caused by sex and recombination confers a fitness advantage sufficiently high to overcome the cost of the recombination load generated by the negative curvature of the fitness function. However, Charlesworth's approximations are only valid when recombination rates are not too low, and our simulation results suggest that even the slightest change in optimum can lead to positive rates of sex at equilibrium, higher rates of sex being maintained when mutations have stronger phenotypic effects (see results for smaller phenotypic effects of mutations in Appendix S3).

2.2. Simulation results

2.2.2 Cyclical change in optimum

We implemented a cyclical change of the phenotypic optimum by: $o(t) = A \sin(2\pi t/P)$, with A , the amplitude of the oscillation in the optimum and P , the period of the environmental cycle. For the particular case of $P = 2$ (switch of optimum every generation), we replaced t by $t + 1/2$ in this expression (otherwise the optimum stays constant).

Figure 2.3 shows that increasing the amplitude A of the oscillations has a positive effect on the mean rate of sex at equilibrium. Intermediate values of the period P maximize selection for sex, sex being completely disfavored for really short or really long environmental cycles ($\bar{\sigma} \approx 0$ when $P = 5000$ in all simulations; data not shown). For really short periods P , and when the amplitude of oscillations is moderate ($A < 10$), increasing the genetic variance is not beneficial and while sexual populations fail to track the optimum fast enough (“lag load”), asexual reproduction benefits from a lower fitness variance around the average phenotypic optimum (Figure 2.3). However, when A is high and for strong mutational effects ($a = 1$), we observed that asexual populations enter a different regime under very fast environmental cycles, in which they maintain a high variance for the selected trait, comparable to that of sexual populations (data not shown). In this case, high rates of sex can be maintained for very low periods of environmental fluctuations ($P < 5$), despite the fact that neither sexual or asexual populations are able to

track the fluctuations of the optimum (see Figure S3.1). On the other hand, when the period is very long, environmental change becomes very slow and only very low rates of sex can evolve.

The result that higher rates of sex are favored for intermediate lengths of the environmental cycle is in accordance with Charlesworth (1993) and Bürger (1999). Increasing the phenotypic effect of mutations ($a = 1$) has the effect of reducing the period maximizing the equilibrium rate of sex, and can more easily lead to rates of sex close to 1 (Figure 2.3, bottom).

2.2.3 Stochastic change in optimum

To consider the case of a stochastically changing optimum, we used the same function as in Charlesworth (1993) to describe the change in optimum: $o(t) = \tau o(t-1) + \epsilon$, where τ ($-1 \leq \tau \leq 1$) corresponds to the autocorrelation between two successive values of the optimum, and ϵ is drawn from a Gaussian distribution with mean zero and variance V_ϵ .

A variance in optimum (V_ϵ) of zero is equivalent to a situation in which the environment stays constant, in which case populations always evolve toward asexuality (Figure 2.4). Positive rates of sex are favored only for high values of the environmental autocorrelation ($\tau > 0.5$), the rate of sex increasing with the variance in optimum (Figure 2.4). In the case of complete negative autocorrelation,

2.2. Simulation results

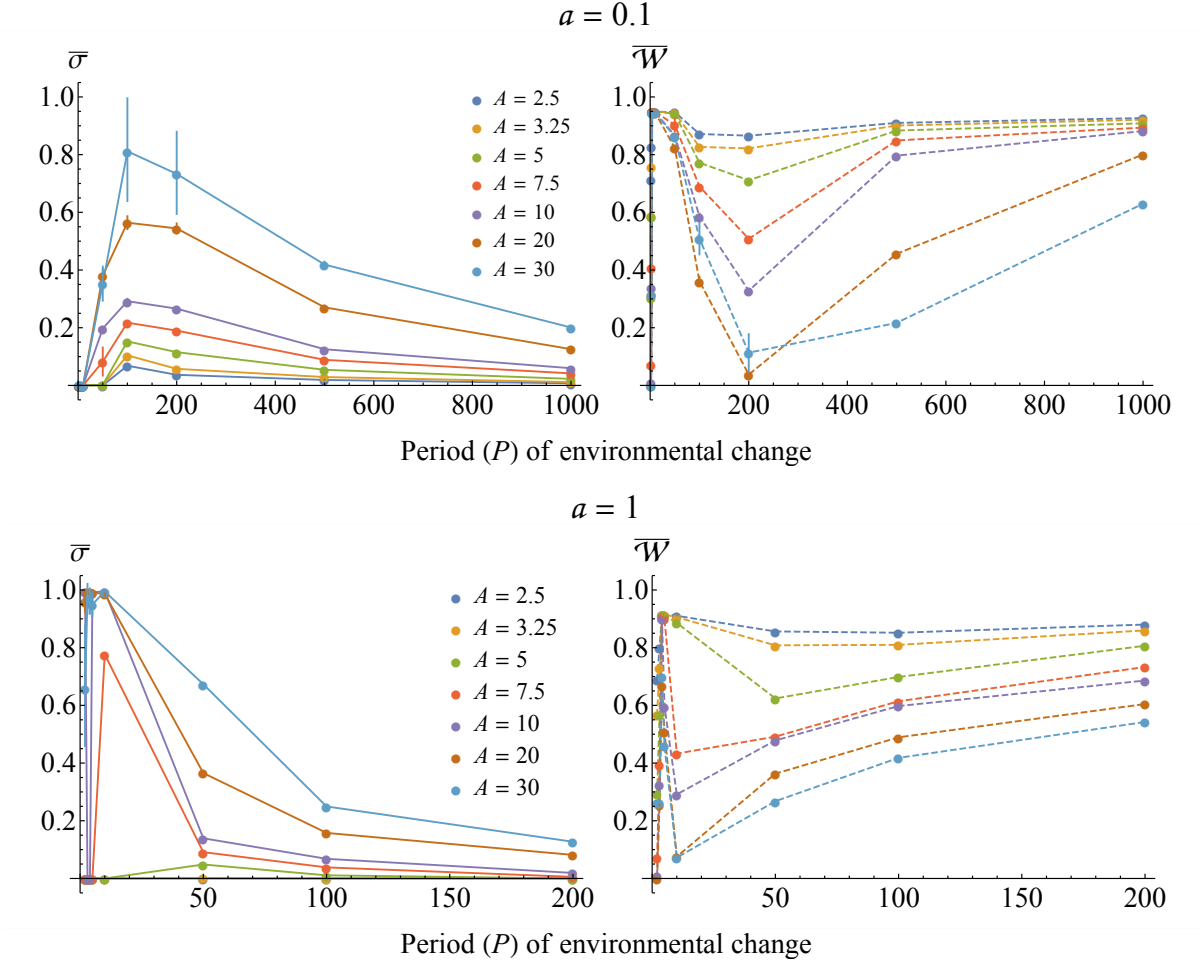


Figure 2.3: Mean rate of sex (left) and mean fitness (right) at equilibrium as a function of the period of the environmental cycle P , for different values of amplitude of the oscillations A , and for $a = 0.1$, corresponding to $\bar{s} \approx 0.00045$ (top) or $a = 1$, corresponding to $\bar{s} \approx 0.045$ (bottom). Parameter values are as in Figure 2.1.

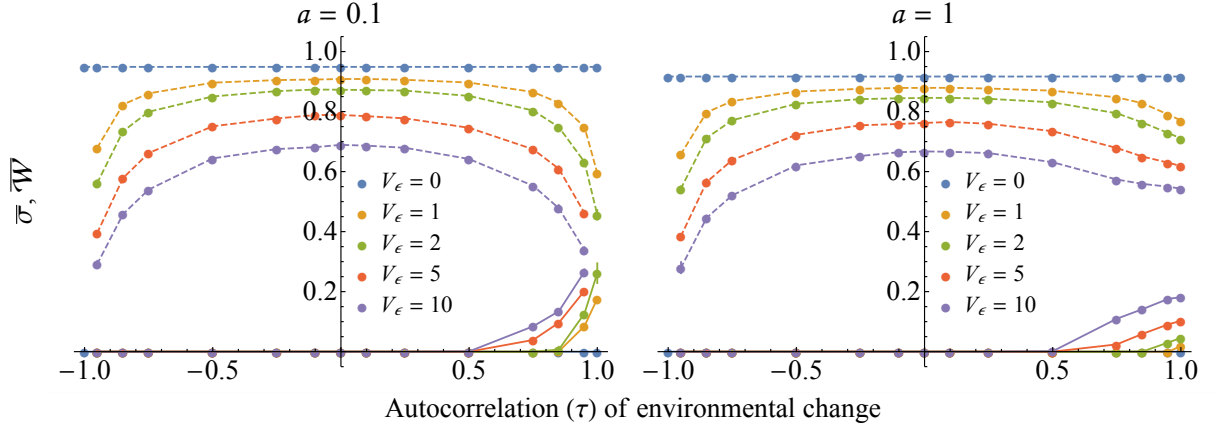


Figure 2.4: Mean rate of sex (plain) and mean fitness (dashed) at equilibrium as a function of the environmental autocorrelation τ , for different values of the variance of random fluctuations V_ϵ and for $a = 0.1$ (corresponding to $\bar{s} \approx 0.00045$, left) and $a = 1$ (corresponding to $\bar{s} \approx 0.045$, right). Parameter values are as in Figure 2.1.

the population did not survive past the 10^5 preliminary generations for the tested values of $V_\epsilon > 0$, probably because the fluctuations of the optimum were too strong (this case is similar to a cyclical change with a very small period).

The results are again in accordance with Charlesworth’s predictions that in a randomly fluctuating environment, increasing the temporal autocorrelation of environmental change favors recombination. Selection for sex is maximal when $\tau = 1$, in which case the trajectory of the optimum becomes a “random walk” (the expected distance of the optimum from its initial value increases over time).

2.3 Discussion

In the previous chapter, we showed that when the environment is stable, directional selection generated by the mutational bias enables the maintenance of positive rates of sex, but those remain rather low. Here we explored the strength of the selection for sexual reproduction when directional selection is generated by a continuous environmental change, using the three types of environmental change considered in Charlesworth (1993). Qualitative results are in accordance with Charlesworth's predictions about the effect of the parameters describing environmental change on selection for recombination: although a steady change in the optimum was the most favorable for the evolution of higher rates of sex, sex is also favored in fluctuating environments with intermediate periods, and in stochastic environments with sufficiently high environmental autocorrelation. From a quantitative perspective, our simulations show that considerably higher rates of sex can be maintained when the environment is changing than in models with a constant environment. Interestingly, higher rates of sex evolve in smaller populations, which may be due to the fact that genetic variation is more limiting, increasing the benefits of recombination, or to the Hill-Robertson effect. The effect of population size would be worth exploring further (in particular, in the case of cyclically or stochastically changing environments).

Chapter 2. Evolution of sex in temporally changing environments

The models presented here included only a single selected trait, and it would be interesting to investigate the effect of environmental change in a model with a higher number of selected traits. Adding more traits under directional selection should increase the strength of selection for sex, while adding traits whose optimal values stays constant should disfavor sex (unless mutational bias is added).

The theory on the evolution of sex and recombination has brought substantial insights into the selective forces that may favor genetic exchange among individuals. However, these predictions have received only little empirical support, as it is difficult to test them in natural populations. Promising advances have been obtained, though, using experimental evolution. For such experiments, facultatively sexual organisms are of great interest. The next part of this thesis presents the results of different experiments performed on the monogonont rotifer *Brachionus plicatilis*, aiming at developing this system as a biological model to explore the possible benefits of sexual reproduction.

Part III

Experimental approach using the
facultatively sexual rotifer

Brachionus plicatilis

Chapter 3

Preliminary experiments on the *Brachionus plicatilis* system

3.1 Monogonont rotifers as an experimental system to explore the benefits of sex

3.1.1 Morphology, life-cycle and reproduction

Rotifers are common small invertebrates, most of which measuring between 50 and 600 μm . They are considered cosmopolitan and live in a wide variety of water bodies ranging from fresh to marine water, including interstitial water in soil, water films covering mosses and lichens, temporary ponds, *etc* (Wallace et al., 2006;

Chapter 3. Preliminary experiments on the *Brachionus plicatilis* system

Segers, 2008; Wallace and Smith, 2009; Fontaneto and Smet, 2014). Up to now, more than 2000 species of rotifers have been described (Segers, 2007). They form the phylum Rotifera and are sorted in three groups: Seisonidea, Bdelloidea and Monogononta. These groups differ by their reproductive mode: Seisonidea reproduce exclusively bisexually; Bdelloidea reproduce exclusively by parthenogenesis; Monogononta use both reproductive modes. Among the monogononts, two species of the genus *Brachionus* (the freshwater species *Brachionus calyciflorus* and the brackish water species *Brachionus plicatilis*) are commonly used in aquaculture (as a source of food for fish larvae), and have recently been used in experimental evolution studies exploring the benefits of sex (Becks and Agrawal, 2010, 2012; Luijckx et al., 2017).

The body of rotifers is divided into head, trunk and foot. The phylum Rotifera is derived from the latin *rota* meaning “wheel”, and *ferre* meaning “to bear”. Some morphological characteristics of the phylum is the presence of a corona – or “wheel organ”, a ciliated structure used both for gathering food and locomotion – on the head, a muscular pharynx called the mastax and a thickened body wall called the lorica (Wallace et al., 2006; Segers, 2008; Wallace and Smith, 2009; Fontaneto and Smet, 2014). Some genera (*e.g.*, *Brachionus*, *Keratella*) create a filtering current that bring particles up to 10 μm in size to their mouth, such as algae, particulate organic detritus, dead bacteria or protozoans. Some genera are detritivores, others

3.1. Monogonont rotifers as an experimental system to explore the benefits of sex

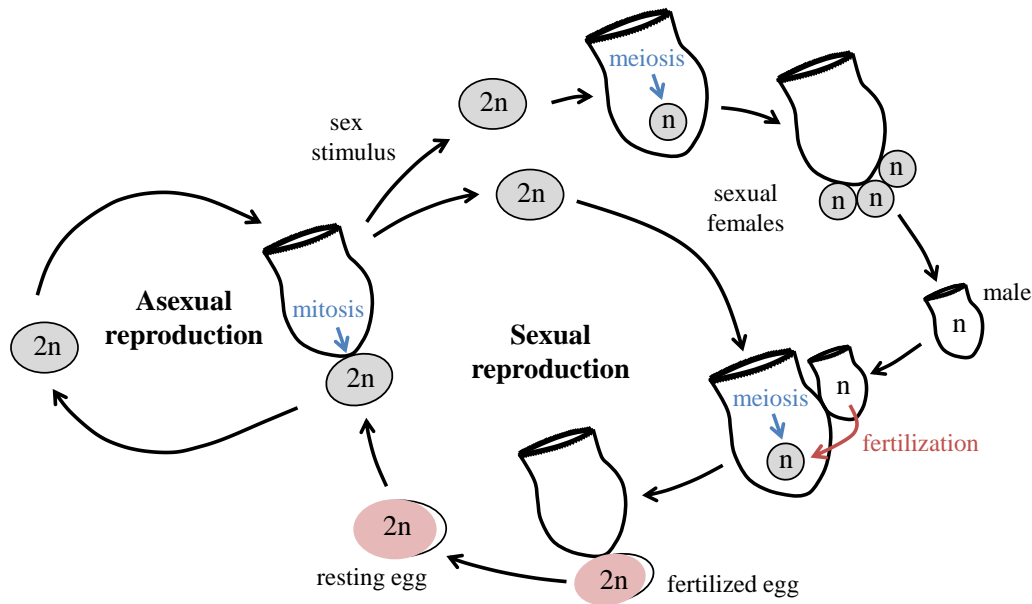


Figure 3.1: Life-cycle of a monogonont rotifer. During the course of its life, there is no possible switch between the two reproductive modes, a female is either asexual or sexual. An asexual female can give birth to both sexual and asexual females, providing there is an appropriate mictic stimulus, whereas a sexual female gives birth either to males (if it has not been fertilized by males in early stages of development), or to fertilized resting eggs. As long as they are not bearing eggs (before sexual maturity), asexual and sexual females cannot be distinguished. In optimal culture conditions, an asexual female can live up to 3 weeks, becomes mature after 2-3 days and produces between 20 and 30 offspring during its life.

grasp and swallow whole preys or scrape food from the surface (Wallace et al., 2006; Wallace and Smith, 2009).

Monogonont rotifers have a cyclically parthenogenetic life cycle, in which asexual reproduction alternates with sexual reproduction. In the absence of males, diploid asexual (amictic) females will produce eggs by mitosis which develop into asexual females (Birky and Gilbert, 1971; Wallace et al., 2006). The mictic phase

is triggered by different environmental cues depending on the species. A given proportion of eggs laid by asexual females, depending on the strength of the mictic stimulus, will develop into sexual (mictic) females. If the sexual females are not fertilized in their early stages of development, they will produce haploid eggs by meiosis, which will develop into males. If fertilization occurs, females will produce resting eggs – multicellular diapausing embryos whose development is arrested – that sink to the bottom of the pond, where they may remain dormant for a period up to several years (Hagiwara and Hino, 1989) before hatching into asexual females (Figure 3.1). Asexual females promote population growth and colonization, whereas sexual reproduction leads to the production of dormant stages, that can survive adverse conditions such as dessiccation.

Monogononts rotifers are sexually dimorphic, females being larger in size than males (Wallace et al., 2006; Fontaneto and Smet, 2014). The former can live from a few days up to three weeks. They hatch as a smaller version of their mother and reach their adult size and sexual maturity after two to three days. The males, usually much smaller, do not have a functional digestive system, they are short-lived and are sexually fertile at birth (Figure 3.2). They swim much faster than females and are easily recognized in cultures.

In rotifers of the genus *Brachionus*, the stimulus for mixis induction has long been described to be density dependent, the first mictic females appearing in high-

3.1. Monogonont rotifers as an experimental system to explore the benefits of sex

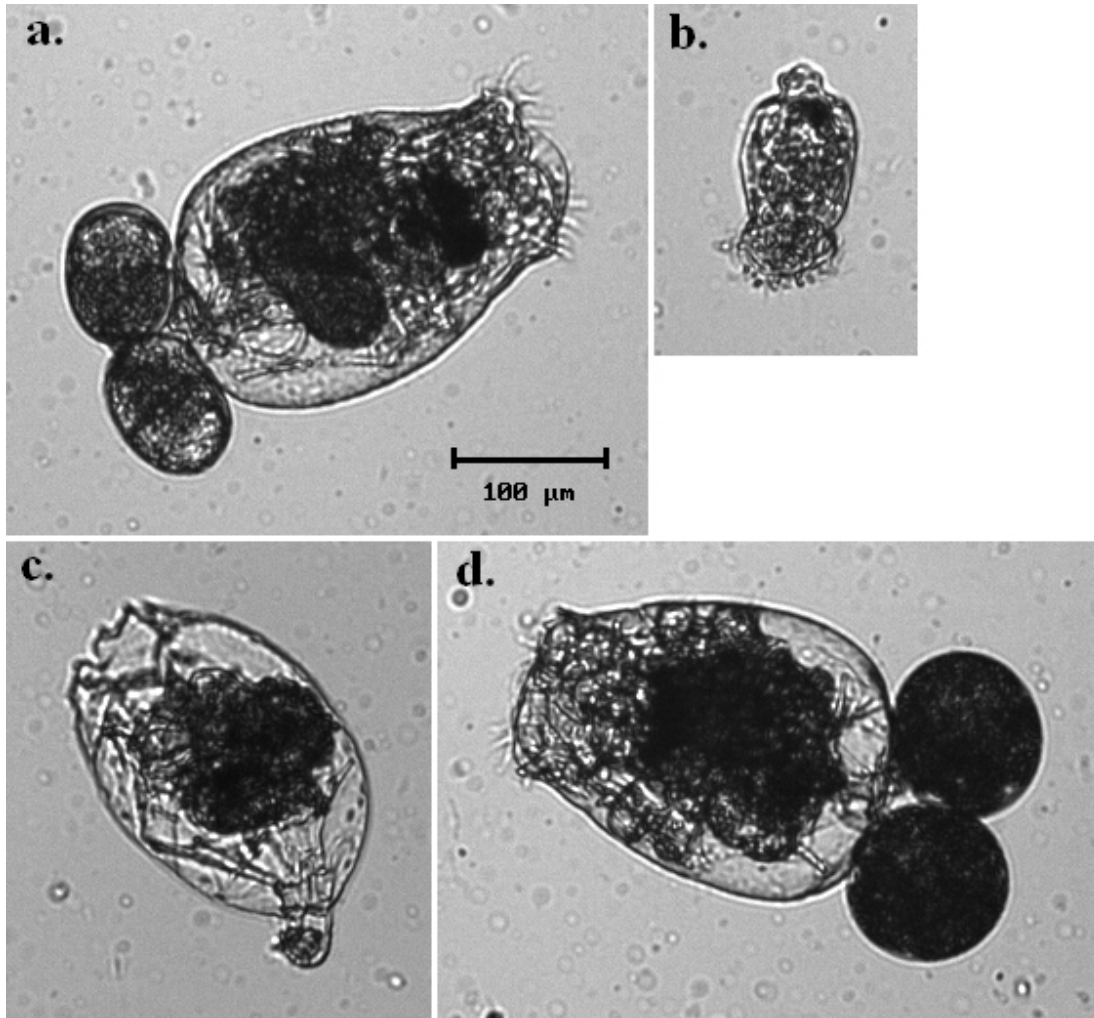


Figure 3.2: Pictures of *Brachionus plicatilis* obtained using a FlowCam®, Fluid Imaging Technologies, Inc. Pictures feature: **a.** an asexual mature female bearing eggs, **b.** a male, **c.** a non-mature female (sexual or asexual undefined) and **d.** a sexual mature female bearing resting eggs. The 100 μm scale is identical for all pictures.

density populations (Gilbert, 1963; Hino and Hirano, 1976; Pourriot and Snell, 1983; Snell and Boyer, 1988). In *Brachionus plicatilis* (Müller), several experiments using preconditioned culture medium confirmed the idea that a chemical signal induces sexual reproduction (Carmona et al., 1993; Stelzer and Snell, 2003, 2006). A study by Snell et al. (2006) has identified this chemical signal (see also Snell, 2017 for a study using *B. manjavacas*, a species of the *B. plicatilis* complex): a mixis-inducing protein (MIP) is produced by the rotifers and its concentration increases as the population density increases, until reaching a threshold triggering the production of mictic females.

3.1.2 Variability in the propensity for sex and mixis initiation

Although a mixis-inducing protein has been described, the production of mictic females is also strongly influenced by other environmental factors such as the availability and quality of food, salinity or temperature. When the concentration of algae is low, the induction of mictic female production is reduced (Snell and Hoff, 1985; Stemberger and Gilbert, 1985, 1987; Snell and Boyer, 1988). Moreover, a decrease in salinity of the culture medium (from 35 g L⁻¹ to lower salinities) increases the number of mictic eggs produced by *B. plicatilis* (Lubzens et al., 1980, 1985) but this tendency is not always significant (Snell and Hoff, 1985). Temper-

3.1. Monogonont rotifers as an experimental system to explore the benefits of sex
ature seems to affect mictic females and eggs production, but the pattern is not
always clear (Snell and Hoff, 1985; Campillo et al., 2009, 2011). It seems that dif-
ferent environmental conditions between natural populations might have selected
for different strategies for sex initiation and investment in sexual reproduction.

Consistent differences in the ability to produce mictic females or in the rates
of mictic reproduction have been observed among *B. plicatilis* strains (Hino and
Hirano, 1977; Snell and Hoff, 1985; Lubzens, 1989), suggesting that the propensity
for sex or the sensitivity to mixis inducing signals is genetically determined. These
results have been confirmed by further studies on *B. plicatilis* (both lab and field
populations), showing significant variations in the level of mixis and mictic response
to density between strains (Carmona et al., 1994, 1995).

A more thorough exploration of rotifer natural populations has been done in
several ponds of the Iberian peninsula. *Brachionus plicatilis* is part of a cryptic
species complex. In some ponds several cryptic species coexist, characterized by
their genetic and reproductive isolation (Gómez et al., 1995; Gómez and Snell,
1996; Gómez et al., 2002; Ortells et al., 2000) and sometimes morphological dif-
ferences (Campillo et al., 2005; Fontaneto et al., 2007; Ciro-Pérez et al., 2001).
Our focus is on *B. plicatilis* sensu stricto, belonging to the large (L-) morphotype.
Strongly variable mictic ratios over time have been observed in populations of Tor-
reblanca Marsh (east Spain) in the field (Carmona et al., 1995). These variations

can partly be linked to the variability of environmental conditions, such as temperature, in the ponds. *B. plicatilis* s.s. clones initiated by single resting eggs from Poza Sur population presented between-clone variability in their propensity for sex – measured as the time and density when the first male appeared in cultures initiated by a single female –, distinguishing roughly between “early” and “late” clones. Mixis initiation occurred from approximately 2 to 10 days after hatching, with rotifer densities within the range of 10–400 ind mL⁻¹ (Aparici et al., 2001; Carmona et al., 2009). Broad-sense heritability for these traits ranged from 0.18 to 0.57, and the propensity for sex between groups of clones qualified as low or high propensity was consistent after a few months (Carmona et al., 2009), confirming the fact that genetic variation for this trait exists in this population. Genetic variability in the propensity for sex – measured as the density at first male appearance – was also detected in Salobrejo population (inland population, east Spain). In this population, density at first male appearance in *B. plicatilis* ranged from 0.55 to 7.15 ind mL⁻¹ (2.91 ind mL⁻¹ on average) and was significantly different among clones, with heritability estimates around 0.51 (Gabaldón and Carmona, 2015). These two populations were screened for genetic diversity at 11 neutral microsatellite markers (Campillo et al., 2009). Levels of heterozygosity within populations were moderate, 0.468 in Poza Sur and 0.238 in the Salobrejo population.

Non-genetic transgenerational effects were also found to affect the propensity

3.1. Monogonont rotifers as an experimental system to explore the benefits of sex for sex in species of the genus *Brachionus*. Indeed, the production of sexual females is inhibited for a number of asexual generations following the last sexual event (*i.e.* hatching from a resting egg). Females from the first parthenogenic generation after hatching have a lower response to the mictic stimulus than females from later parthenogenic generations (Hino and Hirano, 1977; Gilbert, 2002; Schröder and Gilbert, 2004; Hagiwara et al., 2005; Kamizono et al., 2017).

3.1.3 Variability in dormancy and hatching cues

Resting eggs – multicellular embryos with arrested development – are dormant resistant stages, that can survive adverse conditions caused by abiotic (*e.g.*, drought, extreme salinities and/or temperature, etc.) or biotic factors (*e.g.*, competitors, predators, parasites, etc.). When laid, the resting eggs fall into the sediment where they can stay buried for several years. They form a genetically diverse “egg bank” (Kotani et al., 2001; García-Roger et al., 2006). This enables rotifer populations to survive when the environment is rather unpredictable, for instance in temporary pond that can often dry out during summer.

Hatching of diapausing eggs occurs when suitable environmental conditions resume. However, they need to go through a more or less long, dormant phase. Hatching seems triggered by a complex environmental cues, that may vary between species. The cues involve changes in light, temperature and/or salinity conditions

(Minkoff et al., 1983; Pourriot and Snell, 1983; Hagiwara and Hino, 1989; Schröder, 2005). The presence of a food source in the culture medium also influences hatching rates (Minkoff et al., 1983).

The fact that sexually produced (mictic) eggs are not physiologically equivalent to asexually produced ones – especially with the dormancy of mictic eggs – represents a limit of the system for testing potential benefits of sexual reproduction, in particular, benefits due to the production of novel genotypes. Indeed, recombinant genotypes might not contribute to the population’s genetic pool if they are not able to hatch spontaneously in culture conditions. However, recent studies have shown that some resting eggs of *Brachionus* species can hatch rather quickly (within 5 days), without the supposedly mandatory dormancy period, the timing of hatching being just delayed a little (Becks and Agrawal, 2012; Scheuerl and Stelzer, 2013).

3.1.4 Monogonont rotifers as a model system to explore the benefits of sex

Several authors have used experimental evolution to explore the possible benefits of sex. Due to their short generation time, the fact that they can be maintained easily in the lab and their capacity to perform both sexual and asexual reproduction, monogonont rotifers appear to be a suitable model system for this kind of

3.1. Monogonont rotifers as an experimental system to explore the benefits of sex experiments.

In *Brachionus calyciflorus*, Becks and Agrawal (2010) showed that sex was less strongly disfavored in heterogeneous environments compared to homogeneous environments, and intermediate rates of sex could be maintained under spatial heterogeneity (the benefits of sex outweighing its costs). Moreover, the authors later observed an increase of the investment in sexual reproduction during adaptation to novel environments, and showed a decrease in the mean fitness of sexually compared to asexually derived offspring, but an increase in the variance in fitness Becks and Agrawal (2012). Similarly, Luijckx et al. (2017) observed the evolution of a greater investment in sex when adapting to a new environment, increasing with environmental complexity. In these scenarios, an indirect advantage for sex may arise from the production of new genotypes breaking down negative associations and increasing the genetic variance in fitness.

Sexual reproduction of monogonont rotifers is associated with the production of dormant forms (resting eggs) that are resistant to unfavorable conditions. Other experimental evolution studies showed that a greater investment in sex can evolve due to direct selection for resistant forms in populations living in changing, unpredictable environments (Smith and Snell, 2012; Tarazona et al., 2017).

3.2 Preliminary experiments

3.2.1 Standard culture conditions

Over the course of the experiments, a high-density culture of the unicellular green algae *Tetraselmis suecica* (strain provided by the lab of María José Carmona, University of Valencia) was used for food for the rotifers. The algal culture was maintained in a chemostat by the continuous input of artificial seawater (12 g L^{-1} ; Instant Ocean[®], Aquarium Systems) at a dilution rate of 0.2 per day (corresponding to the renewal of 400mL of a 2L culture). The water was enriched with f/2 medium (Guillard and Ryther, 1962; see composition in Appendix S4). The culture was kept at 20 °C under a 12/12-hours light cycle (PAR: approx. $35\text{ }\mu\text{E m}^{-2}\text{ s}^{-1}$). Cultures were supplied in air (with a bubbling system for homogenization) and including 2% CO₂ to boost the growth of algae. The density of algae was regularly estimated from a 1 mL sample fixed with 4 μL of glutaraldehyde solution (Grade II, 25%; Sigma-Aldrich, Inc.) under a microscope, using a Malassez cell (Figure 3.3). A dilution of this culture using 12 g L^{-1} salinity artificial sea water was used as culture medium for the experiments, enabling to control for the concentration of algae. When experimental salinity differed, algae was diluted with osmotic water in order to adjust both salinity and algal concentration. All stocks of artificial seawater and osmotic water were autoclaved before use.

3.2. Preliminary experiments

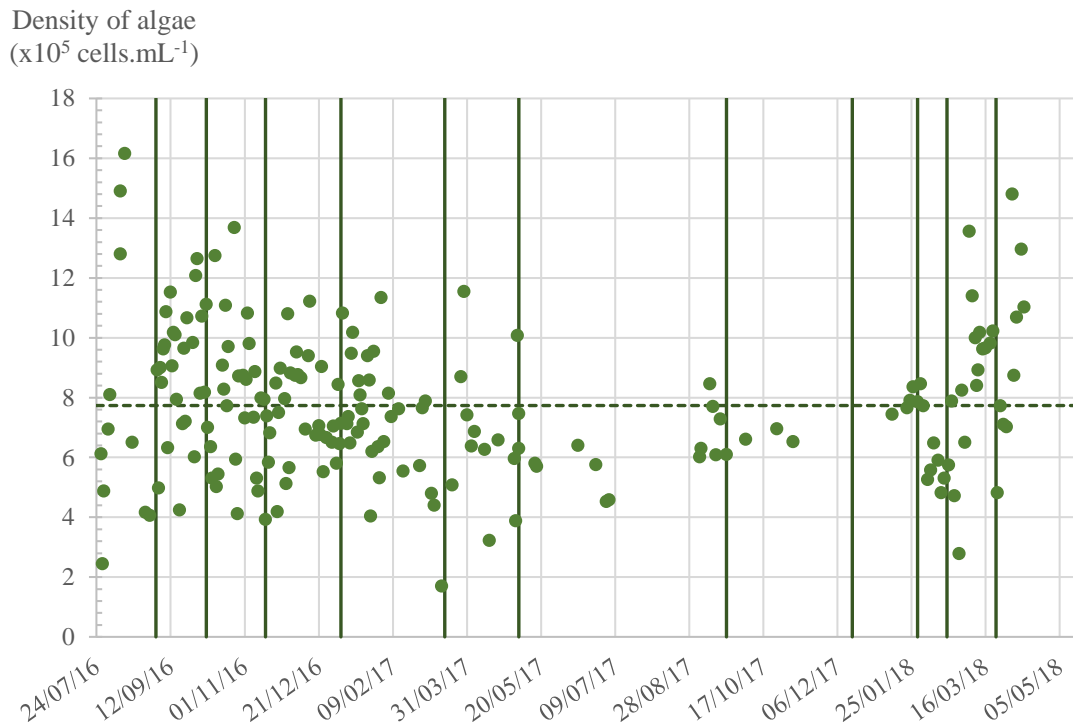


Figure 3.3: Concentrations of *Tetraselmis suecica* in continuous culture over time. Overall mean concentration is 7.7×10^5 cells mL⁻¹ (dashed line). The culture container had to be replaced several times with a clean one due to algae fixing on the walls blocking the light and causing the density of suspended algae to drop (plain lines).

For all the following experiments, the standard culture conditions were practically the same as the culture of algae: a salinity of 12 g L⁻¹, a temperature of 22 °C and a 12/12-hours light cycle.

3.2.2 Isolation of strains

For this study we used sediments kindly sent by María José Carmona and Eduardo García Roger (Cavanilles Institute of Biodiversity and Evolutionary Biology

Chapter 3. Preliminary experiments on the *Brachionus plicatilis* system

in Valencia, Spain; contact: carmona@uv.es, eduardo.garcia@uv.es). The upper layer of the sediment was sampled from an inland shallow, temporary, brakish-water pond (Salobrejo lake) in September 2013 (38° 54.765' N, 1° 28.275' W; see Campillo et al., 2009), and stored at 4 °C. We chose this particular population because it has been extensively explored, and presents features that were interesting for our objectives: genetic variability in investment in sex and evidence for spontaneous hatching of resting eggs in the lab (Campillo et al., 2009; Martínez-Ruiz and García-Roger, 2015). Indeed, Martínez-Ruiz and García-Roger (2015) showed that a reasonable amount of resting eggs from this pound were able to hatch spontaneously after only a few days, most of them hatching within 30 days in culture conditions without a “cold and dark” period.

Isolation of resting eggs from the sediment was done according to Gómez and Carvalho (2000). Approximately 10–15 mL of wet sediment was mixed with 1.75 M sucrose solution in a Falcon tube and made up to 50 mL. The tubes were then centrifuged for 3–5 min at 100 g. The supernatant containing the resting eggs was collected and filtered with a 30 μ m sieve, then washed and put in petri dishes with diluted artificial seawater (12 g L⁻¹). Dishes were wrapped in aluminium foil and stored at 4 °C for future use.

In the Salobrejo lake, the cryptic species *B. manjavacas* and *B. plicatilis* s.s. coexist, the latter being present in minority (Montero-Pau and Serra, 2011). We

3.2. Preliminary experiments

selected *B. plicatilis* s.s. as our model organism for several reasons: this species starts producing mictic females at lower threshold densities, and it appears that the diversity in its propensity for sex is higher (Gabaldón and Carmona, 2015). It is possible to discriminate between the two species using RFLP (restriction fragment length polymorphism) markers (Campillo et al., 2005). To do so, we first needed to establish clonal lineages. Several hundreds of previously stored resting eggs were isolated in individual wells of 24-well plates with 2 mL of artificial seawater (6 g L^{-1}) and kept at 22°C under constant light. Eggs were checked every 24 h for hatching. All female neonates were transferred into new wells of 24-well plates with 2 mL of culture medium at $2.5 \times 10^5 \text{ cells mL}^{-1}$ and standard salinity, and allowed to reproduce. Approximately 10 females in each well were transferred every 2–3 days into fresh medium until the identification of species and the establishment of clones. For DNA extractions, 3 females per well were transferred in $4 \mu\text{L}$ of freshwater into individual wells of 96-well plates, in which we added 2 to 3 volumes of 6% Chelex w/v (Bio-Rad). Rotifers were incubated for 5 min at 56°C , 10 min at 99°C , followed by 30 min at 4°C . The plates were centrifuged for 1 min at 8000 rpm. The supernatant containing DNA was collected in new tubes and stored at -20°C (Papakostas et al., 2005). Using restriction enzymes to differentiate the two cryptic species has proved to be efficient (Campillo et al., 2005). However, our technician S. Mauger designed specific primers using the COIdg sequence,

thus establishing a quicker and less expensive protocol for species identification (see Appendix S5). Polymerase Chain Reaction (PCR) was performed in 96-well PCR plates containing 2 μL of template DNA, 0.15 mM of each nucleotide, 0.5 μM of each primer, 1 X buffer and 0.35 U of Taq-Polymerase (GoTaq[®] Flexi DNA Polymerase, Promega Corporation). PCR was performed in a T100[™] Thermal Cycler (Bio-Rad Laboratories, Inc.) using the following cycling profile: 5 min at 95 °C; 30 cycles of 30 s at 95 °C, 1 min at 56 °C and 1 min at 72 °C; a final step of 10 min at 72 °C after cycling. Products were separated by standard agarose gel electrophoresis using 1.5% (w/v) agarose, for 30 min at 100 V (see Appendix S5).

A total of 20 clones of *B. plicatilis* were identified in July 2016. Clones were then transferred into 30 mL glass tubes and kept at 20 °C and a 12/12-hours light cycle, with culture medium around 5×10^5 cells mL⁻¹ of *T. suecica*, transferred once a week into tubes containing fresh culture medium over the course of the experiments. Later (December 2017), we isolated 3 more clones from sediment samples and also added 32 new clones that had previously been isolated by the laboratory in Valencia, using a similar protocol, giving a total of 55 clones.

3.2.3 Tests on hatching rates

Spontaneous hatching of resting eggs produced by individuals from the Salobrejo population has been observed in the lab. However, hatching rates were also found

3.2. Preliminary experiments

to be genetically variable (Tarazona et al., 2017), and we thus investigated hatching patterns under our culture conditions.

We sampled resting eggs from 8 of our clones by filtering the bottom of the tubes in which those clones were maintained with a $30\text{ }\mu\text{m}$ sieve after letting rotifers grow with a high concentration of algae for a week (note that these eggs were produced by intracloal mating, genetically equivalent to self-fertilization). Twelve eggs per clone were transferred into wells of a 96-well plate for each experimental treatment. In standard hatching conditions, eggs were transferred in 6 g L^{-1} salinity artificial seawater, and the plate was put at $22\text{ }^{\circ}\text{C}$ under constant light directly after the isolation of resting eggs. We tested the effect of salinity by transferring eggs in 12 g L^{-1} salinity water instead. The effect of a dormancy period on hatching of resting eggs was investigated by placing plates at $4\text{ }^{\circ}\text{C}$ in the dark for 1, 2, 3, 4 or 5 weeks prior to placing them in standard hatching conditions. In another treatment, instead of sampling resting eggs from the tubes we sampled mictic females with newly formed resting eggs still attached to the female, in order to test whether a fraction of resting eggs may hatch immediately, as suggested by Martínez-Ruiz and García-Roger (2015) (see a review in García-Roger et al., 2017). Indeed, resting eggs present in the tubes may be up to one week old (as it is unlikely that resting eggs are transferred during the weekly transfer of rotifers to a new tube, since resting eggs fall to the bottom while the upper part of the culture is transferred).

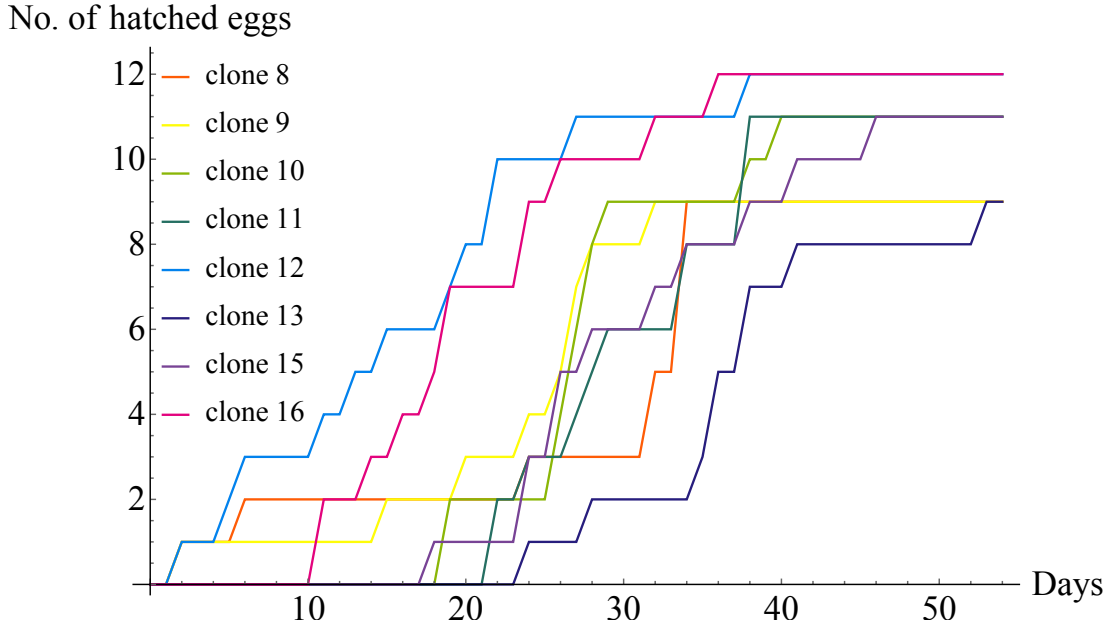


Figure 3.4: Cumulative hatching curves of resting eggs for 8 clones in standard hatching conditions: 6 g L^{-1} salinity water, at 22°C under constant light. Observations were made on 12 eggs per clone. Hatching tests started on August 25th.

Each plate was checked every 24 hours for hatchlings.

In standard conditions – without a period in the dark at 4°C –, we observed hatching of resting eggs in some clones as early as two days after the beginning of the experiment (Figure 3.4). There was strong variation in hatching patterns between clones. For instance, the first egg of clone 13 hatched only after more than three weeks (Figure 3.4). The delay in hatching for clone 13 was consistent when using different hatching conditions (data not shown). Salinity only had little effect on hatching rates (Figure 3.5). Interestingly, similar hatching rates were reached regardless of the length of the simulated dormancy period at 4°C in darkness

3.2. Preliminary experiments

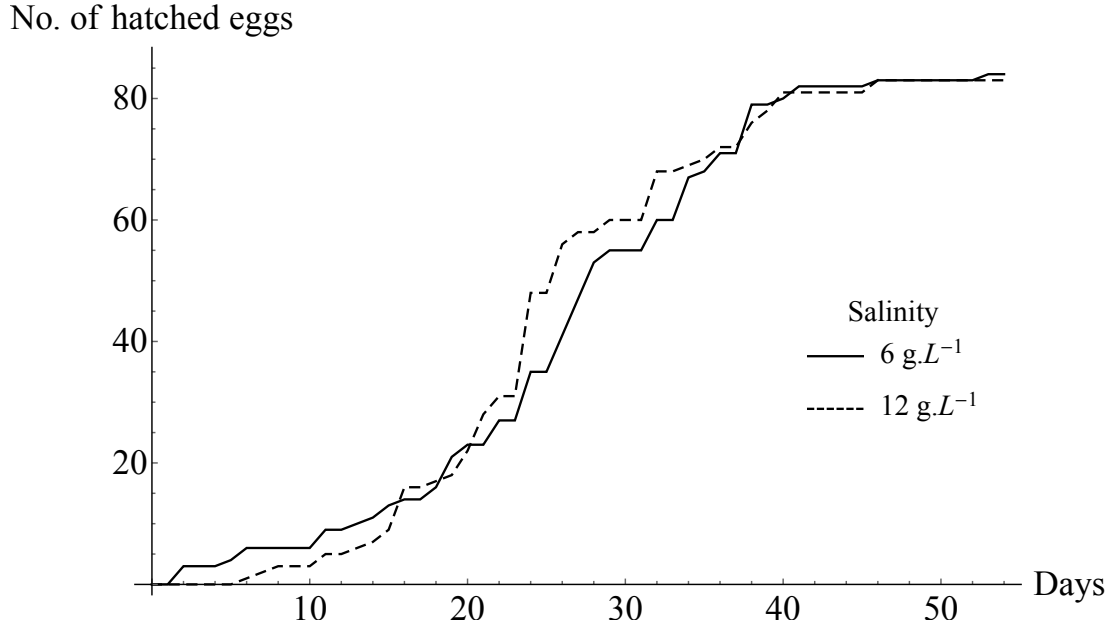


Figure 3.5: Cumulative hatching curves of resting eggs, summed over all 8 clones. Comparison between two different salinity treatments (6 g.L^{-1} and 12 g.L^{-1}). Hatching tests started on August 25th.

(Figure 3.6). Without dormancy, more than 80% of the eggs had hatched after 38 days. This hatching rate was reached in 17 days after 5 weeks in the fridge. A long dormancy period synchronized the hatching of resting eggs. Approximately 45% of the resting eggs had hatched the day after they were put in standard hatching conditions after 5 weeks in the fridge (grey solid line, Figure 3.6). The developmental stage of eggs seemed to have an effect on their hatching rates. Hatching was slightly quicker when the egg was still attached to its mother (Figure 3.7). This supports the hypothesis that some resting eggs may hatch rapidly after being released (bet-hedging strategy): when isolated eggs are sampled, some of these

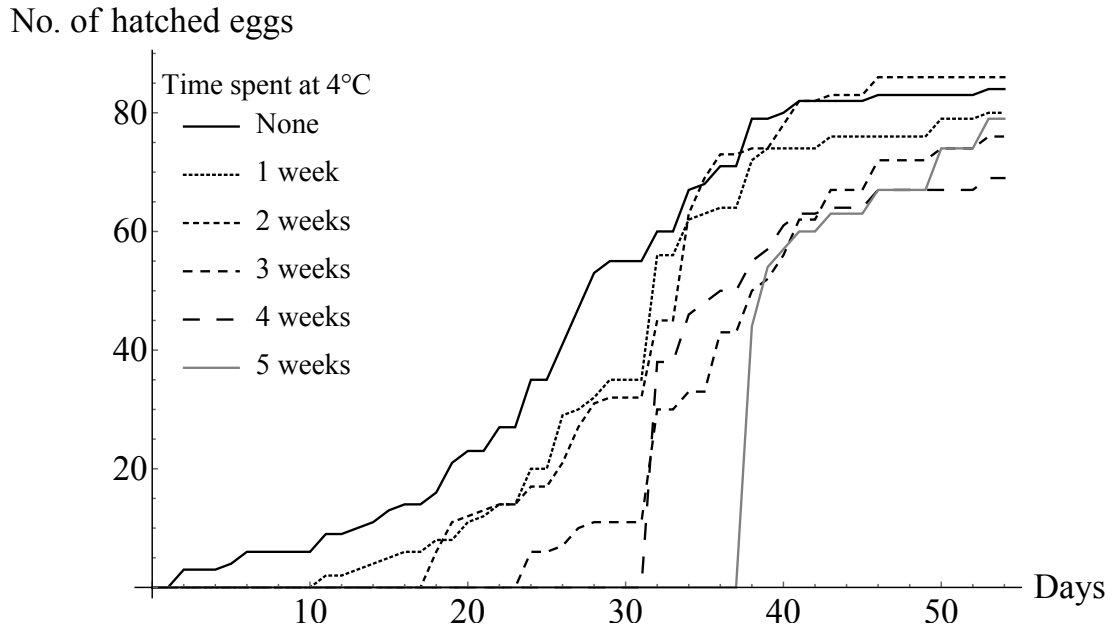


Figure 3.6: Cumulative hatching curves of resting eggs, summed over all 8 clones. Eggs spent 0 to 5 weeks at 4 °C in the dark, prior to hatching in standard hatching conditions. Hatching tests started on August 25th.

early hatching eggs may possibly be missed.

3.2.4 Genetic characterization of strains and reproductive system

All 55 clones were genetically characterized using 11 microsatellite markers (the markers were designed by Gómez et al. (1998) and Campillo et al. (2009); Table 3.1). PCR were done mixing 2 μ L of template DNA (see section 3.2.2 for extraction protocol), 0.2 mM of each nucleotide, 0.5 μ M of each primer and 0.35 U of Taq Polymerase (GoTaq[®] Flexi DNA Polymerase, Promega Corporation). The

3.2. Preliminary experiments

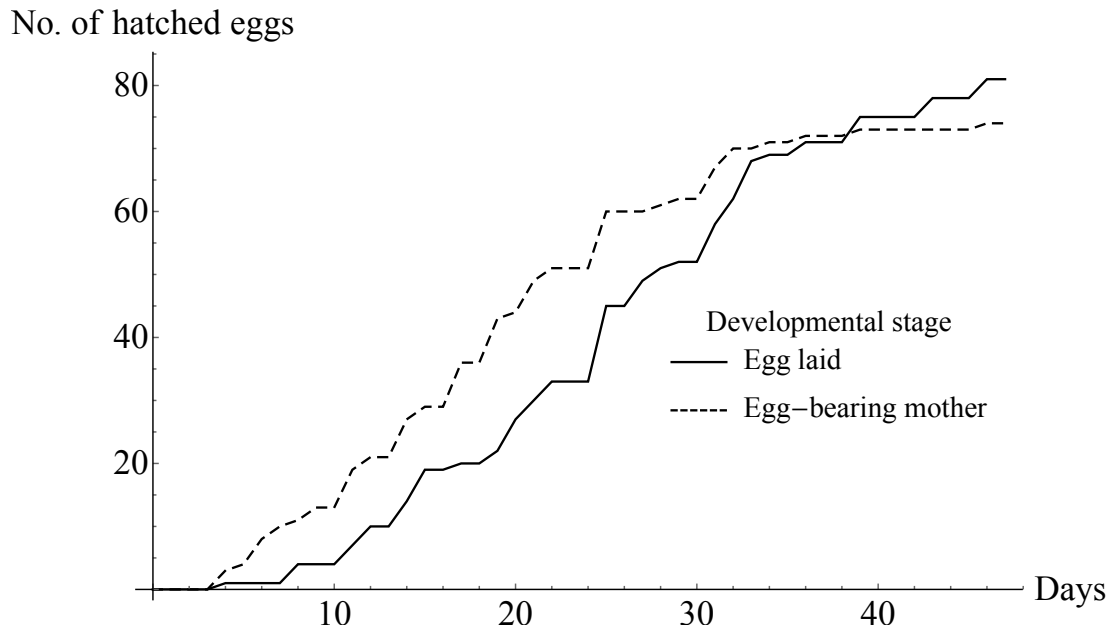


Figure 3.7: Cumulative hatching curves of resting eggs, summed over all 8 clones. The eggs were transferred alone (solid curve) or still attached to their mother (dashed curve) in standard hatching conditions. Hatching tests started on September 1st.

reactions were performed in a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc.). The composition multiplexes, fluorochrome markers used and PCR cycles are detailed in Appendix S6. For each multiplexe, 2 μ L of PCR products were mixed with a 10 μ L solution of formamide and SM594 size marker (Mauger et al., 2012). Products were separated by capillary electrophoresis using an automatic sequencer (ABI prism® 3130xl, Applied Biosystems Inc.). Alleles were then scored and sized using the program GeneMapper®v4.0, Applied Biosystems Inc.).

Genotyping was first performed on 9 or 10 individuals per clone (sampled from the tubes in which clones are maintained) from our initial 20 clones. Multilocus

genotypes for all individuals are presented in table S7.1, in appendix. The last 35 clones were genotyped later (in spring 2018), their multilocus genotypes are summarized in Table S7.2. All individuals within a clone had an identical multilocus genotype, while the few exceptions can be attributed to problems of DNA amplification. This confirms the fact that asexual females reproduce clonally. Three pairs of clones (C6–C16, C7–E15 and E16–E20), had the same multilocus genotype, the other genotypes being unique. Genetic diversity was rather low ($D = 0.261$). Three of the 11 microsatellite loci were monomorphic, while the other loci had only two or three alleles. F_{is} values (calculated using Genepop version 4.7, Rousset, 2008) ranged from -0.080 to 0.333 with an average level of $F_{is} = 0.168$ over all loci (Table 3.2), while a Fisher’s exact test showed a significant excess of homozygotes. This excess may be caused by inbreeding within the Salobrejo population, or to a Wahlund effect, since resting eggs may have been produced during different growing seasons.

Table 3.1: Microsatellite markers and primers sequences (forward and reverse) for *Brachionus plicatilis* developed in Gómez et al. (1998) and Campillo et al. (2009).

Locus	Repeat motif	Primer sequences (5' to 3')	Annealing temp. °C	No. of alleles	Allele size range (bp)
Bp3	(ACC) ₈	F: TTACCCAAGTCAAAGACGGG R: CAAGGCCTGGCTGAATGC	60	4	140–161
Bp2	(CCA) ₄ CTACCA	F: GCTGTCACTCCAAAATCATCC R: CCTGATCGCTCTTAGTGTTGC	58	6	131–167
Bp1b	CAACAG(CAA) ₂ (CAG) ₄ CAA(CAG) ₂ CAACAG	F: CATCAACAAAGACCTGCTCG R: CATGCCGTTAAACATCTGC	58	5	236–251
Bp4a	GTTGATGTTGAT(GTT) ₂ GATGTTGAT(GTT) ₂ GATGTTGAT(GTT) ₂ GAT(GTT) ₄	F: TGGTGTAGGATTCTTTGACGC R: TCAAGGAGACCGTTCAATCG	58	7	177–237
Bp6b	(AGC) ₆ CAAC(AGC) ₂	F: ATATCGGACGAAGAAGAGGC R: CCACCCATGAATGTAGTTGG	58	3	121–127
Bp3c	(GTT) ₆ AATGTTAAT(GTT) ₃ (TG) ₃ ACT (GTT) ₃ ATT(GTT) ₂ ATTGTT	F: GTTAAGCGAGTGGGTCTTGG R: TAGTGTATCTGCCTGCTCCG	58	5	190–205
Bp5d	(CAA) ₆	F: ATATCGTCGTCAATGTCCGC R: TGTGCTCGCGTAGTAGTTGG	58	4	236–245
Bp7	(TC) ₇	F: ATCAACTAATATGTGACAAGACAAC R: TAAAGTATTAAAAGCCAAGATAACG	55	8	165–191
Bp8	(CCAACG) ₇ (CCAACA) ₃	F: GAGTTTTTTTCAACGCTATCGC R: TGCCAAATTGATACTTTTTTGC	55	13	186–301
Bp9	(GA) ₅ CA(GA) ₈	F: AGCAGGTTTTTGTACGTCTGG R: TGCCAAATTGATACTTTTTTGC	55	5	279–287
Bp10	(TG) ₁₀	F: GATCAACTAAAAATGTTCAAGG R: TAGAACAAAACAAAAAGGTG	55	10	392–454

Table 3.2: Summary statistics over all 55 clones, for 11 microsatellite loci: n_a , number of alleles; D , gene diversity (heterozygosity) and F_{is} , inbreeding coefficient (Weir & Cockerham, 1984).

	Bp1b	Bp2	Bp3	Bp3c	Bp4a	Bp5d	Bp6b	Bp7	Bp8	Bp9	Bp10	All
n_a	2	3	1	3	1	1	3	2	3	2	2	
D	0.152	0.645	0	0.464	0	0	0.229	0.323	0.358	0.440	0.264	0.261
F_{is}	-0.080	0.224	–	0.333	–	–	0.205	0.100	0.034	0.173	0.105	0.168
p -value	1	0.017	–	< 0.001	–	–	0.005	0.353	0.267	0.100	0.364	0

In order to further test the hypothesis that asexual reproduction is genetically equivalent to mitosis (instead of some form of automixis or selfing that would lead to increased homozygosity), and test that both sexual and asexual females are produced by the same mechanism, we genotyped females over 3 asexual generations. Two continuous replicate populations (called G and D) were initiated from a mix of our 20 initial clones (the experimental setup will be detailed in Chapter 5). The populations were allowed to grow freely for 3 months. We then sampled asexual females (P_0) from the two populations and transferred them to petri-dishes (\emptyset 90 mm) with 10 mL of culture medium at a concentration around 5×10^5 cells mL⁻¹. Their first offspring (P_1) was isolated in a new petri-dish with fresh medium. The offspring of second generation (F_2 descendants of P_1) were isolated in 48-wells plates with culture medium ($\approx 5 \times 10^5$ cells mL⁻¹) until they reproduced. The individuals giving birth to females or males were typed as asexual and sexual, respectively. We also typed several F_1 offspring (descendants

3.2. Preliminary experiments

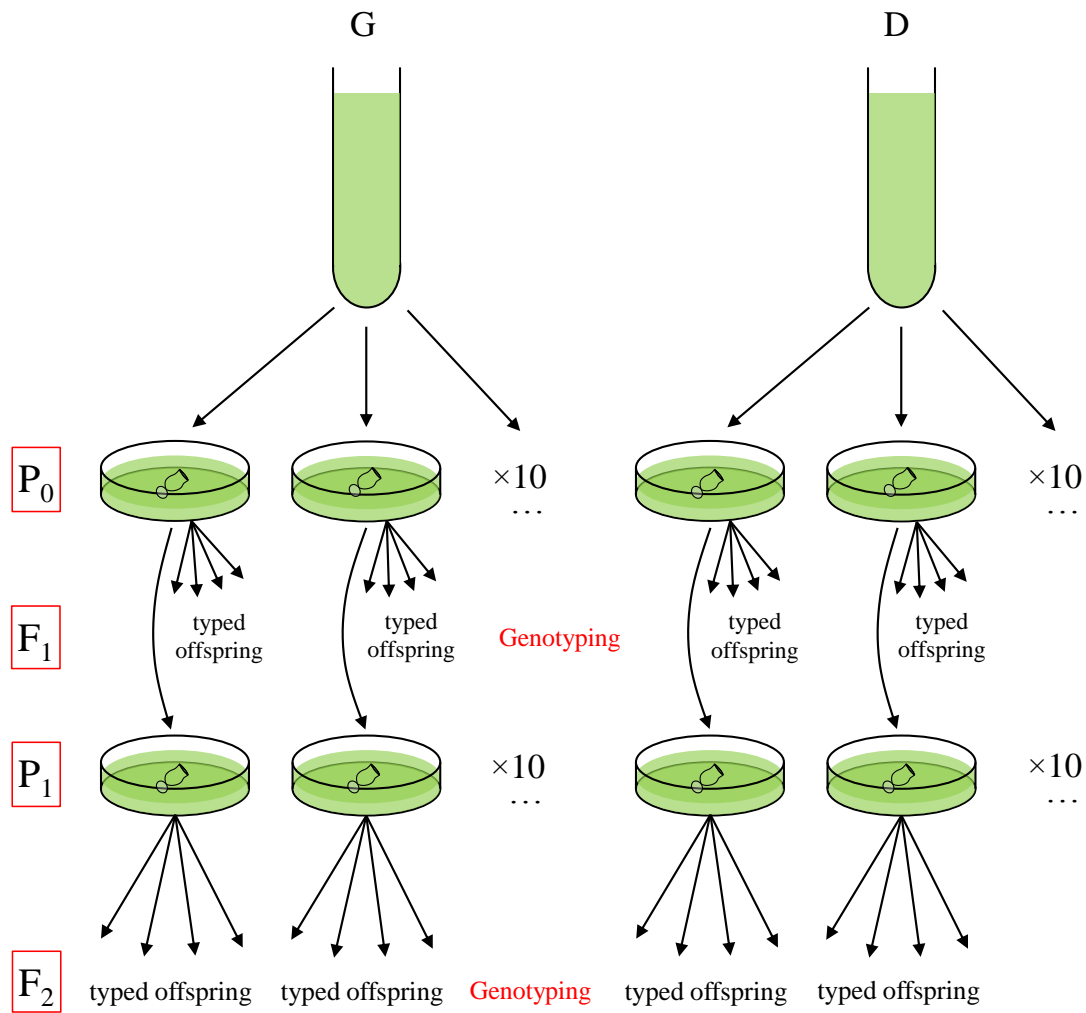


Figure 3.8: Protocol for genotyping over generations, distinguishing sexual and asexual females. The populations G and D were initiated by a mix of 20 clones and allowed to grow for three months in chemostats before the beginning of the assay. Individuals that were genotyped are framed in red.

of P_0) when possible (Figure 3.8). All offspring were then transferred in 96-well plates for genotyping (see section 3.2.2 for DNA extraction protocole, above for microsatellite genotyping), along with the two mothers P_0 and P_1 at the end of the assay.

We maintained and genotyped a total of 10 lineages for each population. Lineages included the parents P_0 and P_1 , 1 to 6 F_1 offspring when possible and 9 to 11 F_2 offspring. Overall, multilocus genotypes were well conserved over the three generations, the few exceptions being more likely to be attributed to sequencing errors due to poor DNA quantity and quality (results not shown). No differences of heterozygosity were found between sexual and asexual offspring. This result confirms that reproduction of asexual females occurs through a mechanism which is genetically equivalent to mitosis.

3.2.5 Effect of different forms of abiotic stress and of reproductive mode on fitness

Several measures may be used to estimate the fitness of individuals or genotypes. During this thesis, fitness was measured either as the number of offspring produced by an asexual female over the course of its life, or as the growth rate of a clone initiated by one individual (the latter being the less tedious to measure).

One of the aim of this thesis was to test the effect of sex on adaptation to a

3.2. Preliminary experiments

new environment (Chapter 5). With this aim in mind, we investigated the effect of different forms of environmental stress on the fitness of individuals (this also allowed us to test different protocols for fitness estimation). We also tested the effect of the reproductive mode (sexual or asexual) on the mean and variance in fitness of offspring in the different environments. The reproductive mode (sexual or asexual) should have an effect on fitness if the initial population presents genetic associations within or between loci affecting fitness (such as linkage disequilibrium between loci, excess of homozygosity or heterozygosity, etc.; see Peters and Otto, 2003) that are maintained by asexual reproduction, but broken by sex. In a similar experiment performed on *Brachionus calyciflorus* under standard culture conditions, Becks and Agrawal (2011) showed that sex between natural isolates decreases both the mean fitness of offspring and the variance in fitness, compared with asexual reproduction. We thus wanted to test if similar effects of the reproductive modes are observed using our clones, under the different tested environments. Additionally, we measured the fitness of offspring produced by intraclonal mating (genetically equivalent to selfing) in order to test for inbreeding depression.

Evidence of inbreeding depression was found in rotifer populations despite their peculiar life-cycle – the production of haploid males should enable the purging of deleterious alleles, at least for genes expressed in both sexes (Tortajada et al., 2009). Moreover, it has been shown in different plant species that the magnitude

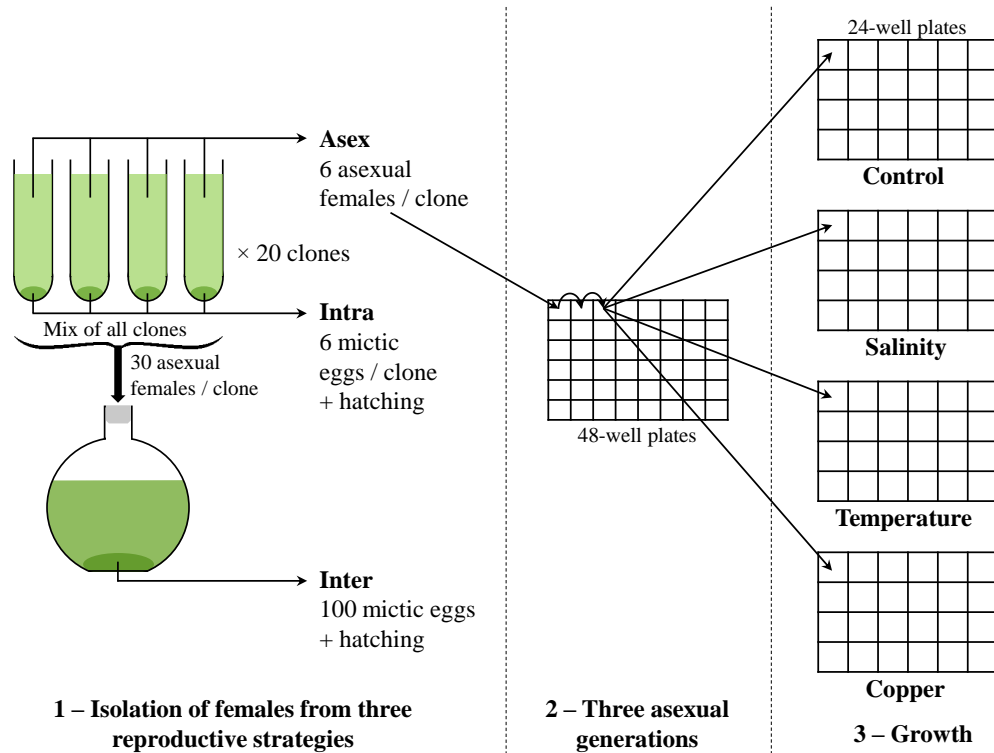


Figure 3.9: Step by step protocol for fitness measures under different forms of environmental stress. 1 – Sampling procedure of individuals produced by the three reproductive modes: asexual reproduction within clones (Asex), sexual crosses within clones (Intra) and sexual crosses between clones (Inter). Resting eggs were hatched after placing them at 4 °C in the dark for 6 weeks. 2 – Each female was let to reproduce for three clonal generations (to avoid maternal effects) in 48-well plates. 3 – Third generation females were transferred into single wells of 24-well plates containing 1 mL of culture medium (2×10^5 cells mL⁻¹) corresponding to each experimental treatment, and were let to reproduce for 5 days.

3.2. Preliminary experiments

of inbreeding depression is often increased in stressful environments (*e.g.*, Cheptou et al., 2000; Armbruster and Reed, 2005), and we thus wanted to test whether inbreeding depression could be stronger under our stressful conditions.

A total of four experimental treatments were carried out simultaneously: a control in standard culture conditions, and in environments with higher salinity (23.5 g L^{-1}), lower temperature (13°C) or under oxidative stress due to the addition of copper (0.1 mg L^{-1}). Thirty mature asexual females from each of our 20 initial clones were mixed together in a flask with *T. suecica* as food, and were allowed to reproduce until reaching high densities. Resting eggs produced by sexual reproduction – crosses between clones (Inter) – were collected and stored for 6 weeks at 4°C in the dark: 100 eggs were then hatched in 96-well plates (one egg per well) with $300 \mu\text{L}$ of 6ppt artificial seawater and algae. Sexual eggs were also collected from the tubes in which the same 20 clones are maintained – crosses within clones, genetically equivalent to selfing (Intra) – and also stored for 6 weeks at 4°C in the dark: 6 eggs per clone were then hatched in the same conditions as above. Finally, 6 asexual females per clone were collected from the tubes of the clones (Asex). The sampling design is detailed in Figure 3.9. Hatchlings and asexual females were let to reproduce for three clonal generations, in order to avoid maternal effects. Four third generation neonates (identical genotype) were transferred for each experimental treatment into single wells of 24-well plates containing 1 mL of

culture medium (2×10^5 cells mL⁻¹) where they were let to reproduce during 5 days. For each treatment, we tested 20×6 offspring from asexual females, 20×6 offspring from within-clone crosses and 78 offspring from between-clones crosses (a proportion of the 100 resting eggs sampled did not hatch or deteriorated). Female density was monitored three times within 5 days. The slope of linear regressions fitted on log-transformed data was used as an estimation of growth rates.

All treatments significantly affected the rotifers by decreasing their growth rates (Kruskall-Wallis non-parametric test, $p < 0.001$). No significant difference was found between Intra and Inter reproductive strategies (and thus no significant inbreeding depression) in the control, salinity and temperature treatments (Figure 3.10) – note however that due to our experimental protocol, some of the eggs of the Inter reproductive scenario may have been produced by intraclonal selfing. The difference in growth rates between Intra and Inter reproductive strategies was significant in the copper treatment, but was rather small (Intra = 0.316 and Inter = 0.354). The fitness of asexually produced offspring was not significantly different from the fitness of outcrossed offspring, except in the low temperature treatment where they had a higher fitness, the growth rate of asexually produced individuals being significantly higher than those of sexually produced individuals. No clear tendency emerged regarding the effect of the reproductive mode on the variance in fitness among offspring: for example, outcrossed offspring had a higher variance

3.2. Preliminary experiments

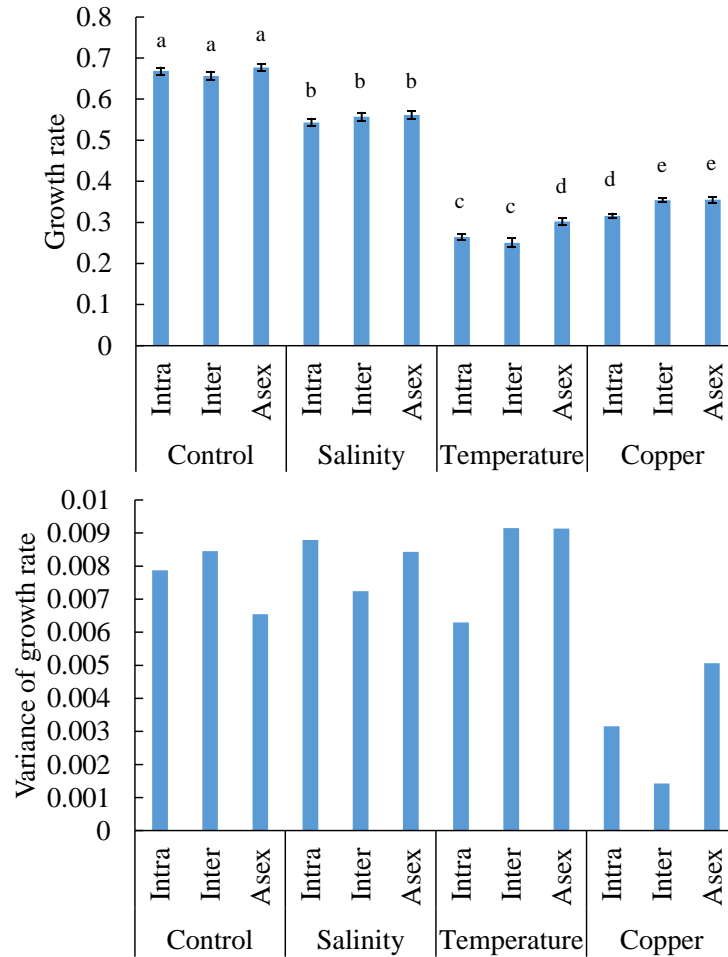


Figure 3.10: Mean ± 1 S.E. (top) and variance (bottom) of growth rates for different stress treatments: control, higher salinity (23.5 g L^{-1}), lower temperature (13°C) and oxidative stress due to the addition of copper (0.1 mg L^{-1}); and different reproductive scenarios: within-clones crosses (Intra), between-clones crosses (Inter) and asexual reproduction (Asex). Different letters represent significantly different groups (non-parametric Kruskal-Wallis tests, $p < 0.05$).

Chapter 3. Preliminary experiments on the *Brachionus plicatilis* system

in fitness than asexually produced or selfed offspring in standard conditions, but a lower variance in the low-salinity or the copper enriched environments.

Overall, this experiment enabled us to set up our protocols for fitness measures and test different environmental stresses. We did not detect any immediate effect of the reproductive mode on the mean fitness of offspring in these different conditions, which suggests that our initial population did not harbor any strong genetic association between loci affecting fitness in these environments. Eventually, we opted for oxidative stress in the adaptation experiment presented in Chapter 5. Indeed, it was rather complicated to maintain populations at different temperatures in the same room, while a high salinity is known to have important effects on the induction of sex in *Brachionus plicatilis* (Lubzens et al., 1980, 1985).

In the following chapter, we will explore endogenous and environmental factors affecting investment in sex in our strains of *Brachionus plicatilis*.

Chapter 4

Variability in the propensity for sex

4.1 Preliminary experiments on the propensity for sex in experimental rotifer populations

4.1.1 Setup of the chemostat system to maintain rotifer populations

In autumn 2016 we set up a chemostat system to maintain rotifer populations in continuous culture. The general set up is similar to the system used in Tarazona et al., 2017 (see Figure 4.1). A first chemostat contains the culture of *Tetraselmis*

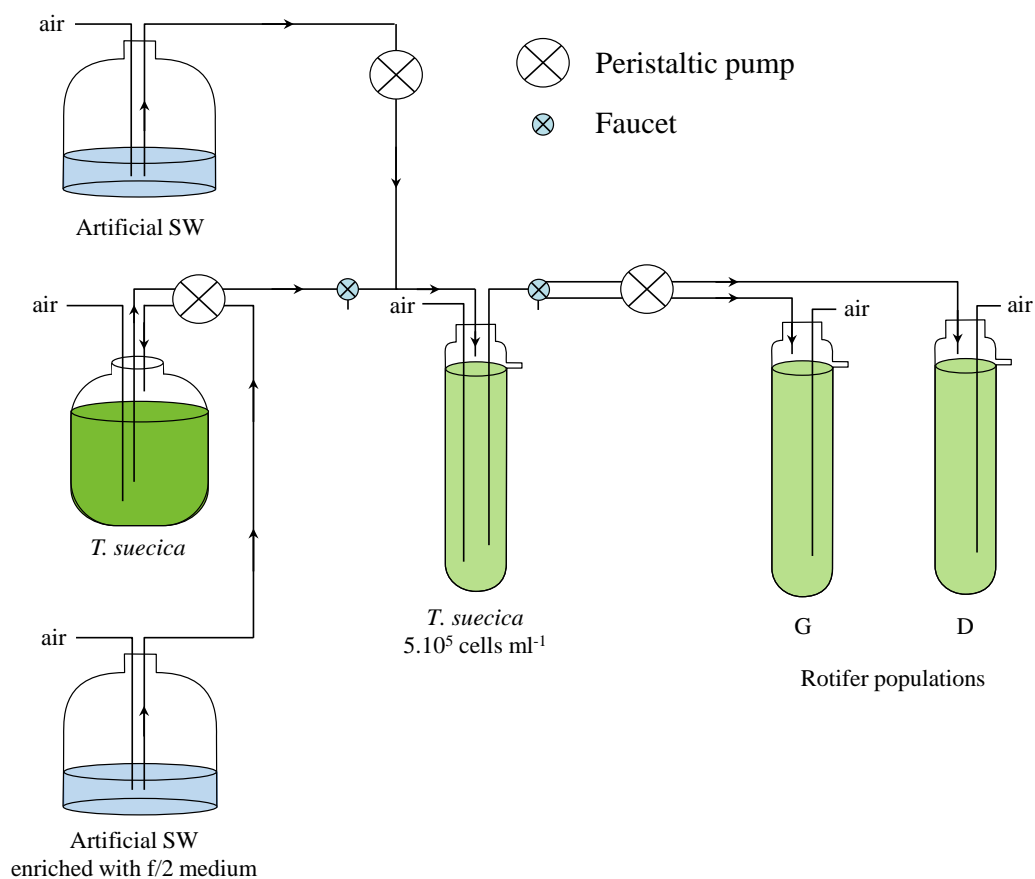


Figure 4.1: Chemostat system to maintain rotifers in continuous culture. A high-density culture of *T. suecica* is maintained by the continuous input of artificial seawater (12 g L^{-1} ; Instant Ocean[®], Aquarium Systems) enriched with f/2 culture medium (Guillard and Ryther, 1962), diluted to a concentration of $5 \times 10^5 \text{ cells mL}^{-1}$, and distributed at a constant rate to both rotifer populations (labeled G and D). The whole system was maintained at 20°C with a 12/12-hours photoperiod. Modified from the internship report of Elisa Leroux.

4.1. Preliminary experiments on the propensity for sex in experimental rotifer populations

suecica used to feed rotifers: a peristaltic pump brings a constant inflow of artificial seawater (12 g L^{-1} , Instant Ocean) enriched with f/2 culture medium (Guillard and Ryther, 1962) to the algal culture. The same pump extracts algal culture at the same rate (400 mL per 24h, for a 2 L culture). A faucet allows to sample algae coming from the culture, in order to estimate their density (using a Malassez hemocytometer). The density is then adjusted to $5 \times 10^5\text{ cells mL}^{-1}$ through a second pump mixing artificial seawater to the extracted algal culture (see Figure 4.1). The resulting mix is stored in a 1 L glass tube wrapped with aluminum foil (to avoid algal growth). A third pump then distributes algae to rotifer populations present in 1 L glass tubes (at a rate of 100 mL per 24h). The same volume is continuously removed from rotifer populations through an overflow system. Two replicate rotifer populations (labeled G and D) were initiated by mixing our 20 initial clones in November 2016, in order to see if we could maintain this continuous culture system over time. The system proved to work well as we maintained our test populations for almost a year (during that time, algae from the algal chemostat were used for the other experiments described in Chapter 3). During that time, we took several samples of individuals from both populations and genotyped them at 11 microsatellite loci, in order to assess to what extent sexual reproduction could contribute to population turnover in our culture conditions. We also estimated the propensity for sex of sampled individuals (as detailed below) in order to check if

propensity for sex could evolve in our populations. Sex is costly as it involves the production of males, and of resting eggs that take more time to hatch than asexually produced eggs, and we thus expected that lower propensities for sex should be favored under our experimental setting.

4.1.2 Propensity for sex

The first measure of propensity for sex was performed in December 2016, approximately one month after the rotifer populations had been initiated. For this, between 30 and 40 asexual egg-bearing females were sampled from each population (replicates) and transferred separately into petri-dishes (\varnothing 55 cm) containing 10 mL of culture medium. Their offspring were transferred into fresh medium for three asexual generations, in order to limit maternal effects (Carmona et al., 2009). Several third-generation newborn females (between 3 and 8) per replicate were isolated in wells of 24-well plates with 500 μ L of 5×10^5 cells mL⁻¹ culture medium. Individuals were allowed to reproduce and were monitored every 24h for several days. Their propensity for sex was measured as the density of females at which we observed the first male. A lower density corresponds to a higher propensity for sex (Aparici et al., 2001; Carmona et al., 2009; Gabaldón and Carmona, 2015).

Three new measures of propensity for sex were then obtained using the same protocol in March, June and September 2017 to investigate the evolution of the

4.1. Preliminary experiments on the propensity for sex in experimental rotifer populations

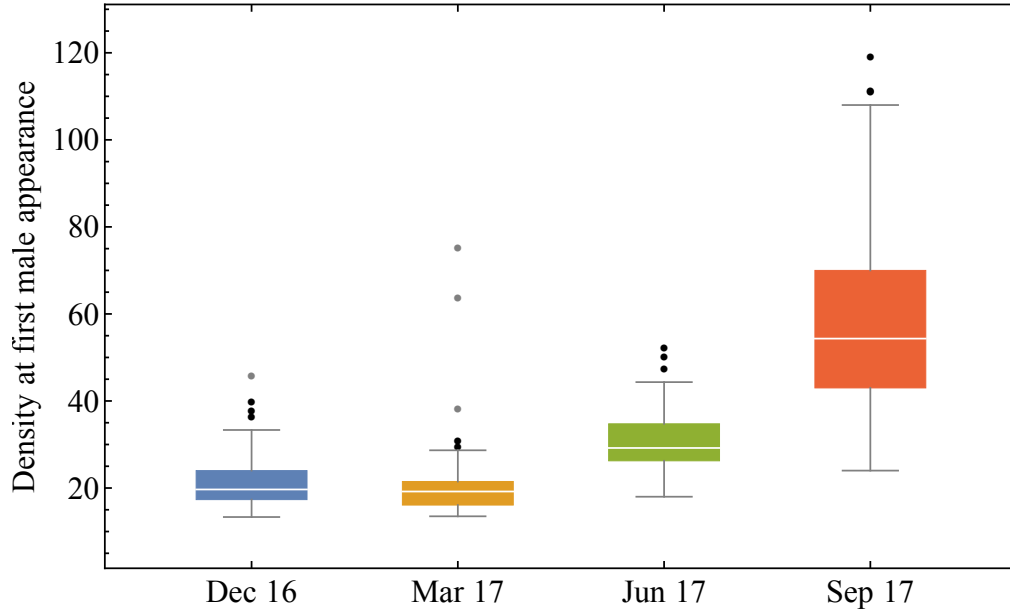


Figure 4.2: Propensity for sex (± 1 S.E.), measured as the density of females (number of females mL⁻¹) at first male appearance, over time in our two experimental populations G and D. The boxes represent the median and the first and third quartiles.

propensity for sex in our laboratory conditions. The difference in propensity over time was analyzed using Kruskal-Wallis non-parametric tests, comparing the different time measures.

We observed that after a few months, the density at first male appearance significantly increased over time in both populations ($\chi^2(3) = 493.61, p < 0.001$; Figure 4.2). This result is in accordance with our expectation that sex should be disfavoured in our constant, optimal environment due to the cost of sex.

However, maternal effects can impact rotifers for several generations after their

birth, particularly when considering mictic female production. Hino and Hirano (1977) observed that some strains of *Brachionus plicatilis* produced mictic females only 20 clonal generations after hatching from a resting egg, with a high variability between strains (we will see in section 4.2 that a similar inhibition of sex during the first clonal generations after hatching from a sexually produced egg occurs in our rotifers). The age (Pourriot and Rougier, 1976; Rougier and Pourriot, 1977) or the environment (Aparici et al., 2001) of the mother have also been shown to influence the reproductive mode of its offspring in various species of rotifers, including species of the genus *Brachionus*. When measuring propensity for sex, we do not know how many generations separate the females tested from the last resting egg in her lineage.

In order to better control this factor, we measured the propensity for sex after 15 asexual generations. In October 2017, we sampled 30 asexual egg-bearing females from each experimental population (G and D), and also 3 from each of our 20 initial clones (in order to compare the propensity for sex of our evolved populations with the propensity of the initial clones), and transferred them into single wells of 48-well plates containing 300 μL of culture medium (2×10^5 cells mL^{-1}). Their offspring were transferred into new wells with fresh medium, until 15 asexual generations were reached. Between 3 and 6 juveniles of the fifteenth generation were isolated in wells of 24-well plates with 500 μL of algae (5×10^5 cells mL^{-1}). The propensity

4.1. Preliminary experiments on the propensity for sex in experimental rotifer populations

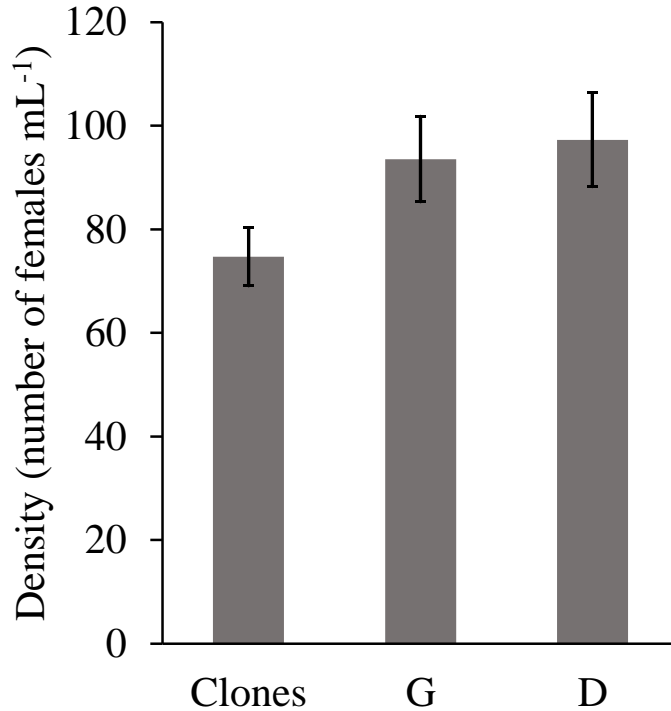


Figure 4.3: Propensity for sex, measured as the density of females (number of females mL⁻¹) at first male appearance, 15 asexual generations after sampling females from experimental populations (G and D) or from the tubes in which clones are maintained. Propensity is averaged over our 20 initial clones (≈ 3 individuals per clone, 6 replicates per individuals), and over 30 individuals sampled from each of our two experimental populations (with 6 measures per individual). The bar represents $\pm 1\text{SE}$. Results for each clone are presented in the next section.

for sex was then again estimated by measuring the density of females at first male appearance.

In the experimental populations G and D, after 15 asexual generations, the average density at first male appearance was 93.58 and 97.30 females mL⁻¹, respectively (Figure 4.3). The propensity for sex is significantly lower (higher density at first male appearance) than the propensity measured in September 2017

($\chi^2(1) = 77.815, p < 0.001$; Figure 4.2). However, the propensity for sex averaged over our clones was also low (density at first male appearance: 74.70 females mL^{-1} ; Figure 4.3). This density is surprisingly high compared to the first measure of propensity in the experimental populations, a month after these populations were initiated from a mix of the same clones (around 20 females mL^{-1} in December 2016; Figure 4.2): indeed, we would have expected that the propensity for sex in the initial populations (consisting in a mix of the 20 clones) should be close to the average propensity of those clones. Three possible (an non exclusive) explanations can be proposed to explain this discrepancy. The first hypothesis is that the propensity for sex was affected by uncontrolled environmental factors that varied between our measurements, causing the higher densities at first male appearance observed in Sept-Oct 2017 (however, it is not clear what these factors may have been). A second possible hypothesis is that the propensity for sex quickly evolved towards higher values during the first month in the experimental populations, either due to a benefit of sex (for example, if sex allows better adaptation to the environmental conditions of these populations, which must differ from natural conditions), or to genetic correlations (if genotypes having higher propensities for sex happened to have higher fitness in this environment). Then, lower propensities for sex would have evolved once the populations are adapted, due to the cost of sex. The last possible hypothesis is that evolution towards lower propensities of sex

4.1. Preliminary experiments on the propensity for sex in experimental rotifer populations

occurred in the tubes in which the clones were individually maintained, between November 2016 and October 2017. This evolution should have occurred through new mutations, since we checked that the microsatellite genotypes of clones had remained identical (data not shown). As we did not measure the propensity for sex of the different clones during the first measurement done on the populations, we cannot discriminate between these hypotheses. However, the observed difference in average propensities for sex of the clones and of the G and D populations (done in the same environmental conditions and after 15 clonal generations) shown on Figure 4.3 shows that the propensity for sex can evolve. In section 4.2 we will show further evidence for genetic variation in the propensity for sex among clones.

4.1.3 Evolution of the genetic composition of experimental populations

To have an idea of whether sexual reproduction contributes to the turnover of individuals in our populations, we also genotyped the individuals used for the propensity tests, using our 11 microsatellite markers (detailed protocol in Chapter 3).

Interestingly, only a month after the initiation of the experimental populations, we observed an important proportion of genotypes that were different from the genotypes of the 20 initial clones (Figure 4.4) and that did not carry any mutant

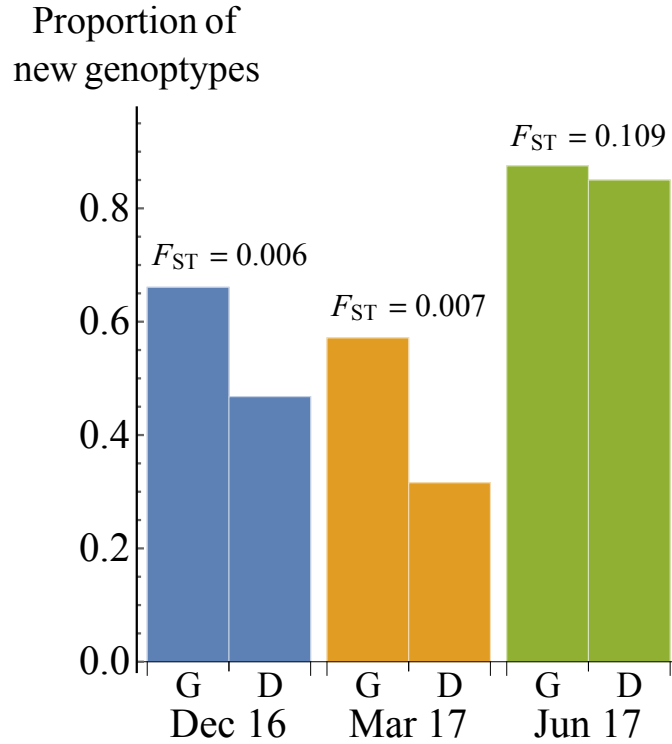


Figure 4.4: Proportion of new genotypes compared to our 20 initial clones over time. The individuals genotyped are those from which we measured the propensity for sex in Figure 4.2. The genetic differentiation between G and D populations (F_{ST}) is indicated above each time sample.

allele. These new genotypes must correspond to individuals produced by sexual reproduction (note that sexual reproduction may also lead to genotypes that are identical to the clone genotypes).

The proportion of new genotypes increased over time (Figure 4.4), along with the genetic differentiation between the two populations ($F_{ST} = 0.006$ in December 2016, $F_{ST} = 0.109$ in June 2017). In the analysis shown in Figure 4.4, we did not take into account possible sequencing errors due to amplification problems. For

4.1. Preliminary experiments on the propensity for sex in experimental rotifer populations

instance, individuals that are identified as homozygous might actually be heterozygous with one of the alleles that was undetected. Individuals thus might have been wrongly identified as new genotypes. We repeated the analysis assuming that at each locus, an homozygous individual (sampled from the populations) may have been heterozygous. Doing so, we still observe between 8 and 32% of new genotypes over time (Figure S8.1, in appendix), indicating that sexual reproduction indeed contributes to population turnover.

Finally, in the last sample (June 2017), two different genotypes had reached high frequency in the two populations, one in frequency 32.5% in population G, and the other in frequency 25% in population D . However, the individuals carrying these genotypes did not have a different propensity for sex than the other tested individuals, and the lower propensity for sex observed in the sample of June 2017 is thus not due to the spread of these genotypes (Figure 4.2).

In the next section (manuscript in preparation), we present the results of our analysis of variation for the propensity for sex among our 20 clones, measured after 15 asexual generations, and of another experiment exploring the effect of the number of generations after fertilization on investment in sex.

4.2 Genetic variability and endogenous regulation of investment in sex in the monogonont rotifer *Brachionus plicatilis*

Océane Seudre*, Eloïse Vanhoenacker*, Jérôme Coudret and Denis Roze, *manuscript in preparation* (* equal contribution of both authors)

4.2.1 Introduction

Many hypotheses have been proposed to explain the widespread occurrence of sexual reproduction among eukaryotes (*e.g.*, Otto, 2009; Hartfield and Keightley, 2012). However, while these hypotheses have stimulated a large number of theoretical studies, our empirical knowledge of the possible evolutionary benefits of sex still remains scarce. This mostly stems from the general difficulty in comparing the effects of sexual and asexual reproduction on the distribution of fitness among offspring in natural conditions. Nevertheless, experimental evolution under controlled conditions has provided important insights, showing in particular that sex increases rates of adaptation to new environments (*e.g.*, Kaltz and Bell, 2002; Colegrave, 2002; Goddard et al., 2005; Lachapelle and Bell, 2012), and that recombination may free novel beneficial alleles from linkage with deleterious mutations

4.2. Regulation of investment in sex in *Brachionus plicatilis*

during phases of adaptation (McDonald et al., 2016). However, to what extent these benefits may allow genotypes coding for higher rates of sex or recombination to increase in frequency remains unclear, given the important costs generally associated with sexual reproduction (*e.g.*, Lewis, 1987; Lehtonen et al., 2012). Experimental evolution on facultatively sexual organisms represents a promising avenue of research: in particular, Becks and Agrawal (2010, 2012) and Luijckx et al. (2017) showed that higher rates of sex may evolve in experimental populations of monogonont rotifers adapting to a new environment or living in a heterogeneous habitat.

Monogonont rotifers are small invertebrates (50 to 2000 μm) living in a variety of aquatic or moist habitats (Wallace et al., 2006). They are cyclical parthenogens, often reaching very large population sizes due to high rates of clonal reproduction. Rotifer populations are typically temporary at temperate latitudes, the growing season starting by the hatching of sexually produced eggs present in the sediment. The hatchlings are diploid asexual females, producing other females by ameiotic parthenogenesis. After an initial phase of population growth, sexual and asexual reproduction co-occur within populations: sex is induced by an environmental factor, causing parthenogenetic females to produce some sexual (mictic) females among their offspring. These sexual females produce haploid eggs by meiosis, which, if not fertilized, develop into dwarf haploid males. If sexual females are

inseminated while they are still young, they produce diploid diapausing eggs (also called resting eggs) formed by regular gamete fusion. These diapausing “eggs” actually consist in multicellular embryos that can resist desiccation and adverse environmental conditions, and may remain viable for several years (Lubzens et al., 2001). After a dormant phase, they can hatch when the environmental conditions become favorable again.

In rotifers from the *Brachionus* genus, the switch from asexual to sexual reproduction is mainly controlled by population density, through a form of quorum sensing mechanism involving a protein (the “misis-inducing protein” or MIP) produced by the females themselves (Carmona et al., 1993; Stelzer and Snell, 2003, 2006; Snell et al., 2006). Investment in sex may be measured by the threshold population density at which sexual females start being produced (called the “misis threshold”), and was shown to vary between species, and between strains from the same species (Gilbert, 2017 and references therein). Variations in the misis threshold between isolates from the same natural population was demonstrated in the brackish-water species *Brachionus plicatilis* (Carmona et al., 2009; Gabaldón and Carmona, 2015), and in the freshwater species *Brachionus calyciflorus* (Becks and Agrawal, 2010). A different measure of investment in sex is the “misis ratio”, corresponding to the proportion of sexual females among offspring (once the misis threshold has been reached); variation for the misis ratio among strains has also

4.2. Regulation of investment in sex in *Brachionus plicatilis*

been demonstrated, but to what extent the mixis ratio correlates with the mixis threshold remains unclear (Gilbert, 2017).

Other environmental factors such as salinity or type of food may also affect investment in sex in monogonont rotifers (*e.g.*, Lubzens et al., 1993). Interestingly, Gilbert (2002, 2003) showed that in *Brachionus calyciflorus*, the mixis ratio is affected by endogenous factors that may persist over several generations: in particular, sexual reproduction is inhibited during the first clonal generations following fertilization, with a gradual increase in the mixis ratio over the first 10-12 clonal generations after hatching of a resting egg. The same pattern was observed in different monogonont species, but was absent in others (*e.g.*, Schröder and Gilbert, 2004; Gilbert, 2017). Although Gilbert (2003) and Schröder and Gilbert (2004) observed variation between genotypes from the same natural population in the rate of increase of the mixis ratio over clonal generations, the heritable component of this variation cannot be assessed from these experiments, as only a single replicate per genotype was performed. Conversely, this type of transgenerational effect may possibly have affected previous estimates of genetic variation for the mixis threshold, as the number of clonal generations since the last fertilization event is generally not controlled in experiments in which this variation is measured.

Understanding the different sources of variation of investment in sex (and the possible contribution of transgenerational maternal effects) is important for the

design and interpretation of evolution experiments using rotifers. In this paper, we quantify genetic variation for the mixis threshold among 20 different strains from the same natural populations of *Brachionus plicatilis*, measured after 15 clonal generations under controlled conditions. In a second experiment, we use a subset of 4 strains with contrasted mixis thresholds, to assess the effect of the number of clonal generations after fertilization on the mixis ratio. The results show important differences between genotypes in their investment in sex, and a correlation between the two measures of investment in sex (mixis threshold and mixis ratio).

4.2.2 Material and methods

Isolation and culture conditions of rotifer strains. Clones of *B. plicatilis* were obtained from the hatching of resting eggs present in sediment sampled from Salobrejo Lake (Eastern Spain) in September 2013, and kindly provided by María José Carmona and Eduardo García Roger (University of Valencia). This particular population was chosen because genetic variation for the mixis threshold had been found in a previous study (Gabaldón and Carmona, 2015). Resting eggs were extracted from the sediment using the sugar flotation technique (Gómez and Carvalho, 2000), and hatched by placing them in artificial seawater (Instant Ocean[®], Aquarium Systems) at 12 g L⁻¹ salinity, under constant illumination and at 22 °C. Upon hatching, individuals were transferred to culture medium (referred hereafter

4.2. Regulation of investment in sex in *Brachionus plicatilis*

as standard culture medium), consisting in f/2-enriched artificial seawater (Guillard and Ryther, 1962) containing 2×10^5 cells mL⁻¹ of the microalgae *Tetraselmis suecica* used for food (our algal culture was maintained in exponential growth in a chemostat throughout the experiment). Because two cryptic species of rotifers (*B. plicatilis* and *B. manjavacas*) coexist in Salobrejo Lake (Gabaldón and Carmona, 2015), the first offspring of each hatched individual was collected for species identification using the RFLP-PCR method described in Gabaldón et al. (2013). Twenty hatched *B. plicatilis* individuals were identified, and individually transferred into 30 mL glass tubes containing standard culture medium to maintain clonal growth.

Estimation of mixis thresholds after 15 clonal generations. Three asexual females from each of the 20 clones were sampled and transferred into single wells of 48-well culture plates (Greiner Bio-OneTM) containing 0.3 mL of standard culture medium, and maintained in a culture chamber at 22 °C. Their first offspring were transferred into new wells with fresh medium, until 15 clonal generations were reached (when a sampled offspring was a sexual female, it was replaced by another offspring from the same mother until obtaining an asexual female). For each clonal line, 6 juveniles of the fifteenth generation were isolated and individually transferred into wells of 24-well culture plates (Greiner Bio-OneTM) containing 0.5 mL of culture medium with an algal concentration of 5×10^5 cells mL⁻¹, where they

were let to reproduce. If the sampled female was sexual, it was replaced whenever possible by another fifteenth generation female from the same mother. Wells were inspected visually every 24h until the first males were observed, in which case the population density was measured by counting the number of females present in the well. This density corresponds to the estimated mixis threshold (Carmona et al., 2009).

Effect of the number of clonal generations following fertilization. A subset of 4 clones with contrasted mixis thresholds was chosen based on the results of the previous experiment (clones 6, 8, 10 and 16, see Results section). Resting eggs were collected from the bottom of the tubes in which those clones were maintained, transferred into Petri dishes containing artificial seawater at 12 g L^{-1} salinity, and maintained in the dark and at 4°C during 3 months. These resting eggs were produced by intraclonal mating, which is genetically equivalent to self-fertilization. Resting eggs were then isolated into single wells of 48-well plates (Greiner Bio-OneTM) with 0.3 mL of standard medium, and placed at 22°C and under constant illumination to induce hatching. For each clone, five hatched females were sampled at random to form our first generation (G1). Note that these five females have been produced by different intraclonal fertilization events, and thus carry different genotypes (however, two females from the same clone are more related

4.2. Regulation of investment in sex in *Brachionus plicatilis*

than females from two different clones). The first three juveniles (G2) produced by each G1 female were collected to initiate clonal lines – note that the three G2 individuals produced by the same G1 female are genetically identical, since they are produced asexually. Clonal lines were maintained in 48-well plates (Greiner Bio-One™) that were inspected daily. When a female of a given generation had produced its first juvenile, the juvenile was transferred into a new well with 0.3 mL of fresh standard culture medium. If the juvenile developed into a sexual female or died before reproducing, it was replaced by another juvenile produced by the same mother. Clonal lines were maintained until the 24th generation (G24), at the exception of clonal lines from clone 8, which took more time as more sexual females were produced, and which were maintained for 18 generations only. At generations 2, 5, 8, 12, 18 and 24, one juvenile female was sampled from each clonal line to measure its mixis ratio. For this, the tested female was placed in a well of a 48-well plate (Greiner Bio-One™) with 0.3 mL of sex-inducing medium, consisting in standard culture medium with an algal concentration of 4×10^5 cells mL⁻¹, mixed in equal proportions with filtrate obtained from a previous rotifer culture that had reached a density of approximately 20 females mL⁻¹ (filtrated on a 0.2 µm mesh). This resulted in an equivalent density of 10 females mL⁻¹, which is well above the density required to induce sex in most populations (Gilbert, 2017). Every day until its death (which generally occurred after 10 to 15 days), the tested female

was transferred into a new well containing 0.3 mL of fresh sex-inducing medium, and its offspring were collected and individually transferred to a single well of a 96-well plate (Greiner Bio-OneTM) containing 0.2 mL of standard culture medium. When offspring started to reproduce, they were typed as asexual (if they produced females) or sexual (if they produced males).

Data analyses. The mixis threshold (measured in number of females per mL at the time of first male appearance) in the first experiment was log-transformed and analyzed by fitting a mixed effects linear model, with ‘clone of origin’ as a fixed effect (with 20 levels corresponding to the different clones) and ‘original sampled female’ (three for each clone) as a random effect. The ‘clone of origin’ effect was further tested using model simplification, reducing the number of levels by pooling clones together, and testing whether the simpler model was significantly worse than the model with more parameters (*e.g.*, p. 374-377 in Crawley, 2007). Mixis ratios in the second experiment were analyzed using a generalized linear mixed effects model (GLMM), in which the numbers of sexual/asexual females produced per day by each tested female was modelled as a binomial variable with a logit link function. The model included ‘clone of origin’ (with 4 levels), ‘tested generation’ (number of clonal generations from the resting egg, treated as a continuous variable), ‘age of mother’ (in days, day 1 corresponding to the first day the tested

4.2. Regulation of investment in sex in *Brachionus plicatilis*

female produced a juvenile, treated as a continuous variable) as fixed effects, as well as an interaction between ‘clone of origin’ and ‘tested generation’, and an interaction between ‘clone of origin’ and ‘age of mother’. Effects of the original G1 female (5 for each clone of origin) and of the clonal line (3 for each G1 female) were included in the model as random effects. The significance of fixed effects and their interactions was assessed by comparing models with or without the tested effect or interaction using likelihood ratio tests. Analyses were carried out using R v. 3.3.3 (R Core Team, 2017), and the *lmer*, *glmer* and *anova* functions from the “lme4” package (Bates et al., 2015). Proportions of variance explained by fixed effects were obtained using the *r.squaredGLMM* function of the “MuMIn” package (Bartoń, 2018).

4.2.3 Results

Variability in mixis threshold. Figure 4.5 shows the average density at first male appearance for the different clones, measured after 15 asexual generations. As explained in the Methods, three clonal lines were maintained for each of the twenty original clones, and 6 measures were performed for each clonal line (yielding 18 measures per original clone). However, some clonal lines were lost (due to the death of individuals that could not be replaced), and as a consequence, we obtained results from only two clonal lines (instead of three) from clones 1, 2

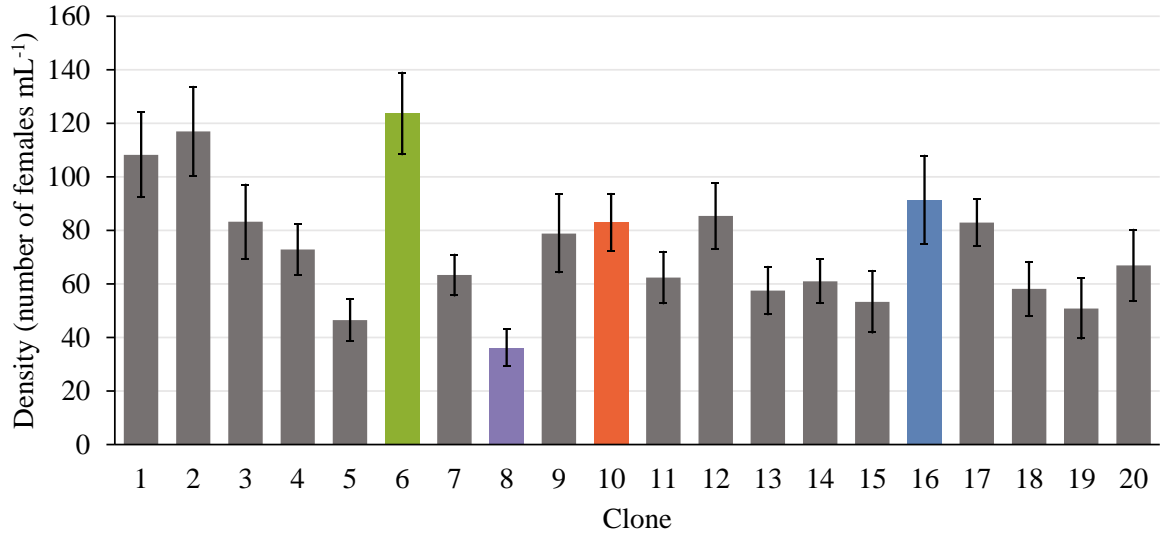


Figure 4.5: Average mixis threshold of the different clones (density at which the first males were observed), measured after 15 asexual generations. Error bars show ± 1 S.E. The colored bars show the clones selected for the second experiment on the effect of the number of generations after fertilization on the mixis ratio.

and 15. Furthermore, only 5 individuals could be tested from one of the clonal lines from clones 4, 8, 9, 13, 17, 18 and 20 (yielding 17 measures for each of these clones). Finally, only 1 (resp. 2) individuals could be tested from one of the clonal lines from clone 19 (resp. 5) due to the early death of the 14th generation mother, yielding 13 (resp. 14) measures for this clone. The statistical analysis showed a significant effect of the clone of origin ($\chi^2(19) = 36.17$, $p = 0.01$). A model in which clones are grouped into three classes (a class comprising clones 2 and 6, another comprising clones 5, 8 and 19, and a third comprising all other clones) was not significantly worse than the model in which a coefficient is assigned to each clone ($\chi^2(17) = 11.60$, $p = 0.82$). The proportion of total variance explained

4.2. Regulation of investment in sex in *Brachionus plicatilis*

by the ‘clone of origin’ effect was 0.16.

Transgenerational effect on the mixis ratio. From the previous results, we selected two clones with high and low mixis thresholds (clones 6 and 8, respectively – see Figure 4.5), and two clones with average mixis thresholds (clones 10 and 16) to perform the experiment on the effect of the number of clonal generations after fertilization on investment in sex. A total of 15 clonal lines were maintained for each of these clones (see Methods), starting from 5 resting eggs produced by intracloal mating (3 clonal lines per resting egg). However, the lines originating from one of the resting eggs from clone 10 had low fitness (high death rates of individuals and low fecundity, which may be caused by inbreeding depression) and these lines could not be maintained: therefore, data from clone 10 consist in measurements from 4 distinct genotypes produced by intracloal mating (instead of 5). Furthermore, one data point was missing for clone 8 at generation 5, and two at generation 18 (leading to 14 and 13 measures instead of 15), due to the premature death of tested females.

Figure 4.6 shows the effect of the number of generations from the resting egg on the mixis ratio (measured at the proportion of sexual females produced among all offspring produced by a female), averaged over each clone (results for each genotype from each clone are shown in Figure 4.7). The results show a significant

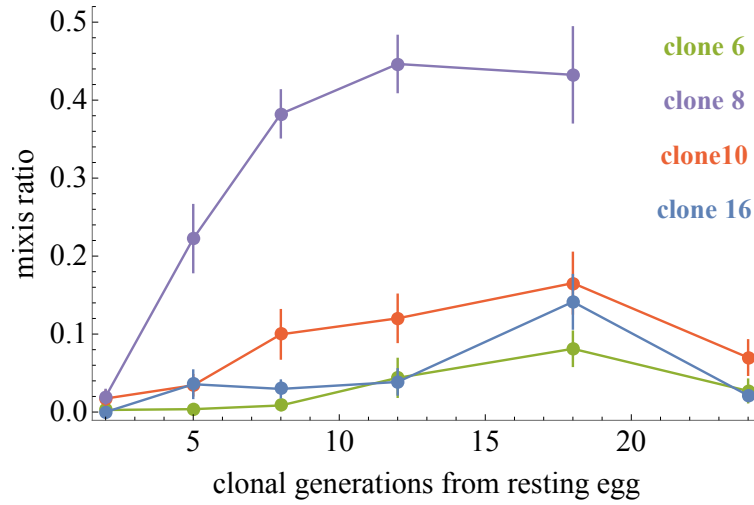


Figure 4.6: Mixis ratio (proportion of sexual females among the whole progeny of an individual) averaged over each clone of origin, and as a function of the number of clonal generations from the resting egg. Error bars show ± 1 S.E.

increase in mixis ratio with the number of clonal generations ($\chi^2(4) = 259.13$, $p < 0.001$), G2 individuals (that is, the offspring of individuals that hatched from resting eggs) producing very few sexual females, while investment in sex increases to reach a plateau after about 8 to 10 clonal generations. The results also show significant differences among clones ($\chi^2(9) = 83.69$, $p < 0.001$), with a much higher investment in sex of individuals from clone 8 (observed over all 5 genotypes, see Figure 4.7), while investment in sex is lowest in individuals from clone 6. The model also detected a significant interaction between the tested generation and clone of origin ($\chi^2(3) = 41.28$, $p < 0.001$), reflecting the fact that the mixis ratio increases more rapidly with the number of clonal generations in some clones than others. The model in which different coefficients were attributed to the four clones

4.2. Regulation of investment in sex in *Brachionus plicatilis*

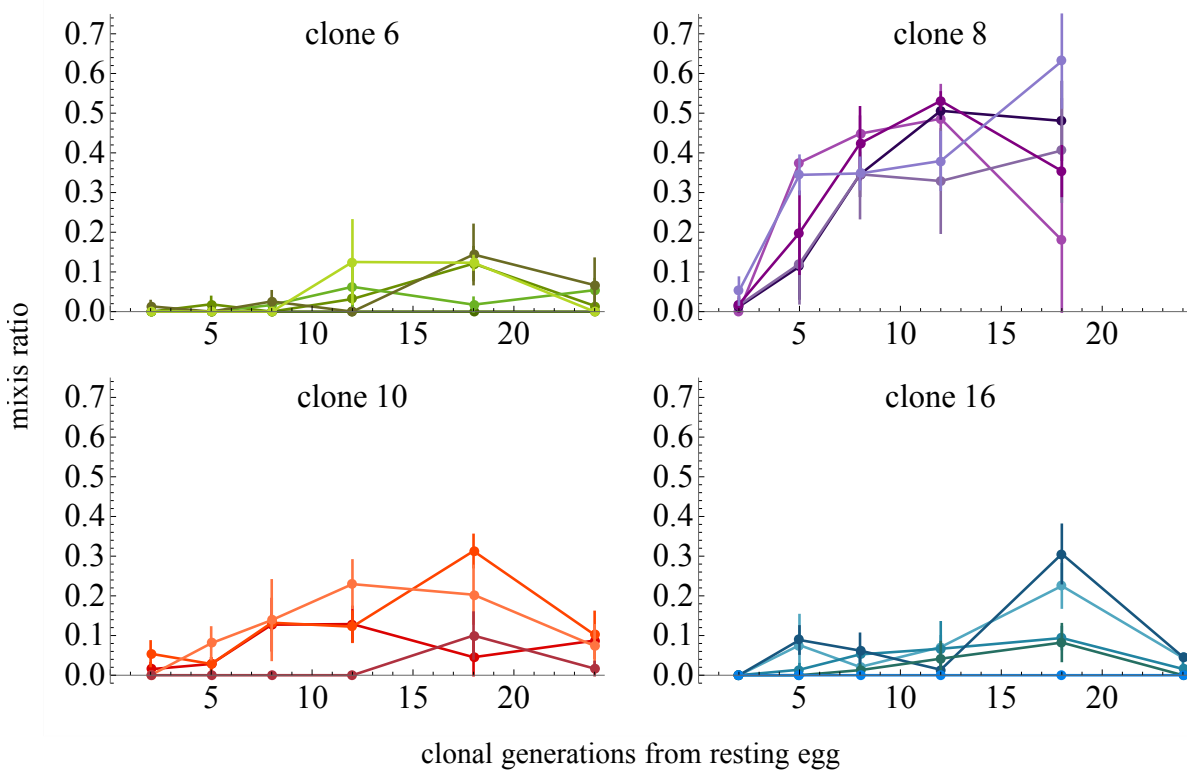


Figure 4.7: Mixis ratio (proportion of sexual females among the whole progeny of an individual) as a function of the number of clonal generations from the resting egg, averaged over each genotype (corresponding to a different resting egg) produced by intraclonal mating within each of the four clones of origin (5 genotypes from clones 6, 8 and 16, and 4 genotypes from clone 10). Error bars show ± 1 S.E.

was significantly better than a model in which clones 6 and 16 were treated as identical ($\chi^2(3) = 13.70$, $p = 0.003$), and was also better than a model in which clones 10 and 16 were treated as identical ($\chi^2(3) = 11.35$, $p = 0.01$), reflecting the fact that the four clones displayed different behaviors.

Effect of maternal age on the mixis ratio. The model detected a significant effect of the age of the tested mother (measured in days, day 1 corresponding to

the day of first reproduction) on the proportion of sexual females produced per day ($\chi^2(6) = 181.67$, $p < 0.001$), and a significant interaction between the clone of origin and the age of the tested mother ($\chi^2(3) = 17.36$, $p < 0.001$). Indeed, Figure 4.8 shows that the tested females tended to produce a higher proportion of sexual offspring during their first days of reproduction, the decline in mixis ratio with the age of the mother being most apparent for clone 8.

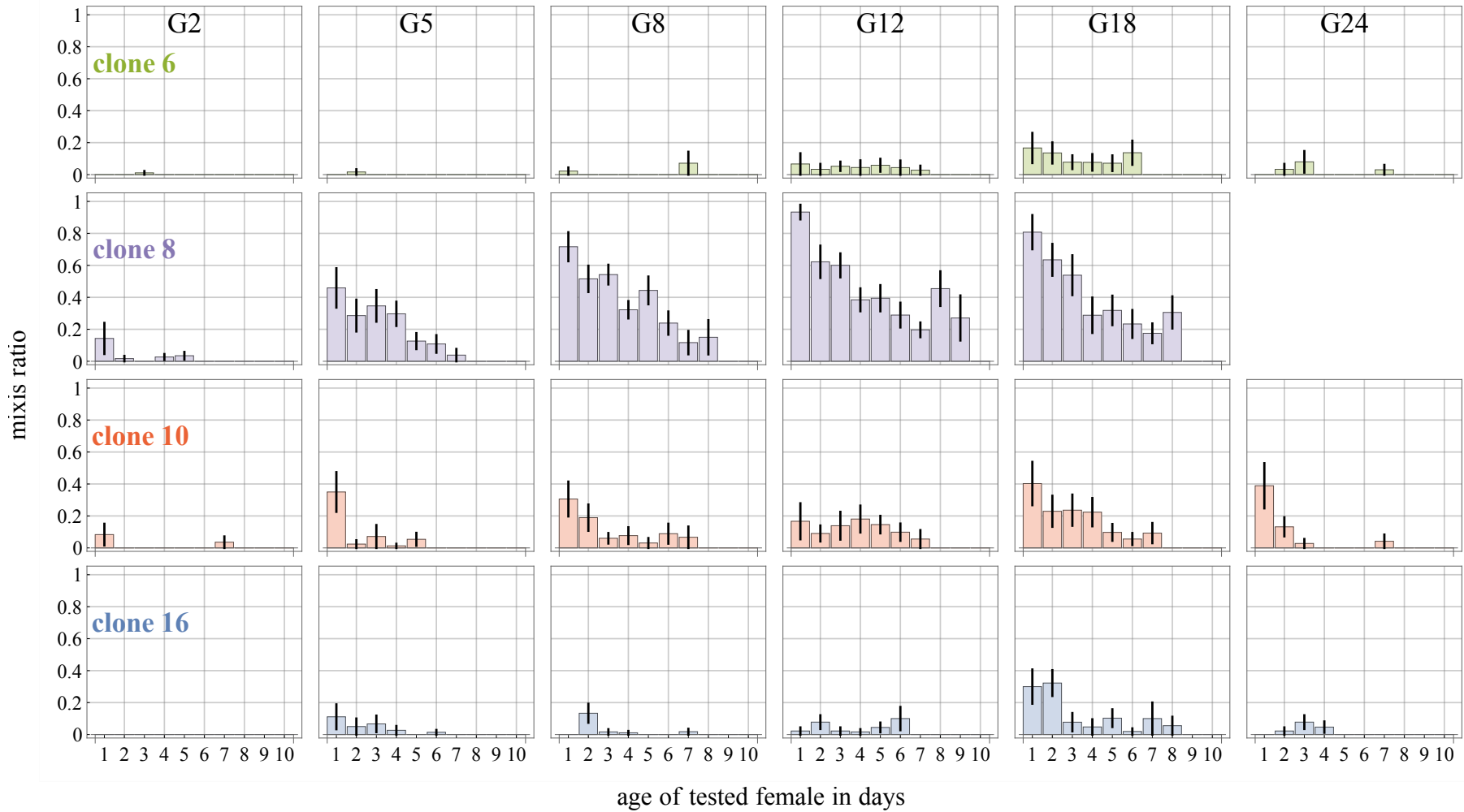


Figure 4.8: Mixis ratio (measured as the proportion of sexual females among offspring produced per day) as a function of the mother's age (in days, day 1 corresponding to the day of first reproduction), for the different clones of origin and numbers of clonal generations after fertilization. Error bars show ± 1 S.E. Averages were computed only when more than 10 juveniles were produced for a given mother's age class, over the whole clone of origin and for a given generation.

4.2.4 Discussion

Investment into sexual reproduction may greatly vary between different species of monogonont rotifers, but also between populations from the same species, and between individuals from the same population (Gilbert, 2017). This variation may be caused by genetic factors, but also by environmental factors and by endogenous effects such as the transgenerational maternal effects that have been described in some species (Gilbert, 2002; Schröder and Gilbert, 2004). Our results confirm the existence of genetic variability for the propensity for sex within a single population of *Brachionus plicatilis*, after controlling for the number of clonal generations since fertilization (at least 15 in our first experiment). Our estimate for the proportion of variance in the mixis threshold explained by the genotype of individuals (0.16) is lower than the heritability estimated by Gabaldón and Carmona (2015) from 16 clones from the same natural population (0.51). This difference may partly be due to the fact that Gabaldón and Carmona (2015) did not control for the number of asexual generations from the last resting egg (although three clonal generations were performed before the estimation of density thresholds). Alternatively, it may be caused by a higher environmental variance in our experiment. In particular, males were observed at densities much higher than in Gabaldón and Carmona's experiment, which is likely due to the fact that densities thresholds were assessed in smaller volumes in our experiment (0.5 mL, vs. 15 mL in Gabaldón and Carmona's

4.2. Regulation of investment in sex in *Brachionus plicatilis*

experiment). Indeed, the mixis threshold is known to be negatively correlated to the culture volume, which may be due to the fact that in smaller volumes, population density may reach higher values before the mixis-inducing protein reaches the concentration needed to induce sex (Carmona et al., 2011). A higher number of reproductive events during the time needed to reach the mixis threshold may possibly have enhanced the effect of environmental factors on the estimated density at the threshold. Furthermore, our test populations were observed once per 24h (vs. twice in Gabaldón and Carmona’s study), which may also have inflated the variance caused by measurement error.

Our second experiment showed important genetic variation in the mixis ratio (proportion of sexual offspring produced), and a correlation between the two measures of propensity for sex: the clone in which the density at first male appearance was the lowest (resp. highest) in the first experiment displayed the highest (resp. lowest) mixis ratio in the second experiment (clones 6 and 8, Figures 4.5 and 4.6). One may notice that our sex-inducing medium was equivalent to a density of 10 individuals mL^{-1} , which is much lower than the mixis thresholds shown in Figure 4.5. However, this discrepancy again stems from the fact that when estimating mixis thresholds by the density at first male appearance in growing populations, the measured density is likely to be much higher than the density that would be required to produce the threshold concentration of mixis-inducing protein in a

steady-state population, particularly when measurements are performed in small volumes (Carmona et al., 2011). In order to test whether a higher density would increase the mixis ratio, we ran additional tests on 10 females from clone 6, after 18 generations from the resting egg, exposing them to a sex-inducing medium corresponding to 25 individuals mL (instead of 10), but we did not observed any significant increase of the mixis ratio (results not shown). Our results also show that young asexual females tend to produce a higher proportion of sexual offspring than older females, in agreement with previous observations on *B. plicatilis* (Carmona et al., 1994), *B. calyciflorus* (Rougier and Pourriot, 1977) and *Synchaeta tremula* (Timmermeyer and Stelzer, 2006) – however, a maximal investment in sex in the middle of the reproductive period of individuals was observed in one study on *B. calyciflorus* (Fussmann et al., 2007).

The selective forces allowing the maintenance of genetic polymorphism for investment in sex within natural populations remain unknown. Carmona et al. (2009) showed that clones investing less in sex tend to increase in frequency during the growing season (since they invest more in parthenogenetic reproduction), and hypothesized that temporal fluctuations in the length of growing seasons may allow the maintenance of polymorphism, as genotypes investing more in the production of resting eggs may be favored when growing seasons are short, while genotypes investing more in parthenogenetic growth may be favored under longer growing

4.2. Regulation of investment in sex in *Brachionus plicatilis*

seasons. Theoretical models have shown that temporal environmental fluctuations coupled with a dormant stage can indeed allow the maintenance of polymorphism (the “storage effect”, *e.g.*, Warner and Chesson, 1985; Ellner and Hairston, 1994; Turelli et al., 2001); however, it would be interesting to further explore to what extent variation in the contribution to the dormant phase can be maintained by such a mechanism.

Finally, our results demonstrate a gradual increase in the propensity for sex over clonal generations following fertilization. This confirms previous indications that a transgenerational maternal effect repressing sexual reproduction, and similar to the one observed in several other monogonont species (Gilbert, 2002; Schröder and Gilbert, 2004) occurs in *B. plicatilis* (Hino and Hirano, 1977; Hagiwara et al., 2005). This delayed-mixis mechanism may have evolved to increase the chances of establishment of newly hatched lineages, by promoting parthenogenetic growth (Serra et al., 2005). Several hypotheses have been proposed concerning the mechanism underlying this transgenerational effect (DNA methylation, cytoplasmic compound present in decreasing concentration over generations, *e.g.*, Gilbert, 2017), but it currently remains unknown. Interestingly, the existence of such a mechanism raises the possibility that the effect of other factors known to affect investment in sex, such as population density or food stress, may persist over a given number of clonal generations (evidence that food stress may affect the mictic response of females

over several generations can be found in Hagiwara et al., 2005; Kamizono et al., 2017). These effects should be explored in order to better understand the selective forces that may act on the evolution of the propensity for sex in monogonont rotifers, in both natural and experimental populations.

Chapter 5

Adaptation of rotifers to stressful environments and the benefits of sex

5.1 Introduction

The existence and maintenance of sex is an evolutionary paradox. The reasons why sexual reproduction is widespread across the eukaryotic kingdom despite its many costs remain to date one of the main questions of evolutionary biology. Indeed, when populations are well adapted to their environment, sex tends to have a negative effect on fitness by breaking up beneficial associations between alleles. Moreover, sexual reproduction comes along high costs: costs of finding a

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex mate, the risks of disease transmission, and so forth. Further, a two-fold cost of sex arises from the existence of unequal-sized gametes, due to the resources used for the production of males, which themselves often do not provide any resource to the next generation.

Theoretical work have lead to the formulation of several hypotheses on the possible benefits of sex. One of the oldest hypotheses stems from the work of Fisher (1930) and Muller (1932). During adaptation, beneficial mutations may arise in different clonal lineages competing against each other, precluding the fixation of all beneficial mutations (clonal interference, see Gerrish and Lenski, 1998). However, recombination can bring together beneficial alleles in a single lineage, increasing their probability of fixation and thus favoring adaptation to novel environments. This advantage of sex implies the existence of negative linkage disequilibrium between loci, beneficial alleles being found more often in different backgrounds than in the same background. Two different forces may generate such negative disequilibrium: stochastic effects occurring in finite populations (the Hill-Robertson effect, Hill and Robertson, 1966; Felsenstein, 1974), or negative epistasis between beneficial alleles (Kondrashov, 1988; Barton, 1995). By reducing negative linkage disequilibrium, recombination increases the variance in fitness and thus the efficiency of the response to directional selection, leading to faster adaptation. Furthermore, alleles coding for more sex or recombination can spread in the popu-

5.1. Introduction

lation along with the better adapted phenotypes they create (hitchhiking effects).

The prediction that sex should improve adaptation has been tested experimentally and received general support. Kaltz and Bell (2002) explored the dynamics of adaptation of *Chlamydomonas reinhardtii* to stressful environments, comparing asexual lineages and lineages that had undergone one or more episodes of sexual reproduction. The authors observed that sexually derived lineages displayed a higher response to selection than asexual lineages – this effect has also been observed in yeast populations (Goddard et al., 2005). Moreover, a sexual episode had the effect of immediately increasing the variance in fitness but decreasing mean fitness of populations (short-term cost, which may reflect negative epistasis between selected loci). The variance in fitness then decreased again during the subsequent asexual generations but this time increasing mean fitness over that of asexual populations (long-term benefit), in agreement with the theoretical predictions mentioned above. Overall, Kaltz and Bell (2002) showed that sexual populations could adapt more rapidly than asexual populations, due to a greater variance in fitness. Yet, populations will only benefit from sex as long as adaptation is incomplete, and the long-term advantage of a single sexual episode might disappear over time (Colegrave et al., 2002). However, the benefits can be maintained by additional sexual episodes, provided that the population still needs to adapt.

In addition to these results, it was shown that the effect of sex in *C. reinhardtii*

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex depends on population size (Colegrave, 2002): in larger populations (where there is a larger supply of beneficial mutations), sex brings more benefits than in smaller populations, where adaptation might be limited by the beneficial mutations supply (in both sexual or asexual populations). Similarly, it was shown in *Escherichia coli* that the increased rate of adaptation enabled by recombination is more important when the mutation rate is higher (Cooper, 2007). Indeed, a higher mutation rate may lead to more competition between beneficial mutations, and recombination brings more benefits. Additionally, recombination may separate beneficial and deleterious alleles that are in linkage disequilibrium (the “ruby in the rubbish” hypothesis, Peck, 1994). More recently, experimental evolution coupled with genomics provided further evidence that sex improves adaptation by reducing clonal interference and decoupling beneficial alleles from linked deleterious mutations (McDonald et al., 2016).

In this study, we investigate the benefits of sex using the monogonont rotifer *Brachionus plicatilis*. Monogonont rotifers have the ability to reproduce using both sexual and asexual reproduction, and species of the genus *Brachionus* have been extensively studied. Genetic variation in the investment in sexual reproduction has been observed in natural populations (Carmona et al., 1995, 2009; Gabaldón and Carmona, 2015), and recent experimental evolution studies have shown that higher rates of sex could evolve in populations adapting to new environments

5.2. Material and Methods

(Becks and Agrawal, 2010, 2012; Luijckx et al., 2017). The aim of this study is to test whether sexual reproduction allows faster adaptation of rotifer populations to a novel environment.

5.2 Material and Methods

5.2.1 Study system

The rotifer *Brachionus plicatilis* is a facultatively sexual metazoan found in shallow brackish waters. It reproduces by mitotic parthenogenesis at low densities and starts producing mictic (*i.e.* sexually reproducing) females at higher densities in response to a chemical stimulus (Snell et al., 2006). If unfertilized, mictic females produce haploid eggs that develop into dwarf haploid males, but if fertilized when juvenile, they produce dormant resting eggs that will develop in amictic (asexual) females. Asexual females are mature within 2-3 days, and may produce 20 to 30 offspring over the course of their life. Resting eggs may hatch spontaneously in culture conditions (Martínez-Ruiz and García-Roger, 2015), but hatching can be synchronized by maintaining the eggs at 4 °C and in the dark during one to two months (see section 3.2.3 page 74). Genetic variability in the propensity for sex (threshold density for production of mictic females, or proportion of mictic females among offspring) has been demonstrated by several studies (see chapter 4; see also Gabaldón and Carmona, 2015).

5.2.2 Experimental populations

Three replicate experimental populations were initiated from a mix of 55 clones previously isolated from Salobrejo Lake (Spain): each clone originated from a resting egg present in the sediment of the pond (sampled in September 2013) and was previously maintained in a test tube transferred every week into fresh medium. These three populations were maintained in 1 L chemostats at a temperature of 20 °C, under 12/12-hours photoperiod, with a dilution rate of 0.1 (10% of the culture was renewed per 24h, and replaced by a culture medium containing the green algae *Tetraselmis suecica* as a food source; Figure 5.1). The culture medium was pumped from a 2 L chemostat in which the algal population was maintained by the continuous input of 12 g L⁻¹ salinity artificial seawater (Instant Ocean[®], Aquarium Systems) enriched with f/2 culture medium (Guillard and Ryther, 1962), with a dilution rate of 0.2. Algal density was measured three times a week and diluted to reach a concentration of 5×10^5 cells mL⁻¹, to be distributed to rotifer populations (Figure 5.1).

After 6 weeks, large numbers (approx. 2000) of resting eggs were sampled from the bottom of the tube of each population and stored in separate Petri dishes with 12 g L⁻¹ seawater at 4 °C in the dark for 5 weeks to synchronize hatching (see Figure 5.2). After this time, the resting eggs were hatched by replacing half the water by culture medium with algae in the Petri dishes ($\approx 5 \times 10^5$ cells mL⁻¹) and

5.2. Material and Methods

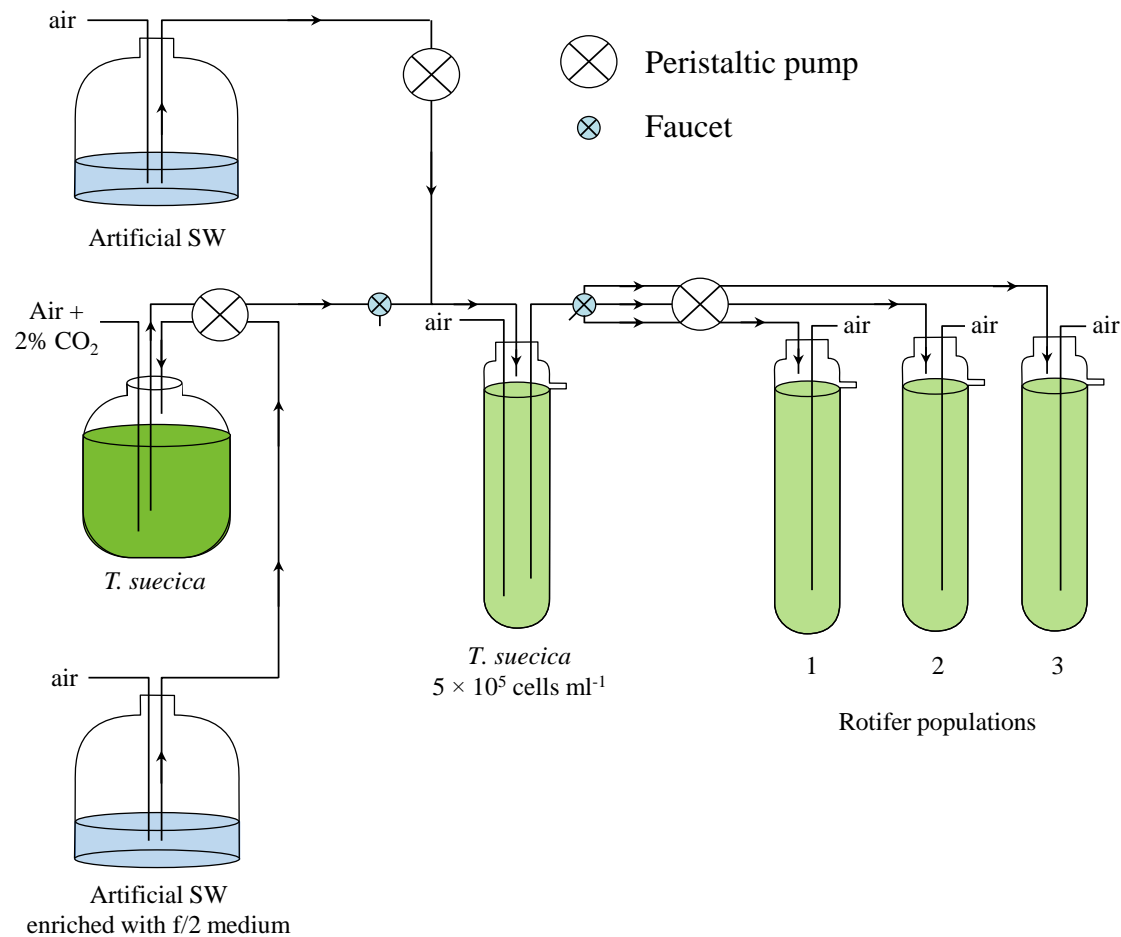


Figure 5.1: Setup of the preliminary experimental populations in chemostat. f/2 culture medium (Guillard and Ryther, 1962; Table S4) in 12 g L⁻¹ salinity artificial seawater (12 g L⁻¹; Instant Ocean[®], Aquarium Systems) is delivered at a constant rate to a high density culture of *Tetraselmis suecica*. This culture is diluted with 12 g L⁻¹ seawater to a concentration of 5 × 10⁵ cells mL⁻¹, which is then dispatched to the experimental populations. Modified from the internship report of Elisa Leroux.

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex

placing them at 22 °C under constant illumination for a week. 500 neonates from each population were sampled to initiate “sexually produced” populations. “Asexually produced” populations were initiated from 500 asexual egg-bearing females sampled from each three initial populations (that had been maintained during the time resting eggs were kept at 4 °C, see Figure 5.2).

Sexually and asexually produced populations were then maintained in chemostats using the same system as described above, either receiving algae grown in standard culture medium, or algae grown in medium to which a solution of copper sulfate (CuSO_4) was added to the artificial seawater at a concentration of 0.05 mg L^{-1} to induce oxidative stress (this concentration was later increased up to 0.2 mg L^{-1} , as explained in the Results section) and from which EDTA (a component of the f/2 medium that chelates Cu^{2+} ions) had been removed. In the last case, the artificial seawater used to dilute algae to achieve a concentration of $5 \times 10^5 \text{ cells mL}^{-1}$ also contained copper sulfate at a concentration of 0.05 mg L^{-1} (see figure of the full experimental setup in Appendix S9). A total of 12 populations were initiated, 4 for each of the 3 initial populations (see Figure 5.2): two sexually produced populations (one maintained in standard medium and one in medium with copper) and two asexually produced populations (one in standard medium and one in medium with copper).

Over the course of the experiment, the density of rotifers in all experimental

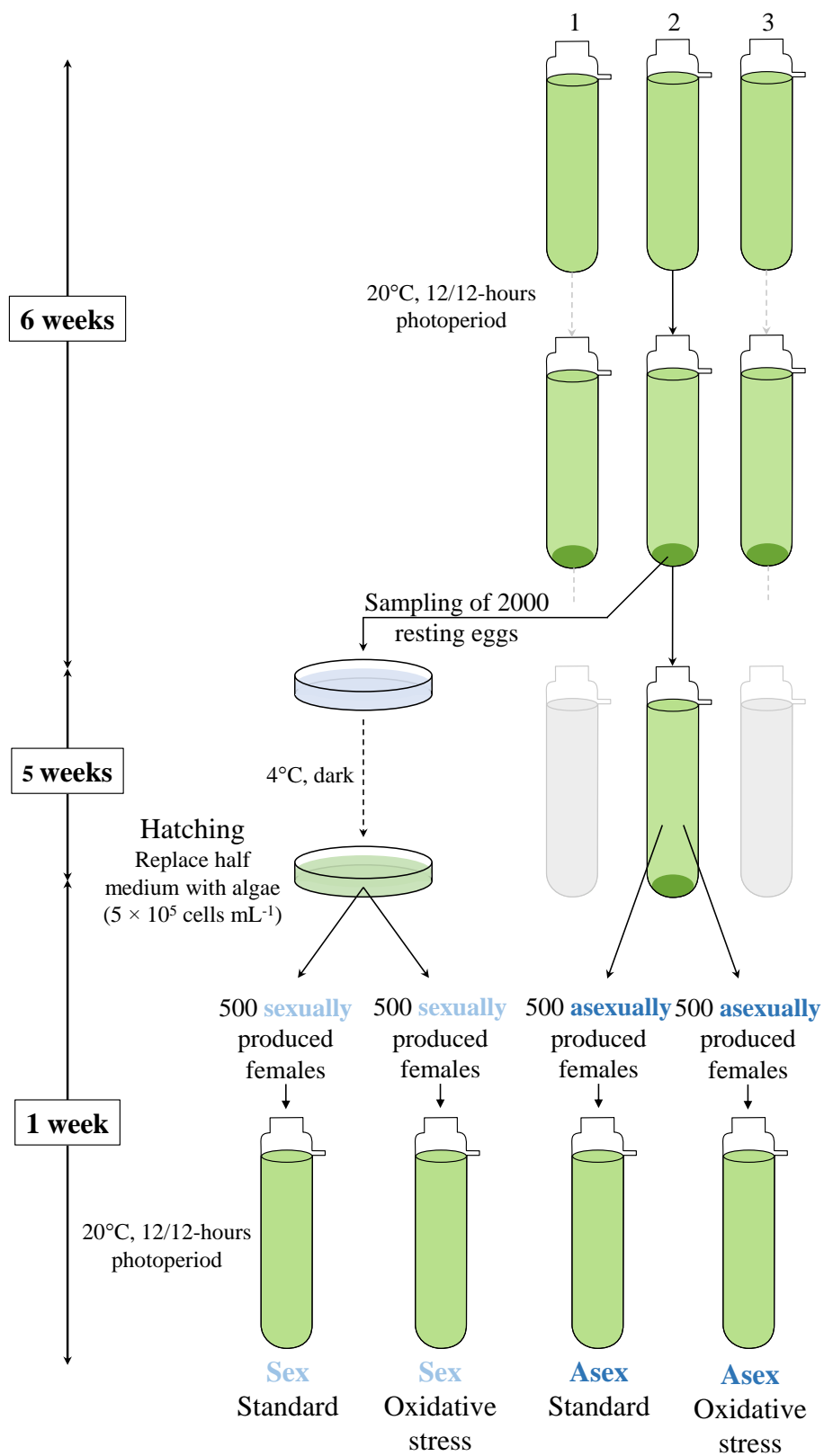


Figure 5.2: Procedure to initiate sexually and asexually produced populations.

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex populations was monitored every 2-3 days. A sample of each population was collected, and the number of females was counted after homogenization in 10 droplets of 100 μL , and extrapolated to the whole population. The counts were averaged over all droplets and converted into a number of females mL^{-1} .

5.2.3 Fitness measures and microsatellite genotyping

Fitness measures were performed every 10 days, starting 2 weeks after the 12 populations were initiated (6 fitness measures were done in total). Fitness was estimated as asexual growth rates. For this, 60 egg-bearing asexual females were sampled from each population and isolated in individual wells of 48-well plates with 300 μL of the culture medium used to feed the source population (*i.e.*, either with or without copper). The following day, juveniles were transferred in wells of 24-well plates with 1 mL of culture medium (either with or without copper depending on the source population, day 0), and were let to grow. The number of females in ~ 30 wells was counted on day 3 and day 6 – from the original 60 females, some did not reproduce, some died, while others gave birth to sexual offspring, but we could obtain 30 fitness measures per population in most cases. On the last day, we also checked if males were present, indicating the presence of sexual females that did not contribute to asexual growth. The growth rate (used as fitness measure) was obtained from the slope of the regression on log-transformed data over time.

5.2. Material and Methods

We genotyped the 55 clones used to initiate our populations at 11 microsatellite loci (of which 8 were polymorphic); the genotypes are given Appendix S7. Individuals used for the first, third and fifth fitness measures were also genotyped to monitor the evolution of the populations genetic composition over time. The protocol for extraction and genotyping is detailed in Chapter 3.

5.2.4 Fitness of sexually produced individuals after adaptation

After the last of our 6 fitness measures, the upper part of each of the 12 experimental populations (containing mostly swimming individuals, as resting eggs fall to the bottom of the tube) was transferred to a flask, supplemented with algal medium (either with or without copper depending on the source population) and maintained for 2 weeks to allow the production of resting eggs. The bottom of each flask was transferred in a Petri dish and maintained in the dark at 4 °C for 7 weeks. 48 resting eggs per source population were then collected and individually placed in a well of a 48-well plate with standard algal medium (without copper) under constant illumination and at 22 °C to induce hatching. 24 hatched individuals per population were collected. These newborn females were let to reproduce for 8 asexual generations in standard medium in order to limit trans-generational maternal effects. From each of these asexual lines, two 8th generation offspring were collected

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex

and transferred to a well of a 48-well plate containing either 0.3 mL of standard culture medium (from an algal culture maintained in standard f/2 medium, diluted to reach a concentration of 2×10^5 cells mL⁻¹), or 0.3 mL of solution from an algal culture maintained in f/2 medium without EDTA, diluted to reach a concentration of 2×10^5 cells mL⁻¹, and to which copper sulfate was added at a concentration of 0.2 µg/L. Individuals exposed to copper were placed in standard medium after 24h, and all individuals were followed throughout their lifetimes, by transferring them every day to a new well with fresh standard medium, and counting the number of offspring produced. Fitness was estimated as the lifetime reproductive success of individuals (total number of offspring produced). For each of our experimental populations, we thus obtained about 20 estimates of fitness after exposure to copper stress, and 20 estimates of fitness without copper stress. Unfortunately, the number of fitness measures was lower for the 3 populations derived from asexually reproducing females and that had evolved under oxidative stress, because very few resting eggs were produced in the flasks (see Results).

5.2.5 Statistical analysis

The different measures of fitness were analyzed separately. For each measure, a linear model was used to test for an effect of the treatment (Standard or Oxidative stress), the reproductive origin (asexually or sexually produced population) or

5.3. Results

the replicate population, and the interaction between the treatments and reproductive origins (growth rate \sim population + reproductive origin \times treatment). In addition, the distribution of growth rates was compared between pairs of sexually and asexually produced populations using Kruskal-Wallis non-parametric tests.

The proportion of populations used for growth rate estimates that had produced males after 6 days was analyzed using a binomial generalized linear model (using a similar formula as above). We also checked for a possible correlation between the proportion of populations that had produced males on day 6 and the mean growth rates of these populations using Pearson's correlation coefficient.

5.3 Results

Over the course of the experiment, the estimated density of rotifers fluctuated strongly in all populations (Figure S11). Note that these fluctuations may partly be due to sampling effects (discrepancies between the densities in the 1 mL samples taken from each population and the true density within each population). During the first weeks of the experiment, densities in populations undergoing oxidative stress were similar to the densities of populations in the standard environment, which made us think that the concentration of copper in the culture medium was not high enough to affect rotifers. The concentration of 0.05 mg L^{-1} of copper sulfate was chosen based on preliminary experiments, in which the same concen-

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex

tration had a significant effect on the fitness of rotifers (data not shown). However, copper may have had less effect in the experimental conditions of the present experiment, because it had more time to fix to organic particles (or being metabolized by algae) before rotifers were exposed to it. For this reason, the concentration of copper sulfate in the culture medium was increased to 0.075 mg L^{-1} on May 11th (approximately 4 weeks after the experimental populations had been initiated), to 0.1 mg L^{-1} on May 23rd and to 0.2 mg L^{-1} on June 11th. Note that the culture medium was distributed drop by drop to the rotifers so that the increase of concentration in the experimental populations was gradual.

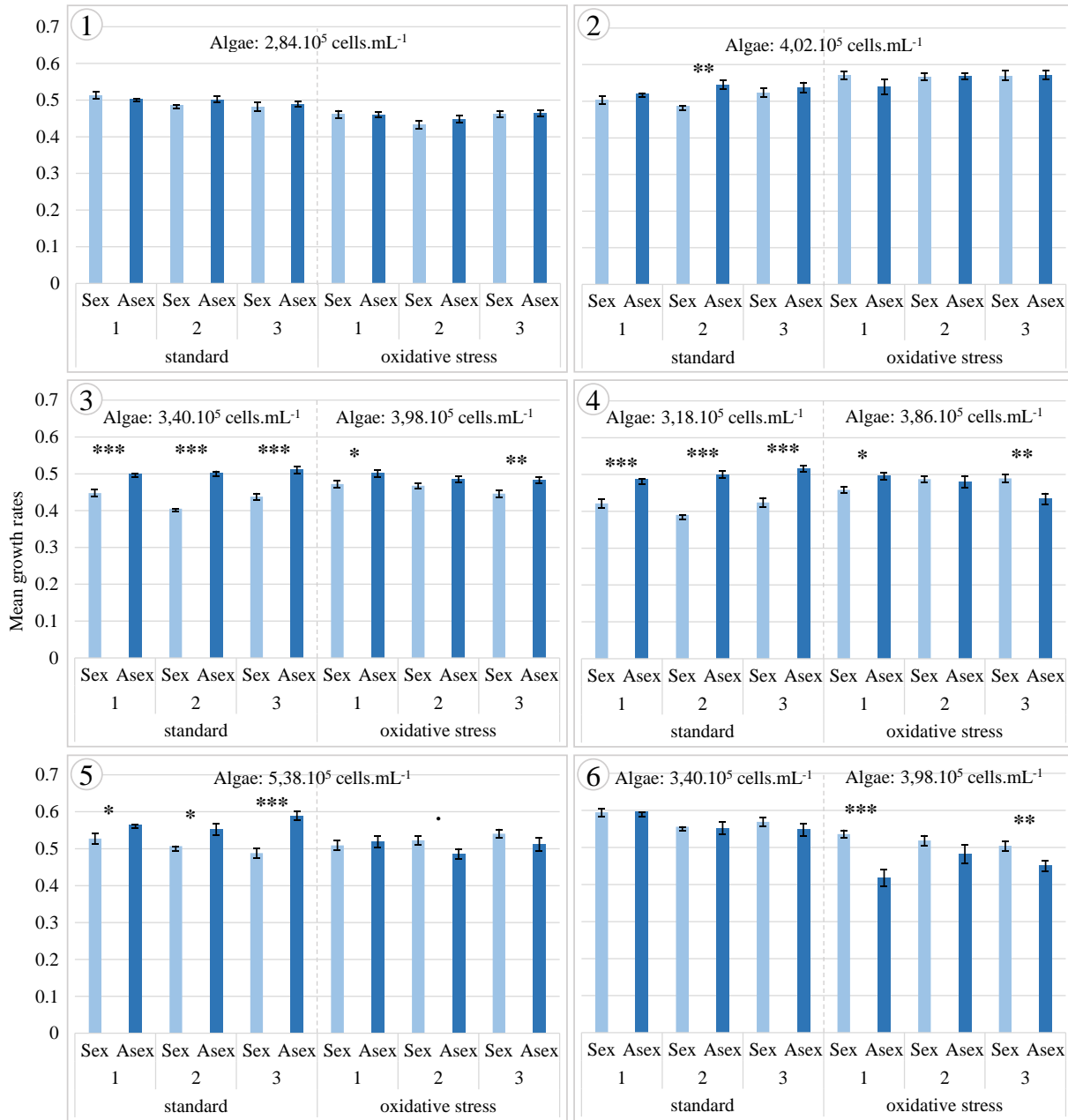


Figure 5.3: Average growth rates (± 1 S.E.) in the experimental populations. Growth rates are estimated by calculating the slope of the linear regression on log-transformed data of density of females over time. Counts were made **1.** from 30/04/18 to 07/05/18, **2.** from 10/05/18 to 17/05/18, **3.** from 21/05/18 to 28/05/18, **4.** from 31/05/18 to 07/06/18, **5.** from 11/06/18 to 18/06/18 and **6.** from 21/06/18 to 28/06/18. Kruskal-Wallis non-parametric tests were used to compare pairs of sexually (Sex) and asexually (Asex) produced populations (** $p < 0.001$, * $p < 0.01$, * $p < 0.05$, . $p < 0.1$). The concentration of algae used for the different fitness measures is indicated.

Table 5.1: Results of the linear statistical model. Effect of populations, reproductive origins (sexually produced vs. asexually produced), treatments (standard vs. oxidative stress) and the interaction between reproductive mode and treatment on the growth rates of populations (** $p < 0.001$, * $p < 0.01$, * $p < 0.05$, . $p < 0.1$, ns non significant).

Formula: Growth rate \sim Population + Reproduction \times Treatment

①	Populations	1 2 3 **	②	Populations	1 2 3 .
	Reproduction (sex vs. asex)	ns		Reproduction (sex vs. asex)	ns
	Treatment (std vs. stress)	***		Treatment (std vs. stress)	*
	Reproduction \times Treatment	ns		Reproduction \times Treatment	**
③	Populations	1 2 3 * .	④	Populations	1 2 3
	Reproduction (sex vs. asex)	***		Reproduction (sex vs. asex)	ns
	Treatment (std vs. stress)	*		Treatment (std vs. stress)	**
	Reproduction \times Treatment	***		Reproduction \times Treatment	***
⑤	Populations	1 2 3	⑥	Populations	1 2 3 .
	Reproduction (sex vs. asex)	.		Reproduction (sex vs. asex)	***
	Treatment (std vs. stress)	***		Treatment (std vs. stress)	***
	Reproduction \times Treatment	***		Reproduction \times Treatment	***

The results from our fitness assays are shown in Figure 5.3. Considering first the asexually produced populations (darker bars in Figure 5.3), we observed significantly lower fitnesses in the stressful environment than in the standard environment in the first fitness measure (Figure 5.3, 1; $p < 0.001$). In the second fitness measure, fitnesses of populations in the standard environment were lower

5.3. Results

than those in the stressful environment. Fitnesses again became significantly lower in the stressful environment in the last 4 fitness measures, the difference becoming more important for the last two fitness measures (probably due to the increase in copper concentration, Figure 5.3, 5–6; $p < 0.001$).

In the standard environment (without copper), we observed that overall, sexually produced populations had a significantly lower fitness than asexually produced populations (Figure 5.3, 2–5). This difference may be caused by the cost of sex, and the fact that sexually derived populations invest more in sex than asexually derived ones. Indeed, we observed that sexually derived populations produced more males during the fitness assays (Figure 5.4), which probably results from the fact that we selected for genotypes that had a higher investment in sexual reproduction by sampling sexually produced resting eggs to initiate our sexually derived populations. Individuals having a higher propensity for sex start producing sexual females at lower densities, which limits population growth. However, we did not observe any effect of the cost of sex in the first and last fitness measures on the populations in the standard environment, while we still observed more males during the fitness measures of sexually derived populations than asexually derived ones (Figures 5.3 and 5.4, 1 and 6). The cost of sex seemed less important in populations facing oxidative stress – there were less differences in fitness between sexually and asexually derived populations (Figure 5.3, 2–5) although males were again more often ob-

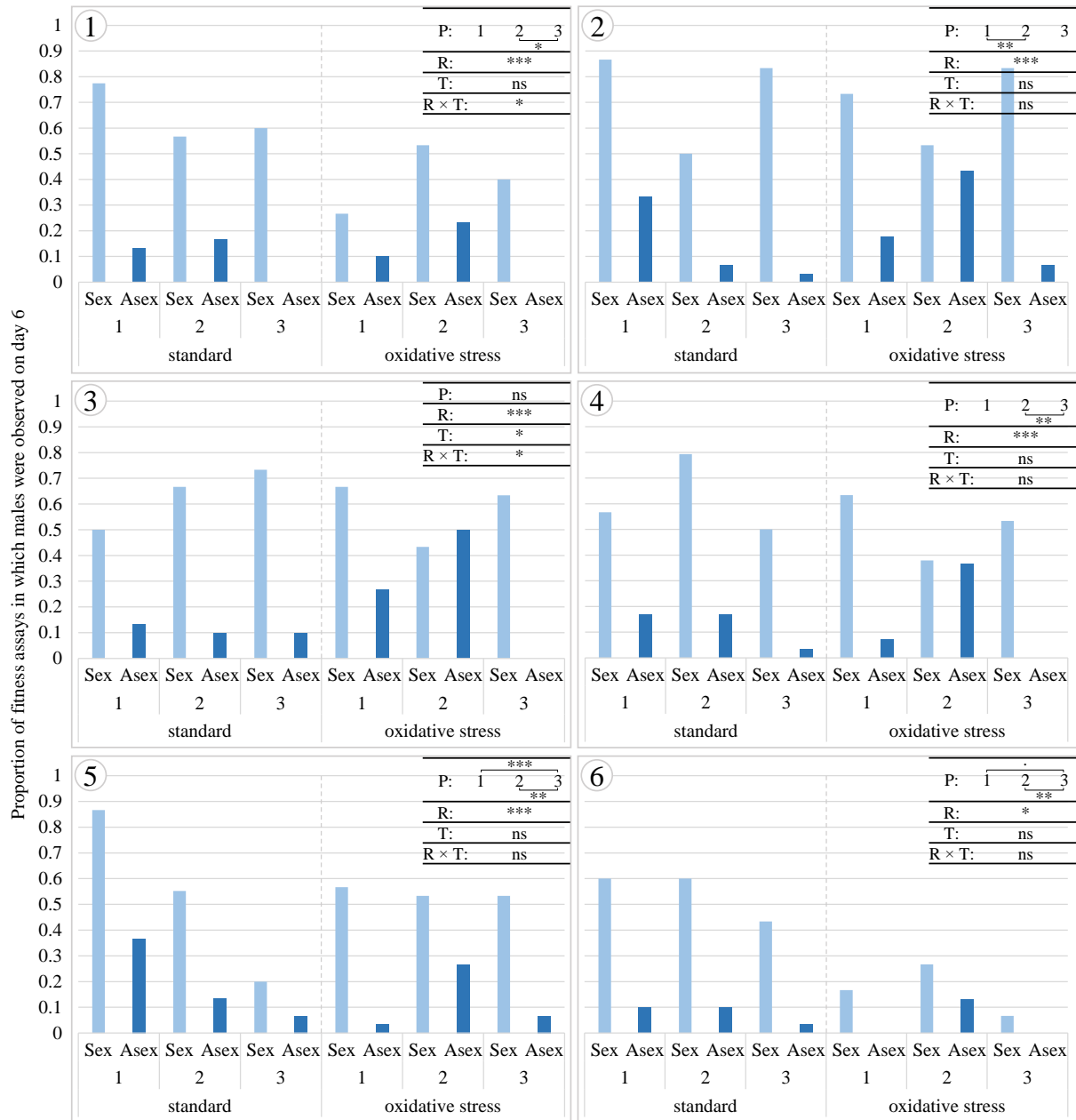


Figure 5.4: Proportion of fitness assays in which males were observed on day 6, for each population. The results of the binomial generalized linear model are indicated. The model tested the effect of the population (P), reproductive origin (R, sexual vs. asexual), treatment (T, standard vs. oxidative stress) and the interaction between reproductive mode and treatment (R x T) (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.1$, ns non significant).

5.3. Results

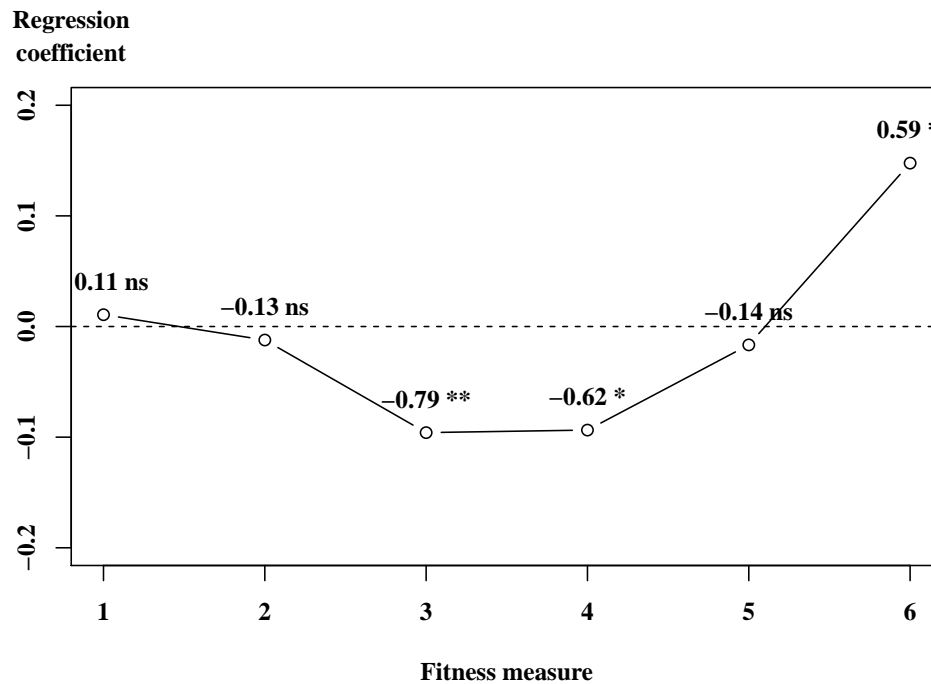


Figure 5.5: Regression coefficient of the proportion of fitness assays in which males were observed on day 6 as a function of the mean growth rate of populations, for each fitness measure. Pearson’s correlation coefficient and its statistical significance are indicated above each point.

served during fitness assays from sexually derived populations than from asexually derived ones (Figure 5.4) – and sexually derived populations had a higher fitness in the last fitness measure (Figure 5.3, 6). As a consequence, the correlation between the proportion of fitness assays in which males were observed and the estimated fitness of the population (which is significantly negative for fitness measures 3 and 4) becomes positive for the last fitness measure (Figure 5.5).

Microsatellite data showed that sexually produced populations maintained a high genetic diversity, regardless of their environment. Almost all individuals

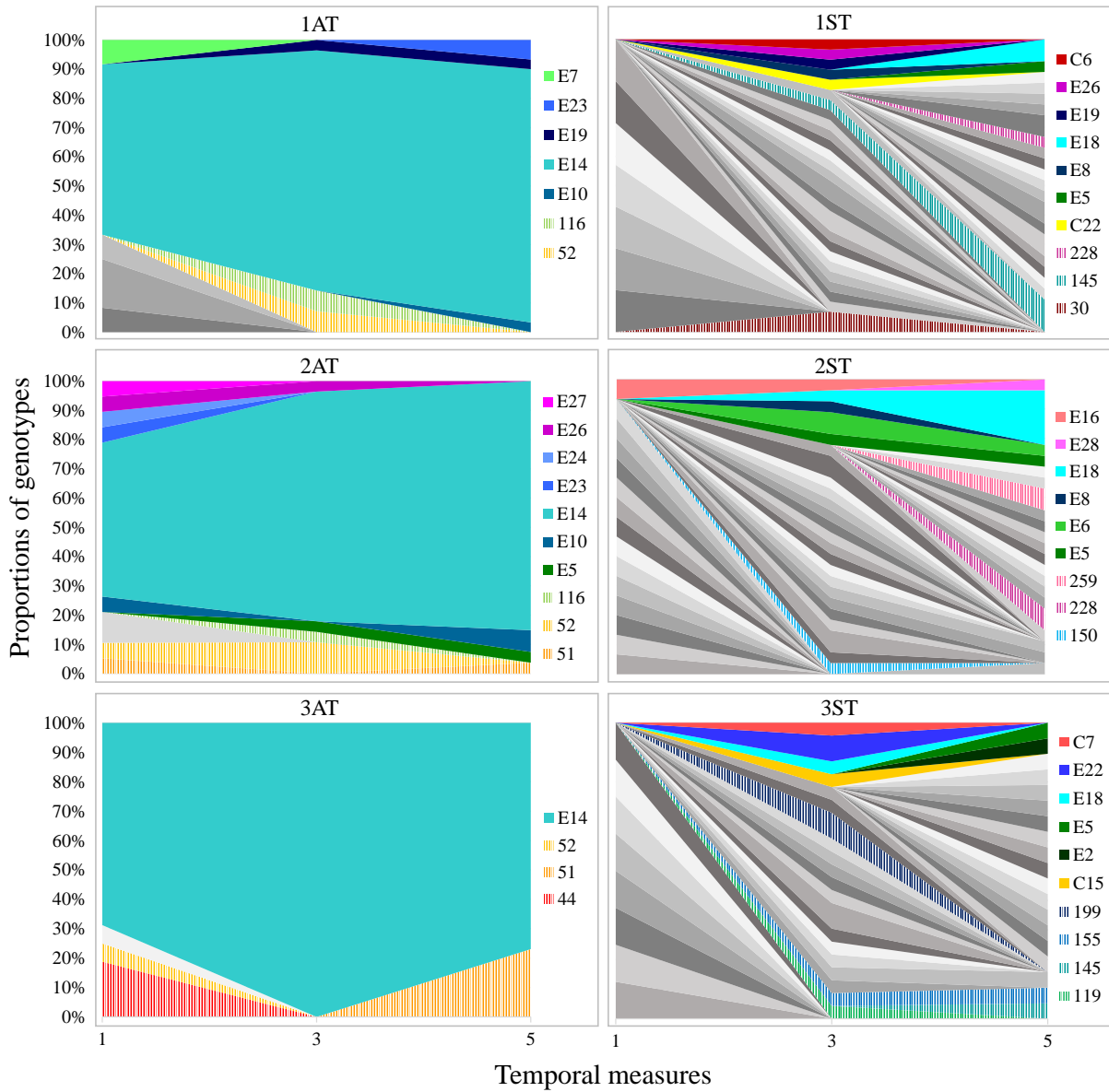


Figure 5.6: Genetic composition of asexually (left) and sexually (right) produced populations in the standard environment, at fitness measures 1, 3 and 5. Each area represents the proportion of a given genotype in the population: genotypes from our 55 initial clones (colored, plain), new genotypes shared between experimental populations (colored, hatched) and new genotypes observed in only one population (gray shades).

5.3. Results

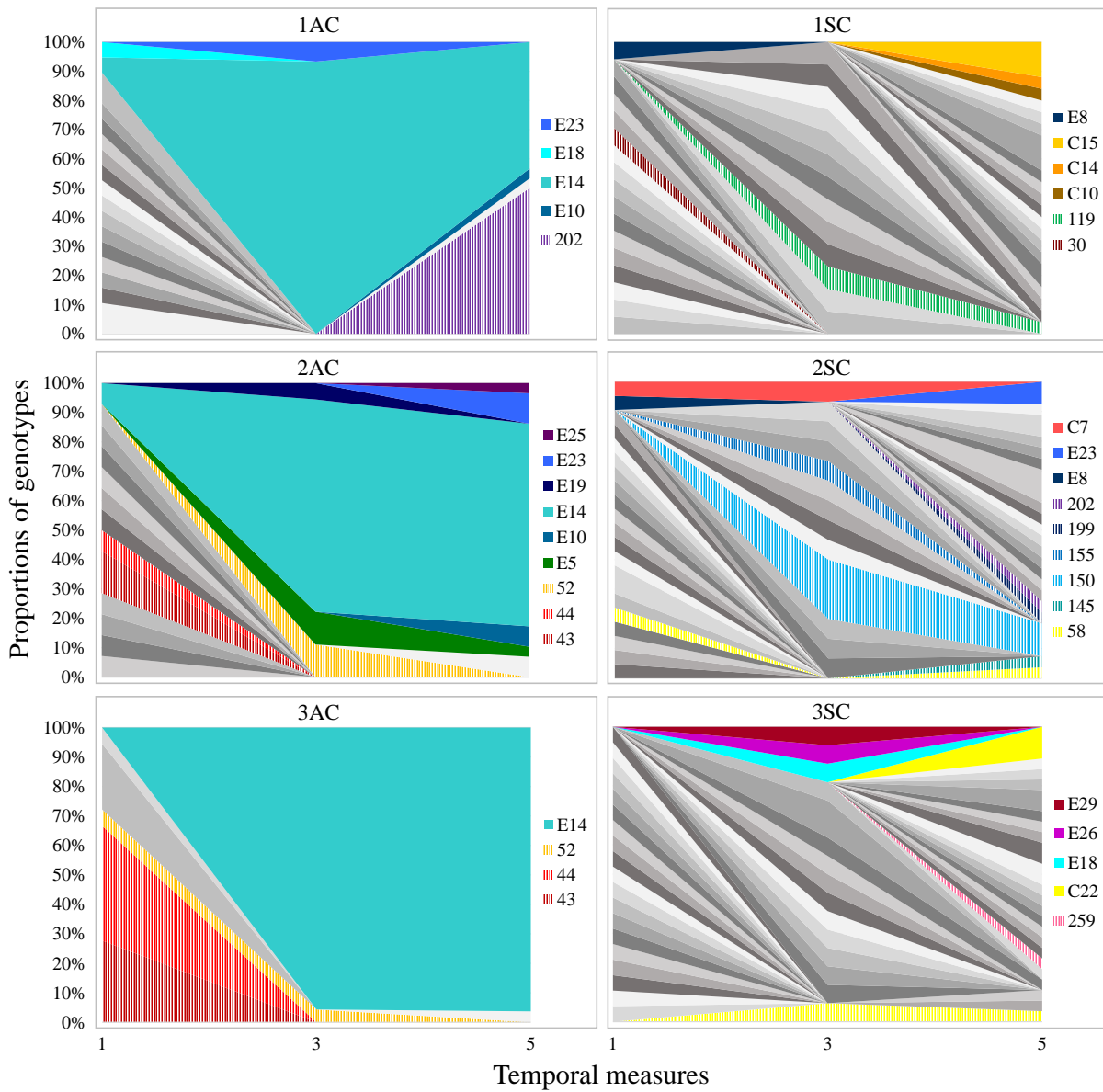


Figure 5.7: Genetic composition of asexually (left) and sexually (right) produced populations in the environment with copper, at fitness measures 1, 3 and 5. Each area represents the proportion of a given genotype in the population: genotypes from our 55 initial clones (colored, plain), new genotypes shared between experimental populations (colored, hatched) and new genotypes observed in only one population (gray shades).

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex

screened had a unique genotype. By contrast, asexually produced populations were a lot less diverse. Interestingly, one genotype of one of our 55 initial clones (the genotype labeled E14) increased in frequency in these populations over time (Figures 5.6 and 5.7). In most asexually derived populations, the fitness of individuals bearing the E14 genotype was higher than the average fitness of all other genotypes (Figure 5.8, top). This may be due to the fact that this genotype invests less in sexual reproduction: indeed, fewer males were observed during the fitness assays on individuals carrying this genotype than averaged over individuals carrying other genotypes from the same population (Figure 5.8, bottom). The higher clonal growth rate of this genotype (possibly caused by a lower investment in sex) could explain its spread within asexual populations.

Additional fitness measures were performed after the end of the adaptation experiment, in order to test to what extent the different populations had adapted to their selection environment. As explained in the methods, fitness was measured on individuals derived from resting eggs produced in each population at the end of the adaptation experiment (8 clonal generations after hatching). Furthermore, we used a different fitness measure (lifetime reproductive success, *i.e.* the total number of offspring produced by an asexual female), which is not affected by the cost of sex. Unfortunately, we obtained very few fitness measures from asexually derived populations that had evolved in copper, due to the fact that very low numbers

5.3. Results

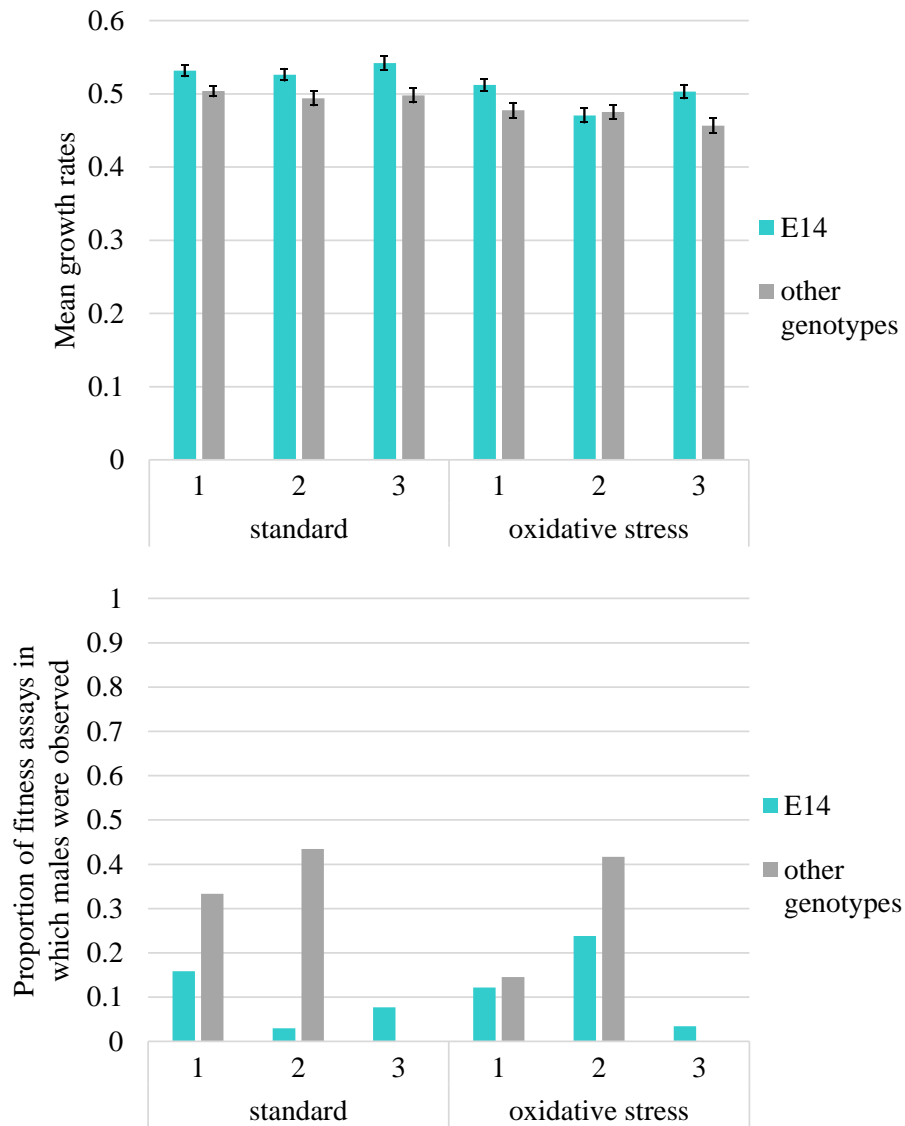


Figure 5.8: Mean (± 1 S.E.) growth rates (top) and proportion of fitness assays in which males were observed on day 6 (bottom) in fitness assays from asexually produced populations (averaged over fitness measures 1, 3 and 5), and for individuals bearing either the E14 or another genotype.

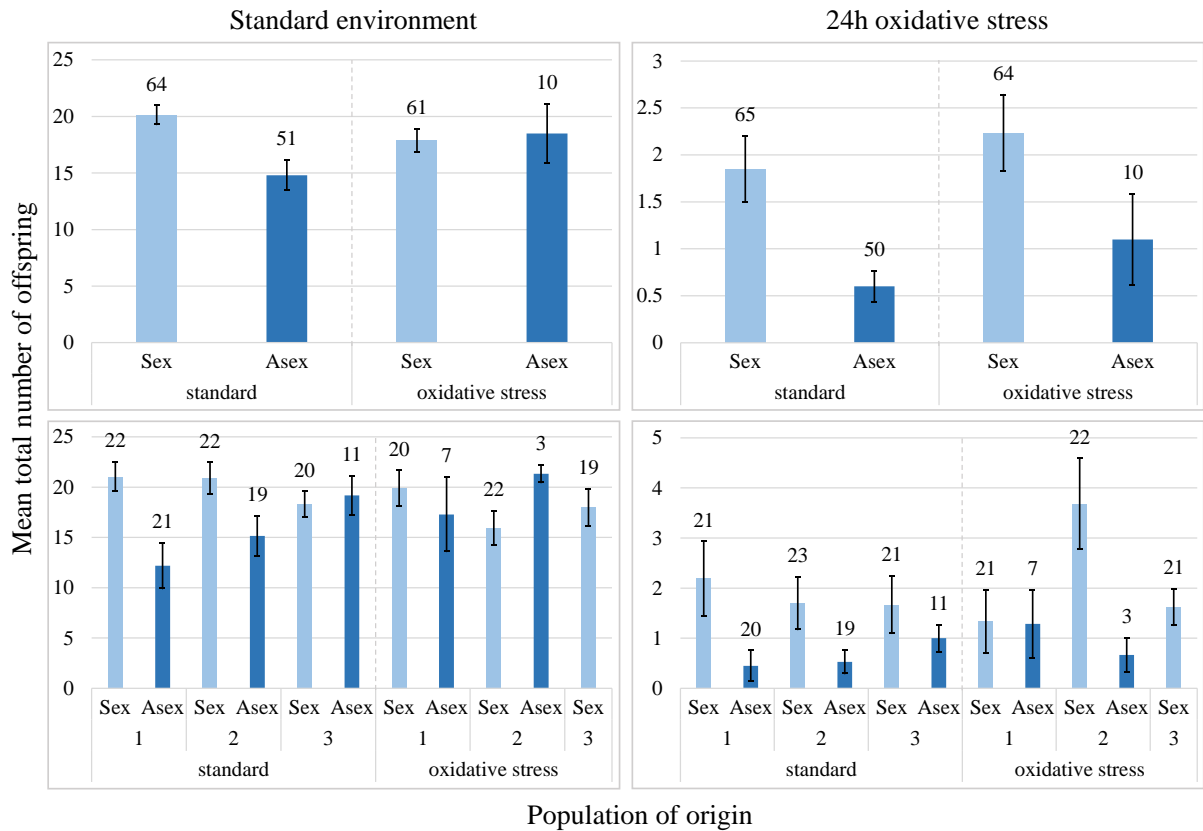


Figure 5.9: Mean (± 1 S.E.) reproductive success (measured as the total number of offspring produced) of all populations in the standard treatment (left), or after undergoing a 24h-oxidative stress (right). In the top panel, fitnesses are averaged over the replicate populations.

of resting eggs were produced in those populations at the end of the adaptation experiment (no resting egg was found in the third population, while we obtained only 7 and 3 fitness measures for the first and second populations, respectively). Furthermore, in this experiment copper was added to the algal medium just before exposing rotifers to the stressful medium, which had much stronger effects than in the adaptation experiment. A range of copper concentrations was tested, but even the lowest concentration ($0.2 \mu\text{g/L}$) had very strong effects. For this reason,

5.3. Results

rotifers were exposed to the stressful medium only during their first 24h (and were then transferred to standard medium). Overall, individuals from asexually derived populations had a lower fitness than individuals from sexually derived populations (Figure 5.9). This may result from a lower adaptation of asexually derived populations, but also potentially from inbreeding depression: indeed, given the high frequency of the microsatellite genotype corresponding to clone E14 in all asexually derived populations, a high proportion of resting eggs from these populations may have been produced by intraclonal mating, which is genetically equivalent to self-fertilization. The reduction in fitness of individuals from asexually derived populations is more important after oxidative stress (Figure 5.9), in agreement with the fact that inbreeding depression is often stronger in more stressful conditions (Cheptou et al., 2000; Armbruster and Reed, 2005). Interestingly, populations that evolved in the stressful environment performed better when stressed than their homologous population that evolved in standard culture medium. However, the difference is mainly driven by the sexually derived population 2 that evolved in the medium with copper, and that resisted significantly better to oxidative stress than the other populations (Figure 5.9, bottom right).

5.4 Discussion

The aim of the experiment was to investigate the benefits of sex by comparing sexually and asexually derived populations facing an environmental stress, and determine whether sexual reproduction allows a faster adaptation. Our results suggest that sex do facilitate adaptation of populations to a new environment. Sexually derived populations had a higher mean fitness than asexually derived populations in the last fitness measure and in the stressful environment, which may come from the fact that they adapted better than asexually derived populations (Figure 5.3, 6).

Interestingly, our sexually and asexually derived populations had a different behavior regarding their investment in sexual reproduction. Indeed, when initiating sexually derived populations, we selected for genotypes investing more in sexual reproduction by sampling sexually produced resting eggs. As a consequence, these populations produced males more frequently during the fitness assays, and maintained higher genetic diversity, compared to asexually derived populations for which males were less frequent, and in which one microsatellite genotype increased in frequency (Figures 5.4, 5.6 and 5.7). Therefore, sexual reproduction may have contributed more to the turnover of sexually derived populations than asexually derived ones.

In the standard environment, the cost of sex had the effect of reducing the fit-

5.4. Discussion

ness (measured as asexual growth rate) of sexually derived populations (investing more in sexual reproduction), compared to that of asexually derived. However, this effect was absent from the first and last fitness measures: a faster adaptation in sexually produced populations may possibly have compensated for the cost of sex, although it is not very clear why indirect benefits of sex should be stronger during the last fitness measure in the standard environment than during the previous ones. Another possible explanation is that the cost of sex was less strong during the first and the sixth fitness measures because sexual females were produced at higher densities, and thus had less impact on growth rates. Indeed, for each fitness measure we counted the number of assays in which males were observed on day 6, in order to evaluate the investment in sexual reproduction of our populations. However, the production of mictic females (and consequently, of males) is influenced by the environment. In the case of the first and last fitness measures, males could possibly have been produced later, and thus have a weaker effect on growth rates, which would explain the absence of any detectable cost of sex.

A major problem encountered in this experiment came from the choice of copper as a source of environmental stress. According to preliminary experiments, we chose a concentration of 0.05 mg L^{-1} of copper sulfate in the culture medium, which did not seem sufficient to stress the populations in the chemostat setup (probably due to the fact that copper had more time to be chelated by organic material or

Chapter 5. Adaptation of rotifers to stressful environments and the benefits of sex be metabolized by algae). Substantial differences in fitness between environments were observed only after we increased the dose of copper to higher concentrations (around 0.2 mg L^{-1}). We then performed a seventh fitness measure to confirm the results, but this time, almost all females died or did not reproduce in the copper treatment. The change in copper concentration within rotifer populations was gradual, and the lethal dose was thus probably exceeded between the 6th and 7th fitness measures.

Similar problems arose when testing for changes in fitness after adaptation. In that case, rotifers were exposed to a culture medium in which copper had just been introduced, and even the smallest concentration of copper had very strong effects on survival and fecundity. For this reason, rotifers were only stressed for 24 hours, and most of them then died without reproducing. As a consequence, the environment in which these last fitness measures were done was different from the stressful environment during the adaptation experiment.

A limit of the chemostat system is that it is difficult to ensure that densities remain constant across populations. Additionally, the results from the fitness measures at the different time points are not easily comparable due to the differences in algal concentrations between measures and sometimes between treatments (see Figure 5.3). The culture medium was sampled from our two algal chemostats (with and without copper sulfate), within which the concentration of algae varied over

5.4. Discussion

time. In order to measure fitness of individuals from the stressed populations in the environment to which they were exposed, we used the culture medium used to feed these populations (*i.e.*, with copper) without diluting it (in order to keep the same copper concentration). However, we adjusted the algal density of the standard culture medium to match that of the medium with copper, but this was not possible when the algal concentration in the standard medium was lower than in copper. Although algal density did not seem limiting in our 6-days fitness assays, the fact that the density varied between measures makes it difficult to compare fitnesses measured at different time points.

Finally, our choice of fitness estimate (asexual growth rate over 6 days) and the fact that investment in sex was higher in sexually derived populations made it difficult to disentangle the cost of sex from potential benefits of sex in terms of better adaptation (although our 6th fitness measure in the stressful environment indicate that these indirect benefits may outweigh the cost). To do so, it would have been better to use a different estimate of fitness, such as individual lifetime reproductive success (total number of individuals produced, as in the last fitness measures). Indeed, this measure is not affected by the cost of sex, and any difference in fitness observed would be solely the result of a better adaptation.

Ideas to improve the system, and perspectives for future experiments will be presented in the general discussion.

Part IV

General discussion

1 Theoretical models on the evolution of sex

Studying the selective forces acting on genes affecting the rate of sex or recombination when selection acts at a potentially large number of loci implies making assumptions on fitness landscapes. Several simulation models have considered the simple scenario in which deleterious alleles occur at a large number of loci, all with the same fitness effect, and with either no epistasis or a fixed epistasis coefficient for all pairs of mutations (*e.g.*, Keightley and Otto, 2006; Roze and Michod, 2010; Roze, 2014). Other simulation models also included a fraction of beneficial alleles, without epistasis (Hartfield et al., 2010). From their simulation results, Keightley and Otto (2006) concluded that epistasis has only minor effect (compared with drift) on the evolution of recombination or sex in finite populations. Other models represent selection acting on quantitative phenotypic traits, with either a fixed or moving phenotypic optimum, and assuming that the effects of mutations on traits are drawn from a Gaussian distribution (*e.g.*, Maynard Smith, 1980, 1988; Charlesworth, 1993; Kondrashov and Yampolsky, 1996). An advantage of this approach is that it enables one to introduce distributions of selection coefficients and epistasis, whose properties seem to match those of empirical distributions obtained from microorganisms (Martin et al., 2007). In Fisher’s geometrical model, with an arbitrary number of quantitative traits under Gaussian stabilizing selection, it can be shown that epistasis is null on

average between any two mutations from any point in phenotypic space (Martin et al., 2007). However, the variance of epistasis can be important (in particular when the dimensionality of the landscape is low), and compensatory mutations may be frequent. Due to this high rate of compensatory mutations, the drift load (fixation of deleterious mutations due to random genetic drift) is quite low, unless population size is very small (*e.g.*, Poon and Otto, 2000; Tenaillon et al., 2007). In this class of models, recombination or sex are expected to be disfavored in populations staying at a phenotypic optimum, due to the fact that recombination disrupts combinations of compensatory mutations (Maynard Smith, 1980; Charlesworth, 1993). By adding a parameter representing mutational bias (in which case mutation tends to displace mean phenotypes away from their optimal value, thus introducing a component of directional selection), the model presented in chapter 1 enabled us to explore a continuum between the classical Fisher’s geometrical model and models in which all mutations have the same deleterious effect and in which epistasis is constant (and negative). The results show that when such a bias is added, drift load may be important, particularly in asexual populations (due to stronger deviations of the mean phenotypes from the optimum). We have also seen that positive rates of sex are favored as soon as there is mutational bias, the equilibrium rate of sex increasing monotonously with the degree of mutational bias. Models with fixed epistasis thus represent a best-case scenario for the evolution of sex (at least in constant environments),

the rate of sex always decreasing as the variance in epistasis increases. With a constant phenotypic optimum, the equilibrium rate of sex remains low when the cost of sex is moderate to high, and the strength of directional selection must be more important in order to enable the evolution of higher rates of sex, which is possible when the phenotypic optimum changes over time (Chapter 2). Higher rates of sex are favored when the speed and regularity (temporal autocorrelation) of the environmental change increase; this matches Charlesworth's results on selection for recombination based on the infinitesimal model of quantitative traits. It would be interesting to extend the models presented in Chapter 2 to the case of multiple selected traits: one expects that selection for sex should increase with the number of traits under directional selection, while adding traits under stabilizing selection around a fixed optimum should disfavour sex (unless mutational bias is added).

The article presented in Chapter 1 provides expressions for selection for sex in terms of measurable quantities (effect of sex on the mean fitness of offspring, and on the additive variance in fitness among offspring). These approximations, however, are only valid when selection is sufficiently weak and recombination rates sufficiently high. In other regimes (*e.g.*, when selection is strong or when there is low recombination) it is difficult to evaluate the relative importance of deterministic (negative epistasis) and stochastic (Hill-Robertson effect) forces selecting for sex. It may be interesting to derive analytical approximations for

the Hill-Robertson effect in a quantitative trait model, but it should again be difficult to obtain useful approximations when the rate of sex is low.

Furthermore, the models that we used only considered haploid populations. In diploid populations, sex may also affect frequency of homozygous or heterozygous individuals at different loci, through segregation (Otto, 2003; Roze and Michod, 2010). Under Gaussian stabilizing selection and assuming additive effects within and between loci on phenotypes, deleterious alleles tend to be partially recessive, in which case sex helps to better purge deleterious alleles (Otto, 2003). It would be interesting to extend the models of chapters 1 and 2 to the case of diploid populations. Another possible extension would be to consider models of spatially structured populations, in which phenotypic optimum varies in space: in such case, local adaptation can maintain more genetic variation within populations, but whether sex should be favored or not is not clear. One could also consider scenarios in which the phenotypic optimum varies both in time and space. Eventually, more complex fitness landscapes could be considered, in which several phenotypic optima could co-occur (instead of a single optimum as in the models considered here), whose position may also vary over time. However, the number of possible scenarios that could be considered is infinite, and more data are needed on the properties of fitness landscapes in real populations.

2 Empirical tests

An initial goal of this thesis was to use monogonont rotifers to test some of the predictions from theoretical models (*e.g.*, on the effect of the type of environmental change on selection for sex). However, the setting up of the experimental system and the preliminary tests took quite some time, and our initial objective could not be achieved over the course of the thesis. Nevertheless, the results show that *Brachionus plicatilis* seems to be an interesting biological system to explore selective forces acting on sex. First, the results clearly show that investment in sexual reproduction is genetically variable and that this variability can be important (Chapter 4). Second, one can easily select for individuals engaging more or less in sex by sampling resting eggs from experimental populations, or by sampling asexual offspring directly (Chapter 5). Furthermore, microsatellite analyses show that sexual reproduction contributes to the turnover of populations maintained in chemostats (Chapter 3), so that genotypes investing more in sexual reproduction might be advantaged when there is a need for the production of new genotypes, for instance, when the environment is changing. Finally, the results of Chapter 5 suggest that sex may facilitate adaptation to a new, stressful environment, since sexually derived populations had higher fitness than asexually derived ones in our stressful environment at the end of the experiment (while the opposite trend was observed in the standard environment). However,

more measures should have been obtained in order to confirm that adaptation was indeed more efficient in sexually derived populations.

Different measures can be used to estimate the fitness of individuals and their propensity for sex, but each of them have their own limitations. In Chapter 5 we used clonal growth rates to estimate fitnesses, but these measures did not allow us to separate the effects of the cost of sex from the possible benefits of sex in terms of higher adaptive rates. In this respect, it could have been better to use a fitness measure that is not affected by the cost of sex, such as the lifetime reproductive success (total number of offspring produced by an asexual female over its lifetime), or even to combine both measures in order to disentangle both effects. However, this type of measure is more time-consuming, as females must be inspected daily and separated from their offspring. Then, estimations of the propensity for sex based on the density at first male appearance (mixis threshold) as was done in chapters 3 and 4, are known to be affected by the experimental protocol, and in particular by the culture volume in which the measures are performed (Carmona et al., 2011): higher density thresholds are usually obtained when experiments are performed in smaller volumes, possibly due to the fact that population density may reach higher values by the time the mixis-inducing protein reached the threshold concentration. This may explain the fact that our estimations of the mixis threshold are much higher than those obtained by Galbaldón and Carmona (2015) from individuals originating from the same natural

population, measured in 15 mL instead of 0.5 mL. Interestingly, Rougier and Pourriot (1977) showed that the production of mictic females decreased when individuals were cultured in medium conditioned by their mother, compared to individuals cultured in daily-renewed culture medium. These results suggest that the effect of density on sex induction might be more complex than usually acknowledged, and that individuals might also produce a substance inhibiting sexual reproduction, to some extent. It would be of particular interest to further explore the effects of density on the induction of sex: previous studies have usually compared 2 densities or a range of moderate densities (*e.g.*, Carmona et al., 1993; Snell et al., 2006), but one may imagine that density could have a non-monotonous effect on sex induction. If this is the case, mixis threshold measurements may yield higher values for clones with higher growth rates, even if the threshold concentration of the mixis-inducing protein stays the same. This may give the false impression that clones with higher growth rate in the medium used for the tests have a lower propensity for sex. Overall, estimating the propensity for sex by measuring the mixis ratio (proportion of sexual individuals over all offspring produced by a female) seems a better solution, but is again more time-consuming.

Better understanding transgenerational maternal effects is also important to better assess to what extent changes in the propensity for sex (such as the ones observed in Becks and Agrawal, 2012 or Luijckx et al., 2017) may be plastic rather

than genetic. The results shown in chapter 4 demonstrate that in *B. plicatilis*, sex tends to be inhibited over the 8 to 10 clonal generations following fertilization, as observed previously in other monogonont species (*e.g.*, Schröder and Gilbert, 2004; Gilbert, 2017). Whether changes in density or in environmental conditions may trigger similar transgenerational maternal effects remains poorly known, and should be explored.

Finally, the use of copper as a source of environmental stress in Chapter 5 proved to be problematic, probably due to the fact that its effect is reduced when it stays longer in the culture medium before rotifers are exposed to it (possibly due to the fact that it has more time to be chelated by organic particles, or that algae have more time to metabolize it). It may have been better to add a small quantity of copper directly into the rotifer populations everyday, to ensure a constant (and better controlled) concentration of copper in the the environment to which these populations were exposed. Alternatively, other forms of environmental challenge may be considered, such as modifying the composition of the culture medium, as in Becks and Agrawal (2010) or Declerck et al. (2015). More experimental tests should thus be performed to evaluate which environmental parameter is easiest to modify, before setting up experiments aiming at comparing different types of temporal or spatial environmental changes, in order to further test experimentally the predictions obtained from theoretical models.

LITERATURE CITED

Agrawal, A. F. 2009. Spatial heterogeneity and the evolution of sex in diploids. *The American Naturalist* 174:S54–S70.

Allen, D. E. and M. Lynch. 2008. Both costs and benefits of sex correlate with relative frequency of asexual reproduction in cyclically parthenogenic *Daphnia pulicaria* populations. *Genetics* 179:1497–1502.

Aparici, E., M. J. Carmona, and M. Serra. 2001. Variability for mixis initiation in *Brachionus plicatilis*. *Hydrobiologia* 446-447:45–50.

Armbruster, P. and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. *Heredity* 95:235–242.

Bartoń, K. 2018. MuMIn: multi-model inference. R package version 1.40.4, <https://CRAN.R-project.org/package=MuMIn>.

Barton, N. H. 1995. A general model for the evolution of recombination. *Genetical research* 65:123–145.

Barton, N. H. and S. P. Otto. 2005. Evolution of recombination due to random drift. *Genetics* 169:2353–2370.

- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4 67:1–48.
- Becks, L. and A. F. Agrawal. 2010. Higher rates of sex evolve in spatially heterogeneous environments. *Nature* 468:89–92.
- Becks, L. and a. F. Agrawal. 2011. The effect of sex on the mean and variance of fitness in facultatively sexual rotifers. *Journal of Evolutionary Biology* 24:656–664.
- Becks, L. and A. F. Agrawal. 2012. The evolution of sex is favoured during adaptation to new environments. *PLoS biology* 10:e1001317.
- Bell, G. 1982. *The Masterpiece of Nature: The evolution and genetics of sexuality*. University of California Press, Berkeley.
- Birky, C. W. J. and J. J. Gilbert. 1971. Parthenogenesis in rotifers: the control of sexual and asexual reproduction. *American Zoologist* 11:245–266.
- Bürger, R. 1999. Evolution of genetic variability and the advantage of sex and recombination in changing environments. *Genetics* 153:1055–1069.
- Campillo, S., E. M. García-Roger, M. J. Carmona, Á. Gómez, and M. Serra. 2009. Selection on life-history traits and genetic population divergence in rotifers. *Journal of Evolutionary Biology* 22:2542–2553.
- Campillo, S., E. M. García-Roger, M. J. Carmona, and M. Serra. 2011. Local adaptation in rotifer populations. *Evolutionary Ecology* 25:933–947.
- Campillo, S., E. M. García-Roger, D. Martínez-Torres, and M. Serra. 2005. Morpholog-

- ical stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. *Hydrobiologia* 546:181–187.
- Carmona, M. J., N. Dimas-Flores, E. M. García-Roger, and M. Serra. 2009. Selection of low investment in sex in a cyclically parthenogenetic rotifer. *Journal of Evolutionary Biology* 22:1975–1983.
- Carmona, M. J., N. Dimas-Flores, J. Montero-Pau, and M. Serra. 2011. Effect of experimental methodology on estimation of density at sex initiation in cyclically parthenogenetic rotifers. *Hydrobiologia* 662:131–139.
- Carmona, M. J., Á. Gómez, and M. Serra. 1995. Mictic patterns of the rotifer *Brachionus plicatilis* Müller in small ponds. *Hydrobiologia* 313/314:365–371.
- Carmona, M. J., M. Serra, and M. R. Miracle. 1993. Relationships between mixis in *Brachionus plicatilis* and preconditioning of culture medium by crowding. *Hydrobiologia* 255-256:145–152.
- . 1994. Effect of population density and genotype on life-history traits in the rotifer *Brachionus plicatilis* O . F. Müller. *Journal of Experimental Marine Biology and Ecology* 182:223–235.
- Charlesworth, B. 1989. The evolution of sex and recombination. *Trends in Ecology and Evolution* 4:264–267.
- . 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genetics Research* 55:199–221.
- . 1993. Directional selection and the evolution of sex and recombination. *Genetical Research* 61:205–224.

- Charlesworth, B. and D. Charlesworth. 1975. An experiment on recombination load in *Drosophila melanogaster*. *Genetics Research* 25:267–274.
- Cheptou, P. O., E. Imbert, J. Lepart, and J. Escarre. 2000. Effects of competition on lifetime estimates of inbreeding depression in the outcrossing plant *Crepis sancta* (Asteraceae). *Journal of Evolutionary Biology* 13:522–531.
- Ciros-Pérez, J., Á. Gómez, and M. Serra. 2001. On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. *Journal of Plankton Research* 23:1311–1328.
- Colegrave, N. 2002. Sex releases the speed limit on evolution. *Nature* 420:664–666.
- Colegrave, N., O. Kaltz, and G. Bell. 2002. The ecology and genetics of fitness in *Chlamydomonas*. VIII. The Dynamics of adaptation to novel environments after a single episode of sex. *Evolution* 56:14–21.
- Cooper, T. F. 2007. Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. *PLoS biology* 5:e225.
- Crawley, M. J. 2007. *The R Book*. Chichester, UK.
- Da Silva, J. and G. Bell. 1996. The ecology and genetics of fitness in *Chlamydomonas*. VII. The effect of sex on the variance in fitness and mean fitness. *Evolution* 50:1705–1713.
- Declerck, S. a. J., A. R. Malo, S. Diehl, D. Waasdorp, K. D. Lemmen, K. Proios, and S. Papakostas. 2015. Rapid adaptation of herbivore consumers to nutrient limitation: eco-evolutionary feedbacks to population demography and resource control. *Ecology Letters* Pp. n/a–n/a.

- Dolgin, E. S. and S. P. Otto. 2003. Segregation and the evolution of sex under over-dominant selection. *Genetics* 164:1119–1128.
- Ellner, S. and N. G. Hairston. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *The American Naturalist* 143:403–417.
- Feldman, M. W., F. B. Christiansen, and L. D. Brooks. 1980. Evolution of recombination in a constant environment. *Proceedings of the National Academy of Sciences of the United States of America* 77:4838–4841.
- Feldman, M. W., S. P. Otto, and F. B. Christiansen. 1997. Population genetic perspectives on the evolution of recombination. *Annual Review of Genetics* 30:261–295.
- Felsenstein, J. 1974. The evolutionary advantage of recombination. *Genetics* 78:737–756.
- Felsenstein, J. and S. Yokoyama. 1976. The evolutionary advantage of recombination. II. Individual selection for recombination. *Genetics* 83:845–859.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford, UK.
- Fontaneto, D., I. Giordani, G. Melone, and M. Serra. 2007. Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. *Hydrobiologia* 583:297–307.
- Fontaneto, D. and W. H. D. Smet. 2014. Rotifera. Pp. 217–300 *in* A. Schmidt-Rhaesa, ed. *Gastrotricha and Gnathifera*. De Gruyter, Berlin, Germany.

- Fussmann, G. F., G. Kramer, and M. Labib. 2007. Incomplete induction of mixis in *Brachionus calyciflorus*: Patterns of reproduction at the individual level. *Hydrobiologia* 593:111–119.
- Gabaldón, C. and M. J. Carmona. 2015. Allocation patterns in modes of reproduction in two facultatively sexual cryptic rotifer species. *Journal of Plankton Research* 37:429–440.
- Gabaldón, C., J. Montero-Pau, M. Serra, and M. J. Carmona. 2013. Morphological similarity and ecological overlap in two rotifer species. *PLoS ONE* 8:e57087.
- Gandon, S. and S. P. Otto. 2007. The evolution of sex and recombination in response to abiotic or coevolutionary fluctuations in epistasis. *Genetics* 175:1835–1853.
- García-Roger, E. M., M. J. Carmona, and M. Serra. 2006. Patterns in rotifer diapausing egg banks: Density and viability. *Journal of Experimental Marine Biology and Ecology* 336:198–210.
- . 2017. Modes, mechanisms and evidence of bet hedging in rotifer diapause traits. *Hydrobiologia* 796:223–233.
- Gerrish, P. J. and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. *Genetica* 102-103:127–144.
- Gilbert, J. J. 1963. Mictic female production in the rotifer *Brachionus calyciflorus*. *Journal of Experimental Zoology* 153:113–123.
- . 2002. Endogenous regulation of environmentally induced sexuality in a rotifer: A multigenerational parental effect induced by fertilisation. *Freshwater Biology* 47:1633–1641.

- . 2003. Environmental and endogenous control of sexuality in a rotifer life cycle: Developmental and population biology. *Evolution and Development* 5:19–24.
- . 2017. Non-genetic polymorphisms in rotifers: Environmental and endogenous controls, development, and features for predictable or unpredictable environments. *Biological Reviews* 92:964–992.
- Goddard, M. R., H. C. J. Godfray, and B. Austin. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature* 434:636–640.
- Gómez, Á. and G. R. Carvalho. 2000. Sex, parthenogenesis and genetic structure of rotifers: Microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology* 9:203–214.
- Gómez, Á., C. Clabby, and G. R. Carvalho. 1998. Isolation and characterization of microsatellite loci in a cyclically parthenogenetic rotifer, *Brachionus plicatilis*. *Molecular ecology* 7:1619–1621.
- Gómez, Á., M. Serra, G. R. Carvalho, and D. H. Lunt. 2002. Speciation in ancient cryptic species complexes: Evidence from the molecular phylogeny of *Brachionus Plicatilis* (Rotifera). *Evolution* 56:1431–1444.
- Gómez, Á. and T. W. Snell. 1996. Sibling species and cryptic speciation in the *Brachionus plicatilis* species complex (Rotifera). *Journal of Evolutionary Biology* 9:953–964.
- Gómez, Á., M. Temprano, and M. Serra. 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. *Journal of Evolutionary Biology* 8:601–622.

- Guillard, R. R. L. and J. H. Ryther. 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Canadian Journal of Microbiology 8:229–239.
- Hagiwara, A. and A. Hino. 1989. Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Hydrobiologia 186-187:415–421.
- Hagiwara, A., Y. Kadota, and A. Hino. 2005. Maternal effect by stem females in *Brachionus plicatilis*: Effect of starvation on mixis induction in offspring. Hydrobiologia 546:275–279.
- Hartfield, M. and P. D. Keightley. 2012. Current hypotheses for the evolution of sex and recombination. Integrative Zoology 7:192–209.
- Hartfield, M., S. P. Otto, and P. D. Keightley. 2010. The role of advantageous mutations in enhancing the evolution of a recombination modifier. Genetics 184:1153–1164.
- Hill, W. G. and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genetics Research 8:269–394.
- Hino, A. and R. Hirano. 1976. Ecological studies on the mechanism of bisexual reproduction in the rotifer *Brachionus plicatilis* - I General aspects of bisexual reproduction inducing factors. Bulletin of the Japanese Society of Scientific Fisheries 42:1093–1099.
- . 1977. Ecological studies on the mechanism of bisexual reproduction in the rotifer *Brachionus plicatilis* - II Effects of cumulative parthenogenetic generation on

- the frequency of bisexual reproduction. *Bulletin of the Japanese Society of Scientific Fisheries* 43:1147–1155.
- Innes, D. J. and P. D. N. Hebert. 1988. The origin and genetic basis of obligate parthenogenesis in *Daphnia pulex*. *Evolution* 42:1024–1035.
- Kaltz, O. and G. Bell. 2002. The ecology and genetics of fitness in *Chlamydomonas*. XII. Repeated sexual episodes increase rates of adaptation to novel environments. *Evolution* 56:1743–1753.
- Kamizono, S., E. O. Ogello, Y. Sakakura, and A. Hagiwara. 2017. Effect of starvation and accumulation of generations on mixis induction in offspring of the monogonont rotifer *Brachionus manjavacas* hatched from resting eggs. *Hydrobiologia* 796:93–97.
- Keightley, P. D. and S. P. Otto. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* 443:89–92.
- Kimura, M. 1956. A model of a genetic system which leads to closer linkage by natural selection. *Evolution* 10:278–287.
- Kondrashov, A. S. 1984. Deleterious mutations as an evolutionary factor. I. The advantage of recombination. *Genetic Research* 44:199–217.
- . 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature* 336:435–440.
- Kondrashov, A. S. and L. Y. Yampolsky. 1996. High genetic-variability under the balance between symmetrical mutation and fluctuating stabilizing selection. *Genetical Research* Vol 68:157–164.

- Kotani, T., M. Ozaki, K. Matsuoka, T. W. Snell, and A. Hagiwara. 2001. Reproductive isolation among geographically and temporally isolated marine *Brachionus* strains. *Hydrobiologia* 446-447:283–290.
- Lachapelle, J. and G. Bell. 2012. Evolutionary rescue of sexual and asexual populations in a deteriorating environment. *Evolution* 66:3508–3518.
- Lehtonen, J., M. D. Jennions, and H. Kokko. 2012. The many costs of sex. *Trends in Ecology and Evolution* 27:172–178.
- Lenormand, T. and S. P. Otto. 2000. The evolution of recombination in a heterogeneous environment. *Genetics* 156:423–438.
- Lewis, W. M. 1987. The cost of sex. Pp. 33–57 in S. Stearns, ed. *The evolution of sex and its consequences*. Birkhauser Verlag, Basel.
- Lubzens, E. 1989. Possible use of rotifers resting eggs and preserved live rotifers (*Brachionus plicatilis*) in aquaculture. Pp. 741–750 in N. De Pauw, E. Jaspers, H. Ackefors, and N. Wilkins, eds. *Aquaculture: a biotechnology in progress: volume 1*. European Aquaculture Society, Bredene, Belgium.
- Lubzens, E., R. Fishler, and V. Berdugo-White. 1980. Induction of sexual reproduction and resting egg production in *Brachionus plicatilis* reared in sea water. *Hydrobiologia* 73:55–58.
- Lubzens, E., G. Minkoff, and S. Marom. 1985. Salinity dependence of sexual and asexual reproduction in the rotifer *Brachionus plicatilis*. *Marine Biology* 85:123–126.

- Lubzens, E., Y. Wax, G. Minkoff, and F. Alder. 1993. A model evaluating the contribution of environmental factors to production of resting eggs in the rotifer *Brachionus plicatilis*. *Hydrobiologia* 255/256:127–138.
- Lubzens, E., O. Zmora, and Y. Barr. 2001. Biotechnology and aquaculture of rotifers. *Hydrobiologia* 446/447:337–353.
- Luijckx, P., E. K. H. Ho, M. Gasim, S. Chen, A. Stanic, C. Yanchus, Y. S. Kim, and A. F. Agrawal. 2017. Higher rates of sex evolve during adaptation to more complex environments. *Proceedings of the National Academy of Sciences* 114:534–539.
- Lynch, M. and H.-W. Deng. 1994. Genetic slippage in response to sex. *The American Naturalist* 144:242–261.
- Martin, G., S. F. Elena, and T. Lenormand. 2007. Distributions of epistasis in microbes fit predictions from a fitness landscape model. *Nature genetics* 39:555–560.
- Martin, G. and T. Lenormand. 2006. A general multivariate extension of Fisher’s geometrical model and the distribution of mutation fitness effects across species. *Evolution; international journal of organic evolution* 60:893–907.
- Martínez-Ruiz, C. and E. M. García-Roger. 2015. Being first increases the probability of long diapause in rotifer resting eggs. *Hydrobiologia* 745:111–121.
- Mauger, S., L. Couceiro, and M. Valero. 2012. A simple and cost-effective method to synthesize an internal size standard amenable to use with a 5-dye system. *Prime Research on Biotechnology* 2:40–46.
- Maynard Smith, J. 1971. The origin and maintenance of sex. Pp. 163–175 *in* G. C. Williams, ed. *Group selection*. Aldine Atherton, Chicago.

- . 1980. Selection for recombination in a polygenic model. *Genetical Research* 35:269–277.
- . 1988. Selection for recombination in a polygenic model - the mechanism. *Genetic Research* 51:59–63.
- McDonald, M. J., D. P. Rice, and M. M. Desai. 2016. Sex speeds adaptation by altering the dynamics of molecular evolution. *Nature* 000:00.
- Minkoff, G., E. Lubzens, and D. Kahan. 1983. Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. *Hydrobiologia* 104:61–69.
- Montero-Pau, J. and M. Serra. 2011. Life-cycle switching and coexistence of species with no niche differentiation. *PLoS ONE* 6:1–7.
- Muller, H. J. 1932. Some genetic aspects of sex. *The American Naturalist* 66:118–138.
- Nei, M. 1967. Modification of linkage intensity by natural selection. *Genetics* 57:625–641.
- Nunney, L. 1989. The maintenance of sex by group selection. *Evolution* 43:245–257.
- Ortells, R., T. W. Snell, Á. Gómez, and M. Serra. 2000. Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. *Arch. Hydrobiol.* 149:529–551.
- Otto, S. P. 2003. The advantages of segregation and the evolution of sex. *Genetics* 164:1099–1118.
- . 2009. The evolutionary enigma of sex. *The American Naturalist* 174:S1–S14.

- Otto, S. P. and N. H. Barton. 2001. Selection for recombination in small populations. *Evolution; international journal of organic evolution* 55:1921–1931.
- Otto, S. P. and M. W. Feldman. 1997. Deleterious mutations, variable epistatic interactions, and the evolution of recombination. *Theoretical Population Biology* 51:134–147.
- Papakostas, S., A. Triantafyllidis, I. Kappas, and T. J. Abatzopoulos. 2005. The utility of the 16S gene in investigating cryptic speciation within the *Brachionus plicatilis* species complex. *Marine Biology* 147:1129–1139.
- Peck, J. R. 1994. A ruby in the rubbish: Beneficial mutations, deleterious mutations and the evolution of sex. *Genetics* 137:597–606.
- Peters, A. D. and C. M. Lively. 1999. The Red Queen and fluctuating epistasis: A population genetic analysis of antagonistic coevolution. *The American Naturalist* 154:393–405.
- Peters, A. D. and S. P. Otto. 2003. Liberating genetic variance through sex. *BioEssays* 25:533–537.
- Poon, A. and S. P. Otto. 2000. Compensating for our load of mutations: freezing the meltdown of small populations. *Evolution; international journal of organic evolution* 54:1467–1479.
- Pourriot, R. and C. Rougier. 1976. Influence de l'âge des parents sur la production de femelles mictiques chez *Brachionus calyciflorus* (Pallas) et *B. rubens* Ehr. (Rotifères). *C.R. Acad. Sci. Paris* 283:1497–1500.

- Pourriot, R. and T. W. Snell. 1983. Resting eggs in rotifers. *Hydrobiologia* 104:213–224.
- Pylkov, K. V., L. A. Zhivotovsky, and M. W. Feldman. 1998. Migration versus mutation in the evolution of recombination under multilocus selection. *Genetical Research* 71:247–256.
- R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Renaut, S., T. Replansky, A. Heppleston, and G. Bell. 2006. The ecology and genetics of fitness in *Chlamydomonas*. XIII. Fitness of long-term sexual and asexual populations in benign environments. *Evolution* 60:2272–2279.
- Rice, W. R. and A. K. Chippindale. 2001. Sexual recombination and the power of natural selection. *Science* 294:555–559.
- Rougier, C. and R. Pourriot. 1977. Aging and control of the reproduction in *Brachionus calyciflorus* (pallas)(rotatoria). *Experimental gerontology* 12:137–151.
- Rousset, F. 2008. GENEPOP’007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8:103–106.
- Roze, D. 2012. Disentangling the benefits of sex. *PLoS Biology* 10:6–9.
- . 2014. Selection for sex in finite populations. *Journal of Evolutionary Biology* 27:1304–1322.

- Roze, D. and N. H. Barton. 2006. The Hill-Robertson effect and the evolution of recombination. *Genetics* 173:1793–1811.
- Roze, D. and R. E. Michod. 2010. Deleterious mutations and selection for sex in finite diploid populations. *Genetics* 184:1095–1112.
- Scheuerl, T. and C. P. Stelzer. 2013. Patterns and dynamics of rapid local adaptation and sex in varying habitat types in rotifers. *Ecology and Evolution* 3:4253–4264.
- Schröder, T. 2005. Diapause in monogonont rotifers. Pp. 291–306 *in* A. Herzig, R. Gulati, C. Jersabek, and L. May, eds. *Rotifera X: Rotifer Research: Trends, New Tools and Recent Advances*, Vol 181. Springer, Dordrecht.
- Schröder, T. and J. J. Gilbert. 2004. Transgenerational plasticity for sexual reproduction and diapause in the life cycle of monogonont rotifers: intraclonal, intraspecific and interspecific variation in the response to crowding. *Functional Ecology* 18:458–466.
- Segers, H. 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclatures, taxonomy and distribution. *Zootaxa* 1564:1–104.
- . 2008. Global diversity of rotifers (*Rotifera*) in freshwater. *Hydrobiologia* 595:49–59.
- Serra, M., T. W. Snell, and J. J. Gilbert. 2005. Delayed mixis in rotifers: An adaptive response to the effects of density-dependent sex on population growth. *Journal of Plankton Research* 27:37–45.
- Smith, H. A. and T. W. Snell. 2012. Rapid evolution of sex frequency and dormancy as hydroperiod adaptations. *Journal of Evolutionary Biology* 25:2501–2510.

- Snell, T. W. 2017. Analysis of proteins in conditioned medium that trigger monogonont rotifer mictic reproduction. *Hydrobiologia* 796:245–253.
- Snell, T. W. and E. Boyer. 1988. Thresholds of mictic female production in the rotifer *Brachionus plicatilis*. *Journal of Experimental Marine Biology and Ecology* 124:73–85.
- Snell, T. W. and F. H. Hoff. 1985. The effect of environmental factors on resting egg production in the rotifer *Brachionus plicatilis*. *Journal of the World Mariculture Society* 16:484–497.
- Snell, T. W., J. Kubanek, W. Carter, A. B. Payne, J. Kim, M. K. Hicks, and C. P. Stelzer. 2006. A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). *Marine Biology* 149:763–773.
- Stelzer, C. P. and T. W. Snell. 2003. Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. *Limnology and Oceanography* 48:939–943.
- Stelzer, C.-P. and T. W. Snell. 2006. Specificity of the crowding response in the *Brachionus plicatilis* species complex. *Limnology and Oceanography* 51:125–130.
- Stemberger, R. S. and J. J. Gilbert. 1985. Body size , food concentration , and population growth in planktonic rotifers. *Ecology* 66:1151–1159.
- . 1987. Rotifer threshold food concentrations and the size-efficiency hypothesis. *Ecology* 68:181–187.
- Tarazona, E., E. M. García-Roger, and M. J. Carmona. 2017. Experimental evolution

- of bet hedging in rotifer diapause traits as a response to environmental unpredictability. *Oikos* 126:1162–1172.
- Tenaillon, O., O. K. Silander, J.-P. Uzan, and L. Chao. 2007. Quantifying organismal complexity using a population genetic approach. *PloS one* 2:e217.
- Timmermeyer, N. and C. P. Stelzer. 2006. Chemical induction of mixis in the rotifer *Synchaeta tremula*. *Journal of Plankton Research* 28:1233–1239.
- Tortajada, A. M., M. J. Carmona, and M. Serra. 2009. Does haplodiploidy purge inbreeding depression in rotifer populations? *PLoS ONE* 4:1–8.
- Turelli, M., D. W. Schemske, and P. Bierzychudek. 2001. Stable two-allele polymorphisms maintained by fluctuating fitnesses and seed banks: Protecting the blues in *Linanthus parryae*. *Evolution* 55:1283–1298.
- Vanhoenacker, E., L. Sandell, and D. Roze. 2018. Stabilizing selection, mutational bias, and the evolution of sex. *Evolution* 72:1740–1758.
- Wallace, R. and H. Smith. 2009. Rotifera. Pp. 689–703 *in* G. E. Likens, ed. *Encyclopedia of Inland Waters*. Elsevier, Oxford, UK.
- Wallace, R. L., T. W. Snell, C. Ricci, and T. Nogrady. 2006. *Rotifera biology, ecology and systematics*. Backhuys Publishers, Leiden.
- Warner, R. R. and P. L. Chesson. 1985. Coexistence mediated by recruitment fluctuations: A field guide to the storage effect. *The American Naturalist* 125:769–787.
- Waxman, D. and J. R. Peck. 1999. Sex and adaptation in a changing environment. *Genetics* 153:1041–1053.

Weismann, A. 1889. The significance of sexual reproduction in the theory of natural selection. Pp. 251–332 *in* E. B. Poulton, S. Schönland, and A. E. Shipley, eds. Essays upon heredity and kindred biological problems. Clarendon, Oxford.

Appendices

Appendix S1

Supplementary files from

Vanhoeenacker et al. (2018)

FILE S1: DESCRIPTION OF SIMULATION PROGRAMS

The genome of each individual consists in a single linear chromosome with map length R (average number of cross-overs at meiosis). The ℓ loci affecting the n traits under stabilizing selection are biallelic and equally spaced along the chromosome, the genome of an individual at these loci being represented by a set of bits (0 or 1). At the beginning of the simulation, the effects of allele 1 at each locus on the different phenotypes are drawn and stored in a table: as explained above, each locus only affects a subset of m randomly sampled traits, the effect on each of these traits being drawn from a Gaussian distribution with standard deviation $a = \sqrt{2V_s(1-\theta)\bar{s}_d/m}$ and average $b = \sqrt{2V_s\theta\bar{s}_d/m}$. At the start of each generation, genetic components g_α are computed for each individual given its genotype, and environmental components e_α are drawn from a Gaussian distribution with mean 0 and variance V_e , fixed to $1/n$ to avoid that fitness reaches very low values when the number of selected traits is large. The fitness of each individual is then computed according to equation 3 in the main text, where ω^2 is fixed to 10; however, as noted above, the values of ω^2 and V_e should have little effect on the results (for given values of \bar{s}_d and θ), since $V_s = \omega^2 + V_e$ may be considered as a scaling factor.

Investment in sexual reproduction s is coded by ℓ_s loci, which are also equally spaced along the chromosome. These loci are multiallelic, investment in sex being given by:

$$s = s_{\text{init}} + \sum_{i=1}^{\ell_s} g_{si} + e_s \quad (1)$$

where s_{init} is the initial investment in sex, g_{si} the effect of locus i on s , and e_s an environmental component drawn from a centered Gaussian distribution with variance

$V_{e,s}$. If the value of s obtained from equation 1 is lower than 0 or higher than 1, it is then set to 0 or 1 (respectively). During a number of preliminary generations, g_{si} is fixed to zero for all loci affecting the rate of sex. Then, mutation occurs at a rate U_s per generation on the whole set of ℓ_s loci. When a mutation occurs at locus i , a quantity drawn from a centered Gaussian distribution with variance a_s^2 is added to g_{si} .

For each of the N individuals of the next generation, a maternal parent is sampled with a probability proportional to $W \left(1 - s + \frac{s}{c}\right)$, where W is its fitness and s its investment into sex. With probability $1 - \sigma$ (where σ is given by equation 13 in the main text), the new individual is produced asexually and carries the same genotype as its mother, except for new mutations (the number of mutations on biallelic loci affecting the traits under stabilizing selection is drawn from a Poisson distribution with parameter U). With the complementary probability, the new individual is produced sexually; in this case a paternal parent is sampled with a probability proportional to Ws , and a recombinant offspring is produced (the number of cross-overs occurring at meiosis is sampled from a Poisson distribution with parameter R , and the position of each cross-over is drawn from a uniform distribution along the chromosome). Every 100 generations, the mean investment in sex, mean rate of sex, mean fitness, mean trait values, genetic variances and covariances among traits and some higher moments of phenotypic distributions are recorded by the program.

FILE S2: APPROXIMATIONS FOR THE EFFECT OF MUTATIONAL BIAS ON
MEAN FITNESS

We explain here the derivation of the approximations given in the main text for the effect of mutational bias on the load, assuming a Gaussian fitness function and biallelic loci. Throughout the following, the notation \overline{X} (also denoted $E[X]$) stands for the average of the quantity X over all individuals of the population, while the notation $\langle Y \rangle$ stands for the expected value of quantity Y in the population at mutation-selection-drift equilibrium. In particular, $\langle \overline{W} \rangle$ is the expected value of the population mean fitness. Assuming that the variance in log-fitness among individuals remains small, we have $\overline{W} \approx e^{\overline{\ln W}}$; furthermore, assuming that the variance in $\overline{\ln W}$ due to drift is small yields:

$$\langle \overline{W} \rangle \approx \langle e^{\overline{\ln W}} \rangle \approx e^{\langle \overline{\ln W} \rangle}. \quad (1)$$

From equations 4 and 18 in the main text, this yields:

$$\langle L \rangle \approx 1 - \exp \left[-\frac{1}{2V_s} \sum_{\alpha=1}^n \langle \overline{g_{\alpha}^2} \rangle \right] = 1 - \exp \left[-\frac{1}{2V_s} \sum_{\alpha=1}^n (\langle V_{g,\alpha} \rangle + \langle \overline{g_{\alpha}^2} \rangle) \right] \quad (2)$$

Equation 1 shows that the load can be decomposed into the two terms, $W_{V_g} = \exp[-\sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle / (2V_s)]$ and $W_{\overline{g}} = \exp[-\sum_{\alpha=1}^n \langle \overline{g_{\alpha}^2} \rangle / (2V_s)]$ representing the decrease in mean fitness due to genetic variance maintained in the population, and to deviations of the mean phenotype from the optimum, respectively. If population size is sufficiently large, the variance of mean phenotypes due to drift should remain small (Lande, 1976; Charlesworth, 2013b), so that $\langle \overline{g_{\alpha}^2} \rangle \approx \langle \overline{g_{\alpha}} \rangle^2$; this is confirmed by simulations (results not shown). In the following, we thus derive approximations for $\langle L \rangle$ by computing expressions for $\langle \overline{g_{\alpha}} \rangle$ and $\langle V_{g,\alpha} \rangle$ in different limit cases.

Genetic associations and decomposition of the genetic variance. Using the notation of Barton and Turelli (1991) and Kirkpatrick et al. (2002), we denote $p_i = \mathbb{E}[X_i]$ the frequency of allele 1 at locus i and define ζ_i as:

$$\zeta_i = X_i - p_i. \quad (3)$$

Furthermore, products of ζ_i variables are denoted:

$$\zeta_{\mathbb{U}} = \prod_{i \in \mathbb{U}} \zeta_i \quad (4)$$

where \mathbb{U} represents a set of loci. For example, for $\mathbb{U} = \{i, j\}$, we have:

$$\zeta_{ij} = (X_i - p_i)(X_j - p_j). \quad (5)$$

Finally, genetic associations $D_{\mathbb{U}}$ are defined as averages of $\zeta_{\mathbb{U}}$ variables over all individuals:

$$D_{\mathbb{U}} = \mathbb{E}[\zeta_{\mathbb{U}}] \quad (6)$$

In particular, D_{ij} is the linkage disequilibrium between loci i and j . As we will see, associations involving repeated indices (such as $D_{iij} = \mathbb{E}[(X_i - p_i)^2(X_j - p_j)]$) sometimes appear in the computations. Using the fact that $X_i^2 = X_i$ (since X_i equals 0 or 1), repeated indices can be eliminated using the relation:

$$D_{\mathbb{U}ii} = p_i q_i D_{\mathbb{U}} + (1 - 2p_i) D_{\mathbb{U}i} \quad (7)$$

with $q_i = 1 - p_i$ (e.g., equation 5 in Kirkpatrick et al., 2002). In particular (and because $D_j = \mathbb{E}[X_j - p_j] = 0$), we have $D_{iij} = (1 - 2p_i) D_{ij}$. Similarly, $D_{iijj} = p_i q_i p_j q_j + (1 - 2p_i)(1 - 2p_j) D_{ij}$, while $D_{ii} = p_i q_i$.

The genetic variance for trait α in the population is given by:

$$V_{g,\alpha} = \mathbb{E}[(g_{\alpha} - \overline{g_{\alpha}})^2] \quad (8)$$

From equations 5 and 6 in the main text:

$$\overline{g_\alpha} = \mathbb{E} \left[\sum_{i=1}^{\ell} r_{\alpha i} X_i \right] = \sum_{i=1}^{\ell} r_{\alpha i} p_i \quad (9)$$

so that $\langle \overline{g_\alpha} \rangle = \sum_i r_{\alpha i} \langle p_i \rangle$. Using the definitions above, we have:

$$\begin{aligned} V_{g,\alpha} &= \mathbb{E} \left[\left(\sum_{i=1}^{\ell} r_{\alpha i} (X_i - p_i) \right)^2 \right] \\ &= \mathbb{E} \left[\left(\sum_{i=1}^{\ell} r_{\alpha i} \zeta_i \right)^2 \right] = \mathbb{E} \left[\sum_{i,j} r_{\alpha i} r_{\alpha j} \zeta_i \zeta_j \right] \end{aligned} \quad (10)$$

where the last sum is over all i and j (including $i = j$). Using equations 4 and 6, one obtains:

$$V_{g,\alpha} = \sum_{i=1}^{\ell} r_{\alpha i}^2 p_i q_i + \sum_{i \neq j} r_{\alpha i} r_{\alpha j} D_{ij}. \quad (11)$$

In the following, we assume that linkage disequilibria remain negligible, so that $\langle V_{g,\alpha} \rangle \approx \sum_{i=1}^{\ell} r_{\alpha i}^2 \langle p_i q_i \rangle$.

Neglecting the effects of selection on mean trait values and genetic variance.

Simple approximations for $\langle \overline{g_\alpha} \rangle$ and $\langle V_{g,\alpha} \rangle$ are obtained for the regime where $\overline{s_d}$ is so low that selection has negligible effects on $\langle p_i \rangle$ and $\langle p_i q_i \rangle$, compared with the effects of mutation and drift. Because drift does not change expected allele frequencies, the change in $\langle p_i \rangle$ over one generation is given by (neglecting the effect of selection):

$$\langle p_i \rangle_{t+1} = u + (1 - 2u) \langle p_i \rangle_t. \quad (12)$$

yielding $\langle p_i \rangle = 1/2$ at equilibrium. Using the change in phenotypic basis given by equations 9 and 10 in the main text, we have:

$$\frac{1}{2V_s} \sum_{\alpha=1}^n \langle \overline{g_\alpha} \rangle^2 = \frac{1}{2V_s} \langle \overline{g_1'} \rangle^2 \quad (13)$$

since only the first trait in the new basis (along which the mutational bias occurs) should differ from zero, on average, at equilibrium. Equation 9 and equation 11 in the main text then yield:

$$\frac{1}{2V_s} \sum_{\alpha=1}^n \langle \overline{g_\alpha} \rangle^2 = \frac{1}{4} \left(\ell \tilde{b}_1' \right)^2 \quad (14)$$

where \tilde{b}_1' is the scaled magnitude of mutational bias.

Neglecting the effects of selection yields the following recursion for $\langle p_i q_i \rangle$:

$$\langle p_i q_i \rangle_{t+1} \approx \left(1 - \frac{1}{N} \right) [u + (1 - 4u) \langle p_i q_i \rangle_t] \quad (15)$$

so that $\langle p_i q_i \rangle \approx Nu / (1 + 4Nu)$ at equilibrium (assuming large N and small u). Noting that $\sum_{\alpha} \sum_i r_{\alpha i}^2 = 2V_s \overline{s_d} \ell$ (see equation 7 in the main text), one obtains:

$$\frac{1}{2V_s} \sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle \approx \overline{s_d} \frac{NU}{1 + 4Nu}. \quad (16)$$

Equations 2, 14 and 16 yield the following approximation for the load (assuming $\langle \overline{g_\alpha^2} \rangle \approx \langle \overline{g_\alpha} \rangle^2$):

$$\langle L \rangle \approx 1 - \exp \left[-\overline{s_d} \frac{NU}{1 + 4Nu} - \frac{1}{4} \left(\ell \tilde{b}_1' \right)^2 \right], \quad (17)$$

equivalent to equation 8 in Roze and Blanckaert (2014) in the absence of mutational bias ($\tilde{b}_1' = 0$). Comparisons with individual-based simulations show that equation 17 does indeed provide correct predictions when $\overline{s_d}$ is very low (see Figures 2-4 in the main text). As $\overline{s_d}$ increases, $\langle \overline{g_\alpha} \rangle$ and $\langle V_{g,\alpha} \rangle$ depart more and more from equations 14 and 16; however, simulations indicate that equation 16 stays valid over a wider range of values of s than equation 14, in agreement with previous observations that selection may have significant effects on mean trait values even when $\langle p_i q_i \rangle$ at each locus is mainly controlled by mutation and drift (Campbell, 1984; Barton, 1989; Charlesworth, 2013a). Based on this, we can derive a better approximation for low $\overline{s_d}$ by taking the

effect of selection on $\langle \bar{g}_\alpha \rangle$ into account, but still neglect the effect of selection on $\langle V_{g,\alpha} \rangle$, as shown in the next subsection.

Effect of selection on mean trait values in the low \bar{s}_d regime. From equation 12, we have:

$$\langle \bar{g}_\alpha \rangle_{t+1} = u \sum_i r_{\alpha i} + (1 - 2u) (\langle \bar{g}_\alpha \rangle_t + \langle \Delta_{\text{sel}} \bar{g}_\alpha \rangle_t) \quad (18)$$

where $\langle \Delta_{\text{sel}} \bar{g}_\alpha \rangle$ is the change in $\langle \bar{g}_\alpha \rangle$ due to selection, given by:

$$\langle \Delta_{\text{sel}} \bar{g}_\alpha \rangle = \left\langle \text{E} \left[\frac{W_g}{\bar{W}} g_\alpha \right] \right\rangle - \langle \bar{g}_\alpha \rangle = \left\langle \text{E} \left[\frac{W_g}{\bar{W}} (g_\alpha - \bar{g}_\alpha) \right] \right\rangle. \quad (19)$$

Assuming weak selection ($V_{g,\alpha}/V_s$ small, of order ϵ), we have from equation 4 in the main text (to the first order in ϵ):

$$\frac{W_g}{W_{g,\text{max}}} \approx 1 - \frac{1}{2V_s} \sum_{\alpha=1}^n g_\alpha^2 = 1 - \frac{1}{2V_s} \sum_{\alpha=1}^n [\bar{g}_\alpha^2 + 2(g_\alpha - \bar{g}_\alpha) \bar{g}_\alpha + (g_\alpha - \bar{g}_\alpha)^2], \quad (20)$$

yielding:

$$\frac{\bar{W}}{W_{g,\text{max}}} \approx 1 - \frac{1}{2V_s} \sum_{\alpha=1}^n [\bar{g}_\alpha^2 + V_{g,\alpha}], \quad (21)$$

and thus:

$$\frac{W_g}{\bar{W}} \approx 1 - \frac{1}{V_s} \sum_{\alpha=1}^n \bar{g}_\alpha (g_\alpha - \bar{g}_\alpha) - \frac{1}{2V_s} \sum_{\alpha=1}^n [(g_\alpha - \bar{g}_\alpha)^2 - V_{g,\alpha}]. \quad (22)$$

From equations 19 and 22, one obtains:

$$\langle \Delta_{\text{sel}} \bar{g}_\alpha \rangle \approx -\frac{1}{V_s} \sum_{\beta=1}^n \langle \bar{g}_\beta C_{g,\alpha\beta} \rangle - \frac{1}{2V_s} \sum_{\beta=1}^n \langle M_{g,\alpha\beta\beta} \rangle \quad (23)$$

where $M_{g,\alpha\beta\beta}$ is the third moment $\text{E}[(g_\alpha - \bar{g}_\alpha)(g_\beta - \bar{g}_\beta)^2]$. Assuming that the distribution of phenotypes in the population stays close to a Gaussian distribution, $M_{g,\alpha\beta\beta}$ should be close to zero. Furthermore, assuming that fluctuations in \bar{g}_β and $C_{g,\alpha\beta}$ due

to drift remain small, $\langle \overline{g_\beta} C_{g,\alpha\beta} \rangle \approx \langle \overline{g_\beta} \rangle \langle C_{g,\alpha\beta} \rangle$. Measuring traits in the phenotypic basis defined by equations 9 and 10 in the main text (so that $C_{g,\alpha\beta} = 0$ for $\alpha \neq \beta$), one obtains the following expression for $\langle \overline{g_1'} \rangle$ at equilibrium (from equations 18 and 23):

$$\langle \overline{g_1'} \rangle \approx \frac{U b_1'}{1 - (1 - 2u) \left(1 - \frac{\langle V_{g,1'} \rangle}{V_s} \right)}. \quad (24)$$

Neglecting the effects of selection on $\langle V_{g,1'} \rangle \approx \sum_{i=1}^{\ell} (r_{1i}')^2 \langle p_i q_i \rangle$ and noting that $\sum_{i=1}^{\ell} (r_{1i}')^2 = \frac{m}{n} (a^2 + m b^2) \ell = 2V_s \overline{s_d} \ell \frac{1}{n} [1 + \theta (m - 1)]$ (using equation 9 in the main text), we have:

$$\langle V_{g,1'} \rangle \approx 2V_s \overline{s_d} \frac{1}{n} [1 + \theta (m - 1)] \frac{NU}{1 + 4Nu}. \quad (25)$$

From equations 24 and 25, one obtains (assuming u and $\overline{s_d}$ are small):

$$\langle \overline{g_1'} \rangle \approx \frac{\ell b_1'}{2 \left[1 + \frac{\overline{s_d}}{n} [1 + \theta (m - 1)] \frac{N\ell}{1 + 4Nu} \right]}. \quad (26)$$

Equations 2, 16 and 26 yield (assuming $\langle \overline{g_\alpha}^2 \rangle \approx \langle \overline{g_\alpha} \rangle^2$):

$$\langle L \rangle \approx 1 - \exp \left[-\overline{s_d} \frac{NU}{1 + 4Nu} - \frac{\left(\ell \tilde{b}_1' \right)^2}{4 \left[1 + \frac{\overline{s_d}}{n} [1 + \theta (m - 1)] \frac{N\ell}{1 + 4Nu} \right]^2} \right]. \quad (27)$$

Comparisons with individual-based simulations confirm that equation 27 provides better predictions than equation 17 in the case of sexual populations, as long as $\overline{s_d}$ is sufficiently small (see Figures 3-5 in the main text). Equation 27 fails when $\overline{s_d}$ is not very small, however, as selection affects genetic variances at equilibrium. Unfortunately, we could not obtain any simple expression for the genetic variance (and mean fitness) in this regime for arbitrary θ , although an approximation can be obtained for $\theta = 1$, as shown in the next subsection.

Effect of selection on genetic variance and approximations for the mutation-selection regime. Neglecting linkage disequilibria, genetic variances can be expressed in terms of the genetic diversities $p_i q_i$ at the different loci (equation 11). Extending equation 15 to include selection yields:

$$\langle p_i q_i \rangle_{t+1} \approx \left(1 - \frac{1}{N}\right) \left[u + (1 - 4u) \langle p_i^{\text{sel}} q_i^{\text{sel}} \rangle_t \right]. \quad (28)$$

Furthermore, noting that $p_i q_i = D_{ii} = \text{E}[\zeta_{ii}]$, we have, to the first order in ϵ :

$$\langle p_i^{\text{sel}} q_i^{\text{sel}} \rangle = \left\langle \text{E} \left[\frac{W}{\overline{W}} \zeta_{ii} \right] \right\rangle. \quad (29)$$

Decomposing g_α , $\overline{g_\alpha}$ and $V_{g,\alpha}$ as sums over loci (using equations 9 and 11) and introducing the centered variables $\zeta_i = X_i - p_i$, we have from equation 22:

$$\frac{W_g}{\overline{W}} = 1 + \sum_{i=1}^{\ell} a_i \zeta_i + \sum_{i,j} a_{ij} (\zeta_{ij} - D_{ij}) \quad (30)$$

with $a_i = -\frac{1}{V_s} \sum_{\alpha=1}^n \overline{g_\alpha} r_{\alpha i}$ and $a_{ij} = -\sum_{\alpha=1}^n (r_{\alpha i} r_{\alpha j}) / (2V_s)$, both of order ϵ (Barton and Turelli, 1991). Using equations 29 and 30, and neglecting linkage disequilibria, one obtains:

$$\langle p_i^{\text{sel}} q_i^{\text{sel}} \rangle = \langle p_i q_i \rangle - \frac{1}{V_s} \sum_{\alpha=1}^n r_{\alpha i} \langle \overline{z_\alpha} (1 - 2p_i) p_i q_i \rangle - \frac{1}{2V_s} \sum_{\alpha=1}^n r_{\alpha i}^2 \langle (1 - 2p_i)^2 p_i q_i \rangle. \quad (31)$$

Equations 28 and 31 lead to the following recursion for the genetic variance:

$$\begin{aligned} \langle V_{g,\alpha} \rangle_{t+1} \approx & \left(1 - \frac{1}{N}\right) \left[2V_s \overline{s_d} \frac{U}{n} + (1 - 4u) \left(\langle V_{g,\alpha} \rangle_t - \frac{1}{V_s} \sum_{\beta=1}^n \langle \overline{z_\beta} C_{\alpha\alpha\beta} \rangle_t \right. \right. \\ & \left. \left. - \frac{1}{2V_s} \sum_{i=1}^{\ell} r_{\alpha i}^2 \sum_{\beta=1}^n r_{\beta i}^2 \langle (1 - 2p_i)^2 p_i q_i \rangle_t \right) \right]. \end{aligned} \quad (32)$$

It is not possible to derive an expression for $\langle V_{g,\alpha} \rangle$ at equilibrium under mutation, selection and drift from equation 32 — one may assume that $\langle \overline{z_\beta} C_{\alpha\alpha\beta} \rangle \approx \langle \overline{z_\beta} \rangle \langle C_{\alpha\alpha\beta} \rangle$ and that $\langle C_{\alpha\alpha\beta} \rangle \approx 0$, and assume that $p_i q_i$ is small at each locus so that $(1 - 2p_i)^2 \approx 1$

(and the second sum on the second line of equation 32 becomes $\sum_{\beta} \langle V_{g,\beta} \rangle$), but the resulting approximation does not work well when $\overline{s_d}$ is small (as $p_i q_i$ may not be small), nor when $\overline{s_d}$ is large and in the presence of mutational bias (as $\langle C_{\alpha\alpha\beta} \rangle \neq 0$, results not shown). Neglecting drift, and in the absence of mutational bias ($\theta = 0$), the change in $p_i q_i$ over one generation is (from equations 28 and 31):

$$\Delta(p_i q_i) \approx u(1 - 2p_i)^2 - \frac{1}{2V_s} \sum_{\alpha=1}^n r_{\alpha i}^2 (1 - 2p_i)^2 p_i q_i \quad (33)$$

so that either $p_i = 1/2$ or $p_i q_i = 2V_s u / (\sum_{\alpha=1}^n r_{\alpha i}^2)$ at equilibrium. When $\overline{s_d} \gg u$, most loci should be at the second equilibrium, in which case $V_{g,\alpha} \approx 2V_s U/n$, and $L \approx 1 - e^{-U}$.

Another approximation can be obtained for the case where $\theta = 1$ (no variance of mutational effects) and when drift is negligible. Indeed, in this case alleles 1 are deleterious, and p_i should thus be small at equilibrium. To the first order in p_i , equations 28 and 31 give for the change in p_i over one generation:

$$\Delta p_i \approx u - \frac{1}{V_s} \sum_{\alpha=1}^n r_{\alpha i} \overline{g_{\alpha}} p_i - \frac{1}{2V_s} \sum_{\alpha=1}^n r_{\alpha i}^2 p_i \quad (34)$$

which may also be written as (using the change in phenotypic basis given by equations 9 and 10 in the main text):

$$\Delta p_i \approx u - \frac{1}{V_s} b_1' \overline{g_1'} p_i - \overline{s_d} p_i. \quad (35)$$

From this, the change in $\overline{g_1'} \approx \sum_i r_{1i}' p_i$ is:

$$\Delta \overline{g_1'} \approx U b_1' - \frac{1}{V_s} b_1' (\overline{g_1'})^2 - \overline{s_d} \overline{g_1'} \quad (36)$$

yielding, at equilibrium:

$$\frac{\overline{g_1'}}{\sqrt{2V_s}} \approx \frac{\sqrt{8\tilde{b}_1'^2 U + \overline{s_d}^2} - \overline{s_d}}{4\tilde{b}_1'}. \quad (37)$$

Finally, we have $\sum_{\alpha} V_{g,\alpha} \approx \sum_i \sum_{\alpha} r_{\alpha i}^2 p_i$, which is also $2V_s \bar{s}_d \ell p_i$ (as p_i should be the same at all loci when $\theta = 1$). Noting that $\bar{g}_1' \approx b_1' \ell p_i$, we thus have:

$$\frac{\sum_{\alpha=1}^n V_{g,\alpha}}{2V_s} \approx \frac{\bar{s}_d}{\tilde{b}_1'} \left(\frac{\bar{g}_1'}{\sqrt{2V_s}} \right). \quad (38)$$

Equations 2, 37 and 38 finally lead to:

$$L \approx 1 - \exp \left[- \frac{4\tilde{b}_1'^2 U + \bar{s}_d \left(\sqrt{8\tilde{b}_1'^2 U + \bar{s}_d^2} - \bar{s}_d \right)}{8\tilde{b}_1'^2} \right] \quad (39)$$

or, in terms of \bar{s}_d , ρ and U :

$$L \approx 1 - \exp \left[- \frac{4\rho U - \bar{s}_d + \sqrt{\bar{s}_d (8\rho U + \bar{s}_d)}}{8\rho} \right]. \quad (40)$$

Simulations confirm that equation 40 provides accurate predictions for $\theta = 1$ (in sexual populations), when \bar{s}_d is sufficiently high (see Figure 4 in the main text).

LITERATURE CITED

- Barton, N. H. 1989. The divergence of a polygenic system subject to stabilizing selection, mutation and drift. *Genet. Res.* 54:59–77.
- Barton, N. H. and M. Turelli. 1991. Natural and sexual selection on many loci. *Genetics* 127:229–255.
- Campbell, R. B. 1984. The manifestation of phenotypic selection at constituent loci. I. Stabilizing selection. *Evolution* 38:1033–1038.
- Charlesworth, B. 2013a. Stabilizing selection, purifying selection, and mutational bias in finite populations. *Genetics* 194:955–971.
- . 2013b. Why we are not dead one hundred times over. *Evolution* 67:3354–3361.
- Kirkpatrick, M., T. Johnson, and N. H. Barton. 2002. General models of multilocus evolution. *Genetics* 161:1727–1750.
- Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30:314–334.
- Roze, D. and A. Blanckaert. 2014. Epistasis, pleiotropy and the mutation load in sexual and asexual populations. *Evolution* 68:137–149.

FILE S3: QLE MODEL FOR THE EVOLUTION OF SEX

We derive here expressions for the change in mean rate of sex in the limit of an infinite population, using a quasi-linkage equilibrium (QLE) argument. For this, we use Turelli and Barton's (1990) method (see also Barton, 1995) to express the effect of selection on genetic associations in terms of partial derivatives of $\ln \bar{W}$ with respect to mean trait values and genetic variances/covariances. Note that the derivations given below are in principle valid for any number of possible alleles at each locus (not necessarily biallelic loci) and any fitness function (not necessarily Gaussian), as long as the distribution of phenotypes affecting fecundity stays approximately Gaussian.

Definitions. Extending the notation of Turelli and Barton (1990) to multiple traits, we define the centered variable $\zeta_{\alpha j}$ as:

$$\zeta_{\alpha j} = g_{\alpha j} - \overline{g_{\alpha j}} \quad (1)$$

(where again $g_{\alpha j}$ is the effect of the allele present at locus j on trait α in a given individual, and $\overline{g_{\alpha j}}$ its average over all individuals). Genetic associations are defined as

$$C_{\mathbb{U}} = E[\zeta_{\mathbb{U}}] \quad (2)$$

where E stands for the average over all individuals, and with $\zeta_{\mathbb{U}} = \prod_x \zeta_x$, each x bearing two elements, the trait α and the locus j . For example, $C_{\alpha j \alpha j} = E[(g_{\alpha j} - \overline{g_{\alpha j}})^2]$ while $C_{\alpha j \alpha k \beta k} = E[(g_{\alpha j} - \overline{g_{\alpha j}})(g_{\alpha k} - \overline{g_{\alpha k}})(g_{\beta k} - \overline{g_{\beta k}})]$. Using these definitions, the genetic

variance for trait α can be written as:

$$\begin{aligned} V_{g,\alpha} &= \mathbb{E} \left[\left(\sum_j \zeta_{\alpha j} \right)^2 \right] = \sum_{j=1}^{\ell} C_{\alpha j \alpha j} + \sum_{j \neq k} C_{\alpha j \alpha k} \\ &= V_{g,\alpha}^0 + \mathcal{D}_{\alpha\alpha} \end{aligned} \quad (3)$$

where $V_{g,\alpha}^0 = \sum_j C_{\alpha j \alpha j}$ is the “genetic variance” for trait α (genetic variance in a population with the same allele frequencies, at linkage equilibrium), and $\mathcal{D}_{\alpha\alpha} = \sum_{j \neq k} C_{\alpha j \alpha k}$ is the effect of linkage disequilibria on the variance. Similarly, the genetic covariance between traits α and β can be decomposed as:

$$\begin{aligned} C_{g,\alpha\beta} &= \sum_{j=1}^{\ell} C_{\alpha j \beta j} + \sum_{j \neq k} C_{\alpha j \beta k} \\ &= C_{g,\alpha\beta}^0 + \mathcal{D}_{\alpha\beta}. \end{aligned} \quad (4)$$

As explained in the main text, we assume that investment in sexual reproduction s is also a polygenic trait with independent genetic and environmental contributions:

$$s = \bar{s} + g_s + e_s \quad (5)$$

where e_s is sampled in a Gaussian distribution with mean 0 and variance $V_{e,s}$, and assuming additive effects of loci affecting s :

$$g_s = \sum_j g_{sj} \quad (6)$$

where g_{sj} is the effect of the allele at locus j on investment in sex (we assume that loci affecting the rate of sex do not affect the other traits). Assuming that the variance in s in the population is sufficiently small (g_s , g_e small, of order η), the rate of sex $\sigma = s / [c(1-s) + s]$ of an individual can also be decomposed into an additive genetic and an environmental component:

$$\sigma = \bar{\sigma} + g_{\sigma} + e_{\sigma} \quad (7)$$

with (to leading order in η):

$$\bar{\sigma} \approx \frac{\bar{s}}{c(1-\bar{s})+\bar{s}}, \quad g_{\sigma} \approx \frac{c}{[c(1-\bar{s})+\bar{s}]^2} g_s, \quad V_{g,\sigma} \approx \frac{c^2}{[c(1-\bar{s})+\bar{s}]^4} V_{g,s}. \quad (8)$$

The expected change in $\bar{\sigma}$ over one generation (denoted $\Delta\bar{\sigma}$) corresponds to the change in \bar{g}_{σ} in the parental generation due to differences in fecundities among individuals and to the cost of sex. In the following, we derive deterministic approximations for $\Delta\bar{\sigma}$, assuming that phenotypic traits affecting fecundity are normally distributed (this implies that the number of loci affecting each of these traits is sufficiently large, each locus having a sufficiently small effect on the trait). We will also use a quasi-linkage equilibrium approximation, assuming that rates of sex and recombination are not too small, so that genetic associations between loci are small and equilibrate fast relative to change in allele frequencies. Finally, we will assume that the genetic variance in the rate of sex in the population ($V_{g,\sigma}$) is small (however, we do not make any assumption on the number of loci affecting the rate of sex). For this, we will decompose a generation into two steps: the first (“selection”) corresponds to the differential reproduction of individuals due to differences in fecundity (according to the values of their phenotypes z_1, \dots, z_n), while the second (“reproduction”) corresponds to the effect of the cost of sex and of sexual recombination (strictly, this second step also involves selection when $c > 1$, since individuals investing more in sex are disfavored). In the next sections, we derive expressions for changes in mean breeding values during these two steps.

Effect of selection on mean breeding values. The effect of selection on \bar{g}_{α} can be written as:

$$\Delta_{\text{sel}} \bar{g}_{\alpha} = \text{E} \left[\frac{W_{\mathbf{g}}}{\bar{W}} (g_{\alpha} - \bar{g}_{\alpha}) \right] \quad (9)$$

where $W_{\mathbf{g}}$ is the mean fecundity of individuals with breeding values $\mathbf{g} = (g_1, g_2, \dots)$ and \bar{W} the mean fecundity of the whole population. Following Barton (1995), we assume that selection is weak and approximate $W_{\mathbf{g}}/\bar{W}$ by:

$$\begin{aligned} \frac{W_{\mathbf{g}}}{\bar{W}} \approx & 1 + \sum_{\alpha} (g_{\alpha} - \bar{g}_{\alpha}) \frac{\partial \ln \bar{W}}{\partial z_{\alpha}} \\ & + \sum_{\alpha \leq \beta} [(g_{\alpha} - \bar{g}_{\alpha})(g_{\beta} - \bar{g}_{\beta}) - C_{\mathbf{g}, \alpha\beta}] \frac{\partial \ln \bar{W}}{\partial C_{\mathbf{g}, \alpha\beta}}. \end{aligned} \quad (10)$$

(see Appendix A), where the last sum includes the terms for $\alpha = \beta$, which involve partial derivatives of $\ln \bar{W}$ with respect to $V_{\mathbf{g}, \alpha}$. From equations 9 and 10, and assuming a Gaussian distribution of breeding values, we recover the classic expression:

$$\Delta_{\text{sel}} \bar{g}_{\alpha} = \sum_{\beta} \frac{\partial \ln \bar{W}}{\partial z_{\beta}} C_{\mathbf{g}, \alpha\beta} \quad (11)$$

(Lande, 1979). The change in \bar{g}_{σ} is obtained similarly:

$$\Delta_{\text{sel}} \bar{g}_{\sigma} = \mathbb{E} \left[\frac{W_{\mathbf{g}}}{\bar{W}} (g_{\sigma} - \bar{g}_{\sigma}) \right]. \quad (12)$$

However, we can no longer assume that the joint distribution of investment into sex σ and of traits affecting fecundity is multivariate normal (indeed, genetic variances and covariances may differ between subgroups of individuals differing in their values of g_{σ} , due to the effect of sexual recombination on genetic associations). From equations 10 and 12, one obtains:

$$\Delta_{\text{sel}} \bar{g}_{\sigma} \approx \sum_{\alpha} \frac{\partial \ln \bar{W}}{\partial z_{\alpha}} C_{\mathbf{g}, \sigma\alpha} + \sum_{\alpha \leq \beta} \frac{\partial \ln \bar{W}}{\partial C_{\mathbf{g}, \alpha\beta}} M_{\mathbf{g}, \sigma\alpha\beta} \quad (13)$$

where $M_{\mathbf{g}, \sigma\alpha\beta}$ is the moment $\mathbb{E}[(g_{\sigma} - \bar{g}_{\sigma})(g_{\alpha} - \bar{g}_{\alpha})(g_{\beta} - \bar{g}_{\beta})]$. Equation 13 is equivalent to Charlesworth (1993)'s decomposition of the selection gradient for a recombination modifier allele into two terms (equation A10 in Charlesworth, 1993, see also Appendix 2 of Barton, 1995). The first part of equation 13 shows that under directional selection acting on trait α , a covariance between g_{α} and g_{σ} generates indirect

selection on σ (this is equivalent to the term in $\delta\bar{z}$ in Charlesworth, 1993). The second part of equation 13 (equivalent to the term in δV_g in Charlesworth, 1993) corresponds to indirect selection on σ due to different genetic variances and covariances for selected traits among subgroups of individuals with different rates of sex. For example, $\partial \ln \bar{W} / \partial V_{g,\alpha} < 0$ under stabilizing selection acting on a single trait α (the immediate effect of increasing genetic variance is to decrease mean fitness) and in this situation we also expect that higher rates of sex tend to increase genetic variance, so that $E[(g_\sigma - \bar{g}_\sigma)(g_\alpha - \bar{g}_\alpha)^2] > 0$, and the second term of equation 13 selects against sex. This term is equivalent to the “short-term effect” in models for the evolution of sex (or recombination) with epistasis (e.g., Agrawal, 2006). Now, if the population mean phenotype $\bar{\mathbf{z}} = (\bar{z}_1, \dots, \bar{z}_n)$ is displaced from the optimum, the higher genetic variance associated with sex will increase the efficiency of selection, generating associations between higher values of g_σ and values of g_α closer to the optimum, that in turn favor sex. This effect is represented by the first term of equation 13 and corresponds to the “long term effect” (favoring sex due to an increased efficiency of selection).

Selection gradients $\partial \ln \bar{W} / \partial \bar{z}_\alpha$ and $\partial \ln \bar{W} / \partial C_{g,\alpha\beta}$ take simple forms in the case of a fully isotropic model with Gaussian stabilizing selection:

$$W = \exp \left[-\frac{\sum_\alpha (z_\alpha - \theta_\alpha)^2}{2\omega^2} \right] \quad (14)$$

where ω^2 represents the strength of selection (the same for all traits), and where the phenotypic optimum is located at $\boldsymbol{\theta} = (\theta_1, \theta_2, \dots, \theta_n)$. A general expression for mean fitness under Gaussian stabilizing selection (and when the maximal possible fitness is 1, as implied by equation 14) is given by:

$$\bar{W} = \sqrt{\det((\mathbf{S} + \mathbf{P})^{-1} \mathbf{S})} \exp \left[-\frac{1}{2} (\bar{\mathbf{z}} - \boldsymbol{\theta})^T (\mathbf{S} + \mathbf{P})^{-1} (\bar{\mathbf{z}} - \boldsymbol{\theta}) \right] \quad (15)$$

(Gomulkiewicz and Houle, 2009), where $\det(\mathbf{A})$ is the determinant of matrix \mathbf{A} , T stands for matrix/vector transpose, \mathbf{S} is a matrix determining the pattern of multivariate stabilizing selection, and \mathbf{P} is the phenotypic variance-covariance matrix. When fitness is given by equation 14, $\mathbf{S} = \omega^2 \mathbf{I}$ (where \mathbf{I} is the identity matrix). Furthermore, our assumption of independent, identically distributed environmental effects yields $\mathbf{P} = \mathbf{G} + V_e \mathbf{I}$, where \mathbf{G} is the genetic variance-covariance matrix (whose diagonal elements are genetic variances $V_{g,\alpha}$, and off-diagonal elements genetic covariances $C_{g,\alpha\beta}$). It is always possible to find an orthonormal basis in which the \mathbf{G} matrix is diagonal, that is, to define new phenotypic traits as linear combinations of the “true” phenotypic traits so that the new traits are independent, and fitness is still given by equation 14. Assuming that phenotypes are measured in this new basis, we show in Appendix B that:

$$\frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} = -\frac{\bar{z}_\alpha - \theta_\alpha}{V_{g,\alpha} + V_s} \quad (16)$$

where $V_s = \omega^2 + V_e$, while:

$$\frac{\partial \ln \bar{W}}{\partial V_{g,\alpha}} = -\frac{1}{2(V_{g,\alpha} + V_s)} + \frac{1}{2} \left(\frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} \right)^2 \quad (17)$$

$$\frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} = \left(\frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} \right) \left(\frac{\partial \ln \bar{W}}{\partial \bar{z}_\beta} \right), \quad (18)$$

for $\alpha \neq \beta$.

Change in mean rate of sex during reproduction. To compute the change in \bar{g}_σ during reproduction (due to the cost of sex), we first compute the change in \bar{g}_s .

We have:

$$\begin{aligned} \Delta_{\text{rep}} \bar{g}_s &= E' \left[\frac{c(1-s)}{c(1-\bar{s}') + \bar{s}'} (g_s - \bar{g}_s') \right] \\ &+ E' \left[\frac{s_\varnothing s_\sigma}{\bar{s}' [c(1-\bar{s}') + \bar{s}']} \frac{(g_{s,\varnothing} - \bar{g}_s') + (g_{s,\sigma} - \bar{g}_s')}{2} \right] \end{aligned} \quad (19)$$

where the primes denote averages among individuals after selection (that is, weighting each individual by its relative fecundity), and where the average on the second line is over all possible pairs of female and male parents, s_{φ} and s_{σ} being the investments in sex of these parents, and $g_{s,\varphi}$, $g_{s,\sigma}$ their value of g_s . Equation 19 can be understood as follows. The term on the first line is the proportion of asexually produced offspring — which is $(1 - \bar{s}') / (1 - \bar{s}' + \frac{\bar{s}'}{c})$ — multiplied by the change in the mean value of g_s among those offspring relative to the parents: for this, each parent is weighted by its relative contribution to the pool of asexually produced offspring, which is $(1 - s) / (1 - \bar{s}')$. The term on the second line is the proportion of sexually produced offspring — which is $\frac{\bar{s}'}{c} / (1 - \bar{s}' + \frac{\bar{s}'}{c})$ — multiplied by the change in the mean value of g_s among those offspring. On average, the mean value of g_s among the offspring of a given female and male is $(g_{s,\varphi} + g_{s,\sigma}) / 2$, where $g_{s,\varphi}$ and $g_{s,\sigma}$ are the values of g_s in the parents. Furthermore, the relative contributions of these parents to the pool of sexually produced offspring are s_{φ} / \bar{s}' and s_{σ} / \bar{s}' . Replacing s by $\bar{s} + g_s - \bar{g}_s' + \bar{g}_s' + e_s$ (and similarly for $g_{s,\varphi}$, $g_{s,\sigma}$) in equation 19, and using $\bar{s}' = \bar{s} + \bar{g}_s'$ finally yields:

$$\Delta_{\text{rep}} \bar{g}_s = - \frac{c - 1}{c(1 - \bar{s}') + \bar{s}'} V_{g,s}' . \quad (20)$$

Equation 20 represents the effect of direct selection against sex (whenever $c > 1$), and is equivalent to the expression derived in Roze (2014) in the case of a single biallelic sex modifier locus. Strictly, \bar{s}' and $V_{g,s}'$ in equation 20 are the mean and genetic variance for investment in sex after selection (weighting each individual by its relative fecundity). However, taking into account the change in \bar{s} and $V_{g,s}$ due to selection would introduce terms in $V_{g,s}^2$ in equation 20; neglecting those terms, \bar{s}' and $V_{g,s}'$ in equation 20 can thus be replaced by their values \bar{s} and $V_{g,s}$ at the start of the

generation (before selection). From equations 8 and 20, one then obtains:

$$\Delta_{\text{rep}} \overline{g_{\sigma}} \approx -\frac{c-1}{1+(c-1)\overline{\sigma}} V_{g,\sigma}. \quad (21)$$

Assuming no mutational bias on σ , the change in the mean rate of sex over one generation is given by:

$$\Delta \overline{\sigma} = \Delta_{\text{sel}} \overline{g_{\sigma}} + \Delta_{\text{rep}} \overline{g_{\sigma}}. \quad (22)$$

In the following, we derive approximate expressions for the moments $C_{g,s\alpha}$ and $M_{g,s\alpha\beta}$ that appear in the expression of $\Delta_{\text{sel}} \overline{g_{\sigma}}$ (equation 13). However before that, we will compute an expression for the contribution of linkage disequilibria to the genetic variances and covariances between traits affecting fecundity ($\mathcal{D}_{\alpha\beta} = \sum_{j \neq k} C_{\alpha j \beta k}$, equations 3 and 4), at quasi-linkage equilibrium.

Genetic associations between selected loci. Neglecting genetic variance in the rate of sex, $C_{\alpha j \beta k}$ at the next generation is given by:

$$C''_{\alpha j \beta k} = (1 - \overline{\sigma}) C'_{\alpha j \beta k} + \overline{\sigma} (1 - r_{jk}) C'_{\alpha j \beta k} \quad (23)$$

where the double prime denotes variables measured at the next generation (after reproduction), and r_{jk} is the recombination rate between loci j and k . The first term of equation 23 is the proportion of asexually produced offspring, multiplied by the genetic association among those offspring, which is the same as among parents. The second term is the proportion of sexually produced offspring, in which $C_{\alpha j \beta k}$ is decreased by a factor $1 - r_{jk}$ due to recombination. Equation 23 can be written under the simpler form:

$$C''_{\alpha j \beta k} = (1 - \rho_{jk}) C'_{\alpha j \beta k} \quad (24)$$

where $\rho_{jk} = \bar{\sigma} r_{jk}$ is the “effective” recombination rate between loci j and k .

The effect of selection on $C_{\alpha j \beta k}$ can be computed as follows (Turelli and Barton, 1990; Barton, 1995). We have:

$$C'_{\alpha j \beta k} = E' [(g_{\alpha j} - \bar{g}_{\alpha j}') (g_{\beta k} - \bar{g}_{\beta k}')] \quad (25)$$

where again the prime denotes averages after selection (weighting each individual by its relative fecundity). Equation 25 can also be written:

$$C'_{\alpha j \beta k} = E' [(g_{\alpha j} - \bar{g}_{\alpha j} - \Delta_{\text{sel}} \bar{g}_{\alpha j}) (g_{\beta k} - \bar{g}_{\beta k} - \Delta_{\text{sel}} \bar{g}_{\beta k})]. \quad (26)$$

In the following, we use the notation $C_{\mathbb{U}}^{\text{sel}}$ for genetic associations measured after selection, but using as “reference values” (the $\bar{g}_{\alpha j}$ in equation 1) allelic averages before selection: in particular, $C_{\alpha j \beta k}^{\text{sel}} = E' [(g_{\alpha j} - \bar{g}_{\alpha j}) (g_{\beta k} - \bar{g}_{\beta k})]$. Expanding equation 26 and noting that $C_{\alpha j}^{\text{sel}} = E' [g_{\alpha j} - \bar{g}_{\alpha j}] = \Delta_{\text{sel}} \bar{g}_{\alpha j}$, one obtains:

$$C'_{\alpha j \beta k} = C_{\alpha j \beta k}^{\text{sel}} - (\Delta_{\text{sel}} \bar{g}_{\alpha j}) (\Delta_{\text{sel}} \bar{g}_{\beta k}). \quad (27)$$

Furthermore, we have:

$$C_{\alpha j \beta k}^{\text{sel}} = E \left[\frac{W_{\mathbf{g}}}{\bar{W}} \zeta_{\alpha j \beta k} \right], \quad \Delta_{\text{sel}} \bar{g}_{\alpha j} = E \left[\frac{W_{\mathbf{g}}}{\bar{W}} \zeta_{\alpha j} \right]. \quad (28)$$

From equation 10, and noting that $g_{\alpha} - \bar{g}_{\alpha} = \sum_j \zeta_{\alpha j}$, while $(g_{\alpha} - \bar{g}_{\alpha}) (g_{\beta} - \bar{g}_{\beta}) - C_{\mathbf{g}, \alpha \beta} = \sum_{j,k} (\zeta_{\alpha j \beta k} - C_{\alpha j \beta k})$ (where the last sum is over all pairs of loci j and k including $j = k$), we have:

$$\frac{W_{\mathbf{g}}}{\bar{W}} \approx 1 + \sum_{\alpha} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\alpha}} \sum_j \zeta_{\alpha j} + \sum_{\alpha \leq \beta} \frac{\partial \ln \bar{W}}{\partial C_{\mathbf{g}, \alpha \beta}} \sum_{j,k} (\zeta_{\alpha j \beta k} - C_{\alpha j \beta k}). \quad (29)$$

Equations 28 and 29 yield:

$$\begin{aligned} C_{\alpha j \beta k}^{\text{sel}} &= C_{\alpha j \beta k} + \sum_{\gamma} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\gamma}} \sum_i C_{\gamma i \alpha j \beta k} \\ &\quad + \sum_{\gamma \leq \delta} \frac{\partial \ln \bar{W}}{\partial C_{\mathbf{g}, \gamma \delta}} \sum_{h,i} (C_{\gamma h \delta i \alpha j \beta k} - C_{\gamma h \delta i} C_{\alpha j \beta k}). \end{aligned} \quad (30)$$

Equation 30 shows that $C_{\alpha j \alpha k}$ is affected by higher-order associations (involving 3 or 4 loci). These associations are in turn generated by the effect of selection, and eroded by recombination. In the following we assume that selection is sufficiently weak relative to recombination, so that between-locus associations remain small (Turelli and Barton, 1990). Ignoring terms involving between-locus associations in the sums on the right-hand-side of equation 30, only the terms for $h = j, i = k$ and $h = k, i = j$ in the last sum remain, giving:

$$C_{\alpha j \alpha k}^{\text{sel}} \approx C_{\alpha j \alpha k} + \sum_{\gamma \leq \delta} \frac{\partial \ln \bar{W}}{\partial C_{g, \gamma \delta}} (C_{\alpha j \gamma j} C_{\beta k \delta k} + C_{\alpha j \delta j} C_{\beta k \gamma k}). \quad (31)$$

Equations 28 and 29 also yield (neglecting between-locus associations):

$$\Delta_{\text{sel}} \bar{g}_{\alpha j} = \sum_{\gamma} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\gamma}} \sum_i C_{\alpha j \gamma j} + \sum_{\gamma \leq \delta} \frac{\partial \ln \bar{W}}{\partial C_{g, \gamma \delta}} \sum_{h, i} C_{\alpha j \gamma j \delta j}. \quad (32)$$

$C_{\alpha j \beta k}$ at QLE is obtained by solving $C''_{\alpha j \beta k} = C_{\alpha j \beta k}$. From equations 24, 27, 31 and 32, this yields:

$$C_{\alpha j \beta k} = \left(\frac{1}{\rho_{jk}} - 1 \right) \Delta_{\text{sel}} C_{\alpha j \beta k} \quad (33)$$

with

$$\Delta_{\text{sel}} C_{\alpha j \beta k} = \sum_{\gamma, \delta} (1 + I_{\gamma \delta}) \frac{\partial \ln \bar{W}}{\partial C_{g, \gamma \delta}} C_{\alpha j \gamma j} C_{\beta k \delta k} - (\Delta_{\text{sel}} \bar{g}_{\alpha j}) (\Delta_{\text{sel}} \bar{g}_{\alpha k}). \quad (34)$$

where $I_{\gamma \delta}$ equals 1 if $\gamma = \delta$, and 0 otherwise. Summing over all loci, one obtains for

$$\mathcal{D}_{\alpha \beta} = \sum_{j \neq k} C_{\alpha j \beta k}:$$

$$\mathcal{D}_{\alpha \beta} \approx \left(\frac{1}{\rho_h} - 1 \right) \Delta_{\text{sel}} \mathcal{D}_{\alpha \beta} \quad (35)$$

where ρ_h is the harmonic mean of ρ_{jk} over all pairs of loci affecting fecundity, and with

$$\Delta_{\text{sel}} \mathcal{D}_{\alpha \beta} \approx \sum_{\gamma, \delta} \left[(1 + I_{\gamma \delta}) \frac{\partial \ln \bar{W}}{\partial C_{g, \gamma \delta}} - \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\gamma}} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\delta}} \right] C_{g, \alpha \gamma}^0 C_{g, \beta \delta}^0. \quad (36)$$

Because $C_{g, \alpha \beta}^0 \approx C_{g, \alpha \beta}$ in the QLE regime (weak linkage disequilibria), we may replace

$C_{g, \alpha \gamma}^0$ and $C_{g, \beta \delta}^0$ in equation 35 by $C_{g, \alpha \gamma}$ and $C_{g, \beta \delta}$. If phenotypes are measured in a

basis that eliminates genetic covariances among traits, one obtains from equations 35 and 36:

$$\mathcal{D}_{\alpha\alpha} = \sum_{j \neq k} C_{\alpha j \alpha k} \approx \left(\frac{1}{\rho_h} - 1 \right) \left[2 \frac{\partial \ln \bar{W}}{\partial V_{g,\alpha}} - \left(\frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} \right)^2 \right] V_{g,\alpha}^2. \quad (37)$$

When the fitness function is given by equation 14, this simplifies to (using equations 16 and 17):

$$\mathcal{D}_{\alpha\alpha} \approx - \left(\frac{1}{\rho_h} - 1 \right) \frac{V_{g,\alpha}^2}{V_{g,\alpha} + V_s} \quad (38)$$

corresponding to the result obtained by Bulmer (1985) under the assumption of exchangeable loci (equations A3c and A6a in Charlesworth, 1993).

Indirect selection for sex: “short-term effect”. As discussed earlier, the “short-term effect” is represented by the term on the second line of equation 13, which depends on moments $M_{g,\sigma\alpha\beta} = E[(g_\sigma - \bar{g}_\sigma)(g_\alpha - \bar{g}_\alpha)(g_\beta - \bar{g}_\beta)]$ (for all traits α, β affecting fecundity). From equation 8, we have:

$$M_{g,\sigma\alpha\beta} \approx \frac{c}{[c(1 - \bar{s}) + \bar{s}]^2} M_{g,s\alpha\beta} \quad (39)$$

with $M_{g,s\alpha\beta} = E[(g_s - \bar{g}_s)(g_\alpha - \bar{g}_\alpha)(g_\beta - \bar{g}_\beta)]$. Furthermore, using our definition of genetic associations, $M_{g,s\alpha\beta}$ can be decomposed as:

$$M_{g,s\alpha\beta} = \sum_{i,j,k} C_{si\alpha j\beta k} \quad (40)$$

where the sum is over all loci i affecting investment sex, and over all pairs of loci j and k affecting traits α, β . A QLE approximation for $C_{si\alpha j\beta k}$ can be obtained as follows.

At the next generation, we have:

$$C''_{si\alpha j\beta k} = E''[(g_{si} - \bar{g}_{si}'')(g_{\alpha j} - \bar{g}_{\alpha j}'')(g_{\beta k} - \bar{g}_{\beta k}'')] \quad (41)$$

where again the double primes denote averages over individuals of the next generation (after reproduction). Equation 41 can also be written:

$$C''_{si\alpha j\beta k} = E'' \left[(g_{si} - \overline{g_{si}}' - \Delta_{\text{rep}} \overline{g_{si}}) (g_{\alpha j} - \overline{g_{\alpha j}}' - \Delta_{\text{rep}} \overline{g_{\alpha j}}) \right. \\ \left. \times (g_{\beta k} - \overline{g_{\beta k}}' - \Delta_{\text{rep}} \overline{g_{\beta k}}) \right] \quad (42)$$

where $\overline{g_{si}}'$, $\overline{g_{\alpha j}}'$, $\overline{g_{\beta k}}'$ are the averages of g_{si} , $g_{\alpha j}$, $g_{\beta k}$ among selected parents (weighting each parent by its relative fecundity), and $\Delta_{\text{rep}} \overline{g_{si}} = \overline{g_{si}}'' - \overline{g_{si}}'$ the change in $\overline{g_{si}}$ during reproduction, due to the cost of sex (and similarly for $\Delta_{\text{rep}} \overline{g_{\alpha j}}$ and $\Delta_{\text{rep}} \overline{g_{\beta k}}$). In the following, we use the notation $C_{\text{U}}^{\text{rep}}$ for genetic associations measured after reproduction, but using as “reference values” (the $\overline{g_{\alpha j}}$ in equation 1) allelic averages after selection ($\overline{g_{\alpha j}}'$): for example, $C_{\alpha j\beta k}^{\text{rep}} = E'' [(g_{\alpha j} - \overline{g_{\alpha j}}') (g_{\beta k} - \overline{g_{\beta k}}')]$. Expanding equation 42 and noting that $E'' [g_{si} - \overline{g_{si}}'] = \Delta_{\text{rep}} \overline{g_{si}}$, one obtains:

$$C''_{si\alpha j\beta k} = C_{si\alpha j\beta k}^{\text{rep}} - (\Delta_{\text{rep}} \overline{g_{si}}) C_{\alpha j\beta k}^{\text{rep}} - (\Delta_{\text{rep}} \overline{g_{\alpha j}}) C_{si\beta k}^{\text{rep}} \\ - (\Delta_{\text{rep}} \overline{g_{\beta k}}) C_{si\alpha j}^{\text{rep}} + 2 (\Delta_{\text{rep}} \overline{g_{si}}) (\Delta_{\text{rep}} \overline{g_{\alpha j}}) (\Delta_{\text{rep}} \overline{g_{\beta k}}). \quad (43)$$

The change in $\overline{g_{\alpha j}}$ during reproduction is generated by the cost of sex and by genetic associations between locus j and loci affecting investment in sex, and is thus proportional to $V_{g,s}$ (the same is true for $\Delta_{\text{rep}} \overline{g_{\beta k}}$). Furthermore, the sum over all i and j of $C_{si\beta k}$ is the genetic covariance between trait β and investment in sex s , which is also proportional to $V_{g,s}$. As a consequence, the last three terms of equation 43 will generate terms in $O(V_{g,s}^2)$, and will thus be ignored, so that:

$$C''_{si\alpha j\beta k} \approx C_{si\alpha j\beta k}^{\text{rep}} - (\Delta_{\text{rep}} \overline{g_{si}}) C_{\alpha j\beta k}^{\text{rep}}. \quad (44)$$

Using a similar reasoning as when deriving equation 19, an expression for $C_{si\alpha j\beta k}^{\text{rep}}$ is

given by (using $\bar{s}' \approx \bar{s}$, and for $j \neq k$):

$$\begin{aligned}
C_{si\alpha j\beta k}^{\text{rep}} = & \mathbb{E}' \left[\frac{c(1-s)}{c(1-\bar{s}) + \bar{s}} \zeta_{si\alpha j\beta k} \right] \\
& + \mathbb{E}' \left[\frac{s_{\varnothing} s_{\sigma}}{\bar{s} [c(1-\bar{s}) + \bar{s}]} \left(r_{ijk, \emptyset} \zeta_{si\alpha j\beta k, \varnothing} + r_{\emptyset, ijk} \zeta_{si\alpha j\beta k, \sigma} \right. \right. \\
& \quad + r_{i,jk} \zeta_{si, \varnothing} \zeta_{\alpha j\beta k, \sigma} + r_{jk, i} \zeta_{\alpha j\beta k, \varnothing} \zeta_{si, \sigma} \\
& \quad + r_{ij, k} \zeta_{si\alpha j, \varnothing} \zeta_{\beta k, \sigma} + r_{k, ij} \zeta_{\beta k, \varnothing} \zeta_{si\alpha j, \sigma} \\
& \quad \left. \left. + r_{ik, j} \zeta_{si\beta k, \varnothing} \zeta_{\alpha j, \sigma} + r_{j, ik} \zeta_{\alpha j, \varnothing} \zeta_{si\beta k, \sigma} \right) \right]. \tag{45}
\end{aligned}$$

In equation 45, $r_{\mathbb{S}, \mathbb{T}}$ is the probability that a meiotic product inherits the set \mathbb{S} of loci from the maternal genome, and the set \mathbb{T} of loci from the paternal genome, while $\zeta_{\mathbb{S}, \varnothing}$, $\zeta_{\mathbb{S}, \sigma}$ variables are measured in the maternal and paternal parent, respectively. Writing s on the first line of equation 45 under the form $\bar{s} + \sum_h \zeta_{sh} + e_s$, and s_{\varnothing} , s_{σ} on the second line as $\bar{s} + \sum_h \zeta_{sh, \varnothing} + e_{s, \varnothing}$ and $\bar{s} + \sum_h \zeta_{sh, \sigma} + e_{s, \sigma}$, one arrives at:

$$\begin{aligned}
C_{si\alpha j\beta k}^{\text{rep}} = & \left[1 - \frac{\bar{s}}{c(1-\bar{s}) + \bar{s}} (1 - r_{ijk, \emptyset} - r_{\emptyset, ijk}) \right] C'_{si\alpha j\beta k} \\
& + \frac{1}{c(1-\bar{s}) + \bar{s}} \left[- (c - r_{ijk, \emptyset} - r_{\emptyset, ijk}) \sum_h C'_{sh si\alpha j\beta k} \right. \\
& \quad + (r_{i,jk} + r_{jk, i}) \left(C'_{\alpha j\beta k} \sum_h C'_{sh si} + \sum_l C'_{sl\alpha j\beta k} \sum_h C'_{sh si} \right) \\
& \quad + (r_{ij, k} + r_{k, ij}) \left(C'_{si\alpha j} \sum_h C'_{sh \beta k} + \sum_l C'_{sl si\alpha j} \sum_h C'_{sh \beta k} \right) \\
& \quad \left. + (r_{ik, j} + r_{j, ik}) \left(C'_{si\beta k} \sum_h C'_{sh \alpha j} + \sum_l C'_{sl si\beta k} \sum_h C'_{sh \alpha j} \right) \right]. \tag{46}
\end{aligned}$$

Many of the terms of equation 46 may be neglected when $V_{g, s}$ is small, using the fact that sums over all loci of associations involving one or several “ s ” indices are proportional to $V_{g, s}$: therefore, the terms on the last two lines of equation 46 and the last term of the third line will generate terms in $V_{g, s}^2$. Furthermore, we will neglect linkage disequilibria between loci affecting investment in sex, so that only the terms

for $h = i$ remain in the sums above, and equation 46 simplifies to:

$$C_{si\alpha j\beta k}^{\text{rep}} \approx (1 - \rho_{ijk}) C'_{si\alpha j\beta k} - \frac{c - r_{ijk,\emptyset} - r_{\emptyset,ijk}}{c(1 - \bar{s}) + \bar{s}} C'_{si\alpha j\beta k} + \frac{r_{i,jk} + r_{jk,i}}{c(1 - \bar{s}) + \bar{s}} C'_{si\alpha i} C'_{\alpha j\beta k} \quad (47)$$

with $\rho_{ijk} = \bar{\sigma} r_{ijk}$, $r_{ijk} = 1 - r_{ijk,\emptyset} - r_{\emptyset,ijk}$ being the probability that at least one recombination event occurs between loci i , j and k . Because $C'_{si\alpha j\beta k} \approx C'_{si\alpha i} C'_{\alpha j\beta k}$ to leading order, equation 47 further simplifies to:

$$C_{si\alpha j\beta k}^{\text{rep}} \approx (1 - \rho_{ijk}) C'_{si\alpha j\beta k} - \frac{c - 1 + r_{jk}}{c(1 - \bar{s}) + \bar{s}} C'_{si\alpha i} C'_{\alpha j\beta k} \quad (48)$$

The term $\Delta_{\text{rep}} \overline{g_{si}}$ in equation 44 is given by:

$$\Delta_{\text{rep}} \overline{g_{si}} = \text{E}' \left[\frac{c(1 - s)}{c(1 - \bar{s}) + \bar{s}} \zeta_{si} \right] + \text{E}' \left[\frac{s_{\varphi} s_{\sigma}}{\bar{s} [c(1 - \bar{s}) + \bar{s}]} \left(\frac{\zeta_{si,\varphi} + \zeta_{si,\sigma}}{2} \right) \right]. \quad (49)$$

Neglecting linkage disequilibria between loci affecting the rate of sex, this yields:

$$\Delta_{\text{rep}} \overline{g_{si}} \approx - \frac{c - 1}{c(1 - \bar{s}) + \bar{s}} C'_{si\alpha i}. \quad (50)$$

From equation 24, $C_{\alpha j\beta k}^{\text{rep}} = (1 - \rho_{jk}) C'_{\alpha j\beta k}$ to leading order (that is, neglecting genetic variation for the rate of sex), so that:

$$(\Delta_{\text{rep}} \overline{g_{si}}) C_{\alpha j\beta k}^{\text{rep}} \approx - \frac{c - 1}{c(1 - \bar{s}) + \bar{s}} (1 - \rho_{jk}) C'_{si\alpha i} C'_{\alpha j\beta k}. \quad (51)$$

Putting everything together, one obtains from equations 44, 48 and 51:

$$C''_{si\alpha j\beta k} \approx (1 - \rho_{ijk}) C'_{si\alpha j\beta k} - \frac{c r_{jk}}{[c(1 - \bar{s}) + \bar{s}]^2} C'_{si\alpha i} C'_{\alpha j\beta k}. \quad (52)$$

The change in $C_{si\alpha i}$ due to selection may be neglected under our assumption that $V_{g,s}$ is small (as it would generate terms in $V_{g,s}^2$). Furthermore, the effect of selection on $C_{si\alpha j\beta k}$ can be neglected when selection is weak, as it involves higher-order associations

between loci i, j, k and other loci, which are themselves generated by the effect of selection at these loci. Using these approximations, equation 52 becomes:

$$C''_{si\alpha j\beta k} \approx (1 - \rho_{ijk}) C_{si\alpha j\beta k} - \frac{c r_{jk}}{[c(1 - \bar{s}) + \bar{s}]^2} C_{si si} C'_{\alpha j\beta k} \quad (53)$$

giving at QLE:

$$C_{si\alpha j\beta k} \approx -\frac{1}{\rho_{ijk}} \frac{c r_{jk} C'_{\alpha j\beta k}}{[c(1 - \bar{s}) + \bar{s}]^2} C_{si si}. \quad (54)$$

From the results of the preceding subsection, $C'_{\alpha j\beta k} \approx (\Delta_{\text{sel}} C_{\alpha j\beta k}) / \rho_{jk}$ (where $\Delta_{\text{sel}} C_{\alpha j\beta k}$ is given by equation 34), so that $r_{jk} C'_{\alpha j\beta k} \approx \Delta_{\text{sel}} C_{\alpha j\beta k} / \bar{\sigma}$. Equation 54 thus simplifies to:

$$C_{si\alpha j\beta k} \approx -\frac{1}{r_{ijk}} \frac{c}{\bar{s}^2} (\Delta_{\text{sel}} C_{\alpha j\beta k}) C_{si si}. \quad (55)$$

The same reasoning as above can be used to compute $C_{si\alpha j\beta j}$, which is found to be negligible. Summing over all loci, one thus obtains:

$$M_{g, s\alpha\beta} \approx -\frac{1}{r_{h,1}} \frac{c}{\bar{s}^2} (\Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}) V_{g, s} \quad (56)$$

where $r_{h,1}$ is the harmonic mean of r_{ijk} over all triplets of loci involving one locus affecting sex and two loci affecting fecundity. From this, we have (using equations 8 and 39):

$$M_{g, \sigma\alpha\beta} \approx -\frac{\Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}}{r_{h,1} \bar{\sigma}^2} V_{g, \sigma}. \quad (57)$$

Indirect selection for sex due to the short-term effect (the second term of equation 13) can thus be written approximately as:

$$-\frac{1}{r_{h,1} \bar{\sigma}^2} \left(\sum_{\alpha \leq \beta} \frac{\partial \ln \bar{W}}{\partial C_{g, \alpha\beta}} \Delta_{\text{sel}} \mathcal{D}_{\alpha\beta} \right) V_{g, \sigma}. \quad (58)$$

We will see later that the term between parentheses can be expressed in terms of the effect of sex on the mean fitness of offspring.

Indirect selection for sex: “long-term effect”. The long-term effect depends on genetic covariances between the rate of sex and traits affecting fecundity ($C_{g,\sigma\alpha}$). From equation 8, we have:

$$C_{g,\sigma\alpha} \approx \frac{c}{[c(1-\bar{s}) + \bar{s}]^2} C_{g,s\alpha} \quad (59)$$

with $C_{g,s\alpha} = E[(g_s - \bar{g}_s)(g_\alpha - \bar{g}_\alpha)]$, which can be decomposed as:

$$C_{g,s\alpha} = \sum_{i,j} C_{si\alpha j}. \quad (60)$$

Using the same approach as above, one obtains for the effect of reproduction on $C_{si\alpha j}$:

$$C''_{si\alpha j} = C_{si\alpha j}^{\text{rep}} - (\Delta_{\text{rep}} \bar{g}_{si})(\Delta_{\text{rep}} \bar{g}_{\alpha j}) \approx C_{si\alpha j}^{\text{rep}} \quad (61)$$

since the term $(\Delta_{\text{rep}} \bar{g}_{si})(\Delta_{\text{rep}} \bar{g}_{\alpha j})$ will generate terms in $V_{g,s}^2$. Neglecting linkage disequilibria between loci affecting the rate of sex and other terms of order $V_{g,s}^2$, we have:

$$C_{si\alpha j}^{\text{rep}} \approx (1 - \rho_{ij}) C'_{si\alpha j} - \frac{c - 1 + r_{ij}}{c(1-\bar{s}) + \bar{s}} C'_{si\alpha j}. \quad (62)$$

To the first order in $V_{g,s}$,

$$C'_{si\alpha j} \approx C_{si\alpha j}^{\text{sel}} - (\Delta_{\text{sel}} \bar{g}_{\alpha j}) C_{si\alpha j} \quad (63)$$

while from equation 29:

$$\begin{aligned} C_{si\alpha j}^{\text{sel}} &\approx C_{si\alpha j} + \sum_{\beta} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\beta}} \sum_k C_{si\alpha j\beta k} \\ &\quad + \sum_{\beta \leq \gamma} \frac{\partial \ln \bar{W}}{\partial C_{g,\beta\gamma}} \sum_{k,l} (C_{si\alpha j\beta k\gamma l} - C_{si\alpha j} C_{\beta k\gamma l}) \end{aligned} \quad (64)$$

$$\Delta_{\text{sel}} \bar{g}_{\alpha j} \approx \sum_{\beta} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\beta}} \sum_k C_{\alpha j\beta k} + \sum_{\beta \leq \gamma} \frac{\partial \ln \bar{W}}{\partial C_{g,\beta\gamma}} \sum_{k,l} C_{\alpha j\beta k\gamma l}. \quad (65)$$

From equations 63 – 65, and using the fact that $C_{si\alpha j\beta k} \approx C_{si\alpha j} C_{\alpha j\beta k}$, $C_{si\alpha j\beta k\gamma l} \approx C_{si\alpha j} C_{\alpha j\beta k\gamma l}$ to the first order in $V_{g,s}$, one obtains that the effect of selection on $C_{si\alpha j}$ is negligible, which finally leads to $C_{si\alpha j} \approx C'_{si\alpha j} \approx 0$ at QLE.

The effect of selection on $C_{si\alpha j}$ is given by:

$$C'_{si\alpha j} \approx C_{si\alpha j}^{\text{sel}} - (\Delta_{\text{sel}} \overline{g_{si}}) (\Delta_{\text{sel}} \overline{g_{\alpha j}}), \quad (66)$$

where

$$\begin{aligned} C_{si\alpha j}^{\text{sel}} \approx & C_{si\alpha j} + \sum_{\beta} \frac{\partial \ln \overline{W}}{\partial z_{\beta}} \sum_k C_{si\alpha j\beta k} \\ & + \sum_{\beta \leq \gamma} \frac{\partial \ln \overline{W}}{\partial C_{g,\beta\gamma}} \sum_{k,l} (C_{si\alpha j\beta k\gamma l} - C_{si\alpha j} C_{\beta k\gamma l}), \end{aligned} \quad (67)$$

while the term $(\Delta_{\text{sel}} \overline{g_{si}}) (\Delta_{\text{sel}} \overline{g_{\alpha j}})$ is of higher order in the strength of selection, and may thus be neglected. Finally, using the same method as in the previous subsection shows that associations $C_{si\alpha j\beta k\gamma l}$ (that appear on the second line of equation 67) are proportional to $C_{si\alpha j} C_{\beta k\gamma l}$. However, 3-locus associations $C_{\alpha j\beta k\gamma l}$ are of higher order in the strength of selection than 2-locus associations, and we will assume that the sum over all loci of these associations is negligible relative to the sum of pairwise associations $C_{\alpha j\beta k}$. This leaves us with the following recursion for $C_{si\alpha j}$:

$$C''_{si\alpha j} \approx (1 - \rho_{ij}) \left[C_{si\alpha j} + \sum_{\beta} \frac{\partial \ln \overline{W}}{\partial z_{\beta}} \sum_k C_{si\alpha j\beta k} \right]. \quad (68)$$

At QLE, and using equation 55, we thus have:

$$C_{si\alpha j} \approx - \left(\frac{1}{\rho_{ij}} - 1 \right) \frac{c}{\bar{s}^2} \sum_k \frac{1}{r_{ijk}} \sum_{\beta} \frac{\partial \ln \overline{W}}{\partial z_{\beta}} (\Delta_{\text{sel}} C_{\alpha j\beta k}) C_{si\alpha i}, \quad (69)$$

and summing over all loci:

$$C_{g,s\alpha} \approx - \left(\frac{1}{r_{h,2} \bar{\sigma}} - \frac{1}{r_{h,1}} \right) \frac{c}{\bar{s}^2} \sum_{\beta} \frac{\partial \ln \overline{W}}{\partial z_{\beta}} (\Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}) V_{g,s} \quad (70)$$

where $r_{h,2}$ is the harmonic mean of $r_{ij} r_{ijk}$ over all triplets of loci i, j and k , where i affects investment in sex while j and k affect fecundity. Equations 8 and 59 then yield:

$$C_{g,\sigma\alpha} \approx - \left(\frac{1}{r_{h,2} \bar{\sigma}} - \frac{1}{r_{h,1}} \right) \frac{1}{\bar{\sigma}^2} \sum_{\beta} \frac{\partial \ln \overline{W}}{\partial z_{\beta}} (\Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}) V_{g,\sigma} \quad (71)$$

and indirect selection for sex due to the long term effect (first term of equation 13) is thus approximately:

$$-\left(\frac{1}{r_{h,2}\bar{\sigma}} - \frac{1}{r_{h,1}}\right) \frac{1}{\bar{\sigma}^2} \left(\sum_{\alpha,\beta} \frac{\partial \ln \bar{W}}{\partial z_{\alpha}} \frac{\partial \ln \bar{W}}{\partial z_{\beta}} \Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}\right) V_{g,\sigma}. \quad (72)$$

Note that the term in the first parenthesis of equation 72 is positive, $1/r_{h,1}$ becoming negligible compared with $1/(r_{h,2}\bar{\sigma})$ as the rate of sex decreases.

Expressing indirect selection in terms of the effect of sex on the fitness of offspring. The terms between parentheses in equation 58 and 72 (involving $\Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}$) provide intuitive understanding of the mechanisms generating indirect selection for sex, but would be difficult to measure in a real population. However, using our hypothesis of weak selection and Gaussian distribution of traits affecting fecundity, these can be expressed in terms of the effect of sex on the fecundity of offspring, that could (at least in principle) be measured in an experimental population. Indeed, a Taylor series on $\ln \bar{W}$ provides the following approximation for the effect of a change in mean breeding values and/or in the genetic variance-covariance matrix on $\ln \bar{W}$:

$$\Delta \ln \bar{W} \approx \sum_{\alpha} \Delta \bar{z}_{\alpha} \frac{\partial \ln \bar{W}}{\partial z_{\alpha}} + \sum_{\alpha \leq \beta} \Delta C_{g,\alpha\beta} \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}}. \quad (73)$$

If we now imagine an experiment where we sample a sufficiently large number of individuals from the population (so that genetic associations within this pool of individuals are representative of associations in the whole population) and let them produce a pool of offspring by sexual reproduction and another pool by asexual reproduction, both pools should have the same mean breeding values (on average), while genetic variances and covariances (measured separately within each pool of offspring) should differ by

an amount:

$$\Delta_{\text{sex/asex}} C_{g,\alpha\beta} = C_{g,\alpha\beta}^{\text{sex}} - C_{g,\alpha\beta}^{\text{asex}} = - \sum_{j \neq k} r_{jk} C'_{\alpha j \beta k} \quad (74)$$

due to the effect of sexual recombination. From equation 33, we have $\Delta_{\text{sel}} \mathcal{D}_{\alpha\beta} \approx \sum_{j \neq k} \rho_{jk} C'_{\alpha j \beta k}$, so that:

$$\Delta_{\text{sex/asex}} C_{g,\alpha\beta} \approx -\frac{1}{\bar{\sigma}} \Delta_{\text{sel}} \mathcal{D}_{\alpha\beta}. \quad (75)$$

Therefore, from equation 73, the difference in $\ln \bar{W}$ between sexually and asexually produced offspring is given by:

$$\Delta_1 = \ln \bar{W}_{\text{sex}} - \ln \bar{W}_{\text{asex}} \approx -\frac{1}{\bar{\sigma}} \sum_{\alpha \leq \beta} \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} \Delta_{\text{sel}} \mathcal{D}_{\alpha\beta} \quad (76)$$

and indirect selection for sex due to the short-term effect (equation 58) thus becomes:

$$\frac{\Delta_1}{r_{h,1} \bar{\sigma}} V_{g,\sigma}. \quad (77)$$

Following Barton (1995) and Charlesworth and Barton (1996), selection for sex due to the long-term effect can be expressed in terms of the effect of sex on the variance in log-fitness among offspring. From equation 10 we have, to leading order in selection gradients:

$$\begin{aligned} \ln W_{\mathbf{g}} - \ln \bar{W} &\approx \sum_{\alpha} (g_{\alpha} - \bar{g}_{\alpha}) \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\alpha}} \\ &+ \sum_{\alpha \leq \beta} [(g_{\alpha} - \bar{g}_{\alpha})(g_{\beta} - \bar{g}_{\beta}) - C_{g,\alpha\beta}] \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} \end{aligned} \quad (78)$$

so that the variance in $\ln W_{\mathbf{g}}$ among individuals is:

$$\begin{aligned} \text{Var} [\ln W_{\mathbf{g}}] &\approx \sum_{\alpha, \beta} C_{g,\alpha\beta} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\alpha}} \frac{\partial \ln \bar{W}}{\partial \bar{z}_{\beta}} \\ &+ \sum_{\alpha \leq \beta} \sum_{\gamma \leq \delta} (C_{g,\alpha\gamma} C_{g,\beta\delta} + C_{g,\alpha\delta} C_{g,\beta\gamma}) \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} \frac{\partial \ln \bar{W}}{\partial C_{g,\gamma\delta}}. \end{aligned} \quad (79)$$

Equation 79 is approximately equivalent to the first two lines of equation A3b in Charlesworth and Barton (1996), corresponding to the additive and epistatic components of the variance in log fitness (denoted hereafter V_A and V_{AA}). Using equations 75 and 79, the sum appearing in the expression for the strength of selection for sex due to the long term effect (equation 72) can be expressed as $-\bar{\sigma} (V_{A,\text{sex}} - V_{A,\text{asex}})$, where $V_{A,\text{sex}}$ and $V_{A,\text{asex}}$ are the additive components of the variance in log fitness (first term of equation 79) among offspring produced by sexual and asexual reproduction, respectively. Selection for sex due to the long term effect thus becomes:

$$\left(\frac{1}{r_{h,2}\bar{\sigma}} - \frac{1}{r_{h,1}} \right) \frac{\Delta_2}{\bar{\sigma}} V_{g,\sigma} \quad (80)$$

with $\Delta_2 = V_{A,\text{sex}} - V_{A,\text{asex}}$. Assuming that epistasis is weak relative to directional selection, Charlesworth and Barton (1996) show that the effect of recombination on V_{AA} may be neglected, in which case the long term effect can be expressed in terms of the effect of recombination on $\text{Var}[\ln W_g]$. However, in situations where epistatic interactions may be of the same order of magnitude as directional selection (as in the present model), the additive component of $\text{Var}[\ln W_g]$ should be estimated, for example from the covariance between parents and offspring (e.g., Lynch and Walsh, 1998). Indeed, under the assumption of a sufficiently large number of loci with weak effects so that the joint distribution of trait values in parents and offspring is approximately multivariate Gaussian, the covariance in log fitness between parents and offspring is:

$$\begin{aligned} \text{Cov}^{\text{PO}}[\ln W_g] &\approx \sum_{\alpha,\beta} C_{g,\alpha\beta}^{\text{PO}} \frac{\partial \ln \bar{W}}{\partial \bar{z}_\alpha} \frac{\partial \ln \bar{W}}{\partial \bar{z}_\beta} \\ &+ \sum_{\alpha \leq \beta} \sum_{\gamma \leq \delta} (C_{g,\alpha\gamma}^{\text{PO}} C_{g,\beta\delta}^{\text{PO}} + C_{g,\alpha\delta}^{\text{PO}} C_{g,\beta\gamma}^{\text{PO}}) \frac{\partial \ln \bar{W}}{\partial C_{g,\alpha\beta}} \frac{\partial \ln \bar{W}}{\partial C_{g,\gamma\delta}} \end{aligned} \quad (81)$$

where $C_{g,\alpha\beta}^{\text{PO}}$ is the covariance between g_α in the parents and g_β in their offspring. Using

$C_{\mathbf{g},\alpha\beta}^{\text{PO}} = C_{\mathbf{g},\alpha\beta}/2$, equation 81 becomes:

$$\text{Cov}^{\text{PO}} [\ln W_{\mathbf{g}}] \approx \frac{V_{\text{A}}}{2} + \frac{V_{\text{AA}}}{4} \quad (82)$$

yielding

$$V_{\text{A}} \approx 4\text{Cov}^{\text{PO}} [\ln W_{\mathbf{g}}] - \text{Var} [\ln W_{\mathbf{g}}] . \quad (83)$$

APPENDIX A: APPROXIMATION FOR $W_{\mathbf{g}}/\overline{W}$

Assuming that selection is weak (meaning that the variance in $W_{\mathbf{g}}$ is small), we can approximate $W_{\mathbf{g}}$ by a Taylor series around $\overline{\mathbf{g}} = (\overline{g_1}, \overline{g_2}, \dots)$:

$$\begin{aligned} W_{\mathbf{g}} \approx & W_{\mathbf{g}}(\overline{\mathbf{g}}) + \sum_{\alpha} (g_{\alpha} - \overline{g_{\alpha}}) \frac{\partial W_{\mathbf{g}}}{\partial g_{\alpha}} \\ & + \frac{1}{2} \sum_{\alpha, \beta} (g_{\alpha} - \overline{g_{\alpha}}) (g_{\beta} - \overline{g_{\beta}}) \frac{\partial^2 W_{\mathbf{g}}}{\partial g_{\alpha} \partial g_{\beta}} \end{aligned} \quad (\text{A1})$$

where the partial derivatives are taken in $\overline{\mathbf{g}}$, and the last sum includes $\alpha = \beta$. Averaging over all individuals yields $\overline{W} \approx W_{\mathbf{g}}(\overline{\mathbf{g}}) + \frac{1}{2} \sum_{\alpha, \beta} C_{\mathbf{g}, \alpha\beta} \partial^2 W_{\mathbf{g}} / (\partial g_{\alpha} \partial g_{\beta})$, so that equation A1 can also be written as:

$$\begin{aligned} W_{\mathbf{g}} \approx & \overline{W} + \sum_{\alpha} (g_{\alpha} - \overline{g_{\alpha}}) \frac{\partial W_{\mathbf{g}}}{\partial g_{\alpha}} \\ & + \frac{1}{2} \sum_{\alpha, \beta} [(g_{\alpha} - \overline{g_{\alpha}}) (g_{\beta} - \overline{g_{\beta}}) - C_{\mathbf{g}, \alpha\beta}] \frac{\partial^2 W_{\mathbf{g}}}{\partial g_{\alpha} \partial g_{\beta}}. \end{aligned} \quad (\text{A2})$$

The derivatives of $W_{\mathbf{g}}$ in equation can be expressed in terms of derivatives of \overline{W} (Barton and Turelli, 1991; Turelli and Barton, 1994). Consider the effect of a slight change in the distribution of breeding values \mathbf{g} on mean fitness: $\overline{g_{\alpha}}$ and $C_{\mathbf{g}, \alpha\beta}$ change to $\overline{g_{\alpha}^*}$ and $C_{\mathbf{g}, \alpha\beta}^*$, causing mean fitness to change from \overline{W} to \overline{W}^* . Replacing $g_{\alpha} - \overline{g_{\alpha}}$ by $g_{\alpha} - \overline{g_{\alpha}^*} + \overline{g_{\alpha}^*} - \overline{g_{\alpha}}$ in equation A2 and averaging over the new state of the population yields:

$$\overline{W}^* \approx \overline{W} + \sum_{\alpha} (\overline{g_{\alpha}^*} - \overline{g_{\alpha}}) \frac{\partial W_{\mathbf{g}}}{\partial g_{\alpha}} + \frac{1}{2} \sum_{\alpha, \beta} (C_{\mathbf{g}, \alpha\beta}^* - C_{\mathbf{g}, \alpha\beta}) \frac{\partial^2 W_{\mathbf{g}}}{\partial g_{\alpha} \partial g_{\beta}}. \quad (\text{A3})$$

Note that terms $(\overline{g_{\alpha}^*} - \overline{g_{\alpha}}) (\overline{g_{\beta}^*} - \overline{g_{\beta}})$ appearing in the second sum have been neglected, as we assume that $\overline{g_{\alpha}^*} - \overline{g_{\alpha}}$ is small for all α . Another expression for \overline{W}^* can be obtained by developing \overline{W} (which is a function of $\overline{g_{\alpha}} = \overline{z_{\alpha}}$ and $C_{\mathbf{g}, \alpha\beta}$ for all α, β) as a Taylor

series:

$$\overline{W}^* \approx \overline{W} + \sum_{\alpha} (\overline{g}_{\alpha}^* - \overline{g}_{\alpha}) \frac{\partial \overline{W}}{\partial \overline{z}_{\alpha}} + \sum_{\alpha \leq \beta} (C_{\mathbf{g}, \alpha \beta}^* - C_{\mathbf{g}, \alpha \beta}) \frac{\partial \overline{W}}{\partial C_{\mathbf{g}, \alpha \beta}} \quad (\text{A4})$$

(note that each (α, β) pair is counted only once in the last sum). From equations A3

and A4, we have

$$\frac{\partial W_{\mathbf{g}}}{\partial g_{\alpha}} \approx \frac{\partial \overline{W}}{\partial \overline{z}_{\alpha}}, \quad \frac{\partial^2 W_{\mathbf{g}}}{\partial g_{\alpha}^2} \approx 2 \frac{\partial \overline{W}}{\partial V_{\mathbf{g}, \alpha}}, \quad \frac{\partial^2 W_{\mathbf{g}}}{\partial g_{\alpha} \partial g_{\beta}} \approx \frac{\partial \overline{W}}{\partial C_{\mathbf{g}, \alpha \beta}} \quad (\alpha \neq \beta) \quad (\text{A5})$$

and equation A2 and A5 yield (after dividing both sides by \overline{W}):

$$\begin{aligned} \frac{W_{\mathbf{g}}}{\overline{W}} &\approx 1 + \sum_{\alpha} (g_{\alpha} - \overline{g}_{\alpha}) \frac{\partial \ln \overline{W}}{\partial \overline{z}_{\alpha}} \\ &+ \sum_{\alpha \leq \beta} [(g_{\alpha} - \overline{g}_{\alpha}) (g_{\beta} - \overline{g}_{\beta}) - C_{\mathbf{g}, \alpha \beta}] \frac{\partial \ln \overline{W}}{\partial C_{\mathbf{g}, \alpha \beta}}. \end{aligned} \quad (\text{A6})$$

APPENDIX B: SELECTION GRADIENTS WITH ISOTROPIC, GAUSSIAN
FITNESS FUNCTION

From equation 15, we have:

$$\begin{aligned}\ln \bar{W} &= \frac{1}{2} \ln [\det((\mathbf{S} + \mathbf{P})^{-1} \mathbf{S})] - \frac{1}{2} (\bar{\mathbf{z}} - \boldsymbol{\theta})^T (\mathbf{S} + \mathbf{P})^{-1} (\bar{\mathbf{z}} - \boldsymbol{\theta}) \\ &= \frac{1}{2} \ln [\det(\mathbf{S})] - \frac{1}{2} \ln [\det(\mathbf{S} + \mathbf{P})] - \frac{1}{2} (\bar{\mathbf{z}} - \boldsymbol{\theta})^T (\mathbf{S} + \mathbf{P})^{-1} (\bar{\mathbf{z}} - \boldsymbol{\theta})\end{aligned}\tag{B1}$$

so that:

$$\frac{\partial \ln \bar{W}}{\partial \bar{\mathbf{z}}} = -(\mathbf{S} + \mathbf{P})^{-1} (\bar{\mathbf{z}} - \boldsymbol{\theta})\tag{B2}$$

and

$$\begin{aligned}\frac{\partial \ln \bar{W}}{\partial \mathbf{G}} &= -\frac{1}{2} \frac{\partial \ln [\det(\mathbf{S} + \mathbf{P})]}{\partial \mathbf{G}} - \frac{1}{2} (\bar{\mathbf{z}} - \boldsymbol{\theta})^T \frac{\partial (\mathbf{S} + \mathbf{P})^{-1}}{\partial \mathbf{G}} (\bar{\mathbf{z}} - \boldsymbol{\theta}) \\ &= -\frac{1}{2} \text{Tr} \left((\mathbf{S} + \mathbf{P})^{-1} \frac{\partial (\mathbf{S} + \mathbf{P})}{\partial \mathbf{G}} \right) \\ &\quad + \frac{1}{2} (\bar{\mathbf{z}} - \boldsymbol{\theta})^T (\mathbf{S} + \mathbf{P})^{-1} \frac{\partial (\mathbf{S} + \mathbf{P})}{\partial \mathbf{G}} (\mathbf{S} + \mathbf{P})^{-1} (\bar{\mathbf{z}} - \boldsymbol{\theta})\end{aligned}\tag{B3}$$

where Tr stands for the trace of a matrix. If phenotypes are measured in a basis that eliminates covariances among traits, $(\mathbf{S} + \mathbf{P})^{-1}$ is a diagonal matrix, with elements $1/(V_{g,\alpha} + V_s)$ on its diagonal (with $V_s = \omega^2 + V_e$). In that case, equations B2 and B3 yield equations 16 – 18.

LITERATURE CITED

- Agrawal, A. F. 2006. Evolution of sex: why do organisms shuffle their genotypes? *Curr. Biol.* 16:R696–R704.
- Barton, N. H. 1995. A general model for the evolution of recombination. *Genet. Res.* 65:123–144.
- Barton, N. H. and M. Turelli. 1991. Natural and sexual selection on many loci. *Genetics* 127:229–255.
- Bulmer, M. G. 1985. *The Mathematical Theory of Quantitative Genetics*, 2nd edition. Oxford University Press, Oxford.
- Charlesworth, B. 1993. Directional selection and the evolution of sex and recombination. *Genet. Res.* 61:205–224.
- Charlesworth, B. and N. H. Barton. 1996. Recombination load associated with selection for increased recombination. *Genet. Res.* 67:27–41.
- Gomulkiewicz, R. and D. Houle. 2009. Demographic and genetic constraints on evolution. *Am. Nat.* 174:E218–E229.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution* 33:402–416.
- Lynch, M. and J. B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.
- Roze, D. 2014. Selection for sex in finite populations. *J. Evol. Biol.* 27:1304–1322.

- Turelli, M. and N. H. Barton. 1990. Dynamics of polygenic characters under selection. *Theor. Popul. Biol.* 38:1–57.
- . 1994. Genetic and statistical analyses of strong selection on polygenic traits: what, me normal? *Genetics* 138:913–941.

SUPPLEMENTARY FIGURES

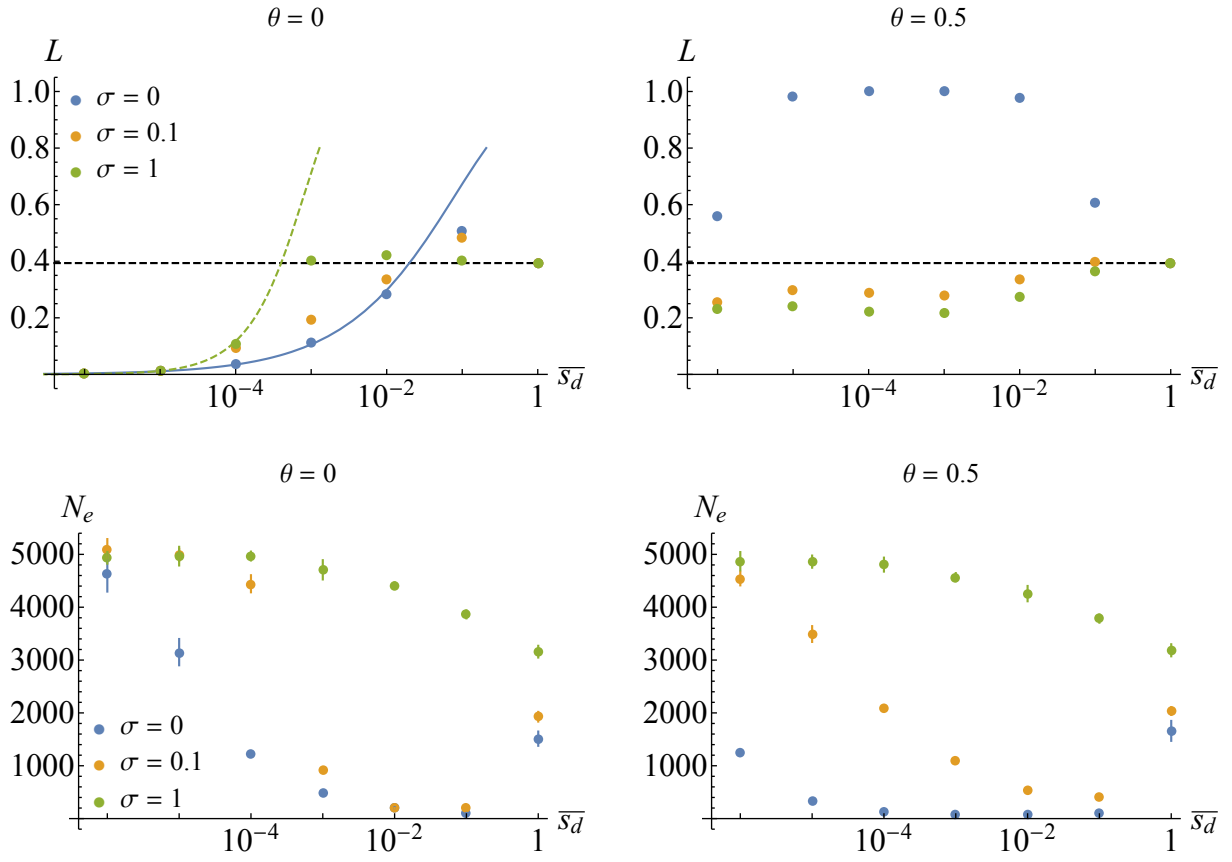


Figure S1. Same as Figure 3 in the main text, comparing $\theta = 0$ and $\theta = 0.5$.

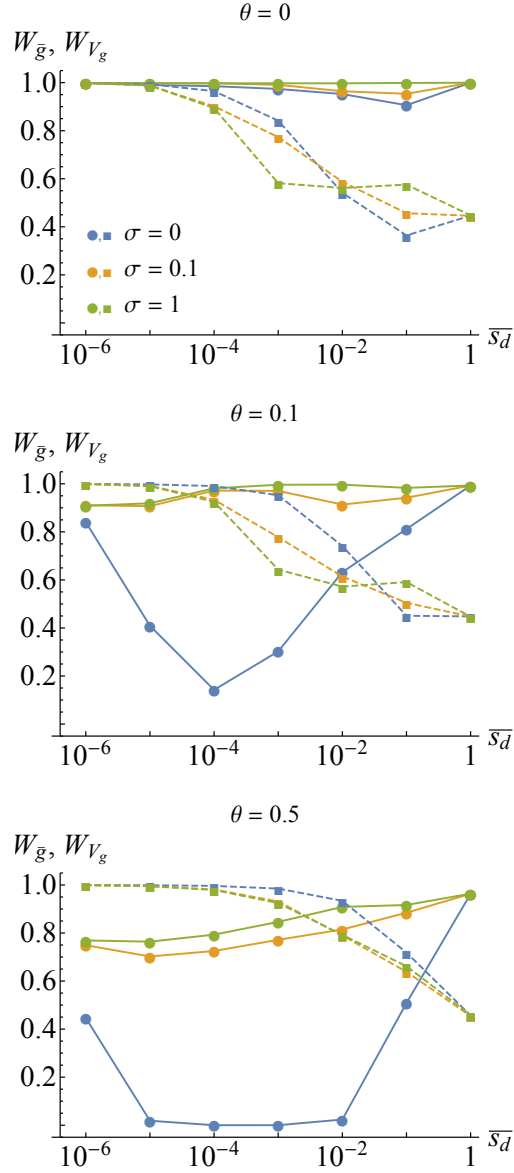


Figure S2. Terms $W_{\bar{g}} = \exp [-\sum_{\alpha=1}^n \langle \bar{g}_{\alpha}^2 \rangle / (2V_s)]$, $W_{V_g} = \exp [-\sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle / (2V_s)]$ representing the effect of departures of mean phenotypes from the optimum ($W_{\bar{g}}$, circles, solid lines) and the effect of genetic variance (W_{V_g} , squares, dashed lines) on the mutation load ($L \approx 1 - W_{\bar{g}} W_{V_g}$, see Supplementary File S2), for different values of \bar{s}_d and θ . Parameter values are as in Figures 3 and S1. Note that the lines simply connect simulation results and do not correspond to analytical approximations. Mutational bias causes an increase in load through $W_{\bar{g}}$.

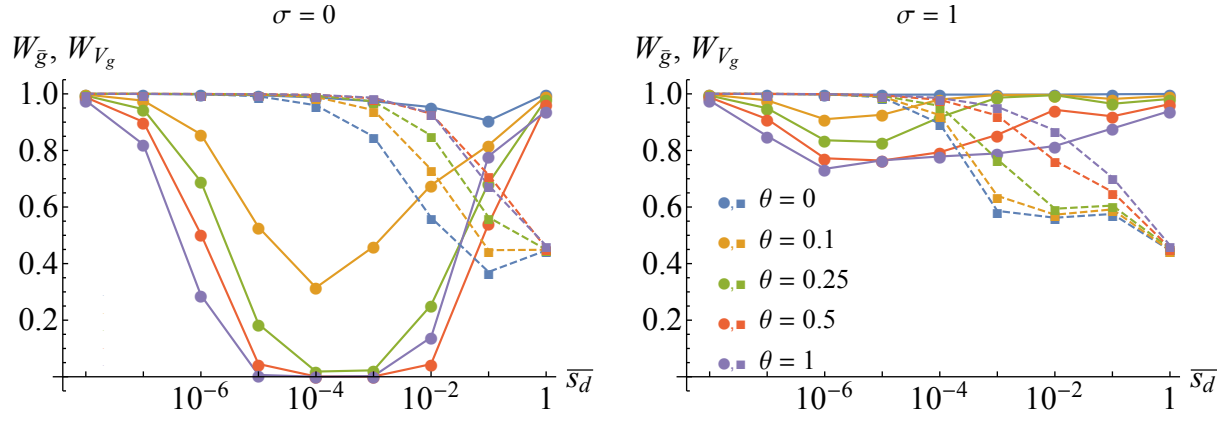


Figure S3. Same as Figure 4 in the main text, showing $W_{\bar{g}} = \exp[-\sum_{\alpha=1}^n \langle \bar{g}_{\alpha}^2 \rangle / (2V_s)]$ (circles, solid lines) and $W_{V_g} = \exp[-\sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle / (2V_s)]$ (squares, dashed lines).

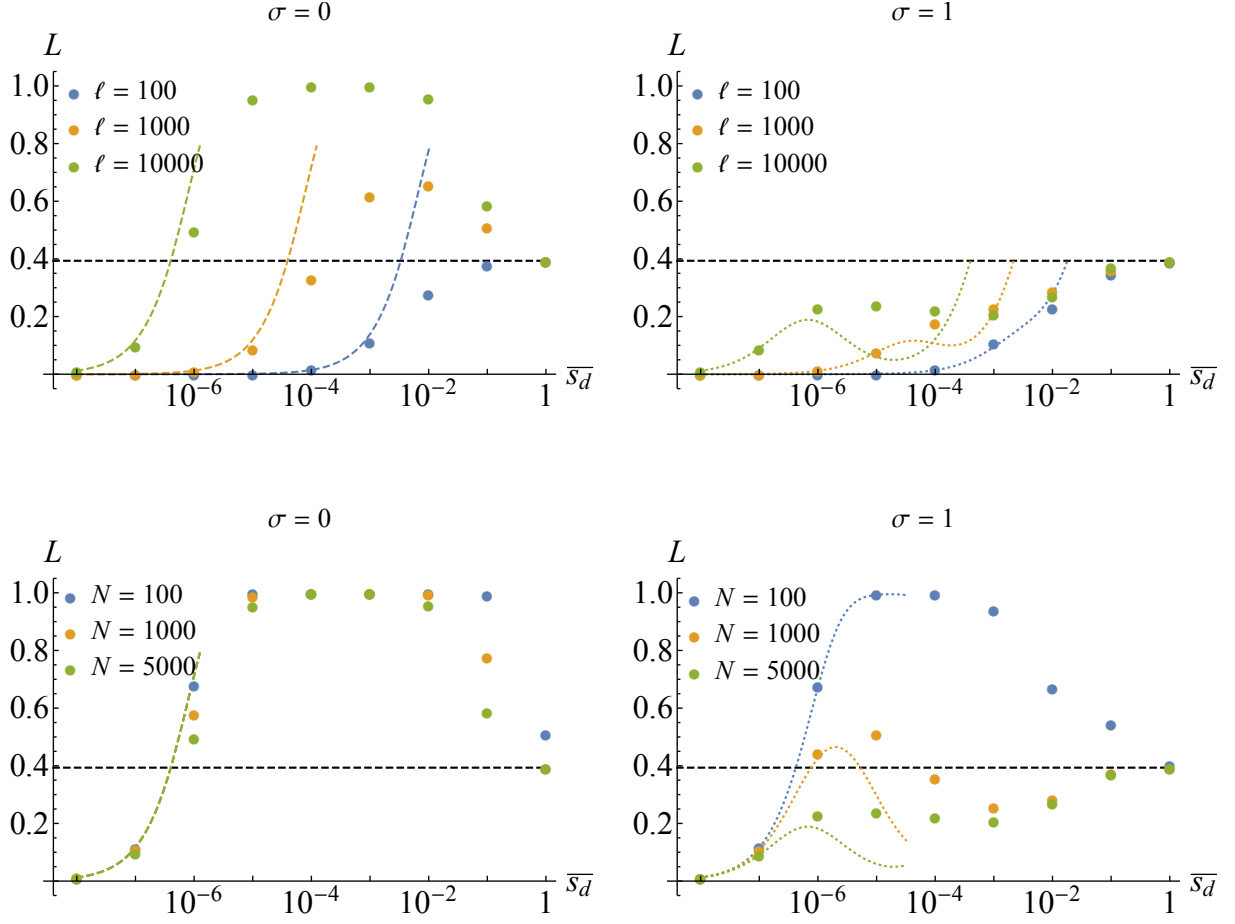


Figure S4. Same as Figure 5 in the main text, with $\theta = 0.5$.

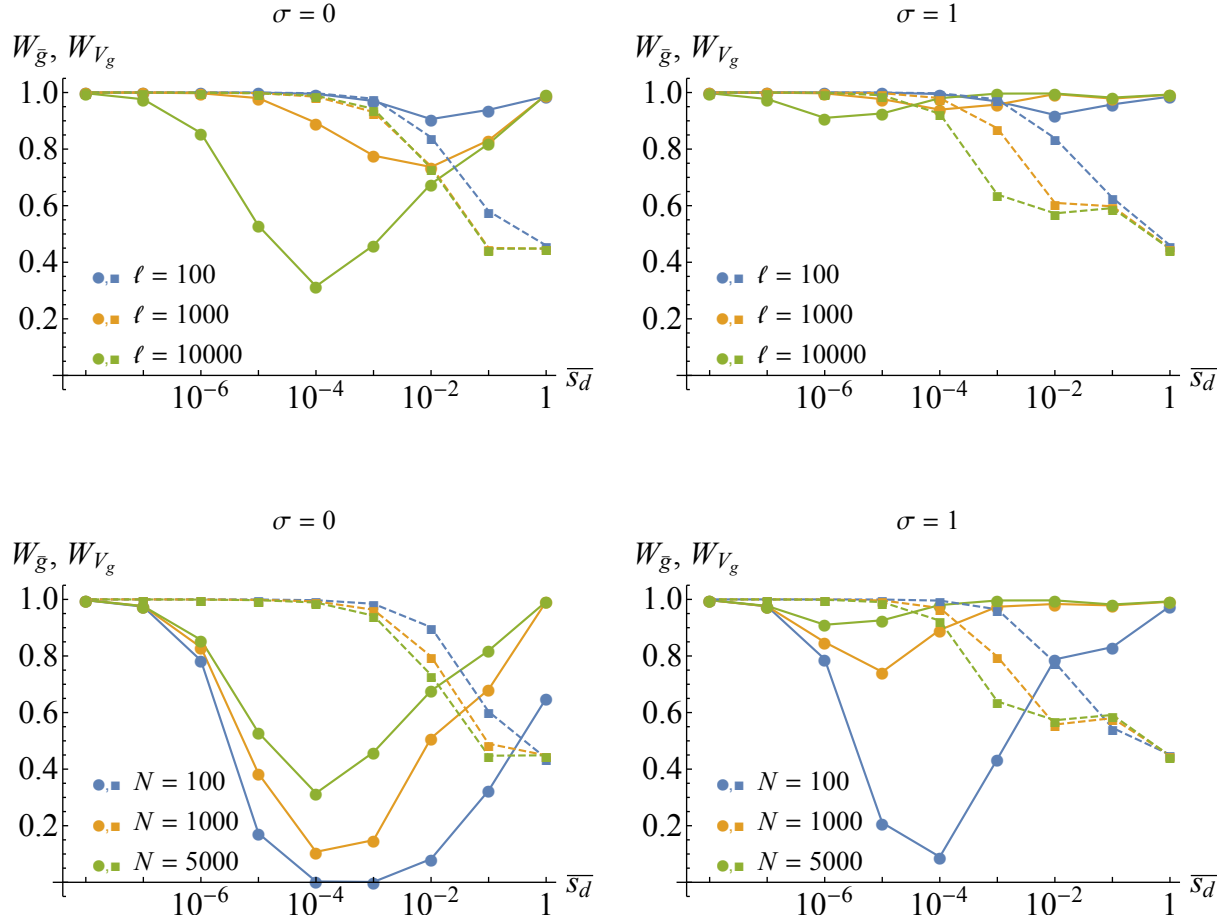


Figure S5. Same as Figure 5 in the main text, showing $W_{\bar{g}} = \exp[-\sum_{\alpha=1}^n \langle \bar{g}_{\alpha}^2 \rangle / (2V_s)]$ (circles, solid lines) and $W_{V_g} = \exp[-\sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle / (2V_s)]$ (squares, dashed lines).

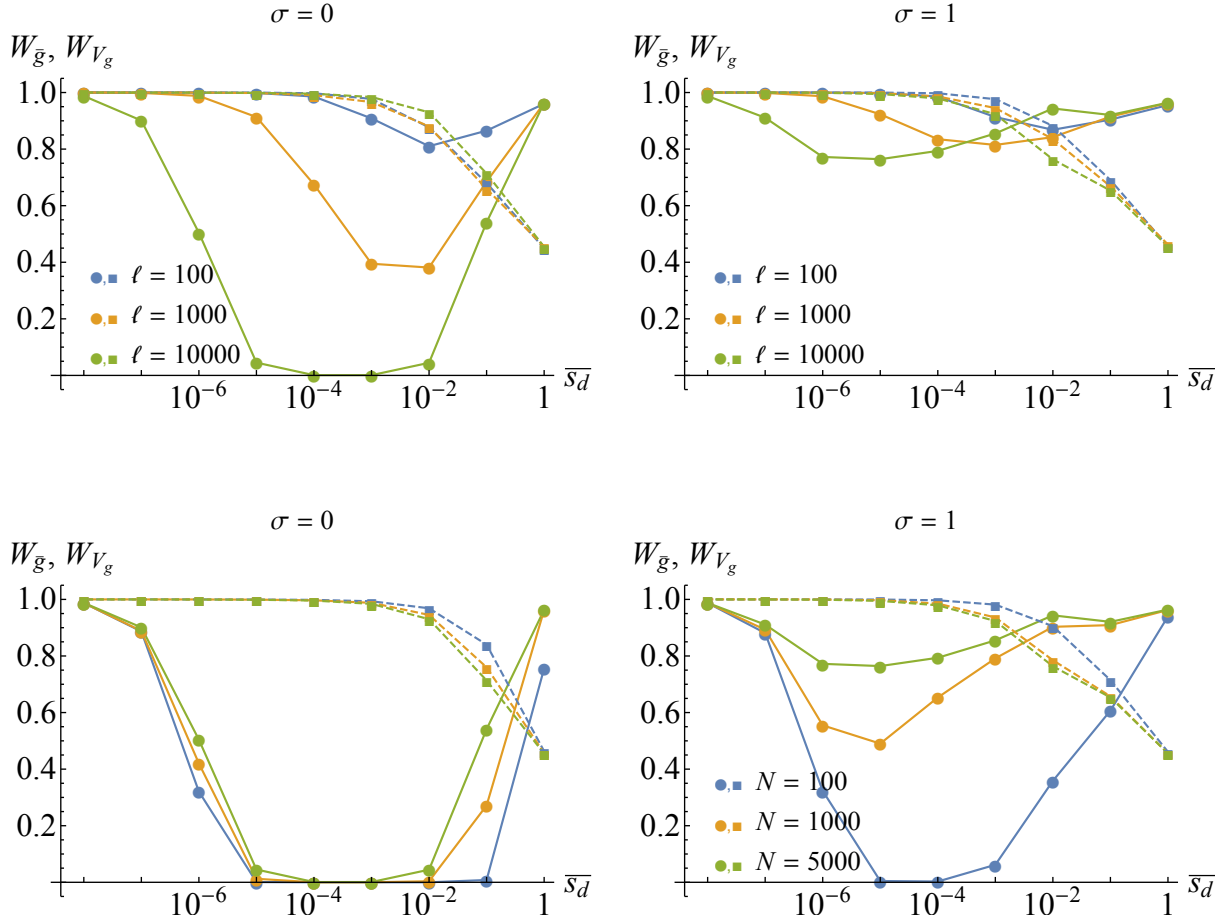


Figure S6. Same as Figure S4, showing $W_{\bar{g}} = \exp[-\sum_{\alpha=1}^n \langle \bar{g}_{\alpha}^2 \rangle / (2V_s)]$ (circles, solid lines) and $W_{V_g} = \exp[-\sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle / (2V_s)]$ (squares, dashed lines).

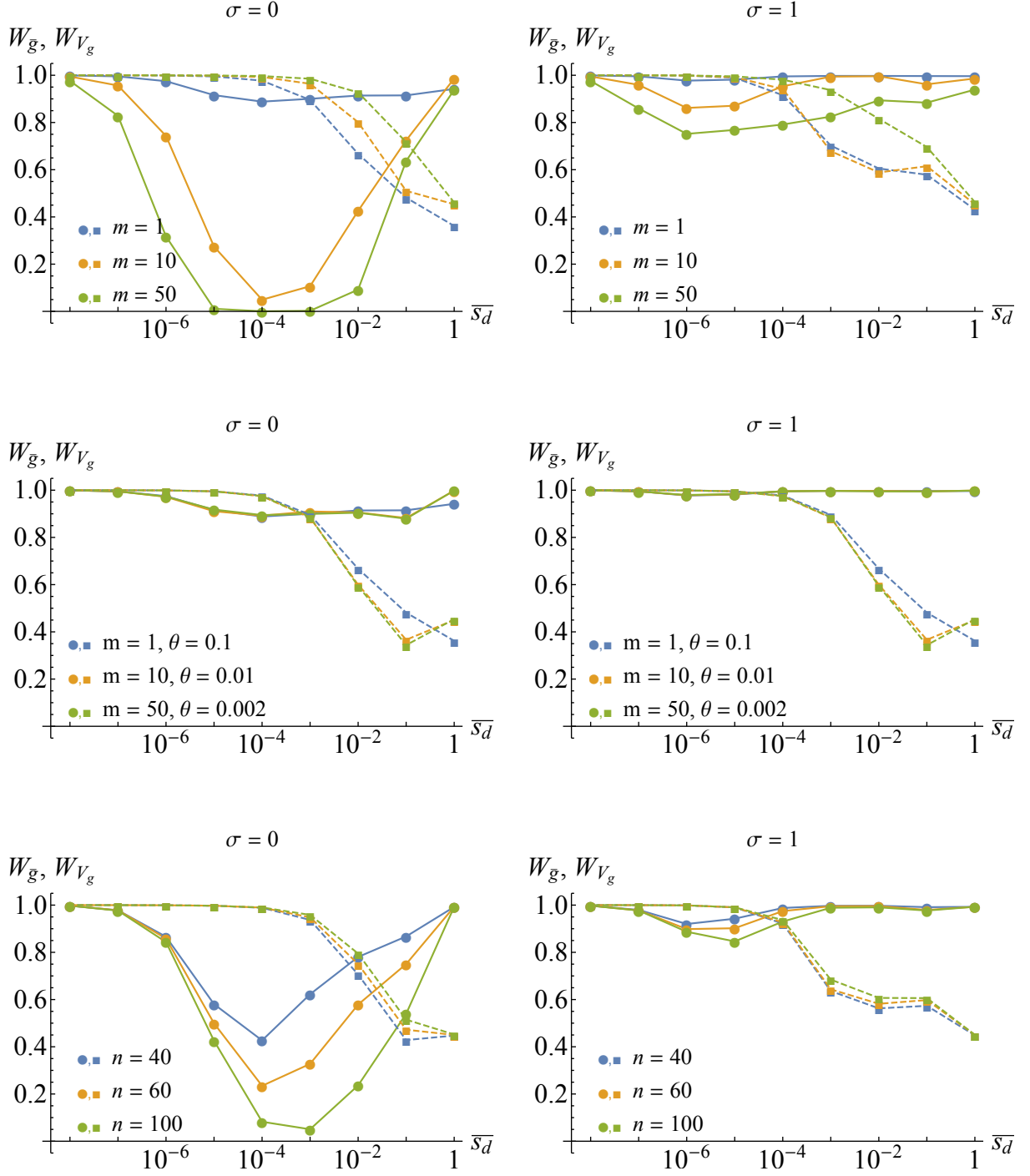


Figure S7. Same as Figure 6 in the main text, showing $W_{\bar{g}} = \exp[-\sum_{\alpha=1}^n \langle \bar{g}_{\alpha}^2 \rangle / (2V_s)]$ (circles, solid lines) and $W_{V_g} = \exp[-\sum_{\alpha=1}^n \langle V_{g,\alpha} \rangle / (2V_s)]$ (squares, dashed lines).

Appendix S2

Mean fitness and rate of sex at
equilibrium when the optimum
changes steadily over time

$(a = 0.1)$

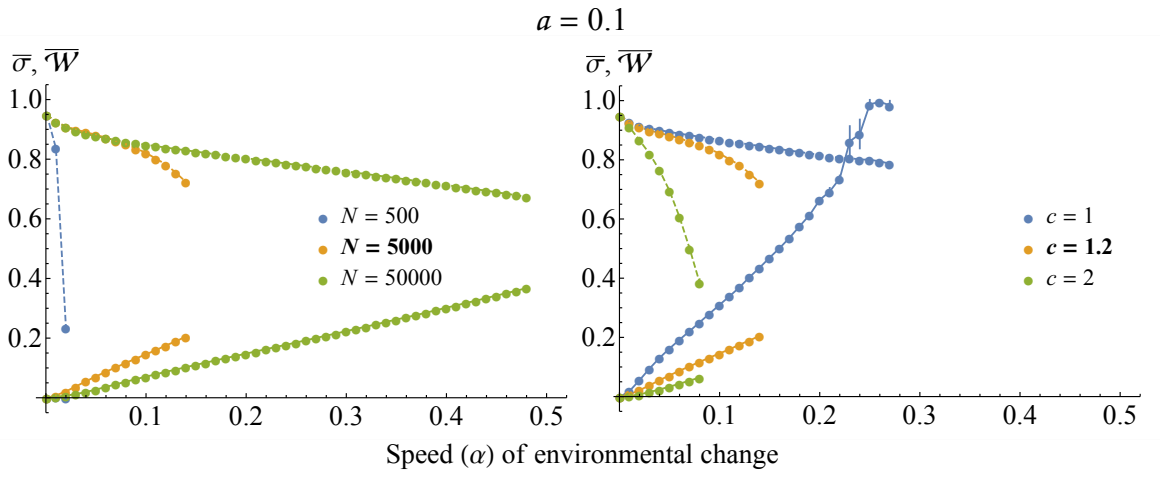


Figure S2.1: Mean fitness (decreasing curves, dashed) and mean rate of sex (increasing curves, solid) at equilibrium as a function of the rate of environmental change α , for different values of population size N and cost of sex c , for $a = 0.1$ (corresponding to $\bar{s} \approx 0.00045$). Parameter values are as in Figure 2.1.

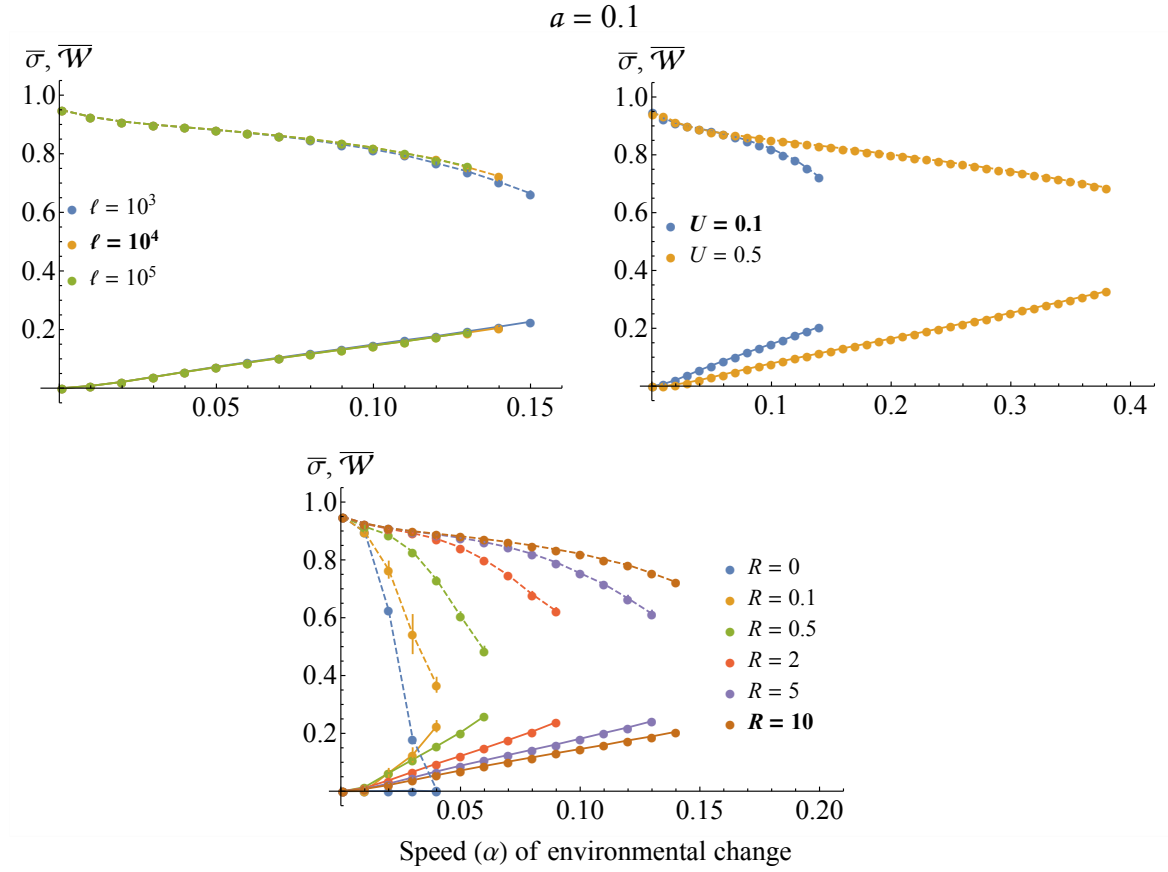


Figure S2.2: Mean fitness (decreasing curves, dashed) and mean rate of sex (increasing curves, solid) at equilibrium as a function of the rate of environmental change α , for different values of the number of selected loci ℓ , the number of loci ℓ_s affecting investment in sex, genome map length R and mutation rate on selected loci U , for $a = 0.1$ (corresponding to $\bar{s} \approx 0.00045$). Parameter values are as in Figure 2.1.

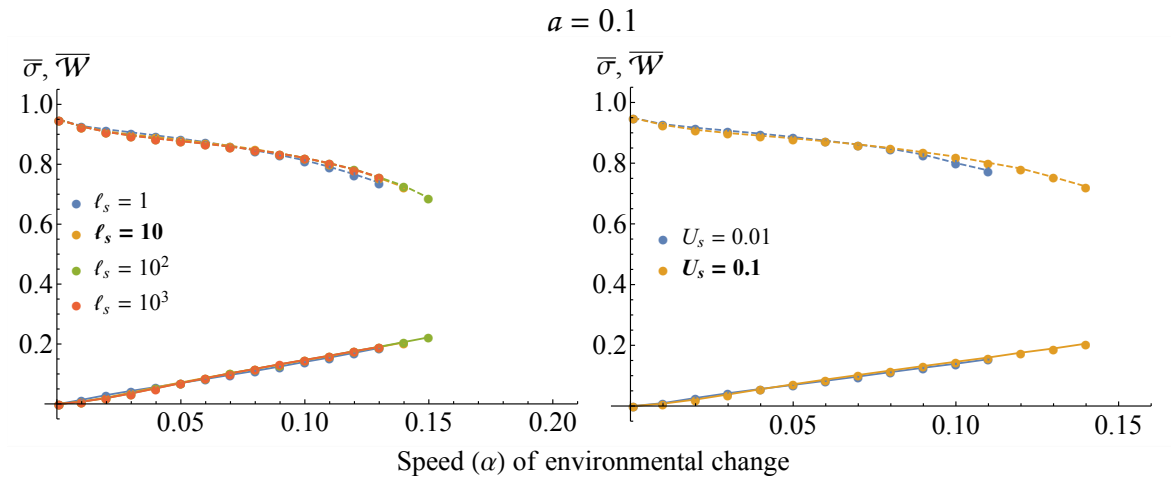


Figure S2.3: Mean fitness (decreasing curves, dashed) and mean rate of sex (increasing curves, solid) at equilibrium as a function of the rate of environmental change α , for different values of the number of loci ℓ_s affecting investment in sex and mutation rate on loci affecting investment in sex U_s , and for $a = 0.1$ (corresponding to $\bar{s} \approx 0.00045$). Parameter values are as in Figure 2.1.

Appendix S3

Mean rate of sex at equilibrium
when the optimum changes
cyclically over time ($P < 10$ and
 $a = 1$)

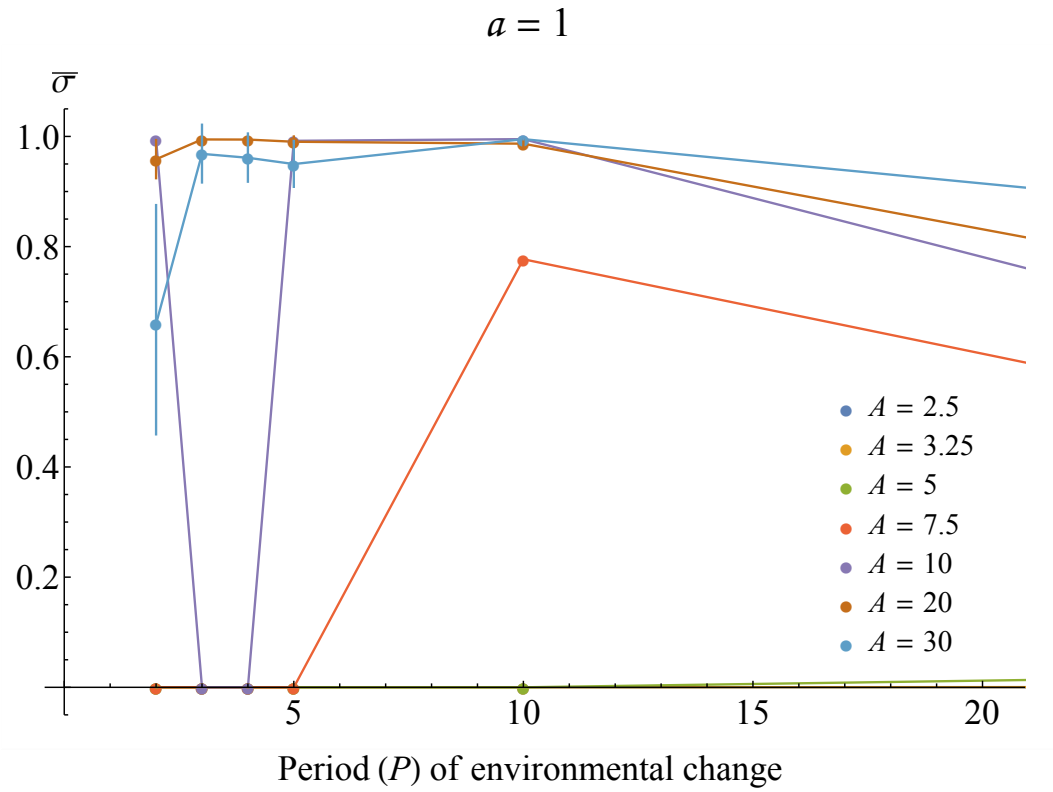


Figure S3.1: Mean rate of sex at equilibrium for small periods of environmental change ($P < 10$), for different values of the amplitude of oscillations A in the case of $a = 1$ (corresponding to $\bar{s} \approx 0.045$). Parameter values are as in Figure 2.1.

Appendix S4

Composition of f/2 culture
medium, from Guillard *et al.*
(1962)

Table S4.1: Composition of the F/2 medium (from Guillard *et al.*, 1962) used in our reactors of *Tetraselmis suecica*. For experiments involving the use of copper sulfate (CuSO_4), this culture medium was modified removing EDTA.

Compounds	Quantity (final concentration)
Main nutrients	
NaNO_3	75 mg L^{-1}
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	5 mg L^{-1}
Trace elements	
$\text{Na}_2 \cdot \text{EDTA}$	4.36 mg L^{-1}
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.15 mg L^{-1}
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.01 mg L^{-1}
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.022 mg L^{-1}
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.01 mg L^{-1}
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.18 mg L^{-1}
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.006 mg L^{-1}
Vitamins	
thiamine $\cdot \text{HCl}$	0.1 mg L^{-1}
biotin	0.0005 mg L^{-1}
B_{12}	0.0005 mg L^{-1}

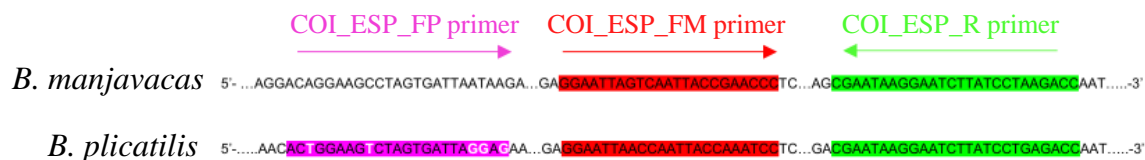
Appendix S5

Protocol for species

discrimination between

Brachionus plicatilis and

Brachionus manjavacas



Primers sequences

- COI_ESP_FP: 5'-ACTGGAAGTCTAGTGATTAGGAG-3'
- COI_ESP_FM: 5'-GGAATTAGTCAATTACCGAACCC-3'
- COI_ESP_R: 5'-GGTCTYAGGATAAGATTCTTATTTCG-3'

Composition of PCR mix

- Taq: GoTaq® Flexi DNA Polymerase, Promega Corporation
- Number of reactions: 96
- Volume: 10 μL

Product	Volume for 1 PCR (μL)	PCR Mix (μL)	Final [C] or quantity	Stock [C]
No. of reactions	1	102		
COI_ESP_FP	0.50	51	500 nM	10 μM
COI_ESP_FM	0.50	51	500 nM	10 μM
COI_ESP_R	0.50	51	500 nM	10 μM
Buffer	2	204	1 X	5 X
MgCl ₂	0.60	61.2	1.5 mM	25 mM
dNTP	0.60	61.2	150 μM	2.5 mM
H ₂ O	3.23	329.5		
Taq	0.07	7.1	0.35 U	5 U/ μL
DNA	2		10 ng	
Total vol. Mix	10	816		

PCR cycle

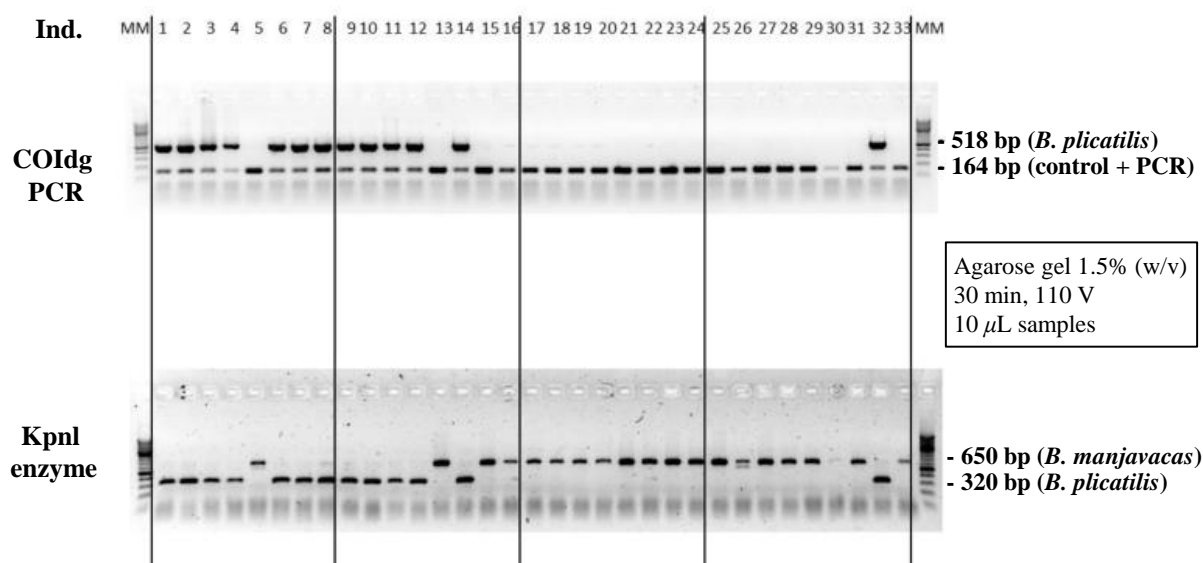
95°C - 5 min
 95°C - 30 sec
 56°C - 1 min
 72°C - 1 min

× 30 cycles

72°C - 10 min

Expected results

- *B. Manjavacas*: 164 bp fragment (COI_ESP_FM and COI_ESP_R)
- *B. plicatilis*: 164 bp and 518 bp fragments (COI_ESP_FP, COI_ESP_FM and COI_ESP_R)



Appendix S6

Composition of PCR mixes and PCR cycles for 11 microsatellite markers

Composition of PCR mixes

- Taq: GoTaq® Flexi DNA Polymerase, Promega Corporation
- Number of reactions: 192
- Volume: 10 μ L

Product	Volume for 1 PCR (μ L)	PCR Mix (μ L)	Final [C] or quantity	Stock [C]
No. of reactions	1	198		
Primer F	0.50	99	500 nM	10 μ M
Primer R	0.50	99	500 nM	10 μ M
Buffer	2	396	1 X	5 X
MgCl ₂	0.60	118.8	1.5 mM	25 mM
dNTP	0.80	158.4	200 μ M	2.5 mM
H ₂ O	3.53	698.9		
Taq	0.07	13.9	0.4 U	5 U/ μ L
DNA	2		10 ng	
Total vol. Mix	10	1584		

PCR cycles

Markers: Bp3, Bp1b, Bp4a, Bp6b, Bp3c and Bp5d

95°C - 3 min	
95°C - 1 min	
58°C - 1 min	× 5 cycles
72°C - 30 sec	
95°C - 30 sec	
58°C - 30 sec	× 35 cycles
72°C - 30 sec	
72°C - 10 min	

Product	Volume for 1 PCR (μ L)	PCR Mix (μ L)	Final [C] or quantity	Stock [C]
No. of reactions	1	198		
Primer F	0.50	99	500 nM	10 μ M
Primer R	0.50	99	500 nM	10 μ M
Buffer	2	396	1 X	5 X
MgCl ₂	1.2	237.6	3 mM	25 mM
dNTP	0.80	158.4	200 μ M	2.5 mM
H ₂ O	2.93	580.1		
Taq	0.07	13.9	0.4 U	5 U/ μ L
DNA	2		10 ng	
Total vol. Mix	10	1584		

Markers: Bp2

95°C - 3 min	
95°C - 1 min	
55°C - 1 min	× 5 cycles
72°C - 30 sec	
95°C - 30 sec	
55°C - 30 sec	× 35 cycles
72°C - 30 sec	
72°C - 10 min	

Product	Volume for 1 PCR (μ L)	PCR Mix (μ L)	Final [C] or quantity	Stock [C]
No. of reactions	1	198		
Primer F	0.50	99	500 nM	10 μ M
Primer R	0.50	99	500 nM	10 μ M
Buffer	2	396	1 X	5 X
MgCl ₂	0.80	158.4	2 mM	25 mM
dNTP	0.80	158.4	200 μ M	2.5 mM
H ₂ O	3.33	659.3		
Taq	0.07	13.9	0.4 U	5 U/ μ L
DNA	2		10 ng	
Total vol. Mix	10	1584		

Markers: Bp7, Bp8, Bp9 and Bp10

95°C - 3 min	
95°C - 1 min	
55°C - 1 min	× 5 cycles
72°C - 30 sec	
95°C - 30 sec	
55°C - 30 sec	× 35 cycles
72°C - 30 sec	
72°C - 10 min	

Multiplexes

	Marker	Label	Color	Dilution
I	Bp3	FAM	Blue	1/2
	Bp7	VIC	Green	1
	Bp3c	NED	Yellow	1
	Bp1b	PET	Red	1/2
	Bp9	FAM	Blue	1

	Marker	Label	Color	Dilution
II	Bp6b	VIC	Green	1
	Bp2	NED	Yellow	1
	Bp4a	PET	Red	1/2
	Bp8	FAM	Blue	1
	Bp5d	VIC	Green	1
	Bp10	NED	Yellow	1

Appendix S7

Multilocus genotypes of the
clones for 11 microsatellite loci

Table S7.1: Multilocus genotypes of the first 20 clones for 11 microsatellite loci. Alleles correspond to the fragment size in base pairs. Different alleles within one locus are highlighted in different colors. Missing values are represented by a 0.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7		Bp8		Bp9		Bp10	
C1	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	0	0	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	0	0	151	151	137	137	0	0	0	0	0	0	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	202	202	229	229	121	121	163	163	203	279	283	283	417	417
	248	248	151	151	137	137	206	212	0	0	229	229	121	121	163	163	203	279	283	283	417	417
C2	248	248	160	160	137	137	212	212	202	202	229	229	121	123	0	0	203	279	278	283	417	426
	0	0	160	160	137	137	212	212	202	202	229	229	121	123	163	165	203	279	278	283	417	426
	248	248	160	160	137	137	212	212	202	202	229	229	121	123	163	165	0	0	278	283	0	0
	248	248	160	160	137	137	212	212	202	202	229	229	121	123	163	165	203	279	278	283	417	426
	248	248	160	160	137	137	212	212	202	202	229	229	121	123	163	165	203	279	278	283	417	426
	248	248	160	160	137	137	212	212	202	202	229	229	121	123	163	165	203	279	278	283	417	426
	248	248	160	160	137	137	212	212	202	202	229	229	0	0	0	0	203	279	278	283	417	426
	0	0	160	160	137	137	0	0	202	202	229	229	121	123	163	165	203	279	278	283	417	426
	248	248	160	160	137	137	212	212	202	202	229	229	0	0	163	165	203	279	278	283	417	426
	248	248	160	160	137	137	212	212	0	0	229	229	121	123	163	165	203	279	278	283	417	426
C3	248	248	133	160	137	137	212	212	202	202	229	229	123	123	163	163	203	273	278	283	417	417
	0	0	133	160	137	137	212	212	202	202	229	229	123	123	163	163	0	0	278	283	417	417
	248	248	133	160	137	137	212	212	202	202	229	229	123	123	163	163	203	273	0	0	417	417
	248	248	133	160	137	137	212	212	202	202	229	229	123	123	163	163	203	273	278	283	417	417
	0	0	133	160	137	137	212	212	202	202	229	229	123	123	163	163	203	273	278	283	417	417
	248	248	133	160	137	137	212	212	202	202	229	229	123	123	163	163	203	273	278	283	417	417
	248	248	0	0	137	137	212	212	202	202	229	229	0	0	0	0	0	0	278	283	0	0
	248	248	133	160	137	137	212	212	202	202	229	229	123	123	163	163	203	273	278	283	417	417
	248	248	133	160	137	137	212	212	202	202	229	229	0	0	163	163	203	273	278	283	417	417
	0	0	133	160	137	137	212	212	0	0	229	229	123	123	163	163	203	273	278	283	417	417

Table S7.1: Continued from previous page.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7		Bp8		Bp9		Bp10	
C4	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	0	0	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	0	0	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	0	0	151	160	137	137	0	0	202	202	229	229	123	123	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	206	212	202	202	229	229	0	0	163	165	203	279	278	283	417	417
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	165	203	279	278	283	417	417
C6	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	0	0	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
C7	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	151	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	283	283	417	417

Table S7.1: Continued from previous page.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7			Bp8		Bp9		Bp10	
C8	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	0	0	278	283	0	0	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	151	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
C9	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	0	0	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	0	0	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
	248	248	133	160	137	137	212	212	202	202	229	229	121	123	163	163	203	203	278	283	417	417	
C10	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	
	248	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	426	

Table S7.1: Continued from previous page.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7		Bp8		Bp9		Bp10	
C11	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	0	0	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	0	0	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	279	278	283	417	426
	239	248	133	133	137	137	206	206	202	202	229	229	0	0	163	163	203	279	278	283	417	426
C12	239	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	0	0	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	0	0	133	160	137	137	206	206	202	202	229	229	0	0	163	163	203	203	278	283	417	417
	239	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	239	248	133	160	137	137	206	206	202	202	229	229	123	123	163	163	0	0	278	283	417	417
	0	0	133	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	239	248	133	133	137	137	206	206	202	202	229	229	0	0	163	163	0	0	278	283	417	417
	239	248	133	160	137	137	206	206	0	0	0	0	123	123	163	163	0	0	278	283	417	417
	0	0	133	160	137	137	206	206	202	202	229	229	123	123	163	163	0	0	278	283	417	417
	248	248	133	160	137	137	0	0	202	202	229	229	123	123	163	163	203	203	0	0	0	0
C13	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	0	0
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	0	0	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417
	248	248	151	160	137	137	212	212	202	202	229	229	126	126	163	163	203	279	278	283	417	417

Table S7.1: Continued from previous page.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7		Bp8		Bp9		Bp10	
C14	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	0	0	163	163	203	203	283	283	417	426
	0	0	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	160	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
C15	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	0	0	0	0	0	0	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	163	203	203	283	283	417	417
C16	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	0	0	0	0	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	248	248	151	151	137	137	206	212	202	202	229	229	123	123	163	163	203	203	283	283	417	426
	0	0	151	151	137	137	206	212	202	202	229	229	0	0	163	163	203	203	283	283	417	426

Table S7.1: Continued from previous page.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7		Bp8		Bp9		Bp10	
C17	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	0	0	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
	248	248	133	133	137	137	206	206	202	202	229	229	123	123	163	163	203	203	278	283	417	417
C18	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	0	0	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	0	0	0	0	203	203	283	283	417	417
	248	248	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	0	0	160	160	137	137	206	206	202	202	229	229	123	123	163	165	203	203	283	283	417	417
C19	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	0	0	0	0	203	203	278	283	417	426
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	0	0
	248	248	151	160	137	137	212	212	202	202	229	229	123	123	163	163	203	203	278	283	417	426

Table S7.1: Continued from previous page.

Clone	Bp1b		Bp2		Bp3		Bp3c		Bp4a		Bp5d		Bp6b		Bp7		Bp8		Bp9		Bp10	
C20	239	248	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	239	248	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	0	0	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	239	248	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	239	248	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	0	0	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	239	248	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	239	248	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
	0	0	133	151	137	137	206	212	202	202	229	229	123	123	163	163	273	273	278	278	426	426
C21	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	0	0	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	202	202	229	229	123	123	163	165	203	203	283	283	417	417
	248	248	133	151	137	137	206	212	0	0	229	229	123	123	163	165	203	203	283	283	417	417

Table S7.2: Multilocus genotypes of clones of *Brachionus plicatilis* for 11 microsatellite loci. Alleles correspond to the fragment size in base pairs. Different alleles within one locus are highlighted in different colors. Missing values are represented by a 0. For each clone, 2 to 6 individuals were genotyped, here are presented the consensus multilocus genotypes.

Clone	Bp1b	Bp2	Bp3	Bp3c	Bp4a	Bp5d	Bp6b	Bp7	Bp8	Bp9	Bp10
C22	248	248 160	160 137	137 212	212 202	202 229	229 123	123 163	163 203	203 283	283 417
C23	239	248 151	151 137	137 212	212 202	202 229	229 123	123 163	165 203	273 283	283 417
C24	248	248 151	151 137	137 206	212 202	202 229	229 123	123 163	165 203	273 283	283 417
E1	248	248 133	160 137	137 218	218 202	202 229	229 123	123 163	165 203	203 278	283 417
E2	248	248 133	133 137	137 212	212 202	202 229	229 123	123 163	163 203	203 278	283 417
E3	248	248 133	133 137	137 206	212 202	202 229	229 121	123 163	163 203	203 283	283 417
E4	248	248 133	151 137	137 212	212 202	202 229	229 123	123 163	163 203	273 283	283 417
E5	248	248 151	160 137	137 212	212 202	202 229	229 123	123 163	163 203	273 283	283 417
E6	248	248 151	160 137	137 206	212 202	202 229	229 123	123 163	163 203	203 283	283 417
E7	248	248 151	160 137	137 0	0 202	202 229	229 123	123 163	163 203	203 283	283 417
E8	248	248 151	151 137	137 206	212 202	202 229	229 123	123 163	163 203	203 278	283 417
E9	248	248 151	160 137	137 212	212 202	202 229	229 123	123 163	163 203	203 278	278 417
E10	248	248 160	160 137	137 212	212 202	202 229	229 121	123 163	165 203	203 278	283 417
E11	248	248 160	160 137	137 212	212 202	202 229	229 123	123 163	163 203	279 283	283 417
E12	239	248 133	151 137	137 206	206 202	202 229	229 121	123 163	163 203	273 283	283 417
E13	248	248 160	160 137	137 212	212 202	202 229	229 123	123 165	165 203	203 278	283 417
E14	239	248 151	160 137	137 212	212 202	202 229	229 121	123 163	165 203	273 278	278 417
E15	248	248 151	151 137	137 212	212 202	202 229	229 123	123 163	163 203	203 283	283 417
E16	248	248 151	151 137	137 212	212 202	202 229	229 123	123 163	165 203	279 283	283 417
E17	248	248 160	160 137	137 206	212 202	202 229	229 123	123 163	165 203	279 283	283 417
E18	248	248 151	160 137	137 206	212 202	202 229	229 123	123 163	163 203	203 283	283 417
E19	248	248 133	151 137	137 206	212 202	202 229	229 123	123 163	163 203	203 278	283 417
E20	248	248 151	151 137	137 212	212 202	202 229	229 123	123 163	165 203	279 283	283 417
E21	248	248 0	0 137	137 206	206 202	202 229	229 123	123 165	165 203	203 278	278 417
E22	239	248 133	160 137	137 212	212 202	202 229	229 123	123 163	163 203	203 283	283 417
E23	248	248 151	160 137	137 206	206 202	202 229	229 121	123 163	163 203	203 283	283 417
E24	248	248 151	160 137	137 206	212 202	202 229	229 123	123 163	163 203	273 283	283 417
E25	248	248 160	160 137	137 212	212 202	202 229	229 123	123 163	165 203	203 283	283 417
E26	248	248 151	151 137	137 212	212 202	202 229	229 123	123 163	163 203	203 278	283 417
E27	248	248 151	151 137	137 206	206 202	202 229	229 123	123 163	163 203	203 278	278 417
E28	248	248 160	160 137	137 212	212 202	202 229	229 123	123 165	165 203	203 283	283 417
E29	239	248 133	160 137	137 212	212 202	202 229	229 123	123 163	165 203	203 278	283 417
E30	248	248 151	151 137	137 206	212 202	202 229	229 121	123 163	163 203	203 283	283 417
E31	248	248 151	151 137	137 212	212 202	202 229	229 121	123 163	163 203	279 283	283 417
E32	248	248 151	160 137	137 212	212 202	202 229	229 121	123 163	165 203	279 283	283 417

Appendix S8

Proportion of new genotypes in
experimental populations G and
D over time

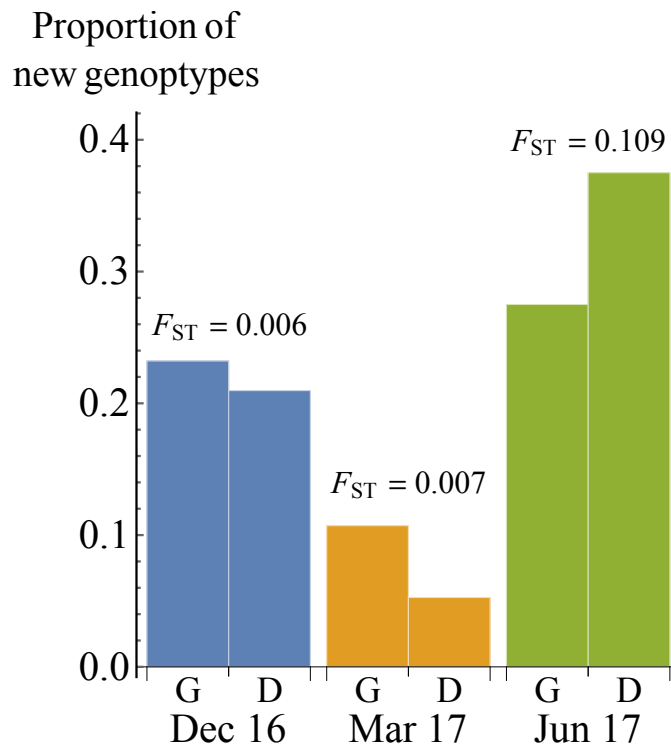
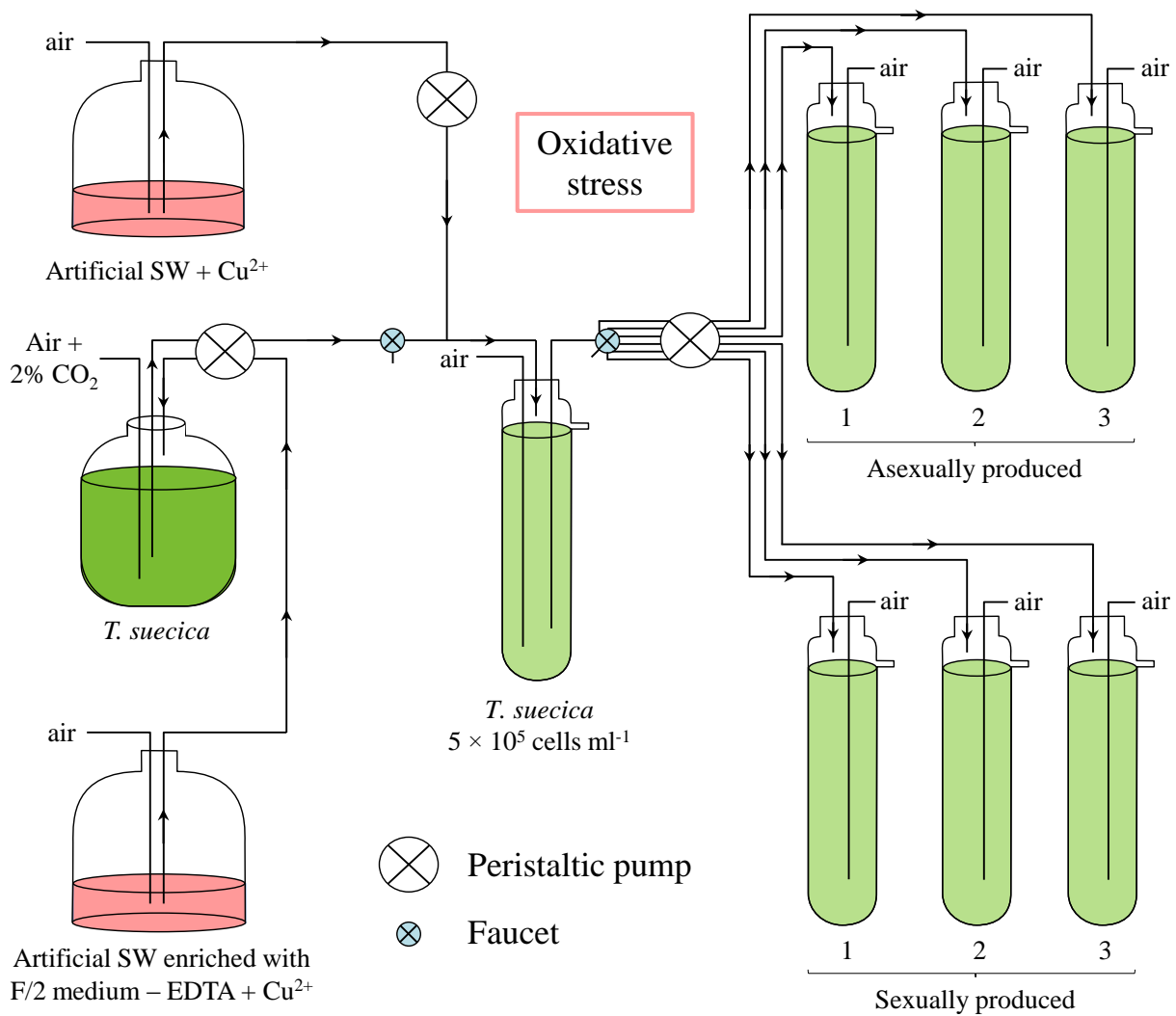
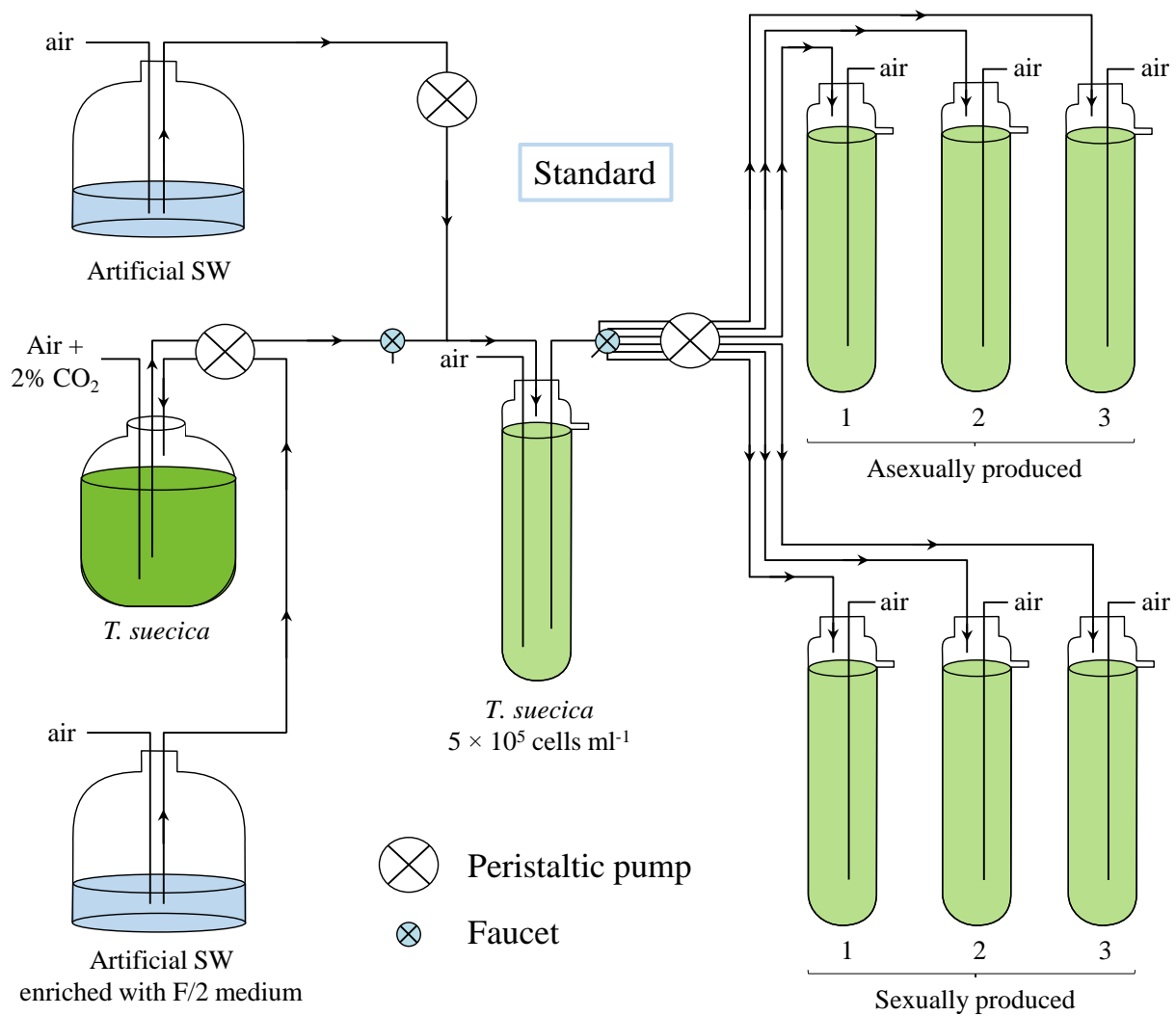


Figure S8.1: Proportion of new genotypes compared to our 20 initial clones over time. The individuals genotyped are those from which we measured the propensity for sex in Figure 4.2. The genetic differentiation between G and D populations (estimated with F_{ST} values) is indicated above each time sample. The method used for comparing genotypes takes into account possible amplification errors, that is to say individuals can be detected as homozygous for one locus while being in fact heterozygous.

Appendix S9

Diagram of the complete
experimental setup to investigate
the effect of the reproductive
mode on the adaptation of
populations to stressful conditions
(adapted from the internship
report of Elisa Leroux)



Appendix S10

Effect of treatment and
reproductive origin on the
variance in fitness of experimental
populations

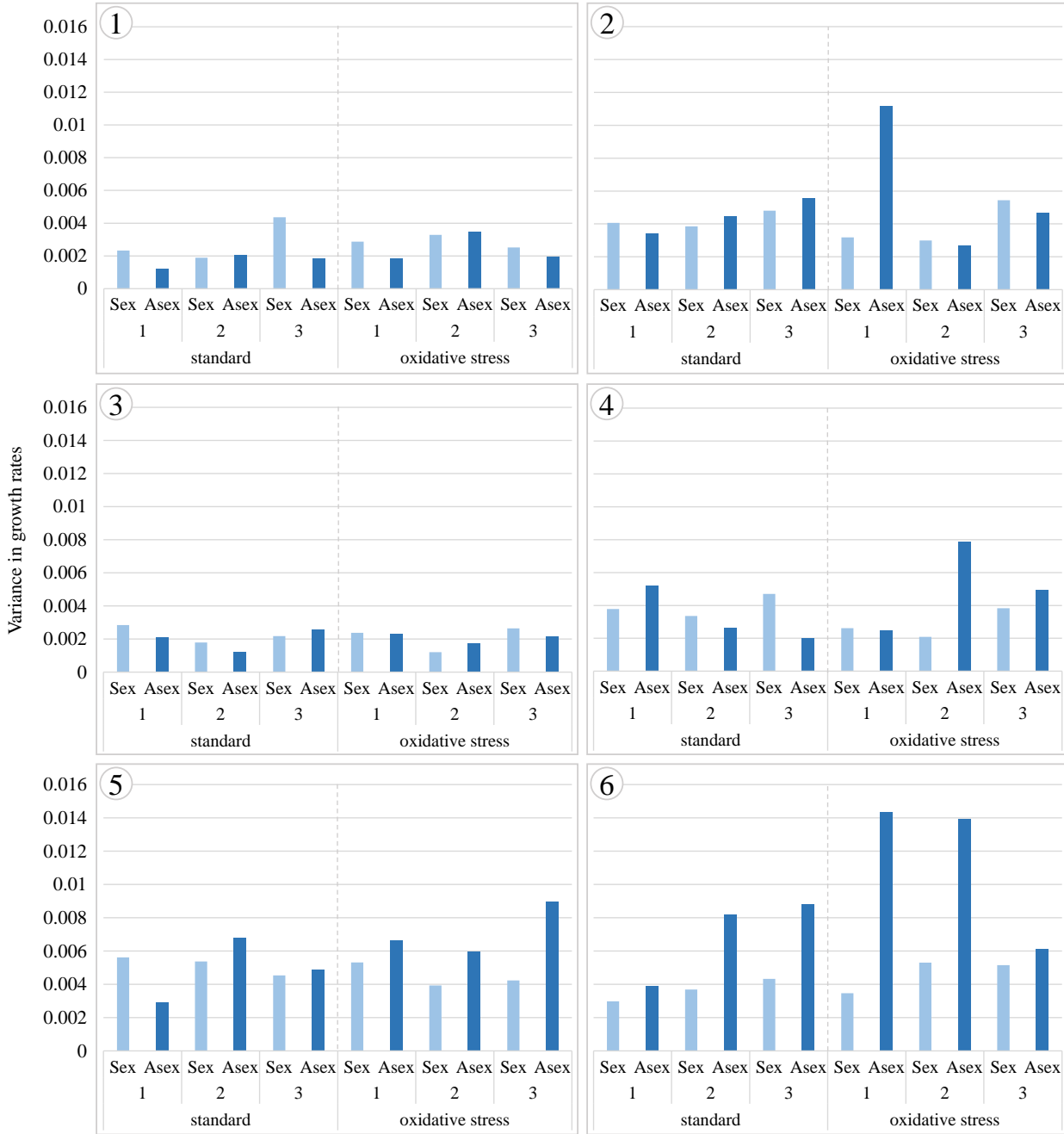


Figure S10.1: Variance in fitness during the adaptive phase of the experimental populations. Each frame corresponds to a different measure of fitness: **1.** from 30/04/18 to 07/05/18, **2.** from 10/05/18 to 17/05/18, **3.** from 21/05/18 to 28/05/18, **4.** from 31/05/18 to 07/06/18, **5.** from 11/06/18 to 18/06/18 and **6.** from 21/06/18 to 28/06/18.

Appendix S11

Density of rotifers over time in
the experimental populations.

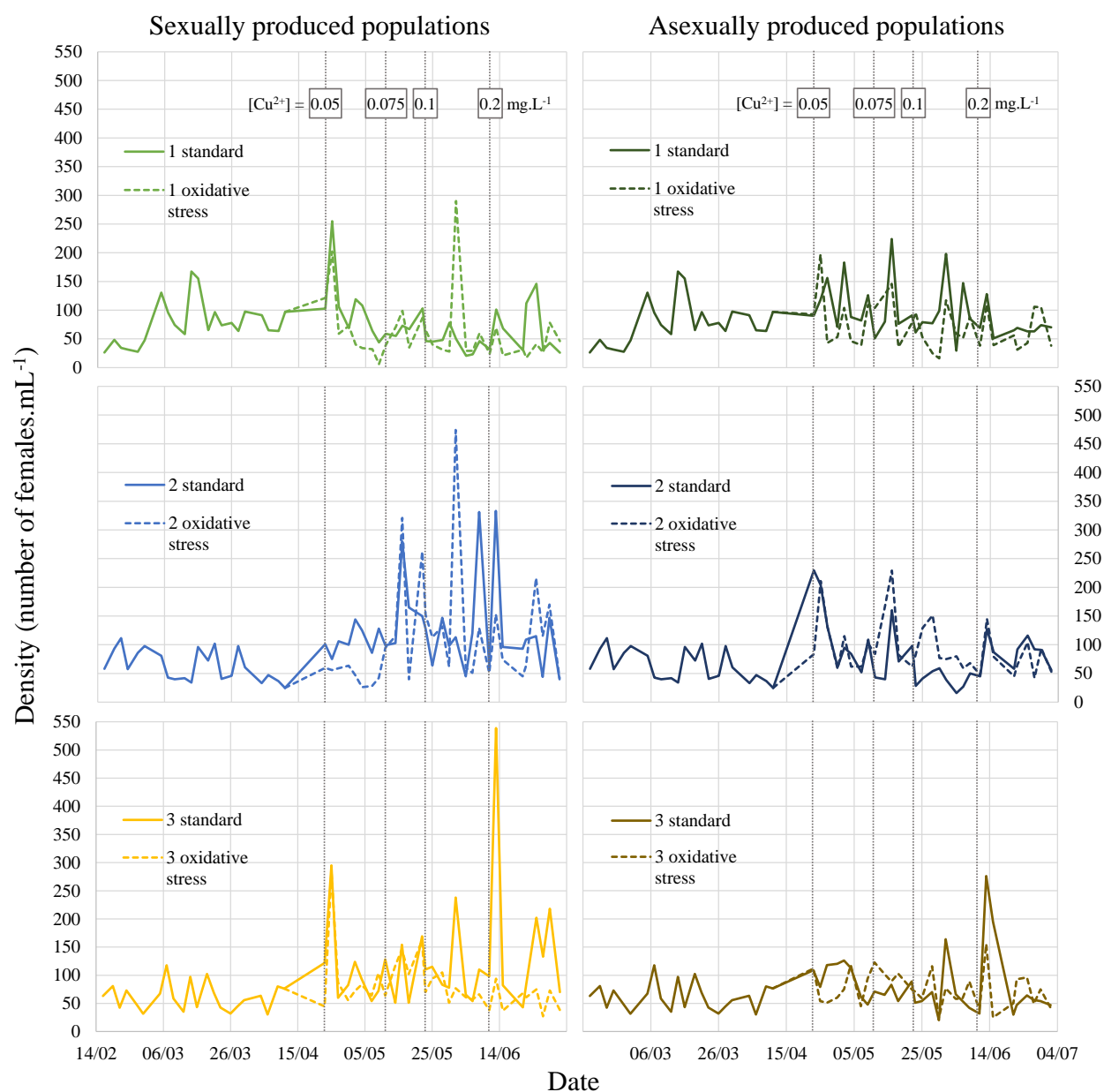


Figure S11.1: Density (number of females mL^{-1}) of populations in standard (solid curves) and stressful (dashed curves) environments over time . The dates when the concentration of copper was increased are indicated.

L'évolution du sexe : Etude théorique s'appuyant sur des modèles de génétique quantitative et approche expérimentale utilisant le rotifère à reproduction sexuée facultative *Brachionus plicatilis*.

L'évolution et le maintien de la reproduction sexuée reste aujourd'hui une des principales questions en biologie évolutive. Ces dernières années, des approches théoriques ont permis la formulation de plusieurs scénarios vraisemblables dans lesquels la recombinaison est favorisée. Cependant, ces modèles supposent souvent une architecture génétique simple de la fitness (*e.g.* épistasie fixée entre loci, pas d'effets compensatoires entre mutations). De plus, il est souvent difficile de relier les prédictions de ces modèles à des quantités mesurables et elles restent peu vérifiées expérimentalement. L'objectif de cette thèse était d'étudier les bénéfices évolutifs possibles de la reproduction sexuée par une approche à la fois théorique et expérimentale. La première approche visait au développement de modèles de génétique quantitative pour l'évolution du sexe. Un premier modèle (s'appuyant sur le modèle géométrique de Fisher) considère un nombre arbitraire de traits phénotypiques sous sélection stabilisante autour d'un optimum fixe, et explore l'effet du biais mutationnel agissant sur les traits. Un deuxième modèle représente un trait phénotypique à la fois sous sélection stabilisante et directionnelle (représentée par un déplacement de l'optimum phénotypique de façon linéaire, cyclique ou stochastique). Des simulations individu-centrées ont montré qu'en l'absence de biais ou de changement environnemental, la population évolue vers l'asexualité. Cependant, avec un biais mutationnel, des taux de sexe intermédiaires peuvent évoluer même lorsque le coût du sexe est élevé. Des taux de sexe plus importants peuvent évoluer lorsque l'optimum phénotypique change dans le temps. La deuxième partie de la thèse consistait à développer un modèle expérimental pour tester les prédictions théoriques sur les éventuels avantages du sexe, en utilisant le rotifère à reproduction sexuée facultative *Brachionus plicatilis*. Les résultats démontrent l'existence de variabilité génétique dans l'investissement pour le sexe, entre des souches d'une même population naturelle, et celle d'effets maternels transgénérationnels qui affectent le taux de sexe. Pour finir, les résultats d'une expérience d'évolution suggèrent que le sexe facilite l'adaptation à un environnement stressant.

The evolution of sex: theoretical approach based on quantitative genetics models and experimental approach using the facultatively sexual rotifer *Brachionus plicatilis*.

The evolution and maintenance of sexual reproduction remain to date some of the major questions in evolutionary biology. Over recent years, theoretical models have generated several plausible scenarios under which sexual recombination is favored. However, these models often make simplistic assumptions about the genetic architecture of fitness (*e.g.*, fixed value of epistasis across loci, no possible compensatory effect among mutations). Furthermore, it is often difficult to relate their predictions to measurable quantities and they have received little experimental support. This thesis aimed at investigating some of the possible evolutionary benefits of sexual reproduction using both a theoretical and an experimental approach. The first approach consisted in developing evolutionary quantitative genetic models for the evolution of sex. A first model (based on Fisher's geometrical model) considers an arbitrary number of phenotypic traits under stabilizing selection around a fixed optimum, and explores the effect of mutational bias acting on the traits. A second model represents a phenotypic trait under both stabilizing and directional selection (modeled by a linear, cyclical or stochastic change in the phenotypic optimum). Individual-based simulations showed that in the absence of mutational bias or environmental change, the population evolves towards asexuality. However, with mutational bias, intermediate rates of sex are maintained in the population even when the cost of sex is strong. Higher rates of sex evolve when the phenotypic optimum changes over time. The second part of the thesis consisted in developing an experimental model to test theoretical predictions on the possible benefits of sex, using the facultatively sexual rotifer *Brachionus plicatilis*. The results demonstrate genetic variation for investment in sex between strains from the same natural population, and the existence of transgenerational maternal effects affecting the rate of sex. Finally, the results from an evolution experiment suggest that sex facilitates adaptation to a stressful environment.
