Mutation, asexual reproduction and genetic load: A study in three parts

by
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Introduction

I am interested in the genetic variation that exists in plant populations and in exploring the different forces that can influence genetic variation, specifically mutation rate, mating systems and asexual reproduction.

Mutation is the ultimate source of genetic variation. The rate at which mutations occur influences the mutation load (the loss of population fitness due to deleterious mutation; Whittle and Johnston 2003), levels of genetic diversity (Drake et al. 1998), and the rate of evolution (Orr 2000; Johnson and Barton 2002). Therefore, estimates of mutation rates are important for many evolutionary theories. Most mutations that influence fitness are thought to be slightly deleterious (Lynch et al., 1999), that is they have a slight negative effect on fitness. However much remains unknown about the distribution and effect of mutations. For example, are most mutations neutral with little to no effect on fitness? How often do beneficial mutations occur and do they have a large effect? Also unknown is the rate at which neutral, beneficial and deleterious mutations are generated.

Mating system, which has been classically defined as the proportion of outcrossing and/or selfing, can also influence levels of genetic variation. However, only considering the degree of outcrossing and/or selfing ignores another type of reproduction that occurs frequently within the flowering plant kingdom, asexual reproduction. There are no exact estimates for the proportion of flowering plants that

asexually reproduce, however general estimates for the percentage of plants that undergo vegetative reproduction are anywhere upwards from 70% (Klimés *et al.*, 1997). How does incorporating asexual reproduction into mixed mating models (models that incorporate outcrossing and selfing) effect genetic variation?

Deleterious mutations are considered to be a main contributor to inbreeding depression, the reduction of fitness due to inbreeding (Willis 1999). If inbreeding depression is due to mutations of deleterious effect, then inbreeding depression itself can be purged from the population (Lande and Schemske 1985; Charlesworth et al. 1990; Schultz and Willis 1995). The purging of inbreeding depression can influence the mating system of the population, resulting in either a completely selfing or completely outcrossing population (Lloyd 1979). However the extent of purging depends upon the nature of the deleterious mutations themselves. If the mutations are strongly deleterious, then natural selection can quickly remove them from the population, but if the mutations are weakly deleterious, substantial inbreeding depression can still be maintained within the population (Lande and Schemske 1985; Charlesworth et al. 1990; Schultz and Willis 1995).

My dissertation addresses mutation and complex mating systems (models of mating systems that include outcrossing, selfing, and asexual reproduction) in three distinct chapters. Chapter 2 discusses the direct estimation of mutation rates for di-nucleotide microsatellite markers in the model genetic organism, *Arabidopsis thaliana*. Chapter

3 describes an empirical study that estimates quantitative genetic variance components, genetic diversity, and inbreeding depression/inbreeding load for a predominately asexual population of *Mimulus guttatus*. This study also fits different evolutionary models to the empirical data to determine which model best describes the population. Chapter 4 is a theoretical investigation of the effects asexual reproduction, outcrossing and selfing on the average number of deleterious mutations per gamete and inbreeding load for infinite and finite populations. The study utilizes both Infinite (an infinite number of genetic loci and an infinite population size) and Finite (a small number of genetic loci and a variety of small population sizes) computer simulations. These simulations incorporate different meiotic and mitotic mutation rates, varying degrees of dominance and differing strengths of selection.

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Chapter 2. Direct estimation of the mutation rate at dinucleotide microsatellite loci in *Arabidopsis thaliana* (Brassicaceae)

Abstract

The mutation rate at fifty-four perfect (uninterrupted) dinucleotide microsatellite loci is estimated by direct genotyping of 96 *Arabidopsis thaliana* mutation accumulation lines. The estimated rate differs significantly among motif types with the highest rate for AT repeats (2.03×10^{-3} per allele per generation), intermediate for CT (3.31×10^{-4}), and lowest for CA (4.96×10^{-5}). The average mutation rate per generation for this sample of loci is 8.87×10^{-4} (SE = 2.57×10^{-4}). There is a strong effect of initial repeat number, particularly for AT repeats, with mutation rate increasing with the length of the microsatellite locus in the progenitor line. Controlling for motif and initial repeat number, chromosome 4 exhibited an elevated mutation rate relative to other chromosomes. The great majority of mutations were gains or losses of a single repeat. Generally, the data are consistent with the stepwise mutation model of microsatellite evolution. Several lines exhibited multiple step changes from the progenitor sequence, but it is unclear whether these are multi-step mutations or multiple single step mutations. A survey of dinucleotide repeats across the entire *Arabidopsis* genome indicates that AT repeats are most abundant, followed by CT, and CA.

Introduction

Microsatellites are simple sequence repeats that frequently display length variation within natural populations. These loci can be classified according to the length and type of repeated motif, where the most common lengths are 2, 3, or 4 bases (di-, tri- and tetra- nucleotide repeats, respectively). Microsatellites are highly polymorphic and are frequently used as genetic markers in ecological and evolutionary studies (Schlötterer and Pemberton 1994). The multi-allelic character of microsatellites makes them ideal for paternity analysis (Chase *et al.* 1996; Dow and Ashley 1998), estimation of parameters in pollination biology (e.g. Kelly and Willis 2002) and studies of dispersal/spatial-genetic structure (e.g. Sweigart *et al.* 1999). If one further assumes that microsatellite variation is selectively neutral, they can be used to estimate the effective population size (e.g. Schug *et al.* 1998).

Polymerase slippage during DNA replication is thought to be the primary source of mutation in microsatellites (Schlötterer *et al.* 1998). However, much remains unknown about the nature of the mutational process. Most studies suggest that mutations are typically gain or loss of a single repeated unit (Thuillet *et al.* 2002; Vigouroux *et al*. 2002), although there are putative examples of multi-repeat gains or losses (Ellegren 2004). The rate of mutation may depend on allele length, i.e. the number of repeat units (Wierdl *et al*. 1997; Vigouroux *et al*. 2002; Thuillet *et al.*

2004), as can the direction of changes, i.e. the relative likelihood of gain versus loss (see Wierdl *et al.* 1997). Finally, the mutation rate and other mutational properties may depend on the repeat motif, i.e. AG vs CG (Bachtrog *et al.* 2000; Kelkar *et al.* 2008). Most data suggest that dinucleotide microsatellites mutate at a rate that is greater than that of trinucleotide and tetranucleotide microsatellites (Chakraborty *et al.* 1997 but see Weber and Wong 1993).

Table 2-1. Mutation rates of wheat, corn and chickpea as estimated from mutation accumulation experiments. All mutation rates are haploid (per allele) with a 95% C.I. on the estimate given in parentheses.

Species	Number of loci	Type of repeat	Number of observed mutations	Average mutation rate	Reference
Wheat	10	di-nt	12	2.4×10^{-4} (1.4×10^{-4}, 4.2×10^{-4})	Thuillet et al. 2002
Corn	88	di-nt	73	7.7×10^{-4} (5.2×10^{-4}, 1.1×10^{-3})	Vigouroux et al. 2002
Corn	42	>2 (compound)	0	0.0 (0.0, 5.1×10^{-5})	Vigouroux et al. 2002
Chickpea Ghab2 var.	15	tri-nt	167	5.0×10^{-3} (4.5×10^{-3}, 6.0×10^{-3})	Udupa and Baum 2001
Chickpea Syrian local var.	15	tri-nt	60	1.95×10^{-3} (1.45×10^{-3}, 2.5×10^{-3})	Udupa and Baum 2001

Microsatellites are distributed non-randomly across plant genomes and are associated with non-repetitive DNA (Zhang *et al.* 2006). In *A. thaliana*, they are often found in regulatory regions, especially 5'UTRs and 5'flanking regions (Zhang *et al.* 2006;

Grover and Sharma 2007). A-rich repeats are prominent in introns and intergenic regions. AG is the most common di-nt motif in exons and 5'flanking regions, while AT is most common in introns, intergenic regions, 3' flanking regions (Zhang *et al.* 2004). Mutation rates have been estimated for a variety of crop plants (Table 2-1). Rate estimates range from 0 to 5 x 10^{-3} per locus per generation. Across these studies, mutations were more frequently observed in loci with long alleles (more repeat units) and most were single repeat changes with gains more frequent than losses. Across all three studies in Table 2-1, smaller loci (fewer repeats) tended to expand while longer loci (more repeats) tended to lose repeats.

Microsatellite mutation rates are directly relevant to hypotheses about genetic diversity in natural populations. Symonds and Lloyd (2003) found that genetic diversity for 20 microsatellite loci across 126 accessions was positively correlated with the number of contiguous repeats in *A. thaliana*. This association is predicted by models where mutation rate increases with repeat number. Direct estimates of mutation rate are also essential for evaluating theories of microsatellite evolution. The simplest model is the Infinite Alleles Model (IAM; Kimura and Crow 1964; Balloux and Lugon-Moulin 2002) where mutations occur at a constant rate and each mutation creates a novel allele. Seemingly, more appropriate for microsatellites is the stepwise mutation model (SMM; Ohta and Kimura 1973) where mutations occur at a constant rate and involve the gain or loss of a single unit. The two-phase model of DiRienzo *et al.* (1994) is a modification of the SMM with most mutations involving a

gain or loss of a single repeat and the remainder of the mutations being multi-step mutations following a geometric distribution. In a survey of variation at five microsatellite loci across 37 populations of *A. thaliana*, Bakker *et al.* (2006) found support for both the SMM (two of the five loci) and the IAM (four of the five loci).

In this paper, we estimate the rate of mutation per allele per generation of dinucleotide repeats in *A. thaliana*. A large panel of Mutation Accumulation (MA) lines is scored for allele length at fifty-four perfect dinucleotide repeat loci. Perfect repeats are uninterrupted strings of a single motif, e.g. AT. The loci examined in this study are not associated with genes or within intergenic regions of gene clusters. As a consequence, natural selection on allele length within these loci is likely to be much weaker than for gene associated microsatellites. All putative mutations were confirmed by multiple independent PCR amplifications. These results corroborate the effect of allele length on mutation rate. They also indicate an important effect of motif type and possibly also chromosomal location. We also conduct a genomic survey of *A. thaliana* and interpret our mutation estimates in relation to the full distribution of repeat lengths and motif frequency in the *Arabidopsis* genome.

Methods

Plant growth and DNA extraction—Shaw *et al.* (2002) maintained 118 independent Mutation Accumulation Lines of *Arabidopsis thaliana* for 30 generations prior to the current study. All lines were initiated from the Columbia accession and each was

propagated by single seed descent. We chose a random subset of this population (96 lines) and grew plants to maturity in the University of Kansas greenhouse in February 2008. The soil was equal parts vermiculite and perlite with potting soil sprinkled on top of seeds. Day length was artificially expanded to 18 hours and plants were fertilized every week with Peat-lite (20-10-20 NPK). Tissue was collected for DNA extraction from the basal rosette when each plant was approximately five weeks old.

Tissue was collected into a 96-well plate with a metal bead in each well. 500 µL of CTAB buffer and 1 µL of β-mercaptoethanol was added to each sample. The plate was then sealed and shaken at high speed for 45s in a bead beater. The plate was then incubated for ~20 min. in 60°C water bath and then centrifuged for ~10 sec (3980 rpm) to separate solids. We transferred 300 µl liquid from each tube to a new 96-well Costar plate and added 300 µl of chloroform to each sample. This was followed by another round of mixing using the "slanted- vortex technique" and centrifuge for 10 min @ 3980 rpm. Each sample was then fully separated into aqueous (upper) and chloroform (lower) layers. We removed the aqueous layer to a new 96-well plate, added 200 µl isopropanol, and mixed well by inverting the plate repeatedly. The new plate was stored at -20°C overnight and then centrifuged for 10 minutes @ 3980 rpm. This produced a gelatinous pellet in each well. We then poured off the supernatant, added 200 µl 70% ethanol, capped the tubes, and repeated the shake and centrifuge steps. We then poured off the ethanol and air-dried the pellet. Each DNA pellet was resuspended in 50 µL of distilled water. All samples were quantified using a

NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and diluted with distilled H_20 to 7-9 ng/μL.

Locus selection for genotyping—Microsatellite loci were identified by searching the *Arabidopsis* genome sequence via The Arabidopsis Information Resource (TAIR) website (www.arabidopsis.org). Microsatellites were found by searching for each motif in a string of 8 repeats, e.g. ATATATATATATATAT or $(AT)_8$. For coverage of the genome, we divided each of 5 chromosomes into four regions and selected one locus per region per motif type. Not all regions contained a microsatellite satisfying our selection criteria. We eliminated microsatellites that were within 200 bp of start/end of gene, in either a UTR or an intron, had more than 30 repeats, or if the repeat sequence of the microsatellite was interrupted. We found no CG repeats that met these conditions and so our sample consisted entirely of AT, CA, and CT repeats. A number of loci failed to amplify, and as a consequence, we ended up with fewer CA loci (14) than AT or CT loci (20 of each). Primers, described in the Appendix A, were designed for the selected loci using the program Primer3 with the default settings (Rozen and Skaletsky 2000).

For each locus, we genotyped 96 individuals using a 3-primer method for polymerase chain reaction (PCR; Boutin-Ganache *et al.* 2001). We used one untagged primer for each pair, a second primer with a 5' tag (CAG sequence: 5'-CAGTCGGGCGTCATCA-3'), and a third CAG-sequence primer with a 5'-6FAM

(Applied Biosystems, Foster City, CA, USA) fluorescent label. The CAG sequence was added to the primer in each pair such that the melting temperature of the tagged primer was approximately 65 ºC. PCRs (15 µl total volume) contained 40ng of template DNA, 0.25 µM untagged primer, 0.025 µM CAG-tagged primer, 0.25 µM 6FAM-labeled CAG primer, 200 µM each dNTP, 0.5 units *Taq* DNA polymerase (Promega, Madison, WI, USA) and 1x PCR buffer (500 mM KCl, 15 mM $MgCl_2$, 100mM Tris-HCl; Promega). For temperature cycling, we implemented a touchdown PCR protocol using an iCycler Thermal Cycler (BioRad, Hercules, CA, USA): 94 ºC for 1 min, 21 cycles of denaturing at 94 ºC for 30 s, annealing for 20 s, and extension at 72 ºC for 20 s; initial annealing temperature (T_a) = 60 ºC and decreased by 0.5 ºC with each cycle until T_a reached 50 ºC, followed by 9 cycles using this T_a, and a final extension at 67 ºC for 45 min. We detected PCR-amplified fragments on an ABI 3130 Genetic Analyzer (Applied Biosystems), and sized fragments using GENEMAPPER 4.0 software (Applied Biosystems) calibrated with the ROX500 size standard (Applied Biosystems). Logistic regression and other statistical analyses of the mutation accumulation data were performed in R (www.r-project.org/).

Genome scan for dinucleotide microsatellite loci—We downloaded entire chromosome sequences as FASTA files from www.arabidopsis.org and used the program Tandem Repeats Finder v. 4.0 for Windows (TRF; Benson 1999) to identify microsatellites. We used the following parameter values within TRF for genome analysis: alignment weights +2, -7, -7 (representing match, mismatch and indel

penalties); matching probability of 0.80 and an indel probability of 0.10 (pM = 0.80 and pI = 0.10, respectively); a minimum alignment score of 20 and a maximum period size of 10. We extracted the dinucleotide repeats of all motif types from the full TRF output by visual inspection. We statistically analyzed the resulting data in Minitab (v. 14.0) for mean repeat length for each repeat motif category.

Results

For all loci, the majority of lines produced fragments that matched the length of the progenitor sequence: the Col-1 genomic sequence length plus the increment due to the primers. Putative mutations were identified as deviations from this progenitor sequence length. Each putative mutant was subsequently re-amplified and re-genotyped two to six times to distinguish real mutations (acquired during mutation accumulation) from those due to PCR error. Approximately 15% (19/124) of all putative mutations identified in the initial screen were determined to be PCR errors.

Across lines and loci, there were 5165 genotypes. Of these, 137 (2.7%) were confirmed mutations (Table 2-2). If we bin all mutant types in Table 2-2, the (haploid) mutation rate, μ, can be estimated as the number of mutations divided by the product of the number of lines (L) and the number of generations of mutation accumulation (G). Each line is expected to produce 2μ mutations per locus per generation but only half of these mutations will fix in subsequent generations of propagation. By this procedure, the estimated μ is 2.03×10^{-3} for the 20 AT repeats,

Table 2-2. The genotypes for all 96 are summarized for each locus Non-mutant genotypes match the progenitor line. Unscored genotypes could not be determined and/or replicated. Het-loss and het-gain denotes lines that were heterozygous for a single repeat mutation and the progenitor allele. The other six categories are homozygous lines that differ from the progenitor by 1, 2, or 3 repeats.

Locus	Non-Mutant	Unscored	Het-Loss	Het-gain	1 Loss	1 Gain	2 Loss	2 Gain	3 Loss	3 Gain
AT.CIW7	85	1			5	5				
AT0101	96									
AT0102	91	1				4				
AT0103	88	1			3	3	1			
AT0104	96									
AT0201	93				1	2				
AT0202	89	3			1	3				
AT0203	95	1								
AT0204	93				3					
AT0301	82		1	1	6	6				
AT0302	93	1			1	1				
AT0303	96									
AT0304	96									
AT0402	66		1		12	11	5		1	
AT0403	76	2			9	7	1			1
AT0404	96									
AT0501	89	1			3	3				
AT0502	93				2	1				
AT0503	96									
AT0504	83	1			5	7				
CA0101	96									
CA0102	95	1								
CA0103	96									
CA0104	95					1				
CA0201	95	1								
CA0202	96									
CA0301	96									
CA0302	96									
CA0401	96									
CA0501	96									
CA0502	95				1					
CA0503	95	1								
CA0504	96									
CA72	95	1								
CT.nga1145	96									
CT.nga172	96									
CT.nga225	95			1						
CT.nga32	95	1								
CT.nga59	96									
CT0101	96									
CT0102	96									
CT0103	96									
CT0104	94					2				
CT0201	96									
CT0301	96									
CT0302	96									
CT0303	95	1								
CT0304	96									
CT0401	89				3	4				
CT0402	90	1				5				
CT0403	96									
CT0501	95					1				
CT0502	96									
CT0503	93					3				

4.96×10^{-5} for the 14 CA repeats, and 3.31×10^{-4} for the 20 CT repeats. For the entire sample, the estimated $\mu = 8.87 \times 10^{-4}$ with a standard error of 2.57×10^{-4}.

The preceding calculations are approximate because the number of mutant lines may not exactly match the number of mutant alleles. Counting het-gain and het-loss as full mutations produces a slight upward bias in mutation rate because we expect that half of these lines will revert to the progenitor sequence with random allele loss due to segregation. However, we are likely underestimating mutation rate by single counting the multi-gain and multi-loss lines. These lines might reflect real multi-step mutations but they might also have fixed multiple single repeat mutations. Also, a small fraction of lines are expected to match the progenitor because of canceling of gains and losses.

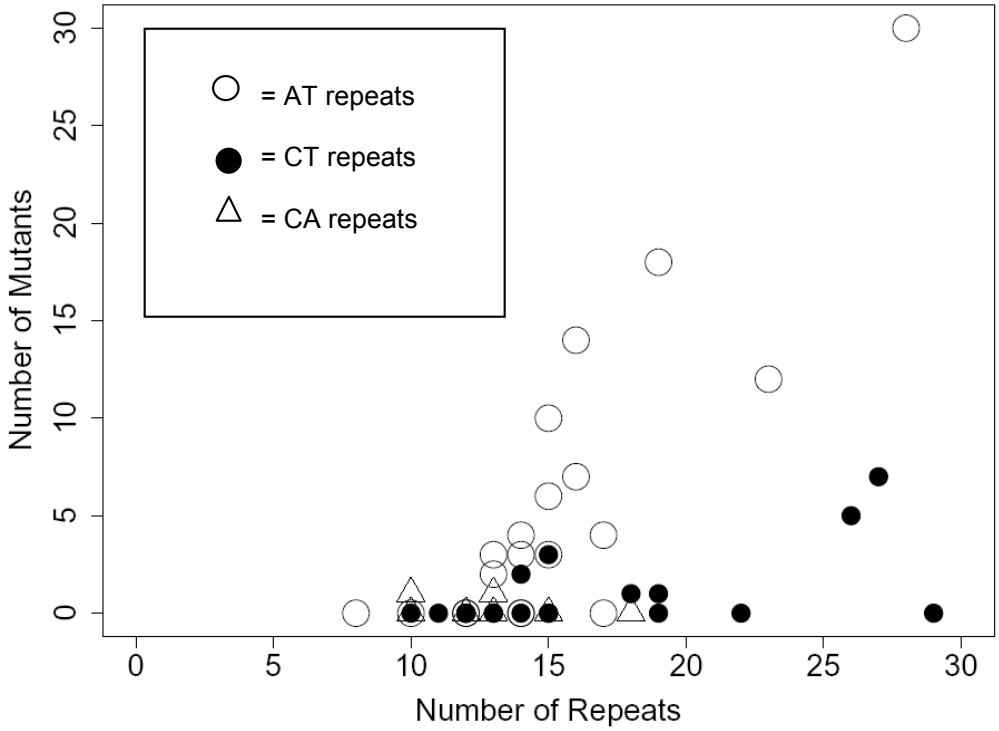

Figure 2-1. The number of lines scored as mutant is given as a function of initial allele length.

There was a great deal of variability among loci in mutation rate (Table 2-2). This is partly due to the difference among motif types. However, within both the AT and CT groups, the variance in mutation count substantially exceeds the mean. Much of this variation can be attributed to the strong effect of initial repeat number (Figure 2-1). For both AT and CT repeats, mutation rate increases substantially with the allele length for that locus in the progenitor line. This is confirmed statistically using a Poisson general linear model with mutant count per locus as the response variable, motif type as a categorical factor, and progenitor repeat number as the covariate. The estimated mutation rate equations for each motif type are:

Mutation rate for AT = -1.086 + 0.165 * (Repeat Number) (1a)

Mutation rate for CA = -4.002 + 0.165 * (Repeat Number) (1b)

Mutation rate for CT = -3.251 + 0.165 * (Repeat Number) (1c)

All coefficients, intercepts and slope, are significantly different from zero ($P < 0.001$). These equations share the same slope estimate because the test for an interaction between motif type and progenitor repeat number (slope heterogeneity) is non significant.

The direction of mutation (gain vs. loss) was related to initial repeat number. Overall, gains were more frequent than losses. For AT loci, there were an equal number of gains and losses (four of each), but gains occurred more frequently in shorter alleles (16.5 vs. 20 repeats on average, respectively). For the AC repeat loci, there was equal number of gains and losses (one of each). The number of repeats in the gain was 10 and the number of repeats in the loss was 13. For the AG repeats, all five mutations were gains. In our second longest locus (AT0402; 28 repeats), six of the mutation accumulation lines differed from the progenitor by two or more repeats and all were losses. This is consistent with the trend noted in other studies for longer loci to contract with mutation.

The loci were chosen to span all five chromosomes of *Arabidopsis*. To test for an effect of chromosome on mutation rate, we added it as a factor in the Poisson regression model. Controlling for the effect of initial repeat number and motif type, the chromosomes were indistinguishable except for chromosome 4, which exhibits an elevated mutation rate ($Z = 2.876$, $P < 0.005$). This is because the most mutable loci within motifs (AT402, AT403 and CT401, CT402) reside on chromosome 4. With chromosome included as a factor in the model, initial repeat number remains the dominant predictor of mutation rate, although the estimated slope is reduced by about 25%.

Table 2-3. The average and standard deviation of repeat length is given for both perfect and imperfect repeats of each motif type.

	Perfect repeats			**Imperfect repeats**		
	Average repeat length	SD of repeat length	Number of loci	Average repeat length	SD of repeat length	Number of loci
AT	7.97	4.332	9433	12.13	5.559	1737
AC	6.60	2.116	2518	9.61	2.494	194
AG	7.71	5.135	7258	12.22	8.066	1780

Results from genome survey—Microsatellites composed of AT repeats were the most frequent followed by AG and then AC microsatellites (Table 2-3). The scan also identified a small number of short GC repeats, but these were excluded from Table 2-3. A greater number of perfect microsatellites (uninterrupted repeat strings) were identified than imperfect microsatellites. The latter category included compound

microsatellites for all repeat motif types. Compound microsatellites comprise more than one repeat type. Some, but not all, compound microsatellites also have insertions between the multiple repeat types and this is likely to affect the mutational pattern.

Discussion

This survey estimates the rate of mutation at 54 dinucleotide microsatellite loci in *A. thaliana*. The average estimated rate across loci is $\mu = 8.87 \times 10^{-4}$ and the great majority of mutations were gains or losses of a single repeat. The mutation rate is heterogeneous across loci and increases with repeat number. Mutations in longer alleles are more frequently losses than gains (e.g. locus AT0402 in Table 2-2). These observations are fully consistent with previous mutational studies of plants (Table 2-1) and other organisms (e.g. Wierdl *et al.* 1997; Schlötterer *et al.* 1998; Dieringer and Schlötterer 2003; Harr and Schlötterer 2004; Seyfert *et al.* 2008).

For a given allele length, mutation rate differed among motif types. Kelkar *et al.* (2008) review a number of reasons why motifs might differ in mutability. The rate of loss and/or formation of hydrogen bonds can differ among motifs with AT potentially more mutable because fewer H bonds must be broken. The relative mutability of motifs could also depend on the stability of hairpin structures formed (ranked by hairpin stability: $AT_n > AG_n > AC_n$) or in other secondary structures. Finally, motifs may be recognized differently by DNA repair mechanisms (see Harr and Schlötterer

2000; Schlötterer *et al.* 2006). We found the AT motif to be most mutable and the CA motif to be least mutable (see difference in intercept estimates in equations 1), which is consistent with each of the first two suggestions (hydrogen bond and hairpin stability). There is also a slight tendency towards greater variability in allele length among *A. thaliana* lines for AT loci than for other motifs in the surveys of Innan *et al.* (1997) and Symonds and Lloyd (2003).

Our overall mutation rate estimate is probably less useful than the calibrated functions predicting rate given locus-specific features (equations 1a, b, and c). The strong dependence on motif and initial length implies that the average genomic mutation rate depends on the relative frequency of the various motif types and on the distribution of allele sizes currently segregating in the population. The AT motif, which had highest mutation rate, is the most frequent motif (Table 2-3; see also Morgante *et al.* 2002). CA is least mutable and least frequent. The overall average mutation rate also depends on the distribution of repeat numbers per motif in the genome. We selected loci with allele sizes in the 8-30 range (Figure 2-1; averages 15.35, 11.86, and 16.35 for AT, CA, and CT, respectively). These average repeat lengths for our sample are higher than the mean for each motif type in our genome survey (Table 2-3). Since mutation rate increases with repeat number, the average rate across our loci within motifs should be elevated relative to the genomic average. However, this bias is counteracted because the most mutable motif (AT) is more frequent in the genome than in our sample.

Equations (1) use a single slope to describe the linear relationships between mutation rate and repeat length across motifs. This is statistically defensible—the test for slope heterogeneity was not significant—but is unlikely to be literally correct. For example, we see essentially no relationship between allele length and mutation rate in CA repeats of our dataset (Figure 2-1), although our sample contains few CA loci with large numbers of repeats. Also, the fact that equations (1) have negative intercept estimates is consistent with the idea that there is a minimum size for microsatellite loci to accrue mutations at their typically high rate. According to our linear model, this minimum is identified by where our lines cross the x-axis. In fact, our estimates suggest that this minimum may differ among motif types. However, we caution that the true relationship between mutation rate and repeat length is likely to be non-linear.

Approximately 15% of all putative mutations identified in our initial screen proved to be PCR mutations and were discarded. This proportion is lower than in other studies that have verified putative mutations with multiple rounds of PCR. In their study of corn, Vigouroux *et al*. (2002) found 166 mutations in their initial screen, but only 72 were confirmed (approximately 43%). Symonds and Lloyd (2003) reported a PCR error rate of 95% for single base pair differences in *A. thaliana* microsatellites. While replicating PCR eliminates 'false positives', it is also possible for PCR to produce false negatives. This occurs if PCR reverts a real mutation back to the allele length of

the progenitor. While we did not directly correct for false negatives, this bias should be minimal.

Estimation of the effective population size—There is great interest in estimating N_e, the effective size of natural populations (Frankham 1995; Leberg 2005). The neutral theory of molecular evolution predicts that the amount of genetic diversity within a population should be a direct function of the product of N_e and the mutation rate, μ (Kimura, 1983). An independent estimate for μ allows these two variables to be disentangled and permits inference of N_e from genetic diversity.

Symonds and Lloyd (2003) surveyed 126 accessions of *A. thaliana* for variation at 20 dinucleotide microsatellite loci. The average gene diversity (G) in this survey was 0.76, similar to a previous estimate (0.79) obtained by Innan *et al.* (1997). Assuming neutrality, the expected G is $1-1/\sqrt{1+8N_e\mu}$ under the Stepwise Mutation Model (Ohta and Kimura 1973). Substituting the average G from Symonds and Lloyd (2003) and our average μ across loci, we find that $N_e \approx 2300$. With $G = 0.79$, $N_e \approx$ 3050. A distinct estimator for N_e is based on V, the variance of allele lengths in a population. The expected value for V is $4\ N_e\ \mu$, assuming stepwise mutation (Moran 1975). Pooling variance estimates from 20 loci (accounting for differences in sample sizes) in Innan et al. (1997) yields an average V of 25.5. Solving, $N_e = 25.5/(4 \times 8.87 \times 10^{-4}) \approx 7200$.

Although reasonable, these N_e estimates are encumbered with a number of notable caveats. First, each is subject to the bias inevitable when substituting point estimates into non-linear functions. Estimation error in either the variation statistics (G or V) or in the mutation rate biases estimation of N_e. Second, these calculations ignore real variation in mutation rate among loci. Finally, microsatellite allele length may not be selectively neutral. Very weak selection can substantially affect species level polymorphism (Akashi 1997). The first two issues could be addressed by applying a more elaborate statistical model to the data. A large population survey focused on the same loci for which we have direct mutation rate information could potentially provide a strong test of the neutrality assumption.

The source of mutations—Plants do not have a segregated germ line. As a consequence, both mitotic and meiotic mutations will accumulate in MA lines. A few studies have attempted to isolate the mitotic rate by comparing genotypes from ancestral and descendent cells within the same plant. Cloutier *et al.* (2003) observed no microsatellite mutations in a total of 12 loci of *Pinus strobus,* allowing the authors to place an upper bound of between 2.3×10^{-7} and 6.9×10^{-8} for the mutation rate per mitotic cell division. Leberg (2005) observed one microsatellite mutation across 8 loci of *Thuja plicata* and from this estimated 3.13×10^{-4} mitotic mutations per allele per generation.

While our study cannot distinguish between meiotic and mitotic mutations, we suggest that meiotic errors are likely to be more important. Whittle and Johnson (2003) found that a greater proportion of mutations in *A. thaliana* are transmitted to progeny via pollen than ovule, implying mutation during gametogenesis. Also, our mutation rate estimate and most of the others in Table 2-1 are much higher than the mitotic rate estimate obtained by Cloutier *et al.* (2003). However, in long-lived species or those with extensive clonal reproduction, mitotic mutations might contribute a larger fraction of the genetic variation. In the future, application of the molecular tools available for this model plant might provide a quantitative estimate for the contribution of meiotic and mitotic mutation.

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Chapter 3. Inbreeding depression in an asexual population of *Mimulus guttatus*

Abstract

The reproductive mechanism, that is whether an organism outcrosses, selfs or asexually reproduces, has a substantial impact on the amount and pattern of genetic variation. In this study, we estimate genetic variation and genetic load for a predominately asexual population of *Mimulus guttatus,* and then compare our results to other studies of predominately sexually reproducing (outcrossing and selfing) populations of *M. guttatus*. The asexual population had low levels of heterozygosity (H_e = 0.03) and very low levels of inbreeding load, especially when compared to other *M. guttatus* populations. All traits except pollen viability exhibited significant inbreeding depression. This varies greatly from the sexual populations of *Mimulus* where male fitness traits display substantial inbreeding depression. We discuss a variety of reasons why we see such low load in this study and suggest future research projects to further explore the questions.

Introduction

For many plant taxa, mating system can be described as the fractional investment in three different modes of reproduction: sexual reproduction via outcrossing, sexual reproduction via self-fertilization, and asexual reproduction. The evolutionary consequences of this partitioning are profound because the reproductive mechanism determines genetic transmission across generations, and the way in which allelic variation is "presented" to natural selection. Mating system influences how frequently an allele occurs in heterozygotes as opposed to homozygotes, and has a pronounced effect on the diversity of genetic backgrounds associated with an allele. These effects are critical to evolution because the *entire* multi-locus genotype determines the fitness of an organism.

There has been extensive theoretical study on the evolution of selfing rate in sexual populations (e.g. Lloyd 1980; Lande and Schemske 1985; Charlesworth *et al.* 1990), as well on the evolutionary consequences of variation in selfing rate (Darwin 1876; Kelly 1999; Charlesworth 2003; Wright *et al.* 2008). Surveys of experimental studies have determined the distribution of selfing rates across taxa (Schemske and Lande 1985; Barrett and Eckert 1990; Vogler and Kalisz 2001), and the relationship between selfing rate and the magnitude of inbreeding depression, as well as other species characteristics (e.g. Husband and Schemske 1996; Scofield and Schultz 2006). Plant evolutionary biologists focused on outcrossing versus self-fertilization in mating system evolution have rarely incorporated asexual reproduction into their studies.

Given that approximately 70% of all flowering plants are capable of asexual reproduction (Klimés *et al.* 1997), it is worthwhile to consider this reproductive mode in these same contexts, thereby investigating the combined influence of selfing, outcrossing and asexual reproduction on inbreeding depression and genetic load.

Muirhead and Lande (1997) considered a theoretical model of the joint effects of outcrossing, selfing, and asexual reproduction on mutation-selection balance. They predict that mutational load, as well as the magnitude of inbreeding depression and the genetic variance in fitness, will usually ***increase*** with the amount of asexual reproduction. Asexual reproduction was found to reduce inbreeding depression only at very high genomic mutation rates. However, the Muirhead and Lande (1997) predictions are contingent on a number of assumptions, perhaps the most important of which is an infinite population size.

Deleterious mutations are rare at mutation-selection equilibrium in an infinite population (Haldane 1927; Kondrashov 1985). As a result, the inbreeding depression and the genetic variance in fitness are simple functions of the average number of deleterious mutations per individual. In a finite population, genetic drift causes deleterious mutations to fix, and these fixations increase the genetic load (Byers and Waller 1999). There is considerable genetic data to suggest that the effective size of local populations may be reduced by asexual reproduction (Orive 1993; Balloux *et al.* 2003). Even if deleterious alleles do not reach fixation, outcrossing will sometimes

unite gametes that share the same deleterious mutation in a finite population. In this situation, asexual reproduction can actually reduce inbreeding depression by lowering the fitness of offspring produced by outcrossing (and thus bringing their average fitness closer to that produced by selfing). If asexual reproduction occurs via stolons or any other sort of sprawling vegetative growth, pollen from neighboring plants may very likely be self-pollen. This can also lead to a reduction in the difference in fitness between inbred and outbred individuals from the same population.

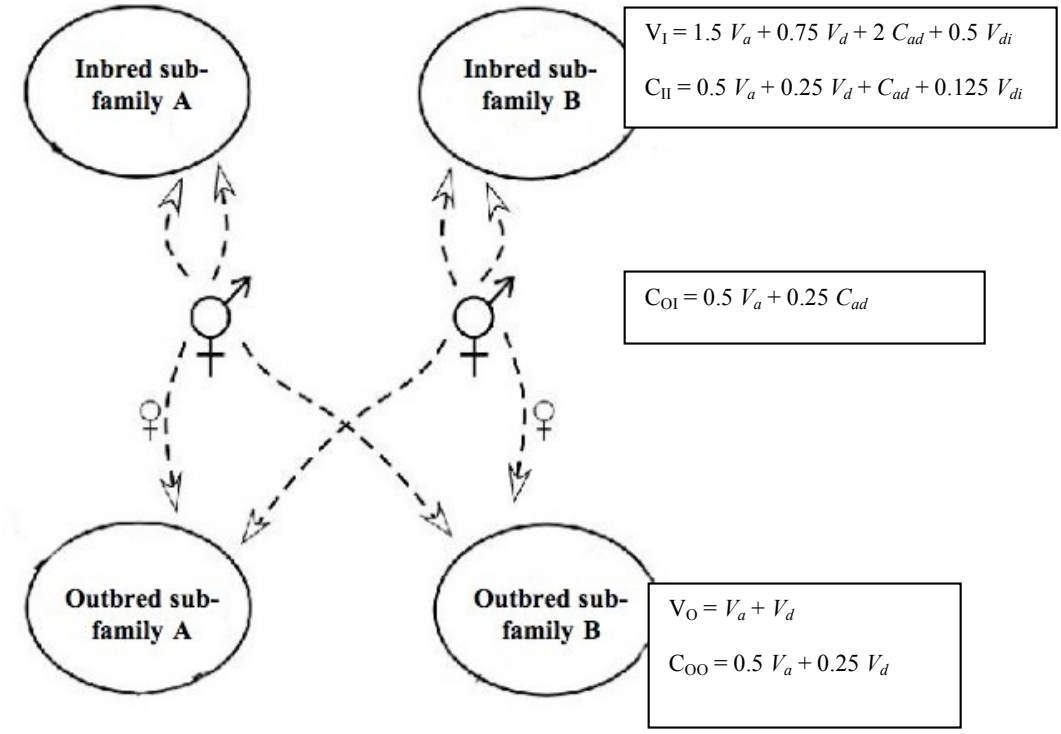
Our purpose in this study is to address the effect of asexual reproduction by comparing populations of *Mimulus* (Monkeyflower) that differ in reproductive system. Populations of the *M. guttatus* species complex are reproductively variable, ranging from fully outcrossing, to largely selfing, to predominantly asexual. We compare estimates of both molecular and quantitative genetic variability among populations of each type. Estimates for polymorphism at microsatellite loci are used as an indicator of effective population size and population structure. Quantitative genetic statistics include measures of inbreeding depression and genetic variability in fitness related traits.

There is a large body of genetic data from predominantly outcrossing and self-fertilizing populations of *M. guttatus* (Willis 1993b; Latta and Ritland 1994; Carr and Dudash 1995; Carr and Dudash 1996; Willis 1996; Carr *et al.* 1997; Kelly and Willis 1998; Sweigart *et al.* 1999; Willis 1999a, b, c; Kelly and Willis 2001; Carr and

Eubanks 2002; Carr *et al.* 2003; Kelly 2003; Kelly and Arathi 2003; Ivey and Carr 2005). Here, we synthesize these results with new data from the predominantly asexual population (Hall and Willis 2006; Hall *et al.* 2006). We estimate variability at the same microsatellite loci that have been used in both outcrossing and selfing populations. Inbreeding depression and genetic variation are estimated using a replicated diallel breeding design. These data are used to evaluate different evolutionary hypotheses for the maintenance of variability. Each hypothesis is formalized as a statistical model and is evaluated by comparing maximum likelihoods.

Statistical theory—We used a replicated diallel breeding design (e.g., Willis 1993a; Lynch 1988) in which outbred parental plants are randomly paired and reciprocally crossed. Each parent is also selfed. This produces four progeny sets per family, two outbred and two inbred (Figure 3-1). Because crosses are done reciprocally, maternal environmental effects can be distinguished from genetic effects as causes for resemblance of relatives. We first estimate the population mean for inbred and outbred plants. Inbreeding depression is most appropriately characterized by the 'inbreeding load', β, which is the slope of the regression of –Ln[fitness] onto f, the inbreeding coefficient of individuals (Morton *et al.* 1956; Charlesworth and Charlesworth 1987). The resemblance among plants within families (or variance

Figure 3-1. Dotted lines represent the transmission of gametes from the parental plants in each diallel (pictured in center). Inbred subfamilies A and B share the same maternal parent as the outbred subfamilies A and B, respectively. The genetic variances among inbred and outbred plants are denoted V_I and V_O, respectively. These variances, as well as the covariances among inbred siblings (C_{II}) among outbred siblings (C_{OO}), and beween inbred and outbred plants within the same diallel (C_{OI}) are each expressed as a function of genetic parameters.



among families) can be used to estimate genetic variance components (Harris 1964). These components include additive and dominance variances, V_a and V_d respectively, and also parameters that emerge from the joint effects of inbreeding and genetic dominance. Because we ignore epistasis and assume that two alleles segregate at each locus affecting measured traits, there are only two additional genetic parameters: the covariance of additive and dominance effects, C_{ad} , and the inbreeding dominance variance, V_{di} (see Cockerham and Weir 1984; Shaw 1987).

The predicted genetic variances and covariances for the contrasting crosses are given in Figure 3-1. These relationships assume that loci affecting our traits are in linkage equilibrium, at least in the experimental population if not the natural population. The phenotypic covariance for individuals that share the same mother is elevated by V_m, the variance of maternal effects. Allowing a general environmental effect with variance V_e, our "full model" has 6 variance components, four genetic (V_a, V_d, C_{ad}, and V_{di}) and two environmental (V_m and V_e).

Specific hypotheses about genetic variability can be directly evaluated as subsets of this full model. We test three distinct hypotheses, the Kondrashov model, the Single Founder model and the No Genetic Variation (No V_g model). Kondrashov (1985) developed a model that is routinely used in theoretical studies for the evolution of mating system and sexuality, and which assumes that genetic variation is caused by rare alleles. The Kondrashov model predicts that the four genetic variance components (V_a , V_d , C_{ad} and V_{di}) can be reduced to two parameters because $V_d = 0$ and $C_{ad} = \sqrt{2V_aV_{di}}/2$ (see Model I of Kelly 2003). A second hypothesis is essentially the opposite of the rare-alleles Kondrashov model. Consider a population that is founded by a single propagule that subsequently expands asexually. Here, all local variation is due to heterozygosity in the founder, apart from that generated *de novo* by somatic mutation. Allele frequency will be ½ at all loci in this Single Founder Model. If we then synthesize an experimental base population by

intercrossing individuals (see Methods), then $C_{ad} = 0$ and $V_{di} = 0$. Finally, according to the No V_g model, all variation is strictly due to environmental components, V_e and V_m. The Kondrashov, Single Founder, and No V_g models are each subsets of our full model and each can be tested using likelihood ratios.

Methods

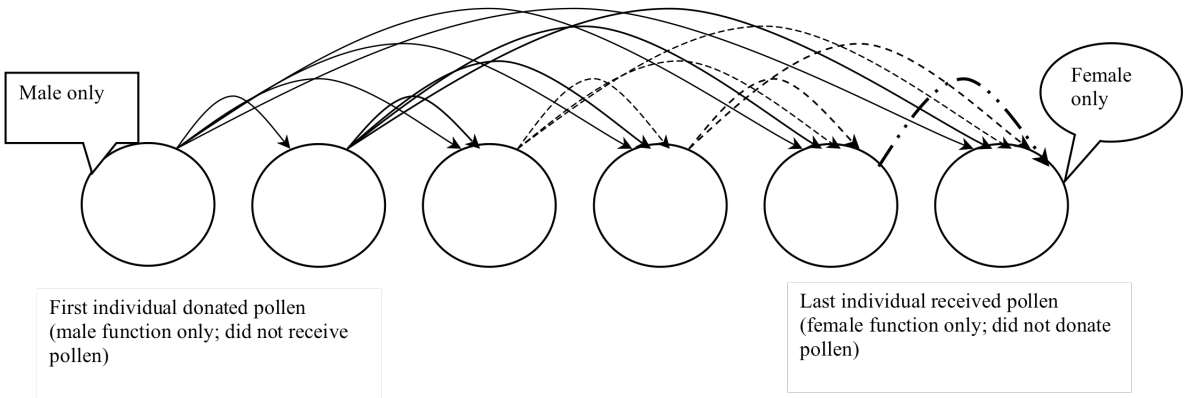
Natural population and sampling regime— Mimulus guttatus is a hermaphroditic wildflower native to western North America. It is usually found growing in moist areas such as stream banks, cliff faces, wet meadows and roadsides. The reproductive system varies extensively among populations with differing proportions of outcrossing, selfing, and asexual propagation. The most substantial data on mating system concerns the relative frequency of outcrossing and selfing—selfing rate estimates range from 0 to 0.75 (Ritland and Ganders 1987; Ritland 1990; Willis 1993b; Awadalla and Ritland 1997; Sweigart *et al*. 1999). The extent of asexual reproduction is more difficult to quantify and there have been no direct estimates. However, reproduction via stolons is frequently observed in perennial populations (Kiang and Libby 1972; Kiang 1973; van Kleunen 2007).

The founding plants for this breeding design were sampled from a natural perennial population of *M. guttatus* growing on the coastal sand dunes of the Oregon Dunes National Recreation Area south of Florence, Oregon (hereafter abbreviated DUN).

DUN is extensively asexual (M. Hall, personal communication). The environment at the DUN population is temperate with year round moisture (Hall *et al.* 2006). Plants at this location have larger flowers, stems and leaves than typical of annual *M. guttatus* populations (Hall and Willis 2006). A total of 40 distinct maternal seed families were collected from the DUN site. Ten maternal seed families were sampled in 2000 by Megan C. Hall and 30 maternal seed families were sampled by Liza M. Holeski in 2002. During each collection period, parents were sampled every 5 meters along a transect. The 2002 transect spanned the entire local population. The experiment consisted of three successive generations of greenhouse growth under the same conditions (see Arathi and Kelly 2004, for a description of our standard protocols).

For generation 1, both sets of field-collected seed were grown simultaneously and were randomly assigned to one of six groups. Each group contained six plants (4 of the 40 noted above were randomly excluded). Crossing within each group follows that diagrammed in Figure 3-2. Plants were hand pollinated and the corolla was removed from each flower that received pollen as a guarantee that no self-fertilization occurred. Plants within each group were randomly assigned to numbers one through six. Plant 1 functioned as sire only and donated pollen to plants 2-6. Plant 2 received pollen from plant 1 and donated pollen to plants 3-6. Plant 3 received pollen from plants 1 and 2 and donated pollen to plants 4-6. Plant 4 received pollen from plants 1,

Figure 3-2. Generation of the outcrossed 'base population' from field collected seed (Arrows indicate movement of pollen). Progeny from these crosses (generation 2) are the parents in the breeding design of Figure 3-1.



2, 3 and donated pollen to plants 5 and 6. Plant 5 received pollen from plants 1, 2, 3, 4 and donated pollen to plant 6. Plant 6 received pollen from plants 1-5. Fifteen outbred individuals were generated from each group of six giving a total of 90 outcrossed unrelated individuals. The progeny from these crosses (generation 2) are a synthetic large outbred population representative of the DUN population in terms of allele frequencies, but not multi-locus genotype frequencies.

The second greenhouse generation involved constructing the diallel families. The 90 outcrossed individuals generated in the first greenhouse generation were randomly paired (45 pairs), excepting pairings between individuals from the same Gen 1 group. Each plant in the pair was selfed and outcrossed (see Figure 3-1), generating families

each comprised of two outbred and two inbred members. Two diallel families were lost because either one plant in the pair did not flower or did not survive to be crossed.

The third greenhouse generation consisted of three independent grow-ups with subsequent measurement of plants from the 43 diallel families (4 replicates per subfamily). All floral measurements were made on the first flower on the day of flowering. The following floral traits were measured to the nearest hundredth of an inch and then measurements were transformed to millimeters: corolla width, corolla length, stigma length, and anther length. Anthers were collected from the first flower at anthesis and were placed into a 1.5 mL centrifuge tube for viability analysis. Growth of all plants proceeded until the majority of plants had flowered. Once a majority of the plants flowered (day 52 for grow ups one and two; day 71 for grow up three), we recorded the number of flowers per plant and harvested plants for above ground biomass. Plant tissue was dried in an oven for approximately 24-48 hours at 65°C. The dried above ground plant material was weighed to the nearest 0.01 g. We used the trait of biomass as a proxy for female fitness because it is highly correlated with flower number (Carr and Dudash 1995; Galloway 1995) and total seed production (Fenster and Ritland 1994) in *M. guttatus*.

Pollen was counted and sized using a Coulter Counter Model Z1 dual (Coulter, Miami, Florida, USA) and the pollen size index (PSI), which can be used as a

measure of pollen viability (Kelly *et al.* 2002), was calculated. From the measurements obtained, we also calculated stigma-anther separation, amount of viable pollen per flower, and total male fitness. The latter estimate was obtained by multiplying total flower number per plant by the estimated viable pollen per flower. All trait means were adjusted for the effect of grow up through one-way analysis of variance (ANOVA) using Minitab v. 14 (State College, Pennsylvania, USA). Traits with right skewed distributions were log base 10 transformed (PSI, viable pollen per flower, total male fitness, and biomass) or square root transformed (flower number).

DNA extraction and microsatellite genotyping—Field collected seed were planted and used to estimate microsatellite diversity in order to maximize our ability to detect heterozygosity. Floral bud and young leaf tissue was collected into a 96-well plate with one metal bead in each well. DNA extraction protocols followed that of Marriage *et al.* (2009).

PCR amplification followed that of a standard touchdown protocol. PCR mixtures (10 μl total volume) contained 2-10 ng of template DNA, 5 μM HEX labeled forward primers and 5 μM reverse primers, 250 μM each dNTP, 0.15 units Taq DNA polymerase (Promega, Madison, WI, USA) and 1x PCR buffer (Promega, Madison, WI, USA). For temperature cycling, we implemented a touchdown PCR protocol using an iCycler Thermal Cycler (BioRad, Hercules, CA, USA): 94 ºC for 3 min, 10 cycles of denaturing at 94 ºC for 30 s, annealing for 30 s, and extension at 72 ºC for

45 s; the initial annealing temperature (Ta) = 62 ºC decreased by 1 ºC with each cycle, followed by 30 cycles of denaturing at 94 ºC for 30 s, annealing using Ta = 52 ºC for 30 s, and extension at 72 ºC for 45 s and a final extension at 72 ºC for 20 min. We detected PCR-amplified fragments on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and sized fragments using GENEMAPPER 4.0 software (Applied Biosystems) calibrated with the ROX500 size standard (Applied Biosystems).

Model fitting— We performed maximum likelihood estimation of variance components and model fitting on adjusted/transformed trait values. Maximum likelihood was used to estimate M_O (the outbred trait mean) and β [as fixed effects, where $\beta = 2(M_I - M_O)$, and M_I is the inbred trait mean] and the variance components for each trait (Shaw 1987). Estimates were obtained by finding the set of values for M_O, β, V_a, V_d, C_{ad}, V_{di}, V_m, V_e that maximize the log likelihood of the model given the data. Maximization was subject to the constraints that (1) V_e must be positive; (2) V_m, V_a, V_d, V_{di} must be greater than or equal to zero; and (3) C_{ad} is constrained in absolute value by V_a and V_{di}. The program to conduct these calculations was written in C and is available upon request (contact JKK).

Hypothesis testing was then conducted in two stages. We first tested for maternal effects and inbreeding depression in each trait. If an effect was significant, we retained the corresponding parameter in the model for subsequent model tests. In the

second stage of testing, we used likelihood ratios to evaluate the Single Founder model, the Kondrashov model, and the No V_g model. Each of these Hypotheses is a simplified case of the general 6 parameter model.

Likelihood ratio statistics are routinely evaluated by comparison to the chi-square distribution with degrees of freedom equal to the difference in the number of parameters between models. However, when a 'free parameter' is constrained in the more general model, e.g. a variance cannot be negative, the appropriate null distribution is a mixture of chi-square densities. Self and Liang (1987) review a number of special cases, but unfortunately, none of these cases correspond exactly to the parameter simplifications associated with our Single Founder model or the Kondrashov model. Thus, we opt for the conservative critical value based on 2 df for each.

Results

Microsatellite variation—Only 3 of 9 loci were polymorphic in the DUN population. Across loci, the average expected heterozygosity for the population is 0.03 (Table 3-1). In contrast, the predominantly outcrossing Iron Mountain population (IM) has an average expected heterozygosity of 0.62 (this combines estimates from previous studies and new data presented here). Each surveyed locus is polymorphic in IM, with most having multiple alleles. Data from another population, Shearer Falls (SF),

a highly selfing population of the closely related *Mimulus nasutus*, is also summarized in Table 3-1. While SF was genotyped at only three of these loci, the average expected heterozygosity is 0.19. Overall, variation is highest in the predominantly outcrossing population, intermediate in the highly selfing population, and lowest in the extensively asexual DUN population.

Table 3-1. A summary is given for polymorphism data at nine microsatellite loci in four populations of the *M. guttatus species* complex. *N* is the number of population chromosomes sampled (twice the number of individuals for outbred diploid genotypes or the actual number of individuals from inbred lines for IM estimates). N_a is the number of distinct alleles and H_e is the unbiased expected heterozygosity (Nei 1978). Two different estimates are given for ATT356 polymorphism in the IM population. Superscript denotes source for estimates extrinsic to this study: [1] Kelly and Willis 1998, [2] Sweigart et al. 1999, [3] Inbred IM lines from Willis 1999a.

	DUN			**IM**			**SF**		
Locus	*N*	N_a	H_e	*N*	N_a	H_e	*N*	N_a	H_e
AAT225	24	2	0.08	60	4	0.43[1]	54	2	0.38[1]
AAT240	40	2	0.14	53	5	0.68[3]			
AAT267	40	1	0.00	60	2	0.12[1]	54	1	0.00[1]
AAT300	24	1	0.00	152	12	0.83[2]			
AAT308	38	1	0.00	53	5	0.56[3]			
AAT356	30	2	0.07	162	25	0.81[2]	54	2	0.20[1]
				52	17	0.87[1]			
AAT367	40	1	0.00	47	4	0.55[3]			
AAT374	40	1	0.00	35	4	0.64[3]			
AAT9	26	1	0.00	176	27	0.93[2]			

Table 3-2. The means for both outbred and inbred plants are given with their respective standard deviations and sample sizes. Units are mm for all floral measurements and Log(g) for Biomass.

Trait	Outbred mean (± 1 sd)	Outbred sample size	Inbred mean (± 1 sd)	Inbred sample size
Flower morphology:				
Corolla width	36.76 (± 3.39)	914	36.30 (± 3.62)	897
Corolla length	40.06 (± 2.75)	946	39.53 (± 3.00)	926
Stigma length	25.06 (± 1.38)	973	24.87 (± 1.45)	965
Anther length	20.38 (± 1.45)	975	19.98 (±1.60)	965
Stigma anther separation	4.69 (± 1.43)	973	4.89 (± 1.54)	964
Male fitness:				
Log(PSI)	–0.24 (± 0.24)	915	–0.24 (± 0.20)	903
Log(Viable pollen per flower)	4.20 (± 0.45)	915	4.15 (± 0.43)	903
Log(Total male fitness)	5.72 (± 0.38)	834	5.62 (± 0.42)	803
Phenology and Plant size:				
Days to flower	35.65 (± 4.13)	976	36.29 (± 3.74)	966
Log(Biomass)	-0.01 (± 0.29)	810	-0.05 (± 0.30)	783
Sqrt(Flower number)	4.71 (± 1.41)	887	4.40 (± 1.41)	859

Inbreeding depression and genetic variance components—The means and standard deviations for each trait are given in Table 3-2. The outbred mean is significantly higher for all traits except stigma anther separation, days to flower and biomass (Table 3-3). There is no difference between outbred and inbred means for pollen size

Table 3-3. Likelihood Ratio Tests (LRT) for inbreeding depression and maternal effects for each trait are summarized in the first two columns. Both LRT are evaluated relative to the chi-square distribution with 1 df. Pollen Size Index, Viable Pollen per Flower, Total male fitness, and Biomass were log-transformed. Flower number was square-root transformed. Significant LRT are bold with the following p-value indicators: * = 0.01< p <0.05, ** = 0.001< p <0.01, *** = p <0.001. [§]See Appendix B for references and details. [‡]Estimates of load given on original scale of measurement instead of transformed values.

	LRT for Inbreeding Depression	**LRT** for Maternal Effects	Inbreeding Load (SE)	Mean and range of inbreeding load from other studies of *Mimulus guttatus*[§]
Flower morphology:				
Corolla width	**6.83****	0.19	-0.024 (0.009)	-0.188 (-0.399, -0.080)
Corolla length	**14.40*****	0.00	-0.026 (0.006)	-0.105 (-0.192, -0.071)
Stigma length	**7.38****	0.00	-0.016 (0.005)	-0.061
Anther length	**21.99*****	0.00	-0.039 (0.008)	n/a
Stigma-anther separation	**9.10****	0.00	0.088 (0.028)	0.056 (0.013, 0.080)
Male fitness:				
Pollen Size Index	1.04	2.58	-0.002[‡] (0.112)	-0.308
Viable pollen per flower	**4.34***	0.00	-0.194[‡] (0.097)	-0.487 (-0.673, -0.299)
Total male fitness	**10.91*****	0.00	-0.398[‡] (0.136)	n/a
Phenology and Plant size:				
Days to flower	**11.15*****	1.31	0.034 (0.010)	0.086 (0.057, 0.112)
Biomass	**7.05****	0.07	-0.179[‡] (0.068)	-0.349 (-1.360, 0.503)
Flower number	**13.18*****	0.04	-0.282[‡] (0.909)	-0.320 (-0.944, 0.756)

index (PSI). There was no evidence of maternal effects for any trait (Table 3-3). After excluding V_m, the full model variance component estimates for all traits are listed in Table 3-4. The environmental variance component is the largest variance component. There is very little to no outbred dominance variance (V_d) for all traits. All traits except the amount of viable pollen per flower (VPF) have substantial V_{di}. Corolla width, stigma length, anther length, stigma anther separation, PSI, days to flower and biomass have negative C_{ad} estimates, although these estimates are low.

Likelihood Ratio tests reject No V_g with high confidence for all traits (Table 3-5). The Kondrashov model can be rejected for PSI, while the Single Founder Model cannot be rejected for any trait.

Table 3-4. Variance component estimates are given from the full model. SE were obtained from the asymptotic dispersion matrix. The coefficient of additive variation (CV_a) is also reported and used for comparisons. All variance component values are standardized so that the outbred variance ($V_e + V_a + V_d$) is equal to 1. [§]Estimates of CV_a are based on non-transformed trait means and V_a (see text for back-transformation details) : [‡]Details for studies are in Appendix C.

	V_e (SE)	V_a (SE)	V_d (SE)	C_{ad} (SE)	V_{di} (SE)	CV_a	Mean and range of CV_a from other Mimulus studies[‡]
Flower morphology:							
Corolla width	0.763 (0.038)	0.237 (0.062)	0.000 (0.059)	-0.060 (0.086)	0.268 (0.269)	0.013	0.054 (0.007, 0.120)
Corolla length	0.803 (0.036)	0.197 (0.056)	0.000 (0.058)	0.007 (0.080)	0.104 (0.256)	0.011	0.048 (0.009, 0.113)
Stigma length	0.616 (0.039)	0.379 (0.079)	0.005 (0.058)	-0.163 (0.101)	0.501 (0.289)	0.025	0.050 (0.033, 0.097)
Anther length	0.830 (0.040)	0.170 (0.054)	0.000 (0.065)	-0.096 (0.079)	0.601 (0.262)	0.020	0.088 (0.063, 0.112)
Stigma-anther separation	0.782 (0.036)	0.218 (0.058)	0.000 (0.057)	-0.006 (0.081)	0.160 (0.256)	0.100	0.284 (0.241, 0.334)
Male fitness:							
Pollen Size Index[§]	0.670 (0.043)	0.190 (0.062)	0.139 (0.060)	-0.125 (0.088)	0.164 (0.272)	0.209	1.186
Viable pollen per flower[§]	0.906 (0.038)	0.042 (0.038)	0.051 (0.056)	0.001 (0.060)	0.000 (0.234)	0.002	n/a
Total male fitness[§]	0.872 (0.046)	0.011 (0.042)	0.118 (0.071)	0.022 (0.068)	0.091 (0.258)	0.060	n/a
Phenology and Plant size:							
Days to flower	0.753 (0.035)	0.207 (0.057)	0.040 (0.050)	-0.074 (0.077)	0.053 (0.246)	0.013	0.071 (0.023, 0.214)
Biomass[§]	0.946 (0.041)	0.054 (0.040)	0.000 (0.061)	-0.015 (0.065)	0.122 (0.257)	0.103	n/a
Flower number[§]	0.838 (0.043)	0.033 (0.043)	0.129 (0.065)	0.010 (0.067)	0.006 (0.246)	0.035	n/a

Table 3-5. The relative fit of different genetic models is compared by Likelihood Ratios. Variable transformations and significance levels are as in Table 3. Pollen Size Index, Viable Pollen per Flower, Total viable pollen, and Biomass were log-transformed. Flower number was square-root transformed. Each Likelihood Ratio Test is against the full model: 2 df for the Kondrashov and Single Founder models and 4 df for the No V_g model.

	Likelihood Ratio Tests		
	Single Founder	**Kondrashov**	**No V_g**
Flower morphology:			
Corolla width	1.22	1.22	**164.96*****
Corolla length	0.75	0.19	**160.49*****
Stigma length	1.72	4.82	**329.33*****
Anther length	5.91	4.91	**102.88*****
Stigma-anther separation	1.20	0.46	**199.26*****
Male fitness:			
Pollen size index	5.90	**13.93*****	**84.56*****
Viable pollen per flower	0.00	0.78	**20.34*****
Total male fitness	1.53	4.05	**19.62*****
Phenology and plant size:			
Days to flower	2.00	4.21	**130.51*****
Biomass	0.48	0.40	**16.89*****
Flower number	0.13	3.57	**27.86*****

Trait correlations—We calculated trait correlations based on outbred and inbred phenotypic trait means, outbred family trait means (Appendix D). Based on outbred phenotypic trait mean correlations, floral traits (corolla width, corolla length, stigma length and anther length) were positively correlated with each other. Biomass and flower number were also positively correlated with floral traits, as well as biomass and flower number were positively correlated with each other. Male fitness traits (pollen size index, viable pollen per flower and total male fitness) were also

positively correlated with each other. Male fitness traits and stigma-anther separation were negatively correlated. Days to flower was negatively correlated with biomass and with flower number. Similar correlations exist for phenotypic trait means and for inbred phenotypic trait means. Correlations based on outbred family trait means were the same as outbred and inbred trait mean phenotypic correlations, except biomass and days to flower were positively correlated (these traits were negatively correlated for outbred and inbred trait mean correlations).

Discussion

Different populations of the *Mimulus guttatus* species complex span the entire range of reproductive variability from nearly wholly outcrossing, to nearly entirely selfing, to predominantly asexual. The DUN population investigated here is thought to be highly asexual, and consistent with this view, it yields estimates for genetic statistics that are clearly distinct from those obtained previously for predominantly outcrossing and selfing populations. With respect to molecular variation, the amount of variability in allele length at microsatellite loci is minimal in DUN (Table 3-1). The DUN population does exhibit significant inbreeding depression (Table 3-3) and genetic variability (Table 3-4) in fitness related traits. However, the magnitudes are substantially lower than in other populations (Table 3-3, and Appendix B). These results are inconsistent with the theoretical prediction (Muirhead and Lande 1997) that asexual reproduction should increase inbreeding depression and genetic load.

We argue that this discrepancy may be explained by considering genetic drift and/or founder effects. Extensive asexual propagation, combined with limited sexual reproduction, may result in a low effective population size for the DUN population.

The distinction between genetic load and inbreeding load is particularly important when considering the genetic characteristics of local populations. The genetic or mutational load is the average total reduction in fitness due to deleterious mutations (Crow and Kimura 1970, pp 297-303). The inbreeding load is the reduction in fitness of inbred individuals relative to outbred individuals within a population (the inbreeding load determines the more standard inbreeding depression statistic given the experimental design, see Charlesworth and Charlesworth 1987). Deleterious mutations that are fixed within a population will contribute to the mutational load but not the inbreeding load. A population with a small effective size can thus have high mutational load but low inbreeding load or depression. The same may also apply to populations that have recently expanded from one (or a few) founding propagules or that have experienced a recent bottleneck in size.

The very low microsatellite variability in DUN is consistent with a recent founding event or bottleneck, but little is known of the demographic history of this population. No direct estimates have been made with respect to the frequency of asexual reproduction within the DUN population, but field observations suggest this phenomenon occurs regularly (M. Hall, pers. comm.). DUN plants produce below

surface stolons and even isolated individuals may be members of the same clonal lineage. Like other *M. guttatus* populations, DUN plants produce large, showy flowers and are capable of effective outcrossing and self-fertilization.

Extensive asexual propagation likely alters the genetic consequences of outcrossing, potentially amplifying the effects of genetic drift. The bee pollinators of *M. guttatus* typically move pollen between neighboring plants. Insofar as neighbors are clonal relatives, outcrossing becomes nearly equivalent to geitonogamous selfing. Indeed, we expect little inbreeding depression in highly selfing species that undergo frequent bottlenecks and/or founder events (Lande and Schemske 1985).

Inbreeding depression, inbreeding load and genetic variation for fitness—We used a diallel breeding design to estimate inbreeding load and genetic variance components. Significant inbreeding depression has been found for many different traits in *Mimulus guttatus* (Willis 1993a, b; Willis 1999a, b, c; Carr and Dudash 1995, 1996, 1997; Dudash *et al.* 1997; Table 3-3 and Appendix B). In DUN, all traits except pollen size index, which is a measure of pollen viability, exhibited statistically significant inbreeding depression. However, the magnitude of inbreeding depression in the DUN population is substantially lower than the amount of inbreeding depression in other *Mimulus* populations (Table 3-3). The absence of inbreeding load for pollen viability is striking. In the predominantly outcrossing IM population, pollen viability is a

major component of inbreeding depression through male fitness (Kelly 2003; Kelly 2005).

Despite the generally low variability evident in DUN, all traits exhibit significantly non-zero additive genetic variation (see No V_g test in Table 3-5). We did not find evidence of maternal effects, despite an experimental design appropriate for detecting such effects (Table 3-3). The coefficient of additive variation (CV_a) is a dimensionless statistic useful for comparisons across studies (Houle 1992). Estimates of CV_a for PSI, VPF, Total male fitness, Biomass and Flower number are given for non-transformed trait means and estimates of V_a. Back-transformations for V_a were based on the following:

$$V_{a\ (rescaled)} = V_{a(transformed)} \times (\text{outbred trait standard deviation})^2 \times g'(x)$$

where $g'(x)$ is the first derivative of the function for the back-transformation of the outbred trait mean.

For morphological traits, sexual *Mimulus* populations typically have CV_a that are at least twice the estimate from DUN. Again, the pollen size index is an exception: CV_a for the sexual IM population is almost six times higher than the estimate for DUN. The differing genetic architectures of pollen viability suggested by these studies is a worthwhile target for future studies.

The inbreeding variance components, C_{ad} and V_{di}, contribute to response to selection when there is non-random mating (Wright and Cockerham 1985; Kelly 1999). Significantly non-zero values for C_{ad} and V_{di} were estimated for the Iron Mountain (IM) population of *M. guttatus* (Kelly and Arathi 2003), for inbred population of *Nemophila menziesii* (Shaw *et al.* 1998), and for certain traits in this study (DUN). Stigma length and anther length had the greatest contribution of V_{di} to the phenotypic variance (Table 3-4). Pollen size index and biomass had negative C_{ad} estimates while the remaining traits in the DUN population had positive C_{ad} estimates. In the IM population, corolla width and age at first flower exhibited negative C_{ad} (although standard errors are large). In aggregate, the variance component estimates suggest different evolutionary trajectories for sexual and asexual *Mimulus* populations.

Finally, the replicated diallel design allows tests of specific hypotheses about genetic variability. We developed the 'Kondrashov model', where variation is caused by rare, equivalent alleles, and the 'Single Founder model', where variation is cause by polymorphisms with 2 equally frequent alleles, as special cases of our more general model of trait variation. We were able to reject the Kondrashov model for only one trait, Pollen Size Index (Table 3-5). We were unable to reject the Single Founder Model for any trait, although likelihood ratio for anther ength and pollen size index nearly reach our conservative critical value. The lack of significant results here is probably a consequence of low power. The limited amount of variation in the DUN population makes it difficult to distinguish models.

Conclusions

Results from this study highlight the need for continued investigations into mating system and the factors influencing its evolution in the *M. guttatus* species complex. This study also emphasizes the need for incorporating asexual reproduction into existing models of mating system evolution and mutation selection balance. Asexual reproduction affects both the overall effective population size and the "effective" amount of outcrossing (since outcrossing between clone mates is tantamount to selfing and is likely to have strong effects on load). Future research will incorporate asexual reproduction in theoretical simulations of mutation selection balance for both finite and infinite populations with varying levels of outcrossing, selfing and asexual reproduction. This will allow a comparison of the results from the current study, as well as provide a means to estimate mutation rates for loci that contribute to load.

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Chapter 4. Effects of asexual reproduction on genetic load in infinite and finite populations.

Abstract

The reproductive system of an organism, whether it outcrosses, selfs or asexually reproduces, can influence levels of genetic load within populations. The effect of increasing the amount of asexual reproduction on genetic load was studied with two distinct sets of computer simulations; deterministic, Infinite simulations, and stochastic, Finite simulations. Two variables were used to estimate genetic load; the average number of deleterious mutations per gamete (Q) and inbreeding load (β). Overall, as the amount of asexual reproduction increased, both Q and β decreased for dominance coefficient (h) greater than 0.2.

Introduction

Mode of reproduction, or reproductive system (defined here as the proportion of selfing, outcrossing and asexual reproduction within a single generation of mating) has great influence on levels of genetic load and inbreeding depression within populations. The mean fitness of a population depends on the reproductive system (Higgs 1994). Under certain conditions, different reproductive systems may be equally fit at equilibrium, yet differ with respect to the number of mutations per locus at equilibrium (Hopf *et al*. 1988). With respect to mutation load and fitness, sexual populations (completely or predominately outcrossing populations) are expected to have lower mutational load and higher fitness than asexual populations (Kondrashov 1982). Selfing populations are expected to have fewer deleterious mutations than asexual populations (Pamilo *et al*. 1987) and selfing can even decrease genetic load within a population (Charlesworth *et al*. 1990) via the purging of deleterious mutations.

Population size also influences levels of load, with small populations predicted to have greater genetic load than large populations (Kimura *et al*. 1963; Lynch *et al*. 1995) due to the fixation of deleterious mutations under genetic drift. However, analytical studies have shown that mating system confounds the simple expectations of load and population size, and other factors, such as mutational effect size and dominance (Glemin 2003) become important. Genetic load increases as population

sizes become smaller but decreases as inbreeding rate increases (Bataillon and Kirkpatrick 2000). Genetic drift has opposing effects on genetic load and inbreeding depression with load being higher in small populations and larger populations having higher inbreeding depression (Glemin *et al*. 2003).

There has been a thorough theoretical treatment of how mixed mating determines the levels of genetic load and inbreeding depression under mutation selection balance (see, for example, Ohta and Cockerham 1974; Kondrashov 1984; Charlesworth *et al*. 1990; Kelly 2007). Ohta and Cockerham (1974) found that the equilibrium number of deleterious mutations decreases as the strength of selection and dominance coefficient increase. Weakly deleterious alleles make a major contribution to inbreeding depression and can lead to increased inbreeding depression, especially for nearly recessive mutations (Charlesworth *et al*. 1990). According to Kelly (2007), simple multi-locus models of mixed mating can even result in deviations from expected numbers of heterozygous and homozygous mutations per individual.

Of the above-mentioned studies, only two (Pamilo *et al*. 1987 and Hopf *et al*. 1988) include asexual reproduction (modeled as either apomixis or parthenogenesis) in their models of mixed mating (selfing vs. outcrossing). Muirhead and Lande (1997) incorporated asexual reproduction in their theoretical simulations of mutation selection balance to see if asexual reproduction could account for high levels of inbreeding depression seen among selfing species. They found an increase in

inbreeding depression with increasing asexuality, but that the equilibrium inbreeding depression depended on the importance of purging (removal of homozygous deleterious mutations) relative to the selection against heterozygotes (Muirhead and Lande 1997).

In this paper, we investigate the effects of asexual reproduction on the number of deleterious mutations per gamete and levels of inbreeding load under mutation selection balance with mixed mating in two separate models of finite and infinite populations. The different models incorporate asexual reproduction with varying levels of outcrossing and selfing, degrees of dominance and selection coefficients. The mean number of deleterious mutations per gamete is calculated and used to estimate genetic load. These estimates are then compared within and among finite and infinite populations to identify the effect of asexual reproduction on levels of load, and how asexual reproduction may interact with genetic drift (population size).

Methods

Simulation structure

The Infinite and Finite simulations have the same order of events with mutation followed by the different modes of reproduction (selfing, outcrossing and asexual reproduction) and then selection. Mutation involves both meiotic and mitotic mutations. For both sets of simulations, mitotic mutations are added to all individuals within the population; however, meiotic mutations are incurred only in selfing or

outcrossing individuals (see details about how individuals/loci are selected for the different modes of reproduction for Infinite and Finite simulations below). Within a single run, mutations have equal effect on fitness for all loci. Loci are unlinked for both Infinite and Finite simulations. As indicated by the simulation names, the Infinite simulations have an infinite number of loci that can acquire mutations. The Finite simulations have a finite number of loci, $L = 100$, that can acquire mutations. Also, the Infinite simulations and Finite simulations differ in population size. As the name implies, the Infinite simulations assume an infinite population size, and as such, the simulations are deterministic. The Finite simulations are limited in population size, and the simulations are stochastic. Fitness is multiplicative, determined each generation by the following equation:

$$w_{ij} = (1 - hs)^i (1 - s)^j \quad (1)$$

where w_{ij} is the fitness value for individuals with i heterozygous and j homozygous mutations, respectively; h is the dominance coefficient; and s is the strength of selection. Both sets of simulations output Q, the average number of deleterious mutations per gamete, and the estimate of Q is used in the estimation of the inbreeding load, β, which is defined as

$$\beta = Qs(1-2h), \quad (2)$$

where s and h are defined above (Kelly 2007). Selection occurs on diploid individuals; therefore, selection for both sets of simulations is zygotic selection. Code for both sets of simulations is written in C and is available from the author by request.

Infinite population simulations

Infinite population simulations are similar to those described by Kondrashov (1985) and Charlesworth *et al*. (1990) in which the fitness of individuals is determined by the number of deleterious mutations in heterozygous and homozygous form. Fitness is multiplicative, mutations have equal effects across loci, and loci are unlinked. Because there are an infinite number of loci, we assume that mutations only occur at loci that previously had no mutations; that is, mutation only generates heterozygotes. Outcrossing does not produce any homozygous offspring. Since mutations are assumed to only occur at loci that were previously mutant free, the only way to generate new homozygosity in this set of simulations is through the production of selfed offspring. The selection coefficient, dominance coefficient, genomic deleterious mutation rate (U), and fraction of meiotic mutations (Fm) are manually set before each run. The value of Fm ranges between zero and one and is used to determine the proportion of mutations that are meiotic (meiotic mutation rate multiplied by Fm) or the proportion of mutations that are mitotic ($1 - Fm$).

The infinite simulation program is written to cycle over all possible outcrossing, selfing, and asexual proportions, based on increments of 0.10, for a set value of h, s, U and Fm. Three arrays are used to keep track of the number of heterozygous and homozygous mutations for the asexually produced progeny, the selfed progeny and the number of deleterious heterozygous mutations for outcrossed progeny,

respectively. These arrays are then used to calculate the fitness of each of the respective reproductive modes and the overall population mean number of deleterious mutations per gamete Q. The program runs until equilibrium is reached or for a minimum of 51 generations. Equilibrium is met if the difference between the outcross fitness and its variance in deleterious mutation for outcrossed individuals, and the difference between the inbred fitness and its variance in deleterious mutation is less than some critical value (defined as 0.00000001). The program outputs the equilibrium Q, Q_O (average number of deleterious mutations per gamete for outcrossed progeny), Q_S (average number of deleterious mutations per gamete for selfed progeny), and Q_A (average number of deleterious mutations per gamete for asexually produced individuals) for each combination of reproductive proportions.

Mutation

Initial conditions for the mutation frequency follow that of Ohta and Cockerham (1974) with q, the initial probability of being heterozygous, equal to u/hs, where u is the per locus, per generation meiotic mutation rate and h and s are as defined above. Mutations are Poisson distributed across loci. Mitotic mutations are added to all members of the population. Meiotic mutations are added to selfed progeny (diploid mutation rate, calculated as the mutation array for selfed progeny times the deleterious mutation frequency array) and to outcrossed gametes (haploid mutation rate, calculated as the mutation array for outcrossed progeny times the gamete deleterious mutation frequency array).

Reproduction

Asexual reproduction

Mitotic mutations are added to all members of the population and this new mutational array represents the asexual progeny array.

Selfing

The program uses multinomial probabilities to calculate the probability of a parental genotype with *i* heterozygous mutations and *j* homozygous mutations, creating *x* heterozygous and *y* homozygous offspring. These multinomial probabilities are then multiplied by the deleterious mutation frequencies for the parental genotype. Meiotic mutations are added to the recently produced diploid selfed progeny as described above.

Outcrossing

The program uses binomial probabilities to calculate the probability of a parental genotype with *i* heterozygous and *j* homozygous mutations producing gametes with *x* mutant alleles. Meiotic mutations are added to the gametes, as described above, and then the gametes are combined to produce the outcrossed progeny. After selfed and outcrossed progeny are produced, the program combines the selfed, outcrossed and asexual deleterious mutation arrays into one population-level array, using the proportion of each reproductive mode and multiplying by the individual reproductive mode array. This population level array is then used in the selection loop.

Selection

Once mutations have been added, and reproduction has occurred, the fitness of each individual is calculated based on Equation 1, and the population level array. The resulting heterozygous and homozygous deleterious allele frequencies represent the starting population level allele frequencies for the next generation.

Finite population simulations

The Finite population simulations are comprised of 5 replicates of 50,000 generations per replicate. Mutations can occur at any one of the 100 loci. Values for h, s, u, v, N_a, N_s, N_o (where h and s have been defined previously, u is the per locus per generation meiotic mutation rate, v is the per locus per generation mitotic mutation rate, N_a is the number of asexually reproducing individuals in the population, N_s is the number of selfing individuals in the population, and N_o is the number of outcrossing individuals in the population, respectively) are manually set at the start of each set of runs (5 replicates for 50,000 generations). The finite simulation keeps track of the frequency of mutant alleles at each locus (Q_0). Simulations were run for total population sizes of N=30, N=60, N=90, N=150, and N=300.

The number of unlinked loci (L) stayed constant for all sets of simulations, with L=100. At the end of each generation, the program prints the average number of

deleterious mutations per gamete (Q_0), the average number of heterozygous (Q_1) and homozygous mutations per gamete (Q_2) to a data file. The estimate of Q_0 is used in Equation(2) to calculate inbreeding load.

Mutation

As for the Infinite simulations, initial conditions for the mutation frequency follow that of Ohta and Cockerham (1974) with q, the initial probability of being heterozygous, equal to u/hs, where u, h, and s are as defined above. An individual is chosen at random based on a random number. If the value of the random number is less than q, a mutation is added, making the selected locus heterozygous. These initial genotype frequencies are assigned prior to the start of looping over generations and at the start of each new replicate.

Mutations do not follow an exact distribution but rather are assigned based on random numbers and comparisons of the random number to the mitotic mutation rate. Each position in the genome is designated as being in one of three states; 0 = no mutations (or homozygous normal); 1 = one mutation (heterozygote); 2 = two mutations (homozygous mutant). A position in the genome is selected at random from a random number generator. Any of the 100 total loci can receive mutations. If the value of the random number is less than the mitotic mutation rate (v) and the position at the genome currently has no mutations, a mutation is added. Also if the value of

the random number is less than half the mitotic mutation rate and the position at the genome has one mutation, another mutation is added. The model is one of one locus with two alleles so if the locus is homozygous for the mutant allele, no further mutations are added.

Reproduction

Asexual reproduction

Once mitotic mutations have been added to all individuals in the population, the program cycles over the number of individuals manually designated for each type of reproduction. Asexual reproduction was modeled after plants that reproduce asexually through bulbil production, as found in the asexual *Mimulus gemmiparus* (Moody *et al*. 1999). Other models that incorporate asexual reproduction (Pamilo *et al*. 1987 and Hopf *et al*. 1988) assume that asexual reproduction occurs through either apomixis or parthenogenesis. With these two forms of asexual reproduction, unreduced gametes (gametes that for some reason fail to fully completed meiosis) are produced. Modeling asexual reproduction through bulbil production is a simpler situation to model because new zygotic progeny are produced through mitotic growth, as opposed to incomplete meiotic reproduction. A random number is used to randomly select an individual from the $N = 0$ to $N = N_a$ possible individuals for asexual reproduction. If the value of the random number is less than zero or greater than N_{tot} (where $N_{tot} = N_a + N_s + N_o$, the total population size), a new random number, and a new individual are selected. Otherwise, the genotype of the selected asexual individual becomes the genotype of this individual for the next generation.

Meiotic mutations are then added to the remaining individuals in the population. A position in the genome is selected at random from a random number generator. Any of the 100 total loci can receive meiotic mutations. If the value of the random number is less than the meiotic mutation rate (u) and the locus currently has no mutations, or if the value of the random number is less than half the meiotic mutation rate and the locus has one mutation, a mutation is added.

Selfing

A random number is used to randomly select an individual from the $N = N_a$ to $N = N_a + N_s$ for selfing. If the value of the random number is less than zero or greater than N_{tot}, a new random number, and a new individual, are chosen for selfing. If the random number is valid (between zero and N_{tot}), the chosen individual's genotype is taken into consideration. If the genotype is homozygous for no mutants, or homozygous for two mutations, then the genotype of the chosen individual remains the same for the next generation. If the chosen individual's genotype is heterozygous for a mutation, then a new random number is selected to determine the genotype for this individual for the next generation. If the value of the randomly selected number is less than 0.25, then the genotype of this individual for the next generation is homozygous for no mutations. If the value of the randomly selected number is greater than 0.75, then the genotype of this individual for the next generation is homozygous for two mutations. If the value of the randomly selected number is between 0.25 and 0.75, the individual's genotype will remain heterozygous. These

equalities are based on the expected proportion of offspring generated by a one-locus, two allele double heterozygote mating: 0.25 of the offspring will be homozygous for no mutations, 0.5 of the offspring will be heterozygous for one mutation, and 0.75 of the offspring will be homozygous for two mutations.

Outcrossing

The outcrossing loop cycles over the $N = N_a + N_s$ to $N = N_{tot}$ individuals remaining in the population. Two random numbers are drawn to select the individuals involved in outcrossing. Genotypes for each of the selected parents are considered separately in order to produce progeny. For a heterozygous parent, if the value of a randomly selected number is less than 0.5 (because half of the gametes produced by this individual will include the mutant locus), then a mutation is added to the progeny genotype at this locus. For a parent that is homozygous for two mutations, one mutation is added to the progeny genotype at this locus.

Selection

Once mutations have been added, and reproduction has occurred, the fitness of each individual is calculated (Equation 1) and a new generation begins with the deleterious allele frequencies from the previous generation representing the deleterious allele frequencies for the current generation. Once the program cycles over all generations and all replications, the Q values for generation 50,000 are averaged across the 5 replicates and are used in Equation 2 to calculate inbreeding load.

Results

Results are presented both in terms of Q and in terms of β, the inbreeding load. Presenting the results in terms of Q shows how the frequency of deleterious alleles changes as the strength of selection and dominance coefficient change; presenting these results illustrates how many mutations accumulate and fix over a complete run. Presenting the results in terms of β displays how inbreeding load changes as the strength of selection and dominance coefficient change. These results illustrate how the number of deleterious mutations and the different proportions of reproduction influence load.

Infinite population simulations

As the strength of selection increases, there is a decrease in Q, the average number of deleterious mutations per gamete (Figures 4-1a,b; Table 4-1). Likewise, for a given strength of selection, increasing the amount of asexual reproduction increases Q, but only for $h \leq 0.1$ (Table 4-1). For $h \geq 0.2$, increasing the amount of asexual reproduction decreases Q, for a given strength of selection (Table 4-1).

Figure 4-1. Comparison of average deleterious mutations per gamete (*Q)* for two dominance levels ($h = 0.1$ and $h = 0.5$) and different levels of asexual reproduction as the strength of selection increases (Infinite simulations).

Fig. 4-1a.

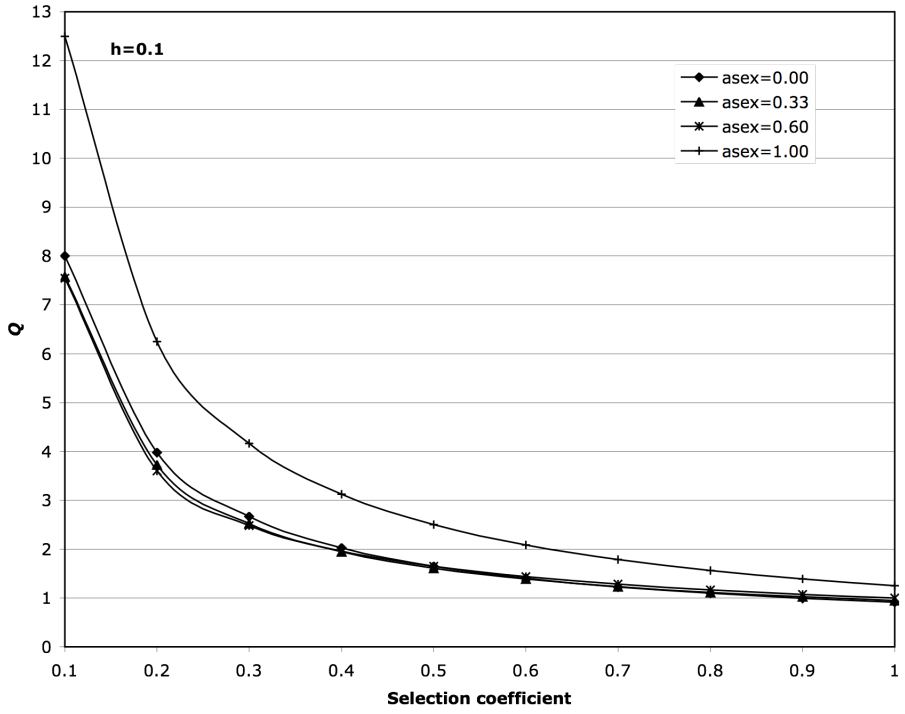
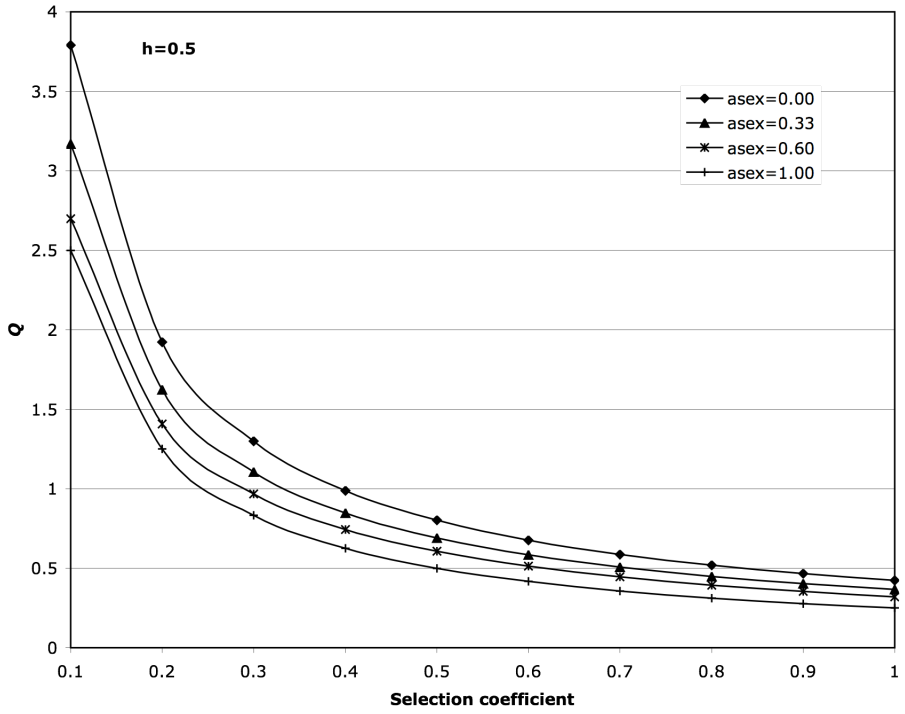


Fig. 4-1b.



For the infinite case, there appears to be conflicting effects of dominance and selection. For strongly recessive alleles, (h = 0.05) increasing asexuality leads to higher levels of inbreeding load (Table 4-2). This result supports that found by Muirhead and Lande (1997): for highly recessive lethal alleles, increasing asexuality results in an increase in load.

Table 4-1. Average deleterious mutations per gamete (Q) for increasing selection and dominance across different amounts of asexual reproduction (Infinite simulations).

h	*s*	asex=0	asex=0.2	asex=0.33	asex=0.4	asex=0.6	asex=0.8	asex=1.0
0.05	0.1	10.1168	10.3651	11.5900	11.8333	19.731	16.0191	25
0.05	0.2	4.9458	4.9464	5.2720	5.1906	6.136	7.404	12.5
0.05	0.3	3.2731	3.2564	3.4130	3.3569	3.7218	4.6058	8.3333
0.05	0.4	2.465	2.4651	2.5777	2.5541	2.8331	3.5223	6.25
0.05	0.5	1.9949	2.0135	2.1117	2.1079	2.3555	2.9273	5
0.05	0.6	1.6895	1.7233	1.8157	1.8238	2.0517	2.5405	4.1667
0.05	0.7	1.476	1.5218	1.6107	1.6264	1.8385	2.2636	3.5714
0.05	0.8	1.3187	1.3739	1.4610	1.4808	1.679	2.053	3.125
0.05	0.9	1.1983	1.2609	1.3457	1.3688	1.5542	1.8858	2.7778
0.05	1	1.1033	1.1719	1.2550	1.2798	1.4533	1.749	2.5
0.1	0.1	8.0023	7.6534	7.5760	7.4411	7.5446	7.9518	12.5
0.1	0.2	3.9798	3.7877	3.7273	3.6425	3.5996	3.8301	6.25
0.1	0.3	2.6686	2.5526	2.5227	2.4761	2.4849	2.7116	4.1667
0.1	0.4	2.0273	1.9564	1.9463	1.9214	1.9579	2.1606	3.125
0.1	0.5	1.6496	1.6074	1.6100	1.597	1.6457	1.8214	2.5
0.1	0.6	1.4014	1.3786	1.3890	1.3832	1.4361	1.5868	2.0833
0.1	0.7	1.2262	1.2169	1.2320	1.2308	1.2838	1.4126	1.7857
0.1	0.8	1.0959	1.0964	1.1143	1.1162	1.1672	1.2768	1.5625
0.1	0.9	0.9954	1.0031	1.0227	1.0263	1.0742	1.1674	1.3889
0.1	1	0.9153	0.9286	0.9490	0.9538	0.998	1.0768	1.25
0.2	0.1	6.0081	5.5328	5.2440	5.0765	4.6633	4.3892	6.25
0.2	0.2	3.0288	2.7993	2.6667	2.589	2.4234	2.3857	3.125
0.2	0.3	2.0489	1.9083	1.8317	1.7866	1.7028	1.7085	2.0833
0.2	0.4	1.5643	1.4689	1.4190	1.3899	1.3405	1.3525	1.5625
0.2	0.5	1.2757	1.207	1.1720	1.1518	1.1187	1.1278	1.25
0.2	0.6	1.0842	1.0327	1.0073	0.9917	0.967	0.9709	1.0417
0.2	0.7	0.9478	0.908	0.8880	0.876	0.8556	0.8542	0.8929
0.2	0.8	0.8456	0.8141	0.7977	0.788	0.7696	0.7636	0.7812
0.2	0.9	0.7661	0.7406	0.7270	0.7185	0.701	0.691	0.6944
0.2	1	0.7024	0.6814	0.6697	0.6619	0.6447	0.6313	0.625
0.3	0.1	4.9576	4.5072	4.2190	4.0677	3.6562	3.3472	4.1667
0.3	0.2	2.5116	2.2958	2.1630	2.0941	1.9222	1.8257	2.0833
0.3	0.3	1.7025	1.5672	1.4860	1.4444	1.3444	1.2917	1.3889
0.3	0.4	1.3	1.2045	1.1483	1.1189	1.0496	1.0096	1.0417
0.3	0.5	1.059	0.9867	0.9440	0.9218	0.868	0.8323	0.8333
0.3	0.6	0.8984	0.8409	0.8067	0.7886	0.7437	0.7096	0.6944
0.3	0.7	0.7836	0.7361	0.7073	0.6921	0.6525	0.6191	0.5952
0.3	0.8	0.6973	0.6569	0.6320	0.6186	0.5825	0.5496	0.5208
0.3	0.9	0.6299	0.5948	0.5723	0.5605	0.5269	0.4943	0.463
0.3	1	0.5758	0.5447	0.5240	0.5133	0.4815	0.4493	0.4167
0.4	0.1	4.2777	3.8656	3.5983	3.463	3.084	2.7817	3.125
0.4	0.2	2.1701	1.9717	1.8470	1.7844	1.6182	1.5	1.5625
0.4	0.3	1.4704	1.3436	1.2653	1.2259	1.1229	1.0473	1.0417
0.4	0.4	1.121	1.0294	0.9727	0.9445	0.8693	0.8098	0.7813
0.4	0.5	0.9113	0.8401	0.7957	0.7736	0.7133	0.6619	0.625
0.4	0.6	0.7712	0.7131	0.6767	0.6581	0.6069	0.5605	0.5208
0.4	0.7	0.6708	0.6217	0.5903	0.5744	0.5292	0.4864	0.4464
0.4	0.8	0.5953	0.5526	0.5250	0.5108	0.4699	0.4298	0.3906
0.4	0.9	0.5362	0.4984	0.4733	0.4607	0.423	0.3852	0.3472
0.4	1	0.4888	0.4547	0.4317	0.4201	0.3849	0.349	0.3125
0.5	0.1	3.7902	3.4137	3.1683	3.046	2.6982	2.4078	2.5
0.5	0.2	1.9218	1.739	1.6227	1.5651	1.4067	1.2795	1.25
0.5	0.3	1.2999	1.1814	1.1067	1.0697	0.9681	0.883	0.8333
0.5	0.4	0.9887	0.9018	0.8470	0.8197	0.7438	0.677	0.625
0.5	0.5	0.8017	0.7332	0.6897	0.6679	0.6064	0.5499	0.5
0.5	0.6	0.6766	0.62	0.5837	0.5654	0.5131	0.4634	0.4167
0.5	0.7	0.587	0.5386	0.5070	0.4914	0.4454	0.4007	0.3571
0.5	0.8	0.5195	0.4771	0.4490	0.4353	0.3939	0.353	0.3125
0.5	0.9	0.4668	0.4289	0.4040	0.3911	0.3534	0.3156	0.2778
0.5	1	0.4244	0.3901	0.3673	0.3555	0.3206	0.2855	0.25

There is no change in load for fully asexual populations (asex = 1.0) as mutations become more and more deleterious, approaching lethality (Figure 4-2a).

Figure 4-2. Comparison of inbreeding load (β) across different dominance levels and for different levels of asexual reproduction as the strength of selection increases.

Fig. 4-2a.

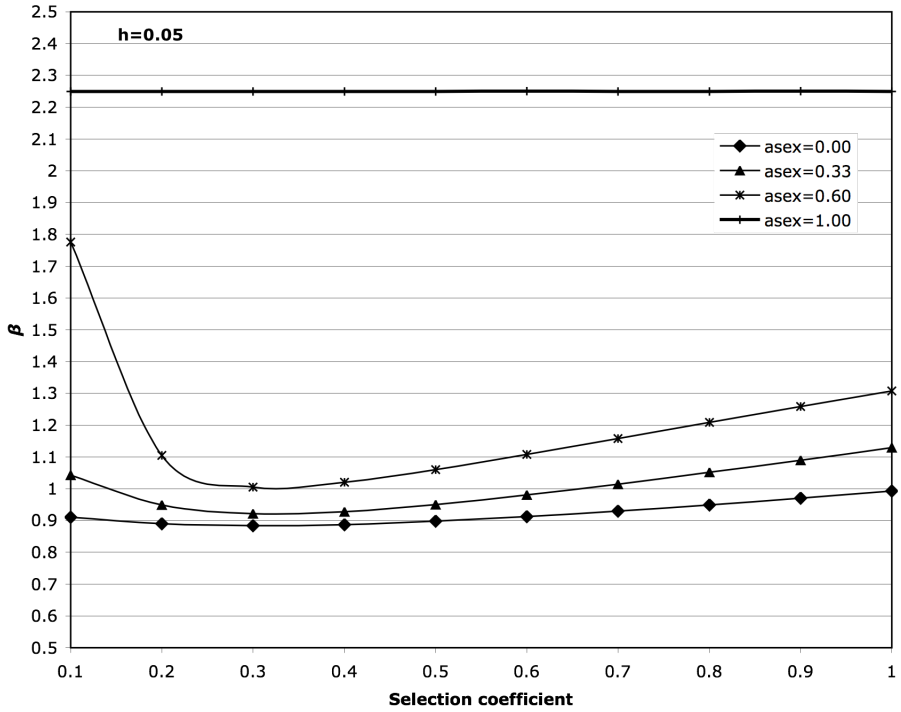


Fig. 4-2b.

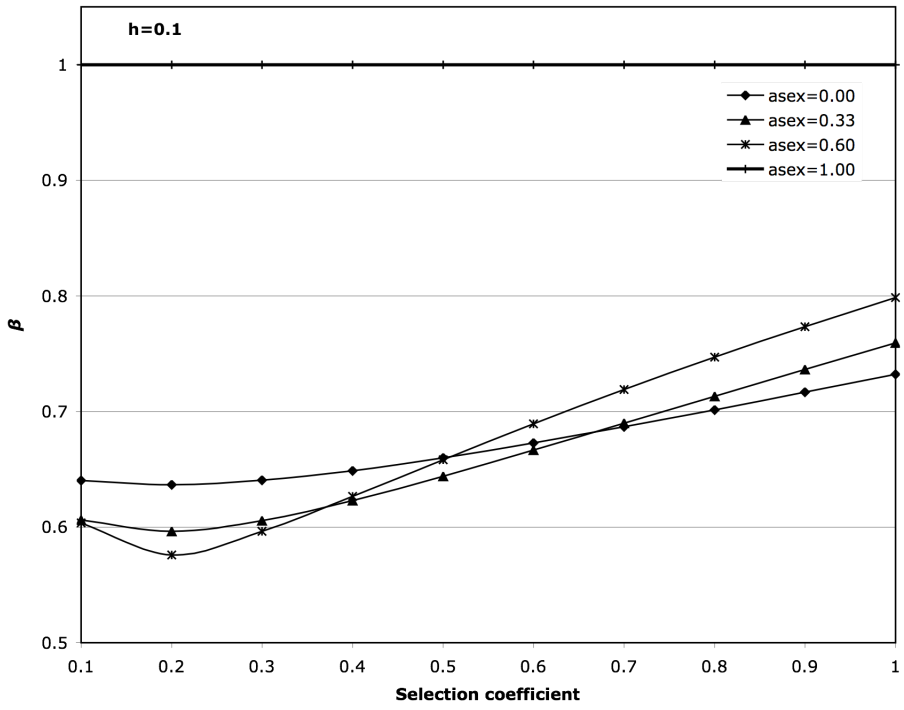

h=0.1
asex=0.00
asex=0.33
asex=0.60
asex=1.00
β
1
0.9
0.8
0.7
0.6
0.5
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Selection coefficient

Fig. 4-2c.

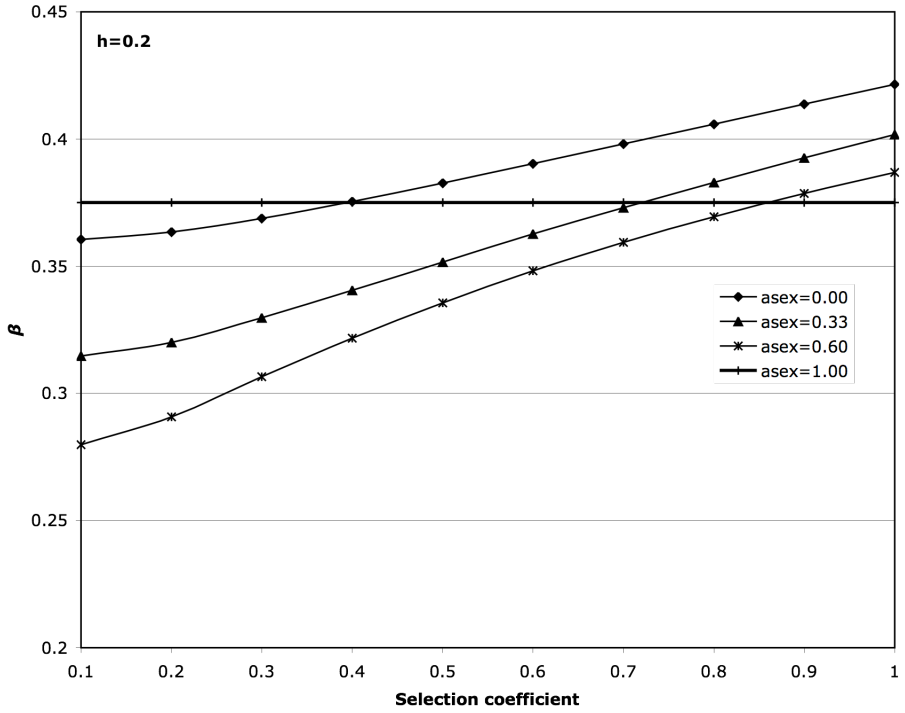

h=0.2
0.45
0.4
0.35
0.3
0.25
0.2
β
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Selection coefficient
asex=0.00
asex=0.33
asex=0.60
asex=1.00

Fig. 4-2d.

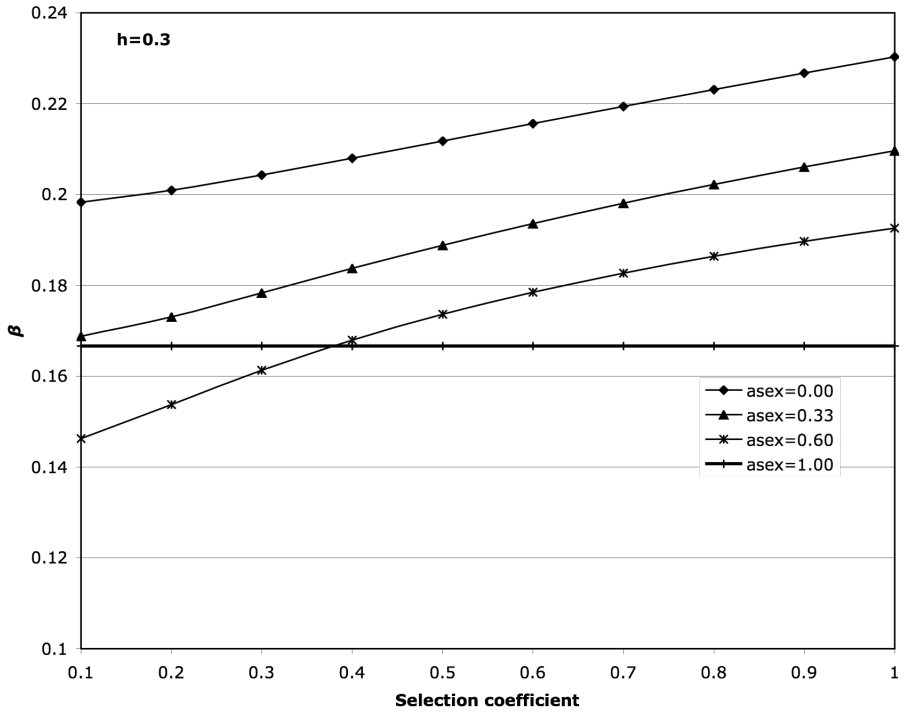
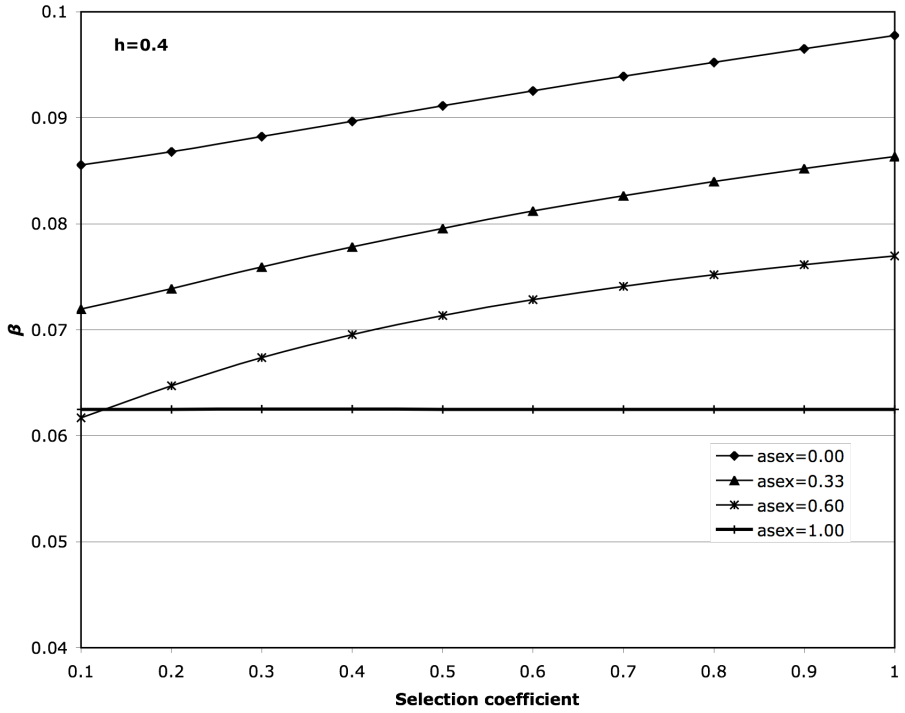

h=0.3
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
β
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Selection coefficient
asex=0.00
asex=0.33
asex=0.60
asex=1.00

Fig. 4-2e.



As mutations become less recessive, specifically for $h \geq 0.20$, there is a decrease in load as asexuality increases. The highest amount of inbreeding load is seen for the case of no asexual reproduction (Figures 4-2c-4-2e). For $h = 0.20$, complete asexual reproduction has the lowest load only for almost lethal and completely lethal alleles (Fig. 4-2c). For $h = 0.30$, complete asexual reproduction has the lowest amount of load for $s \geq 0.50$ (Fig. 4-2d). For $h = 0.40$, complete asexual reproduction has the lowest load for $s \geq 0.30$ (Fig. 4-2e).

Table 4-2. Inbreeding load (β) for increasing selection and dominance across different amounts of asexual reproduction (Infinite simulations).

h	s	asex=0	asex=0.2	asex=0.33	asex=0.4	asex=0.6	asex=0.8	asex=1.0
0.05	0.1	0.9105	0.9329	1.0431	1.0650	1.7758	1.4417	2.2500
0.05	0.2	0.8902	0.8904	0.9490	0.9343	1.1045	1.3327	2.2500
0.05	0.3	0.8837	0.8792	0.9215	0.9064	1.0049	1.2436	2.2500
0.05	0.4	0.8874	0.8874	0.9280	0.9195	1.0199	1.2680	2.2500
0.05	0.5	0.8977	0.9061	0.9503	0.9486	1.0600	1.3173	2.2500
0.05	0.6	0.9123	0.9306	0.9805	0.9849	1.1079	1.3719	2.2500
0.05	0.7	0.9299	0.9587	1.0147	1.0246	1.1583	1.4261	2.2500
0.05	0.8	0.9495	0.9892	1.0519	1.0662	1.2089	1.4782	2.2500
0.05	0.9	0.9706	1.0213	1.0900	1.1087	1.2589	1.5275	2.2500
0.05	1	0.9930	1.0547	1.1295	1.1518	1.3080	1.5741	2.2500
0.1	0.1	0.6402	0.6123	0.6061	0.5953	0.6036	0.6361	1.0000
0.1	0.2	0.6368	0.6060	0.5964	0.5828	0.5759	0.6128	1.0000
0.1	0.3	0.6405	0.6126	0.6054	0.5943	0.5964	0.6508	1.0000
0.1	0.4	0.6487	0.6260	0.6228	0.6148	0.6265	0.6914	1.0000
0.1	0.5	0.6598	0.6430	0.6440	0.6388	0.6583	0.7286	1.0000
0.1	0.6	0.6727	0.6617	0.6667	0.6639	0.6893	0.7617	1.0000
0.1	0.7	0.6867	0.6815	0.6899	0.6892	0.7189	0.7911	1.0000
0.1	0.8	0.7014	0.7017	0.7132	0.7144	0.7470	0.8172	1.0000
0.1	0.9	0.7167	0.7222	0.7363	0.7389	0.7734	0.8405	1.0000
0.1	1	0.7322	0.7429	0.7592	0.7630	0.7984	0.8614	1.0000
0.2	0.1	0.3605	0.3320	0.3146	0.3046	0.2798	0.2634	0.3750
0.2	0.2	0.3635	0.3359	0.3200	0.3107	0.2908	0.2863	0.3750
0.2	0.3	0.3688	0.3435	0.3297	0.3216	0.3065	0.3075	0.3750
0.2	0.4	0.3754	0.3525	0.3406	0.3336	0.3217	0.3246	0.3750
0.2	0.5	0.3827	0.3621	0.3516	0.3455	0.3356	0.3383	0.3750
0.2	0.6	0.3903	0.3718	0.3626	0.3570	0.3481	0.3495	0.3750
0.2	0.7	0.3981	0.3814	0.3730	0.3679	0.3594	0.3588	0.3750
0.2	0.8	0.4059	0.3908	0.3829	0.3782	0.3694	0.3665	0.3750
0.2	0.9	0.4137	0.3999	0.3926	0.3880	0.3785	0.3731	0.3750
0.2	1	0.4214	0.4088	0.4018	0.3971	0.3868	0.3788	0.3750
0.3	0.1	0.1983	0.1803	0.1688	0.1627	0.1462	0.1339	0.1667
0.3	0.2	0.2009	0.1837	0.1730	0.1675	0.1538	0.1461	0.1667
0.3	0.3	0.2043	0.1881	0.1783	0.1733	0.1613	0.1550	0.1667
0.3	0.4	0.2080	0.1927	0.1837	0.1790	0.1679	0.1615	0.1667
0.3	0.5	0.2118	0.1973	0.1888	0.1844	0.1736	0.1665	0.1667
0.3	0.6	0.2156	0.2018	0.1936	0.1893	0.1785	0.1703	0.1667
0.3	0.7	0.2194	0.2061	0.1981	0.1938	0.1827	0.1733	0.1667
0.3	0.8	0.2231	0.2102	0.2022	0.1980	0.1864	0.1759	0.1667
0.3	0.9	0.2268	0.2141	0.2060	0.2018	0.1897	0.1779	0.1667
0.3	1	0.2303	0.2179	0.2096	0.2053	0.1926	0.1797	0.1667
0.4	0.1	0.0856	0.0773	0.0720	0.0693	0.0617	0.0556	0.0625
0.4	0.2	0.0868	0.0789	0.0739	0.0714	0.0647	0.0600	0.0625
0.4	0.3	0.0882	0.0806	0.0759	0.0736	0.0674	0.0628	0.0625
0.4	0.4	0.0897	0.0824	0.0778	0.0756	0.0695	0.0648	0.0625
0.4	0.5	0.0911	0.0840	0.0796	0.0774	0.0713	0.0662	0.0625
0.4	0.6	0.0925	0.0856	0.0812	0.0790	0.0728	0.0673	0.0625
0.4	0.7	0.0939	0.0870	0.0826	0.0804	0.0741	0.0681	0.0625
0.4	0.8	0.0952	0.0884	0.0840	0.0817	0.0752	0.0688	0.0625
0.4	0.9	0.0965	0.0897	0.0852	0.0829	0.0761	0.0693	0.0625
0.4	1	0.0978	0.0909	0.0863	0.0840	0.0770	0.0698	0.0625

Finite population simulations

All results presented for Finite simulations are based on generation 50,000 Q values averaged across all 5 replications. Increasing asexuality results in a slight decrease in Q, the average number of deleterious mutations per gamete, for all finite population sizes (Fig. 4-3a, 4-3b; Table 4-3). Table 4-3 gives results in terms of both the absolute selection coeffecient (s) and the scaled selection coefficient (Ns). Increasing the total population size also results in a decrease in Q (Fig. 4-3a, 4-3b; Table 4-3). Results are presented in Figure 4-3 are for h=0.1. Results for increasing h (decreasing recessivity) follow a similar trend as h = 0.1, except that with increases in h, there are fewer average deleterious mutations per gamete (decrease in Q with increasing h, Table 4-3).

Simulations for a finite population size of N= 30 have the greatest amount of inbreeding load (Fig. 4-4a, b, c; Table 4-4) and the amount of inbreeding load decreases as the population size increases, as expected. Once the finite population size exceeds approximately 100, the amount of inbreeding load is similar to the load for an infinite population size. Increasing asexuality results in a decrease of β (Fig. 4-4d, e, f; Table 4-4).

Figure 4-3. Effect of increasing selection on *Q*, average number of deleterious mutations per gamete, for varying levels of asexual reproduction and finite population sizes. Error bars in Fig. 3a, 3b represent ± 1 standard deviation.

Fig. 4-3a. Values of *Q* for N=90, N=150 are similar to that for N=300 (Table 4-3).

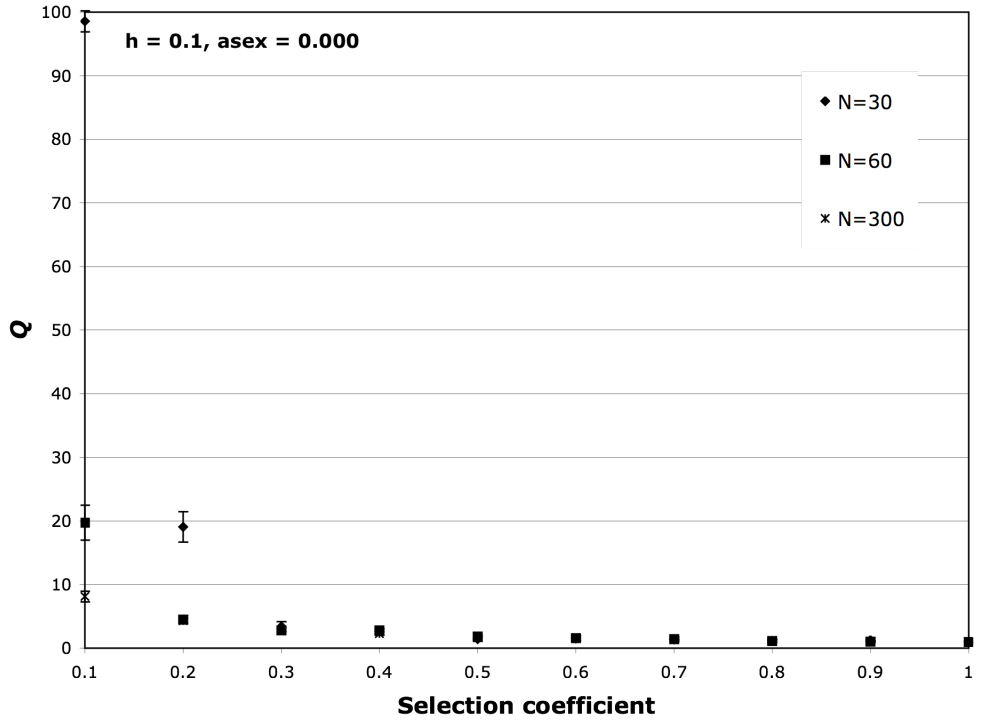


Fig. 4-3b. Values of *Q* for N=90, N=150 are similar to that for N=300 (Table 4-3).

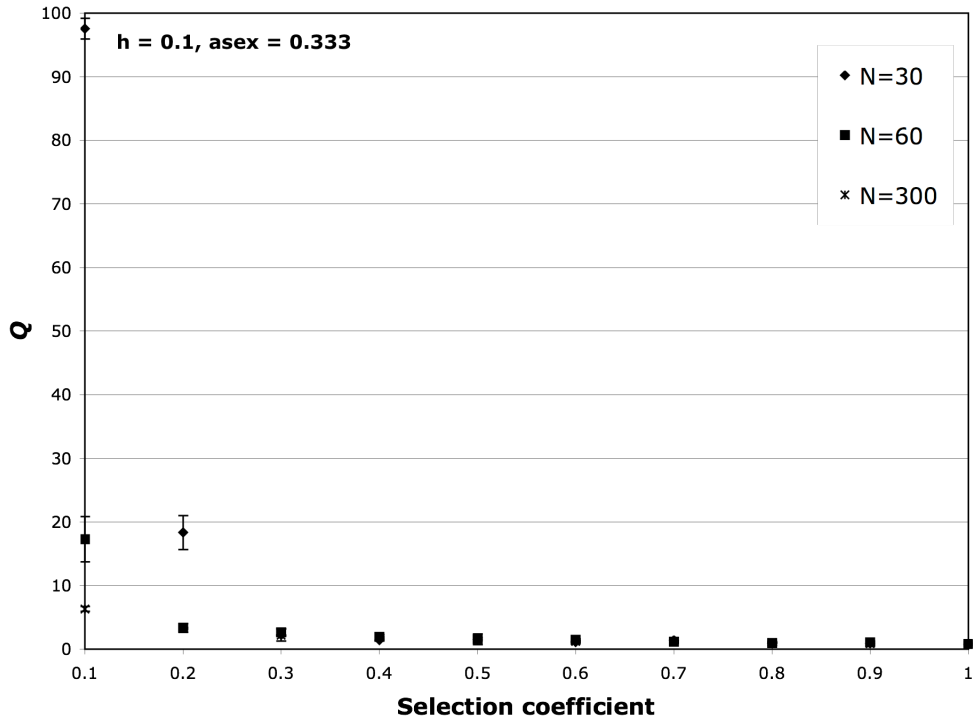


Table 4-3. Effect on *Q* for different population sizes, varying dominance and selection. Values of *Q* for asex=0.000 are given. The values in parentheses represent the change in *Q* with asex=0.333.

			***N* = 30**	***N* = 60**	***N* = 90**	***N* =150**	***N* = 300**
s	***N*s***	***h***					
0.1	3	0.1	98.57075				
			(-0.99575)				
		0.2	99.0066				
			(-1.8266)				
		0.3	98.8468				
			(-2.9068)				
		0.4	99.3468				
			(-3.3768)				
		0.5	99.12				
			(-3.2966)				
	6	0.1		19.7484			
				(-2.4684)			
		0.2		26.3664			
				(-8.4328)			
		0.3		24.1666			
				(-1.5282)			
		0.4		29.5982			
				(-4.7048)			
		0.5		28.415			
				(-0.385)			
	9	0.1			8.5524		
					(-1.6556)		
		0.2			7.181		
					(-1.4276)		
		0.3			6.9534		
					(-2.1432)		
		0.4			5.6366		
					(-0.3688)		
		0.5			5.1454		
					(-0.291)		
	15	0.1				8.0612	
						(-1.6784)	
		0.2				6.5274	
						(-1.261)	
		0.3				6.1754	
						(-1.7412)	

		0.4				5.588	
						(-1.5178)	
		0.5				5.39	
						(-2.16)	
	30	0.1					8.1232
							(-1.8026)
		0.2					6.8396
							(-1.9738)
		0.3					5.3842
							(-1.3768)
		0.4					5.3956
							(-1.6994)
		0.5					4.8872
							(-1.7182)
0.2	6	0.1	19.06675				
			(-0.73325)				
		0.2	22.5402				
			(-1.9236)				
		0.3	25.94				
			(-4.5)				
		0.4	31.8166				
			(-5.5998)				
		0.5	34.4668				
			(-2.5534)				
	12	0.1		4.4882			
				(-1.1582)			
		0.2		3.7468			
				(-1.1836)			
		0.3		3.4218			
				(-1.0636)			
		0.4		3.0302			
				(-1.0354)			
		0.5		2.86			
				(-0.8282)			
	18	0.1			4.8644		
					(-1.5246)		
		0.2			3.5276		
					(-0.9898)		
		0.3			3.2234		
					(-0.9744)		
		0.4			2.3812		
					(-0.691)		
		0.5			2.3232		
					(-0.753)		

	30	0.1				4.5022	
						(-1.0616)	
		0.2				3.328	
						(-0.8086)	
		0.3				3.1142	
						(-0.911)	
		0.4				2.7028	
						(-0.9242)	
		0.5				2.3394	
						(-0.6628)	
	60	0.1					4.3326
							(-1.0564)
		0.2					3.2978
							(-0.7188)
		0.3					3.0844
							(-0.8472)
		0.4					2.5882
							(-0.6352)
		0.5					2.3872
							(-0.8002)
0.3	9	0.1	3.3875				
			(-1.1665)				
		0.2	2.9532				
			(-0.9064)				
		0.3	3.84				
			(-1.57)				
		0.4	3.2434				
			(-0.2434)				
		0.5	5				
			(-1.88)				
	18	0.1		2.8016			
				(-0.1748)			
		0.2		2.66			
				(-0.8052)			
		0.3		2.1168			
				(-0.665)			
		0.4		1.8898			
				(-0.5466)			
		0.5		1.785			
				(-0.715)			
	27	0.1			2.8156		
					(-0.397)		
		0.2			2.2064		
					(-0.3586)		

		0.3			1.96		
					(-0.4156)		
		0.4			1.6912		
					(-0.4722)		
		0.5			1.4878		
					(-0.371)		
	45	0.1				3.0446	
						(-0.847)	
		0.2				2.494	
						(-0.6928)	
		0.3				1.9354	
						(-0.4554)	
		0.4				1.684	
						(-0.3586)	
		0.5				1.6788	
						(-0.6242)	
	90	0.1					2.9216
							(-0.7522)
		0.2					2.3
							(-0.5416)
		0.3					1.956
							(-0.575)
		0.4					1.817
							(-0.6328)
		0.5					1.6262
							(-0.458)
0.4	12	0.1	2.5915				
			(-1.18325)				
		0.2	1.43				
			(0.0068)				
		0.3	1.6334				
			(-0.4536)				
		0.4	1.5468				
			(-0.0768)				
		0.5	1.25				
			(-0.26)				
	24	0.1		2.7984			
				(-0.8734)			
		0.2		2.025			
				(-0.5982)			
		0.3		1.3784			
				(-0.2016)			
		0.4		1.125			
				(0.0166)			

		0.5		1.3434			
				(-0.3784)			
	36	0.1			2.4188		
					(-0.562)		
		0.2			1.7788		
					(-0.3758)		
		0.3			1.4578		
					(-0.1988)		
		0.4			1.3056		
					(-0.231)		
		0.5			1.1434		
					(-0.3088)		
	60	0.1				2.4488	
						(-0.7414)	
		0.2				1.7576	
						(-0.4282)	
		0.3				1.4614	
						(-0.278)	
		0.4				1.4374	
						(-0.4888)	
		0.5				1.146	
						(-0.3146)	
	120	0.1					2.2732
							(-0.3806)
		0.2					1.86
							(-0.415)
		0.3					1.477
							(-0.3394)
		0.4					1.2962
							(-0.2824)
		0.5					1.1294
							(-0.2516)
0.5	15	0.1	1.44575				
			(0.10425)				
		0.2	1.37				
			(-0.1534)				
		0.3	1.2966				
			(-0.2834)				
		0.4	0.9132				
			(0)				
		0.5	1.12				
			(-0.2266)				
	30	0.1		1.8484			
				(-0.2782)			

		0.2		1.6468			
				(-0.4136)			
		0.3		1.2934			
				(-0.4484)			
		0.4		1.125			
				(-0.3332)			
		0.5		1.1216			
				(-0.3216)			
	45	0.1			1.8768		
					(-0.268)		
		0.2			1.5934		
					(-0.4978)		
		0.3			1.3634		
					(-0.5834)		
		0.4			0.9966		
					(-0.102)		
		0.5			0.9798		
					(-0.2932)		
	75	0.1				1.9132	
						(-0.2932)	
		0.2				1.5614	
						(-0.366)	
		0.3				1.1586	
						(-0.2358)	
		0.4				1.0092	
						(-0.2784)	
		0.5				0.882	
						(-0.1828)	
	150	0.1					1.8564
							(-0.2018)
		0.2					1.4034
							(-0.3072)
		0.3					1.2582
							(-0.347)
		0.4					1.013
							(-0.2288)
		0.5					0.9576
							(-0.3004)
0.6	18	0.1	1.54575				
			(-0.40425)				
		0.2	1.3468				
			(-0.33)				
		0.3	0.99				
			(-0.1566)				

		0.4	0.8034				
			(-0.08)				
		0.5	0.8932				
			(-0.1066)				
	36	0.1		1.5616			
				(-0.09)			
		0.2		1.3416			
				(-0.3848)			
		0.3		1.1366			
				(-0.3082)			
		0.4		0.88			
				(-0.2516)			
		0.5		0.8716			
				(-0.225)			
	54	0.1			1.5346		
					(-0.3524)		
		0.2			1.268		
					(-0.3192)		
		0.3			1.2276		
					(-0.453)		
		0.4			0.8602		
					(-0.297)		
		0.5			0.8368		
					(-0.3434)		
	90	0.1				1.6742	
						(-0.4568)	
		0.2				1.174	
						(-0.2954)	
		0.3				1.002	
						(-0.3022)	
		0.4				0.8992	
						(-0.2312)	
		0.5				0.8526	
						(-0.3274)	
	180	0.1					1.5558
							(-0.3146)
		0.2					1.2682
							(-0.3194)
		0.3					1.0584
							(-0.2696)
		0.4					0.9094
							(-0.2542)
		0.5					0.7722
							(-0.206)

0.7	21	0.1	1.3832				
			(0.0202)				
		0.2	0.96				
			(-0.0534)				
		0.3	0.89				
			(-0.3198)				
		0.4	0.8768				
			(-0.3034)				
		0.5	0.7832				
			(-0.2898)				
	42	0.1		1.4164			
				(-0.248)			
		0.2		1.2082			
				(-0.2584)			
		0.3		0.86			
				(-0.205)			
		0.4		0.7466			
				(-0.1116)			
		0.5		0.6966			
				(-0.15)			
	63	0.1			1.2266		
					(-0.1886)		
		0.2			1.0456		
					(-0.2534)		
		0.3			0.921		
					(-0.1974)		
		0.4			0.8212		
					(-0.2858)		
		0.5			0.6344		
					(-0.0776)		
	105	0.1				1.3368	
						(-0.1708)	
		0.2				1.0294	
						(-0.0926)	
		0.3				0.9214	
						(-0.228)	
		0.4				0.8298	
						(-0.2692)	
		0.5				0.6374	
						(-0.1466)	
	210	0.1					1.3026
							(-0.1514)
		0.2					1.0548
							(-0.2758)

		0.3					0.8486
							(-0.186)
		0.4					0.7692
							(-0.1498)
		0.5					0.681
							(-0.1818)
0.8	24	0.1	1.1534				
			(-0.23)				
		0.2	0.7866				
			(-0.1466)				
		0.3	0.7666				
			(-0.1534)				
		0.4	0.6168				
			(-0.1534)				
		0.5	0.4198				
			(-0.0398)				
	48	0.1		1.1068			
				(-0.1168)			
		0.2		1.1532			
				(-0.2614)			
		0.3		0.8318			
				(-0.1618)			
		0.4		0.5802			
				(-0.1118)			
		0.5		0.71			
				(-0.2684)			
	72	0.1			1.2154		
					(-0.0988)		
		0.2			1.0546		
					(-0.3804)		
		0.3			0.9278		
					(-0.2734)		
		0.4			0.6744		
					(-0.0934)		
		0.5			0.5946		
					(-0.159)		
	120	0.1				1.15	
						(-0.1692)	
		0.2				0.8706	
						(-0.152)	
		0.3				0.7514	
						(-0.1588)	
		0.4				0.7566	
						(-0.2468)	

		0.5				0.5806	
						(-0.1366)	
	240	0.1					1.1618
							(-0.1438)
		0.2					0.9208
							(-0.1932)
		0.3					0.7374
							(-0.1254)
		0.4					0.6904
							(-0.2312)
		0.5					0.6586
							(-0.2094)
0.9	27	0.1	1.2868				
			(-0.3832)				
		0.2	0.8866				
			(-0.16)				
		0.3	0.81				
			(-0.2566)				
		0.4	0.7366				
			(-0.4732)				
		0.5	0.4734				
			(-0.0534)				
	54	0.1		1.0348			
				(0.0168)			
		0.2		0.8632			
				(-0.1682)			
		0.3		0.5748			
				(-0.0564)			
		0.4		0.6666			
				(-0.2932)			
		0.5		0.5584			
				(-0.1718)			
	81	0.1			1.16		
					(-0.2056)		
		0.2			0.809		
					(-0.0956)		
		0.3			0.7368		
					(-0.2068)		
		0.4			0.6166		
					(-0.1232)		
		0.5			0.52		
					(-0.149)		
	135	0.1				1.0818	
						(-0.208)	

		0.2				0.7964	
						(-0.1072)	
		0.3				0.7304	
						(-0.2436)	
		0.4				0.6162	
						(-0.1508)	
		0.5				0.5396	
						(-0.1102)	
	270	0.1					1.0866
							(-0.2068)
		0.2					0.8716
							(-0.2018)
		0.3					0.6788
							(-0.1438)
		0.4					0.5862
							(-0.148)
		0.5					0.5246
							(-0.1486)
1.0	30	0.1	1.07				
			(-0.3134)				
		0.2	0.6566				
			(0.0466)				
		0.3	0.6766				
			(-0.2134)				
		0.4	0.44				
			(-0.0634)				
		0.5	0.5666				
			(-0.1868)				
	60	0.1		0.9666			
				(-0.1332)			
		0.2		0.6918			
				(-0.1018)			
		0.3		0.6986			
				(-0.1236)			
		0.4		0.4584			
				(-0.0816)			
		0.5		0.4218			
				(-0.0338)			
	90	0.1			0.9822		
					(-0.0932)		
		0.2			0.8178		
					(-0.16)		
		0.3			0.6834		
					(-0.2066)		

		0.4			0.6456		
					(-0.2546)		
		0.5			0.4622		
					(-0.142)		
	150	0.1				1.0592	
						(-0.1796)	
		0.2				0.7394	
						(-0.1382)	
		0.3				0.6388	
						(-0.133)	
		0.4				0.5946	
						(-0.1992)	
		0.5				0.49	
						(-0.1638)	
	300	0.1					0.9198
							(-0.0838)
		0.2					0.74
							(-0.1314)
		0.3					0.6406
							(-0.152)
		0.4					0.5254
							(-0.1078)
		0.5					0.4718
							(-0.1358)

Figure 4-4. Effect of increasing strength of selection on inbreeding load, for different finite population sizes and different amounts of asexual reproduction (a-c, asex=0.000; d-f, asex=0.333). Error bars indicate ± 1 standard deviation.

Fig. 4-4a.

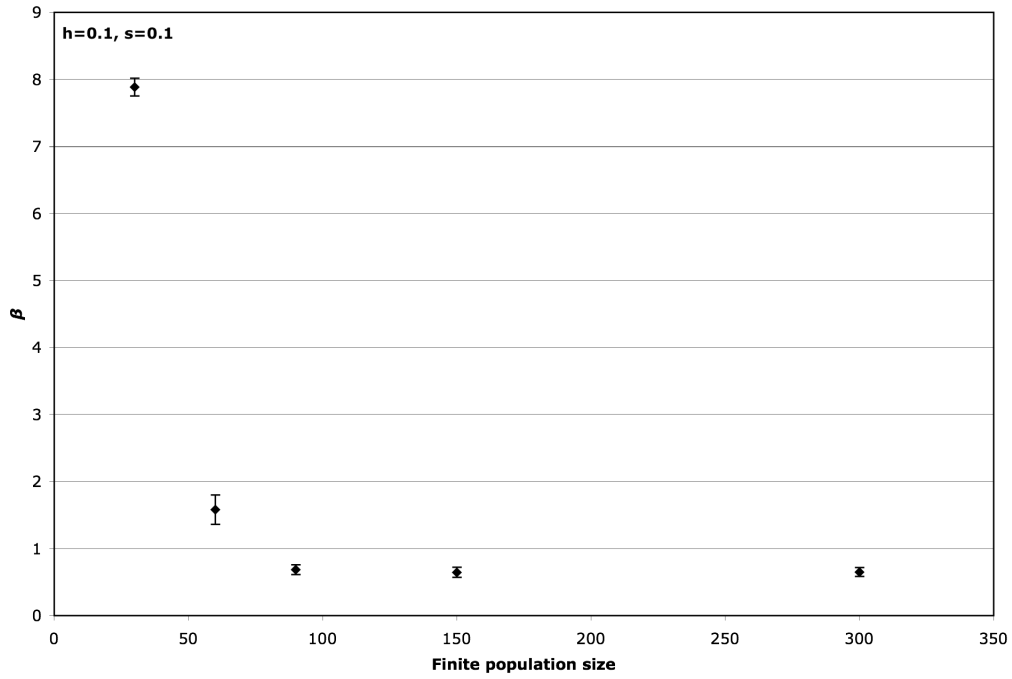


Fig. 4-4b.

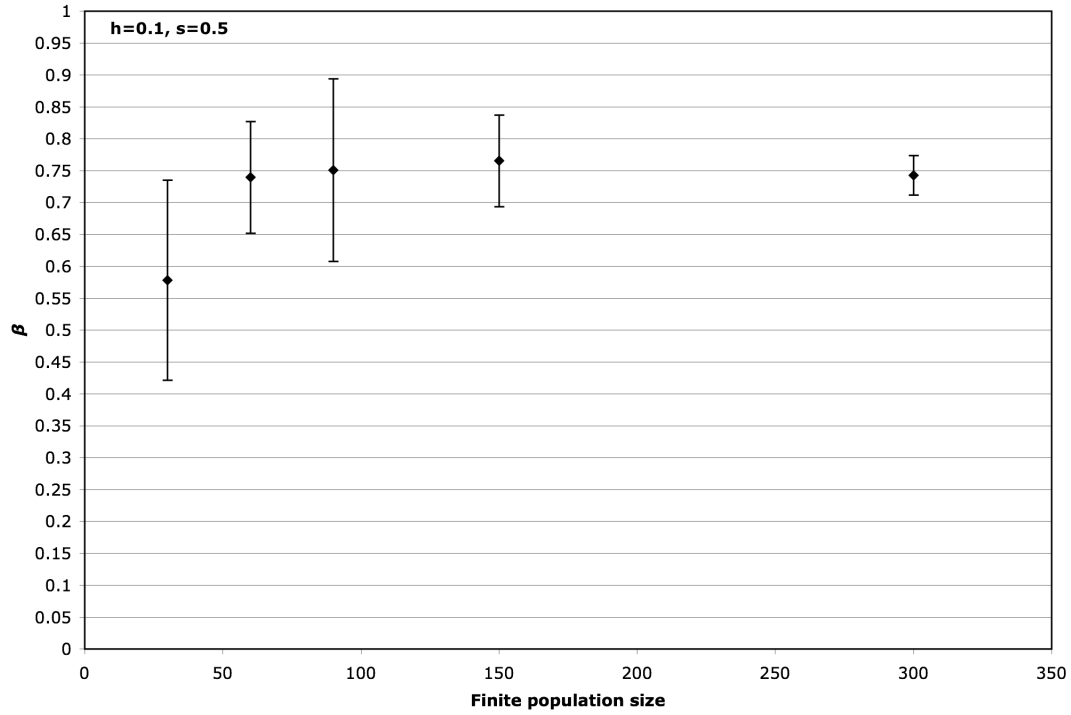

h=0.1, s=0.5
β
Finite population size
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1
0
50
100
150
200
250
300
350

Fig. 4-4c.

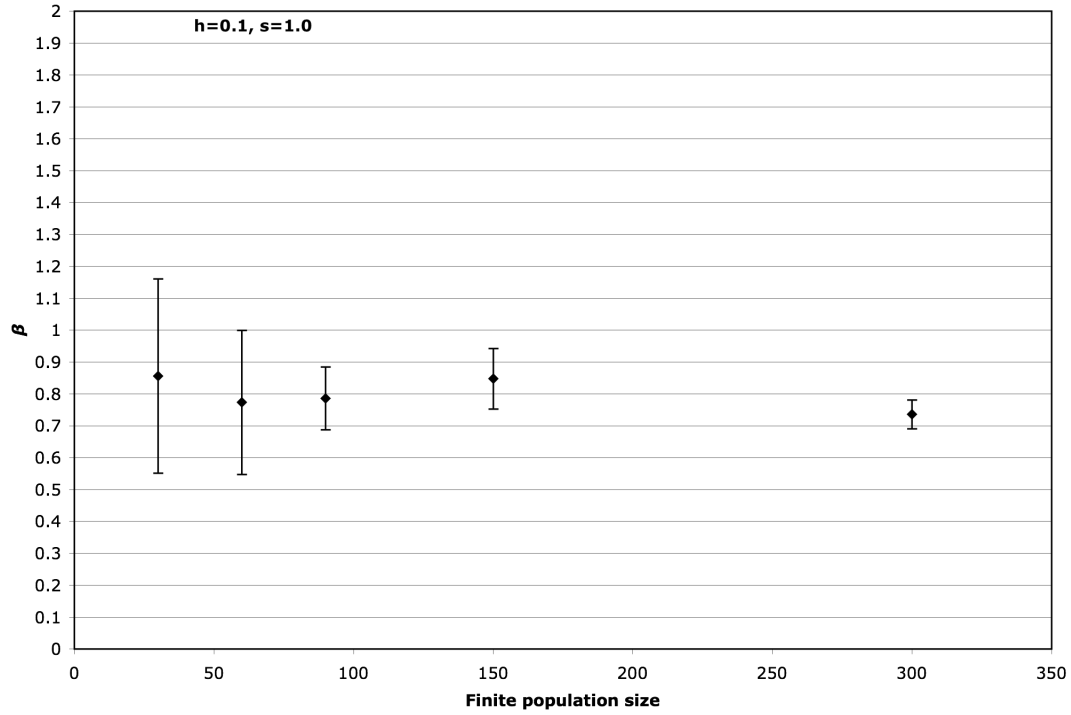

h=0.1, s=1.0
β
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
350
Finite population size

Fig. 4-4d.

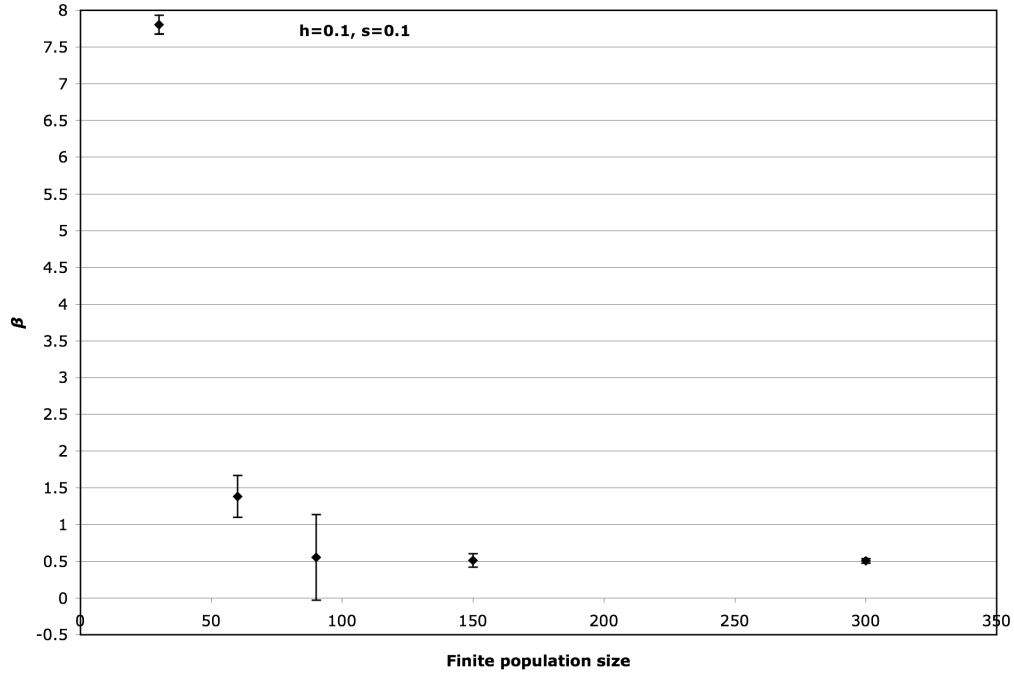

h=0.1, s=0.1
β
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
50
100
150
200
250
300
350
Finite population size

Fig. 4-4e.

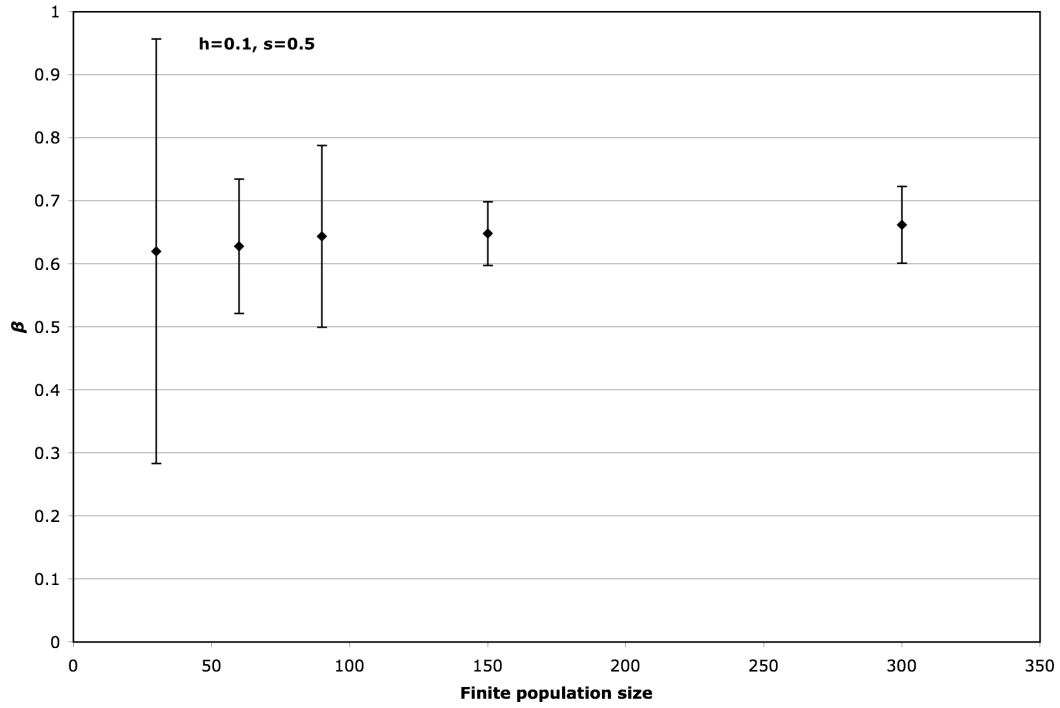

h=0.1, s=0.5
β
Finite population size
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
50
100
150
200
250
300
350

Fig. 4-4f.

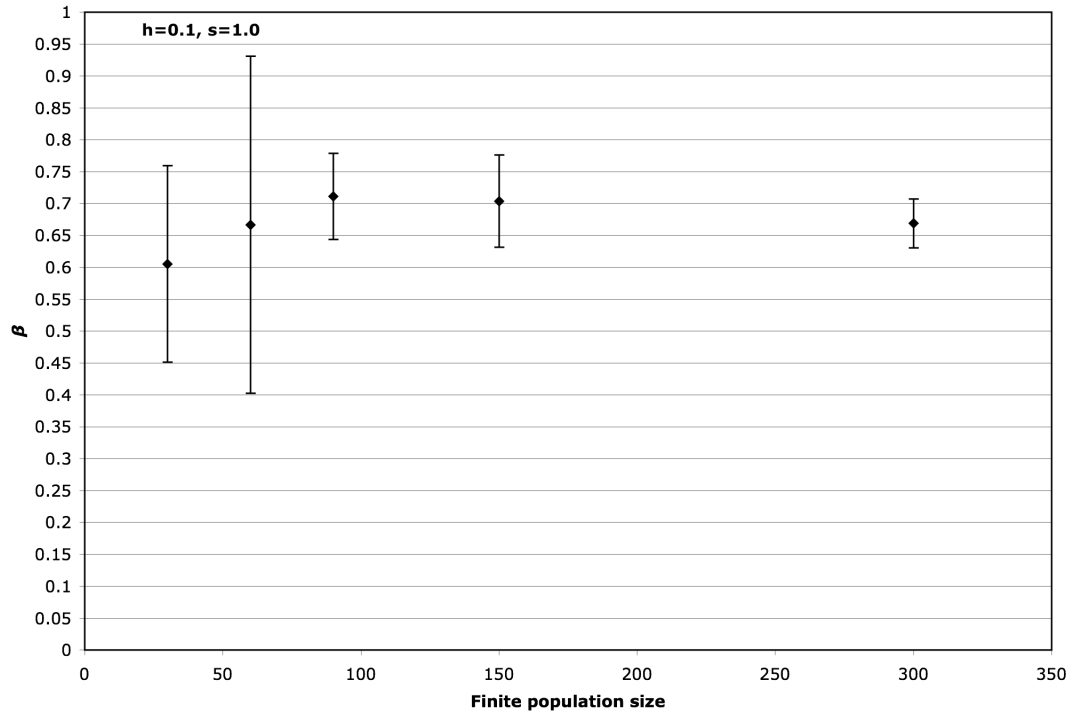

h=0.1, s=1.0
β
Finite population size
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1
0
50
100
150
200
250
300
350

Table 4-4. Inbreeding load for different finite population sizes, strengths of selection, dominance coefficients and levels of asexual reproduction.

asex=0.000

	s=0.1				
h	*N*=30	*N*=60	*N*=90	*N*=150	*N*=300
0.1	7.88566	1.579872	0.684192	0.644896	0.649856
0.2	5.940396	1.581984	0.43086	0.391644	0.410376
0.3	3.953872	0.966664	0.278136	0.247016	0.215368
0.4	1.986936	0.591964	0.112732	0.11176	0.107912
	s=0.5				
h	*N*=30	*N*=60	*N*=90	*N*=150	*N*=300
0.1	0.5783	0.73936	0.75072	0.76528	0.74256
0.2	0.411	0.49404	0.47802	0.46842	0.42102
0.3	0.25932	0.25868	0.27268	0.23172	0.25164
0.4	0.09132	0.1125	0.09966	0.10092	0.1013
	s=1.0				
h	*N*=30	*N*=60	*N*=90	*N*=150	*N*=300
0.1	0.856	0.77328	0.78576	0.84736	0.73584
0.2	0.39396	0.41508	0.49068	0.44364	0.444
0.3	0.27064	0.27944	0.27336	0.25552	0.25624
0.4	0.088	0.09168	0.12912	0.11892	0.10508

asex=0.333

	s=0.1				
h	*N*=30	*N*=60	*N*=90	*N*=150	*N*=300
0.1	7.806	1.3824	0.551744	0.510624	0.505648
0.2	5.8308	1.076016	0.345204	0.315984	0.291948
0.3	3.8376	0.905536	0.192408	0.177368	0.160296
0.4	1.9194	0.497868	0.105356	0.081404	0.073924
	s=0.5				
h	*N*=30	*N*=60	*N*=90	*N*=150	*N*=300
0.1	0.62	0.62808	0.64352	0.648	0.66184
0.2	0.36498	0.36996	0.32868	0.35862	0.32886
0.3	0.20264	0.169	0.156	0.18456	0.18224
0.4	0.09132	0.07918	0.08946	0.07308	0.07842
	s=1.0				
h	*N*=30	*N*=60	*N*=90	*N*=150	*N*=300
0.1	0.60528	0.66672	0.7112	0.70368	0.6688
0.2	0.42192	0.354	0.39468	0.36072	0.36516
0.3	0.18528	0.23	0.19072	0.20232	0.19544
0.4	0.07532	0.07536	0.0782	0.07908	0.08352

Finite vs. infinite population simulations

Simulations with large finite population size ($Ns = 30$) show similar inbreeding load values as the infinite population simulations. As the level of asexual reproduction increases, we see a slight decrease in β. The finite population size of $N = 30$ has the highest amount of inbreeding load (β) for both levels of asexual reproduction, and also harbors the greatest number of deleterious mutations per gamete (Q).

Discussion

This study investigated the effects of asexual reproduction on two measures of genetic load (average deleterious mutations per gamete and inbreeding load) for two distinct sets of mutation selection balance simulations, deterministic Infinite simulations and stochastic Finite simulations. Both sets of simulations recover the classic result that as the strength of selection and the dominance coefficient increase, the amount of load decreases (Ohta and Cockerham 1974). For the Infinite simulations, the effect of increasing the proportion of asexual reproduction has different effects on the inbreeding load for different dominance and selection coefficients. For recessive mutations ($h < 0.2$), inbreeding load increased as the strength of selection and amount of asexual reproduction increased. However, for $h > 0.2$ and $s < 0.4$, increasing the amount of asexual reproduction resulted in decreased

inbreeding load. For the Finite simulations, increasing the amount of asexual reproduction results in a slight decrease in the inbreeding load.

The results for the Infinite simulations reflect those found by Muirhead and Lande (1997). For recessive, lethal mutations (h =0.05 and s =1.0), increasing asexual reproduction leads to an increase in inbreeding load. However, as mutations become less and less recessive (h increases from 0.1 to 0.5), the amount of inbreeding load decreases as the level of asexual reproduction increases. With increasing dominance, the amount of inbreeding load is the greatest for complete outcrossing and selfing (no asex), and decreases as the proportion of asexual reproduction increases.

The results from the Infinite simulation can also be compared to that of Kelly (2007), where the Kondrashov model was used to estimate Q, the average number of deleterious mutations per gamete. However, Kelly (2007) does not incorporate asexual reproduction so only the results for no asex (asex = 0.00, equal amounts of outcrossing and selfing) are comparable. The values for the average number of deleterious mutations per gamete (Q) are similar but results from current study have higher values of Q for the same values of U, h, s. The differences in Q between the two studies is likely due to the way in which mutations are added, since all other portions of the simulations are the same; (same order of mutation, reproduction, followed by selection; same assumptions concerning loci- loci unlinked- and fitness is multiplicative). Kelly (2007) does not have separate meiotic and mitotic mutation

rates; whereas the current study does. This suggests the need to investigate how varying meiotic and mitotic mtuation rates influences the average number of deleterious mutations (Q) and inbreeding load (β), which future extensions of the simulation will determine.

Kondrashov (1982) also investigated the difference between sexual and asexual populations and found that the sexual population had fewer deleterious mutations on average than an asexual population. This phenomenon is also true for the Infinite simulations, but only up to a certain point. Once mutations are slightly to increasingly dominant ($h \geq 0.2$, and $s \geq 0.4$; Table 4-1, comparing asex = 0.00 to asex = 1.00) then asexual reproduction has fewer deleterious mutations on average.

The Finite simulation results match previous results regarding the effects of drift on mutational load: that load is much higher in small than large populations (Kimura *et al*. 1963; Charlesworth *et al*. 1992; Bataillon and Kirkpatrick 2000), and that increasing the strength of selection (and increasing the dominance coefficient) result in fewer deleterious mutations (Charlesworth *et al*. 1993). As with the Infinite simulations, increasing the amount of asexual reproduction resulted in a decrease in inbreeding load (Table 4-3).

We expected that increases in asexual reproduction would lead to an increase in load, however the results suggest the opposite; that increases in asexual reproduction may

lead to a decrease in load. However, this expectation was with the assumption of only asexual reproduction. In this study, asexual reproduction, outcrossing and selfing are occurring within the population every generation. This suggests that including sexual reproduction and asexuality reduces load; this result has been noted previously. For example, Bell (1988) found that as little as 10% sexual reproduction, in a predominately asexually reproducing species (i.e., yeast and *Chlamydomonas*) will reduce the genome of almost all of its load.

Empirical estimates of inbreeding load in a predominately asexual *Mimulus guttatus* population , hereafter referred to as the DUN population, revealed very little to no inbreeding load (see Chapter 3; Marriage and Kelly, submitted). Comparison from the empirical study to the results obtained for the Finite simulations does not suggest a small population size for DUN population, because the Finite simulation results with small population sizes have the greatest amounts of load. This suggests that mutations contributing to load in the DUN population are almost completely additive (near $h = 0.5$). However, differences in amount of load between the empirical study and simulations could be due to the fixation of mutations. Mutations are free to accumulate and fix in the finite simulations; however, mutations have the opportunity to be purged in natural populations.

Future work will involve more thorough investigation of the effect of increasing asexual reproduction in finite populations. Also, it would be interesting to investigate

numbers of deleterious mutations per gamete and inbreeding load with unequal levels of outcrossing and selfing, while also including asexual reproduction. Results presented thus far were for cases with equal amounts of outcrossing and selfing, while only changing the level of asexual reproduction. All individuals in the simulations receive mitotic mutations and only sexually produced progeny (progeny generated via outcrossing or selfing) receive meiotic mutations. With equal amounts of meiotic and mitotic mutations, increasing the amount of asexual reproduction results in an overall decrease of the mutation rate. Results of this study suggest the need to examine cases where the total mutation rate is held constant.

As mentioned earlier, it would also be worthwhile to investigate the impact of mutation rate on inbreeding load in finite and infinite populations. Results presented are for $U = 0.5$ and an equal magnitude of meiotic to mitotic mutation rates. What happens to the number of deleterious mutations per gamete and levels of load when U is greater than or less than 0.5, and for orders of magnitudes of difference between meitotic vs. mitotic mutations? The information gained from the future work could be used to provide theoretical estimates of the significance of mitotic (somatic) mutation rates to asexually reproducing organisms.

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asexual populations. Genetical Research 49: 135-146.

Appendix A The forward and reverse primer sequence is given for each locus in our survey.

Locus	Repeat composition	Forward primer sequence	Reverse primer sequence	PCR product length (bp)
AT.CIW7	$(AT)_{15}$	aatttggagattagctggaat	ccatgttgatgataagcacaa	144-148
AT0101	$(AT)_{14}$	ttgtcaaaatgcactcttcattatc	ctagttacccgccaatccaa	220-222
AT0102	$(AT)_{16}$	cgtgatattgatcactcgtcaga	ggcacatccgttttgaagat	182-184
AT0103	$(TA)_{16}$	tcaattctacaagaaaaatgctga	gcccatataatgtgcatcacg	121-127
AT0104	$(AT)_{10}$	aacataaagggcgtgaggtg	tttaaagtaagcattttcattgcat	237
AT0201	$(AT)_{13}$	gcaaaactgcctaaataacacc	tcgtttgaggtcaatttttgaa	181-185
AT0202	$(AT)_{14}$	gggttagacaattcaaatgttttt	aaacccaagatcaatattttctttaca	180-184
AT0203	$(AT)_{14}$	tgcgatatattatgcacggatt	caaaacgtgttcgattttggt	161
AT0204	$(AT)_{14}$	ttctcaaagtctccaagtatggtg	aaagcttttgttaggcaagca	215-217
AT0301	$(AT)_{16}$	ttggcctaacctaaccatcaa	ctaaaaacaacaatagaagccaca	213-217
AT0302	$(AT)_{12}$	catcaatatgatatgttcctattttca	aagccgtattgacaggagaa	192-196
AT0303	$(AT)_{12}$	ccatgatttcattcacaacca	ttccatgatccaccacttctc	211
AT0304	$(AT)_{17}$	tgaaatgaacagaagaagaaacca	agaagcaccatgattcaaaga	165
AT0402	$(AT)_{28}$	acatggttttgctcccaagt	tgcagcccagaactttctct	198-204
AT0403	$(AT)_{23}$	ttttcccgacagctcgtagt	tctcacatggttagggaaacaa	182-190
AT0404	$(AT)_{8}$	ggtctctttagtctttaagtttgtcca	tgccgttatagcggtcattt	178
AT0501	$(AT)_{15}$	aagaaagtgctgaatgttgatga	tgcataagccaaatgaatttttt	168-172
AT0502	$(AT)_{15}$	tgtacgtaaaatataagaaggacgatt	gaatgaaccatttcgcacct	198-200
AT0503	$(AT)_{12}$	atcctacccgaattccgaac	ccatgccaaaatttacacga	229
AT0504	$(AT)_{23}$	tttggatcttcaacaaatgctc	ttacccaaaccaagcaaagc	257-261
CA0101	$(AC)_{14}$	acgaggacttcgcctgtcta	cggaaacacagtactgcttga	180
CA0102	$(TG)_{10}$	ttatgagactggtcgactgga	catgtcgagaccgatttcaag	164
CA0103	$(TG)_{12}$	tcacatcaaggtttgctcca	cgtgtttccttatccggtgt	202
CA0104	$(TG)_{10}$	gacaaacaaaatccgttctgg	tatcgtgacgctctcacctg	202-204
CA0201	$(TG)_{10}$	ccatgcatgtaaataatgaatagtga	ttgatgcttgtttgttttcca	190
CA0202	$(TG)_{12}$	aatactgcttcggtggcatc	tggaaatcccgtgttaccat	222
CA0301	$(TG)_{10}$	tccagcatttctttgcctttt	aagctgaaaaatttcccttaatgt	224
CA0302	$(TG)_{12}$	aatggctggccatcaaact	ttgggtgtcattctcctcgt	263

CA0401	$(CA)_{12}$	atcacatacgccgtcctaca	tgtagctccgaatcctactcc	174
CA0501	$(TG)_{10}$	catcgtttctcaattcgatgg	gggtgcacagggatttaaca	263
CA0502	$(TG)_{13}$	ttcccttcaccgaacttgag	aaagccttcttcaatcaaagc	165
CA0503	$(TG)_{10}$	tttttctacacattttctctcaatttc	atgaactatctttgatccaatgc	166
CA0504	$(TG)_{13}$	aaaacgggaaaggtggaagt	gcctcgtgaggagtttggta	233
CA72	$(CA)_{18}$	aatcccagtaaccaaacacaca	cccagtctaaccacgaccac	168
CT.nga1145	$(GA)_{14}$	ccttcacatccaaaacccac	gcacatacccacaaccagaa	229
CT.nga172	$(GA)_{29}$	agctgcttccttatagcgtcc	catccgaatgccattgttc	175
CT.nga225	$(CT)_{18}$	gaaatccaaatcccagagagg	tctccccactagttttgtgtcc	134-136
CT.nga32	$(GA)_{13}$	ggagacttttgagattggcc	ccaaaacaattagctcccca	275
CT.nga59	$(CT)_{19}$	gcatctgtgttcactcgcc	ttaatacattagcccagacccg	124
CT0101	$(CT)_{11}$	cagagacgaaagaggtgatgg	tcgaagagagagaaaatcccttt	169
CT0102	$(AG)_{15}$	agacctccacctccaagacc	tcttccacgatccttatcgaa	228
CT0103	$(CT)_{10}$	caacactgtgaaaccaaaaacc	ccaacctcatgaaacaaagga	198
CT0104	$(AG)_{14}$	ttgttcggctctgcttcttt	ttgccctccaaacatggtat	211-213
CT0201	$(AG)_{12}$	tgtgcgtgtaattttgttgct	tcagaaacgtgggtgtgtgt	223
CT0301	$(AG)_{12}$	gggctctgtgttttgaggaa	ggatttccgcaatcatcatc	230
CT0302	$(CT)_{12}$	gcactcgcaagtgtgaacat	tcgtttgcttcttctgtttgtc	266
CT0303	$(CT)_{15}$	caatggtgatgtggcattgt	aaagaagaggagcagcgtgt	193
CT0304	$(AG)_{13}$	caatttccgatggaggaaga	ccctttttctcaatgcccttt	167-169
CT0401	$(AG)_{27}$	aacaatgaggcgtatgtgagg	tgaaacttttgttgtttgggttt	193-197
CT0402	$(AG)_{25}$	gccgctgacacttgtcacta	tcagatttccttggctttcg	229-231
CT0403	$(CT)_{12}$	cttaggggccagctttctct	ccgaggcgtattttgtcatc	215
CT0501	$(AG)_{19}$	gaagaagcgtgggatatgga	ggcctcacatgaaaccctaa	204-206
CT0502	$(CT)_{22}$	cccgactcggaattcactaa	ctggcccaaccactactcat	218
CT0503	$(AG)_{15}$	cttccatttttggcttagca	tgctttttcctcggtaatgaa	212-214

Appendix B. Detailed information for inbreeding load estimates given in Table 3-3.

Trait	Parent inbreeding coefficient (F)	Population	Estimated inbreeding load (β)*	Study
Flower morphology:				
Corolla width	1.0[1]	IM[a]	-0.097	Kelly and Arathi 2003
	0.0[2]	IM[a]	-0.399	Kelly and Willis 2001
	0.039[3]	M13W[b]	-0.288**	Ivey and Carr 2005
	1.0[4]	IM[a]	-0.093	Kelly 2003
	0.0[5]	IM[a]	-0.182	Willis 1996
	0.0[5]	CP[c]	-0.080	Willis 1996
	0.0[6]	IM[a]	-0.177	Willis 1999a
Corolla length	0.039[3]	M13W[b]	-0.192**	Ivey and Carr 2005
	1.0[1]	IM[a]	-0.071	Kelly and Arathi 2003
	0.0[5]	IM[a]	-0.076	Willis 1996
	0.0[5]	CP[c]	-0.081	Willis 1996
Stigma length	1.0[1]	IM[a]	-0.061	Kelly and Arathi 2003
Stigma-anther separation	1.0[1]	IM[a]	0.080	Kelly and Arathi 2003
	0.0[5]	IM[a]	0.013	Willis 1996
	0.0[5]	CP[c]	0.074	Willis 1996
Male fitness:				
Pollen size index	1.0[4]	IM[a]	-0.308	Kelly 2003
Viable pollen per flower	0.0[6]	IM[a]	-0.673	Willis 1999a

	1.0[7]	IM[a]	-0.488	Willis 1999b
	1.0[8]	IM[a]	-0.299	Willis 1999c
Phenology and Plant size:				
Days to flower	1.0[1]	IM[a]	0.075	Kelly and Arathi 2003
	0.0[5]	IM[a]	0.101	Willis 1996
	0.0[5]	CP[c]	0.112	Willis 1996
	0.0[6]	IM[a]	0.057	Willis 1999a
Biomass	0.0[9]	S, T[d]	-0.740**	Carr and Dudash 1995
	0.0[9]	S, T[d]	-1.360**	Carr and Dudash 1996
	0.0[10]	M5[e]	-0.040**	Carr and Eubanks 2002
	0.0[10]	M13[f]	-0.220**	Carr and Eubanks 2002
	0.0[9]	S, T[d]	-0.360**	Carr et al.1997
	0.0[10]	M5[e]	-0.380**	Carr et al. 2003
	0.0[10]	M13[f]	-0.460**	Carr et al. 2003
	0.35[11]	127[g]	-0.459**	Latta and Ritland 1994
	0.19[11]	133[h]	-0.370**	Latta and Ritland 1994
	0.56[11]	137[i]	-0.731**	Latta and Ritland 1994
	0.57[11]	138[j]	0.433**	Latta and Ritland 1994
	0.47[11]	153[k]	0.503**	Latta and Ritland 1994
Flower number	0.0[10]	M5[e]	-0.240**	Carr and Eubanks 2002
	0.0[10]	M13[f]	-0.360**	Carr and Eubanks 2002
	0.0[9]	S, T[d]	-0.400**	Carr et al. 1997

	0.0[10]	M5[e]	-0.180**	Carr et al. 2003
	0.0[10]	M13[f]	-0.560**	Carr et al. 2003
	0.039[3]	M13W[b]	-0.462**	Ivey and Carr 2005
	0.35[11]	127[g]	-0.148**	Latta and Ritland 1994
	0.19[11]	133[h]	-0.790**	Latta and Ritland 1994
	0.56[11]	137[i]	0.756**	Latta and Ritland 1994
	0.57[11]	138[j]	0.471**	Latta and Ritland 1994
	0.47[11]	153[k]	-0.531**	Latta and Ritland 1994
	0.0[12]	IM[a]	-0.944	Willis 1993b
	0.0[12]	CP[c]	-0.816	Willis 1993b
	0.0[6]	IM[a]	-0.511	Willis 1999a
	1.0[7]	IM[a]	-0.272	Willis 1999b
	1.0[8]	IM[a]	-0.129	Willis 1999c

*β = $(W_I - W_O)/ (\Delta f * W_O)$ where W_I is the inbred trait mean, W_O is the outbred trait mean, and $\Delta f = \frac{1}{2} f_{parent} + \frac{1}{2}$

**β calculated from δ where $\delta = (W_I - W_O)/ W_O$ and $\beta = 1/\Delta f * \delta$

[1]Parents derived from over 1000 independent highly inbred lines of J. Willis
[2]Parents derived from 1200 outbred families
[3]Estimation of F from six allozyme loci; D. Carr, personal communication
[4]Parents derived from over 1200 highly inbred lines of J. Willis
[5]Parents derived from field collected seed, assumed to be outbred
[6]Parents derived from unrelated field collected seed
[7]Parents derived from over 1200 highly inbred lines
[8] Parents derived from approximately 300 highly inbred lines
[9]Parents derived from field collected seed; estimated outcrossing rate of population T is 0.70, no estimate available for population S, assumed to be outbred for both populations.

[10]Parents derived from field collected seed, no outcrossing estimates available for populations (D. Carr and C. Ivey, personal communication), assumed to be outbred
[11]Parents derived from field collected seed; estimates of F obtained from Table 1, Latta and Ritland 1994
[12]Parents derived from field collected seed, assumed to be outbred

[a] IM = Iron Mountain, Linn Co., OR
[b] M13W (38°33' N, 122°22' W), Napa Co., CA
[c] CP = Cone Peak, Linn Co., OR
[d] S and T, Tuolome Co., CA
[e] M5 (37°17' N, 122°09' W), Santa Clara Co., CA
[f] M13 (38°33' N, 122°22' W), Napa Co., CA
[g]127, Mariposa Co., CA
[h]133, Josephine Co., OR
[i]137, Jackson Co., OR
[j]138, Klickitat Co., OR
[k]153, Riverside Co., CA

Appendix C. Detailed information for coefficient of additive variation estimates from Table 3-5.

Trait	Parent inbreeding coefficient (F)	Population	CV_a	V_a	V_e	Study
Flower morphology:						
Corolla width	0.0	DUN	0.013	0.237	0.763	Current study
	1.0[1]	IM [a]	0.023	0.25		Kelly and Arathi 2003[‡]
	0.0[2]	S [b]	0.120			Carr and Fenster 1994
	0.0[2]	T [c]	0.064			Carr and Fenster 1994
	0.0[2]	Copperopolis mine [d]	0.007	0.04		Roberston et al. 1994
Corolla length	0.0	DUN	0.011	0.197	0.803	Current study
	1.0[1]	IM [a]	0.015	0.11		Kelly and Arathi 2003
	0.0[2]	S [b]	0.113			Carr and Fenster 1994
	0.0[2]	T [c]	0.055			Carr and Fenster 1994
	0.0[2]	Copperopolis mine [d]	0.009	0.05		Roberston et al. 1994
Stigma length	0.0	DUN	0.025	0.379	0.616	Current study
	1.0[1]	IM [a]	0.033	0.24		Kelly and Arathi 2003
	0.0[2]	S [b]	0.097			Carr and

	0.0[2]	S [b]	0.097			Carr and Fenster 1994
	0.0[2]	T [c]	0.048			Carr and Fenster 1994
	0.0[2]	Copperopolis mine [d]	0.021	0.17		Roberston et al. 1994
Anther length	0.0	DUN	0.020	0.170	0.830	Current study
	0.0[2]	S [b]	0.112			Carr and Fenster 1994
	0.0[2]	T [c]	0.063			Carr and Fenster 1994
Stigma-anther separation	0.0	DUN	0.100	0.218	0.782	Current study
	1.0[1]	IM [a]	0.241	0.36		Kelly and Arathi 2003
	0.0[2]	S [b]	0.277			Carr and Fenster 1994
	0.0[2]	T [c]	0.334			Carr and Fenster 1994
Male fitness:						
Pollen size index	0.0	DUN	1.816	0.190	0.670	Current study
	0.0[2]	Copperopolis mine [d]	1.186	0.49		Roberston et al. 1994[§]
Viable pollen per flower	0.0	DUN	0.049	0.042	0.906	Current study
Total male fitness	0.0	DUN	0.018	0.011	0.872	Current study
Phenology and Plant size:						
Days to						Current

	1.0[1]	IM [a]	0.023	0.32		Kelly and Arathi 2003[†]
	0.0[2]	S [b]	0.158			Carr and Fenster 1994
	0.0[2]	T [c]	0.079			Carr and Fenster 1994
	0.0[2]	Copperopolis mine [d]	0.023	0.27		Roberston et al. 1994
Biomass	0.0	DUN	23.238	0.054	0.946	Current study
Flower number	0.0	DUN	0.039	0.033	0.838	Current study

[1] Parents derived from over 1000 independent highly inbred lines of J. Willis
[2] Parents derived from field collected seed, assumed to be outbred

[a] IM = Iron Mountain, Linn Co., OR
[b] S, Tuolome Co., CA
[c] T, Tuolome Co., CA
[d] Copperopolis mine, Calveras Co., CA

[‡]Estimate based on non-transformed corolla width
[§]Estimate based on pollen viability. Pollen viability = PSI + 0.1
[†]Estimate based on non-transformed age at first flower

Appendix D

D1. Phenotypic correlation matrix for outbred plants. Significant correlations are in bold with the following p-value indicators:
* = 0.01< p <0.05, ** = 0.001< p <0.01, *** = p <0.001.

	Corolla length	Stigma length	Anther length	Stigma anther separation	Log (PSI)	Log (Viable pollen per flower)	Log (Total male fitness)	Days to flower	Log (Biomass)	Sqrt (Flower number)
Corolla width	**0.811*** **	**0.619*** **	**0.466*** **	**0.126*** **	**0.076***	0.065	**0.124****	–0.043	**0.249*** **	**0.150*** **
Corolla length		**0.741*** **	**0.559*** **	**0.148*** **	**0.090****	**0.078***	**0.114****	–0.054	**0.246*** **	**0.152*** **
Stigma length			**0.494*** **	**0.466*** **	0.064	0.059	**0.076***	0.024	**0.236*** **	**0.129*** **
Anther length				**–0.540*****	**0.317*** **	**0.328*** **	**0.223*** **	–0.014	**0.181*** **	**0.094****
Stigma anther separation					**–0.256*****	**–0.274*****	**–0.153*****	0.037	0.042	0.027
Log(PSI)						**0.766*** **	**0.326*** **	0.060	0.012	0.001
Log(VPF)							**0.684*** **	–0.026	0.020	0.043
Log (Total male fitness)								**–0.399*****	**0.351*** **	**0.610*** **
Days to flower									**–0.181*****	**–0.504*****
Log (Biomass)										**0.491*** **

D2. Phenotypic correlation matrix for inbred plants. Significant correlations are in bold with the following p-value indicators:
* = 0.01< p <0.05, ** = 0.001< p <0.01, *** = p <0.001.

	Corolla length	Stigma length	Anther length	Stigma anther separation	Log (PSI)	Log (Viable pollen per flower)	Log (Total male fitness)	Days to flower	Log (Biomass)	Sqrt (Flower number)
Corolla width	**0.838*****	**0.650*****	**0.480*****	**0.125*****	**0.133*****	**0.140*****	**0.140*****	**–0.086****	**0.204*****	**0.085***
Corolla length		**0.752*****	**0.576*****	**0.122*****	**0.166*****	**0.163*****	**0.155*****	**–0.101****	**0.206*****	**0.111****
Stigma length			**0.490*****	**0.428*****	**0.084***	**0.088****	**0.127*****	–0.052	**0.183*****	**0.126*****
Anther length				**–0.579*****	**0.322*****	**0.347*****	**0.244*****	–0.051	**0.155*****	**0.074***
Stigma Anther separation					**–0.252*****	**–0.274*****	**–0.133*****	0.006	0.008	0.039
Log(PSI)						**0.756*****	**0.337*****	–0.015	0.064	0.037
Log(VPF)							**0.683*****	–0.059	**0.121****	**0.117****
Log (Total male fitness)								**–0.404*****	**0.402*****	**0.672*****
Days to flower									**–0.101****	**–0.516*****
Log (Biomass)										**0.460*****

D3. Family mean correlation matrix for outbred plants. Significant correlations are in bold with the following p-value indicators:
* = 0.01 < p < 0.05, ** = 0.001 < p < 0.01, *** = p < 0.001

	Corolla length	Stigma length	Anther length	Stigma anther separation	Log (PSI)	Log (Viable pollen per flower)	Log (Total male fitness)	Days to flower	Log (Biomass)	Sqrt (Flower number)
Corolla width	**0.802*****	**0.596*****	**0.393****	**0.353***	–0.023	**–**0.030	0.051	0.230	**0.343***	0.081
Corolla length		**0.775*****	**0.607*****	**0.371***	0.061	0.075	0.023	0.190	**0.329***	0.031
Stigma length			**0.566*****	**0.673*****	–0.011	–0.015	–0.044	0.229	**0.427****	0.113
Anther length				–0.229	0.171	0.198	–0.081	0.131	0.111	–0.159
Stigma anther separation					–0.168	–0.197	0.020	0.154	**0.405****	0.274
Log(PSI)						**0.812*****	0.266	0.236	0.250	0.103
Log(VPF)							**0.646*****	0.039	0.237	0.265
Log (Total male fitness)								**–0.420****	0.222	**0.731*****
Days to flower									**0.395****	**–0.568*****
Log (Biomass)										0.144