How do different *Wolbachia* strains influence *Aedes aegypti* virus transmission?

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Abstract

With a large proportion of the human population at risk of becoming infected by arthropod-borne diseases, and vaccines for most of these diseases not existing yet, consequences for public health are substantial. These diseases can be caused by several types of pathogens, most of which are viruses. It is imperative that action is taken to reduce the number of people infected with these viruses. These viruses are primarily carried by the mosquito vector *Aedes aegypti*. Therefore the actions taken have been against these mosquitoes, in an attempt to limit the amount of infections. Most of the actions are population control, but other limiting strategies have also been deployed. An alternative method of reducing the amount of infections would be reducing the capability of the mosquitoes to carry the viruses. In an attempt to do both methods at once, a bacteria species that is present in roughly 60% of insect species called *Wolbachia* has been introduced. These bacteria have been found to impact host fitness, as well as interact with viruses. In this thesis, I will compare three strains of *Wolbachia* in their ability to both control the mosquito population size and reduce viral transmissions. In summary, all three compared strains serve their purpose, and have their situation in which they would be preferred over the others. The wMel strain however does show the best overall results, making it the most viable strain in the majority of situations. Further research should be conducted to not only find new *Wolbachia* candidates, but also as preventive measures, by keeping an eye out for unwanted actions by the viruses.

Table of contents

Background

With over half the human population at risk, the importance of arthropod-borne diseases, which account for almost one million deaths annually, has never been more evident (WHO). Unfortunately, vaccines for most of these diseases are currently unavailable and challenging to develop. Most of these diseases, often viruses, are transmitted by mosquitoes. One of the most studied species is the yellow fever mosquito, *Aedes aegypti*, a vector for multiple arboviruses, such as dengue, Zika, chikungunya, and yellow fever viruses (Powell, 2018). Besides its ability to transmit a broad range of viruses, this invasive species is also one of the most widespread mosquitoes. It has settled in many ecosystems it did not naturally occur in, allowing the spread of diseases to new regions (Juliano et al., 2005). Thus, measures are crucial to prevent the transmission of arboviruses by *A. aegypti*.

To prevent this mosquito from spreading these diseases many actions have been thought of, and many have been implemented. These actions fall into two categories: population reduction and influencing the ability to transmit the viruses. The former is oftentimes being done by the use of insecticides, which frequently harm more than just the mosquitoes. Although a useful temporary solution, the unintended damage caused by this calls for a new solution. This frequently results in harm to other insect species, but it can also spread to humans through the food chain. Furthermore, resistance against these insecticides can arise, calling for either more of the same insecticide to be used, or used a different one instead. The second action has primarily been discussed in theoretical models (Ferguson et al., 2015), although it is rarely addressed in reality, mainly due to practical reasons.

There is however a bacterium called *Wolbachia* to come to the rescue. This bacterium is capable of controlling the populations, as well as influencing the virus transmission, tackling both issues at once. *Wolbachia* is present in roughly 60% of all insect species, and although it is found in many mosquitoes, it is not naturally present in *A. aegypti* (Johnson, 2015). Because it is naturally present in closely related species, attempts have been made to introduce the bacterium into these mosquitos, which ended up quite successful (Lambrechts et al., 2019). The introduction of these bacteria influences the spread of the viruses in two ways:

- Firstly the bacteria are capable of controlling the mosquito population by influencing the fitness of the mosquito, therefore serving as a population controller of the mosquitoes. The mosquito spreads through the population vertically, specifically maternally, meaning that in order to spread, the fitness costs cannot be so high that the mosquitoes are unable to reproduce. As they spread through the population of mosquitoes, *Wolbachia* induces a mechanic which favors reproduction of its own strain, called cytoplasmic incompatibility. Most males are only able to fertilize viable eggs when mating with a partner that has the same strain of *Wolbachia*. When the female has a different strain, or no strain at all, this will induce an incompatibility. This latter part is less strong in a reverse situation, where only the female carries the *Wolbachia* (Zabalou et al., 2004).
- Secondly, *Wolbachia* has been shown to influence both the viral resistance and viral load in related mosquito species, as well as in other less related insect species, however, in which way this is being influenced depends on several factors. For disease control, only a decrease of the load and an increase in resistance would be desired, but as this link depends on many factors, it is unpredictable. As there are examples of *Wolbachia*

increasing virus susceptibility in related mosquito species, care should be taken when dealing with this (Atlini et al., 2020).

From the point of view of only a reduction of the virus transmission, *Wolbachia* would do this without impacting the fitness of the host at all. This is however not the case. There is therefore a tradeoff between these two, in which a balance has to be found: not too harmful to the host, while still being able to impact the transmission of viruses sufficiently. It is important to look at both the viral resistance and the viral load, as these two both influence the competence of the mosquito.

Intention

Most research focuses on 3 main strains, although many more variations of these strains do exist. In this thesis I will compare how these strains influence the phenotype of the *A. aegypti*, in an attempt to get more clarity on when these strains can be best implemented. Furthermore, I will aim to show current gaps in the knowledge, and suggest follow-up research that could be relevant to the advances in the field.

Research findings

Why the different genotypes are important

As a result of the presence of *Wolbachia* in many different species, there is a large variety of genotypes present. Not all these strains however are fit to be introduced into *A. aegypti*. All strains currently being researched in *A. aegypti* have been previously known to reduce virus transmission competence in other species. This however does not mean that strains which are not known to do this would not be viable in *A. aegypti*. The influence on the virus transmission can be either positive or negative, with only negative being desirable. Besides this, not all *Wolbachia* strains are capable of spreading through the population. All tested strains so far have shown a decrease in fitness of the mosquitoes, however not all to the same extent. Strains such as wPip (Figure 1) have a too large fitness consequence to be seen as viable means to control the population, therefore not all strains will be reviewed in this thesis.

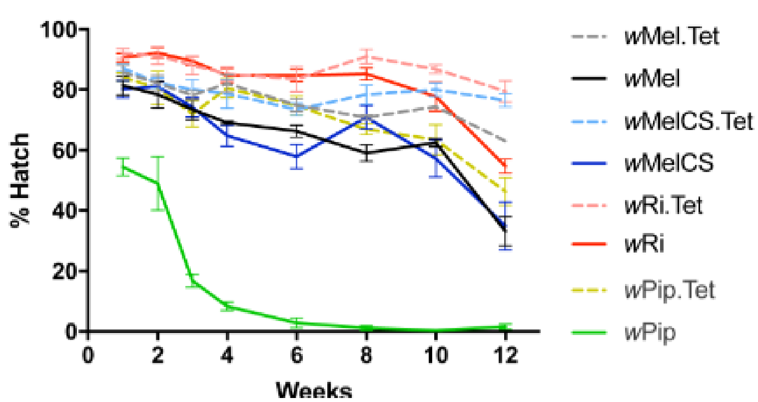

Figure 1 Hatch rate larvae (figure from Fraser et al., 2017)

The currently most used *Wolbachia* strains are wMel (*Wolbachia Melanogaster*), wMelPop (*Wolbachia Melanogaster*) and wAlb (*Wolbachia Albopictus*). wMel and wMelPop both originate from *Drosophila melanogaster*, but they differ greatly in one aspect: whereas most strains range from mutualists to reproductive parasites, wMelPop overreplicates in the host, causing severe life-shortening. This life shortening however is not severe enough that it is unable to spread through the population, therefore it is a viable option to control the population size. Both these strains are interesting candidates, as they have been shown to slightly suppress pathogenic viruses in their original hosts (Osborne et al., 2009)

The third strain originates from a close relative of the *A. aegypti*, the Asian tiger mosquito, *Aedes albopictus*. As this strain lives in a symbiotic relationship with a much more related species than the other two strains, it would be expected that this strain would not impact the fitness of the mosquitoes as severely. This mosquito is however also a virus vector for several viruses also found in *A. aegypti*. This suggests there would be a lower impact on the virus transmission too. Despite its apparent benefits over the other two strains, much research remains to be done on this strain, compared to the two very well-studied strains. It could be argued that wAlb should not impact the vector competence to a high degree, as it does not do so in its original host. This strain however behaves differently in *A. aegypti*, being present in somatic tissues in much higher densities than in its original host (Lu et al., 2012).

Population control

Egg viability

Mosquito egg viability decreases over time. The three strains behave as would be expected when it comes to egg viability, with wAlb showing the least decrease in egg viability. This is likely a result of the strain being present in a very related species, in which the fitness impacts are also minimal. The wMel shows moderate levels of decrease, with about a 75% hatch rate up to roughly a month, after which the viability rapidly decreases (Farnesi et al., 2019). wMelPop shows a very severe decrease in viability of the eggs, and the hatch rate drops below this same 75% after a matter of days already.

Despite the severe effect the strains have on the egg viability, once the eggs have been hatched different results are found. Both the larval survival and growth are barely affected by the introduction of *Wolbachia* at all (Axford et al., 2016).

Adult survival

Once reached adulthood, a distinction must be made between males and females. Not only is this distinction important because of the large differences in natural survival time of the two sexes, but in males, the wMel strain did not have any fitness effect. The wMelPop and wAlb however showed a slight decrease in fitness. In females however, the effect is much more pronounced. wMelPop shows a very clear decrease in survival straight away. wMel and wAlb both also show a decrease in survival when compared to mosquitoes without *Wolbachia*, but they do show an interesting pattern when compared to themselves. Up to the age of roughly 50 days, the survival of wAlb is higher than that of wMel. However, at this point the survival of the wAlb rapidly decreases, whereas wMel has a slower decrease in fitness starting earlier, and ending much later (Axford et al., 2016). This adult survival can be largely attributed to the different loads of *Wolbachia* present in the hosts. The adult survival is negatively correlated to the viral load in the mosquitoes. The previously mentioned overreplication of the wMelPop is the likely cause of these severe fitness costs, whereas wMel is barely present in the mosquitos, therefore not requiring nearly as many resources. The reduced lifespan of wAlb cannot be explained as easily however. As this strain originates from a closely related species, which has roughly the same lifespan as the wildtype *A. aegypti*, close to no reduction would be expected. Instead, the lifespan decreases by roughly 20 days. The severe fitness consequences of wMelPop also translate to increased difficulty in spreading through populations. Whereas the wMel strain can spread through populations without many problems, wMelPop has the potential to cause extinction of local communities (Rasić et al., 2014). Despite lab infections for wAlb proving quite successful, unlike the other two strains, no studies have been done regarding the actual spread through populations.

Cytoplasmic incompatibility

The previously mentioned cytoplasmic incompatibility linked with *Wolbachia* is also found when introduced into *A. aegypti*. There is a difference between the different strains, however, the difference between the sexes is much larger. Releasing infected males has severe effects on the compatibility, bringing the hatch rate down to 0% in females where no *Wolbachia*, or a different strain of *Wolbachia* is present. Infected females however have a much smaller effect on the eggs. Not only can females infected with wAlb breed with males with this same strain, but if the male does not have a *Wolbachia* strain at all, the hatch rate is unaffected (Axford et al., 2016).

Virus Transmission

Viral resistance

For virus transmission, the strains are compared in both their impact on the virus resistance and the viral load for the four largest arthropod-borne viruses present in *Aedes aegypti*: dengue, Zika, yellow fever (*Flavivirus*), and chikungunya virus (*Alphavirus*). Although many more viruses can be vectored, these four are currently of the highest interest to public health. Three of the four viruses compared are closely related, with chikungunya being a more distant relative. This suggests that the interaction between the strains and the more related viruses could be relatively similar.

After being fed blood meals infected with DENV-2 while 80.2% of the wildtype mosquitoes were positive for this virus, versus 4.2% in wMel, and 0.0% in wMelPop-CLA (Walker et al., 2011; Moreira et al., 2009). Although not as much of a reduction, wAlb also managed to reduce the amount of infected mosquitoes by 76%

When fed blood meals infected with chikungunya instead, the same pattern arose. wMel showed a significant reduction in the amount of infected individuals, and the virus was unable to establish in any of the wMelPop infected mosquitoes (Moreira et al., 2009). Although data for wAlb is yet to be collected, mathematical models have predicted this to be right in between wMel and the wildtype (Xue et al., 2018).

Although the effect of *Wolbachia* is not as strong on yellow fever virus, the reduction of infections is still present. An 89% infection rate of wild-type mosquitoes was reduced to 32% in wMel, with further reductions again in wMelPop (van den Hurk et al., 2012). Studies on this virus using the wAlb strain remain to be done, so no conclusive things about its effect on it can be said.

When infected mosquitoes were fed Zika-infected blood meals, effects of the *Wolbachia* strains were found as well. wMel showed a decrease in infection, paired with a delay. This delay however does raise questions about the true infection rate of the mosquitoes. There was a linear increase of the infections as a proportion of time, with 10 days post blood meal 30% of the wMel mosquitoes were infected with the virus. This could still increase with more time, requiring more research to be done to say if there is a reduction, or simply a delay (Aliota et al., 2016). Despite the lack of studies, it is likely that wMelPop will once again show better results than wMel, as it has been doing for every virus thus far. This delay is not present in wAlb, where there is a roughly 50% decrease in the amount of infected mosquitoes (Chouin-Carneiro et al., 2020).

Overall, all three strains appear to reduce the amount of infections in the mosquitoes, with some differences being present between the strains. From only the point of view of a reduction of vector competence, wMelPop appears to be much more effective, this is however not the only point of view that should be taken into account, as fitness and load consequences must also be considered. More research remains to be done, not only as there is missing data, but also because many more viruses are capable of infecting these mosquitoes.

Viral load

When a mosquito is not resistant to a particular virus, and it does get infected, it is not always able to spread it. The virus first has several barriers to overcome within the mosquito for the mosquito to be able to spread it. Usually a mosquito will be infected orally, after which it has to pass through several internal barriers for it to reach the saliva and spread further. These barriers usually create a situation where the virus needs to be present above a certain threshold in the mosquito to be able to spread. The wMel is an example of this: mosquitos with this strain were not completely resistant to dengue virus, but the small percentage of individuals that did get infected, did not reach a high viral load (Walker et al., 2011). Figure 2 shows the amount of DENV-2 copies present in the legs of mosquitoes that have been exposed to this virus via a blood meal. Whereas in the mosquitoes without *Wolbachia* the load reaches up to 50,000 genome copies per leg, wMel infected individuals only had a few copies. wMelPop did not have any load, as these were not infected (Walker et al., 2011). Despite the wAlb strains ability to reduce the amount of mosquitoes infected with dengue, the load in these infected mosquitoes did not change at all (Lu et al., 2012).

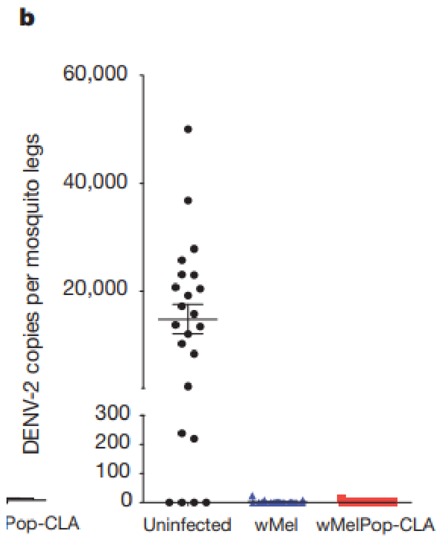

Figure 2: Viral load of dengue in the thorax of uninfected and infected mosquitos (figure from Walker et al., 2011)

Just like with dengue virus, as wMelPop prevented all infections of chikungunya virus, there was no load to be measured. wAlb shows a roughly 50% decrease in the amount of virus copies present in the midgut, however, this decrease is enough to prevent the virus from overcoming one of the barriers. Salivary gland loads for the virus are near zero in these mosquitoes (Ahmad et al., 2021).

The effect of *Wolbachia* on the yellow fever virus was previously noted to be subpar compared to the other viruses. This can again be found when looking at the viral load. Although with wMelPop, the amount of virus copies decreased roughly a thousandfold, the wMel barely impacted load at all, being comparable to the wildtypes (van den Hurk et al., 2012). However, this load is achieved at a delayed rate. Much like with the virus resistance, studies using the wAlb strain remain to be done.

Although the amount of mosquitoes infected with Zika virus decreased significantly, the viral loads in the wMel and wMelPop mosquitoes did not change much as with the other viruses (Dutra et al., 2016). In wAlb however, there is evidence to suggest otherwise: although no studies have been done on purely this, when studying the infection rate with this strain, it was noted the amount of mosquitoes with a virus infection in the wings was significantly lower than the amount of total infections. This suggests the load has been decreased and has become unable to cross one of the internal barriers (Hugo et al., 2022).

Furthermore, in dengue virus infections, it has been observed that the viral load per cell is negatively correlated with the load of *Wolbachia* (Frentiu et al., 2010). Although this has only been tested using the wMelPop strain, this trend is likely to occur in the other strains too. This density dependent load is a very important component of the ability to transmit viruses. If one of the barriers for the virus to overcome would have an increased density, this would severely impact its ability to overcome this. This is the case with the wMel strain, which tends to be present in very high densities in the salivary glands, whereas the other two are much more spread throughout the body. wAlb has the highest density present in the oocytes, and wMelPop has over replicated throughout the body (Hugo et al., 2022). This is likely the reason for the strong decrease of dengue copies as a proportion of *Wolbachia* replicates when compared to the other strains.

When looking at the viral load the viral resistance should always be kept in mind. If a virus would be present in a very high load, but in very few mosquitoes, the strain might still be viable as a means to prevent that virus. Therefore when deciding which strain would be best in a certain situation, both of these factors must be taken into consideration. Just based on the relatedness of the viruses, no detailed predictions can be made for other viruses that might be carried. As previously mentioned, chikungunya virus is less related to the other viruses, yet the interactions with the strains are very similar to Zika and dengue viruses, whereas the more closely related yellow fever virus reacts quite differently.

Conclusion

The three different strains discussed in this thesis all have their strengths and weaknesses, shown in Figure 3. They all have their situations in which they would serve as the best solution. All three strains are capable of controlling the size of the population, as well as limiting the transmissibility of viruses in these mosquitos. There is a correlation between the amount of *Wolbachia* present in a mosquito and both its lifespan, and the viral load.

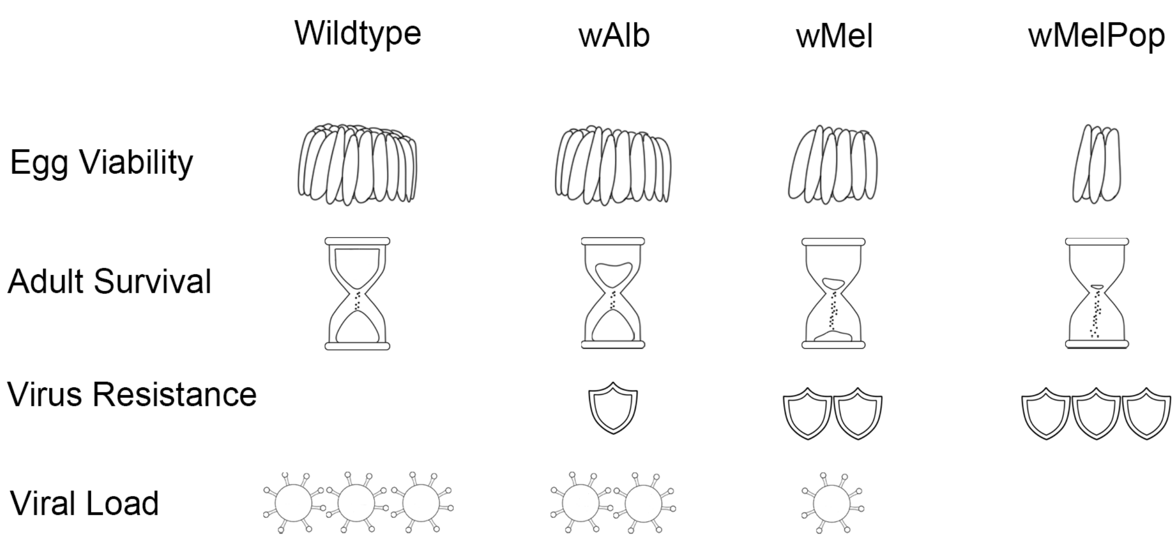

Figure 3: Summary of the strains

Eliminating mosquitoes also has its downsides. Although this might seem at first like a good idea, mosquitoes do serve a role in the ecosystems. If a certain kind of mosquito is not able to fulfill this role, another mosquito might be able to invade the ecosystem, creating opportunities for other diseases. It is therefore important to find a balance between a limited amount of mosquitoes to limit the disease transmission and having too few mosquitoes, resulting in empty ecological niches and risk of new species invasion.

Although the *Wolbachia* strains have a negative effect on the ability of the mosquitoes to transmit dengue virus, this does not mean that it has this same effect on other viruses. As *Wolbachia* has been found to influence the transmissibility of viruses in both directions, it is possible that these strains might facilitate the transmission of other viruses. This can create a false sense of security, with the local population taking down other vector control or protective measures thinking the risk of disease is almost gone. However, if the mosquitoes end up being more capable of transmitting other diseases, this would be detrimental.

The introduction of *Wolbachia* seems like a very good solution to the problem, this might not be a sustainable solution. Viruses might be able to adapt to the presence of *Wolbachia*, evolving to be able to withstand or even thrive in its presence. This does call for continuous monitoring of the virus transmission after the introduction of *Wolbachia*. Not only to acquire more knowledge about it, but also to identify and tackle unwanted side effects early.

For the control of the population size, many studies have been done already using these different *Wolbachia* strains. There are not many studies however linking population control to viral transmission. The release of infected males serves as a much better controller of population size, whereas the release of infected females allows for the spread of the strain through the population, therefore serving as a better control for viral load (Beebe et al., 2021). This makes it important to also study in what sex ratio the infected mosquitoes should be released, depending on the situation.

For the virus transmission however, much research remains to be done. Currently researched strains appear to be very effective at controlling virus transmissions; spending time looking for completely new strains would therefore not be ideal for follow-up research. Rather, I argue that future research should focus on existing variations of the strains presented in this paper. As there is a lot of variation within *Wolbachia* strains originating from the same organism, these should be the focus of future research. Much like with antibiotics, other strains that appear as efficient in suppressing the virus transmission should be kept as a backup, instead of used simultaneously, in case the viruses evolve to handle the particular strain of *Wolbachia*. This way the situation of no more strains being available to handle the situation will not arise. However, if better strains are characterized these could replace the ones currently in use. Although researching variations of what is known to work will likely yield the fastest results, this might not always be ideal. If a virus evolves to withstand a particular mechanism used by several strains, this could result in a multi-strain resistance. Therefore research on strains that differ greatly from the currently used ones should not be left out.

Different *Wolbachia* strains all interact with *A. aegypti* in unique ways, and not all strains are viable to use. The wMel strain is very fit for a lot of cases, as it serves a nice balance between fitness costs and transmission of diseases. The wMelPop strain does outperform it when it comes to preventing transmission, but as a result of the severe fitness costs, it can not be used in as many cases. The wAlb strain is unable to control virus transmission to the degree of the other two strains, but thanks to its low fitness costs this too can be a viable strain for certain cases.

Fieldwork studies have been done using all three strains on a moderate (city sized) scale. In these studies wAlb and wMel have been shown to be effective control agents the the number of dengue cases in the area (Nazni et al., 2019; O'Neill et al., 2019). Both of these strains were able to spread through the population with relative ease after being released in several stages. Similar study for the effect of wMelPop on Zika and dengue has shown its difficulty to spread through the population as a consequence of its fitness costs (Nguyen et al., 2015). Despite this, it should not be ruled out as a possible virus control agent under different circumstances. Further studies have shown that this trait can be used to control local population size, when released on a weekly basis (Ritchie et al., 2015). Although the effects of these trials on other viruses are yet to be measured, the ability of wAlb and wMel to spread through the population gives a bright outlook.

These released mosquitoes are modified organisms, which has been a controversial topic over the last decades. Furthermore these mosquitoes could be capable of carrying not yet researched, or even currently unknown diseases better than the current mosquitoes. Because of these reasons, despite the introduction of *Wolbachia* having a seemingly good effect on the virus transmission, there is an ethical dilemma at play. This dilemma could be solved by engaging the community in an attempt to obtain public acceptance (O'Neill et al., 2019). Because larger scale trials would face both these ethical dilemmas, as well as costs a significant amount of money, these can only be conducted if both the scientific community and the public support this.

With *Wolbachia* being present in so many different species, and so many strains of it existing, searching for the perfect strain to tackle virus transmissions might never stop. Some of the strains found however are already very good transmission suppressors, so there might not be much of a rush to find new strains. Larger scale trials of the release of infected mosquitoes have proven to be very successful, which gives a bright outlook on the future. If this release would happen on a global scale, maybe one day arthropod-borne viruses will no longer be a threat.

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