

## Some Suggestions for How to Think About This System

A lot of work has been done with the *P.Flu* strain SBW25. The wild-type strain forms smooth, round colonies when plated. These smooth (SM) genotypes form the majority of bacteria within a microcosm and are free-floating within a liquid (planktonic). Studies have shown that in microcosmic settings such as a test-tube, a common mutation arises that causes some of the bacteria to form a biofilm mat. When these bacteria divide, the biofilm they create causes them to stick together, so the clones of the mutated bacteria will spread outwards in a single mat. When plated, these mutated bacteria form wrinkly, irregular colonies and are thus referred to as Wrinkly Spreaders (WS).

As we will see in more detail below, *P.Flu* bacteria contain a few genes that cause them to produce a small amount of this biofilm. These genes have “on” and “off” switches that constantly flicker back and forth, creating the biofilm in small bursts and regulating its overproduction. Essentially, the mutations that lead to the WS phenotype represent loss-of-function mutations specifically for the “off switch” of this gene system. When the proteins lose their off switch, they are stuck in the “on” position and instead of creating biofilm in small bursts they end up producing it all the time.

The mat produced by the WS sometimes allows the bacteria to stay at the surface of the liquid in a test tube. This colonization of the surface happens in a very specific way. Initially, the biofilm produced by a WS bacterium will cause it to stick to the side of the glass. As it divides, the clone bacteria will stick together in a mat, spreading outwards. If the place where the bacterium initially sticks and spreads out happens to be at the surface of the liquid, these WS bacteria will be at an advantage because they will have greater access to oxygen.

If WS mats form at a location other than the surface of the liquid, the bacteria will be at a disadvantage because they may sink to the bottom, where they will have much less access to oxygen than the planktonic SM types. Also, overproduction of a biofilm is energetically costly; creating the biofilm requires precious nutrients and energy that would have been used instead for growth and reproduction. So for the most part, mutations causing the WS phenotype are not actually beneficial. In a natural system, only the WS that happen to mutate at the surface will likely survive and reproduce.

What we are doing in this experiment is creating an artificially selective advantage for the WS phenotype. If we did not perform the bead transfer, if a WS phenotype emerged (via a random mutation process) and happened to stick to the bead, it would be at a disadvantage and would be unlikely to outcompete the SM. However, when we transfer the beads from one tube to the next, we are creating a distinct advantage for bacteria that can better stick to the beads. If they don't stick to the beads, they are not brought into the new environment (basically, they don't “survive”). In this case, the WS have a much greater ability to outcompete the SM.