27. J. M. Cronshaw, A. N. Krutchinsky, W. Zhang, B. T. Chait, M. J. Matunis, *J. Cell Biol.* **158**, 915–927 (2002).
28. A. Ori *et al.*, *Mol. Syst. Biol.* **9**, 648 (2013).

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Supplementary Materials
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Polyploids Exhibit Higher Potassium Uptake and Salinity Tolerance in *Arabidopsis*

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Genome duplication (or polyploidization) has occurred throughout plant evolutionary history and is thought to have driven the adaptive radiation of plants. We found that the cytotype of the root, and not the genotype, determined the majority of heritable natural variation in leaf potassium (K) concentration in *Arabidopsis thaliana*. Autopolyploidy also provided resistance to salinity and may represent an adaptive outcome of the enhanced K accumulation of plants with higher ploidy.

Polyploidy, the quality of possessing multiple complete sets of chromosomes, is pervasive within land plants, suggesting an adaptive benefit though no mechanisms have been established (*1*). Soil discontinuities, such as boundaries between soil types, may underlie plant-selective constraints. In an analysis of the elemental composition of leaves from a set of 349 *Arabidopsis thaliana* accessions (*2*), the autotetraploid accession Wa-1 (from Warsaw, Poland) had the highest concentration of leaf potassium (K) (Fig. 1A) and the K analog rubidium (Rb) (fig. S1A). Recombinant inbred lines (RILs) between the diploid accession Col-0 and the autotetraploid Wa-1 (*3*) contain diploids and tetraploids with recombinant genotypes (*4*). All 89 RILs were phenotyped for the leaf concentration of K and Rb by inductively coupled plasma mass

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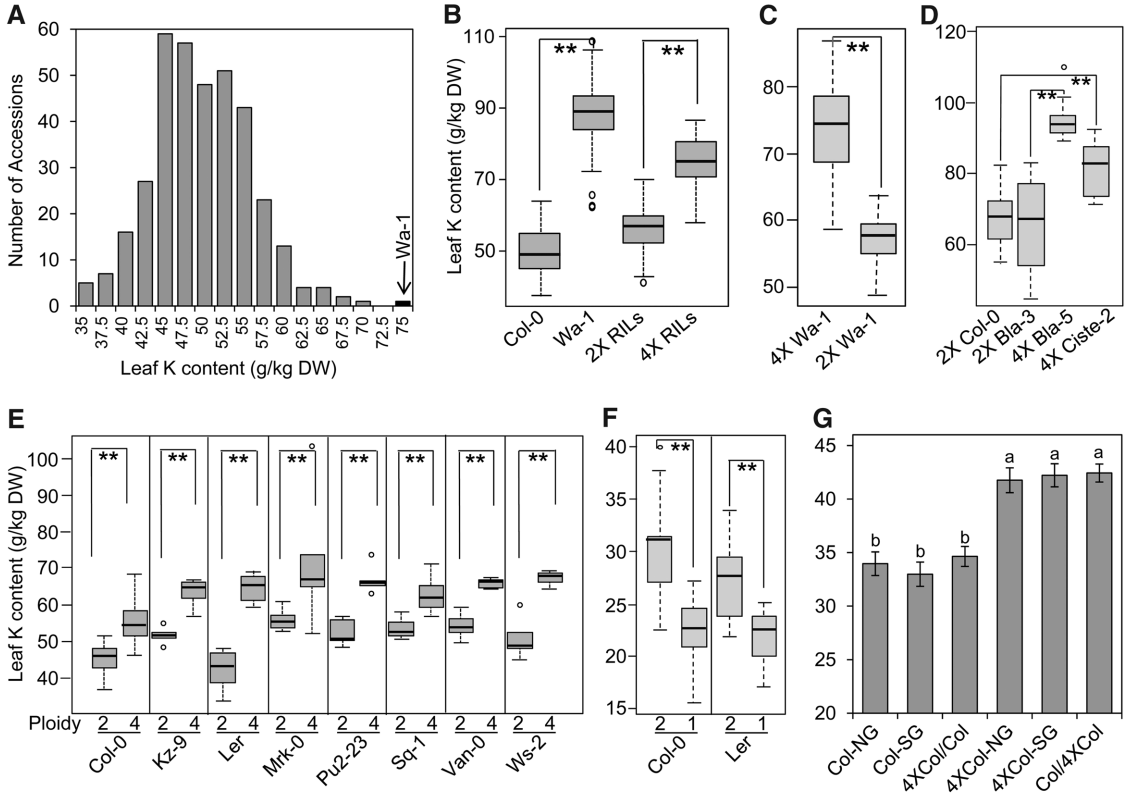

Fig. 1. Ploidy contributes to K accumulation in *A. thaliana* leaves. (**A**) Leaf K concentration among 349 accessions. The arrow and back bar indicate the tetraploid, Wa-1. DW, dry weight. (**B** to **F**) Box plots (the minimum, first quartile, median, third quartile, and maximum are shown, with data >1.5 interquartile ranges denoted with circles) for leaf K concentration in Col-0 x Wa-1 RILs (B), Wa-1 and diploid Wa-1 (C), natural tetraploid accessions (D), natural diploids and derived tetraploids (E), and natural diploids and derived haploids (F). (**G**) Leaf K concentration of grafted diploid and tetraploid plants. NG, nongrafted; SG, self-grafted; Col/4XCol, grafted with Col-0 as scion and 4XCol-0 as rootstock; 4XCol/Col, grafted with 4XCol-0 as scion and Col-0 as rootstock. Asterisks in (B) to (F) indicate the significance of pairwise comparisons (Student's *t* test; $*P < 0.05$; $**P < 0.01$). Letters above the bars in (G) indicate statistically different groups (one-way analysis of variance with groupings by Tukey's HSD with a 95% confidence interval). 2X, diploid; 4X, tetraploid. Data were collected from 6 to 18 biological replicates for each accession, cytotype or graft, and represented in (G) as mean ± SE. All leaf K concentration data are accessible using the digital object identifiers (DOIs) 10.4231/T9H41PBV and 10.4231/T93X84K7 (see http://dx.doi.org/).

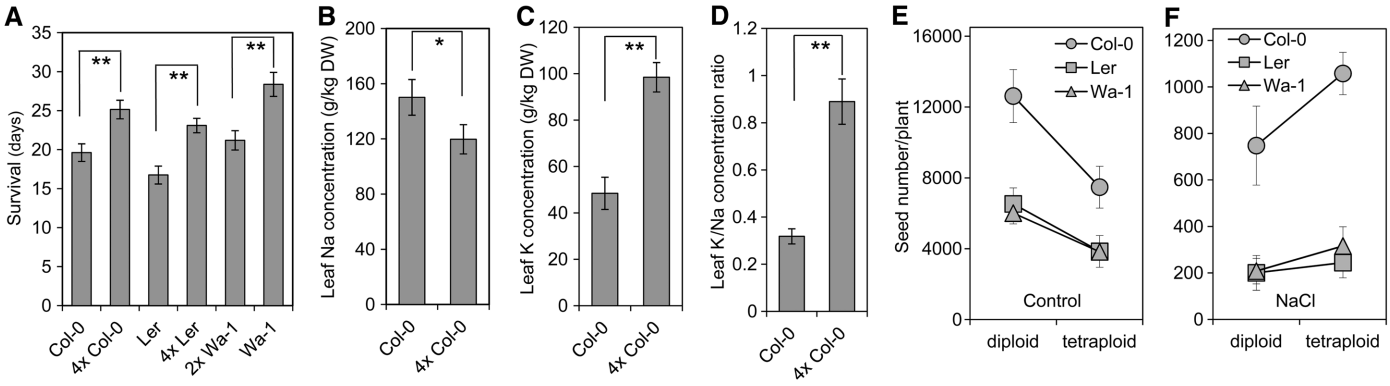

Fig. 2. Tetraploidy promotes *A. thaliana* salinity tolerance. (**A**) Survival of diploid and tetraploid plants after irrigation with 200 mM NaCl. Leaf concentrations of Na (**B**) and K (**C**) and the K/Na concentration ratio (**D**) of plants after 2 weeks of irrigation with 200 mM NaCl. (**E** and **F**) Seed production of diploid and tetraploid plants without (E) and with irrigation with 100 mM NaCl (F). 2X, diploid; 4X tetraploid. Data represent means of measurements from biological replicates [(A) $n = 40$ replicates; (B) to (D) $n = 12$; (E) and (F) $n = 5$ to 6] ± SE. (A to D) Data were analyzed with a Student's t test; $^{*}P < 0.05$; $^{**}P < 0.01$. (E and F) Data were analyzed with a log-normal Poisson generalized linear mixed model (table S2), which identifies a significant two-way interaction between salinity treatment and cytotype ($P < 0.001$), but no significant interaction between salinity and genotype ($P = 0.051$).

spectrometry (ICP-MS). Ploidy was a significant determinant (logarithm of the odds ratio for linkage = 28) of leaf K concentration, accounting for 57.2% of the variation in the RILs, and increased K by 32% compared with the diploids (Fig. 1B). A diploid Wa-1, derived by haploid induction (*5*), had reduced leaf K and Rb concentrations (Fig. 1C and fig. S1B), confirming that tetraploidy increased leaf K. A minor portion (9.9%) of the variation in leaf K in the RILs was accounted for by a quantitative trait locus (QTL) on chromosome 2. Wa-1 contributed the allele for increased leaf K. No obvious candidate genes are present in the genomic region of this QTL.

We used flow cytometry to identify two additional natural autotetraploids, Bla-5 (from Blanes, Spain) and Ciste-2 (from Cisterna di Latina, Italy), from a screen of 344 accessions (table S1) (*4*) (see also supplementary materials and methods). These autotetraploids also had elevated leaf K and Rb compared with the diploid Col-0 and with Bla-3 (from Blanes, Spain), a close diploid relative of Bla-5 (Figs. 1D and fig. S1B). To test for genotype by ploidy interaction, eight diploid and colchicine-doubled tetraploid pairs were evaluated for leaf K and Rb. All autotetraploids were phenotypically similar to their diploid progenitor, and all eight had elevated leaf K and Rb (Fig. 1E and fig. S1B). Examination of haploid Col-0 and Ler, prepared by haploid induction (*5*), revealed that haploidy reduced leaf K compared with diploid progenitors (Fig. 1F). Thus, leaf K in *A. thaliana* was directly related to ploidy level. We used reciprocal grafting (*6*) to show that the elevated leaf K and Rb observed in tetraploid Col-0 was present in diploid leaves on shoots grafted to tetraploid roots (Fig. 1G and fig. S2), whereas leaves from tetraploid shoots grafted to diploid roots showed the same leaf K as diploid Col-0 (Fig. 1G and fig. S2). These results establish that leaf K is controlled by root ploidy, independently of the ploidy of the shoot.

Increased K/Na ratios enhance salinity tolerance in plants (*7*). We grew diploid and tetraploid Ler, Col-0, and Wa-1 with nutrient media supplemented with 200 mM NaCl. Plant survival after NaCl treatment was used as a measure of salinity tolerance (*6*). Tetraploids showed an increased rate of survival compared with diploids (Fig. 2A). In a separate experiment, tetraploid NaCl-treated plants were shown to have elevated leaf K and reduced Na compared with diploids (Fig. 2, B to D). This suggests that enhanced salinity tolerance in tetraploids was associated with both elevated leaf K and reduced leaf Na accumulation. To assess the reproductive success of tetraploids and diploids in a saline environment, Ler, Col-0, and Wa-1 plants were grown to maturity with sublethal salinity treatment (100 mM NaCl) and seed production was determined as a proxy for fitness. In the untreated controls, the diploid cytotypes for all three genotypes produced more seeds than the tetraploids ($P < 0.001$) (Fig. 2E), as observed previously (*4*). As expected, salinity treatment reduced the seed production of all genotypes, regardless of cytotype ($P < 0.001$), though there was no significant interaction between genotype and salinity treatment. On the other hand, the interaction between cytotype and salinity treatment was significant ($P < 0.001$), with tetraploids of all genotypes producing significantly more seeds than diploids under elevated salinity ($P < 0.001$) (Fig. 2F). Thus, polyploidy can provide a reproductive advantage in saline environments.

Soils with high Na concentration are prevalent in many regions of the world. We propose that under saline conditions, polyploids, with their improved ability to accumulate K and exclude Na, may have a fitness advantage that could contribute to the establishment and persistence of polyploid populations.

References and Notes

1. C. Parisod, R. Holderegger, C. Brochmann, *New Phytol.* **186**, 5–17 (2010).
2. I. Baxter *et al.*, *PLoS Genet.* **6**, e1001193 (2010).
3. C. L. Schiff, I. W. Wilson, S. C. Somerville, *Plant Pathol.* **50**, 690–701 (2001).
4. I. M. Henry *et al.*, *Genetics* **170**, 1979–1988 (2005).
5. M. Ravi, S. W. Chan, *Nature* **464**, 615–618 (2010).
6. A. Rus *et al.*, *PLoS Genet.* **2**, e210 (2006).
7. R. Munns, M. Tester, *Annu. Rev. Plant Biol.* **59**, 651–681 (2008).

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Supplementary Materials

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