How might highly resilient plant immunity have evolved? A “multiverse” hypothesis

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SUMMARY

Flowering plants have highly resilient core immunity, which is likely key to withstanding assaults from fast-evolving pathogens for millions of years. A major means of enabling resilience is acquisition of backup immune signaling mechanisms, which effectively conceal evolutionary goals from pathogens. However, it seems impossible to acquire backup mechanisms via incremental adaptation under constant pressure from faster-evolving organisms. Here I propose a hypothesis for how a system with backup mechanisms could have evolved using concepts borrowed from a multiverse hypothesis in cosmology.

Pathogens can evolve much faster than plants

Many plant pathogens are microbial and can evolve much faster than plants. Two factors that positively affect the rate of evolution (the nucleotide substitution rate) in the neutral theory of molecular evolution are mutation rate and effective population size [1]. The mutation rate is typically defined on a per-generation basis. The mutation rates of DNA viruses, bacteria, to Arabidopsis are in a similar range (10^{-9} to 10^{-6} substitutions/nucleotide/generation) whereas those of RNA viruses are higher (10^{-6} to 10^{-4} substitutions/nucleotide/generation) [2-4]. Therefore, the evolution rate in a fixed time interval, such as a year, is also highly dependent on generation time. Generally, the larger the effective population size and the shorter the generation time, the faster evolution is. Viruses could have generation times on the order of 10 minutes, and bacteria could have generation times on the order of 1 hour, while the fastest-cycling flowering plants have generation times on the order of a month. While the population size of a single species of bacterium could easily be many orders of magnitude larger than that of a plant species, their effective population size could be even larger due to frequent horizontal gene transfer across different species of microbes [5]. Thus, in the race of evolution, plants have lopsided disadvantages relative to their microbial pathogens. In contrast, we, as vertebrates, have a big advantage against fast-evolving pathogens. We have adaptive immunity, which is a super evolution machine inside each of us [6]. Plants do not have adaptive immunity. How have plants managed to stay in this world under pressure from fast-evolving pathogens?

Backup mechanisms provide resilience to plant immunity and buy time for plants

One key factor for plants to withstand pathogen evolution despite their lopsided disadvantages is resilience of the plant immune system. The core part of immunity of flowering plants is highly resilient. A pathogen effector can target immune signaling components and compromise the immune functions of the components, but the immunity is not strongly affected. One of the simplest ways to achieve such resilience is to acquire multiple backup mechanisms in the system: while one signaling mechanism could be shut down by a pathogen effector, a backup mechanism can take over the signaling task; consequently, the immune system does not suffer a strongly negative impact from the effector [7].

For example, we have demonstrated that at least three signaling mechanisms can back each other up in Arabidopsis Effector-Triggered Immunity (ETI) elicited by the *Pseudomonas syringae* effector AvrRpt2

(AvrRpt2-ETI) [8-10]. Simultaneous removal/inactivation of five major immune signaling sectors, the jasmonate (JA), ethylene (ET), PAD4, and salicylate (SA) sectors and ETI-mediating, PTI-inhibited sector (EMPIS), results in almost complete loss of AvrRpt2-ETI. From this state of almost no AvrRpt2-ETI, any one of the three mechanisms is sufficient to restore AvrRpt2-ETI: the PAD4 sector only; the JA and SA sectors together, and EMPIS only. None of the three mechanisms is necessary, and thus the mechanisms back up one another and conceal themselves from pathogens, i.e., the backup mechanisms conceal the evolutionary goals from pathogens [7].

Another example we found is that coevolution of homologous immune regulators can confer resilience to immunity [11]. In the CBP60 protein family, members of two subfamilies, the CBP60g and SARD1 subfamilies, are positive, partially functionally redundant immune regulators, while those of the third CBP60a subfamily are negative regulators [12-15]. We hypothesized two coevolutionary mechanisms: (a) the members of two positive regulator subfamilies in a single species are selected to be divergent from each other to avoid simultaneous targeting by a single pathogen effector (which ensures that a member in the other subfamily can back up the signaling function); and (b) the negative regulator subfamily member in the same species is selected to be similar to one of the positive regulators so that simultaneous targeting of positive and negative regulators by a pathogen effector moderates the negative impact of the effector on immunity. We detected coevolutionary signatures consistent with these hypotheses in each of 12 diverse eudicot linages we examined. Two positive regulators that cannot be targeted by a single pathogen effector (coevolutionary mechanism (a)) can conceal themselves from a pathogen as discussed in the previous paragraph. However, since the two positive regulators are only partially functionally redundant, the effect of concealing the evolutionary goals is partial as well - effector targeting of one of the positive regulators has some (but not a large) negative impact on immunity. Protection of one of the positive regulators by a negative regulator (coevolutionary mechanism (b)) can complement this partial weakness. Coevolutionary mechanism (b) can suddenly change which positive regulator is more susceptible to effector targeting when the identity of the positive regulator that is more similar to the negative regulator suddenly flips.

Concealing evolutionary goals for pathogens and changing them suddenly can confer resilience to core plant immunity. Resilient core immunity can buy time for plants to evolve specific solutions to particular pathogen effectors, such as evolution of specific R genes [7]. Thus, resilient immunity is likely a main reason flowering plants have been faring quite well in the evolutionary war against pathogens.

Evolution of multiple backup mechanisms appears impossible for plants

OK. Flowering plants are doing fine now, but how did they get here? How have flowering plants acquired such resilient immunity, or more specifically, backup mechanisms in the immune system? It is difficult to imagine that incremental adaptation could innovate multiple backup mechanisms: pathogen evolution would rapidly negate whatever single evolutionary innovation a plant acquired; in this way, plants would never be able to evolve a system that requires multiple innovations, i.e., multiple backup mechanisms, under pathogen pressure. Multiple backup mechanisms must have been invented almost simultaneously (in the evolutionary time scale).

In addition to components of the backup mechanisms, the parameter values that control the backup mechanisms, particularly those that control relationships among the backup mechanisms, also need to

be right. A parameter of a system describes how one component of the system interacts with another component or with itself (e.g., the self-decay rate). Generally, immunity is expensive energetically and resource-wise [16] and has a negative impact on plant growth when immunity is not needed, i.e., when plants are not attacked by pathogens [17]. For better fitness, immunity needs to be suppressed when it is not needed. On the other hand, when immunity is needed, it should be induced rapidly and sufficiently strongly (but not more strongly than needed). Thus, the multiple backup mechanisms needed to be sufficiently well-controlled at the moment of their invention. If the parameter values at the beginning were sufficiently good, incremental adaptation could optimize the parameter values.

We can describe acquisition of new mechanisms and different parameter values of an existing system in a consistent manner using a parameter space of the system. Acquisition of new mechanisms can be described as dimensions that previously had zero values (one newly acquired mechanism usually corresponds to more than one new non-zero dimensions). Thus, evolution of a system can be described as changes in the coordinates in a parameter space.

These discussions revealed a requirement for plants to acquire multiple backup mechanisms in the immune system: almost simultaneous changes in multiple dimensions of the parameter space. We need an evolutionary mechanism different from incremental adaptation to meet this requirement.

A multiverse hypothesis in cosmology

Since I will use an analogy from a multiverse hypothesis in cosmology, I will briefly explain the cosmological hypothesis and the concepts I borrow from it. There are about 20 fundamental physical constants that cannot be derived from the current physics theories, the standard model of particle physics and general relativity [18]. Instead, the values of the constants need to be measured and the theories can use these constant values to make many other precise predictions. It appears that the values of the physical constants are fine-tuned [19]. If the physical constant values were a little different, the universe would rapidly collapse or rapidly become very flat (no complexities) after its birth (i.e., a Big Bang). Since our universe had the physical constant values just right, it was able to have a long lifetime (about 14 billion years so far [20]) and to have evolved complexities, such as galaxies, stars, planets, and lives. One possibility is that the ultimate theory of everything would reveal that the physical constants must have these values – we just do not yet know such a theory. Another possibility is that we are merely agents in a computer simulation and the creator of the simulation determined the constant values of our (simulated) universe ("simulation hypothesis"; [21]). A third possibility is that we are just extremely lucky. A superstring theory predicts an extremely high, but finite, number of possible physical constant values [22], and a set of constant values for a universe is randomly chosen from the numerous possible sets during a Big Bang [23]. With an extremely low but non-zero probability, a universe could have the right physical constant values to be long-lasting with complexities. Another part of the third possibility is that numerous Big Bangs are happening all the time [24], i.e., numerous universes are being born all the time, hence the name of the multiverse hypothesis. When an extremely high number of trials occur, even an outcome with an extremely low probability should happen. For example, even if the probability that one particular outcome occurs is 10^{-10}, the probability this outcome never occurs after 10^{11} trials is pretty much 0 ($\approx e^{-10} < 0.0001$). Extremely lucky universes may have evolved intelligent lives that observe their own universes ("anthropic principle"), which has happened in our universe. The concepts I borrow from the multiverse hypothesis of cosmology are: (1) numerous trials, each of which

makes big, fundamental changes are happening all the time; (2) with numerous trials, an outcome with an extremely low probability happens; (3) only the very few successful outcomes are recognized retrospectively.

Whole genome duplication followed by extensive genome reorganization is a Big Bang of plant evolution

A Big Bang in plant evolution must be a massive genome reorganization event. It has been pointed out that a whole genome duplication (WGD) is often phylogenetically associated with the base of a major plant clade [25, 26]. For example, ζ-WGD and *ε*-WGD are shared by all seed plants and all flowering plants, respectively [27]. However, WGD alone is unlikely to lead to big evolutionary innovations. Natural and artificial homo-polyploidization (i.e., WGD) of flowering plants is generally not associated with large phenotypic changes (e.g., [28]) – taxonomists uninformed about homo-polyploidization would not classify plants with homo-polyploidy into taxonomic families different from those of the starting plants. The big innovation opportunities are in the following processes [29, 30]. Duplicated genes could undergo neofunctionalization. For example, innovation of major immune regulator subfamilies, CBP60g/SARD1 positive and CBP60a negative immune regulator subfamilies, occurred through a gene duplication event around the time of *ε*-WGD [11]. Similarly, a pivotal immune signaling mechanism comprised of the EDS1/PAD4/SAG101 protein family appears to have arisen around the time of ζ-WGD [31]. No protein molecules that have the overall structural organization of the EDS1/PAD4/SAG101 protein family, which contains both a lipase-like domain and an EP domain, have been observed in any organisms outside seed plants while essentially every seed plant has the EDS1/PAD4/SAG101 protein family. This innovation may have been possible when a lipase gene duplicated in ζ-WGD – a duplicated lipase gene might have been free to be fused to an EP domain gene. Thus, neofunctionalization could add new dimensions to the parameter space.

Duplicated genes could also undergo specialization. For example, a segmental genome duplication of the *isochorismate synthase* (*ICS*) gene around the time of α- or β-WGD resulted in two copies of the gene, *ICS1* and *ICS2* [32]. The duplication allowed the expression of *ICS1* to be highly specialized for an immune function as the ICS product isochorismate is a major precursor for salicylate synthesis in the plastid in Arabidopsis [33]. A low but constitutive level of *ICS2* expression in Arabidopsis maintains a minimal level of isochorismate production for phylloquinone biosynthesis, which is essential for Photosystem I [34]. Such specialization could drastically change some parameter values. In addition, deletion of one of the duplicated genes could also change such parameters. For example, in a simple case, the activity level of the gene product could be cut in half when one of the duplicated genes is deleted. It is easy to imagine much more complicated parameter value changes caused by deletion of one of the duplicated genes when two genes have been specialized. Thus, neofunctionalization, specialization, and deletion of the duplicated genes could have large impacts on the system parameter space.

There are many small-scale gene duplication events, such as tandem duplication of a single gene, which also allow neofunctionalization, specialization, and deletion of the duplicated genes. Then what is special about WGD compared to small scale gene duplication? It is that WGD provides innovation opportunities to many genes almost simultaneously. This allows system-level evolutionary innovation.

Outcomes of neofunctionalization, specialization, and deletion of multiple genes following WGD are affected by stochasticity (e.g., where in the genome mutations have occurred in what order?) and selection imposed by pathogens and other environmental factors (including drastic ones that led to a mass extinction). As pathogen selection could change drastically in a short time, such as by invasion of completely new pathogens into an area, every WGD followed by large genome reorganization would broadly survey a massive parameter space. Parameter subspaces that can represent resilient immune systems could be very small within a massive parameter space. This is why the probability of acquiring successful parameter sets for resilient immunity is extremely small.

A multiverse hypothesis of evolution of resilient plant immunity

I have argued above that massive genome reorganization by WGD and subsequent neofunctionalization, specialization, and deletion have a chance to evolve a resilient immune system, but with an extremely low probability. To realistically observe an outcome with an extremely low probability, the number of trials must be extremely high. In our case, this translates to the idea that the frequency of WGD events must be very high. One thing we should remember is that evolution of a very new resilient immune system does not need to happen very often. For example, major evolutionary innovations for plant immunity are associated with ζ-WGD and *ε*-WGD, i.e., emergence of seed plants and flowering plants, respectively. We are talking about hundreds of millions of years here.

Let's try a thought experiment. Assume the following: on average 1 in 10,000 seeds of a single Arabidopsis individual had WGD; an Arabidopsis individual set 10,000 seeds once a year; and there are 10 million individuals in the world all the time. Then, in 100 million years, 10^{15} WGD events happen. And we are only considering a single plant species. WGD events in any extant land plant species could lead to a new land plant clade with a more resilient immune system. Thus, with this time scale and number of plants, the frequency of WGD per plant does not have to be very high to achieve an extremely high number of trials.

In summary, a multiverse hypothesis of evolution of resilient plant immunity consists of three parts: (1) the frequency of WGD is sufficiently high to have an extremely high number of trials in hundreds of millions of years; (2) with an extremely low probability, an WGD event followed by neofunctionalization, specialization, and deletion can evolve a new resilient immune system; (3) Only an extremely small number of cases resulted in species with highly resilient immune systems. Retrospectively, these species are recognized as the most recent common ancestors (MRCAs) of highly divergent clades of successful modern plant species.

Can we test the multiverse hypothesis of resilient plant immunity evolution?

One major criticism of the multiverse hypothesis of cosmology is that the hypothesis is fundamentally untestable as we cannot observe outside of our own universe [35]. Is the multiverse hypothesis of resilient plant immunity evolution testable? Yes and no. It is not possible to test a specific case, such as whether the acquisition of highly resilient immunity that occurred in the MRCA of flowering plants resulted from WGD followed by neofunctionalization, specialization, and deletion. This is because we cannot obtain information regarding the genome organization and the level of immune resilience right

before and right after the acquisition event. The acquisition event including neofunctionalization, specialization, and deletion following WGD may span many generations although it may appear to be a very short time span when compared to the evolutionary time scale of flowering plants. However, it is possible to collect data that support the probabilistic argument in the hypothesis. We could survey the frequency of WGD in several diverse plant species. Such a survey is feasible with current technologies. The estimated trial number per year across multiple species will give a ballpark idea of the order of magnitude of the probability necessary to acquire resilient immunity in hundreds of millions of years.

Did acquisition of highly resilient immunity help radiation of flowering plants?

Flowering plants are the dominant form among land plants these days [36, 37]. Is it likely that acquisition of resilient immunity helped flowering plants radiate to the current level? Flowering plants have characteristic traits that separate them from other groups of land plants: (i) many of them have intimate interactions with highly mobile animals, e.g., flying insects, for pollination and seed dispersion; (ii) plants that rapidly spread into new environments are often flowering plants. Both these characteristics of flowering plants tend to increase the possibility of encountering new pathogens. In such cases, plants would not have evolved specific solutions to new pathogens as the plant species would never have encountered the pathogens. It is conceivable that acquisition of resilient core immunity may have allowed flowering plants to evolve these characteristic traits.

Concluding remarks

How can a system that appears to require the values of many of its parameters to fall into the right values almost simultaneously evolve in nature? The resilient plant immune system is one such system. I propose a multiverse hypothesis that assumes occurrence of an extremely low probability outcome of sufficiently good multiple parameters through an extremely high number of trials resulting from a very large number of individuals and a very long time-scale. With numerous massive genome reorganizations via WGD followed by neofunctionalization, specialization, and deletion, the extremely low probability event of acquisition of highly resilient immunity may have occurred.

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REFERENCES

[1] M. Kimura, Evolutionary rate at the molecular level, Nature 217(5129) (1968) 624-6.
[2] K.M. Peck, A.S. Lauring, Complexities of Viral Mutation Rates, J Virol 92(14) (2018).

[3] A. Chevallereau, S. Meaden, S. van Houte, E.R. Westra, C. Rollie, The effect of bacterial mutation rate on the evolution of CRISPR-Cas adaptive immunity, Philos Trans R Soc Lond B Biol Sci 374(1772) (2019) 20180094.
[4] S. Ossowski, K. Schneeberger, J.I. Lucas-Lledó, N. Warthmann, R.M. Clark, R.G. Shaw, D. Weigel, M. Lynch, The rate and molecular spectrum of spontaneous mutations in Arabidopsis thaliana, Science 327(5961) (2010) 92-4.
[5] H. Ochman, E. Lerat, V. Daubin, Examining bacterial species under the specter of gene transfer and exchange, Proc Natl Acad Sci U S A 102 Suppl 1(Suppl 1) (2005) 6595-9.
[6] V. Müller, R.J. de Boer, S. Bonhoeffer, E. Szathmáry, An evolutionary perspective on the systems of adaptive immunity, Biol Rev Camb Philos Soc 93(1) (2018) 505-528.
[7] F. Katagiri, Review: Plant immune signaling from a network perspective, Plant Sci 276 (2018) 14-21.
[8] K. Tsuda, M. Sato, T. Stoddard, J. Glazebrook, F. Katagiri, Network properties of robust immunity in plants, PLoS Genet 5(12) (2009) e1000772.
[9] N. Hatsugai, D. Igarashi, K. Mase, Y. Lu, Y. Tsuda, S. Chakravarthy, H.L. Wei, J.W. Foley, A. Collmer, J. Glazebrook, F. Katagiri, A plant effector-triggered immunity signaling sector is inhibited by pattern-triggered immunity, EMBO J 36(18) (2017) 2758-2769.
[10] F. Katagiri, An averaging model for analysis and interpretations of high-order genetic interactions, bioRxiv (2022) https://doi.org/10.1101/2022.02.21.481332.
[11] Q. Zheng, K. Majsec, F. Katagiri, Pathogen-driven coevolution across the CBP60 plant immune regulator subfamilies confers resilience on the regulator module, New Phytol 233(1) (2022) 479-495.
[12] L. Wang, K. Tsuda, M. Sato, J.D. Cohen, F. Katagiri, J. Glazebrook, Arabidopsis CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against Pseudomonas syringae, PLoS Pathog 5(2) (2009) e1000301.
[13] L. Wang, K. Tsuda, W. Truman, M. Sato, l.V. Nguyen, F. Katagiri, J. Glazebrook, CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling, Plant J 67(6) (2011) 1029-41.
[14] Y. Zhang, S. Xu, P. Ding, D. Wang, Y.T. Cheng, J. He, M. Gao, F. Xu, Y. Li, Z. Zhu, X. Li, Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors, Proc Natl Acad Sci U S A 107(42) (2010) 18220-5.
[15] W. Truman, S. Sreekanta, Y. Lu, G. Bethke, K. Tsuda, F. Katagiri, J. Glazebrook, The CALMODULIN-BINDING PROTEIN60 Family Includes Both Negative and Positive Regulators of Plant Immunity, Plant Physiology 163(4) (2013) 1741-1751.
[16] M.D. Bolton, Primary Metabolism and Plant Defense-Fuel for the Fire, Molecular Plant-Microbe Interactions 22(5) (2009) 487-497.
[17] A.J. Heidel, J.D. Clarke, J. Antonovics, X. Dong, Fitness costs of mutations affecting the systemic acquired resistance pathway in Arabidopsis thaliana, Genetics 168(4) (2004) 2197-206.
[18] J.P. Uzan, Varying Constants, Gravitation and Cosmology, Living Rev Relativ 14(1) (2011) 2.
[19] Eco-Phenomenology: Life, Human Life, Post-Human Life in the Harmony of the Cosmos, 2018.
[20] P. Collaboration, Planck 2018 results. VI. Cosmological parameters, A*stronomy & Astrophysics* 641 (2020) A6.
[21] N. Bostrom, Are You Living in a Computer Simulation?, Philosophical Quarterly 53(211) (2003) 243-255.
[22] J. Read, B. Le Bihan, The landscape and the multiverse: What's the problem?, Synthese 199 (2021) 7749-7771.
[23] M. Tegmark, Parallel universes, Scientific American 288(5) (2003) 40-51.
[24] A.D. Linde, Eternally Existing Self-Reproducing Chaotic Inflation Universe, Physics Letters B. 175(4) (1986) 395-400.
[25] P.S. Soltis, D.E. Soltis, Ancient WGD events as drivers of key innovations in angiosperms, Curr Opin Plant Biol 30 (2016) 159-65.

[26] J.W. Clark, P.C.J. Donoghue, Whole-Genome Duplication and Plant Macroevolution, Trends Plant Sci 23(10) (2018) 933-945.
[27] J.W. Clark, P.C.J. Donoghue, Constraining the timing of whole genome duplication in plant evolutionary history, Proc Biol Sci 284(1858) (2017).
[28] H. Tsukaya, Does ploidy level directly control cell size? Counterevidence from Arabidopsis genetics, PLoS One 8(12) (2013) e83729.
[29] G.C. Conant, J.A. Birchler, J.C. Pires, Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time, Curr Opin Plant Biol 19 (2014) 91-8.
[30] A.I. Teufel, L. Liu, D.A. Liberles, Models for gene duplication when dosage balance works as a transition state to subsequent neo-or sub-functionalization, BMC Evol Biol 16 (2016) 45.
[31] D. Lapin, V. Kovacova, X. Sun, J.A. Dongus, D. Bhandari, P. von Born, J. Bautor, N. Guarneri, J. Rzemieniewski, J. Stuttmann, A. Beyer, J.E. Parker, A Coevolved EDS1-SAG101-NRG1 Module Mediates Cell Death Signaling by TIR-Domain Immune Receptors, Plant Cell 31(10) (2019) 2430-2455.
[32] Y. Yuan, J.D. Chung, X. Fu, V.E. Johnson, P. Ranjan, S.L. Booth, S.A. Harding, C.J. Tsai, Alternative splicing and gene duplication differentially shaped the regulation of isochorismate synthase in Populus and Arabidopsis, Proc Natl Acad Sci U S A 106(51) (2009) 22020-5.
[33] M.C. Wildermuth, J. Dewdney, G. Wu, F.M. Ausubel, Isochorismate synthase is required to synthesize salicylic acid for plant defence, Nature 414(6863) (2001) 562-565.
[34] C. Garcion, A. Lohmann, E. Lamodière, J. Catinot, A. Buchala, P. Doermann, J.P. Métraux, Characterization and biological function of the ISOCHORISMATE SYNTHASE2 gene of Arabidopsis, Plant Physiol 147(3) (2008) 1279-87.
[35] P. Davies, The Goldilocks Enigma: Why is the Universe Just Right for Life?, Mariner Books2008.
[36] P.R. Crane, E.M. Friis, K.R. Pedersen, The origin and early diversification of angiosperms, Nature 374 (1995) 27-33.
[37] M.J.M. Christenhusz, J.W. Byng, The number of known plants species in the world and its annual increase, Phytotaxa 261(3) (2016) 201-217.