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Chromosome Counts for Packera paupercula Variety Gypsophila

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Chromosome Counts for *Packera paupercula* variety *gypsophila*

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ABSTRACT

New plant species develop when diploid plants (having two sets of chromosomes) spontaneously double their chromosomes and the resulting tetraploids (having four sets of chromosomes) are no longer able to back cross to diploid members of the population. The North American plant species *Packera paupercula* (balsam-leafed ragwort), is widespread and morphologically diverse. An isolated group of populations from the chalky soils of west-central Alabama was recently named *Packera paupercula* variety *gypsophila* (the chalk-loving balsam ragwort). The variety's large diameter pollen grains suggest it may be tetraploid. This project attempts the first chromosome count for the variety by examining cells undergoing meiosis in the anthers (male sex organs) of flower buds. A "squash" is performed by dissecting out, staining, and pressing the anthers between a slide and cover slip and viewing the result with a microscope. If variety *gypsophila* is tetraploid, it may merit recognition as a new species.

INTRODUCTION

Unlike most animal species, plants can and do spontaneously double their sets of chromosomes (Raven et al. 1999). This mutation probably occurs because plants don't have complex internal organs like animals, so this kind of "mistake" doesn't create problems in their development. When a population of one species becomes a "polyploid" (having more than two sets of chromosomes), it should be named as a new species because members of this population can no longer interbreed with diploid individuals (having two sets of chromosomes – like people and most animals) (Raven et al. 1999).

Packera paupercula (the "balsam-leafed ragwort") is a member of the sunflower family (Figure 1). Members of the sunflower family are characterized by a unique kind of inflorescence (arrangement of flowers on the plant) called a "head" (Figure 2). What appears to be a single flower is actually a dense "head" of tiny individual flowers (Raven et al. 1999).

Figure 1. *Packera paupercula*, the balsam-leafed ragwort.

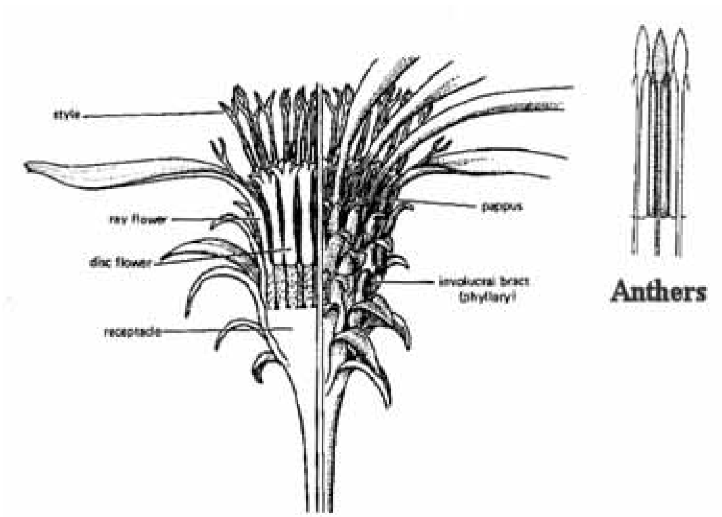

Figure 2. The head of a sunflower and anthers dissected out of disc floret.

Packera paupercula occurs across northern North America except in the Great Plains and desert Southwest (Barkley, 1978; Kowal, 1975) (Figure 3). Some populations are diploid and some are polyploid (most commonly tetraploids with four sets of chromosomes, or rarely hexaploids with six sets of chromosomes and octaploids with eight sets of chromosomes) (Kowal et al., unpublished ms). Groups of populations from different regions are quite variable in appearance and may actually be different species.

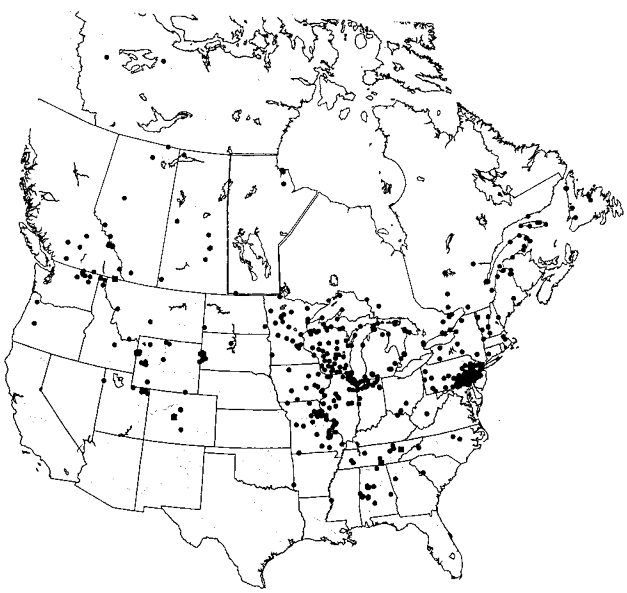

Figure 3. Distribution of *Packera paupercula*

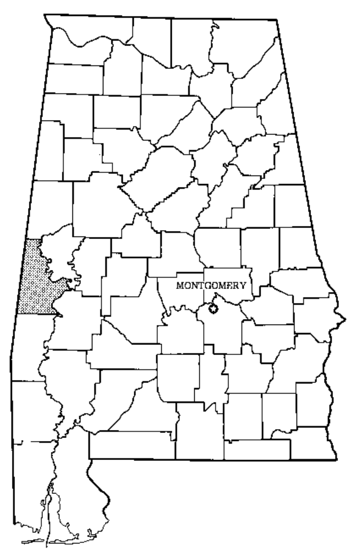

Figure 4. Distribution of *Packera paupercula* var. *gypsophila*.

Isolated populations of *Packera paupercula* occur in west central Alabama on the unusual “Black Prairie” soils in and around Sumpter County (Mahoney 2000) (Figure 4). These soils developed from a very fine textured chalk and have a very high clay content and high pH (Harris 1989). This “race” will soon be named as a new variety of the species: *P. paupercula* var. *gypsophila*, “the chalk-loving balsam-leafed ragwort,” (Mahoney and Kowal in revision). The pollen grain diameters of var. *gypsophila* are larger than we expect for diploid members of the species (Mahoney 2000). Our hypothesis is that these populations are tetraploid and perhaps this group of populations should not be considered a variety of *P. paupercula* but as a new species.

One of the best ways to count chromosomes is to isolate and examine cells undergoing meiosis (Kowal 1975). During this stage the nucleus dissolves and the chromosomes condense so that they can be viewed with a phase-contrast light microscope at high power (Figures 6 and 7). As flowers mature, spore-mother cells undergo meiosis in the anthers (male sex organs) of flowers to produce pollen (Raven et al. 1999).

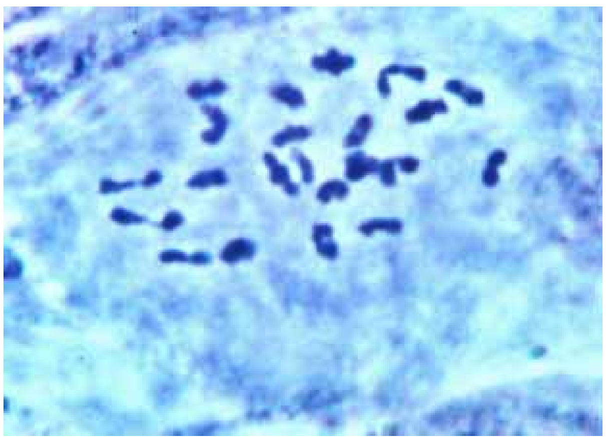

Figure 6. Diploid cell in Metaphase of Meiosis I with 22 chromosomes.

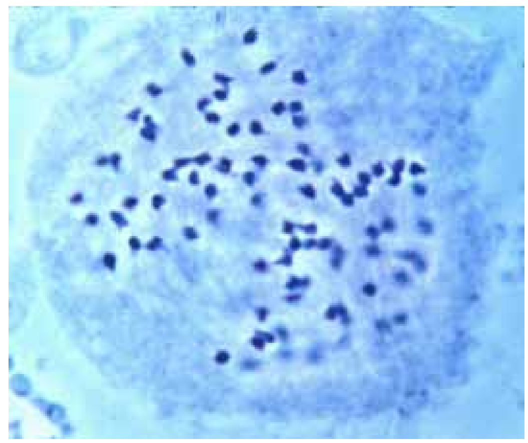

Figure 7. Tetraploid cell in late Anaphase of Meiosis I with approximately 44 chromosomes.

METHODS

Immature heads from garden-grown *Packera paupercula* var. *gypsophila* were preserved in Jackson's Solution (4 parts absolute ethyl alcohol [EtOH], 2 parts absolute methyl alcohol, 2 parts chloroform, 1 part propionic acid, and 1 part acetone). The solution "fixes" the plant tissues by quickly killing them and preserving cells undergoing meiosis (Kowal 1975). After 24 hours, the heads were transferred to 70 percent EtOH and stored in a refrigerator.

The squash was performed using the methods of Beeks (1956) and Mahoney, Kong, and Kowal (2002). A fixed head of approximately 4-5 mm in diameter was placed on a slide in a drop of Aceto-carmine solution (heat 45 ml glacial acetic acid, 55 ml distilled water; add1 gm carmine powder a little at a time; boil 2-10 minutes; decant and strain; add a few drops of dilute ferric chloride until dye changes from deep red to dark purple)(Figure 7). Two to four florets were removed from the head and the five anthers dissected out of each while staining in Aceto-carmine solution.

Anthers were never allowed to dry out and once they were stained to a dark black color all debris was removed and the anthers washed in 45% glacial acetic acid. The stained anthers were placed in a drop of Hoyer's Medium (50 ml distilled water, 30 gm gum Arabic, 200 gm chloral hydrate, and 16 ml glycerine) and squashed with a cover slip as hard as physically possible on a smooth surface using the eraser of a pencil on each individual anther while taking care not to let the cover slip slide around. The edges of the

cover slip were sealed with clear fingernail polish and the slides were scanned at 40x and cells of interest were examined and images were captured digitally at 100x under oil emersion on a phase contrast microscope.

Figure 7. Two fixed heads (to left and right below eraser) with a floret between them.

RESULTS

Four squashes were prepared and one slide had a few meiotic figures. No one cell was countable, however, it was possible to tell that the individual was a polyploid – probably a tetraploid (Figure 8).

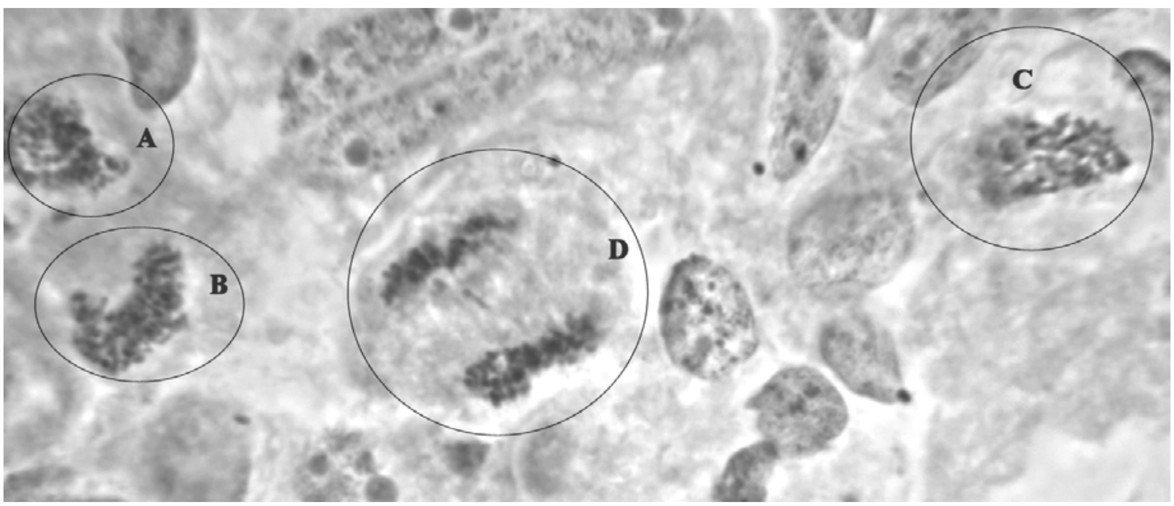

Figure 8. Four cells from an anther squash of var. *gypsophila*. A, B, and C show uncountable clusters of chromosomes in cells in late Prophase or early Metaphase of Meiosis I; D shows a cell in telophase of Meiosis I.

DISCUSSION

Although we were unable to get a clear chromosome count, we are reasonably sure that the individual from a population in west central Alabama is tetraploid. The buds were probably too young and not enough spore-mother cells were at the proper stage of meiosis. We still have a single individual of var. *gypsophila* from Alabama alive in Dr. Mahoney's research garden on the east side of South Trafton Science Center. More young heads have already been collected so that more squashes can be attempted. Perhaps next fall, the perfect squash will allow us to count the chromosomes exactly.

CONCLUSION

My work indicates that *Packera paupercula* var. *gypsophila* is tetraploid. Squashes from more individuals from more populations from the Black Prairie soils of Alabama will be required to be sure that all populations from the region are tetraploid. However, this study provides evidence that the variety should be named as a new species.

REFERENCES CITED

Barkley, T.M. 1978. *Senecio.* In Rogerson, C.T., ed. *North American Flora*, series II, part 10. New York Botanical Garden, pp. 50-139.

Beeks, R. M. 1955. Improvements in the squash technique for plant chromosomes. *El Aliso* 3: 131-133.

Harris, M.C. 1989. *Soil Survey of Sumpter County, Alabama*. United States Department of Agriculture, Soil Conservation Service.

Kowal, R.R. 1975. Systematics of *Senecio aureus* and allied species on the Gaspé Peninsula, Quebec. *Mem. Torrey Bot. Club* 23: 1-113.

Kowal, R. R., C. C. Freeman, and T. M. Barkley. Unpubl. ms. Chromosome numbers of the genus *Packera* (Asteraceae: Senecioneae): a summary.

Mahoney, A.M. 2000. *Contributions to the systematics of the* Packera paupercula *complex (Asteraceae: Senecioneae).* (PhD dissertation, University of Wisconsin-Madison.)

Mahoney, A.M., V.W. Kong, and R.R. Kowal. 2002. Documented chromosome numbers 2002:3. Chromosome counts for *Packera paupercula* var. *appalachiana* (Asteraceae: Senecioneae). *SIDA* 20:605-610.

Mahoney, A.M. and R.R. Kowal. Ms. in revision. Races of the *Packera paupercula* complex (Asteraceae: Senecioneae) in the upper Midwest and southeastern United States. *SIDA*.

Raven, P.E., R.F. Evert, and S.E. Eichhorn. 1999. *Biology of Plants*, 6th ed. N.Y.: W.H. Freeman & Company.

Biographical sketches

Chad Larson grew up in Worthington, MN and graduated from Worthington High School in May of 2001. He started attending Minnesota State University-Mankato in the Fall of

2002. He is currently majoring in Clinical Laboratory Sciences, Medical Technology with a minor in Chemistry.

Dr. Alison Mahoney grew up in northern California and received a Bachelor of Science in Art with a minor in French from the University of Wisconsin-Oshkosh. She worked as a graphic artist and ran her own typesetting business in San Francisco and in Madison, WI from 1978 to the early 1990s. She returned to the University of Wisconsin-Madison to study Botany and received her PhD in Plant Systematics from that institution in 2000. She has been an Assistant Professor in the Department of Biological Sciences at Minnesota State University-Mankato since August 1999. She studies the systematics of the *Packera paupercula* complex, a herbaceous perennial plant species native to North America.