**Appendix S2.** Supplemental description of flow cytometry methodology and example data.

Widely used to determine the DNA content of cell nuclei in plants (Kron et al. 2007), including *L. tridentata* (Laport et al. 2012; Laport and Ramsey 2015), flow cytometry has only relatively recently been optimized to estimate pollen movement within plant communities, and has not been widely applied to examine pollen exchange among intraspecific ploidies (Kron and Husband 2012). We used flow cytometry to infer the ploidy of pollen removed from bees collected on diploid and tetraploid *L. tridentata* following the procedure of Kron et al. (2014). We gently rinsed pollen loads from silica-preserved bees with 1 ml of LB01 buffer (15 mM Tris, 2 mM Na2EDTA, 0.5 mM Spermine Tetrahydrochloride, 80 mM KCl, 20 mM NaCl, 0.1% Triton X-100, 1ul/ml β-mercaptoethanol; Doležel et al., 2007) by vortexing and/or pipetting up and down for 10-15 sec. Resuspended pollen was passed through a 100 µm filter (Partec CellTrics, Görlitz, Germany) to remove large debris. The filtered pollen grains were then gently rubbed against a 10 µm “bursting” filter (Partec CellTrics, Görlitz, Germany) using a glass stir rod to extract nuclei. Extracted nuclei were gently rinsed through the filter with 500 µl of LB01 buffer containing 50 µl of propidium iodide at 1 mg/ml and 25 µl RNase A at 1 mg/ul, and allowed to stain for 30 min. on ice.

To infer the DNA content of our unknown pollen samples, we used flow cytometry to infer DNA content of external standard specimens. We used *L. tridentata* tissue of previously determined DNA content (Laport et al. 2012), or plant tissue recommended in Doležel et al. (2007) as external standards that were run at the beginning of each flow cytometry session (*Raphanus sativus* cv. Saxa, 2C DNA content = 1.11 pg; *Glycine max* cv. Polanka, 2C DNA content = 2.50 pg). For each sample, ~10 *L. tridentata* leaves, or ~1 cm2 of leaf tissue for other species, was placed in 1 mL of LB01 buffer and were chopped by hand for 30-60 sec. with a razor blade in a plastic petri plate. The resulting slurry was filtered through a 30 µm filter (Partec CellTrics, Görlitz, Germany) into a 1.5 ml microcentrifuge tube to remove debris and centrifuged for 10 min. at 2,000 ×g. After removing the supernatant, nuclei were resuspended in 500 µl of Tris-MgCl2 buffer (200 mM Tris, 4 mM MgCl2 · 6H2O, 0.5% Triton X-100; Doležel et al., 2007) containing 50 µl of propidium iodide at 1 mg/ml and 25 µl of RNAse A at 1 mg/ml, and allowed to stain for 30 min. on ice. Stained samples containing conspicuous debris were passed through a second filter.

All samples were run at the University of Nebraska-Lincoln Flow Cytometry Service Center on a B-D FACSCalibur flow cytometer (B-D Biosciences, San Jose, California, USA). Samples were run for ~2-3 min. at medium speed, or nearly until sample exhaustion if event acquisition was slow. Settings were chosen to detect *L. tridentata* nuclei based upon size expectations from the external standards. Using CellQuest Pro Software (version 5.2.1; B-D Biosciences, San Jose, California, USA) we inferred ploidy from the relative fluorescence (FL2-A) of each sample compared to the external standards ensuring the DNA contents of our unknowns were of the expected genome size (+/- ~15%) for diploid and tetraploid *L. tridentata*. Since *L. tridentata* pollen is binucleate (Brewbaker 1967), and corresponding fluorescence histograms are bimodal (one mode represents the haploid vegetative cell nuclei while the other mode represents the undivided generative cell nuclei) the relationship between the bimodal diploid and tetraploid histograms is similar to that for somatic cells: tetraploid fluorescence is approximately twice that of diploid fluorescence (Figs. S1 and S2). When the fluorescence histograms for pollen samples were unimodal, bimodal with higher or lower than expected inferred DNA content, contained more than three modes, or were non-modal we excluded those samples as non-*L. tridentata* or as representing low quality data (e.g., Fig. S2e-h). Additionally, nuclei can sometimes adhere to each other (i.e., “doublets”) producing a peak at twice the fluorescence, but usually of smaller magnitude. In binucleate pollen analyses, these doublets could lead to incorrect pollen load composition inference. For example, pollen from a trinucleate species (producing unimodal fluorescence histograms) with many doublets and a similar DNA content to the focal species (see below) could be inferred as pollen from the binucleate focal species. Determining which nuclei are doublets can be challenging unless flow cytometry samples are free from cell wall debris (Wersto et al. 2001). Since our focus was not on the ratio of diploid and tetraploid pollen in each pollen load, we generally adopted the approach of Kron et al. (2014) by assuming that fluorescence peaks occurring in a 1:2 ratio represented binucleate pollen if they were roughly similar in size (e.g., Fig. S1). We inferred asymmetrically mixed pollen loads (i.e., trimodal histograms) in a similar way, with small third peaks “counting” as indicating a mixed pollen load if the size of the peak appeared approximately large enough to account for any difference in size between the other two peaks (because it is one of a pair of peaks, one of which overlaps with another peak; e.g., Fig. S2a). While this approach may potentially slightly overestimate the number of mixed pollen loads (and rates of inter-cytoytpe pollen/gene flow), it could also potentially slightly underestimate single cytotype pollen loads (and the strength of reproductive isolation), and we felt that it was appropriately conservative for our analyses.

Despite being a potentially powerful tool for investigating polyploid reproductive interactions, the use of flow cytometry to investigate inter-cytotype pollen movement faces challenges associated with ensuring analyzed pollen is from the species of interest and that sufficient pollen can be obtained from bee-collected pollen loads for analysis. The *L. tridentata*-bee pollinator system is particularly well-suited to investigate questions about cytotype specialization and pollinator-mediated inter-cytotype pollen movement while minimizing some concerns associated with pollen contamination from non-focal species. *Larrea tridentata* generally initiates flowering earlier than co-occurring (non-focal) species in the spring, produces abundant floral displays, and throughout broad swaths of the southwestern deserts (including our research site) is numerically much more abundant than co-occurring species (Barbour et al. 1977; Benson and Darrow 1981; Turner et al. 1995; Whitford et al. 1996; Laport and Ramsey 2015). Although, queries of the Kew Plant C-Values Database (Pellicer and Leitch 2020) suggest at least a few co-occurring species have DNA contents roughly similar to that of diploid (2C DNA content = 1.5pg) and tetraploid (2C DNA content = 2.4pg) *L. tridentata* (e.g., *Prosopis velutina* 2C DNA content = 0.86pg, *Carnegiea gigantea* 2C DNA content = 2.87pg, *Fouqueria splendens* 2C DNA content = 1.06pg), many of these co-occurring species flower later than *L. tridentata*, have trinucleate pollen (Brewbaker 1967), or have genome sizes/DNA contents that would usually be distinguishable from *L. tridentata* fluorescence histograms.The earlier bloom and greater abundance of *L. tridentata* at the study site furtherreduces opportunities for non-*L. tridentata* bee foraging. Moreover, the bee pollinators of *L. tridentata* are well-studied, and the intimacy of their relationships to *L. tridentata* as pollen specialists or generalists are well-characterized with a large proportion of the pollinator assemblage representing *L. tridentata-*specialists that do not often collect pollen from other species when *L. tridentata* flowers are available (Hurd and Linsley 1975; Minckley et al. 2000). For evaluating bee-mediated pollen movement within and between ploidies, we assumed the abundant floral displays and numerical abundance of *L. tridentata* at the study site, combined with the documented specialist associations of bee pollinator species would minimize opportunities for undetected pollen contamination from non-focal species.

Obtaining a sufficient quantity of pollen from typical bee-collected pollen loads for high-quality flow cytometry is a pervasive challenge, especially from small native bees. However, by employing the conservative approach described here and in the main text, we think that even low numbers of pollen nuclei could be reasonably scored as representing diploid, tetraploid, or mixed pollen loads (Figs. S1 and S2) with appropriate consideration of limitations. Namely, the identity of analyzed pollen relies on assumptions (or observations) about which plants are being visited by the pollinators, and non-focal pollen contamination from species with similar pollen type (binucleate, trinucleate) and genome size/DNA content could bias inferred pollen load compositions. Additionally, the presence of doublet nuclei could confuse inferred pollen load compositions, and appropriate standards and caution should be adopted to adequately contextualize results. Thus, while this methodology for investigating the reproductive interactions between cytotypes can be challenging and is not always successful, we feel confident that this approach allowed us to reliably detect single-ploidy and mixed-ploidy pollen loads as shown in Figs. S1 and S2.

**Figure S1**. Example flow cytometry histograms showing relative fluorescence values (FL2-A) for pure diploid and tetraploid bee-collected *L. tridentata* pollen loads. a) Pollen load containing only diploid pollen, b) pollen load containing only tetraploid pollen, c) small pollen load containing only diploid pollen, d) small pollen load containing only tetraploid pollen. Insets in (c) and (d) show the same histogram with the counts (y-axis) rescaled to enhance the small mode sizes. Although the number of events recorded for the small pollen loads was typically rather low (often <50 events), the modal peaks were typically observable with fairly high confidence. Pollen loads collected from honeybees were often much larger than those collected from native bees and often resulted in better quality fluorescence histograms. Yet, histograms from both honeybees and native bees provided interpretable data. e) Honeybee-derived pollen load containing only diploid pollen, f) honeybee-derived pollen load containing only tetraploid pollen, g) native bee-derived pollen load containing only diploid pollen, h) native bee-derived pollen load containing only tetraploid pollen. We scored the ploidy of pollen loads using the modal peaks in the fluorescence histograms. The relationship between the bimodal diploid and tetraploid histograms is similar to that for somatic cells: tetraploid fluorescence is approximately twice that of diploid fluorescence, though slight differences can arise due to the condition of the analyzed pollen, variation in propidium iodide binding, the amount of pollen exine debris, and instrument settings. The gray bar in each histogram represents the approximate location of the external control (e.g., diploid *L. tridentata* leaf tissue) run at the beginning of each flow cytometry session.

**Figure S1.**

**Figure S2**. Example flow cytometry histograms showing relative fluorescence values (FL2-A) for mixed diploid and tetraploid bee-collected *L. tridentata* pollen loads. a) Asymmetrically mixed pollen load originating from a bee collected on diploid *L. tridentata* exhibiting an excess of diploid vs. tetraploid pollen. b) Mixed pollen load originating from a bee collected on tetraploid *L. tridentata* with similar quantities of diploid and tetraploid pollen. c) Asymmetrically mixed pollen load originating from a bee collected on tetraploid *L. tridentata* exhibiting an excess of tetraploid pollen. d) Mixed pollen load originating from a bee collected on tetraploid *L. tridentata* with similar low quantities of diploid and tetraploid pollen. Inset in (d) shows the same histogram with the counts (y-axis) rescaled to enhance the small mode sizes. As in Fig. S1, the relationship between the bimodal diploid and tetraploid histograms is similar to that for somatic cells: tetraploid fluorescence is approximately twice that of diploid fluorescence and mixed pollen loads are trimodal. We inferred all trimodal histograms as indicating mixed pollen loads if the ratios between peak locations occurred in a roughly 1:2:4 ratio, though slight differences can arise due to the condition of the analyzed pollen, variation in propidium iodide binding, the amount of pollen exine debris, and instrument settings. e-g) Examples of suspected non-*L. tridentata* pollen that were excluded from our dataset. All histograms exhibit a single mode (often very small as shown by insets with rescaled y-axes) that sometimes does not occur at the expected relative fluorescence for *L. tridentata* pollen. h) An example of a fluorescence histogram that shows no discernible *L. tridentata* pollen, even at low levels as shown in the inset. Samples like those in e-h were excluded from our analyses. The gray bar in each histogram represents the approximate location of the external control (e.g., diploid *L. tridentata* leaf tissue) run at the beginning of each flow cytometry session.

**Figure S2.**

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