Paramutation in evolution, population genetics and breeding

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Abstract

Paramutation is a fascinating phenomenon in which directed allelic interactions result in heritable changes in the state of an allele. Paramutation has been carefully characterized at a handful of loci but the prevalence of paramutable/paramutagenic alleles is not well characterized within genomes or populations. In order to consider the role of paramutation in evolutionary processes and plant breeding, we focused on several questions. First, what causes certain alleles to become subject to paramutation? While paramutation clearly involves epigenetic regulation it is also true that only certain alleles defined by genetic sequences are able to participate in paramutation. Second, what is the prevalence of paramutation? There are only a handful of well-documented examples of paramutation. However, there is growing evidence that many loci may undergo changes in chromatin state or expression that are similar to changes observed as a result of paramutation. Third, how will paramutation events be inherited in natural or artificial populations? Many factors, including stability of epigenetic state, mating style and ploidy, may influence the prevalence of paramutation states within populations. Developing a clear understanding of the mechanisms and frequency of paramutation in crop plant genomes will facilitate new opportunities in genetic manipulation, and will also enhance plant breeding programs and our understanding of genome evolution.

Keywords: Paramutation, population genetics, evolution

Introduction

Paramutation refers to epigenetic phenomena by which the expression of one allele is heritably changed upon exposure to and interaction with another allele of the same gene [1]. In well-defined examples, such as the *b1* locus of maize, paramutation is a *trans*-communication event in which the epigenetic state of one allele is communicated to another allele of the same gene resulting in stable, heritable alterations of expression for the receptive allele [2]. Five different loci in maize meet a definition of paramutation which specifies that interaction between two specific loci results in a heritable change in expression of one allele, that can then communicate the new expression change to another paramutable allele in a subsequent generation [3-7]. These five loci are not uniform in penetrance, transgenerational behavior, stability of expression, or cis requirements (reviewed by [1]). In addition, there are several well-characterized examples of paramutation in other species (reviewed [8-9]). However, performing the rigorous genetic experiments to show that the allelic communication actually occurred is quite difficult and time consuming [10] and many additional examples may be better described as paramutation-like phenomena. One of the challenges associated with determining that an allelic interaction and expression change is in fact paramutation lies in the fact that the exact mechanism of paramutation has not been clearly defined, and there may be multiple types of paramutation with distinct underlying mechanisms. In order to fully consider the role that paramutation might play in evolution and breeding of crop plants we review the available research on many examples of paramutation and paramutation-like events.

Mechanisms of paramutation

Paramutation must include a series of events, including communication, initial silencing and maintenance of the silencing. It is easiest to describe these events by discussing paramutation at the *b1* locus of maize and our knowledge of the molecular basis for paramutation at this locus. At *b1*, paramutation occurs between *B'*, which is lowly expressed, and *B-l*, which is highly expressed [4,17]. The protein encoded by the *b1* locus, B1, is a transcription factor that activates anthocyanin biosynthesis,

the highly expressed *B-I* allele confers deeply pigmented sheath and husk tissues [18] which enables simple visual observation of pigment levels that are tightly correlated with specific transcription levels [17]. This has facilitated forward genetic screens and recombination mapping strategies that identified *cis* and *trans* factors required for *b1* paramutation that have helped to shed light upon the molecular events that underlie paramutation and are discussed at length in separate chapters, we only briefly revisit them here.

Recombination-based analysis of paramutagenic alleles revealed the importance of a tandem hepta repeat sequence (*b1TR*) located ~100kb upstream of *B'* and *B-I* [19]. Other alleles of *b1*, like *b* and *B-Peru* that do not participate in paramutation only contain a single copy of this sequence. Transgenic experiments have shown that the *b1* tandem repeats can induce paramutation and receive the signal of paramutation, when located in non-allelic genomic positions [20]. While the *B-I* and *B'* alleles are genetically identical there are differences in chromatin, including DNA methylation, nucleosome position, and chromatin modifications at *b1TR* [21-23]. There is also evidence for small RNAs produced by the *b1TR* [24].

Genetic analysis identified the *mediator of paramutation* mutants (*mop1*, *mop2* and *mop3*) based upon their inability to establish and maintain paramutation dependent silencing of *B1* [25]. Molecular cloning of each mutation revealed that *mop1* encodes a predicted RNA-dependent RNA polymerase [26], *mop2* encodes the second largest subunit of plant specific DNA dependent RNA polymerases IV and V (Pol IV and Pol V) [27], and *mop3* encodes the largest subunit of Pol V [28]. MOP1, MOP2, and MOP3 are each orthologous to Arabidopsis proteins involved in RNA-directed DNA methylation (RdDM) and gene silencing, and physically associate with one another in Pol IV complexes [29]. Similarly, several of the *required to maintain repression (rmr)* genes that were identified based on their role in maintaining the silenced state for the *Pl'* paramutated allele also encode components of PolIV or associated proteins [30-31]. The involvement of RdDM provides a temptingly simplistic model for paramutation, with *b1TR* derived siRNAs directing chromatin modifications and *B1* silencing in *trans*. However, while it is clear that paramutation-induced silencing bears the hallmarks of RdDM, it is still not clear that RdDM or siRNAs are the sole determinants or communicating factors that define paramutation [23]. We distinguish transcriptional silencing and paramutation on the basis that paramutation involves allele communication that is transgenerationally stable. Silencing can be induced by RdDM-like mechanisms, but does not always persist in the absence of the inducing factor [23], indicating that transient silencing can occur in the absence of paramutation. When paramutation-induced silencing is disrupted in mutants that lack RdDM-like machinery, crossing away the mutation allows silencing to return, indicating that silencing has been interrupted but the paramutated condition persisted [25]. Thus, transcriptional silencing associated with RdDM may be how silencing results from paramutation, but may not itself constitute paramutation.

The *b1* locus and several others in maize have been extremely useful in documenting paramutation and beginning to probe the underlying mechanisms. However, this is only one example. As at *b1*, *p1*, and *r1* [25], paramutation at *p1* requires the activity of MOP1. Unlike the other loci, silencing at *P1-rr* is not alleviated after a single generation in a *mop1-1* homozygous mutant, and only partial release of silencing is observed after two generations [32]. While transcriptional silencing of both *B'* and *Pl'* is released in *mop1-1* homozygous mutants, upon outcrossing after multiple generations of exposure to *mop1-1*, silencing of *B'* is efficiently reestablished, while the transcriptionally active condition of *Pl'* can persist [25]. These distinctions may be indicative that disparate mechanisms with some overlapping genetic requirements function at distinct paramutable loci.

Why do some alleles become subject to paramutation?

At loci that undergo paramutation, alleles can be described as paramutagenic, paramutable or neutral. Thus, fully defining paramutation requires the evaluation of an allelic series. Paramutagenic alleles have the ability to communicate to paramutable alleles. In most cases, the paramutagenic allele has a low expression state while the paramutable allele has a higher expression state and the communication event results in heritably reduced expression for the paramutable allele. The paramutable allele will then adopt a paramutagenic state and is able to influence a naïve paramutable allele when present in the heterozygous condition. The neutral alleles do not participate in paramutation.

While paramutation, specifically the heritable conversion of the paramutable allele into a paramutagenic allele, is clearly an epigenetic phenomenon that does not depend upon DNA sequence changes, there are genetic requirements for paramutation. The difference between a neutral allele and an allele that participates in paramutation often includes specific sequence changes and these changes are required for paramutation. For example at the *b1* locus the presence of multiple tandem repeats at a region ~100kb upstream of the coding sequence is required for paramutation [18]. Alleles of *r1* that induce or respond to paramutation have multiple tandem or inverted repeat copies of the *R1* gene, respectively [33-34]. In the case of *p1*, paramutation of *p1-rr* was invoked by transgenes containing enhancer sequences that are tandemly repeated in the endogenous allele [6].

Interestingly, some of the other alleles for which paramutation-like silencing has been described are transgenes that may have a complex insertion [9]. It is quite possible that these loci have tandem or inverted repeats of either coding or regulatory sequences. There are also several examples of complex interactions involving small gene families in Arabidopsis that are similar to paramutation. Arabidopsis encodes at least three members of the *PAI* gene family that are located in three different genomic regions [35-37]. Some Arabidopsis accessions contain an additional *PAI* copy, *PAI4* that forms an inverted repeat with *PAI1*. This locus can generate siRNAs that lead to the accumulation of DNA methylation and silencing of all *PAI* genes. However, this silencing does not appear to be heritable in the absence of the *PAI1-PAI4* inverted repeat. The *AtFOLT* genes provide another example of interactions within small gene families [38]. In this case, *AtFOLT1* can be silenced by exposure to a complex *AtFOLT2* locus and this silencing is stably heritable even in the absence of the inducing locus [38].

The shared features of loci that are subject to paramutation or paramutation-like phenomena suggest can be invoked to propose an explanation of why these particular alleles are subject to paramutation. In most cases there are either tandem or inverted repeats of coding or regulatory sequences or insertion of transposons into regulatory regions for the gene. These types of sequences, or sequence rearrangements, have the potential to create small RNAs. Given the potential for small RNAs to provide a mobile communication signal between genomic loci and the evidence for a role of small RNA pathways in paramutation it seems likely that an allele must have the potential to generate small RNAs, likely 24nt, in order for the allele to trigger paramutation. It is not clear that the paramutable allele will be the source of siRNAs, but it does appear that the paramutable allele must contain sequences homologous to the small RNAs in order for the signal to be received. There may also be additional requirements for the chromatin state of the paramutable allele to facilitate initiation and maintenance of silencing. It is worth noting that the types of sequences that may be important for generating paramutagenic potential, complex arrangements of duplicated sequences or presences of transposons, may be more common in crop species with complex genomes than in species with simpler genome structure such as Arabidopsis.

While the expression levels of many different genes are disrupted in mutants defective for RdDM-associated proteins like *mop1-1* [39], and MOP1-dependent siRNAs map to many locations in the genome [40], it is clear that not all of these loci are regulated directly by MOP1-siRNA dependent silencing [41]. It is also apparent that not all of these loci are subject to paramutation, suggesting that paramutation occurs at a subset of genes subject to RdDM. This may indicate that the mechanism of paramutation is a subspecialization of RdDM in maize, perhaps related to the specific activity of distinct complexes of Pol IV and or Pol V with different subunit compositions [28-29].

What is the prevalence of paramutation?

Understanding whether paramutation is a rare phenomenon that occurs at a handful of loci or a more wide-spread phenomenon that affects a number of traits is critical in estimating the potential importance of paramutation in evolutionary processes and breeding. Addressing this question may be limited by the factors that limit our ability to observe and document paramutation. Nearly all of the loci for which paramutation has been described encode loci that are dispensable but result in a visibly scored qualitative trait. For example, in maize, the majority of research on paramutation has centered on loci that encode regulators of anthocyanin synthesis. Alleles with reduced expression have limited or no effect upon fitness. However, they do result in visible phenotypes and some of these phenotypes may have been selected by humans due to the novel color traits. Many other examples of paramutation-like behavior involve transgenes with easily scored markers.

Our ability to detect paramutation is therefore subject to two confounding issues: the ability for paramutagenic alleles to be tolerated and our ability to detect paramutation. Typically, a paramutated allele would result in reduced or no expression for a gene. Interestingly, many well documented examples of paramutation in maize occur at loci that exhibit regulatory diversity and tissue-limited expression patterns [34, 42-44]. It is possible that these attributes make a gene amenable to paramutation. For many other genes, reduced expression might result in strong fitness consequences or even lethality. These traits would be easy to observe but they would be unlikely to be tolerated in plant populations. These deleterious alleles would be purged due to selective pressures in natural or domesticated populations. For other genes, reduced expression might have limited phenotypic impact. How might we detect paramutation in these examples? Mapping of quantitative trait loci (QTL) using either bi-parental populations or GWAS approaches would likely fail to detect loci with paramutation. Stable paramutation in a bi-parental population would remove all segregation as all off-spring would contain paramutated alleles with low expression. A GWAS population may include examples of both epigenetic states at a paramutable locus but these would have identical sequence and therefore would provide no SNP that could be associated with phenotypic differences. These complications likely result in our ability to easily detect paramutation only for dispensable loci with easily scored phenotypic impact. This may suggest that the documented cases of paramutation are only the tip of the iceberg.

If we had a detailed understanding of the mechanism of paramutation it might be possible to know the molecular signatures of paramutation. However, our understanding of the mechanism of paramutation is incomplete and many of the surrogate phenotypes, such as siRNA abundance or CHH methylation, are not perfectly correlated with paramutation. In spite of this imperfect correlation, it does seem that paramutation occurs at loci that are targeted by siRNAs and RdDM and we can therefore use genomic approaches to identify paramutation-like inheritance patterns by observing gene / siRNA expression patterns and DNA methylation patterns. The analysis of gene expression in a bi-parental mapping population of maize identified 145 genes that exhibit paramutation-like expression patterns [45]. These genes exhibit differences in expression in the parents but all of the progeny exhibit expression similar to

one of the two parents. In most cases (124/145) the off-spring had gene expression levels that were similar to that of the lower expressed parent and in many cases the F_1 plants also exhibit expression levels similar to that of the low parent [45]. The analysis of 24nt siRNAs in Arabidopsis [46-47], rice [48] and maize [49] revealed many loci with non-additive expression patterns in the F_1 hybrids and in some cases these non-additive expression levels of 24nt siRNAs are associated with non-additive DNA methylation patterns that could be inherited in later generations. The analysis of small RNAs in tomato hybrids and introgression lines also provided evidence for potential paramutation-like expression patterns [50]. The genome-wide analysis of DNA methylation patterns has also provided evidence for potential paramutation-like inheritance patterns. A series of studies in Arabidopsis have defined trans-chromosomal methylation (TCM) patterns based on studies of DNA methylation profiles in parents and F_1 hybrids [51]. They find that a subset of regions with differential levels of DNA methylation between the parents will exhibit non-additive patterns of DNA methylation in the F_1 off-spring [51] and that in some cases these non-additive patterns can be inherited in subsequent generations [52]. The analysis of maize DNA methylation profiles also provides evidence for potential paramutation-like inheritance of DNA methylation profiles at a subset of genomic regions [53]. However, while some differentially methylated loci can lead to paramutation-like inheritance patterns there is also evidence that the majority of differentially methylated loci can be inherited in Mendelian patterns [54-57]. While these studies of the inheritance of gene expression, small RNA abundance and DNA methylation profiles provide evidence for the potential of paramutation to affect many loci it is not clear that these examples do in fact represent paramutation-like mechanisms.

How will paramutation influence inheritance in populations?

Paramutation has the potential to result in novel inheritance patterns in populations. Highly penetrant paramutation will result in the uniform expression of one particular trait in F_2 or subsequent generations as opposed to the expected segregation of two traits. Although the parental alleles vary, the occurrence of paramutation would homogenize the epigenetic state at this locus in all off-spring. This would prevent the ability to use QTL mapping approaches to identify all of the loci that influence a trait. A related problem could affect GWAS approaches. A SNP might be shared by both paramutable and paramutated alleles that differ in their impact upon the phenotype and this would reduce the ability to associate the SNP with the trait. This suggests the potential for paramutation to create problems related to missing heritability of traits [58]. However, it is also likely that evolutionary pressures will maintain a low prevalence of paramutation in most populations.

There have been limited attempts to develop models for how paramutation would influence population genetics [59-61]. In one sense, paramutation is similar to a selfish genetic element and may act in a fashion similar to meiotic drive or other genetic conflicts [62]. Paramutation involves an allele with two states, the paramutable and paramutant states, and in general the paramutable state is expected to be highly expressed while the paramutant state is silent or lowly expressed. There are a number of factors to consider about how paramutation may spread through a population including mating systems, effects of the different allele states upon fitness and penetrance of the paramutant state.

In a very simple sense, paramutation would affect allele frequencies by converting all heterozygotes (paramutable / paramutant) to homozygous paramutant alleles. This means that the remaining frequency of paramutable alleles is halved each successive generation which would quickly result in the elimination of this allele state unless other forces act to maintain the paramutable allele. Species that reproduce primarily through self-pollination would have a very low potential for paramutation because

heterozygosity is quite low in selfing species. In contrast, out-crossing species tend to have much higher heterozygosity and a paramutant allele state could more easily spread throughout the population.

The effect of the paramutable and paramutant allele states upon fitness will influence the expected outcome in populations. In some cases, the low expression associated with the paramutant state might be highly deleterious and selection would act strongly to eliminate this allele from a population. This would act to maintain the paramutable state in the population and may push the locus towards fixation for the paramutable allele. There might be some on-going spontaneous paramutation at this locus, but selective pressures would maintain this at a low allele frequency. Conversely, the high and low expression for the two allele states may have no impact upon fitness at other loci. In these cases, it is likely that the paramutant allele would quickly spread through the population and become fixed. Only in cases in which there are minor deleterious consequences associated with the paramutant allele would you expect to maintain both allele states. Weak deleterious effects would result in selection against the paramutant allele state while the process of paramutation would act to increase the frequency of this allele. In some cases, these opposing forces may be balanced and would act to maintain both allele states within a population. Geoghegan and Spencer [61] provide a much more complex and in-depth modeling of these factors.

The discussion above assumes full penetrance of paramutation and stable inheritance of the paramutant state. However, the previously characterized examples of paramutation are not homogenous in terms of penetrance of paramutation or heritability of the paramutant state [9]. If the paramutant state is unstable, on-going reversion of the paramutant state back to paramutable state would be expected to create the potential for on-going paramutation within a population. Reductions in the penetrance of the paramutation event would slow the rate of decrease for the paramutable allele within populations. There is also evidence that some examples of paramutation may depend upon other factors. For example, some transgenes exhibit paramutation activity only after being exposed to changes in ploidy [63]. Exposure to altered ploidy could reveal novel epigenetic states for some alleles that could trigger paramutation.

Paramutation could be important in several aspects of plant breeding and artificial selection. Interestingly many of the paramutable alleles that have been characterized in maize like reflect alleles that were selected for novel high expression states of a gene with variably expressed alleles. These regulatory attributes may facilitate the development of paramutation at a locus. In ancestral maize populations anthocyanins tend to be expressed in a limited set of tissues [64-65]. However, rare novel alleles that provided pigmentation to seed or vegetative tissues were selected by humans. In some cases, these alleles with novel tissue-specific expression exhibit unstable behaviors such as paramutation [4,65-66].

It is also possible that strong selective pressure for increased expression of genes may produce unstable alleles. In these cases, the advantageous traits conferred by these alleles may be unstable when crossed to other individuals resulting in a loss of the selected trait, meaning that regulatory instability may become more common in hybrid individuals. Thus, there is also potential for paramutation or related phenomena to play a role in heterosis. In heterosis the F_1 hybrids exhibit superior phenotypes relative to the parents that are not maintained in subsequent generations. The decay in phenotypes observed in F_2 and subsequent generations may largely be due to segregation of beneficial alleles but paramutation could also play a role in reducing segregation of beneficial alleles. Some of the molecular phenomena associated with paramutation such as 24nt small RNAs and trans-chromosomal methylation patterns

have been studied in heterotic F₁ plants [46-49, 51-52]. However, there is limited evidence that these patterns are critical for the heterotic phenotype [49].

Concluding remarks:

Paramutation is a fascinating phenomenon that violates the Mendelian principle of segregation and will result in difficulties for introgressing or mapping certain traits. This may pose real problems in plant breeding as important selected traits may resist efforts to cross them into other genetic backgrounds. It is difficult to know the exact number of loci subject to paramutation within particular species or crosses. However, as we gain a better understanding of the exact mechanism of paramutation and develop the abilities to profile molecular events related to paramutation, such as small RNAs, DNA methylation, histone modifications or enhancer activity, we are likely to have much better understanding of the prevalence of paramutation. Our view is that the number of segregating loci subject to paramutation is likely to be quite low at any specific point in time. Over longer periods of time, it is quite possible that paramutation arises at a moderate frequency and that either the paramutable or the paramutant allele will be fixed, giving rise to a new expression pattern for a gene. We are excited about the prospects for developing a better understanding of the prevalence of paramutation and its role in phenotypic variation in plant species in the coming years.

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