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Evolution

# Genotypic variation within asexual lineages of *Taraxacum officinale*

**(rRNA-encoding DNA/alcohol dehydrogenase/evolution)**

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**ABSTRACT** **Restriction site variation in DNA that encodes rRNA (rDNA) was surveyed among 714 offspring within 31 lineages (26 genotypes) of obligate asexually reproducing *Taraxacum officinale* (dandelions). Although clonal offspring are expected, plants with nonparental rDNA were produced from two parents that were themselves siblings (same genotype). The variation is best characterized by the loss of an *Eco*RI restriction site that maps to the spacer region in the parental rDNA and is most likely involved in amplification of rare or unique rDNA repeats. In one family, 41 surveyed offspring lacked the *Eco*RI site. In the other family, only 1 of 26 offspring lost the *Eco*RI site. Other classes of DNA surveyed, chloroplast DNA and the alcohol dehydrogenase 2 gene (*Adh2*), showed no variation. However, offspring with nonparental rDNA also had nonparental alcohol dehydrogenase 1 (*Adh1*) restriction fragments. Because somatic mutations in plants can be incorporated into reproductive tissue, we propose that somatic events affecting at least both multicopy rDNA and DNA homologous to the maize *Adh1* gene occurred at different developmental times in the two families. An event early in development would result in all variant offspring; an event late in development would result in a single variant offspring. These results support the view that mutation (in the broad sense) influences the level of genotypic variation in asexual organisms, which may facilitate adaptive evolution of asexual species.**

Sexual reproduction and recombination creates genotypic variation, which is necessary for the evolution of populations and species. Asexual organisms lack this mechanism of genetic reassortment and produce lineages of genetically identical offspring. Although asexual reproduction is advantageous in preserving genotypes that are well adapted to the immediate environment, sexual recombination is thought to be necessary for long-term evolutionary success. Therefore, asexual species traditionally have been regarded as having extremely limited evolutionary potential. However, in the absence of sexual recombination, somatic mutation and intrachromosomal (mitotic) mechanisms of recombination can potentially become important processes that generate genetic variability in asexual plants. In North American asexual *Taraxacum officinale* Wigg. (sensu lato) (Asteraceae), we demonstrate genotypic variation among asexually produced offspring. The rate at which these new genotypes arise in *T. officinale* is higher than expected by random mutation alone. These results show that plants that are solely or predominantly asexual in their mode of reproduction may generate genotypic variation by nonmeiotic processes, albeit at a lower rate than by sexual recombination. Obligate asexual plants therefore do not necessarily consist of invariant lineages. Rather, clonal lineages most likely accumulate appreciable genotypic variation, which may become the basis for subsequent adaptation and evolution.

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The members of the genus *Taraxacum* (dandelions) are geographically widespread and predominantly asexual polyploids that comprise thousands of morphologically variable asexually reproducing lines or clones (1). About 40 diploid sexual species are found in Eurasia (2). North American *T. officinale* is typically triploid and asexual (3, 4).

Asexual reproduction in dandelions is through parthenogenetic development of unreduced eggs (agamospermy). Unreduced eggs are produced following the formation of a restitution nucleus during the first meiotic division of the egg cell and involves little or no chromosome pairing (5). Barring mutation and autosegregation, offspring are expected to be genetically identical to the parent. The clustered inflorescence of the dandelion (capitulum) consists of numerous ligulate florets. Asexual seed production is demonstrated by nearly full seed set following removal of the anthers and stigmas of the florets before the capitulum opens (6).

Other studies have described considerable morphological, chromosomal, electrophoretic, and ecological variation in asexual *Taraxacum* (discussed in refs. 6 and 7). In this study, genotypic variation within asexual lineages of *T. officinale* was surveyed with three classes of DNA: midrepetitive rRNA-encoding DNA (rDNA), single-copy *Adh1* and *Adh2*, (the genes encoding alcohol dehydrogenases 1 and 2, respectively), and cytoplasmically inherited chloroplast DNA (cpDNA). We describe several cases in which novel rDNA and *Adh1* genotypes were produced between asexual generations of *T. officinale*.

## MATERIALS AND METHODS

**Plant Material.** Thirty-one plants or seeds (achenes) were collected from several sites across the U.S. (location of sites are listed in ref. 8) and were grown in the greenhouse. The plants set seed after flower emasculation, and root-tip chromosome counts on 12 plants were $2n = 24$, confirming that the plants were triploid. The plants (parents) represent 26 genotypes characterized by restriction enzyme analysis of rDNA and *Adh1*. Seeds were collected from single emasculated inflorescences of 23 genotypes and from nonemasculated inflorescences of 7 genotypes (5 genotypes had both treatments). The seeds were germinated on moist filter paper in covered Petri dishes, and 22–36 seedlings per parent were grown in the greenhouse. After 6 weeks, the rDNA genotypes of surviving offspring were determined. Additional surveys for restriction site variation in *Adh1* and *Adh2* and in a portion of the cpDNA were done in 12, 2, and 14 of the families, respectively.

**DNA Manipulation.** The protocols for individual DNA isolations and rDNA and cpDNA survey conditions are described elsewhere (8). The DNA isolation yields about 2 μg of DNA per 1–2 g of tissue. For cleaner and larger amounts

Abbreviations: rDNA, DNA that encodes rRNA; IGS, intergenic spacer; cpDNA, chloroplast DNA.

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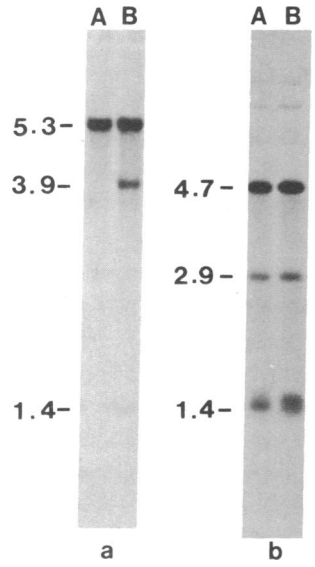


FIG. 1. Ribosomal DNA variation found in two families of asexual *Taraxacum*. Lane A, nonparental rDNA genotype. Lane B, parental rDNA genotype. (*a*) An *Eco*RI digest shows the loss of a restriction site in the nonparental genotype that produces 3.9- and 1.4-kb fragments in the parental genotype. (*b*) Double digestions with *Dra* I and *Bst*EII show that the nonparental genotype has small changes in fragment length (<50 base pairs) compared to the ≈1.4-kb fragments of the parental genotype. These fragments were visualized by hybridization with pTEE5.

of DNA, the components of the isolation procedure were increased 10-fold and two steps were added. After isopropanol precipitation, the DNA pellet was suspended in 1 ml of 1× TE (1 mM Tris, pH 7.2/0.1 mM EDTA, pH 8.0) and digested with RNase for 30 min at 37°C. An equal volume of 7.5 M $NH_4OAc$ was added; the suspension was placed at 4°C for 15 min and centrifuged at 10,000 rpm (Sorvall SS34 rotor) for 20 min. The supernatant was recovered and precipitated with 95% (vol/vol) ethanol, and the pellet was resuspended in 1× TE.

The restriction enzymes *Eco*RI, *Dra* I, *Xba* I, and *Xmn* I were chosen for the survey based on previously mapped restriction site and length polymorphisms that distinguish the parental genotypes (8). The enzymes were purchased from either International Biotechnologies or New England Biolabs and were used according to the supplier's recommended conditions. Approximately 0.5 g of DNA per individual was digested, electrophoresed on 0.8–1.2% agarose gels, transferred to nylon membranes (MSI), and hybridized with rDNA probes pTEE3 and pTEE5. These two clones represent about 98% of a single *Taraxacum* rDNA repeat (8). The chloroplast probes were 21-kilobase (kb) and 15.3-kb *Pst* I fragments of the large single-copy region of the petunia chloroplast genome. The probes were radioactively labeled for hybridization with [$\alpha$-$^{32}$P]dCTP by the random primer method (9). Conditions for electrophoresis, gel blotting, filter hybridization, filter washing, and autoradiography are described by Learn and Schaal (10).

For the *Adh* surveys, 8 $\mu$g of DNA per individual was digested with *Hin*dIII and *Eco*RV and electrophoresed on 0.9% agarose gels. Filters were prehybridized in hybridization solution [5× SSPE (0.9 M NaCl/50 mM $NaH_2PO_4$, pH 7/5 mM EDTA/1% $NaDodSO_4$), 0.5% nonfat dry milk (Carnation), and denatured salmon sperm DNA at 0.5 mg/ml] for 2 hr and hybridized in fresh solution at 65°C overnight to either the maize cDNA probe pZML841 (*Adh2*) (11) or agarose gel-isolated *Pst* I inserts of pZML793 (*Adh1*) (12). The filters were washed according to Reed and Mann (13) and then treated as above.

## RESULTS

The rDNA genotypes of 714 total offspring were characterized. In 2 of the 31 families (6.45%), offspring had nonparental rDNA repeat types. These two families were grown from seeds collected from emasculated flowers, so the nonparental genotypes were found in offspring that must have been asexually produced. The parents of these two families (referred to as P1 and P2) were themselves siblings, grown from seeds collected in the field from a single inflorescence. Parents P1 and P2 had the same genotype based on rDNA restriction sites for 12 mapped enzymes and the same *Eco*RI and *Eco*RV restriction fragments when probed with maize pZML841 (*Adh2*) and pZML793 (*Adh1*).

The difference in rDNA between parents P1 and P2 and their offspring is best characterized by the loss of two restriction fragments in the offspring (Fig. 1*a*) corresponding to the loss of an *Eco*RI site that maps to the intergenic spacer (IGS) region of the parental rDNA. In family P1, 35 offspring surveyed showed the *Eco*RI site loss in the IGS. Because we were interested if seeds produced in other inflorescences had these rDNA changes, 6 offspring from a second emasculated inflorescence were surveyed. These plants also lacked the parental *Eco*RI restriction site. Double digestions with *Dra* I and *Bst*EII, two enzymes that are insensitive to DNA methylation (14), also showed restriction fragment length differences between parent and offspring (Fig. 1*b*), which map near the parental *Eco*RI site in the IGS (Fig. 2). Scanning densitometry on these autoradiograms did not reveal large differences in rDNA amounts between parent and offspring. Chromosome counts on two offspring were $2n = 24$, so the rDNA changes presumably did not involve changes in chromosome number.

In family P2, only 1 of 26 offspring had a nonparental rDNA genotype, which was also characterized by the loss of the diagnostic IGS *Eco*RI restriction site. Root-tip chromosome

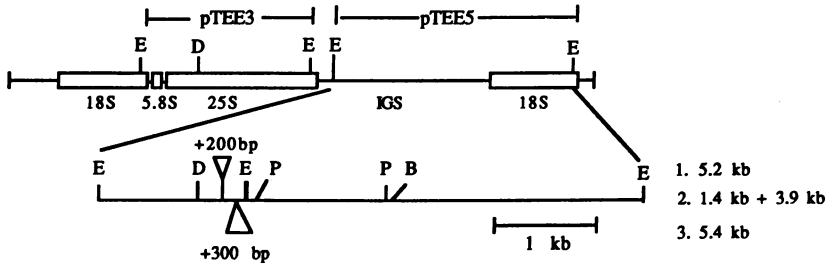


FIG. 2. A *Taraxacum* rDNA restriction map showing the location of two cloned *Eco*RI fragments, pTEE3 and pTEE5, above the map and the location of the variable restriction sites and fragment lengths between parents and offspring. Based on densitometry, the *Eco*RI (E) site within the IGS is found in approximately one-third of the rDNA of the parental genotype. Two other parental repeat types are indicated by 200- and 300-base-pair inserts, which map between the *Dra* I (D) and *Pvu* II (P) sites. The variant offspring of parents P1 and P2 lack the IGS *Eco*RI restriction site and show different length polymorphisms between the *Dra* I and the *Bst*EII (B) sites.

counts on this single variant offspring were $2n = 24$; the change did not involve gross aneuploidy.

Further surveys for variation in cytoplasmically inherited cpDNA and nuclear, presumably single-copy, unlinked *Adh1* and *Adh2* (11) genes were done to see if changes in other classes of DNA occurred. No cpDNA variation was found in any of the families surveyed, as shown by reprobing the filters with the petunia cpDNA probes. Hybridizations with *Adh1* showed variation only in the offspring of families P1 and P2. The parents (P1 and P2) and a nonvariant offspring of P2 had the same *Eco*RI and *Hin*dIII *Adh1* restriction fragments. The single variant of parent P2 showed several nonparental restriction fragments with *Adh1* hybridization (Fig. 3) but parental restriction fragments with *Adh2* hybridizations (Fig. 4). The variant offspring in either family do not share the same *Adh1* restriction fragments (Fig. 5). Under these hybridization conditions, there was an overlap in the restriction fragments visualized by pZML793 and pZML841, which may partly explain the large number of fragments detected with *Adh1*. Also, pZML793 may reproducibly hybridize to a non-*Adh1* portion of the *Taraxacum* genome. Six offspring from the single variant of family P2 were also surveyed; this second generation showed inheritance of both the nonparental rDNA and *Adh1* restriction fragments.

In summary, the rDNA and *Adh1* restriction data show genotypic changes that arose within one asexual generation of two related families, P1 and P2, which were stably inherited in a second asexual generation of offspring. The diagnostic DNA changes in the variant offspring were the loss of a parental *Eco*RI site in the rDNA of both families, which was not accompanied by large changes in rDNA amount, and nonparental restriction fragments visualized by *Adh1* hybridization. With *Adh1*, the variants in each family had a unique set of nonparental restriction fragments. Possible phenotypic effects of the *Adh1* restriction fragment variation were assayed by enzyme electrophoresis and showed no differences in alcohol dehydrogenase band migration.

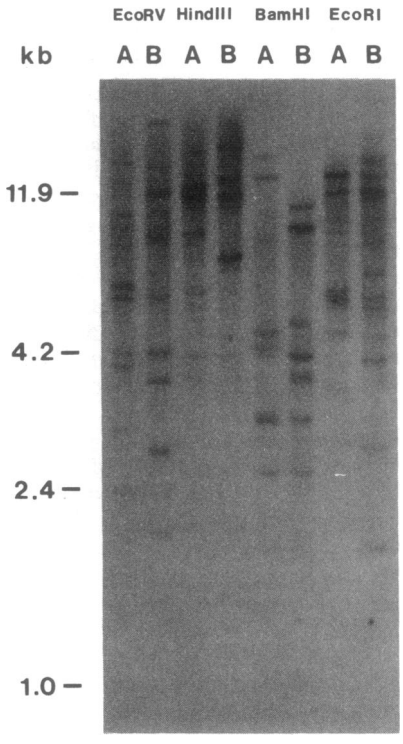


FIG. 3. A comparison of the *Eco*RV, *Hin*dIII, *Bam*HI, and *Eco*RI restriction fragments between the single P2 variant offspring (A) and a nonvariant sibling (B) (B has parental rDNA, *Adh1* and *Adh2* genotypes) detected by hybridizing with the *Pst* I inserts of the maize cDNA clone pZML793 (*Adh1*). The observed variation cannot be entirely due to DNA methylation because *Eco*RV and *Hin*dIII are not sensitive to DNA methylation (14).

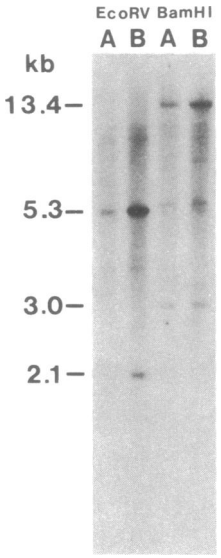


FIG. 4. Hybridization with pZML841 shows no *Adh2* variation between the variant A and nonvariant B offspring. A subset of the restriction fragments hybridizing with pZML793 also hybridize with pZML841 in *Taraxacum* as well as a maize control (not shown). Under these conditions, pZML793 hybridizes to an ≈7.2-kb *Bam*HI fragment in the maize line B37, as expected for the standard *Adh1-1F* allele (15) and also hybridizes with 5.8- and 2.6-kb fragments that are detected with *Adh2* and are similar to *Bam*HI fragment sizes in other lines of maize (12).

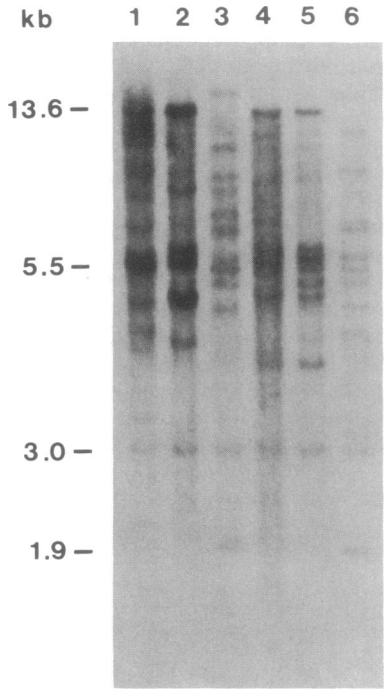


FIG. 5. Restriction fragment length variation among six individuals digested with *Eco*RI and hybridized with the *Pst* I inserts of pZML793 (*Adh1*). Lanes 1 and 2, *T. officinale* collected from Nevada and Kentucky, respectively, shown for comparison. Lanes: 3, offspring of P1; 4, offspring of the single P2 variant offspring; 5, P2 variant offspring; 6, sibling of the P2 variant offspring, which represents an individual with parental rDNA. Although the individuals in lanes 3 and 5 have the same nonparental rDNA genotype, their *Adh1* genotypes differ.

## DISCUSSION

The different numbers of offspring with nonparental genotypes in families P1 (all 41 surveyed) and P2 (1 out of 26 surveyed) suggest that somatic DNA changes occurred at different stages of development of the two parents. In higher plants, the germ line is not set aside early in development; it is the undifferentiated meristematic cells that give rise to floral buds. Because of this developmental pattern, mutations in meristematic tissue can be incorporated into the germ line. Therefore, it is possible that a somatic event occurred in parent P1, was incorporated into meristematic cell lines, and was subsequently passed on to offspring produced within at least two inflorescences. The lack of homologous chromosome pairing during the nonreductional meiosis of asexual *Taraxacum* supports the idea that the DNA changes resulted from mitotic events early in development. In family P2, an event most likely occurred during the formation of one seed. Although the seeds were produced asexually, in this case it is more difficult to exclude the possibility that homologous recombination occurred during the formation of a single embryo.

The DNA variation between asexual generations of *T. officinale* is consistent with both electrophoretic and morphological observations of variation found among clonal offspring of other asexual members of the genus (16–18). Also, variation within asexual *Taraxacum* clones has been characterized by karyotypic changes in satellited regions of chromosomes associated with the nucleolar-organizing regions (NOR) and by chromosomal rearrangements and bridges at mitosis (6, 19). The somatic chromosomal rearrangements have been proposed to be due to transposable element activity (19). The cytological studies on *Taraxacum* show that separate asexual lineages may have different levels of chromosomal instability. In this study, the parent–offspring variation involved two families from a single genetic lineage. This also suggests that some asexual *Taraxacum* genotypes may be more predisposed to undergo genomic changes than other asexual genotypes.

The restriction fragments visualized with presumably single-copy *Adh1* suggests that the maize gene is hybridizing with *Adh1*, *Adh2*, and/or other portions of the *Taraxacum* genome that show restriction site variation between generations of asexual reproduction. Although the relationship, if any, between rDNA and *Adh1* is not known, the *Adh1* hybridizations show that genomic changes other than in rDNA have occurred. Although we do not know how the changes occurred, they may be explained by various mutational events associated with molecular recombination. Studies on flax have shown that somatically induced DNA changes affect copy number variation in several repeated DNA sequences, including rDNA (20).

Changes in DNA methylation between asexual generations will influence restriction enzyme analysis if the enzymes do not digest methylated sequences. However, DNA methylation does not sufficiently explain the observed *T. officinale* variation because several restriction enzymes that are not sensitive to DNA methylation showed both rDNA and *Adh1* restriction fragment variation. Because of the number of *Adh1* restriction fragment differences (approximately five when the *Adh2* homologous fragments are accounted for), the changes probably involved more than one site and more than one event.

The number of rDNA repeats in *T. officinale* has been estimated to be about several thousand copies per haploid genome (8). The turnover of rDNA repeats in *Taraxacum* therefore must have involved large changes in numbers of particular repeat types. Of possible mechanisms to explain the multicopy rDNA changes, mitotic sister chromatid exchange is known to cause rDNA variation in *Drosophila melanogaster* (21), yeast (22), and possibly in wheat (23), and gene amplification is thought to be involved in the somatic induction of ≈2-fold variation in rDNA and other repeated sequence DNA in flax (20). Other studies also suggest that non-Mendelian mechanisms may influence rDNA recombination and variation, although the mechanisms causing this variation are not known. Novel rDNA repeats detected by restriction enzyme analysis have been observed in hybrids of maize and *Tripsacum* (24) in somatic cell hybrids of *Nicotiana* and *Atropa* (25) and in asexually produced offspring of *Poecilia formosa* (26).

In *Taraxacum*, the fixation of rDNA variants must have been accompanied by loss of a class of repeats and amplification of the rare rDNA repeat types unless many of the rDNA units simultaneously underwent the same changes. Densitometry shows that variation for rDNA repeat types occurs within individuals, so a subset of novel repeats may have been present in very low copy number and remained undetected within the parents but amplified and passed on to the offspring. It is necessary to assume that the rDNA rearrangement in *Taraxacum* was amplified. Otherwise the novel repeats would not have been detected by the hybridization conditions used in this study. The loss of the rDNA *Eco*RI site appeared to involve changes in fragment lengths, also shown by the *Dra* I and *Bst*EII double digests, so the site loss may have been accompanied by small DNA insertions and/or deletions. Genomic rearrangements are often associated with gene amplification (27, 28), and the observations in *Taraxacum* are consistent with this process.

Tandem arrangements of subrepeated DNA sequences have been proposed to facilitate insertion and deletions during DNA replication and repair (29). The rDNA restriction site changes in the *Taraxacum* offspring are located about 1300 bp from the *Eco*RI site at the 3′ end of the 25S gene. This region of the ribosomal cistron is known to consist of subrepeating elements that contribute to rDNA repeat length variation in several plant species (30–35). In *Taraxacum*, length polymorphisms ranging from 100 to 1000 bp map to this region (8). Thus, the rDNA of *T. officinale* most likely has repeated elements in this region, which may facilitate recombination events.

Whatever the precise mechanisms of origin, this work documents genotypic variation arising between generations of *T. officinale*, which suggests that nonmeiotic events create genetic variation within asexual *Taraxacum*. We do not know the extent of nonmeiotic recombination, and other processes may also contribute to genetic variation in asexual species. Our data confirm that nonsexual species can generate genotypic variation, although at a lower rate than would sexual species. The processes that generate genetic variation may themselves be under selection; selection for optimization of mutation rates has been proposed for asexual organisms (36–38). Further, asexual *T. officinale* has high fecundity and viability, and large numbers of individuals colonize widespread habitats. Therefore, even a low rate of nonmeiotic recombination would result in the accumulation of genotypic variation. The process of clonal selection among the asexual genotypes may subsequently influence the distribution of clonal genotypes in different environments over time, and such processes may facilitate adaptive evolution.

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