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Current Biology

Polyploidy and microbiome associations mediate similar responses to pathogens in *Arabidopsis*

Highlights

- Polyploids are more resistant to DC3000 than diploids in general
- Polyploids are more resistant, regardless of microbial associations
- Diploid plants are reliant on their microbiome to mount a defense response

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In brief

Mehlferber et al. employ a synthetic microbial community to investigate the differences in microbiome recruitment and pathogen defense across diploid and polyploid accessions of *Arabidopsis*. Diploids are reliant on their microbiome to mount a defense response to *P. syringae*, but polyploids are more resistant, regardless of microbial associations.

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Polyploidy and microbiome associations mediate similar responses to pathogens in *Arabidopsis*

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SUMMARY

It has become increasingly clear that the microbiome plays a critical role in shaping the host organism's response to disease. There also exists mounting evidence that an organism's ploidy level is important in their response to pathogens and parasites. However, no study has determined whether or how these two factors influence one another. We investigate the effect of whole-genome duplication in *Arabidopsis thaliana* on the above-ground (phyllosphere) microbiome and determine the interacting impacts of ploidy and microbiome on disease outcome. Using seven independently derived synthetic autotetraploid *Arabidopsis* accessions and a synthetic leaf-associated bacterial community, we confirm that polyploids are generally more resistant to the model pathogen *Pseudomonas syringae* pv. Tomato DC3000. Polyploids fare better against the pathogen than diploids do, regardless of microbial inoculation, whereas diploids harboring an intact microbiome have lower pathogen densities than those without. In addition, diploids have elevated numbers of defense-related genes that are differentially expressed in the presence of their phyllosphere microbiota, whereas polyploids exhibit some constitutively activated defenses, regardless of colonization by the synthetic community. These results imply that whole-genome duplication can enhance immunity, resulting in a decreased dependence on the microbiome for protection against pathogens.

INTRODUCTION

Whole-genome duplications (WGDs), or "polyploidizations," are evolutionary events where the entire genome is doubled. Despite its dramatic nature, WGD is a common mutation in plants[1] and is found throughout the tree of life.[2] Polyploidy is associated with many novel and potentially adaptive phenotypes including changes to biomass, photosynthesis, water- and nitrogen-use efficiencies, and secondary metabolism,[3–6] with polyploids having larger cells and organs and more chloroplasts per cell.[4] For these reasons, polyploidy is often considered to be a mechanism by which short-term adaptations may arise in response to changes to the environment or stress.[7] Polyploidy is also implicated in an increased resistance to parasites and pathogens,[8] and there is some experimental evidence that supports this conclusion. For example, in *Actinidia chinensis* (kiwifruit), hexaploids are the most resistant to pathogenic *Pseudomonas syringae*, followed by tetraploids and then diploids,[9] and inducing polyploidy in *Impatiens walleriana* (cultivated impatiens) confers increased resistance to mildew.[10]

Another way that plants can achieve increased pathogen resistance is via the associated microbial community (the microbiome), which has also been found to play a critical role in defense against pathogens.[11,12] Both the root and shoot systems of plants host diverse microbial communities, including bacteria, fungi, and other eukaryotes, but these plant systems associate with only a subset of all environmentally available microbes. These associations play important functions in disease or nutrient acquisition.[13] Which taxa successfully colonize a given plant can be mediated by the host both directly and indirectly, including through immune responses,[14] coordination of stress and immune system functions,[15] or the production of secondary chemicals.[6] Host reliance on the microbiome for disease resistance is now considered a key determinant of immune system evolution,[16–18] and thus, ploidy-induced changes in microbiome-mediated defense could have important consequences for subsequent host evolution. For example, both the employment of the microbiome as well as WGD could potentially allow plants to circumvent the trade-off between growth and defense, but it is not known if WGD would disrupt the recruitment of a beneficial microbiome and perhaps incur negative consequences for growth and/or defense.[19,20] As such, a current open, yet critical, question is how whole-genome duplication impacts the interaction between plants and their associated microbiota.

To determine how WGDs alter the interactions between the plant and its above-ground microbiota and the consequences of these altered interactions on pathogen growth, we used seven

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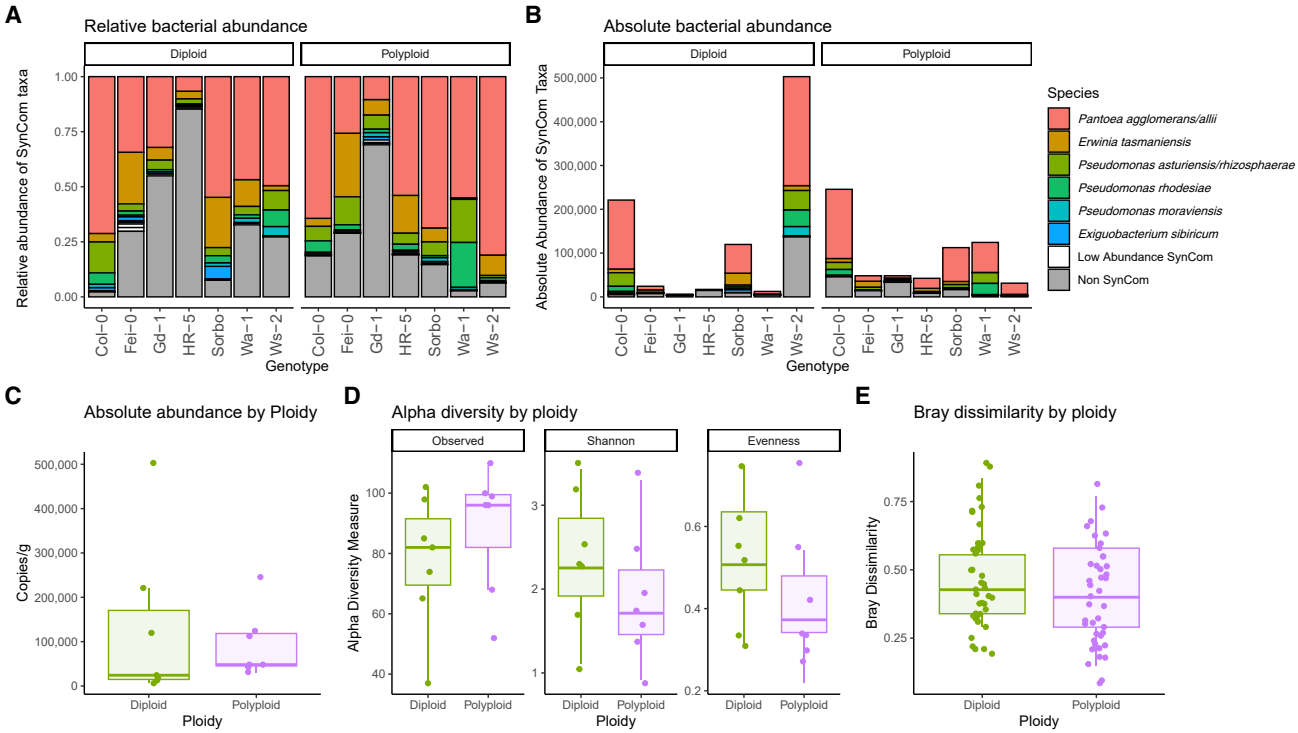

Figure 1. Ploidy level does not impact microbiome composition

The effect of ploidy on microbiome composition and structure.

(A) The relative abundance of the SynCom inoculated ASVs across treatments as well as the residual community.

(B) The absolute abundance of the microbial communities across treatments.

(C) There is no significant difference in the absolute abundance of phyllosphere bacteria between the two treatments.

(D) There is no significant difference in the tested alpha diversity metrics, inducing observed diversity, Shannon diversity, and evenness. However, there is a non-significant trend toward lower Shannon diversity in the polyploid plants, which is driven primarily by their lower evenness.

(E) Bray-Curtis dissimilarity between plants of each treatment is not significantly different.

See also Data S1.

lines of synthetic autotetraploid accessions of *Arabidopsis thaliana* and their corresponding diploid progenitors (so each of the seven *Arabidopsis* genotypes is included as both diploid and autotetraploid lines), inoculated them with a synthetic community (SynCom) comprising microbial taxa common to the leaf habitat. Although the *Arabidopsis* phyllosphere is naturally composed of hundreds of different bacterial species,[21] the SynCom is composed of only 16 species to remain tractable. We then determined whether there was a conserved change in bacterial community composition across ploidy level and if plants of differing ploidy had different transcriptional responses to these bacteria, in particular with respect to priming a plant for defense. To further investigate any effects of these changes on microbiome-mediated pathogen protection, we inoculated these plants, along with untreated controls (plants without the synthetic microbiome), with the model *Arabidopsis* pathogen *Pseudomonas syringae* pv. tomato DC3000 and measured growth during early establishment. By using synthetic autotetraploid accessions of *Arabidopsis* in conjunction with a controlled, synthetic microbial community, we were able to assess the associations between genotype, ploidy level, and the microbiome; determine the extent to which these interactions are mediated through shared transcriptional responses; and quantify the effect of these interactions on pathogen defense.

RESULTS

Ploidy level does not impact microbiome composition

To determine if there was a difference in the extent to which diploids versus polyploids recruited their microbiome from the inoculated synthetic community, we assayed the communities from 14 SynCom-inoculated plants (one diploid and one tetraploid for each of the seven *Arabidopsis* lines) inoculated with the SynCom by 16S amplicon sequencing 1 week after their inoculation (immediately prior to pathogen introduction).

The majority (65.8% in diploids and 77.3% in polyploids) of bacteria that we found associated with the plants were from the synthetic community, with *Pantoea*, *Pseudomonas*, and *Exiguobacterium* showing consistently high relative and absolute abundance across samples (Figures 1A and 1B). The absolute abundance of bacteria from the synthetic community on the leaves 1 week after inoculation, as ascertained by qPCR, was not significantly different across ploidy levels (Figure 1C; standardizing for sample weight: Welch two-sample t test, $t = -0.11455$, $df = 10.076$, $p = 0.911$; Figure S3). Further, using DESeq2, we concluded that there were no SynCom-associated bacteria with significantly different abundances across ploidy levels (Data S1A).

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Table 1. Time since exposure to the pathogen and the presence of the SynCom significantly impacted pathogen abundance

	DC3000 abundance (log_{10})		
Predictors	Estimates	CI	p
(Intercept)	1.15	0.18 to 2.11	0.033[a]
Time [2]	2.34	1.03 to 3.64	0.002[a]
Ploidy [4]	0.06	−1.25 to 1.36	0.935
Treatment [C]	2.2	0.89 to 3.51	0.003[a]
Time [2] * ploidy [4]	−1.73	−3.59 to 0.12	0.088
Time [2] * treatment [C]	−1.99	−3.83 to −0.14	0.052
Ploidy [4] * treatment [C]	−1.4	−3.25 to 0.44	0.164
(Time [2] * ploidy [4]) * treatment [C]	0.55	−2.13 to 3.24	0.702
Random effects			
σ^2	1.46	–	–
τ_{00eco}	0.14	–	–
ICC	0.09	–	–
N_{eco}	7	–	–
Observations	54	–	–
Marginal R^2/ conditional R^2	0.404/0.456	–	–

Linear mixed-effects model (nlme) of DC3000 abundance as a function of the explanatory variable time (2 = time point 2, 48 h postinoculation), ploidy (4 = tetraploid), treatment (SynCom inoculation, C = control), and their interactions.
[a]Significant at the p = 0.05 threshold

Table 2. Time, treatment (SynCom application), and their interaction impact pathogen abundance in diploids

	DC3000 abundance (log_{10})		
Predictors	Estimates	CI	p
(Intercept)	1.15	0.48 to 1.82	0.004[a]
Time [2]	2.34	1.39 to 3.29	<0.001[a]
Treatment [C]	2.2	1.25 to 3.15	<0.001[a]
Time [2] * treatment [C]	−1.99	−3.33 to −0.64	0.01[a]
Random effects			
σ^2	0.71	–	–
τ_{00eco}	0	–	–
ICC	0	–	–
N_{eco}	7	–	–
Observations	28	–	–
Marginal R^2/ conditional R^2	0.607/0.607	–	–

Linear mixed-effects model (nlme) for diploids, with DC3000 abundance as a function of the explanatory variables time (2 = time point 2, 48 h post-inoculation), treatment (SynCom inoculation, C = control), and their interactions. See also Table S1.
[a]Significant at the p = 0.05 threshold

Alpha diversity of the established microbiome (measured as species richness, Shannon index, or species evenness) did not differ significantly between diploids and polyploids (pairwise ANOVA, p > 0.05; Figure 1D). Likewise, we found no significant differences in beta diversity (community composition measured with Bray-Curtis dissimilarity) across ploidy level (ADONIS nonparametric multivariate analysis of variance, p > 0.05; Figure 1E).

Polyploids are less susceptible to pathogen establishment

To determine how ploidy level and microbiome inoculation impacted pathogen growth, we used droplet digital PCR (ddPCR) to measure the abundance of DC3000 over time, normalized for the mass of the plant.[22] Plants were inoculated with the pathogen one week after their treatment with the SynCom (or buffer control). We first sampled 14 plants (both ploidy levels for each line) immediately after inoculation with the pathogen to provide our initial densities (T0). Subsequently, we sampled 28 plants (full factorial sampling across ploidy, *Arabidopsis* line, and SynCom inoculation) at 24 (T1) and 48 (T2) h after inoculation.

Both time since exposure to the pathogen and the presence of the SynCom significantly impacted pathogen abundance. Analyzing log_{10} transformed abundance (linear mixed-effects model, p = 0.0018 and p = 0.0031, respectively; lines 2 and 4 in Table 1), we found a marginally significant interaction between the two (p = 0.0517; line 6 in Table 1). When analyzing the diploid samples alone we found a significant impact of time, treatment (SynCom application), and their interaction on pathogen abundance (p = 0.0001, p = 0.0003, p = 0.01, respectively; lines 2, 3, and 4 in Table 2). We performed a Tukey HSD post hoc test, finding significant differences between the pathogen densities of SynCom-treated samples across time points one and two (p = 0.0008; line 1 in Table S1), as well as between SynCom-treated samples from time point one and control (buffer inoculated) samples from time point two (p = 0.0003; line 2 in Table S1), and between SynCom-treated and control samples in time point one (p = 0.0014; line 3 in Table S1). For the polyploids, there was no significant improvement to model fit through the addition of any terms when compared with a null model including only the intercept, indicating that there was no effect of SynCom application on pathogen density in this ploidy level. In addition, there was significantly lower pathogen abundance (log_{10} transformed) when comparing the polyploid with diploid plants at the second time point for both the control (buffer inoculated) plants (Welch two-sample t test, t = 2.809, df = 4.9939, p = 0.03765) and the treated (SynCom inoculated) plants (Welch two-sample t test, t = 2.4295, df = 8.211, p = 0.04048; Figure 2), indicating that pathogen reduction in the polyploids was microbiome independent.

Diploid plants exhibit greater response to synthetic community colonization, while polyploids constitutively express certain defense genes

To test whether plants differed in their responses to inoculation with the SynCom, we sampled leaves from three *Arabidopsis* lines (Col-0, Ws-2, and Sorbo) across ploidy level (diploid and polyploid) and both treatments (SynCom versus buffer inoculation) for RNA sequencing. Leaf samples for transcriptomic analysis were collected at the same time as the samples for 16s amplicon sequencing, prior to pathogen inoculation, but from separate replicate plants (i.e., for each accession, ploidy level

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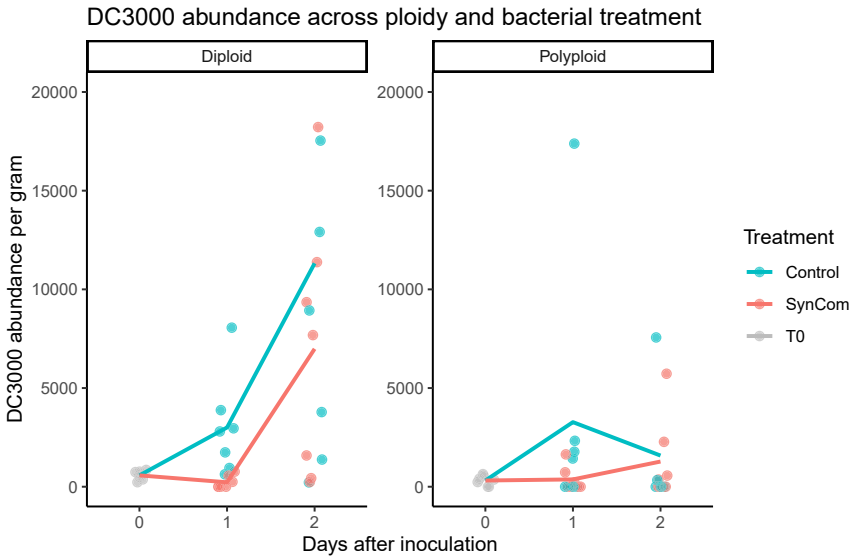

Figure 2. Polyploids are less susceptible to pathogen establishment

Abundance of the pathogen (normalized as DC3000 count per gram of plant mass) across time points and treatments in the diploid and polyploid plants. Samples were taken immediately after inoculation from a subset of plants in each ploidy (n = 14) to establish initial densities (T0), and from each genotype and ploidy across treatments (n = 28) at 24 h (T1) and 48 h (T2) after inoculation to determine pathogen growth. Pathogen density was established using ddPCR and was $\log_{10}$ transformed prior to statistical analysis to yield a more normal distribution. Two days after inoculation, there was significantly less DC3000 detected in polyploids when compared with diploids, across both SynCom inoculated ($p = 0.04048$) and control plants ($p = 0.03765$).

See also Figure S3.

and bacterial treatment, two plants were grown, with one being sampled for RNA sequencing and the other for amplicon sequencing).

Analyzing RNA sequences prior to pathogen inoculation, using DESeq2, we found 220 up- or downregulated genes between SynCom-treated and untreated diploid plants, whereas polyploid plants had only 35 significantly differentially expressed genes (at the 0.1 p value cutoff; Figures 3A and 3B). We chose the 0.1 p value cutoff to ensure that we captured patterns that might be important but not rise to the standard 0.05 significance level; the results are qualitatively identical when evaluated at a 0.05 p value cutoff, and those genes that are significant at $p < 0.05$ are indicated in the figure. In general, there is a greater range for differentially expressed genes in the polyploids compared with the diploids (Figures 4A and 4B). Diploid plants showed several clusters of significantly differentially expressed genes when those genes were grouped by function. Many of these groups of genes are associated with defense functions, including, for example, genes associated with the well-characterized phytohormone abscisic acid (ABA; Figure 3A). Genes associated with hypoxia as well as defense response to bacteria were also significantly up- or downregulated. Furthermore, several genes associated with ethylene signaling were upregulated in the SynCom-treated diploids when compared with the control (Figure 3A), and although it is unclear if ethylene response is essential for *P. syringae* defense in *Arabidopsis*,[23] it has been implicated in resistance.[24] When comparing the polyploid treated and control plants, we saw a pattern of primarily increased gene expression, with the majority of these being defense related, including ABA, hypoxia, and ethylene signaling-related genes (Figure 3B). When comparing polyploid and diploid plants directly, we see that all significantly differentially expressed genes are upregulated in the polyploids. Several of these genes are associated with stress- or defense-related functions, including cellular response to hypoxia, general defense response, and negative regulation of defense response to bacteria (Figure 3C).

To focus specifically on the plant responses to the microbial community that might underly the differences in *P. syringae* resistance we observed, we identified genes that are both known to function in *Arabidopsis* response to *P. syringae* infection (by searching the UniProt database[25]) and that are expressed at a significantly higher level in the diploids after exposure to the SynCom. When comparing the expression patterns of these genes in the polyploids (Figure 5), we find for four of the six genes (AMC4, CYP19–1, STP4, and VDAC1) a pattern of elevated expression, regardless of exposure to the SynCom. Further, for the additional two genes (NADK1 and WRKY53), which show significantly reduced expression in the diploids in response to SynCom application, we find that their expression is not significantly reduced in the polyploids.

DISCUSSION

Effects of polyploidy on microbiome diversity

Overall, we found no significant differences in microbiome establishment—either in composition or diversity—between the diploid or polyploid plants. Although it is possible that there are ploidy-dependent effects within certain genotypes, our use of multiple accessions allowed us to rule out a generalized response of the microbiome in this system. The polyploids weighed significantly more across all accessions than did the diploids, consistent with patterns observed in some groups[26] but in contrast to previous work on autotetraploid *Arabidopsis*,[27,28] and supported a higher total number of commensal bacteria (once we normalized for the weight of the plant, this difference was not significant). Likewise, we did not find any significant differences in the relative abundance of any of the synthetic community members across the two ploidy levels, both of which were primarily colonized by *Pantoea*, *Pseudomonas*, and *Exiguobacterium*. The lack of conserved impact on community composition is in line with work on wheat, where ploidy was found to play a weak and inconsistent role in shaping the below-ground microbiome[29] but contrasts with previous work on *Arabidopsis* that did find a signature of ploidy in shaping microbial communities.[30] Regardless of the impacts of ploidy on microbiome composition, our study highlights the importance of

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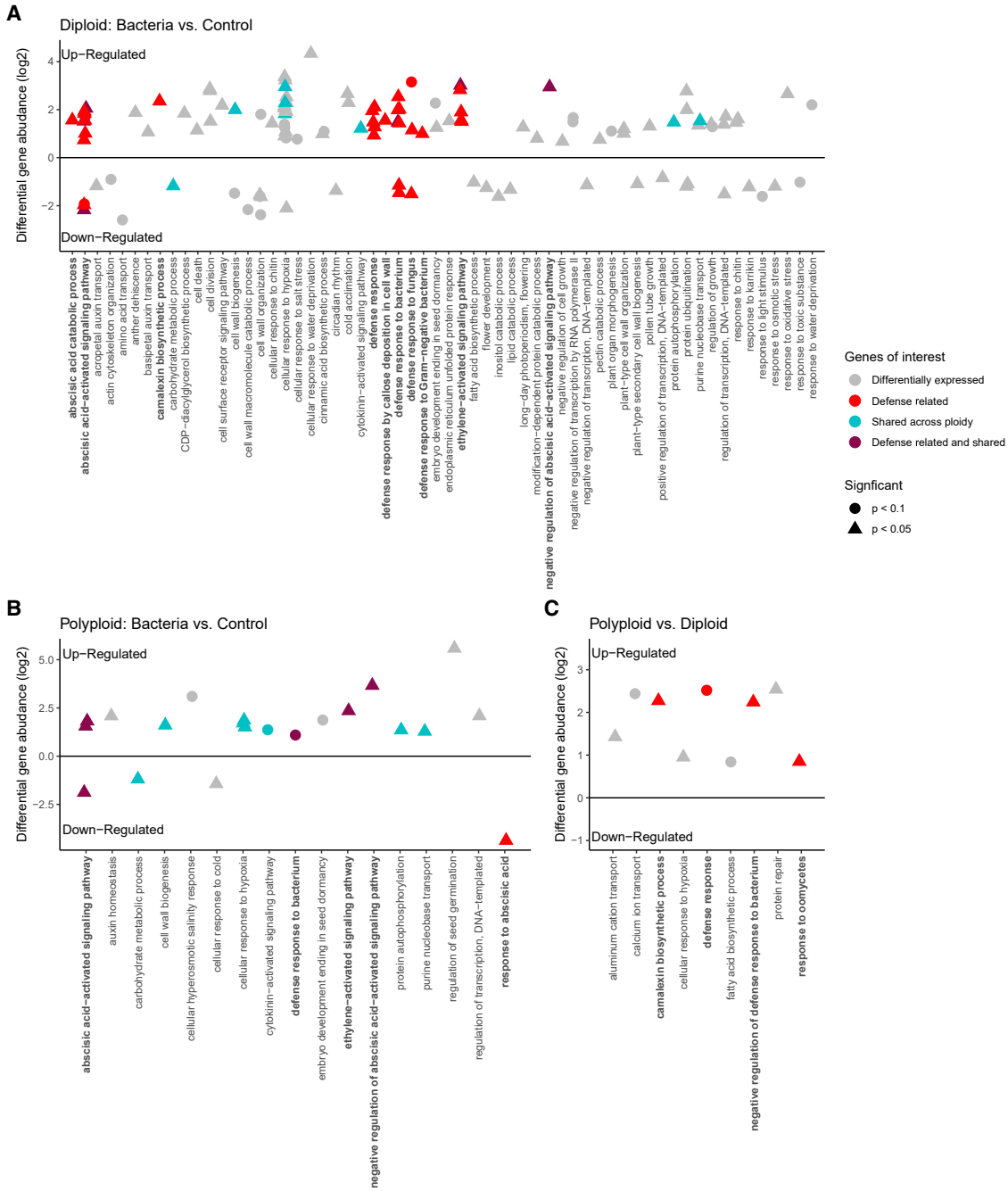

Figure 3. Diploid plants exhibit greater response to synthetic community colonization, whereas polyploids constitutively express certain defense genes

Differential expression of genes highlighted by association with defense response.

(A) Genes that are significantly differentially expressed ($p < 0.1$) when comparing the microbiome inoculated and control (buffer inoculated) diploid plants. Genes that are associated with defense functions are noted in bold and highlighted in red, whereas genes that are also differentially expressed in the polyploids are highlighted in blue, and genes that are both defense-associated and shared are highlighted in purple.

(B) Significantly differentially expressed genes between microbiome inoculated and control polyploids, using the same colors from the previous panel.

(C) Significantly differentially expressed genes between all polyploid and diploid plants, regardless of treatment, using the same colors as the previous two panels.

See also Figure S2.

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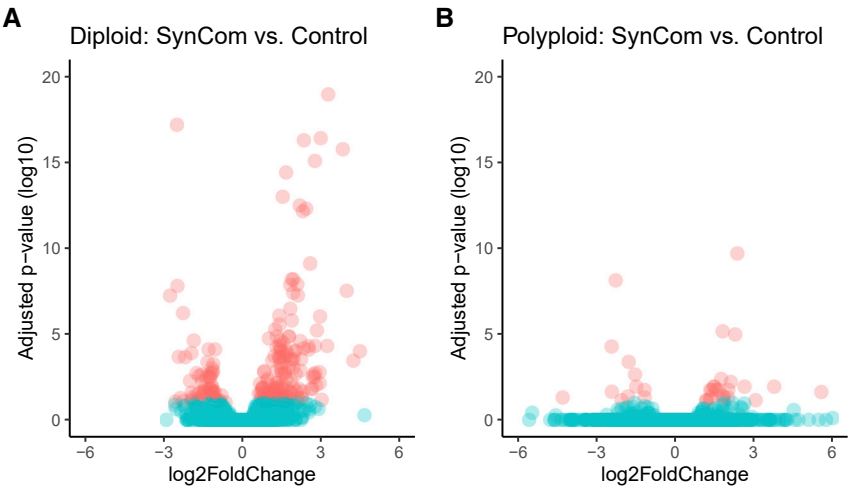

Figure 4. Polyploids have fewer significant differentially expressed genes than diploids when treated with the SynCom

Variability of gene expression between treated (SynCom inoculated) and control (buffer inoculated) plants of both ploidies.

(A and B) Volcano plots showing proportion of total genes that are significantly differentially expressed (red dots) at the level of adjusted p value of 0.1 (to account for multiple testing) plotted against their log_2-fold changes. Genes that were not statistically significantly differentially expressed are in blue. Comparisons are drawn across SynCom treatment (buffer inoculation versus SynCom inoculation) for both diploid samples and for polyploid samples. See also Figure S2 and Data S1.

considering the impact of the microbiome as a function of ploidy, given that although we do not see a conserved shift in the community, we do see changes in the way the plants respond to these same bacteria.

Effects of ploidy on pathogen response

To date, there has been little study of polyploidy on pathogen response. Although polyploids have been proposed to be more resistant to pathogens,[6,8] empirical studies have generally been inconclusive, i.e., they find evidence for both increased resistance and increased susceptibility.[31,32] Our study leveraged multiple accessions of *Arabidopsis* to discern general patterns between ploidy level and pathogen defense. Overall, we found a trend toward lower pathogen abundance in the polyploid plants, regardless of association with a bacterial community as well as a significant decrease in the abundance of the pathogen in the second time point (Figure 2).

Autotetraploids may be more resistant than diploids due to higher expression of defense genes as a consequence of their doubled genome.[33] For example, tetraploid *Arabidopsis* accessions acquired increased resistance to copper stress by having increased activation of antioxidative defense.[34] A buttressing of the antioxidant defense system was also found in synthetic tetraploid plants of *Dioscorea zingiberensis* where antioxidant enzymes were over-produced and maintained at high concentration.[35] These elevated defense responses generally come with a trade-off—for example, elevated expression of stress-response genes is associated with a fitness cost and slowed growth.[28] In contrast, our autotetraploids exhibit both greater biomass (Figure S3) and higher defensive capacity (Figure 2). Although autotetraploids have double the copy number for all genes, the effect of copy number on gene expression is not necessarily linear. Differences in relative expression may therefore potentially reduce sensitivity to growth-defense trade-offs, although more work would need to be done to test this hypothesis.

Interaction between ploidy and the microbiome on pathogen response

When assessing the effectiveness of the microbiome in protecting the plants of different ploidy levels, we found that the microbiome temporarily arrests pathogen growth on the diploids, whereas polyploids are protected, regardless of exposure to their microbiome (Figure 2). This result is particularly interesting in light of previous work on microbiome-mediated protection by a synthetic microbiome in tomato in which the phyllosphere microbiome was protective against pathogen growth in the absence of fertilizer application but unimportant when plants had been fertilized prior to microbiome and/or pathogen inoculation.[36] Plant response to commensal bacterial organisms are complicated, often showing an overlap with the response to pathogens.[37] This overlap can be explained in part through the broadly conserved plant responses to common microbial-associated molecular patterns (MAMPs), such as flagellin,[38] although even these responses can be modulated by a host of commensal interactions, such as repression of conserved epitopes.[39] These responses can be beneficial through the early activation of broad defense responses (priming) that will then respond more effectively to pathogen exposure.[40,41] It is possible that this phenomenon plays a role in the increased protection afforded to the diploid plants that have been inoculated with the SynCom, as it may provide a mechanism of priming against potential future pathogens. It is important to note, however, that *Pseudomonas syringae* represents only one plant pathogen, and further study with a variety of other pathogens, representing alternative infective patterns, is needed to determine if there is a generalized effect of ploidy on pathogen defense and microbiome reliance.

Both polyploids and diploids modulate defense pathways in response to inoculation with the synthetic community, but in different ways

As expected, we found that the broad-scale transcriptional profiles of the samples grouped strongly together by accession (Figure S2). Nonetheless, when looking across accessions, we saw that many of the significant changes in gene expressions associated with microbiome treatment were linked to defense-associated genes (Figures 3A and 3B). These genes include those associated with ABA regulation, response to hypoxia, general defense response, and ethylene signaling. ABA is a well-studied plant signaling hormone that is linked to a variety of processes ranging from plant growth to development and stress response.[42] The function of ABA in defense response is

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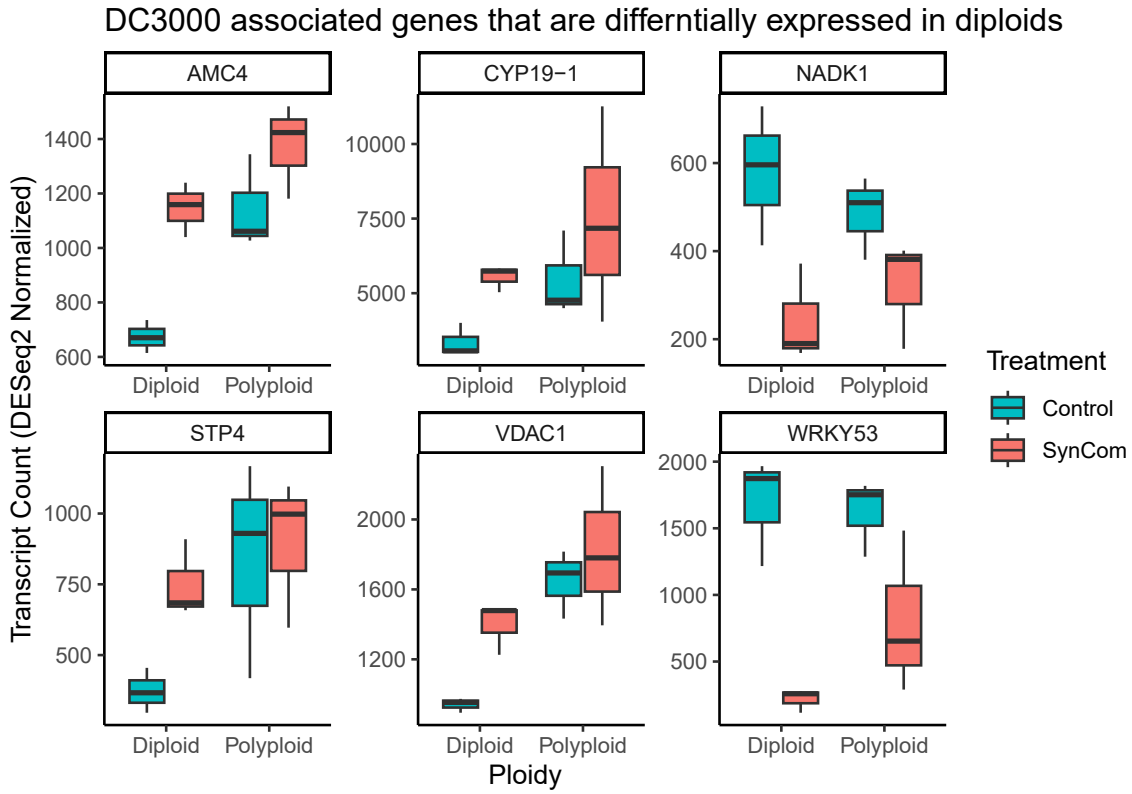

Figure 5. Polyploids maintain defense expression, regardless of exposure to the synthetic community

Expression levels of genes that were identified in UniProt as associated with *P. syringae* response and that are significantly differentially expressed between the treated (SynCom inoculated) and control (buffer inoculated) diploid plants. For four of the six genes (AMC4, CYP19–1, STP4, and VDAC1), we see a pattern of increased expression in the polyploids, regardless of treatment with the SynCom, and for the other two genes (NADK1 and WRKY53), we see that their expression, which is significantly reduced in the diploids, is not significantly altered in the polyploids.

See also Data S1.

multifaceted and has been shown to be important in preinvasion defense, through the closing of stomata in response to MAMPs,[43] as well as negatively regulating postinvasion defense through the suppression of callose deposition[44] and SA-dependent resistance.[45,46] For all plants that received the synthetic microbiome expression responses were also significantly enriched for GO terms associated with cellular response to hypoxia when compared with the control group. The response to hypoxia requires the ethylene pathway in plants,[47] which is involved in the hormonal control of programmed cell death[48] and has been shown to influence the composition of the leaf microbial community.[21] Responses to pathogens involve increased respiration that creates local hypoxia around the leaf which is otherwise aerobic.[49] Similarly, alcohol dehydrogenase, which in plants is involved in NAD^+ production, is not only over-expressed in times of low oxygen but is also induced in response to biotic and abiotic stress and improves responses to pathogens.[50] All synthetic microbiome-treated plants showed differential expression for several WRKY transcription factors that are linked with defense signaling,[51] as well as CCR4-associated factor 1, which has been shown to play a role in susceptibility to *P. syringae* infection.[52] Finally, SynCom-treated plants also show a pattern of increased expression in ethylene-activated signaling pathways. Ethylene is another well-characterized phytohormone that is responsible for regulation of plant growth, development, and senescence[53] as well as response to pathogen invasion and modulation of defense response.[54] These results demonstrate that there are multifaceted defense responses to inoculation with the SynCom by both diploids and polyploids.

Polyploids maintain defense expression regardless of exposure to the synthetic community

The trade-offs between growth and defense in plants are often metabolic but can also be due to antagonistic cross talk between hormones involved in both processes.[19] One way to mitigate this trade-off could be through the association with microbiota that perform similar defenses.[19] Consistent with these ideas, the microbiome-treated diploids significantly slowed the progression of DC3000 growth. When comparing the control and inoculated plants, diploids showed nearly six times more differentially expressed genes than did the polyploids, with a mix of up- and down-regulated defense-associated genes. In contrast to the diploids, the differentially expressed genes in the polyploid samples were primarily upregulated, including several defense-related genes (Figure 3B).

In total, we saw a greater range of genes differentially expressed in the polyploids compared with the diploids, but with fewer reaching significance (Figures 4A and 4B), indicating that

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their expression was more variable between lines and that genome duplication events may have had line specific effects on gene expression. Despite this variation, we see that several genes known to function in response to *P. syringae*, which are expressed at higher levels in the SynCom-inoculated diploids compared with the controls, show elevated expression in the polyploids, regardless of their exposure to the SynCom (Figure 5). These genes are AMC4, which plays a role in programmed cell death in response to *P. syringae* exposure;[55] CYP19–1, involved in the production of reactive oxygen species after infection by *P. syringae*;[56] STP4, which is induced by pathogen wounding,[57] although its link to *P. syringae* response is contested;[58] and VDAC1, which is involved in the maintenance of reactive oxygen species homeostatis and stress response and has been shown to be upregulated following *P. syringae* infection.[59] This result suggests that the polyploid plants may be protected from *P. syringae* colonization by the constitutive expression of some subset of their defense responses. Further, for the additional two genes, NADK1, which phosphorylates NADH to produce the antioxidant factor NADPH,[60] and WRKY53, which promotes *P. syringae* resistance through salicylic acid response pathways,[61] we see a pattern of decreased expression in the SynCom-treated diploids but not the treated polyploids. It has been shown that commensal species sometimes downregulate defense-associated genes to successfully colonize,[62] which does not appear to be happening in the polyploids.

This conclusion makes sense in light of our pathogen growth results, as the polyploids are broadly protected, regardless of their microbiome, whereas the diploids require the microbiome to arrest pathogen growth. Consistent with this mechanism, seven of the 24 *A. thaliana* general non-self-response (GNSR) genes,[63] which have conserved expression changes in the presence of different bacteria, were constitutively expressed in the polyploids, regardless of their exposure to the commensal microbiome. Wang et al.[41] show how polyploidy "potentiates" stress-responsive gene expression in response to abiotic stress, which gives some precedence to the finding that polyploids demonstrate some improved defense responses. Our study extends this pattern to include biotic stress. Whether this alteration is adaptive or not would be highly dependent on the environmental context of the plant. For example, in a familiar environment where they could reliably source beneficial bacteria, constitutive activation of certain defense genes could be a waste of otherwise better utilized resources, whereas in a novel environment, this decoupling of defense from microbial associations could be a boon.

Future work could elaborate on the mechanistic underpinnings of these results by inducing autopolyploidy in known *Arabidopsis* defense mutants and determining their responses to pathogen invasion, for example, using the bak1/bkk1 mutant identified by Vogel et al.[37] to be deficient in priming response to commensal species or by using CRISPR to knock out the *P. syringae*-associated genes that we identified in our study.

Conclusions

Our work highlights the important role that polyploidization plays in the interplay between plants, their associated phyllosphere microbiota, and an invading foliar pathogen. Although the presence of the synthetic phyllosphere microbiome was always associated with a pattern of decreased *P. syringae* growth, the effect was only significant in the diploid plants; the polyploids appeared to be broadly protected, regardless of the presence of these beneficial bacteria. Our transcriptional results suggest this result is due to a microbiome-independent regulation of defense genes in the polyploids, whereas the diploids required exposure to the microbiome to induce a sufficient defense response. It is possible that as a consequence of gene dosage doubling due to WGD, polyploids may have a higher baseline activation of certain defense genes, thus decoupling their defense responses from those induced only by prior microbial associations, although the benefit of this may be context dependent. These results are particularly relevant to understanding the role that domestication, which often involves polyploidization, has played in altering interactions between plants and their associated microbes in agricultural settings. Likewise, the protective effects of the SynCom in diploid plants have important implications for the role of phyllosphere bacterial communities in managing plant disease, both naturally and as an applied supplement. Finally, we note that our results show that a lack of differences in microbiome composition need not imply absence of differences between ploidy levels.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.05.015.

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AUTHOR CONTRIBUTIONS

E.C.M., M.J.S., J.N.P., and J.J. designed the experiment; E.C.M., M.J.S., J.N.P., and J.J. performed the research; E.C.M. and M.J.S. analyzed the data; and E.C.M., M.J.S., J.N.P., J.J., J.E.C., B.K., and C.J.R. contributed to the writing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Pseudomonas syringae pv. tomato DC3000	This Study	N/A
Brevibacterium frigoritolerans	This Study	N/A
Bacillus wiedmannii	This Study	N/A
Curtobacterium herbarum	This Study	N/A
Curtobacterium pusillum	This Study	N/A
Erwinia tasmaniensis	This Study	N/A
Exiguobacterium sibiricum	This Study	N/A
Frigoribacterium endophyticum	This Study	N/A
Microbacterium oleivorans	This Study	N/A
Pantoea aurea	This Study	N/A
Pantoea agglomerans	This Study	N/A
Pantoea allii	This Study	N/A
Pseudomonas asturiensis	This Study	N/A
Pseudomonas rhizosphaerae	This Study	N/A
Pseudomonas rhodesiae	This Study	N/A
Pseudomonas moraviensis	This Study	N/A
Rathayibacter festucae	This Study	N/A
Chemicals, peptides, and recombinant proteins		
$MgCl_2$	Sigma Aldrich	Cat: 7786-30-3
phosphate buffer	Sigma Aldrich	Cat: 1219
MS medium	Sigma Aldrich	Cat: M5519
sodium hypochlorite (7% available chlorine)	Sigma Aldrich	Cat: 13440
Triton X-100	Sigma Aldrich	Cat: 9036-19-5
Critical commercial assays		
Spectrum Plant Total RNA Kit	Merck/MilliporeSigma, MO, USA	Cat: STRN50
MoBio PowerMag Soil DNA Isolation Bead Plate	Mp Biomedicals, LLC, CA, USA	Cat: 116560200-CF
FastPrep-24 Classic bead beating grinder and lysis system	Mp Biomedicals, LLC, CA, USA	Cat: 116004500
BIO-RAD QX 200 Droplet Reader	Bio-Rad Laboratories, Hercules, CA, USA	Cat: 1864003
Mini-BeadBeater 8	BioSpec Products, Bartlesville, OK, USA	Cat: 23998
Deposited data		
BioProject	PRJNA817596	N/A
Experimental models: Organisms/strains		
Arabidopsis thaliana Columbia (Col-0)	Adrienne Roeder, Cornell University	N/A
Arabidopsis thaliana Warschau (Wa-1)	Luca Comai, UC Davis	N/A
Arabidopsis thaliana Wassilewskija (Ws-2)	Luca Comai, UC Davis	N/A
Arabidopsis thaliana Gudow (Gd-1)	Brian Husband, U. of Guelph	N/A
Arabidopsis thaliana HR (HR-5)	Brian Husband, U. of Guelph	N/A
Arabidopsis thaliana Sorbo (Sorbo)	Brian Husband's, U. of Guelph	N/A
Arabidopsis thaliana St. Maria d. Feiria (Fei-0)	Brian Husband's, U. of Guelph	N/A

(*Continued on next page*)

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
DC3000 specific primer (ddPCR): Forward GACCAAGGATGCAGCAGAAA	N/A	N/A
DC3000 specific primer (ddPCR): Reverse GCCGTTACGGATATCAACGA	N/A	N/A
Bacterial-specific primer (qPCR): Forward AGAGTTTGATCCTGGCTCAG	N/A	N/A
Bacterial-specific primer (qPCR): Reverse ATTACCGCGGCTGCTGG	N/A	N/A
16S V4 sequencing: Forward GTGCCAGCMGCCGCGGTAA	N/A	N/A
16S V4 sequencing: Reverse GGACTACHVGGGTWTCTAAT	N/A	N/A
Software and algorithms		
Trimmomatic 0.40	Trimmomatic	https://github.com/usadellab/Trimmomatic
HISAT 2.2.1	HISAT2	http://daehwankimlab.github.io/hisat2/
HTSeq 0.13.5	HTSeq	https://htseq.readthedocs.io/en/master/
DESeq2	DESeq2	https://github.com/mikelove/DESeq2
Phyloseq	Phyloseq	https://joey711.github.io/phyloseq/
vegan 2.5-7	Vegan	https://cran.r-project.org/web/packages/vegan/index.html
DADA2 1.16	DADA2	https://benjjneb.github.io/dada2/tutorial.html
Other		
Sequencing done at Microbiome Insights	The University of British Columbia, 2405 Wesbrook Mall #6206, Vancouver, BC V6T 1Z3, Canada	N/A
Sequencing done at Novogene USA	8801 Folsom Blvd #290, Sacramento, CA 95826	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Elijah Mehlferber (emehlferber@berkeley.edu).

Materials availability

The synthetic community used in this study is available upon request.

The seeds for the diploid and colchicine-inducted tetraploid lines are available upon request.

This study did not generate new unique reagents.

Data and code availability

- 16S data and RNA-seq data have been deposited at BioProject and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Arabidopsis accessions

14 total lines were used from seven Arabidopsis diploid accessions from natural populations and their colchicine induced autotetraploids: Columbia (Col-0), Warschau (Wa-1), Wassilewskija (Ws-2), Gudow (Gd-1), HR (HR-5), Sorbo (Sorbo), St. Maria d. Feiria

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(Fei-0). All the autotetraploids produce viable seeds. All experiments were performed on a single cohort of plants that were grown and maintained together.

Plant growth conditions

Seeds were surface sterilized by treatment with 70% ethanol v/v for 2 min and then sodium hypochlorite solution (7% available chlorine v/v) containing 0.2% Triton X-100 v/v for 8 min. Samples were then washed seven times with sterile double distilled H2O.[64] Seeds were then placed on MS media with.8% agar m/v and cold stratified for two to three days at 4°C in the dark.[64] After germination, seedlings were transferred to a controlled environment in a Conviron growth chamber (Model E15) with a long-day photoperiod (16-h photoperiod) at 22°C and 55% relative humidity with cool white fluorescent light[64] set at 25% (approximately 200 μmol photons m^{-2} s^{-1}). After seven days the seedlings were transferred to sterile peat and the lighting was changed to short-day conditions (9-h photoperiod).[65]

METHOD DETAILS

Inoculation with synthetic community (SynCom) and infection with pathogen DC3000

The synthetic community is composed of 16 taxa that span the microbial diversity of tomato (key resources table). The synthetic community is prepared by growing each bacterial species in a Panasonic incubator (Model MIR-554) at 28.2°C, shaking at speed setting 140 (VWR Advanced Digital Shaker) for 3 days in Kings Medium B (KB) broth,[66] after which the bacteria are centrifuged for 10min at 2500g. The supernatant is removed, and the bacteria are resuspended in 10 mM $MgCl_2$. Bacterial density is measured and adjusted to a density OD600 nm = 0.2. Each species is added in equal volume, and the assembled community is diluted 10 fold in 10 mM $MgCl_2$ to yield a final concentration of OD600 nm = 0.02. Two weeks after germination, each plant was inoculated with either the synthetic community suspended in 10 mM $MgCl_2$ buffer at a density of OD600 nm = 0.02 or just the 10 mM $MgCl_2$ buffer alone. The plants were inoculated by spraying the plant until the leaves were fully saturated, as indicated by runoff. Three weeks after germination (one week post synthetic community inoculation), the plants were spray-inoculated with either the pathogen (*Pseudomonas syringae* pv. tomato DC3000) or a 10 mM $MgCl_2$ buffer. The pathogen was inoculated at a density of OD600 nm = 0.0001.[65]

Sample collection

Four sets of samples were collected, the first set one week post inoculation with the SynCom, but immediately prior to inoculation with DC3000 to determine the commensal community composition, the second immediately after inoculation with DC3000, the third 24 hours post inoculation, and the fourth 48 hours post inoculation. All of the plants were approximately at the same stage of development and no plants that showed signs of inflorescence emergence were used in the assay. To sample the aerial portion of the plants, plants were cut just above the roots and transferred the total above-ground biomass into a tube with either 10 mM $MgCl_2$ (for sequencing the SynCom), or into 100mM phosphate buffer (pH 7), for the pathogen inoculated samples. Samples for sequencing were sonicated for 15 minutes in a Branson M5800 sonicating water bath. The resulting leaf wash was then pelleted, the supernatant removed, and frozen at -20°C until sequencing. Pathogen inoculated samples were bead homogenized using the FastPrep-24 Classic bead beating grinder and lysis system (MP Biomedicals, CA, USA) and frozen at -20°C until ddPCR sequencing was performed.

Amplification and sequencing of microbial 16S rDNA

Samples were snap frozen on liquid nitrogen and kept at -20°C and sent to Microbiome Insights for 16S V4 sequencing and qPCR analysis within one month of freezing. Amplification and sequencing were performed according to Microbiome Insights standard protocol: Specimens were placed into a MoBio PowerMag Soil DNA Isolation Bead Plate. DNA was extracted following MoBio's instructions on a KingFisher robot. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5′ -GTGCCAGCMGCCGCGGTAA-3′, and 806R 5′ -GGACTACHVGGGTWTCTAAT-3′), as per the protocol of Kozich et al.[67] Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3). The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from template-free controls (negative control) and extraction kit reagents processed the same way as the specimens. A positive control from 'S00Z1-' samples consisting of cloned SUP05 DNA, was also included. The only modification to this standard protocol was the addition of PNAs according to the method developed in Lundberg et al.,[68] in brief (mPNA, to knock out mitochondria and pPNA to knock out chloroplast) into the PCR step during library prep at a concentration of 5uM per PNA. The PCR reaction was then modified with the addition of a PNA annealing step at 78°C for 10s.

qPCR assay of microbial abundance

From the standard methods of Microbiome Insights: Bacterial-specific (300 nM 27F, 5′ -AGAGTTTGATCCTGGCTCAG-3′) forward primers coupled to (300 nM 519R, 5′ -ATTACCGCGGCTGCTGG-3′) reverse primers were used to amplify bacterial 16S rRNA. 20 μl reactions using iQ SYBR Green Supermix (Bio-Rad), with 10μl Supermix, 0.6μl Primer F, 0.6μl Primer R, 6.8μl H2O and 2μl template, were run on Applied Biosystems StepOne Plus instrument in triplicate using the following cycle conditions; 95°C for 3 min., 95°C 20 sec., 55°C for 20 sec., 72°C for 30 sec., return to step two 45 times. For standards, full-length bacterial 16S rRNA gene was cloned into a pCR4-TOPO vector, with Kanomycin-Ampicillin resistance. The total plasmid fragment size is expected to be 5556 bp. A bacterial standard was prepared via. 10-fold serial dilutions, and the copies of 16S was determined by the

following: Copy# = (DNA wt. x 6.02E23)/(Fragment Size x 660 x 1E9). Linear regression was used to determine copy numbers of samples, based on CT of standards. Reaction specificity was assessed using a melt curve from 55°C to 95°C, held at 0.5°C increment for 1s.

ddPCR assay of pathogen abundance

Absolute bacterial abundance was estimated by performing digital droplet PBR (ddPCR) on homogenized whole plant samples randomized within plate columns using the BIO-RAD QX 200 Droplet Reader (Bio-Rad Laboratories, Hercules, CA, USA) and custom primers to specifically target and amplify Pseudomonas syringae pv. tomato DC3000. The PCR protocol is as follows: 95°C for 5 min., 95°C for 30 sec., 60°C for 100 sec., return to step two 40 times., 4°C for 5 min., 90°C for 5 min., keep at 4°C overnight.

RNA sample collection and sequencing

For each of three accessions (Columbia (Col-0), Wassilewskija (Ws-2), Sorbo (Sorbo)), six plants of each ploidy level (diploids and induced autotetraploids) were grown in randomized blocks with three plants treated with the synthetic community and three treated with the control buffer, for a total of 36 plants. Single leaves from the largest developmental node of plants at Stage 1.10 (ten rosette leaves >1 mm in length[69]) were collected and directly froze them in liquid nitrogen before subsequent storage at -80C. Tissue was homogenized using a Mini-BeadBeater 8 (BioSpec Products, Bartlesville, OK, USA) following the manufacturer's instructions. RNA was extracted using the Spectrum Plant Total RNA Kit (Merck / MilliporeSigma, MO, USA) according to the manufacturer's recommendations.Three samples per accession were pooled. Samples were sent to Novogene USA (Sacramento, CA) for library prep (Poly(A) capture, ligation-based addition of adapters and indexes) and sequencing (Illumina NovaSeq 6000, 150bp paired-end reads, 20M reads per sample).

Test for euploidy

Tetraploid samples were tested to assess aneuploidy or euploidy by calculating fold change in relative expression (transcripts per million; TPM) per gene for every pairwise comparison of biological replicates following the methods outlined in Song et al.[70] If there is aneuploidy, the expectation would be to see a large coordinated increase or decrease in TPM for genes on that chromosome, which would be reflected in a shift in fold change of expression relative to the other biological replicates (Figure S1). No shift was found and therefore it can be concluded that all tetraploid individuals were euploid.

QUANTIFICATION AND STATISTICAL ANALYSIS

Microbiome data analysis

Forward and reverse paired-end reads were filtered and trimmed to 230 and 160 base pairs (bps), respectively using the DADA2 pipeline with default parameters.[71] Following denoising and merging reads and removing chimeras, DADA2 was used to infer amplicon sequence variants (ASVs), which are analogous to operational taxonomic units (OTUs), and taxonomy was assigned to these ASVs using the DADA2-trained SILVA database. Using the negative samples from 16s sequencing the decontam package was implemented using default settings to identify and remove potential contamination from the samples.[72] The assigned ASVs, read count data, and sample metadata were combined in a phyloseq object[73] for downstream analyses. Differential microbial changes were calculated using DESeq2[74] and the phyloseq package was implemented in R to calculate changes in alpha and beta diversity. For a permutational analysis of variance (PERMANOVA), data was rarified to 90% of the reads of the least abundant sample and the test was performed using the adonis function in the vegan package[75] in R with 999 permutations to test whether ploidy or genotype had an effect on beta diversity measures.

The test statistics and p values for the Welch Two-Sample t test, pairwise ANOVA, and nonparametric multivariate analysis of variance can be found in the results section.

ddPCR assay of pathogen abundance

To assay the pathogen abundance using ddPCR, the default thresholds for identifying positive samples on the Biorad analysis software was used and then the weight of each sample was used to calculate a normalized per gram density of bacteria present on the above-ground plant. The absolute abundance of polyploids and diploid accession pairs across each timepoint was compared to assay how the pathogen interacted with ploidy and microbiome treatment.

The test statistics and p values for a Linear Mixed Effects Model (nlme) of DC3000 Abundance as a function of the explanatory variables time, ploidy, treatment (SynCom inoculation), and their interactions can be found in Table 1.

The test statistics and p values for a Linear Mixed Effects Model (nlme) for Diploids, with DC3000 Abundance as a function of the explanatory variables Time, Treatment (SynCom inoculation), and their Interactions can be found in Table 2.

The test statistics and p values for a Post hoc Tukey HSD (emmeans) for the Diploid Linear Mixed Effects Model can be found in Table S1.

RNA-seq data processing and analysis

Raw FASTQ files were trimmed and filtered to remove low-quality reads and technical sequences using Trimmomatic[76] with the default settings. Filtered reads were aligned to the Arabidopsis reference sequence[77] (TAIR10), with HISAT2.[78] HTSeq[79] was

used to determine read counts per gene for the test for euploidy and DESeq2 was used to analyze differential gene expression[74] for different experimental comparisons. For DESeq2 analysis gene ontology was assigned using UniProt.[25] Links to the DESeq2 output data for each of these comparisons can be found in Data S1B. *P. syringae* associated genes were identified using UniProt[25] and were analyzed using the rstatix package t_test function.[80]