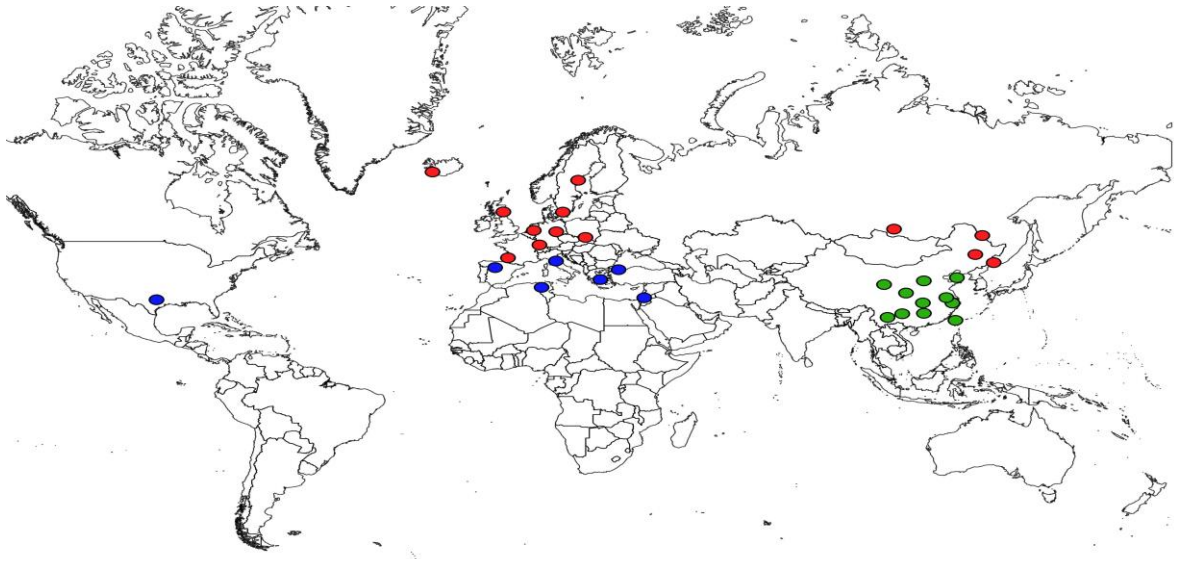
UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

CHARACTERIZING THE GENETIC LOAD IN *CAPSELLA BURSA-PASTORIS* ACROSS ITS RANGE



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Front page illustration: Origin of the Capsella bursa-pastoris samples analysed, coloured according to the three genetically distinct clusters. Asia (ASI) = Green, Europe and Russia (EUR) = Red and Middle East (ME) = Blue.

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Abstract

In a fast changing world, where human activities disturb and change many habitats, species being able to exploit these changed areas have an opportunity to rapidly expand their range. During range expansion, the edge of a population may undergo repeated bottlenecks reducing the genetic variation and the effective population size (N_e). Leading to a stronger effect of genetic drift and thereby allowing deleterious alleles to accumulate. The accumulation of deleterious alleles contributes to the genetic load, the fraction mean fitness diverges from a theoretical optimum. The amount of genetic load can shape the future of a population's ability to adapt and survive in its environment. It is therefore crucial to understand how demography and deleterious mutations interact across a population. Here, I used the allotetraploid *Capsella bursa-pastoris*, with a known population structure and demographic history, to address if and how genetic load varies across its range. *Capsella*. *bursa-pastoris* originated somewhere between Europe and the Middle East and later spread to Asia. Additionally, I investigated whether there is an effect of polyploidy. In particular, I tested whether one subgenome buffers the other. *Capsella bursa-pastoris* is a great candidate to address these questions because it is an allopolyploid with two distinct subgenomes and disomic inheritance. I used two separate approaches to assess the genetic load. The first was to simply count the number of predicted deleterious alleles in coding regions. The second approach was to estimate the distribution of fitness effects (DFE) from sequence polymorphism data. In agreement with expectations, I found that the subpopulation at the edge of the expansion front, in Asia, exhibited the highest genetic load. However, in disagreement with the expectations, the European subpopulation displayed the lowest genetic load. The main conclusion at this stage is that genetic load varies across the range of this species, however, the variation could not be entirely explained by the range-expansion hypothesis first assumed.

Introduction

The majority of mutations arising in populations are deleterious by nature and effect fitness negatively, therefore contributing to the genetic load. The concept of genetic load was first formulated by J. B. S. Haldane (Haldane 1937). James F. Crow built on Haldane's ideas and formulated genetic load in three separate ways, one being, as the reduction in mean fitness of a population due to the presence of deleterious alleles compared to an ideal population free of deleterious mutations (Crow 1970).

Changes in a species habitat, either directly induced by humans (such as deforestation) or indirectly by the climate (such as drought), can force a species to expand its range in order to survive. It has been shown that range expansion is generally accompanied by an increase in genetic load (Peischl et al. 2013; Peischl et al. 2015). As a result of low genetic diversity and genetic drift, the amount of genetic load can critically reduce the viability and adaptive potential of a population (Lynch and Gabriel 1990; Lynch et al. 1995; Lohr and Haag 2015). It is therefore of importance to understand the underlying dynamics behind the accumulation of deleterious alleles, more specifically that due to range expansion.

Accumulation of deleterious mutations

A multitude of causes have been invoked in the literature to explain how the accumulation of deleterious alleles contributes to genetic load. Firstly, all populations experience mutations which are deleterious and will lead mean fitness to be less than what the optimal fitness theoretically could be (Muller 1950). Secondly, in small populations, fixation of deleterious alleles can occur at random through genetic drift (Crow 1970; Lynch et al. 1995; Whitlock 2000; Lohr and Haag 2015). Finally, expanding populations often undergo multiple colonization events at the forefront of the expansion range causing repeated bottlenecks and increasing the frequency of deleterious alleles (Hallatschek and Nelson 2010; Peischl et al. 2013; Peischl et al. 2015).

The mating system of a species also affects its genetic load, especially when comparing strictly outcrossing species with highly self-fertilizing ones (Husband and Schemske 1996; Arunkumar et al. 2015). In outcrossing species, deleterious alleles are maintained at low frequencies because selection is efficient and they are mostly masked within heterozygotes. Selfing on the other hand unmasks deleterious alleles by increasing homozygosity and strongly deleterious alleles can be purged from the genome (Husband and Schemske 1996). However, selection is

less efficient in selfing species due to a reduction in the effective population size (N_e) and thereby an increased effect of genetic drift (Charlesworth and Wright 2001). Because selection is less effective weakly deleterious alleles behave as neutral or nearly neutral ones and can therefore reach higher frequency and even go to fixation (Bataillon and Kirkpatrick 2000; Charlesworth and Willis 2009).

A common phenomenon in plants is whole genome duplication (WGD), which is a duplication of the genomes by either fusing unreduced gametes or doubling the genome of somatic tissue. WGD can occur in two ways, either by allopolyploidy, meaning through hybridisation where the progeny inherits the full set of one or both parental genomes or autopolyploidy where the duplication happens within the same taxon. Little is known about the relationship between the amount of deleterious alleles and ploidy level. By just going from a haploid genome to a diploid genome the accumulation of deleterious alleles changes. In haploid genomes, deleterious alleles will quickly be purged because they are always expressed and therefore will result in lower fitness and be removed by natural selection (Mable and Otto 2001). On the other hand, in a diploid genome, deleterious alleles will persist longer and at low frequencies in the genome due to masking in heterozygotes.

Inbreeding depression

Populations with varying degrees of inbreeding will increase the proportion of homozygotes. This increase in homozygous sites can in turn lead to inbreeding depression (i.e. the reduction in fitness of inbred individuals compared to outbred ones). Two main processes leading to more homozygous individuals having lower fitness, hence explaining inbreeding depression: (i) overdominance or heterozygote advantage (i.e. the heterozygotes having a higher fitness than either homozygotes) and (ii) partially recessive deleterious alleles.

Partially recessive deleterious alleles can create inbreeding depression by being expressed at a higher rate. This increase in expression is due to the increased frequency of homozygous sites and selection being less efficient in removing them. Overdominance will not be discussed further since the focus here lies on deleterious mutations.

Characterisation of deleterious mutations

There are numerous ways to characterise deleterious alleles in a genome. A common method is to estimate the distribution of fitness effects (DFE). The DFE is the distribution of the allelic fitness effects, a gradient ranging from lethal, through neutral, to strongly beneficial. There are

different approaches to estimate the DFE: one method is based on mutation accumulation (MA) experiments and has been used in model organisms with short generation time, including *Saccharomyces cerevisiae* (Zeyl and DeVisser 2001; Joseph and Hall 2004) *Caenorhabditis elegans* (Keightley and Caballero 1997; Vassilieva et al. 2000), *Drosophila melanogaster* (García-Dorado et al. 1998; Loewe and Charlesworth 2006; Keightley and Eyre-Walker 2007) and *Arabidopsis thaliana* (Schultz et al. 1999; Shaw et al. 2002)**.** These experiments are limited for practical and theoretical reasons. For example, not all organisms of interest are easy to study over numerous generations due to long generation times—nor easy to keep in a constant and benign environment to minimize the effects of selection. Additionally, only alleles that have a high or moderate impact on fitness can be identified (Davies et al. 1999; Bataillon and Bailey 2014). Therefore, much information is lost since the majority of mutations have a low impact on fitness (Davies et al. 1999; Keightley and Eyre-Walker 1999; Boyko et al. 2008).

Alternative approaches to estimate the DFE are based on DNA sequence data and can be used to get around the limits of MA experiments. A variety of models have been presented through the years, a few that only estimate the DFE of deleterious alleles (Loewe and Charlesworth 2006; Eyre-Walker and Keightley 2009) and lately models that also consider both deleterious and beneficial alleles (Keightley and Eyre-Walker 2007; Boyko et al. 2008; Gronau et al. 2013). Many models build upon generating the site frequency spectrum (SFS) of both neutral alleles and alleles under selection, fitting this spectrum to a model and finding the most likely parameter values to represent the data. The DFE are typically modelled using a gamma distribution, since it is flexible and can take different forms. Typically, the DFE is expected to be L-shaped, meaning that most mutations have low or neutral effects on fitness and relatively few have strong effects.

A complementary approach to DFE for characterising deleterious alleles is to assign the mutation a prediction regarding the effect it might have on protein function. There are software packages that do this, the most commonly used being PolyPhen2 (Polymorphism Phenotyping v2) (Adzhubei et al. 2010), SIFT (Sorting Intolerant from Tolerant) (Kumar et al. 2009) and PROVEAN (Protein Variation Effect Analyzer) (Choi et al. 2012). They all use different approaches to make predictions. The SIFT algorithm uses alignments of orthologous genes to calculate and assign a conservation score to mutations at each site—the rarer the mutation is at a site, the more likely it is to be deleterious and to get a low score. Polyphen2 on the other hand builds its predictions with a sequence and structure-based algorithm using a naïve Bayes

classifier. PROVEAN makes predictions based on a calculated PROVEAN score. The score is inferred by aligning homologous sequences and calculating a sequence score before and after the mutation is introduced. Software comparisons have shown that they produced equivalent results (Flanagan et al. 2010; Choi et al. 2012; Renaut and Rieseberg 2015; Zhang et al. 2016). Recently, SIFT4G (SIFT for Genomes) has been developed (Vaser et al. 2016). This is a faster version of the original SIFT and will therefore be the software used here to predict the effect of mutations on protein function.

Study system and goal

The self-fertilizing species *Capsella bursa-pastoris* is a suitable candidate to use as a study system to tackle the questions on genetic load in an expanding and structured population and effects of polyploidy. Firstly, *C. bursa-pastoris* has been shown to be an allopolyploid (Hurka et al. 1989; Douglas et al. 2015; Roux and Pannell 2015) with strictly disomic inheritance (Hurka et al. 1989), meaning that it is a hybrid with no recombination occurring between the subgenomes. This gives the opportunity to treat and analyse the two subgenomes as two separate diploid genomes while providing information about polyploidy.

Secondly, the parental species to *C. bursa-pastoris* are believed to be the ancestors of the outcrossing *C. grandiflora* and the self-fertilizing *C. orientalis* (Douglas et al. 2015). The different mating systems in the parental species in combination with *C. bursa-pastoris* being a self-fertilizing species provides the possibility to investigate the genetic load from different starting conditions.

Thirdly, *C. bursa-pastoris* has a wide distribution (Hurka and Neuffer 1997; Hurka et al. 2012) and is genetically structured in at least three subpopulations (Cornille et al. 2016; Kryvokhyzha et al. 2016): Asia (ASI), Middle East (ME) and finally Europe and Russia (EUR). Further, Cornille et al. (2016) suggests that the origin of *C. bursa-pastoris* lies in the Middle East and that there have been two main colonization events, the first colonization happened towards Europe and the second in the direction of Asia. The recent spread of *C. bursa-pastoris* into Europe and Asia will allow examination of the accumulation of deleterious alleles during range expansion.

In order to address the issues of how polyploidy in combination with range expansion affect genetic load, I characterized the genetic load in the three subpopulations of *C. bursa-pastoris* by estimating the DFE and by using SIFT4G to characterize deleterious alleles.

Materials and methods

Genomic data

The data used for the analyses consisted of an alignment of 1.9 million single polymorphism sites within coding regions across 31 *C. bursa-pastoris*, 10 *C. orientalis* and 13 *C. grandiflora* samples. Whole genome DNA sequences for 10 *C. bursa-pastoris* accessions and all *C. orientalis* and *C. grandiflora* samples were downloaded from GenBank (PRJNA268827, PRJNA245911, PRJNA254516). The other 21 *C. bursa-pastoris* accessions were sequenced using the same method as the downloaded *C. bursa-pastoris* accessions (100-bp paired-end reads, Illumina HiSeq 2000 platform, SciLife, Stockholm, Sweden) (Kryvokhyzha et al. unpublished). The DNA reads were mapped to the *Capsella rubella* reference genome (Slotte et al. 2013) using Stampy v1.0.22 (Lunter and Goodson 2011), Picard Tools 1.115 (http://picard.sourceforge.net) was used to mark PCR duplicates which were ignored during genotyping. *HaplotypeCaller* from the Genome Analysis Toolkit (GATK) v3.5 (McKenna et al. 2010) was used for genotyping and HapCUT version 0.7 (Bansal and Bafna 2008) for phasing each sample. The fragments HapCUT were joined into continuous sequences descendant from either *C. grandiflora* or *C. orientalis* using custom scripts (Kryvokhyzha).

Reconstructed ancestral sequences were obtained by using the empirical Bayes joint reconstruction method with PAML v4.6 (Yang 1997) on the tree assumed to best reflect the true history of *Capsella* (Kryvokhyzha et al. unpublished).

Watterson's theta

Estimations of Watterson's theta were obtained with Equation 1, where *s* is the number of segregating sites in a sample of *n* sequences.

$$\theta = \frac{s}{1 + \frac{1}{2} + \frac{1}{3} + \cdots + \frac{1}{n-1}} \quad (1)$$

SIFT characterising of deleterious mutations

Two different approaches were used to assess the genetic load. First, I identified deleterious mutations within each sample. A reference prediction file was made by classifying all variants (A, T, G, C) for all sites within coding regions as tolerated or deleterious, hereafter referred to

as the SIFT annotation file. The classifications were made by the software SIFT4G using the *C. rubella* database and mutations were polarized with three reference points (Figure 1).

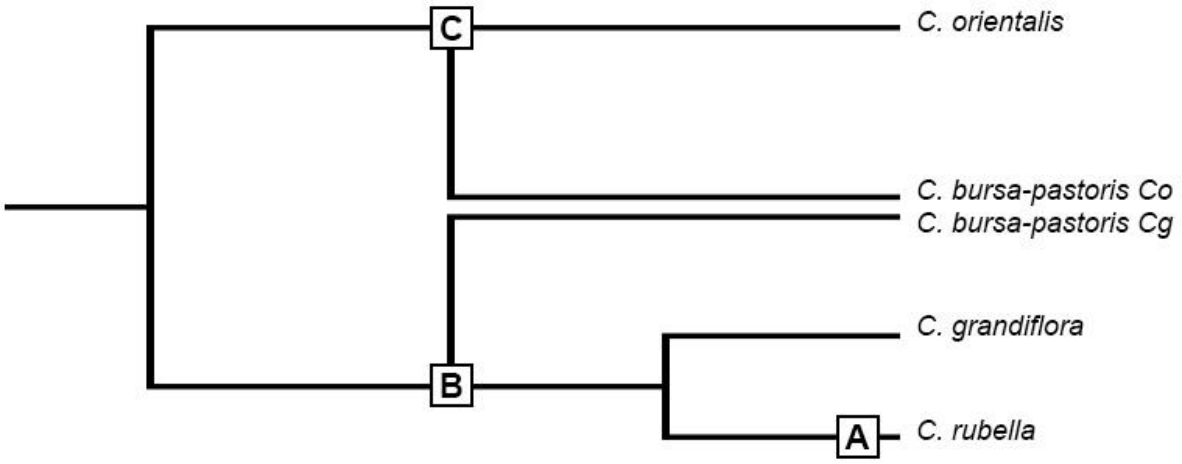

Figure 1. Phylogenetic relationships of Capsella species. The three different reference points used are marked A-C. A represents C. rubella. B represents the reconstructed ancestral sequence between C. bursa-pastoris and C. grandiflora and C, the reconstructed ancestral sequence between C. bursa-pastoris and C. orientalis.

Comparison between the subpopulations (ASI, EUR, ME), the subgenomes within *C. bursa-pastoris* (*CbpCg*, *CbpCo*) and the parental species (*C. grandiflora* and *C. orientalis*) were made by referencing all the SNP sites towards the SIFT4G annotation file and count variants predicted to have a deleterious effect within each sample. This was done in four separate sets. The first, to count all deleterious alleles within each sample. The second, to count all fixed deleterious alleles in each subpopulation. The third, counting derived deleterious alleles in each sample by using the reconstructed ancestral sequence (Figure 1B, Figure 1C) to polarize the alleles. Lastly, the count of the number of fixed derived deleterious alleles in each subpopulation. Common to all sets were that deleterious predictions with low confidence (SiFT4G annotation: "*WARNING! Low confidence") were ignored. However, including low confidence predictions in the analysis did not change the outcome (Table A1, Figure A1 and Figure A2, Appendix).

As the coverage level of the genome varied among individuals, I normalized the absolute number of deleterious and fixed deleterious alleles by the total number of annotated sites. Similarly, the derived deleterious and derived fixed deleterious alleles were normalized by the total number of derived annotated sites.

To test whether subpopulations differed from one another a Kruskal-Wallis test and a Dunn's test were performed due to unequal variance among subpopulations. Each subgenome was considered separately.

Site frequency spectrum

The site frequency spectrum (SFS) for derived mutations was constructed with the ancestral references (Figure 1B, Figure 1C) to identify derived mutations and the SIFT4G annotation file for classification. Each derived mutation was classified as either synonymous or nonsynonymous, with the nonsynonymous mutations further divided into tolerated and deleterious.

To estimate the total number of nonsynonymous and synonymous sites, a simplified method that assumes the same mutations probability between all variants at a site was used. Synonymous and nonsynonymous mutations were counted at all SIFT4G annotations where the alternate allele did not equal the reference allele. The absolute numbers were then divided by 3 since three different mutations can occur at a site.

The distribution of fitness effects

The second approach used to assess the genetic load was to estimate the full distribution of fitness effect (DFE) of mutation, based on the comparison of the synonymous and nonsynonymous mutations SFS. Here, polyDFE was used since it can estimate the full DFE by using polymorphism data alone (Tataru et al. 2016). Within the framework of polyDFE there are four different models that can be used to describe the DFE. The first model (A) predicts only deleterious alleles and uses a gamma distribution to describe their distribution. The second (B) and third (C) models consider both deleterious and beneficial alleles. Both use a gamma distribution to model deleterious alleles. For beneficial alleles, the second model (B) uses a discrete distribution and the third model (C) uses an exponential distribution. The last model (D) uses a discrete distribution and K number of selection coefficients (S_1, S_2,… S_K) with probabilities (p_1, p_2,… p_K).

PolyDFE was run with the Broyden-Fletcher-Goldfarb-Shanno (bfgs) algorithm to estimate the parameters that maximize the likelihood function. To start, all models (A, B, C, D) were run for each data set. To be able to run the four models, the range within which the parameter values can be estimated needs to be provided (Table A2, Appendix). Additionally, to run Model D the number of selection coefficients to consider (K) is needed and K was set to 4. Further, Model

D also requires initial values for the parameters (Table A3, Appendix), each parameter value is accompanied by a flag signalling whether the parameter value should be estimated (0) or be kept fixed (1). Both the range values and initial parameter values were obtained from the example files accompanying polyDFE. An additional run was performed using only model A and fixing the maximum values the selection coefficient can take (*Smax)* to 0.

In choosing the model that best represents the data, the Akaike information criterion (AIC) score was calculated and the model with the lowest score chosen. For data set where model A had the lowest AIC score but a skewed distribution the values from the run with Smax set to 0 was used.

Results

The proportion of segregating sites differed among subpopulations between the two subgenomes of *C. bursa-pastoris.* In *CbpCg* the highest proportion was found in EUR, and the lowest in ASI, while in *CbpCo,* the highest proportion was also found in EUR but the lowest in ME (Table 1). Notably, the *CbpCo* ASI subgenome had a somewhat higher proportion of segregating sites than the *CbpCg* ASI (Table 1). Similarly, the Watterson's theta estimates were higher for *CbpCo* than for *CbpCg*, however, marginally in ME (Table 1).

Table 1. Proportion of segregating sites and Watterson's theta estimates for each subpopulation of *C. bursa-pastoris* and the parental species *C. grandiflora* and *C. orientalis.*

	Proportion of segregating sites	*Watterson's theta*
CbpCg ASI	0.009	0.0024
CbpCo ASI	0.015	0.0038
CbpCg EUR	0.022	0.0054
CbpCo EUR	0.023	0.0057
CbpCg ME	0.012	0.0035
CbpCo ME	0.012	0.0036
C. grandiflora	0.077	0.0159
C. orientalis	0.008	0.0018

Comparing *C. bursa-pastoris* subpopulations to their parental species show that all *CbpCg* subpopulations have less genetic variation in comparison to *C. grandiflora* (Table 1). In contrast, all *CbpCo* subpopulations have a higher genetic variation than *C. orientalis* (Table 1).

SIFT characterising of deleterious mutations

By using SIFT4G to make predictions about the state of mutations (tolerated or deleterious) the genetic load in the three subpopulations (ASI, EUR and ME) and the parental species (*C. grandiflora* and *C. orientalis*) was estimated. The overall genetic load, meaning all deleterious alleles, was significantly higher in *CbpCg* ASI than in *CbpCg* EUR (p-value 8.97E-06) (Figure 2A). Additionally, all three *CbpCg* subpopulations were significantly different from *C. grandiflora* (p-values: ASI 1.13E-10, EUR 2.36E-04, ME 1.18E-04) (Figure 2A). Furthermore, similarly as in the *CbpCg* subgenome, all three *CbpCo* subpopulations significantly differ from their parental species *C. orientalis* (p-values: ASI 1.43E-04, EUR 3.44E-10, ME 8.76E-03) (Figure 2A). The significantly different subpopulation in the *CbpCo* subgenome was EUR (p-values: ASI 4.83E-02, ME 2.06E-02) showing the lowest genetic load, however, still higher than the *CbpCg* counterpart (Figure 2A).

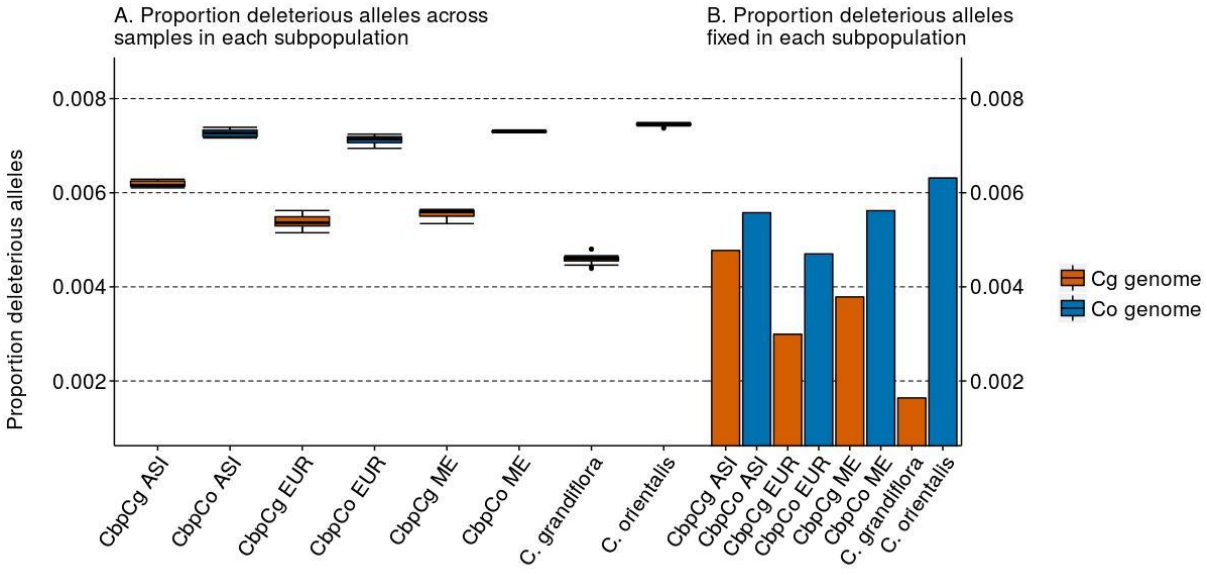

Figure 2. A comparison of the proportion of deleterious alleles between subgenomes of *C. bursa-pastoris* and genomes of *C. grandiflora* and *C. orientalis*. (A) The proportion of deleterious alleles within all samples. (B) Proportion of fixed deleterious alleles within each subpopulation and parental species. Orange represents *C. grandiflora* and the *C. bursa-pastoris* Cg subgenome. The blue indicates *C. orientalis* and the *C. bursa-pastoris* Co subgenome.

The pattern observed in the overall genetic load also hold true for the fixed number of deleterious alleles. The *CbpCg* ASI subpopulation showed more fixed deleterious alleles than

either of the *CbpCg* EUR and *CbpCg* ME subpopulations. Similarly, *CbpCo* EUR displayed the fewest fixed deleterious alleles out of the three *CbpCo* subpopulations (Figure 2B).

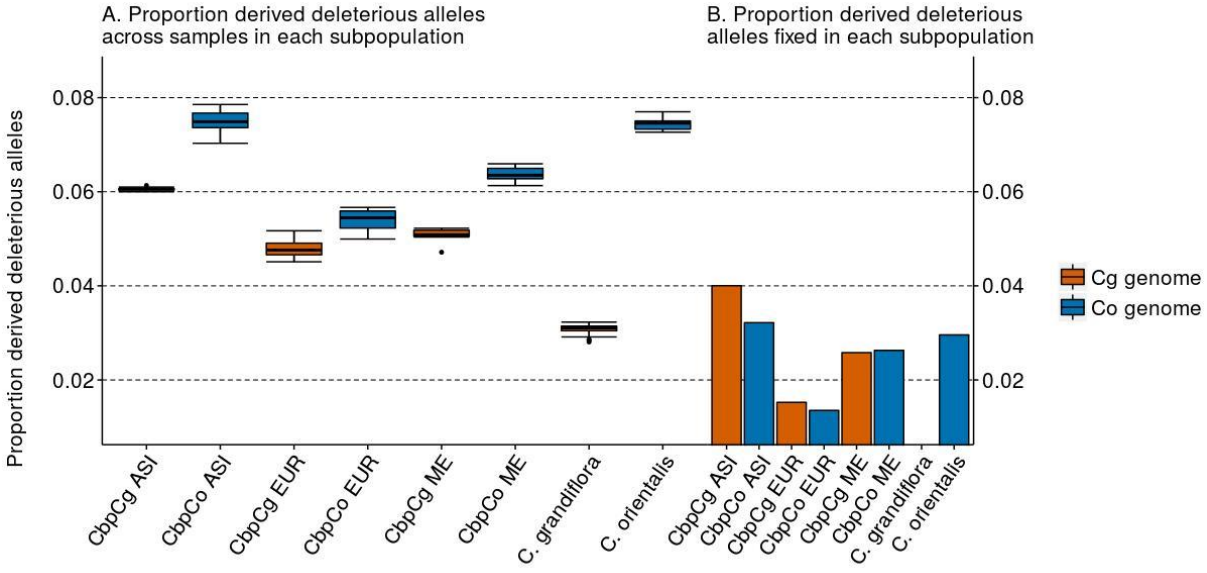

Figure 3. A comparison of the proportion of derived deleterious alleles between subgenomes of *C. bursa-pastoris* and genomes of *C. grandiflora* and *C. orientalis*. (A) The proportion of derived deleterious alleles within all samples. (B) Proportion of fixed derived deleterious alleles within each subpopulation and parental species. Orange represents *C. grandiflora* and the *C. bursa-pastoris* Cg subgenome. The blue indicates *C. orientalis* and the *C. bursa-pastoris* Co subgenome.

Adding the reconstructed ancestral sequence as a reference in addition to the SIFT4G predictions made it possible to identify derived deleterious alleles specific to the two subgenomes (CbpCg and CbpCo) and the parental species (*C. grandiflora* and *C. orientalis*). These derived deleterious alleles in the polyploid subgenomes are the ones that have occurred after *C. bursa-pastoris* speciation, rather than being inherited from the parental lineages. Within the *CbpCg* subgenome, the subpopulation ASI showed a significantly higher genetic load than EUR (p-value 8.68E-06) (Figure 3A). While in the *CbpCo* subgenome ASI was significantly different from both EUR (p-value 5.08E-08) and ME (p-value 1.26E-03). Hence ASI showed the largest genetic load (Figure 3A). In comparison with the parental species, all three *CbpCg* subpopulations were significantly different from *C. grandiflora* (p-values: ASI 1.13E-10, EUR 2.49E-04, ME 1.09E-04) but only *CbpCo* EUR (p-value 4.62E-07) and *CbpCo* ME (p-value 1.05E-02) differ significantly from *C. orientalis* (Figure 3A).

Regarding the fixed derived deleterious mutations, there was barely any difference between the two subgenomes in EUR nor ME (Figure 3B). On the other hand, in ASI the *CbpCg* subgenome showed a higher genetic load than the *CbpCo* (Figure 3B).

The distribution of fitness effects

The result from polyDFE gave that model B (a full DFE) best explained the two non-synonymous tolerated data sets in EUR. All other data sets were best explained by model A (with only a deleterious distribution). To better compare the populations and because there was just one population found to be best described by a full DFE model, the first model (A) was used on all sets. Additionally, it may be a convergence issue in the software, because the result with beneficial mutations appeared unrealistic.

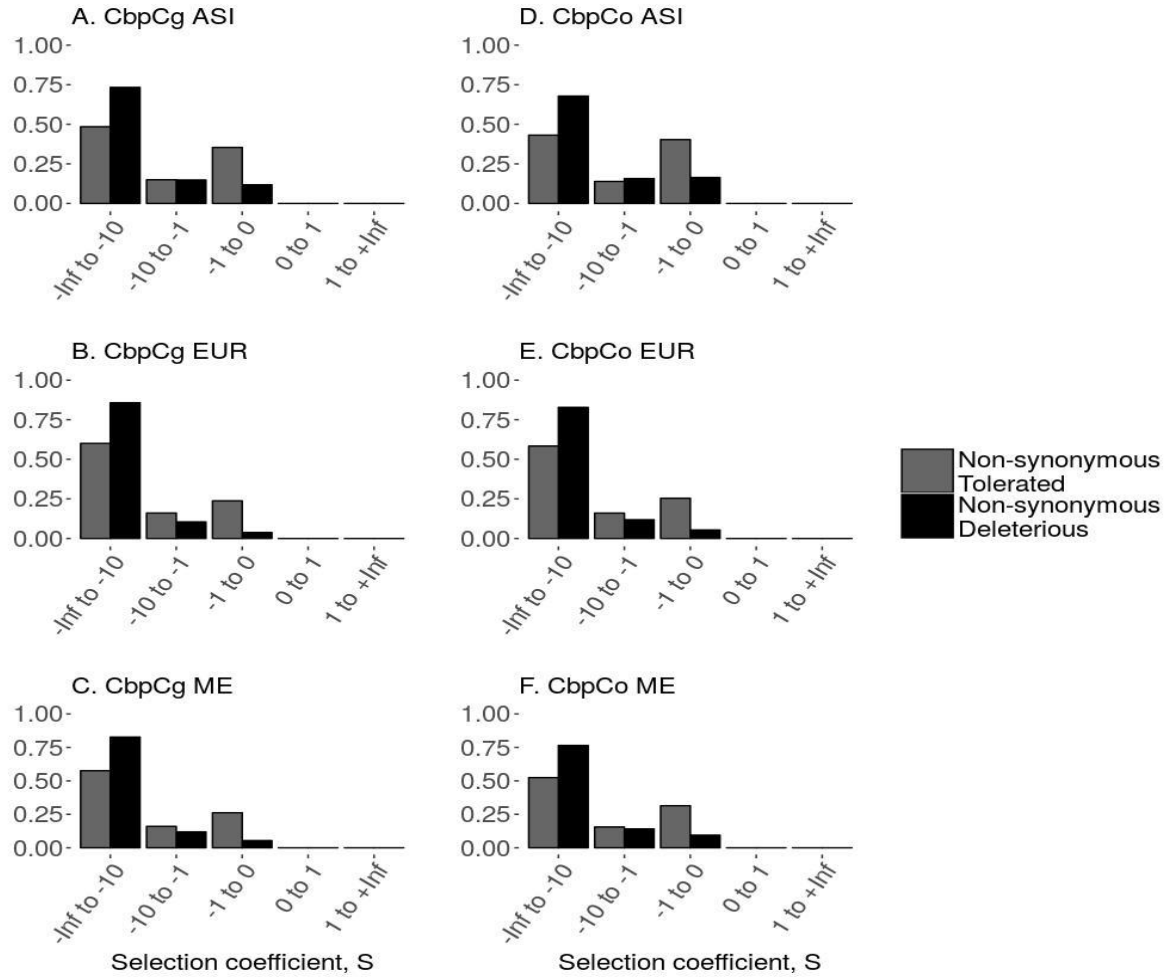

Figure 4. An estimation of the proportions of non-synonymous tolerated (grey) and non-synonymous deleterious (black) alleles occurring under varying selection coefficients (S). (A-C) *C. bursa-pastoris Cg* subgenome and subpopulations. (D-F) *C. bursa-pastoris* Co subgenome and subpopulations. S < 0 = deleterious, S = 0 = neutral and S > 0 = beneficial.

In the ASI subpopulation, most mutations are estimated to be strongly deleterious, followed by nearly neutral and with the lowest proportion of intermediate effect alleles (Figure 4A and Figure 4D). There are slightly less strongly deleterious and more nearly neutral in the *CbpCo* subgenome in comparisons to *CbpCg*. Both of these two patterns are also observed in ME (Figure 4C and Figure 4F). Continuing with the EUR subpopulation, most mutations are estimated to be strongly deleterious and a similar proportion of mutations in the intermediate and nearly neutral effects. Additionally, there is no striking difference between the two subgenomes (Figure 4B and Figure 4E).

Discussion

By characterising the genetic load of the allotetraploid *C. bursa-pastoris* I aimed to investigate a possible difference in the accumulation of deleterious alleles between the two subgenomes (*CbpCg* and *CbpCo*) due to polyploidization. Further, I wanted to explore if there was an effect of range expansion across its three subpopulations (ASI, EUR and ME).

The C. bursa-pastoris subgenomes differ from their parental genomes

Comparing the *CbpCg* subpopulations and *C. grandiflora* in the overall genetic load (Figure 2), I found that all *C. bursa-pastoris* subpopulations have a higher load than *C. grandiflora*, both regarding fixed and all deleterious alleles. This becomes even clearer when considering derived deleterious alleles. There are barely any fixed alleles in *C. grandiflora* and notably more in all subpopulations of *CbpCg*. This pattern, that *CbpCg* has a higher load than *C. grandiflora*, agrees with estimates of genetic diversity (the proportion of segregating sites and Watterson's theta), given that *C. grandiflora* has a higher genetic diversity in comparison to *CbpCg* (Table 1). However, this is not surprising considering that *C. grandiflora* is an out-crosser, with a much larger effective population size (N_e) than *C. bursa-pastoris* and is therefore more likely to keep its genetic variation and less likely to fix deleterious alleles due to genetic drift. On the other hand, *C. bursa-pastoris* is mainly a selfer and is therefore expected to experience a higher level of genetic drift with deleterious alleles more likely to go to fixation.

Another notable observation concerns the subpopulations for *CbpCo* and *C. orientalis*. The relationship here is almost reversed to the one between *CbpCg* and *C. grandiflora*. The

subpopulations have slightly less load than *C. orientalis* (Figure 3). This is most apparent when considering fixed deleterious alleles (Figure 3B). Similarly, as with *CbpCg* and *C. grandiflora,* the genetic diversity estimates correlates with the SIFT results. *Capsella. orientalis* displays the highest genetic load and the lowest diversity.

One can only speculate as to why *C. orientalis* and *CbpCo* differ in this way. One explanation could be a difference in population size. *Capsella orientalis* is a self-fertilising species that has a much smaller range than *C. bursa-pastoris* (Hurka et al. 2012), both of which should inevitably result in a smaller N_e, which in turn would affect the rate at which alleles go to fixation. Additionally, keeping in mind the larger range of *C. bursa-pastoris* and its population structure. Theory tells us that genetic diversity can decline in the subpopulations of a structured population. Although, the genetic diversity in the population as a whole can still be somewhat preserved. This is the result of different alleles getting lost due to genetic drift in the subpopulations. However, the allele is still present in the population and can therefore be restored in a subpopulation through gene flow.

Another source of the difference in genetic load between *CbpCo* and *C. orientalis* could be that *C. bursa-pastoris* has reduced more of its genetic load than *C. orientalis.* One can only assume that a single factor, such as purging deleterious alleles due to inbreeding, is not a sufficient explanation as this is a factor present in both *C. bursa-pastoris* and *C. orientalis.*

One of the expectations is that the additional ploidy level would buffer the genetic load of the *Cbp* subgenomes. Thus, polyploidy cannot be overlooked as a possible source. It has been indicated that a neotetraploid can show an increase in mean fitness for generations due to masking and a lower proportion of genome wide deleterious alleles, especially in comparison to a diploid parental species (Otto and Whitton 2000). However, even if *C. bursa-pastoris* is a relatively new species (100-300 kya (Douglas et al. 2015)) it might be that it has already passed the window during which neopolyploids display a lower proportion of genome-wide deleterious alleles. As I do not have estimates of the fitness it is hard to assess the likelihood of this explanation but it seems plausible.

Difference between the two subgenomes of the allotetraploid C. bursa-pastoris

I also discovered that the genetic load differed between the two subgenomes of tetraploid *C. bursa-pastoris*. The *CbpCo* subgenome displayed a higher proportion of deleterious alleles than the *CbpCg* subgenome (Figure 2, Figure 3). This difference is likely partly reflecting the

difference between the loads inherited from the two different parental species. *Capsella orientalis* provided a subgenome with a higher load and lower genetic diversity since the genome had evolved under higher selfing regime. In contrast, *C. grandiflora,* as an outcrosser, contributed a subgenome with a lower genetic load and higher genetic variation. Therefore, it is apparent that the difference in mating system of the parental species has influenced the amount of genetic load in the two subgenomes. However, considering the derived deleterious alleles (mutations arisen in the two subgenomes after polyploidization) there is still a difference when considering derived deleterious alleles within each sample (Figure 3A). This difference between the derived deleterious alleles between *CbpCg* and *CbpCo* can possibly indicate that one subgenome buffers the other, but further information is needed.

Subpopulations of C. bursa-pastoris are different

My analysis revealed that there were differences in genetic load between the different subpopulations. The first expectation was that the amount of genetic load should increase along the waves of range expansion. I therefore expected the load to be highest in the ASI subpopulation and lowest in ME. Additionally, the polymorphism should decrease following the expansion wave due to repeated bottlenecks and increased genetic drift. In this case ME should be the most polymorphic and ASI the least.

The result found with SIFT does not follow these two hypotheses, therefore, range expansion alone cannot explain the patterns of genetic load across the *C. bursa-pastoris* range. Firstly, ME does not have the lowest genetic load. Actually, EUR has a significantly lower load than both ME and ASI (Figure 2 and Figure 3). Secondly, EUR is the most polymorphic subpopulation (Table 1) in both subgenomes followed by ME and then ASI in *CbpCg* and reversed (ASI then ME) in *CbpCo.* This reversed relationship between the level of polymorphisms between the subgenomes of ASI and ME can be explained by higher values in the *CbpCo* ASI subpopulation due to introgression between *CbpCo* and *C. orientalis* (Kryvokhyzha et al. unpublished).

A second expectation was to find a shift towards more deleterious alleles with nearly neutral effects in the subpopulations with smaller N_e (however, the majority should still be deleterious mutations with strong effects). There is a notable tendency towards this; there are more nearly neutral mutations in ASI than in EUR and more in *CbpCo* than in *CbpCg* (Figure 4).

The most striking deviation from expectations is the low genetic load and higher genetic diversity found in EUR rather than ME. Connecting the DFE result (where EUR is estimated to have a higher occurrence of alleles with a strong deleterious effect) to the SIFT result (EUR having a lower genetic load), suggest that selection is more efficient in EUR.

Reasons why EUR deviates from my expectations based one the range-expansion scenario could be as follows. Firstly, purging deleterious alleles in this population could be more effective than both ASI and ME. It has been suggested that purging occurs in two distinct ways: by non-random mating and by genetic drift (both which are sensitive to population size), with non-random mating expected to be more efficient (Glémin 2003). Bearing this in mind and that EUR displays the larger N_e, one can hypothesise that genetic drift might acts as the principal force in purging in ASI and ME due to smaller population sizes.

The second possibility for why EUR deviates from expectations is that the demographic scenario assumed may be too simple and in need of refinement to better explain why N_e appears lower in ME than in EUR. Similarly, as to the explanation for the reverse polymorphism in *CbpCo* and *CbpCg*, signs of introgression have been found between *CbpCg* EUR and *C. rubella* (Slotte et al. 2008; Kryvokhyzha et al. unpublished).

Further explanations for the deviation is that only alleles with nearly neutral effects adds to the expansion load whereas strongly deleterious alleles do not (Peischl et al. 2013). Additionally, simulations have indicated that range expansion with cyclic gene flow can effectively purge the genetic load (Marchini et al. 2016). It is hard to say whether one of these explanations can compensate for the deviation alone or whether the two can apply here.

Beyond C. bursa-pastoris

To summarise my findings, first I found that there was a difference in genetic load across the range of C. *bursa-pastoris* and that it could not be explained solely by the simple range-expansion scenario initially proposed or the available demographic model. This finding can be applicable to all expanding species, whether they are invasive or species that are slowly extending within an existing habitat.

In today's world, it is becoming increasingly common for habitats to rapidly change due to human activities. Species that can take advantage of this have an opportunity to expand their range. For research in such species, it is therefore crucial to be aware of the genetic load, especially since both fragmentation and habitat loss can lead to reduced N_e, thereby contributing

to the genetic load. The observation that genetic load varies across a population is therefore something to consider when conducting experiments or population genetic related analyses, whether it is to get a better understanding of evolutionary aspects or for conservation purposes.

My second finding was that there was a difference between the two subgenomes of the polyploid species. The observed differences could possibly be a simple consequence of polyploidization itself but part of the difference simply reflects the different mating systems of the two parental species.

In summary, the current study illustrated well the interaction between genomic properties, selection and demography in shaping standing genetic variation in a species.

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Appendix I, Tabel and plots comparing SIFT4G result with and without low confidence predictions for deleterious alleles.

Table A1. P-values from the group comparison made by Dunn's test for including and excluding low confidence deleterious alleles. Black values are below 0.05.

	All deleterious		*Derived deleterious*	
Groups tested	**excluding**	**including**	**excluding**	**including**
CbpCg_ASI - CbpCg_EUR	8,97E-03	7,85E-03	8,68E-03	6,40E-03
CbpCg_ASI - CbpCg_ME	1,60E-01	1,82E-01	1,65E-01	2,19E-01
CbpCg_EUR - CbpCg_ME	4,05E-01	3,44E-01	3,89E-01	2,65E-01
CbpCg_ASI - Cg	1,13E-10	1,13E-10	1,13E-10	1,13E-10
CbpCg_EUR - Cg	2,36E-04	2,92E-04	2,49E-04	3,99E-04
CbpCg_ME - Cg	1,18E-04	8,45E-05	1,09E-04	5,07E-05
CbpCo_ASI - CbpCo_EUR	4,83E-02	3,40E-02	5,08E-08	3,79E-05
CbpCo_ASI - CbpCo_ME	5,68E-01	6,82E-01	1,26E-03	3,58E-02
CbpCo_EUR - CbpCo_ME	2,06E-02	2,29E-02	1,51E-01	1,51E-01
CbpCo_ASI - Co	1,43E-04	2,67E-04	2,45E-01	2,78E-01
CbpCo_EUR - Co	3,44E-10	3,43E-10	4,62E-07	4,06E-09
CbpCo_ME - Co	8,76E-03	7,72E-03	1,05E-02	1,20E-03

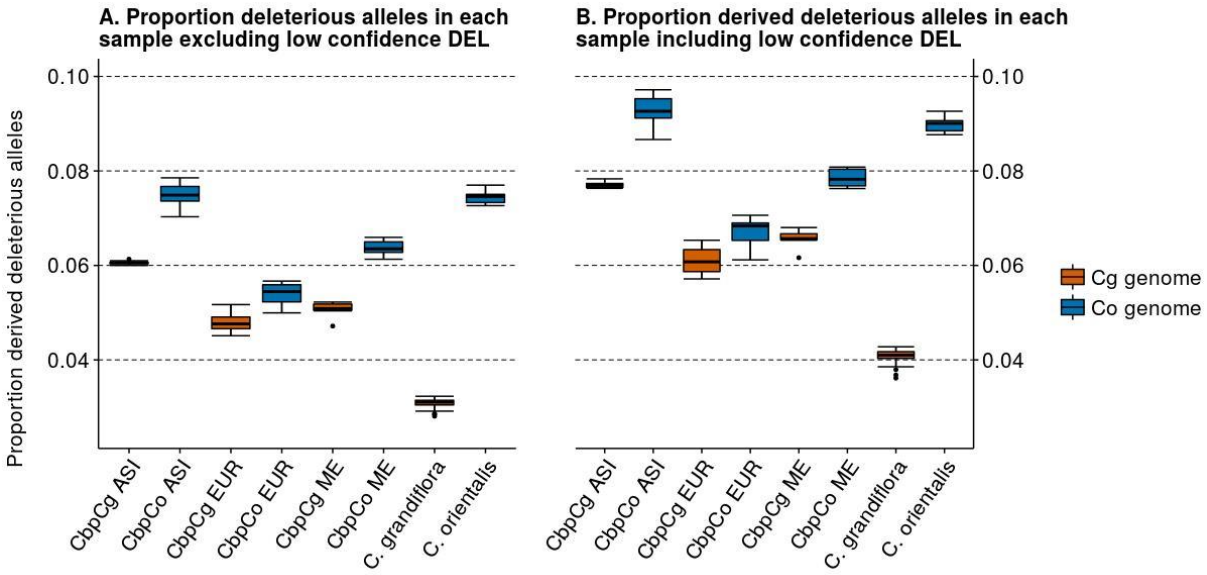

Figure A1. A comparison of the proportion of deleterious alleles when excluding (A) or including (B) low confidence deleterious alleles. Orange represents *C. grandiflora* and the *C. bursa-pastoris* Cg subgenome. The blue indicates *C. orientalis* and the *C. bursa-pastoris* Co subgenome.

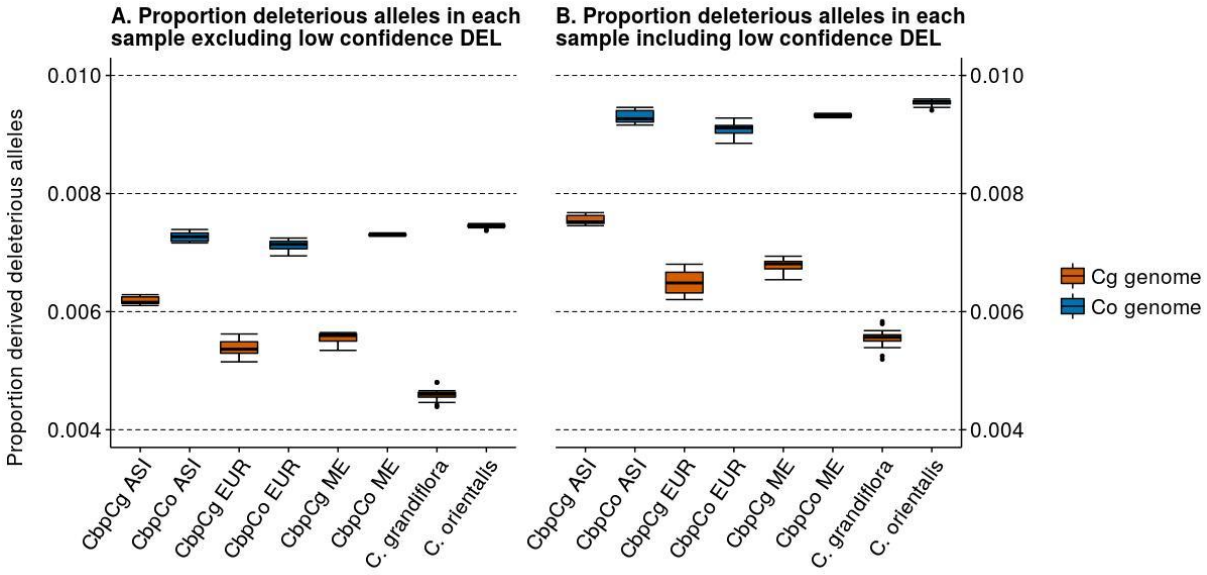

Figure A2. A comparison of the proportion of derived deleterious alleles when excluding (A) or including (B) low confidence deleterious alleles. Orange represents *C. grandiflora* and the *C. bursa-pastoris* Cg subgenome. The blue indicates *C. orientalis* and the *C. bursa-pastoris* Co subgenome.

Appendix II, Tables with range and initial values provided in order to run polyDFE.

Table A2. Parameters estimated in the different polyDFE models and the values of the range to estimate the parameters within.

Parameter	*Range*	*Description*
k	0.01	Value that controls the transformation of the range each parameter should be estimated within.
eps an	0 0.1	Ancestral miss identification error in the SFS under selection
lambda	0 1	Accounts for the number of neutral mutations that goes to fixation during divergence
theta bar	0 1	Scaled mutation rate per site per generation
r	0 10	The density of the gamma distribution in model A and B. The density of the Exponential distribution in model C
beta	0 10	Shape of the gamma distribution
S bar	-200 0	Specific to model A, mean of the DFE
S max	0 100	Specific to model A, the maximum value S can take
Sd	0 0.5	Specific to model B and C, mean of the DFE for S < 0
pb	0 50	Specific to model B and C, probability that S > 0
Sb	0 1	Specific to model B and C, shared selection coefficient of all positively selected mutations

Table A3. Parameter values and flags provided in the init file for model D, polyDFE. The flags represent, estimate parameter value (0) or that the parameter is kept fixed (1).

Parameter	*Flag*	*Value*	*Parameter*	*Flag*	*Value*
eps an	0	0.02	*S2*	0	-10
eps cont	1	0.00	*p2*	-	0.35
lambda	0	0.005	*S3*	0	0
theta bar	0	0.003	*p3*	-	0.1
a	1	-1	*S4*	0	3
S1	0	-50	*p4*	-	0.05
p1	-	0.5	*r*	0	1 1 1 1 1 1 1 1