X Linkage of AP3A, a Homolog of the Y-Linked MADS-Box Gene AP3Y in Silene latifolia and S. dioica

Rebecca H. Penny*, Benjamin R. Montgomery‡, Lynda F. Delph

Department of Biology, Indiana University, Bloomington, Indiana, United States of America

Abstract

Background: The duplication of autosomal genes onto the Y chromosome may be an important element in the evolution of sexual dimorphism. A previous study reported on a putative example of such a duplication event in a dioecious tribe of Silene (Caryophyllaceae): it was inferred that the Y-linked MADS-box gene AP3Y originated from a duplication of the reportedly autosomal ortholog AP3A. However, a recent study, also using cytological methods, indicated that AP3A is X-linked in Silene latifolia.

Methodology/Principal Findings: In this study, we hybridized S. latifolia and S. dioica to investigate whether the pattern of X linkage is consistent among distinct populations, occurs in both species, and is robust to genetic methods. We found inheritance patterns indicative of X linkage of AP3A in widely distributed populations of both species.

Conclusions/Significance: X linkage of AP3A and Y linkage of AP3Y in both species indicates that the genes’ ancestral progenitor resided on the autosomes that gave rise to the sex chromosomes and that neither gene has moved between chromosomes since species divergence. Consequently, our results do not support the contention that inter-chromosomal gene transfer occurred in the evolution of SIA3Y from SIA3A.

Introduction

Sex-chromosome evolution in dioecious Silene has received considerable attention because the sex chromosomes are recently evolved, allowing exploration of early events in the process of sex chromosome evolution [1]. Chromosomal rearrangements can have important implications for sex-chromosome evolution [2]. Intragenomic translocations may limit recombination, although in Silene the role of chromosomal rearrangements in the cessation of X-Y recombination is yet uncertain [3]. In the absence of recombination, genes linked to male traits could accumulate on the sex chromosomes, allowing differential expression between the sexes and facilitating the evolution of sexual dimorphism [4,5]. Consequently it is of interest to understand the origin of Y-linked genes responsible for male traits. Such genes may have initially been located on autosomal ancestors of sex chromosomes, with male-specific roles evolving after Y-chromosome differentiation. In this case, for recently derived sex chromosomes, homology of male-specific genes would be expected on the X chromosome. Alternatively, genes with male-specific function may have migrated to the Y chromosome after the chromosome’s differentiation. In this case, such genes would lack a homolog on the X chromosome [6,7].

A previous study using flow cytometry found evidence of autosomal inheritance of the AP3A gene in Silene latifolia, which is homologous to the Y-linked gene AP3Y, which would indicate that AP3Y’s location on the Y chromosome was attributable to a translocation event [8]. Both AP3A and AP3Y contain MADS-box sequence motifs involved in floral development, with AP3A expressed in petals of both male and female flowers and AP3Y expressed in petals and stamens of male flowers [8,9]. Many studies have cited Matsunaga et al. [8] as a unique example of gene duplication onto the Silene Y chromosome that has implications for understanding the evolutionary processes involved in the origination of heterogametic sex chromosomes [2,10,11].

We were motivated to investigate X-linkage of the AP3A gene because of preliminary results from a previous study [12], in which we attempted to identify hybrids of S. latifolia and S. dioica following mixed-species pollen applications by the presence of an amplification product of the heterospecific sire’s AP3A gene. We successfully amplified the S. latifolia specific product from males and females of S. latifolia but not S. dioica, and we were only able to successfully amplify the S. latifolia specific product from male but not female F1 hybrids [12]. Similar results were found for the S. dioica specific AP3A product for crosses in the other direction, leading to the hypothesis that AP3A is X-linked in both species.

In this study, we used interspecific crosses between Silene latifolia and S. dioica, species that share a homologous Y chromosome, to further investigate X linkage of AP3A across multiple populations. The structure of the Y chromosome has been shown to vary within
populations, which makes the study of multiple populations important for the generalization of possible translocation events [3]. It has also been suggested that segregation experiments represent the most reliable approach to investigate sex-linkage of genes [13]. Given two cytological-isolation studies yielding conflicting results [8,14], our segregation study presents a logical method to conclusively determine the genomic location of the focal gene. By crossing pure species S. latifolia and S. dioica individuals in both cross directions, X-linked genes from maternal species were isolated in male F1 individuals. The expectation is that if the AP3A/X gene (designated SlAP3A in S. latifolia) and SdAP3A in S. dioica) is X-linked, the paternal species copy would be absent in male F1s and present in female F1s. Our results suggest that AP3A is X-linked in both species, indicating that the location of AP3Y on the Y chromosome is consistent with its ancestral condition and predates the divergence of S. latifolia and S. dioica.

Results and Discussion

The presence of SlAP3A in all S. latifolia individuals, SdAP3A in all S. dioica individuals, and AP3Y in all male individuals of both species was confirmed as were the reciprocal absences of these genes in the alternate species/sex. In S. dioica x S. latifolia (dam x sire) crosses all male F1s (N = 9) amplified AP3Y and not SdAP3A, whereas all female F1s (N = 12) amplified SlAP3A (Table 1). Reciprocal crosses between the species showed a similar pattern: all male F1’s (N = 15) amplified AP3Y and only one of fifteen males amplified a product determined to be SdAP3A. All female F1s in this cross direction (N = 19) amplified SdAP3A and not AP3Y. These results are consistent with X-linked, and not autosomal, inheritance of the AP3A gene in both S. latifolia and S. dioica.

As a result of high sequence similarity, some misamplifications of SdAP3A occurred in male F1s from S. latifolia x S. dioica crosses. Amplification with SdAP3A primers resulted in a product for two male F1s in each of crosses B, D, and E and one male F1 in cross D. These products, as well as the species-specific products from each parent, were sequenced and compared to determine the identity and origin of the F1 products. In all seven cases, the F1’s sequence corresponded with the dam’s SdAP3A sequence and differed from the sire’s SlAP3A sequence at each of four sites with one base-pair substitution. This clearly suggests that the observed product in these F1 males resulted from misamplification of SlAP3A, and not the presence of SdAP3A.

Overall, out of 24 F1 males, only one (from cross C) showed a pattern of inheritance consistent with autosomal inheritance. The SlAP3A sequence of this male showed evidence of double peaks, indicative of amplification of two similar products. There were present two insertions (2bp and 4bp) identical to the SdAP3A sequence of the sire but not the dam. Furthermore, at the four polymorphic loci previously discussed, dominant peaks corresponded to the sire’s sequence while lesser peaks were consistent with the dam’s sequence. This outcome could be explained by aneuploidy, with inherition of X and Y from the sire.

For further comparison, the sire from cross C was crossed to another S. latifolia female (Cross C1). One male F1 was present among the 8 seeds planted for this cross. Under relaxed PCR conditions for the amplification of SdAP3A, products were obtained from all 8 F1 individuals. Consistent with the hypothesis of X-linked inheritance, sequences of products from the 4 F1 females sequenced all corresponded with the sire’s SdAP3A sequence, whereas the sequence of the F1 male corresponded to the dam’s SlAP3A sequence. Thus, for this cross, 5 of 5 individuals showed patterns consistent with X linkage of AP3A.

The evidence that AP3A is located on the X chromosome in both S. latifolia and S. dioica suggests that the current location of this gene predates the divergence of these species. Additionally, the pattern of X-linked inheritance of AP3A was consistent among several geographically distinct populations. Another recent study similarly detected an X-linked homolog but no autosomal homolog for AP3Y for one accession of S. latifolia using laser microdissection [14]. Given our finding across multiple populations and matching results from a different seed source, it is unlikely that population-level differences are sufficient to account for the discrepancies in results between these findings and those of Matsunaga et al. [8]. Instead, impurities in flow cytometry may account for the detection of an AP3 gene associated with autosomes in Matsunaga et al. [14]. While the presence of an X-linked homolog does not diminish the importance of AP3Y in understanding sex-specific evolution of sex-linked genes, it does suggest that autosomal gene duplication was not an element in its specialization.

Methods

Five half-sib families were formed by crossing five Silene dioica males from geographically distinct populations in Skeppsvik Island, Sweden, Roscoff and Alençon, France, and Graubünden and Wallis, Switzerland (Crosses A–E) with the same S. latifolia female (from Virginia). Another S. latifolia female from France) was crossed with the S. dioica sire from cross C to yield a sixth family (Cross C1). Four half-sib families were formed in the reciprocal direction from one S. dioica dam from Roscoff, France and four S. latifolia sires from populations in Virginia, Italy, Portugal and France (crosses F–H). Eight seeds from each cross were planted. Sets of individuals from these plantings were genotyped for each family until either results were obtained for at least two males and two females or all plants had been genotyped. Larger samples were obtained for several crosses, but due to mortality and skewed sex-

| Table 1. Status of AP3Y and AP3A genes in hybrids of Silene latifolia and S. dioica. |
|---------------------------------|---|---|---|---|---|---|---|
| **A. Silene latifolia dam by S. dioica sires** | **Female offspring** | **AP3Y** | **SdAP3A** | **Male offspring** | **AP3Y** | **SdAP3A** |
| | | | | | | |
| | **A** | **B** | **C** | **D** | **E** | **C** |
| | | | | | | |
| **SlAP3A** | | | | | | |
| **Male offspring** | | | | | | |
| | | | | | | |
| **SdAP3A** | | | | | | |
| **B. Silene dioica dam by S. latifolia sires** | **Female offspring** | **AP3Y** | **SdAP3A** | **Male offspring** | **AP3Y** | **SdAP3A** |
| | | | | | | |
| | **F** | **G** | **H** | **I** | **Total** | **Total** |
| | | | | | | |
ratios, only one female in cross D and one male in cross G were genotyped. Seeds were planted in sterilized potting soil and housed at the Indiana University greenhouses.

PCR amplification was performed using DNA extracted from young leaves (QiagenDNEasy kit). The same program was utilized for the amplification of SlAP3A and SdAP3A (95°C for 2.5 min, 56.7°C for 30 s, 72°C for 30 s (30 cycles); 72°C for 5 min). A different program was used for the amplification of AP3Y (95°C for 2 min; 94°C for 20 s, 58°C for 10 s (decrease by 2°C every other cycle); 65°C for 45 s (8 cycles); 94°C for 20 s, 50°C for 10 s, 65°C for 45 s (30 cycles); 65°C for 10 min).

The oligonucleotide primer sets used for PCR were as follows: 5’-AGAAAGTAAAGAACCTTGGAAG-3’ and 5’-ATACTGGA-GATAACACAGCCT-3’ for SdAP3A, 5’-TGCAAGAGCGAGAAAAGT-3’ and 5’-GGTCCAAAACAGATTITAT-3’ for SlAP3A, 5’-AGATTTAGCTGAAAGGTATG-3’ and 5’-ATATTCCGAGCACACTTG-3’ for AP3Y. Using the above primers, agarose gel electrophoresis yielded single fragment products for each amplification. The lengths of the fragments for SlAP3A, SdAP3A, and AP3Y were approximately 400 bp, 900 bp, and 700 bp, respectively. The identity of PCR products from parental individuals was verified through a nucleotide BLAST search (NCBI) of AP3A sequences from each dam used and the seven paternal individuals that we sequenced. The presence of AP3Y in F1 individuals was used to determine the sex of these plants, and these results were later verified by flowering observations.

Two male and two female plants from each full-sib family were then amplified for the putatively autosomal gene specific to the paternal species (SlAP3A or SdAP3A).

PCR products were verified by sequencing for paternal individuals used in crosses A-I (not cross C1), for seven of nine paternal individuals, and for 16 out of 35 F1 individuals in the S. latifolia x S. dioica crosses (Indiana Molecular Biology Institute, Applied Biosystems 3730 automated sequencing system, Applied Biosystems BigDye Terminator ver3.1). Sequencing was also completed in instances where PCR products were suspected to be misamplifications of the homologous sequence from the maternal genome. Consensus sequences were assembled, viewed and edited using CodonCode Aligner (Version 3.5.7, CodonCode Corporation) and were compared to parental sequences for both genes using CLUSTAL X (version 2.0.10).

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Author Contributions

Conceived and designed the experiments: RHP BRM LFD. Performed the experiments: RHP BRM. Analyzed the data: RHP BRM. Contributed reagents/materials/analysis tools: LFD. Wrote the paper: RHP BRM LFD.

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