Metapopulation dynamics and the influence of the killer system in a natural S. cerevisiae population

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Metapopulation dynamics and the influence of the killer system in a natural *S. cerevisiae* population

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Abstract

Populations and their genetic makeup are ever changing. Metapopulations of interconnected small populations, observed over many years, can give us an insight into large-scale shifts in their genetic composition. Here we use a natural metapopulation of *Saccharomyces cerevisiae* to explore changes in its genetic composition over time. We further examine a possible cause for genetic shifts using the killer-yeast system. We found a large turnover of genotypes over the course of four years with initially few dominant genotypes being replaced by a greater diversity over time. An analysis of dsRNA viruses encoding the killer-yeast genes showed strong changes over time and space. We show partial success with the standard methods to examine the killer-yeast system but also reveal the limits of the current killer assay method when applied on a larger assemblage of natural populations.

Introduction

Ecological communities were once thought to be static – with nature being in a stable equilibrium state. We have since learned that nature is an ever-changing system, with species locally disappearing, reappearing, and colonising new habitats (Urry et al., 2021). This concept can also be applied to populations of a single species (metapopulation) and the multitude of genotypes contained within. Changes in the biotic and abiotic components of an environment influence the relative fitness of co-occurring genotypes, leading to changes in their relative frequencies over time. We can study metapopulations to get a sense of the overall shift in genotypes and genotype diversity. If we look at the diversity at each site (alpha diversity) and relate it to the metapopulation wide diversity (gamma diversity), we get a new measurement called beta diversity (Whittaker, 1960). Changes in beta diversity over time can inform us on the direction the relative frequencies of genotypes shift.

A useful model organism to observe changes in population composition over time is *Saccharomyces cerevisiae*. With possible clonal reproduction, established genetic tools, and visually distinguishable phenotypes through the killer yeast phenomenon, it posesses many usefull traits for laboratory experiments. In the 1960s, scientists discovered that some strains of S. cerevisiae were able to kill other, sensitive, strains by secreting toxins into their shared environment (Bevan & Makower, 1963; Makower & Bevan, 1963; Woods & Bevan, 1968). The genetic information for toxin production and self-immunity are encoded on double-stranded RNA (dsRNA) viruses, fittingly named “killer” or “M”-viruses. These M-viruses lack the ability to replicate themselves and are therefore dependent on the presence of a second dsRNA virus, the so called “L-A helper” virus. The L-A helper virus, itself autonomous, provides the genes for the RNA polymerase and capsid proteins (Boynton, 2019), necessary for replication and maintenance of both viruses. Both viruses persist in the host cell’s cytoplasm in virus-like particles without causing cell lysis (Chang et al., 2015). They are cytoplasmically inherited and are not known to possess an extracellular state (Schmitt & Breinig, 2006). Both the killer toxins and resistance to them can also be chromosomally encoded (Wickner, 1974). Any natural *S. cerevisiae* population can consist of genotypes that possess killer phenotypes and those that do not, creating a network of killing and resistance interactions. To assess these interactions between genotypes, a method called “killer assay” can be used. In this assay, different genotypes are grown together and the killing, if occurring, is made visible through cell staining. As of yet, it is unknown how much the killer yeast phenomenon contributes to the population structure of natural *S. cerevisiae* populations. Linking genetic data and killer phenotype data could shed light on this.

Here, I make use of a dataset - provided by Dr. Sarah Knight of the University of Auckland - of *S. cerevisiae* strains from Sauvignon Blanc vineyards across New Zealand (*figure 1*), to investigate the population dynamics and their possible causes in a natural system. For this we first examined whether there are indeed shifts in genotype diversity over multiple years and geographical regions. Then we assessed the killer phenotypes of the different genotypes to determine whether killer activity is driving the change in genotype diversity.

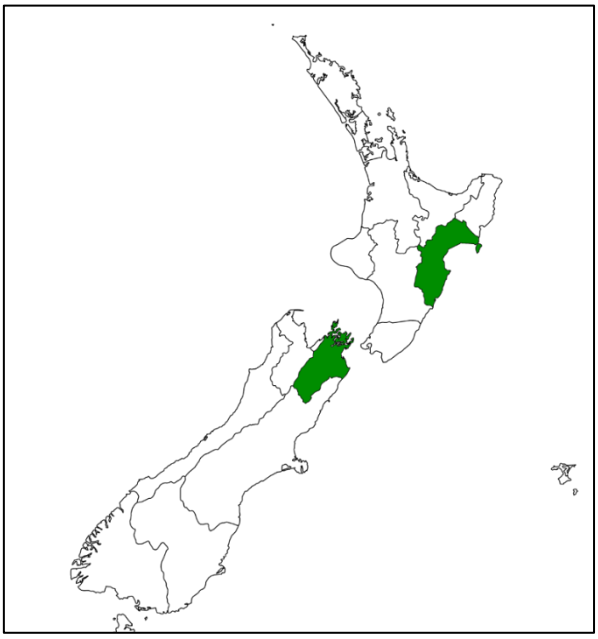

Figure 1 | Sample regions of Hawke's Bay (north) and Marlborough (south) in New Zealand

Methods

The dataset consists of 536 *S. cerevisiae* strains collected from Hawkes Bay and Marlborough in New Zealand in the years 2018, 2019 and 2021. 2020 was not sampled due to the COVID pandemic. Dr. Sarah Knight and colleagues had previously barcoded the yeast with nine microsatellites (single sequence repeats) (Richards et al., 2009).

Samples were clustered into multi-locus genotypes (MLGs) based on microsatellite data. Genetic data was analysed using "poppr" (Kamvar et al., 2015) to form MLGs. Due to some samples not yet being analysed and poor reading quality in others, the microsatellite data was reduced to three loci excluding missing data and poor-quality assignments. Samples were clustered into MLGs if they shared 100% similarity. This resulted in 50 MLGs.

From this data we determined the most common MLGs (>40 occurrences). MLG distribution over time was assessed to explore whether there were appearances or disappearances of genotypes from sites that might be explained by killer activity. Due to the discovery of dominant genotypes appearing and disappearing, we chose to see whether these strains were or were not killers and whether their presence was dictated by their ability to kill or resist other strains found in their environment at that time. As these overall dominant MLGs also dominate their individual samples, we chose to take only one individual of that MLG per sample.

As killing is often a product of endosymbiont-encoded toxins, strains were assessed for dsRNA viruses using an adapted method of Okada et al. (2015) and Fredericks et al. (2021). Gel electrophoresis was used to separate the L-A helper virus, at ~4000kb and the M-virus at ~1500kb. Strains were categorised according to the number of visible bands at those locations: 0 (no viruses), 1 (L-A helper virus only), and 2 (L-A helper virus and M-satellite virus).

To assess the realised killing potential of each strain, a reciprocal all-against-all killer assay was conducted, which resulted in 129x129 (16'641) interactions. The killer assay included both the natural strains and some laboratory strains as controls.

S. cerevisiae strains were grown over night in 5mL standard YPD (10g/L yeast extract, 20g/L casein peptone, 20g/L glucose) in Erlenmeyer flasks on a shaker deck at 30°C and 120 rpm. The samples were diluted with standard YPD to an OD of 0.6. Three methylene blue 4% (MB) YPD-agar (pH 4.5) petri dishes per strain were seeded under a flame with 600µL of the diluted yeast each. The liquid was distributed with a glass cell spreader. The spreader was cleaned with water and ethanol, which was burned off, after each strain. The petri dishes were then left to dry for 2.5 hours in a sterile hood.

10µL of the test and control strains were grown in monoculture in 200µL of standard YPD in wells of 96-well plates. The strains were incubated for three days at 25°C and thereafter stored at 4°C. These plates provided the basis from which strains could be inoculated.

Six 96-well plates (base plates) were filled with 150µL YPD-agar (2%, buffered to pH 4.5 with 0.3M citric acid) and 50µL standard YPD. The combination of liquid and solid YPD was chosen due to the greater effectiveness of transferring semi-solids with the stamp than either liquid or solid. After cooling, 20µL of each strain was added from the liquid plates. The plates were kept at 25°C and topped up with 10µL from the liquid plates after each day of use.

The top strains were transferred to the background dishes with a 48-pin metal stamp under a flame. The stamp was cleaned with a 10% bleach solution and then dried by pressing on kitchen paper. This process was then repeated with water. Finally, the stamp was dipped in ethanol which was then burned off. After cooling, the stamp was inserted into one of the three segments of the base plates (Plate A, column 1-6, A7-12 & B1-6) under its own weight for about two seconds. The stamp was then put on the background dish under its own weight for

about two seconds. This resulted in three background dishes per strain, one for each segment of the base plates (*appendix figure 3*). Each dish was labelled with the background strain and the base plate segment. The dishes were then stored in an incubator at 25°C for 5 days.

Photographs were taken after three and five days. Photos were taken with the main sensor of an iPhone 12 mini from a distance of 14.5cm. The dishes were backlit during the process. After day three, the plates were returned to the 25°C incubation chamber for another two days. Photos were taken at the same time each day to ensure consistency.

Statistical analysis was done in R (R Core Team, 2022) v.4.2.1. Multi-locus genotype data was converted into a binary presence/absence matrix for all years and regions. This was used to create a dissimilarity matrix accounting for spatial turnover (replacement), measured as Simpson pair-wise dissimilarity. A permutational multivariate analysis of variance (PERMANOVA) was then used to test the influence of the year and region on the dissimilarity between region-year pairs. The analysis of beta diversity was done with “betapart” (Baselga et al., 2022) and the PERMANOVA with “vegan”(Oksanen et al., 2022). Figure 2 was created using “ggplot2” (Wickham, 2016).

Results

The analysis of beta diversity showed that beta diversity was significantly influenced by years (PERMANOVA: $F_{1,29} = 15.44$, $p < 0.001$) and to a lesser extent by regions ($F_{1,29} = 3.73$, $p < 0.05$). (table 1).

Table 1 | PERMANOVA results: Permutational Multivariate Analysis of Variance Using Distance Matrices "adonis2" from "vegan". Year and region show a significant effect on beta diversity, with the year effect being ~5 times stronger. Vineyards were used as strata. Distance matrices were created with “betapair”.

	Df	Sum Sq	F	P-Value
year	1	2.4101	15.4445	0.001 ***
region	1	0.5824	3.7324	0.014 *
year : region	1	0.0825	0.5286	0.696
Residual	29	4.524	-	-

In 2018, MLG diversity is dominated by MLG 5 and MLG 49, accounting for 81.4% of samples. By 2019, MLG 5 has almost completely disappeared, to the benefit of previously less common MLGs. In 2021, MLG diversity is no longer dominated by one or two MLGs but distributed more evenly between many common MLGs (*figure 2*).

An analysis of dsRNA viruses showed that overall, 32% of samples (n= 253) had neither the L-A helper nor the M-virus, 43% only the L-A helper virus, and 25% both viruses. However, these percentages varied significantly between years and regions (*figure 3*).

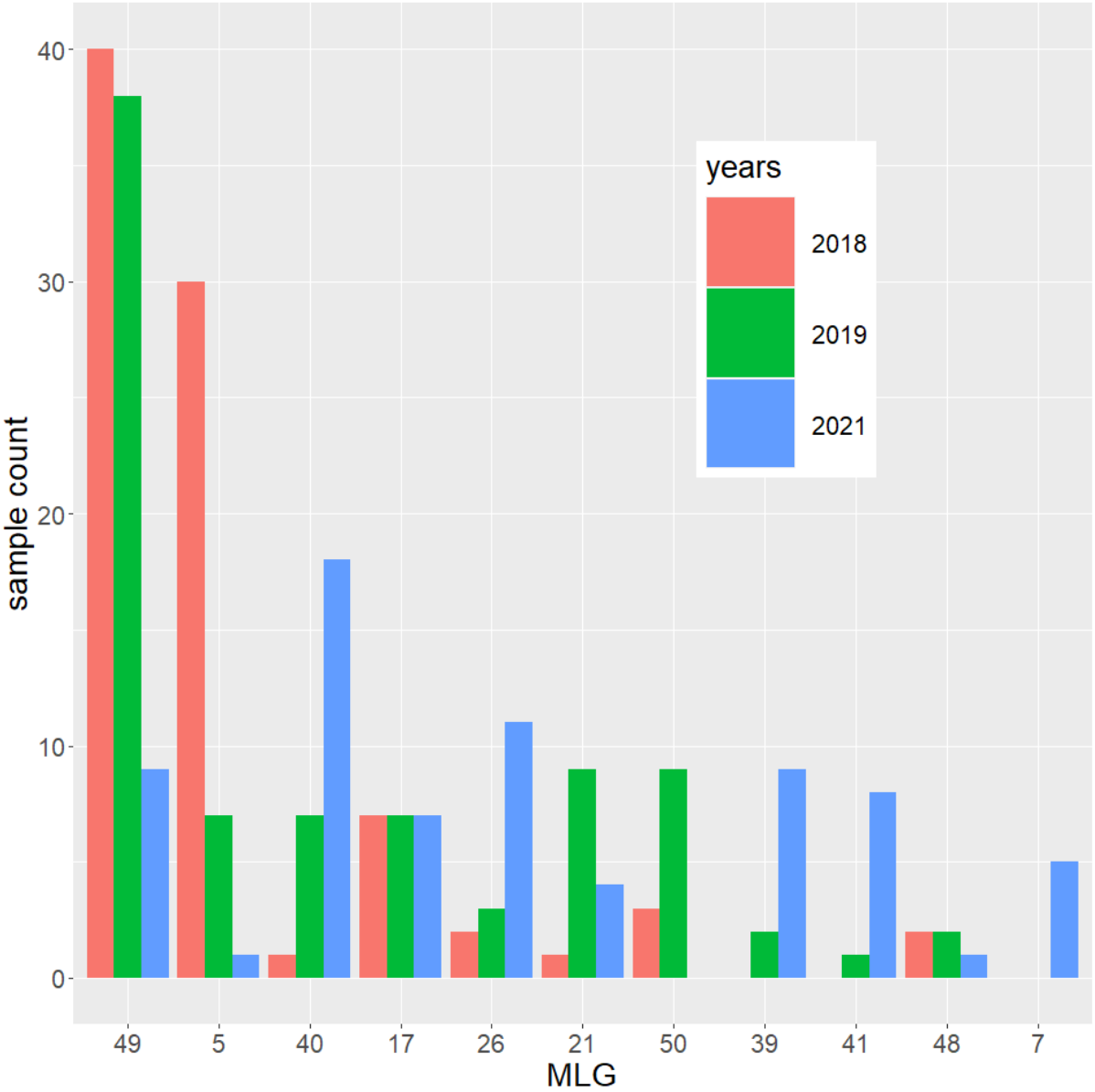

Figure 2 | Common MLGs (>= 5 samples) over both regions throughout the years, ordered by total sample count.

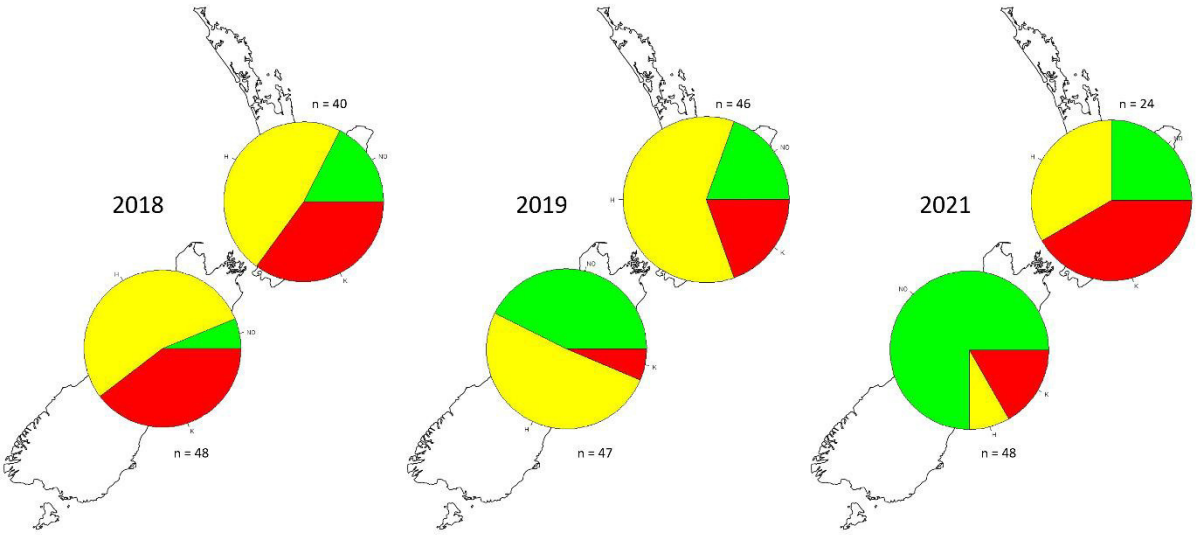

Figure 3 | Killer virus prevalence over regions and years. green = no dsRNA viruses (NO) | yellow = L-A helper virus only (H) | red = L-A & M-viruses (K, killer)

The quality of the killer assays was overall lacklustre. While some killer interactions were easily identifiable as such, many other interactions showed a mix of killing aspects and non-killing aspects (*appendix figure 4*). Using a laboratory sensitive strain (5X47), the killing capacity of three MLGs could be confirmed. Four MLGs that tested positive for killer virus dsRNA did not show any killing activity. One MLG (MLG 14) that tested positive for only the L-A helper virus showed killing activity (*figure 4*).

MLG 3	MLG 4	MLG 5	MLG 7	MLG 26	MLG 43	MLG 45	MLG 14
K	K	K	K	K	K	K	H

Figure 4 | MLGs with killer virus dsRNA found (K) and MLG14 (L-A helper virus only, H), stamped on sensitive lab strain 5X47.

Discussion

Examining the beta diversity of the *S. cerevisiae* populations showed that there was a significant shift in the genetic makeup of these populations over the years. The most dominant MLGs decreased in frequency while other genotypes became more common. The overall diversity increased. These findings answer our first question, showing that there are major shifts in genotype composition across space and time. Future measurements will show, if the new, increased diversity will prevail, or if new, dominant MLGs will emerge.

The strong fluctuations in the dsRNA virus percentages are directly linked to the change in MLGs. MLG 5, which tested positive for both viruses in all samples seems to have disappeared between 2018 and 2019 which corresponds to a sharp drop in the frequency of killer phenotype. The collapse of MLG 49, which tested positive only for the L-A helper virus, between 2019 and 2021 accounts for the strong relative decline in the share of its category. These changes are highly region specific. Hawke's Bay 2018 and 2021 look almost identical, while the composition at Marlborough changes completely.

The most likely explanation for the disappearance of the dominant killers is the evolution of resistance in the other genotypes. In Hawke's Bay, killers are first replaced by L-A virus positive genotypes before their relative share increases again through new killer genotypes. As the dataset of 2021 is only half the size of the other years, this bounce back could just be a random statistical event. In Marlborough, killer genotypes are predominantly replaced by virus-free genotypes between 2018 and 2019. Between 2019 and 2021, L-A positive genotypes get replaced by virus-free genotypes. This could have two causes: Either the cost of infection of the L-A positive genotypes was enough to give an advantage to virus-free genotypes, allowing them to outcompete their infected counterparts, or there was an increased loss of viral infection due to these evolutionary pressures. Our data shows almost no genotypes that have samples with multiple infection statuses (*appendix figure 2*), indicating a greater likeliness for the first explanation. However, since the MLGs are a very condensed form of the raw genetic data, they cannot capture if MLG A in an early year has evolved into MLG B in a later year, meaning viral loss could have co-occurred with genetic changes, without being captured by our data. dsRNA data is also only partially reliable for killer phenotypes, since the M-virus genes can become chromosomally encoded, allowing both L-A only and virus negative genotypes to express a killer phenotype (Meinhardt & Klassen, 2009).

A possible - though entirely speculative - explanation for the regional variation could be the COVID pandemic, specifically the lockdowns it caused. There are different dispersal methods for *S. cerevisiae* between vineyards, one of which is human traffic and exchange of tools

(barrels) between vineyards (Goddard et al., 2010). Due to the COVID lockdowns in NZ, genetic exchange between the regions might have been impeded, thus leading to significantly different developments. It is of note however, that a smaller difference between regions already started to develop in 2019, before any lockdowns.

Overall, our genetic findings fit well into our current understanding of ecological systems being highly dynamic. However, the genetic data as it exists now cannot fully explain these dynamics. A complete sequencing of the genomes could show possible incorporation of M-virus' genetic data into the chromosomal DNA of the genotypes and help trace the evolutionary lines of the MLGs.

The overall killer assay quality did not meet expectations, with interactions being very varied in their colour, shape, and size. This made the interpretation of the results extremely difficult. While some interactions showed the expected blue halo or nothing, many displayed varying degrees between these two states. A trial with ten petri dishes evaluated by three different people showed a mere 38% agreement rate for killer interactions. With sensitive lab strain 5X47 we were however able to show the killer system of some MLGs, showing that the system could be a factor in the genetic structure of our metapopulation. It is unclear why one MLG with only the L-A helper virus and no M virus showed clear signs of a killer. Possible causes are a wrong readout of the dsRNA analysis or potentially chromosomal encoding of the killer genes.

While killer assays have been in use since the 60s (Woods & Bevan, 1968), it has only been used on small samples, allowing for liquid transposition by hand. At the interaction count of this study, this would take weeks to months - not to mention the thousands of plastic pipette tips - hence the stamp method. There are multiple factors that can, and need to be, fine-tuned for future experiments, such as incubation temperature, media pH and initial cell concentrations on the plates, or a different transfer method all together. If readability can be greatly improved, automatic readouts might also be possible in the future.

The collapse of metapopulation dominating genotypes and development to a more diverse genetic population strongly indicates a direct, causal relation between the presence and absence of genotypes in one year compared to the next. A better working killer assay methodology will allow us to assess the influence of the killer yeast interactions on this dynamic in possible future projects.

In conclusion, the *S. cerevisiae* populations from New Zealand proved to be as dynamic as expected. The severe drop in abundance of the two most common, community shaping MLGs hints at some significant changes in the system. Determining whether the killing phenotypes present were (one of) the driving force(s) of that change remains a challenge for future projects and will ultimately require the development of a new large-scale killer assay methodology.

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Figures

Figure 1: https://vemaps.com/new-zealand/nz-01, last visited 12.09.2022 (attribution license)

Figure 3: https://vemaps.com/new-zealand/nz-02, last visited 12.09.2022 (attribution license)

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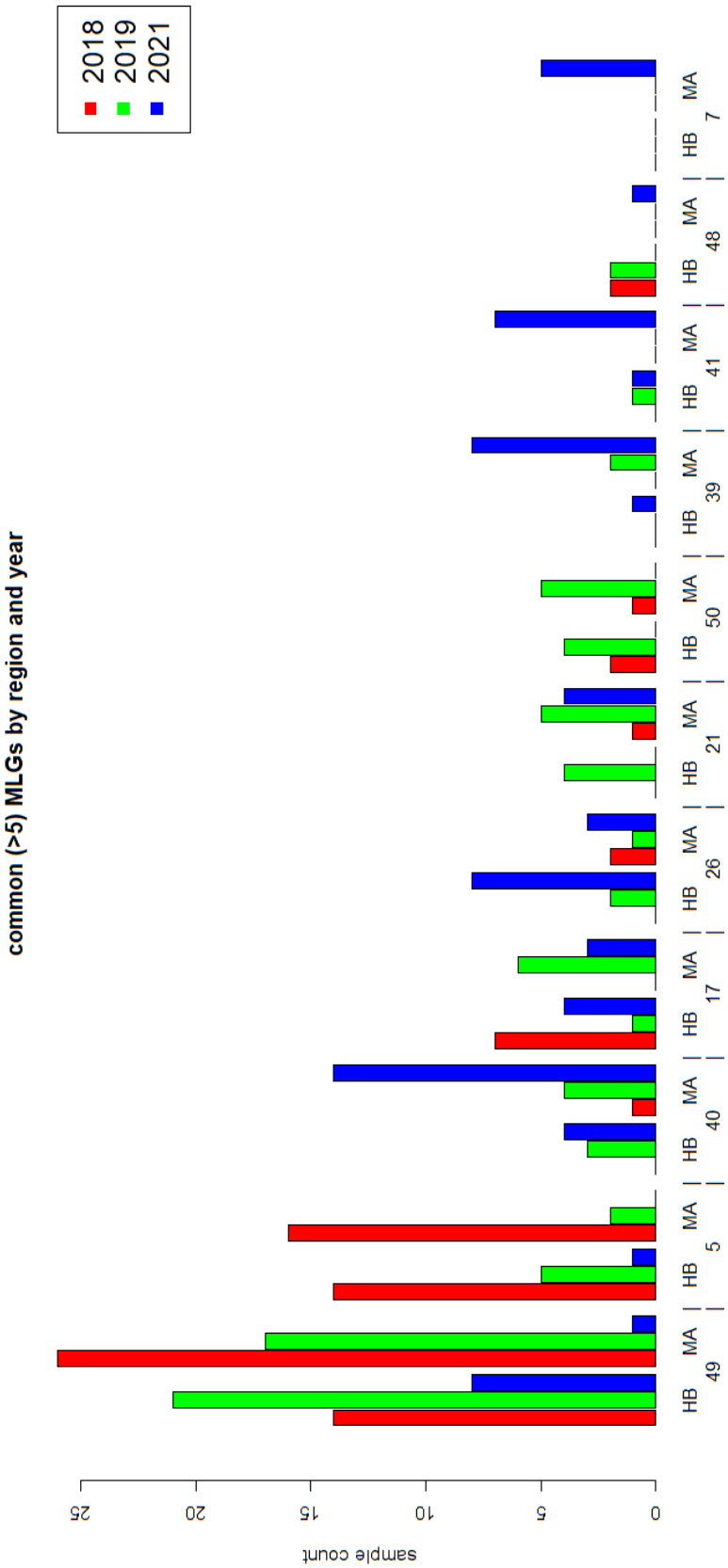
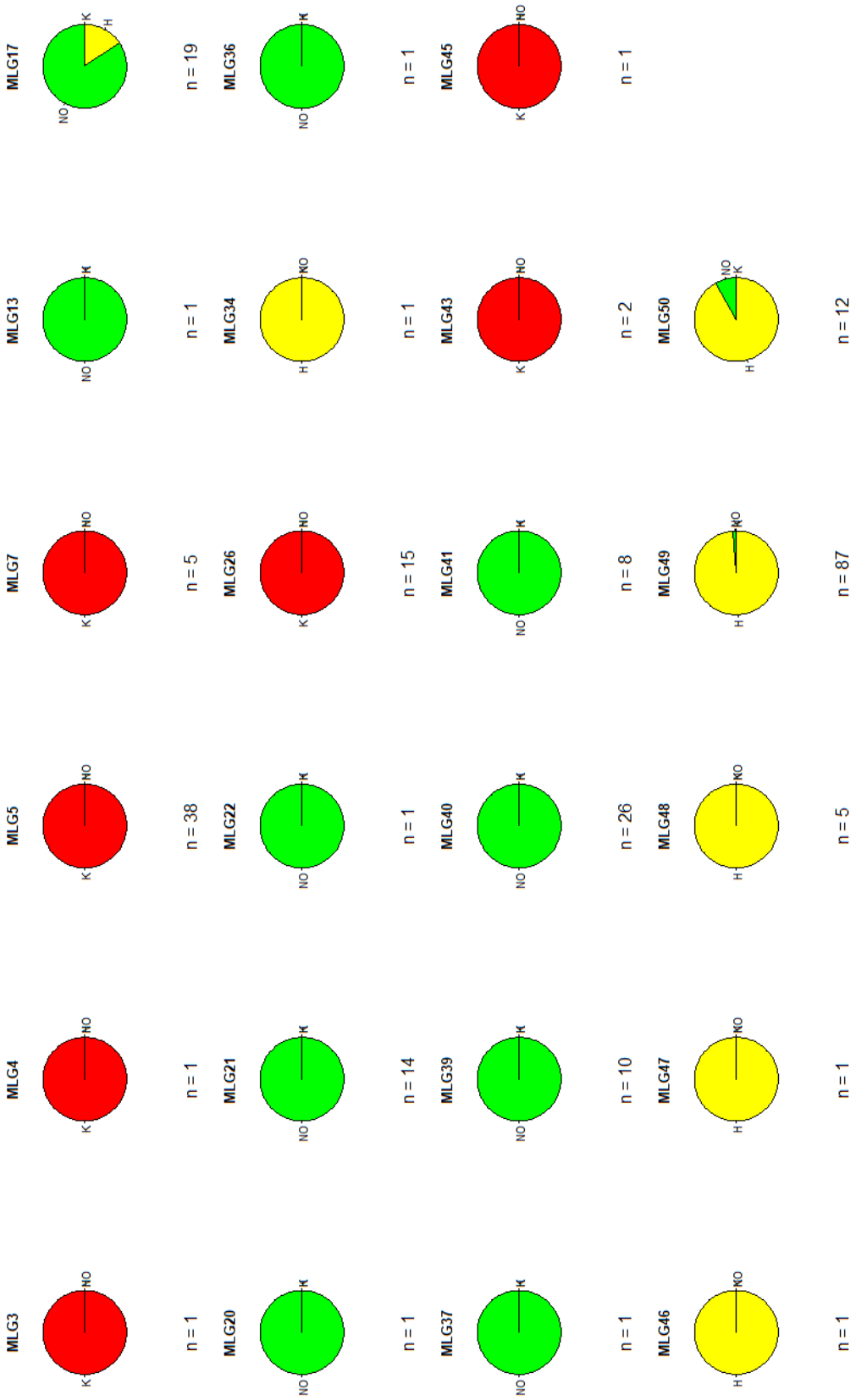
Cornelia Schneider and **Rebecca Oester** for creating that chance

Appendix



Appendix Figure 1 | MLG sample count for common MLGs (>5 samples) by both years (colour) and region (x-axis)



Appendix Figure 2 | dsRNA results of all MLGs found. green = no dsRNA viruses (NO) | yellow = L-A helper virus only (H) | red = LA & M-viruses (K, killer)

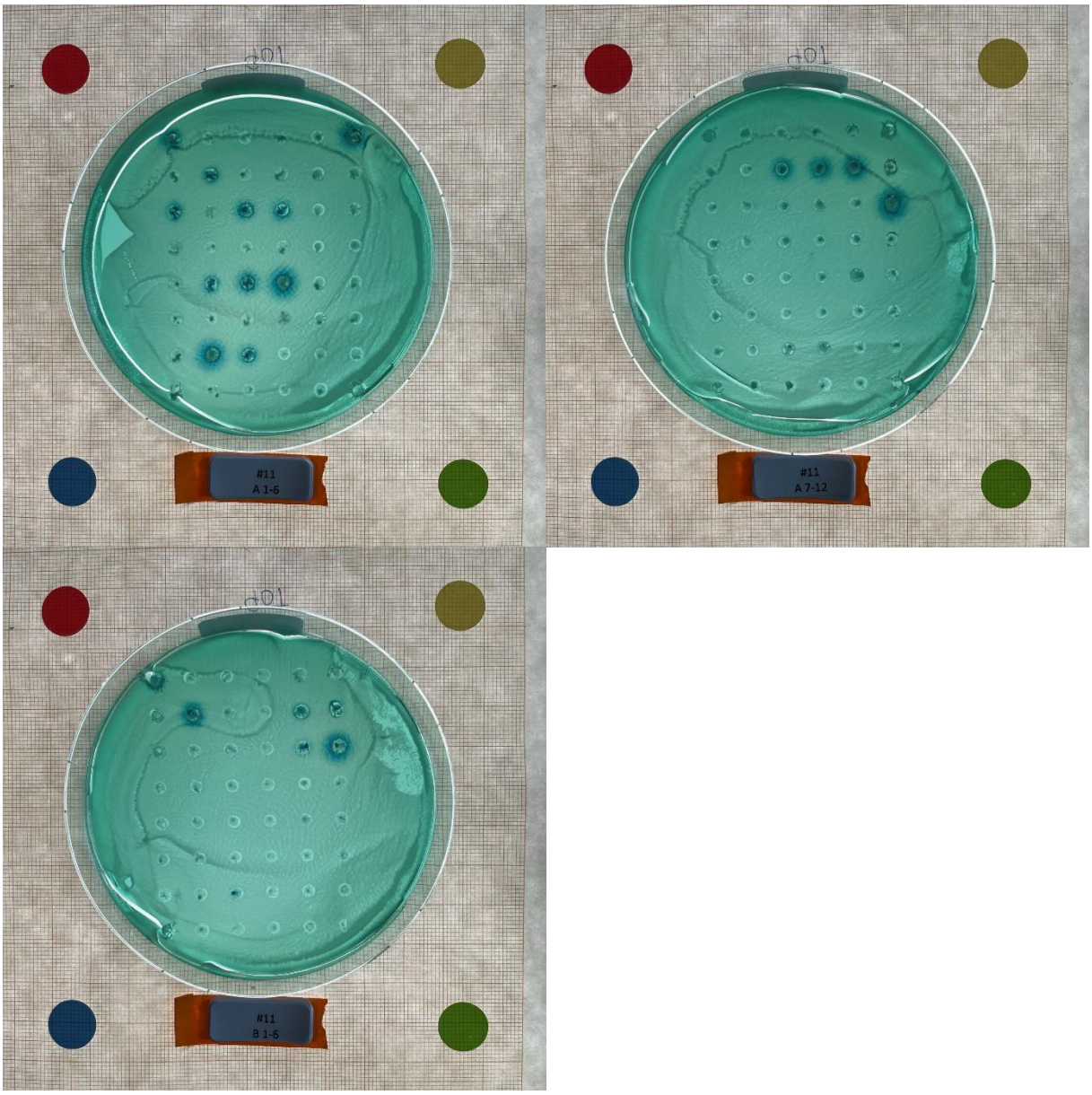


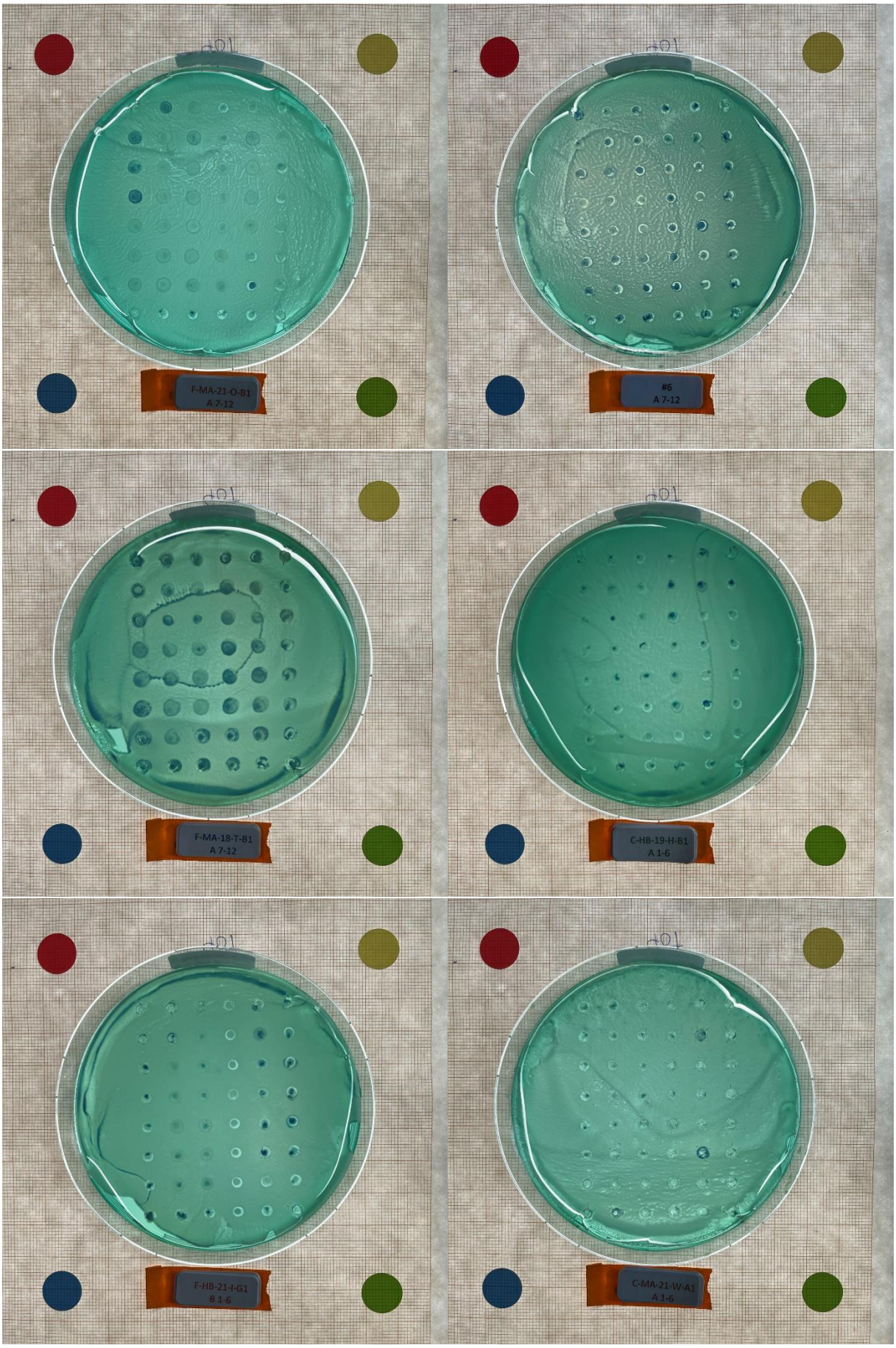
Plate A

	1	2	3	4	5	6	7	8	9	10	11	12
A	MLG5	MLG49	MLG40	MLG50	MLG21	MIG5	MLG17	MLG26	MLG40	MLG49	MLG49	MLG45
B	MLG49	MLG5	MLG49	MLG21	MLG26	MLG17	MLG26	MLG39	MLG7	MLG5	MLG5	MLG49
C	MLG5	MLG17	MLG5	MLG3	MLG39	MLG26	MLG43	MLG40	MLG17	MLG43	MLG50	MLG5
D	MLG17	MLG49	MLG49	MLG49	MLG41	MLG39	MLG26	MLG41	MLG20	MLG48	44 or 31	MLG50
E	MLG47	MLG5	MLG5	MLG5	MLG49	MLG41	MLG37	MLG39	MLG39	kont	31 or 44	MLG40
F	MLG49	MLG50	MLG49	empty	MLG26	MLG45	MLG39	MLG40	MLG40	MLG50	MLG21	MLG41
G	MLG50	MLG5	MLG5	empty	MLG4	27 or 15	MLG40	MLG41	MLG49	MLG17	MLG26	MLG46
H	MLG48	MLG26	MLG49	MLG49	MLG40	27 or 15	MLG41	MLG48	MLG49	MLG21	MLG49	MLG49

Plate B

	1	2	3	4	5	6	7	8	9	10	11	12
A	MLG5	MLG50	MLG49		sens	sens						
B	MLG13	MLG14	MLG50		K1	K1						
C	MLG17	MLG21	MLG21		K2	K2						
D	MLG21	MLG22	MLG22		Klus	Klus						
E	MLG40	MLG49	MLG36		K28	K28						
F	MLG49	MLG34	MLG40		sens	sens						
G	MLG17	MLG39	MLG5									
H	MLG26	MLG40	MLG39									

Appendix Figure 3 | complete killer assay of 5X47 with corresponding MLGs and dsRNA results (A1-6 = Plate A, columns 1-6, etc) green = no dsRNA viruses (NO) | yellow = L-A helper virus only (H) | red = LA & M-viruses (K, killer) | white = NA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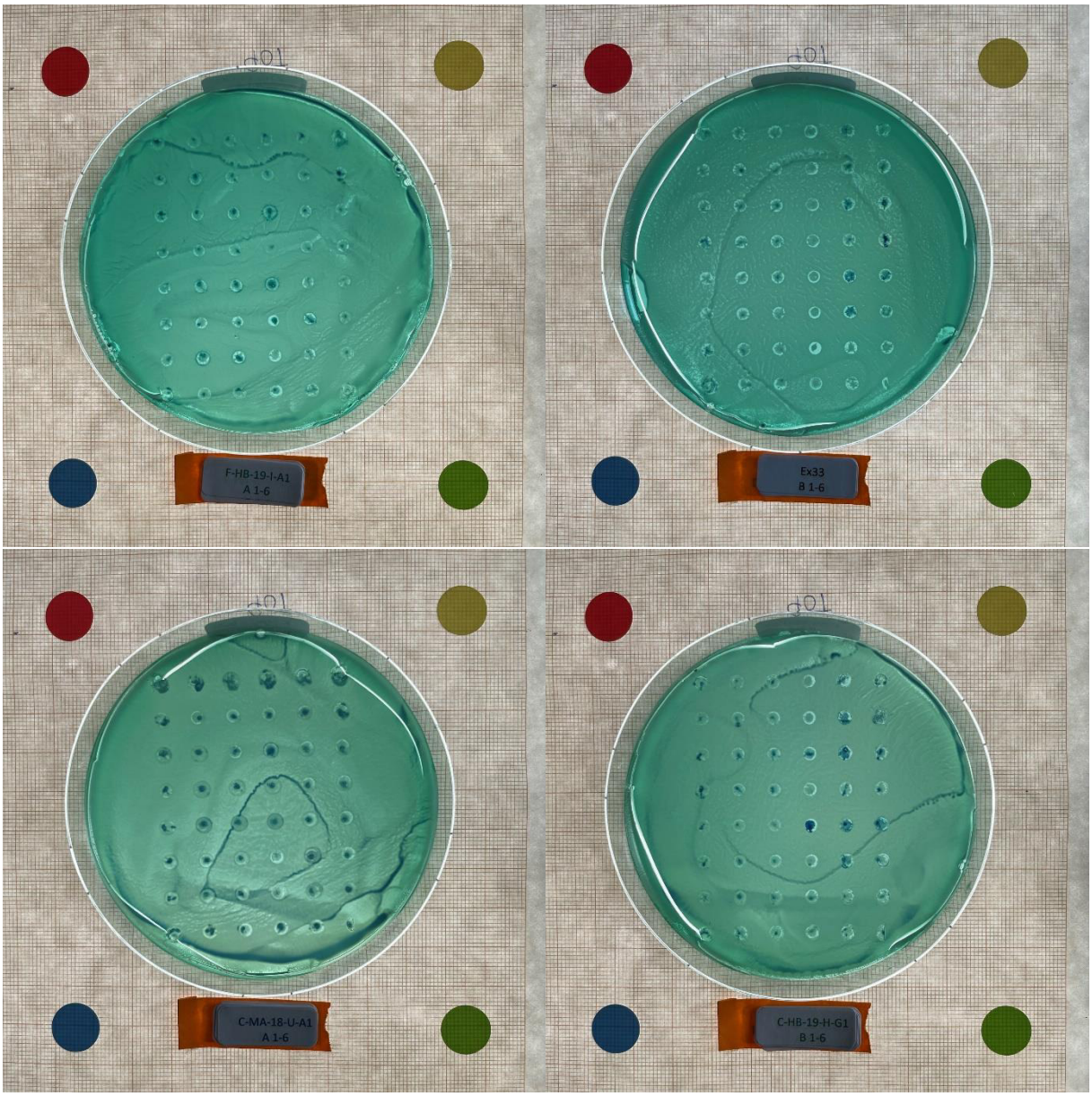
C-MA-21-W-A1
A 1-6



Appendix Figure 4 | A selection of killer assays to demonstrate the variety of killer interaction phenotypes