STUDIES ON THE BACTERIAL SYMBIONT WOLBACHIA IN DROSOPHILA:

Fecundity measurements of *D. mauritiana* infected with Wolbachia 01 and 07
&
Wolbachia localization in Klar embryos of *D. melanogaster*

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STATUORY DECLARATION

I declare in lieu of an oath that I have written this bachelor thesis myself and that I have not used any sources or resources other than stated for its preparation. I further declare that I have clearly indicated all direct and indirect quotations. This bachelor thesis has not been submitted elsewhere for examination purposes.

Date : 1/13/2010

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It was a great experience working with Drosophila flies. Especially *D. melanogaster* offers a lot of possibilities to investigate the impact of genes in a cheap and fast way. For the first time I realised that Drosophila Genetics is an excellent alternative way to explore many functions in cells and I want to continue the work with Drosophila flies. It was a great atmosphere in the laboratory and I felt comfortable there.

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ABBREVIATIONS

CI…cytoplasmic incompatibility

KASH…klarsicht, ANC-1, Syne-1 homology

LD…lipid droplet

#...identification number of the Drosophila library at the Boston University

ABSTRACT

About 16% to 76% of insect species worldwide are infected with the endoparasitic gram-negative bacterium Wolbachia. It manipulates the host insect reproductive machinery for its own benefit. The transmission of Wolbachia is maternally. Infection with Wolbachia can lead to parthenogenesis, male killing, feminization of male progeny or cytoplasmic incompatibility (CI). Many studies about positive fitness costs caused by Wolbachia like fecundity, sex ratio, reproductivity and lifespan in the host were already made in many different insect species. However it is not clear in how far the host benefits from the endoparasitic bacteria Wolbachia until today.

In the first project of this thesis we tried to find out if there are differences in fecundity of *D. mauritiana* infected with Wolbachia 01 or 07. The genetic background of the used Drosophila was the same apart from the infecting Wolbachia strains.

The fecundity data shows that there is a difference between the F1 hybrids infected with Wolbachia 01 and the F1 hybrids infected with Wolbachia 07. Our observations lead to the conclusion that the difference of the fecundity shows that each Wolbachia strain manipulates the reproductive machinery of the host differently.

Although not much is known about the transmission of Wolbachia on a molecular level it was already shown that Wolbachia uses the motor proteins attached on the microtubules on the host.

Kinetic measurements with lipid droplets in *D. melanogaster* embryos in which Klar is mutated showed altered behavior concerning the movement and localization of lipid droplets. The conclusion was that the protein Klar must be an important regulator for motor proteins.

So in the second project of this thesis we investigated if a mutation of the Klar protein affects the Wolbachia localization in *D. melanogaster* in fertilized and unfertilized embryos. Additionally we want to see if the obtained results can strengthen the theory that Wolbachia uses motor proteins for its transmission.

Three different Klar alleles (Klar A, Klar ΔS-15 and Klar YG3) were used for the investigation. The localization of Wolbachia in Klar embryos was estimated.

The quantification results showed that the Klar mutation has an effect concerning the Wolbachia localization in fertilized and unfertilized embryos of *D. melanogaster*. Nine different Wolbachia localization patterns were observed during the quantification. The strongest effect showed the allele Klar YG3. In Klar YG3 homozygous big ring structures were observed which means that Wolbachia is not located in the germ line at the posterior region of the embryo whereas in Klar YG3 heterozygous those big ring structures were not observed.

ZUSAMMENFASSUNG

Weltweit sind ungefähr 16% bis 76% auf der Erde lebenden Insekten mit dem intrazellulären Bakterium Wolbachia infiziert. Wolbachia ist gram negativ und es manipuliert den Ablauf der Oogenese in den Eierstöcken sowie die Spermatogenese in den Hoden. Außerdem kann Wolbachia nur über das Weibchen übertragen werden. Eine Infektion mit Wolbachia hat zur Folge, dass es zur Parathogenese oder zur Geschlechtsumwandlung von männlichen Nachkommen zu weiblichen Nachkommen kommen kann. Weiters kann die Teilung der Chromosomen am Beginn der Embryogenese durch Wolbachia abgebrochen werden auch genannt cytoplasmatische Inkompatibilität (CI).

Es wurden bereits viele Studien über die Fruchtbarkeit, Geschlechterverteilung, Reproduktion und Lebensdauer von einzelnen Insekten Arten gemacht, die mit Wolbachia infiziert sind. Bis heute weiß man nicht genau in wie fern ein Tier, das mit Wolbachia infiziert ist, von Wolbachia profitiert.

Im ersten Teil der These wird untersucht, ob die Infektion von *D. mauritiana* mit zwei verschiedenen Wolbachia Typen zu Unterschieden in der Fruchtbarkeit führt. Dazu wird die Anzahl der gelegten Eier von *D. mauritiana* Weibchen, die entweder mit Wolbachia 01 oder mit Wolbachia 07 infiziert sind verglichen. Wichtig war, dass die Weibchen, die mit Wolbachia 01 oder Wolbachia 07 infiziert sind, alle genetisch identisch sind.

Unsere Daten zeigten tatsächlich, dass es Unterschiede in der Anzahl der gelegten Eier gibt. Daraus lässt sich schließen, dass Wolbachia 01 als auch Wolbachia 07 die Produktion von Eiern im Weibchen unterschiedlich beeinflussen.

Der zweite Teil dieser These behandelt die Lokalisation von Wolbachia in Eier in denen das Gen Klar mutiert ist. Es ist nicht viel bekannt über die molekularen Mechanismen des Infektionsprozesses während der Oogenese. Jedoch wurde gezeigt, dass Wolbachia die Motor Proteine der Mikrotubuli verwendet, um in das sich entwickelnde Ei zu gelangen und so die nächste Generation zu infizieren.

Eine Studie über Fetttröpfchen in Embryos von *D. melanogaster*, in denen das Klar Gen mutiert wurde, zeigte Unterschiede in der Bewegung und der Verteilung

der Fettröpfchen im Vergleich zum Wildtyp. Daraus wurde geschlossen, dass das Klar Protein ein wichtiger Regulator der Motor Proteine ist.

Wir untersuchten, ob das Klar Protein auch eine wichtige Rolle bei der Infektion von *D. melanogaster* Eieren durch Wolbachia spielt.

Außerdem wollten wir auch zeigen, dass Wolbachia tatsächlich die Motorproteine des Wirtes benutzt. Wir benutzten drei verschiedene Mutationen des Klar gens. Diese sind Klar A, Klar ΔS-15 und Klar YG3. Die Lokalisation von Wolbachia in den Embryos wurde unter dem Mikroskop bewertet.

Unsere Quantifizierungsergebnisse zeigten, dass das Klar Protein auch eine wichtige Rolle für Wolbachia in den befruchteten und unbefruchteten Eiern von *D. melanogaster* spielt. Insgesamt wurden neun unterschiedliche Erscheinungsmuster von Wolbachia in den Eiern beobachtet. Den größten Effekt konnte man in den Eieren sehen, die das Allel Klar YG3 homozygot trugen. In diesen wurde beobachtet, dass Wolbachia den hinteren Teil des Eies nicht erreichen konnte. Dieses Muster wurde nicht in den Eiern gesehen, die das Allel Klar YG3 heterozygot besaßen.

1 PART 1

FECUNDITY MEASUREMENT BETWEEN WOLBACHIA 01 AND WOLBACHIA 07 IN *D. MAURITIANA* IN THE SAME GENETIC BACKGROUND

1.1 Introduction

The endoparasitic bacterium Wolbachia infects about 16% to 76% of insect species worldwide. It manipulates the reproductive machinery of the host and is transmitted maternally (Ferree *et al.,* 2005).

Wolbachia infection can lead to parthenogenesis, feminization, male killing and cytoplasmic incompatibility (CI). Cytoplasmic incompatibility (CI) is the most widespread phenotype caused by a Wolbachia infection in a host. It is expressed if a male which is infected with Wolbachia mates with a female which is not infected with Wolbachia. This leads to an embryonic death (Werren *et al.*, 2008; Charlat *et al*., 2004). Interestingly cytoplasmic incompatibility occurs also if the female and male are infected with two different Wolbachia strains. But if these two Wolbachia strains are similar to each other the strains can rescue the fertilization (Dobson *et al.*, 2002; Poinsot and Mercot, 1997).

Three parameters which could influence the infection spread of Wolbachia were developed. These parameters are based on the population models of Wolbachia infection dynamics of one Wolbachia strain in *D. simulans*. The first parameter is the level of cytoplasmic incompatibility (CI), the second one the maternal transmission rate and the third one are possible deleterious effects on the host (Poinsot and Mercot, 1997).
The population models showed that a stable polymorphic equilibrium between both uninfected ones and infected ones which can coexist in a population is only possible if the maternal transmission of Wolbachia is not complete. So in a

population a second unstable equilibrium exists which means that on a certain threshold cytoplasmic incompatibility (CI) compensates for incomplete maternal transmission of Wolbachia and any deleterious effects on the female host (Poinsot and Mercot, 1997).

So with cytoplasmic incompatibility (CI) it is ensured that Wolbachia is spread and maintained in the host population. Especially a high level of cytoplasmic incompatibility (CI) and a high transmission rate make it possible that Wolbachia is maintained in a host population (Dobson *et al.,* 2002; Charlat *et al.,* 2004).

But it was hypothesized that long-term Wolbachia in a population would lead to a reduced cytoplasmic incompatibility level (Charlat *et al.,* 2004).

For example in a population of *D. yakuba* from Gabon, West Africa it was found that in this population Wolbachia did not induce cytoplasmic incompatibility (CI) but the transmission of Wolbachia to the offspring is 100%. Although the transmission of Wolbachia is 100% and it does not cause cytoplasmic incompatibility there were not found any positive effects like the female fecundity (Charlat *et al.,* 2004).

Also in *D. mauritiana* Wolbachia does not induce cytoplasmic incompatibility. So the conclusion was that this species has a neutral strain of Wolbachia. But interestingly in *D. simulans* it was found that this Wolbachia strain induces cytoplasmic incompatibility (Giordano *et al.,* 1995). It could be also possible that positive fitness effects are not identified yet because in *D. melanogaster* cytoplasmic incompatibility (CI) can be detected in the laboratory but not in the field (Charlat *et al.,* 2004).

Recently it was found that Wolbachia makes *D. melanogaster* more resistant to the Drosophila C virus and to two other RNA viruses called Nora virus and Flock House virus. However the Wolbachia infected *D. melanogaster* flies did not show any resistance to a DNA virus called Insect Iridescent Virus 6. But this was the first report which showed a strong beneficial effect of Wolbachia in *D. melanogaster* (Teixeira *et al.,* 2008).

Interestingly it was also hypothesized that an increase of the host fecundity resulting from Wolbachia means a decrease of the maternal transmission rate of

Wolbachia or a low expression of cytoplasmic incompatibility (CI). (Dobson *et al.,* 2002).

Poinsot and Mercot reported the following in 1997. Three *D. simulans* strains which were infected with Wolbachia were compared with those of replicate stocks. These stocks were treated with antibiotics. It was observed that after three generations after antibiotic treatment a significant decrease in the productivity was observed. But in two of three strains five generations after antibiotic treatment the effect disappeared. For the third strain the effect disappeared 14 generations after antibiotic treatment in a third measurement (Poinsot and Mercot, 1997).
The conclusion was that the Wolbachia in *D. simulans* which is also known to express a high level of cytoplasmic incompatibility (CI) did not show a higher productivity in the host (Poinsot and Mercot, 1997).

The opposite was reported by Hoerauf *et al.* concerning gaining benefits due to the Wolbachia infection in the filarial nematode *L. sigmodontis*. It was observed that the tetracycline therapy which targets the intracellular bacteria in which the 16S rRNA gene of this intracellular bacterium is related to Wolbachia resulted in infertility and growth retardation (Hoerauf *et al.,* 1999).

In 2002 the first evidence was reported that Wolbachia which induces cytoplasmic incompatibility (CI) in a high level and increases the female fecundity too was published by S.L. Dobson *et al.* It was shown that the females of the mosquito species *A. albopictus* which are infected with Wolbachia have a high fecundity than the uninfected mosquitos. Moreover the infected mosquitos live longer and the hatching rate is higher compared with the uninfected ones (Dobson *et al.,* 2002).

We tried to figure out if there is a difference of fecundity between two Wolbachia subtypes 01 and 07 in the same genetic background of *D. mauritiana*. Dependent on the magnitude of the fecundity difference between these two Wolbachia

subtypes can we say that every Wolbachia strain manipulates the reproductive machinery of the host differently?

1.2 Materials and methods

1.2.1 Collecting of virgins

In order to gain F1 hybrids which carry 50% of the female genome and 50% of the male genome crosses have to be established.
From the stock bottles *Dmau* 07 w+ #1 and *Dmau* 01 w+ #24 which are kept in the incubator at 25°C and 60% RH virgins were collected for the crosses. An egg hatching test was performed for testing the virginity of the collected female flies from the stock *Dmau* 07 w+ #1 and *Dmau* 01 w+ #24. The collected female flies from the stocks *Dmau* 07 w+ #1 and *Dmau* 01 w+ #24 which are supposed to be virgins were put into bottles separately. On an egg hatching test bottle an agar plate with grape juice covered with active yeast was put on the top of the bottle. The egg hatching test bottles were incubated at 25°C and 60 % RH for two days. After two days the plates were examined under to microscope if eggs hatched. If none of the laid eggs hatched or no eggs were laid the female flies are real virgins.

1.2.2 Establishing the crosses

To gain F1 hybrids in the same genetic background and are infected with the Wolbachia subtype 01 or 07 the following crosses were established.
The virgins from *Dmau* 07 w+ #1 which are infected with the Wolbachia subtype 07 were crossed with males from the stock *Dmau* 01 w+ #24. Also the virgins from *Dmau* 01 w+ #24 which are infected with the Wolbachia subtype 01 were crossed with the males from *Dmau* 07 w+ #1. The flies which were crossed with each other were put into bottles (crossing bottles) with fly food medium and incubated in the incubator at 25°C and 60% RH for three days. After three days the flies were transferred into a new bottle with fly food and incubated in the incubator at 25°C and 60% RH for three days. Totally up to five bottles in which the F1 flies eclose were established.

1.2.3 Collecting F1 females born on the same day & counting the eggs

After ten days the first F1 females eclose in the crossing bottles. It is important to collect F1 females which eclosed on the same day because the age can influence the fecundity. Therefore it is important to check the crossing bottles every three hours.

After five F1 female flies which were eclosed on the same day were collected from the crossing bottles (*Dmau* 07 x *Dmau* 01) and (Dmau 01 x Dmau 07) they were put into empty bottles. On the top of the bottles agar plates with grape juice and covered with active yeast were put on the top. The bottles with the five female flies which were born on the same day were put into the incubator at 25°C and 60% RH. For the 1st set from the cross (*Dmau* 07 x *Dmau* 01) three bottles in which each contained five females eclosed on the same day were established. From the other cross (*Dmau* 01 x *Dmau* 07) four egg counting bottles were established.
The counting bottles were incubated at 25°C and 60% RH for one day and then the egg counting bottles were replaced by new agar plates with grape juice and active yeast. The old agar plates were taken for counting the eggs under the microscope. The plates were fixed with a tape on a sheet with lines. This was done for 20 days. All egg counting bottles from both crosses (*Dmau* 07 x *Dmau* 01) and (*Dmau* 01 x *Dmau* 07) were replaced by new agar plates with grape juice covered by active yeast together in one turn. Every day it was also examined how many female flies were alive in each counting bottle. The number of female flies alive from each counting bottle is included in the fecundity data.

For the second set new crosses were established (*Dmau* 07 x *Dmau* 01) and (*Dmau* 01 x *Dmau* 07) in order to have some new F1 females eclosed on the same day. Additionally it is ensured that the 2nd set is independent from the 1st set. From each cross (*Dmau* 07 x *Dmau* 01) and (*Dmau* 01 x *Dmau* 07) three counting bottles in which each contained five female flies born on the same day were established. The same counting procedure was done which was described above.

1.2.4 Mann-Whitney U-test

It is assumed that the cumulative of laid eggs per female in percent of both Wolbachia subtypes from the 1st and 2nd set are not normal distributed. Therefore a non parametric test called U-test was chosen. The cumulative of laid eggs per female in percent from the same Wolbachia subtypes of the 1st and 2nd set were tested against each other. The U-test was performed online on the homepage http://faculty.vassar.edu/lowry/utest.html .

1.3 Results

After the crossing and collecting female flies eclosed on the same day from the crossing bottles five F1 females from the same cross were put into an egg counting bottle as already described. Every day the plates were replaced by new ones. The laid eggs on the old plates were counted under the microscope. It was also examined how many F1 females are alive in the egg counting bottles. Out of these data the fecundity was measured.

1.3.1 Fecundity difference between F1 hybrids in the 1st set

The cumulative of laid eggs per female in percent of the 1st set (Fig. 1.1) shows clearly that the F1 hybrids which are infected with the Wolbachia subtype 07 produce more eggs than the F1 hybrids which are infected with the Wolbachia subtype 01. At the 20th counting day the ratio between the F1 hybrids which are infected with the Wolbachia subtype 07 and the F1 hybrids which are infected with the Wolbachia subtype 01 is nearly 60% and 40% respectively. On the 1st counting day no eggs were counted. Interestingly looking at the 2nd and 3rd counting day the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 07 increases dramatically. It can be also observed that in the mid counting days the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 01 increases slightly but it goes back in the late counting days.

1.3.2 Fecundity difference between F1 hybrids in the 2nd set

Comparing the cumulative of laid eggs per female in percent of the 2nd set (Fig. 1.2) it can be seen that the ratio between the F1 hybrids which are infected with the Wolbachia subtype 07 and the F1 hybrids which are infected with the Wolbachia subtype 01 is about 55% and 45% at the 20th counting day respectively. It is obvious that from the mid counting days the cumulative of laid eggs per female of the F1 hybrids which are infected with the Wolbachia subtype

01 increases slightly until the end of the counting period whereas the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 07 goes back. Additionally comparing the 2nd and 3rd counting days from the 1st set (Fig. 1.1) and from the 2nd set (Fig. 1.2) it can be seen that the increase of the cumulative of laid eggs per female of the F1 hybrids which are infected with the Wolbachia subtype 07 is much lower. But also at the 2nd set it is obvious that the F1 females which are infected with the Wolbachia subtype 07 have a higher cumulative of laid eggs per female in percent than the F1 females which are infected with the Wolbachia subtype 01.

1.3.3 Comparison of the cumulatives of laid eggs per female between Wolbachia 01 and 07

Comparing the figures 1.3 and 1.4 also here it can be seen that the F1 hybrids which are infected with the Wolbachia subtype 07 have a higher cumulative of laid eggs per female than the F1 hybrids which are infected with the Wolbachia subtype 01. But on figure 1.4 it can be seen that the difference of the cumulative of laid eggs per female between the F1 hybrids which are infected with the Wolbachia subtype 07 and Wolbachia subtype 01 is lower.

1.3.4 Overall average cumulative of laid eggs per female within 20 days

Looking at figure 1.5 it is obvious that the overall average cumulative of laid eggs per female within 20 days of the F1 hybrids which are infected with the Wolbachia subtype 07 is higher. The difference of the overall average cumulative of laid eggs per female within 20 days between both Wolbachia subtypes 01 and 07 is about 50 or 13%.

1.3.5 Total average of laid eggs per female within 20 days

In figure 1.6 the difference of the total average of laid eggs per female within 20 days between the F1 hybrids which are infected with the Wolbachia subtype 01 and 07 is about 120 or 10%. Also in figure 1.6 it can be seen clearly that the F1

hybrids which are infected with the Wolbachia subtype 07 produce more eggs than the F1 hybrids which are infected with the Wolbachia subtype 01.

1.3.6 Comparison of the cumulative of laid eggs per female at the 20th day

Table 1.1 shows that the cumulative of laid eggs per female at the 20th counting day of the F1 hybrids which are infected with the Wolbachia subtype 07 differs between the 1st and 2nd set. At the 1st set the cumulative of laid eggs per female at the 20th counting day is 401.3 and at the 2nd set the cumulative of laid eggs per at the 20th counting day is 289.9 For the Wolbachia subtype 01 this is not the case. The difference between these two sets is 44.6 only.

1.3.7 Comparison of cumulative of laid eggs per female in percent

Surprisingly the difference of the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 07 between the 1st and 2nd set is not big which can be seen at figure 1.7. But looking at the late counting days it can be seen that the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 01 increases slightly. The reason for the slight increase of the cumulative of laid eggs per female in percent between the F1 hybrids which are infected with the Wolbachia subtype 01 from the 1st and 2nd set can be seen on Figure 1.2.

1.3.8 Mann-Whitney U-Test

Although figure 1.7 do not show a big difference between both Wolbachia subtype 01 and 07 a non parametic test called Mann-Whitney U-test was performed. With this test it can be proofed that the cumulative of laid eggs per female in percent from Wolbachia 01 and 07 of the 1st and 2nd set are related to each other. It is assumed that H_0 is the following: The cumulative of laid eggs per female in percent from the same Wolbachia subtype of the 1st and 2nd set are related to each other although they are independent.

In the U-test where the cumulative of laid eggs in precent from the same Wolbachia subtypes of the 1st and 2nd set were tested against each other the overall result is that they are related to each other and therefore H_0 is true. The U-test results of the cumulative of laid eggs per female in percent of the Wolbachia subtype 01 from the 1st set and 2nd set are (*MR* = 17.6, n = 20) and (*MR* = 23.4, n = 20) respectively with $z = -1.54$, $p < .05$.

The U-test results of the cumulative of laid eggs per female in percent of the Wolbachia subtype 07 from the 1st and 2nd are (*MR* = 23.4, n = 20) and (*MR* = 17.6, n = 20) respectively with $z = 1.54$, $p < .05$.

1.4 Discussion

The gained results show that there is a difference of the fecundity between the F1 hybrids which are infected with the Wolbachia subtype 01 and the F1 hybrids which are infected with the Wolbachia subtype 07 (see figure 1.5 and 1.6). The difference of the fecundity is about 10% between Wolbachia 01 and 07 where the infection with Wolbachia 07 results in a higher fecundity than with Wolbachia 01. This difference is high enough that we can say that every Wolbachia strain manipulates the reproductive machinery of the host differently. However it has to be mentioned that there was not a negative control (Wolbachia non infected flies) available. So with this study it can not be answered if Wolbachia increases the fecundity in *D. mauritiana*. It could be possible that the negative control has a higher cumulative of laid eggs per female in percent than the two Wolbachia subtypes.

A higher fecundity for the Wolbachia subtype 07 was observed within the 20 counting days except for the 2nd day in the 1st set. Infection with Wolbachia 07 leaded to a later egg deposition. So the increase of the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 07 from the 2nd counting day to the 3rd counting day in the 1st set is about 20% (Fig. 1.1). A reason for this could be that the Wolbachia subtype 07 reduces the success in sperm competition in non-virgin males stronger than the Wolbachia subtype 01. It was observed that Wolbachia infected males sired about 11% less of the offspring than the uninfected ones. A key factor for the reduced success in sperm competition in non-virgin males is the transmission rate of Wolbachia. For example in *D. melanogaster* using very young males the transmission rate of Wolbachia ranges from 83% to 99.2% (Charlat *et al.,* 2004; Champion de Crespigny and Wedell, 2006). However the transmission rate of Wolbachia 01 and Wolbachia 07 in *D. mauritiana* is not known.

So in the crossing bottles there could be some F1 hybrids (female and male) which are not infected with the Wolbachia subtype 07 or 01. This can lead to a stable polymorphic equilibrium (Poinsot and Mercot 1997)

So then it is possible that the females are able to exercise choice of sperm. In this case this would mean that the infected female in a stock bottle looks for a male which is not infected with Wolbachia and therefore the mating time is later. So the cumulative of laid eggs per female from the Wolbachia subtype 07 increases from the 2nd to the 3rd counting day dramatically. The reason for this explanation is that in *D. melanogaster* females which are infected with Wolbachia and mate with males which are not infected with Wolbachia produce more eggs. With this behavior a dramatic fitness cost which is associated with cytoplasmic incompatibility (CI) can be avoided (Champion de Crespigny and Wedell, 2006).

Based on this knowledge it could be possible that the used F1 hybrid females did not mate or were not infected with the Wolbachia subtype 07 or mated very late and therefore produced eggs later.
The possible explanation that some females mated later could be true because the same observation like in figure 1.1 was not made in the 2nd set (Figure 1.2).
Despite the fact that the increase of the cumulative of laid eggs per female of Wolbachia 07 is smaller in figure 1.2 compared with figure 1.1 also there a slight increase is visible. This could show us that the Wolbachia subtype 07 has a lower transmission rate than the Wolbachia subtype 01 and influences the mating behavior of *D. mauritiana*.

Looking at figure 1.2 it is obvious that the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 01 increases slightly from the 10th counting day. This observation was not made in figure 1.1. Here it was the reverse case. The observation which was made in figure 1.2 is the reason why the overall average cumulative of laid eggs per female between both Wolbachia subtypes 01 and 07 is only about 13%. Taking the results only from the 1st set the difference between both Wolbachia subtype 01 and 07 would be about 17%. The reason why the cumulative of laid eggs per female in percent of the F1 hybrids which are infected with the Wolbachia subtype 01 in the 2nd set increased slightly from the 10th counting day (figure 1.2) is that these females mated early for example. Additionally it should not be excluded that also

the Wolbachia subtype 01 has a certain transmission rate in *D. mauritiana* and therefore also influences the mating behaviour.

Another critical paramter which should be mentioned is the expression level of cytoplasmic incompatibility (CI) which could also explain the obtained results which are shown in table 1.1. However it has to be mentioned that in *D.mauritiana* a detectable amount of cytoplasmic incompatibility (CI) has not been found yet (Poinsot and Mercot, 1997).

Concerning the obtained results which are shown in table 1.1 many factors can influence these numbers. One possibility is the mating behavior which was already discussed.

It was observed that those Wolbachia infected females mated with males which are not infected with Wolbachia produced significantly more eggs than those Wolbachia infected females which mated with Wolbachia infected males (Fry *et al.,* 2004).

So this might be an explanation why in the 1st set the cumulative of laid eggs per female of the F1 hybrid females which are infected with the Wolbachia subtype 07 is much higher compared with the 2nd set.

But the most important thing is that the cumulative of laid eggs per female in percent for both Wolbachia subtypes 01 and 07 is nearly the same which can be seen in figure 1.7. The only exception where a big difference can be seen is at the 2nd counting day. This result also shows us that there must be a difference between the Wolbachia subtype 01 and 07 concerning the transmission rate, expression level of cytoplasmic incompatibility and other fitness effects. Additionally a statistical test called U-test was chosen.

Table 1.2 which summarized the U-test results shows clearly that the cumulative of laid eggs per female in percent of the Wolbachia subtype 01 of the 1st and 2nd set are related to each other. An U value of 257.5 was obtained and assuming that α= 0.05 and therefore the upper limit of U is 273. So the obtained U value tells us that the cumulative of laid eggs per female in percent from the Wolbachia subtype 01 of the 1st and 2nd set did not occur casually and H_0 can not be rejected. The

same thing can be said for the Wolbachia subtype 07. Here an U value of 142.5 was obtained and assuming that α= 0.05 and therefore the lower limit is 127.

However the question remains why the Wolbachia subtype 07 shows a higher fecundity (~13%) than the Wolbachia subtype 01. As already discussed the transmission rate, the level of cytoplasmic incompatibility and the choice of sperm might play an important role. It would not be a surprise if the level of cytoplasmic incompatibility and the transmission rate of Wolbachia 01 and Wolbachia 07 are different. These parameters could explain the different fecundity results.
The reason is that according to the study of Zabalou *et al.* Wolbachia has the ability to modify (mod) and to rescue (resc) functions in the host. *wMau* which is the Wolbachia strain in *D. mauritiana* do not have a mod function but it can rescue the modification of the Wolbachia strain *wNo* (Zabalou *et al.,* 2008).
Moreover table 6 of the study from Poinsot *et al.* could support our observations because it is obvious that the two Wolbachia strains *wHa* and *wNo* have a different effect concerning the fecundity. However it has to be mentioned that the two Wolbachia strains were in different stocks. An interesting observation was that the density of the Wolbachia strain *wHa* was at least three times lower than of Wolbachia strain *wRi* in the egg. Moreover the transmission in laboratory of *wHa* is less efficient than of the Wolbachia strain *wRi* (Poinsot and Mercot, 1997). So this could mean that the density of Wolbachia 01 is lower than of Wolbachia 07. This could also explain why there is a fecundity difference between the Wolbachia subtypes 01 and 07.

2 PART 2

WOLBACHIA LOCALIZATION IN KLAR EMBRYOS OF DROSOPHILA MELANOGASTER

2.1 Introduction

A still unsolved problem in cell biology is the regulation of intracellular transport along microtubules. It is known that cargoes like organelles, proteins and RNAs use the bidirectional transport service of microtubules. Two motor protein families that make the bidirectional transport possible are known. One motor protein family is called the kinesins and the other motor protein family is called the dyneins. (Welte and Gross, 2008)

Seven possible motility regimes were introduced for cargo transport assuming that the number of kinesins and dyneins is equal. The first motlity regime is called (0). In this case the cargo can not move because both kinesin and dynein are attached on the microtubules. The second motility regime is called (+). In this case the cargo has the plus motion where only the kinesins are attached on the microtubules. The third motility regime is called (-). In this case the cargo has the minus motion and only dynein is attached on the microtubules (Müller *et al.,* 2008).
For bidirectional movement like the lipid droplets in Drosophila embryos a combination of (0) and (-) and (+) were introduced by Müller *et al*.
The first motility state is (-+) where the cargo has the minus motion and plus motion without pauses. The second motility state is (-0+) where the cargo has the minus motion and plus motion with pauses. So during the plus or minus motion only one motor type is activated (Müller *et al.,* 2008).

As already mentioned in case of a bidirectional movement many copies of different motor protein families are attached. If the multiple copies of the different motor protein families have the same strength a tug of war is established. This has the consequence that the cargo does not move because every single copy from the

different motor protein families are activated and pull against each other with the same strength (Welte and Gross, 2008).

Mutations in motor protein subunits showed that in case of bidirectional transport a tug of war is avoided in vivo. Therefore there must be a regulation for the different copies of different motor protein families (Welte and Gross, 2008).

Some regulators have been identified in the lipid droplet system of Drosophila embryos like halo, LDS 2 and Klar (Müller *et al.,* 2008).

The Klar gene is about 100kb in the genome and the molecular weight of the Klar protein itself is about 250 kDa. It has 19 exons and it has only one conserved region which is the C-terminal 60-amino acid Klarsicht, ANC-1, Syne-1 homology domain (KASH). This conserved domain can be found also in actin-binding proteins. Moreover with the KASH domain it is possible to target the Klar protein to the nuclear envelope. Indeed immunostaining studies showed that Klar is not only present in the droplet system of embryos it is also present in the region where photoreceptor nuclei migrate apically in the Drosophila fly. Recently Klar was also found in larval brains and adult ovaries. Therefore Klar must play an important role in the Drosophila development (Guo *et al.,* 2005).

Interestingly kinetic measurements showed that mutations in the Klar gene resulted in an altered distribution of lipid droplets at the onset of the gastrulation phase compared with the wild type embryos of Drosophila. (Welte *et al.,* 1998)

Additionally the data of the kinetic measurement showed a big reduction of travel distances, travel velocities and stall forces for the minus and plus motion of lipid droplets. It was observed that the switch from the net inward to net outward motion of lipid droplets in phase III (gastrulation) in Drosophila embryos which carry mutations in the Klar gene did not change correctly. (Guo *et al.,* 2005)

So the conclusion is that the protein Klar is an important regulator for the motor proteins in order to avoid a tug of war in case of a bidirectional movement. (Guo *et al.,* 2005)

Further studies with the Klar protein showed that there are three Klar isoforms called α, β, γ. The Klar isoform α has a molecular weight of 250 kDa and carry the KASH domain. This isoform is the original published cDNA. The Klar isoform β has a molecular weight of 202 kDa. This isoform is associated with the lipid droplets and does not contain the KASH domain but a new identified domain called lipid droplet domain LD. The reason is that the isoform β has an extension of the exon 15 of about 640 bases. In this extension an in-frame stop codon can be found. Therefore this isoform is smaller than the isoform α. With the LD domain the Klar isoform β is able to target the lipid droplets (Guo *et al.,* 2005).

The last Klar isoform is the γ isoform. The molecular weight of this isoform is only 62 kDa and can be found in the ovaries. It contains only the exons G, 16, 17 and 18 which contain the KASH domain (Guo *et al.,* 2005).

With the discovery of the three different Klar isoforms it is suggested that Klar is an important bridge between the identity of the cargo and the motor complexes (Guo *et al.,* 2005).

According to Veneti *et al.* in the wildtype embryos the density and distribution of the Wolbachia in *D. melanogaster* is the strongest in the posterior region.

Based on this knowledge we tried to figure out if a mutation in the Klar gene affects the Wolbachia localization in the embryos of *D. melanogaster* and therefore Klar could also target Wolbachia.

The reason for this study is that it was already shown that the anterior localization of Wolbachia in the oocyte of Drosophila is disrupted by manipulations of microtubules, cytoplasmic dynein and dynactin (Ferree *et al.,* 2005)

Additionally we want to strengthen the theory that Wolbachia uses the motor proteins of the host.

We used three different Klar alleles for our investigation. The three alleles are Klar ΔS-15, Klar A and Klar YG3. The allele Klar ΔS-15 is an unpublished allele but no info about this allele is available.
The published Klar A allele induces a stopcodon on exon 3 which expresses a truncated protein. The published Klar YG3 allele induces a 11kb deletion around exon 0 and expresses truncated protein. (Guo *et al.,* 2005).

These alleles were tested in homozygous flies as well as in heterozygous flies with the wildtype Klar gene.

2.2 Materials and Methods

2.2.1 Establishing crosses

The first step was to bring Wolbachia indicated by w+ and the Klar alleles in one fly together. The first cross was the following (female x male).

$$\frac{APC\ \Delta S}{TM6\ TUHU}^{w+} \times \frac{\text{Klar A}}{\text{Klar A}}$$

The second cross was the following (female x male).

$$\frac{APC\ \Delta S}{\text{TM6 TUHU}}^{w+} \times \frac{\text{Klar } \Delta\text{S-15}}{\text{TM3 SB}}$$

The Klar YG3 homozygous flies which are infected with Wolbachia and Klar YG3 heterozygous which are infected with Wolbachia were already established. Therefore no cross was necessary.

Out of these two crosses the Klar A heterozygous flies which are infected with Wolbachia and the Klar delta S-15 heterozygous flies which are infected with Wolbachia were collected and many living cultures (plastic bottles) were established.

$$\frac{Klar\ \Delta\text{S-15}}{TM6\ \text{TUHU}}^{w+} \qquad \frac{Klar\ \text{A}}{TM6\ \text{TUHU}}^{w+}$$

Out of these living cultures (Klar A and Klar delta S-15) many pupae with the wild type form were collected and put into new plastic bottles in order to establish own living cultures with Klar A homozygous and Klar delta S-15 homozygous.

Out of these living cultures the Klar A homozygous embryos which are infected with Wolbachia and the Klar delta S-15 homozygous embryos which are infected with Wolbachia could be collected.

2.2.2 Collecting fertilized and unfertilized embryos

After the crossing the next step was to collect the fertilized embryos from the homozygous (Klar delta S-15, Klar A and Klar YG3) and from the heterozygous females (Klar delta S-15, Klar A and Klar YG3). The heterozygous females were collected under the microscope using the dominant marker humeral as an indicator. For every embryo collecting turn about 60 till 100 heterozygous females were collected. These flies were put into a clean bottle and an agar plate with grape juice or apple juice covered with active yeast was put on the top. The bottle with the plate was incubated for one hour in the incubator at 25°C and 60% RH. Afterwards the plate was replaced by a new one. The old plate was incubated further in the incubator at 25°C and 60% RH for one hour till one hour and 30 minutes for gaining fertilized embryos at different cycles of the cellularization phase. For collecting homozygous fertilized embryos (Klar delta S-15, Klar A and Klar YG3) it was only necessary to empty some living cultures with the homozygous flies (Klar delta S-15, Klar A and Klar YG3). Also the homozygous flies were put into a bottle. The bottles with the homozygous flies were put into the incubator at 25°C and 60%RH. After one hour the agar plate with grape juice or apple juice covered with active yeast was replaced by a new one. The old plate was incubated at 25°C and 60% RH for one hour till one hour and 30 minutes in order to have embryos at different cycles in the cellularization phase.

For collecting unfertilized embryos female flies which are supposed to be virgins were collected and an egg hatching test was performed which was already described in chapter 1. The virgins were put into bottles where on the top an agar plate with apple or grape juice covered with active yeast was placed. These bottles were kept at room temperature. Every day the plates were replaced one till five times a day by a new one. About three days unfertilized embryos were collected in order to have enough unfertilized embryos for the immunofluorescence staining procedure of Wolbachia.

2.2.3 Fixation of the collected embryos

The next step was to fix the collected embryos. For this the protocol Heat Fixation of Embryos was used. The plate which contained *Drosophila melanogaster* embryos was poured by bleach. The plate was shaked about five seconds with the bleach and afterwards the liquid was spilled into a stainless steel mash basket. Then bleach was added to the plate again and the liquid was spilled into the same stainless steel mash basket again. This was done four times within one minute. After that distilled water was poured on the plate and spilled on the same steel mash basket. The steel mash basket was put onto a clean paper towel. About five ml 1x triton salt (10x Triton salt: 40g NaCl, 3ml Triton X-100 and 1l H_2O) was added into a clean glass vial. The glass vial with the 1x triton slat was heated in the microwave with the loosen cap for about five seconds. Then the steel mash basket with the embryos was put into the glass vial. Five seconds were counted and then about 10 till 15 ml cold 1x triton slat (4°C) was added to the glass vial. Then the glass vial was put on ice for at least five minutes. During this time a fresh eppendorf tube was taken and labeled. After about five minutes the glass vial was removed from the ice. Taking a clean forceps the stainless steel mash basket was removed with the forceps by shaking the basket several times in the glass vial in order to remove the last number of embryos from the basket. When the basket was removed from the glass vial the basket was washed again upon the glass vial with 1x triton salt to get the last embryos. Then some seconds were waited until most of the embryos were on the bottom of the glass vial. Afterwards a small piece of the P1000 tip was cut by a razor in order to soak in all the embryos from the glass vial. The soaked embryos were put into the labeled eppendorf tube.
After the embryos were on the bottom of the tube most of the 1x triton salt liquid was removed. Then 500µl heptane (O3008-1 provided by Fisher scientific) and immediately 500µl methanol (A433P-4 provided by Fisher scientific) were added. The tube was vortexed until most of the embryos were on the bottom. Most of the supernatant was removed and it was washed three times with 500µl methanol. Then the tube with the fixed embryos was put into the freezer and was ready for the immunofluorescence staining procedure.

2.2.4 Immunofluorescence staining of Wolbachia

For the immunofluorescence staining procedure of Wolbachia about 300µl methanol was left in the tube. Then 200µl PBT (1x PBS and 0.2% Triton X-100) was added very slowly. Then two times 200µl PBT was added very slowly again. After this step most of the supernatant was removed and 600µl PBT was added again. Then the tube was incubated for 20 min. on the shaker at room temperature. Again most of the supernatant was removed and 600µl PBT was added again and incubated for 20min. on the shaker at room temperature.

Afterwards most of the supernatant was removed and 500µl PBANG (PBT plus 0.2% BSA and 5% normal goat serum) was added and incubated on the shaker for one hour at room temperature. Then most of the supernatant was removed again and 200µl till 250µl primary antibody (Heat Shock Protein 60 Mouse LK 2 ascite fluids provided by the company Sigma Aldrich) diluted 1:100 in PBANG was added and incubated at 4°C on the shaker overnight.

The next day most of the supernatant was removed and kept for the next immunofluorescence staining procedure of Wolbachia.

Then it was washed six times with 500µl PBT for ten minutes on the shaker at room temperature. Afterwards 500µl PBANG was added and incubated for 30 min. on the shaker at room temperature. Removing the supernatant and 200µl till 250µl secondary antibody (Alexa Fluor R 488 Goat Anti-Mouse Ig provided by the company Invitrogen) diluted 1:500 in PBANG was added. The tube with the secondary antibody solution was incubated 2 hours on the shaker at room temperature or incubated at 4°C on the shaker overnight.

After this step it was washed six times with 500µl PBT on the shaker at room temperature. Then it was incubated with 300µl Hoechst (5µg/ml final) for 30 min. on the shaker at room temperature. Afterwards it was washed four times with PBT for 10 min. on the shaker at room temperature.

If the stained embryos were not mounted in Aqua-Polymount on the same day 500µl PBT was added again and the embryos can be kept at 4°C.

For the mounting procedure most of the PBT is removed and 500µl PBS was added. Most of the supernatant was removed and 500µl PBS was added again.

Then as small part of the P200 tip was cut away with a razor and the stained embryos were soaked in. The stained embryos were put on a cover slide. With a new tip most of the PBS was removed on the cover slide. Then one drop of the Aqua Polymount solution was added and the cover slide was put on the stained embryos very carefully. The coverslide was dried in a dark room for some hours.

The stained embryos were examined with the spinning disk Olympus BX61 and the software Slidebook 4.2 was used.

The distribution of Wolbachia in the embryos was estimated.

2.3 Results

Overall nine different Wolbachia localization patterns were observed. Figure 2.1 shows all nine Wolbachia localization patterns which were observed. According to Veneti *et al.* Wolbachia is accumulated at the posterior region in *D. melanogaster*. Totally 251 homozygous fertilized embryos (Klar ΔS-15, Klar A and Klar YG3) were examined and out of the 251 embryos 196 embryos showed that Wolbachia is accumulated at the posterior region in a normal way. (see Figure 2.1A, B and C). For the heterozygous fertilized embryos (Klar ΔS-15, Klar A and Klar YG3) 182 were totally examined and 171 showed that Wolbachia is accumulated at the posterior region in a normal way. (see also Figure 2.1A, B and C).

The interesting thing was that also other different patterns were observed which were not observed in the study of Veneti *et al.* Our results showed that two embryos of 251 total examined homozygous fertilized embryos show homogenous distribution of Wolbachia (see figure 2.1D). Whereas only one embryo of 182 total examined heterozygous fertilized embryos showed this pattern. Interestingly 39 of 251 total examined homozygous fertilized embryos showed the small ring pattern of Wolbachia whereas only 9 of 182 total examined heterozygous fertilized embryos showed this pattern. Looking at figure 2.1E it can be seen that Wolbachia is not able to enter the germ line. Moreover totally five embryos of 251 total examined homozygous fertilized embryos showed the medium ring pattern (see figure 2.1F) whereas in the heterozygous ones this pattern was not observed. Looking at figure 2.1F it can be seen that Wolbachia is more far away from the germ line. Also the big ring structure was observed (see Figure 2.1G). Total three embryos of 251 total examined homozygous fertilized embryos showed this pattern where in the heterozygous ones this pattern was not observed. Looking at figure 2.1G it can be seen that Wolbachia far away from the posterior region.
Additionally also two other patterns were observed (see Figure 2.1 H and I). In the homozygous (Klar ΔS-15, Klar A and Klar YG3) fertilized embryos two embryos showed that Wolbachia is strong accumulated at the cortex. Whereas in the heterozygous fertilized embryos only one embryo showed this pattern.

The last pattern which was observed is that Wolbachia is not accumulated at the top of the posterior region (see Figure 2.1 I). Only in the homozygous fertilized embryos 4 of 251 total examined embryos showed this pattern.

33 homozygous unfertilized embryos (Klar ΔS-15, Klar A and Klar YG3) of 37 total examined embryos showed that Wolbachia is accumulated at the posterior region in a normal way. Two of the 37 total examined homozygous unfertilized embryos showed that Wolbachia is homogenous distributed. Moreover another two embryos showed the small ring pattern of Wolbachia in the homozygous ones.
All 60 heterozygous unfertilized embryos (Klar ΔS-15, Klar A and Klar YG3) which were examined showed that Wolbachia is accumulated at the posterior region in a normal way.

2.3.1 Klar ΔS-15 fertilized embryos

Figure 2.5 shows the overall quantification results of Wolbachia in fertilized embroyos of Klar ΔS-15 homozygous and heterozygous. Totally 77 Klar ΔS-15 homozygous fertilized embryos were examined. It can be seen that 6.49% (see table 2.1) of 77 total examined embryos showed a small ring pattern at the posterior region whereas in Klar ΔS-15 heterozygous only 0.97% of 103 total examined fertilized embryos showed a small ring pattern at the posterior region. Looking at figure 2.5 and also looking at the tables 2.1 and 2.2 it can be seen that 2.60% of 77 examined Klar ΔS-15 homozygous fertilized embryos showed homogenous distribution whereas only 0.97% of 103 examined Klar ΔS-15 heterozygous fertilized embryos showed a homogenous distribution of Wolbachia.
Interestingly 5.19% of 77 total examined Klar ΔS-15 homozygous fertilized embryos showed that Wolbachia is not at the top at the posterior region. Additionally 1.30% of 77 total examined Klar ΔS-15 homozygous fertilized embryos showed that Wolbachia is accumulated at the cortex. These two patterns were not observed in Klar ΔS-15 heterozygous fertilized embryos.
For the other categories in figure 2.5 +++, ++, + it can be seen that there is a big difference between Klar ΔS-15 homozygous fertilized embryos and Klar ΔS-15

heterozygous fertilized embryos at the category ++. Looking at the tables 2.1 and 2.2 it can be seen that Wolbachia is slightly more accumulated in the posterior region with an average of 1.8 in the heterozygous ones than in the homozygous ones with an average of 1.6. However it has to be mentioned that the different sizes of the ring pattern, the cortex accumulation and no Wolbachia localization at the top of the posterior region are not included in the calculation of the averages.
So overall it can be said that the big difference between Klar ΔS-15 homozygous fertilized embryos and Klar ΔS-15 heterozygous fertilized embryos is that in the heterozygous ones Wolbachia is mainly medium accumulated at the posterior region whereas for the homozygous ones no real trend is visible.

2.3.2 Klar ΔS-15 fertilized embryos at early cycles

Looking at figure 2.6 which shows the quantification results of Wolbachia in fertilized embryos of Klar ΔS-15 homozygous and Klar ΔS-15 heterozygous fixed at early cycles. It can be clearly seen that the two patterns c and n which only occurred in the homozygous ones were seen only at early cycles.
Interestingly looking at table 2.3 11.11% of 36 Klar ΔS-15 homozygous fertilized embryos which are fixed in one of the early cycles of the cellularization phase showed a small ring pattern of Wolbachia at the posterior region. Compare it with figure 2.7 none of the homozygous ones which are fixed in one of the mid cycles of the cellularization phase showed this small ring pattern. But in the late cycles 2.56% of 39 examined Klar ΔS-15 homozygous fertilized embryos which are fixed in one of the late cycles of the cellularization phase showed this small ring pattern. So it can be said that the small ring pattern of Klar Klar ΔS-15 homozygous fertilized embryos occurred only at the early and late cycles. The calculated averages which are shown on the tables 2.3 and 2.4 show that the heterozygous ones with an average of about 1.8 have a stronger Wolbachia accumulation at the posterior region than the homozygous ones with an average of about 1.5.
Like in figure 2.5 also figure 2.6 shows that the heterozygous ones show a trend in which Wolbachia is medium accumulated at the posterior region whereas the homozygous ones do not show a trend.

2.3.3 Klar ΔS-15 fertilized embryos at mid and late cycles

In figure 2.7 there can not be seen a difference between the homozygous ones and heterozygous ones which are fixed at the *mid cycles*. The reason is that only two homozygous ones were fixed at mid cycles compared with the heterozygous ones where 32 were fixed at the mid cycles of the cellularization phase.

The quantification results of the tables 2.8 and 2.9 show with the calculated averages 1.64 in the homozygous ones and 1.58 in the heterozygous ones fixed at the late cycles that Wolbachia is stronger accumulated in the homozygous ones than in the heterozygous ones. Apart from the calculated averages there is no big difference between the homozygous ones and heterozygous ones at the late cycles of the cellularization phase because only one embryo of 39 homozygous ones fixed at the late cycles showed a small ring pattern at the posterior region. The rest of the embryos (homozygous and heterozygous) fixed at the late cycles showed strong, medium or weak Wolbachia accumulation at the posterior region.

2.3.4 Klar A fertilized embryos

The overall quantification results of Wolbachia in fertilized embryos of Klar A homozygous and heterozygous show that in Klar A homozygous about 23.7% of 114 examined embryos show a small ring pattern at the posterior region (see table 2.9 and figure 2.9). Whereas in Klar A heterozygous only about 4.8% of 41 examined embryos showed this small ring pattern at the posterior region (see table 2.10). It is also interesting that in table 2.9 and figure 2.9 it can seen that in the homozygous ones about 0.9% of 114 examined embryos showed a medium ring pattern at the posterior region. Although the medium ring structure at the posterior region was only observed in the homozygous ones another pattern was only observed in the heterozygous ones. Looking at table 2.10 and figure 2.9 it is obvious that about 2.5% of 41 examined embryos showed that Wolbachia is accumulated at the cortex. An interesting thing is that the differences in the categories + and ++ between the homozygous ones and heterozygous ones is really big. But it has to be mentioned that only 41 heterozygous ones were

quantified comparing with the number of quantified embryos of the homozygous ones with 114 (see table 2.9 and 2.10). Although the number of the total quantified Klar A homozygous fertilized embryos and Klar A heterozygous embryos are different it can be said that in Klar A homozygous everything is spreaded through the categories whereas in Klar A heterozygous it can be said that it is concentrated in the categories +++,++ and +. This can be also seen with the calculated averages of 1.73 and 1.93 respectively where overall Wolbachia is weaker accumulated in the posterior region in Klar A homozygous.

2.3.5 Klar A fertilized embryos at early cycles

Looking at figure 2.10 two big differences can be observed between Klar A homozygous and heterozygous. About 30% of 37 examined Klar A homozygous embryos showed the small ring pattern at the posterior region. For the heterozygous ones only about 4.9% of 9 examined Klar A heterozygous embryos showed the small ring pattern at the posterior region (see table 2.11 and 2.12). The second big difference can be seen at the category +. Nearly 44.5% of 9 examined Klar A heterozygous embryos were quantified as + and only about 13.5% of 37 examined Klar A homozygous embryos were quantified as +.

The cortex accumulation of Wolbachia in Klar A heterozygous was only observed at early cycles.

Although only 9 Klar A heterozygous embryos were quantified compared with 37 Klar A homozygous about 3.5% more embryos showed strong Wolbachia accumulation (+++) in Klar A heterozygous. But no Klar A heterozygous embryo was quantified as ++ (see figure 2.10).

Looking at the calculated averages (table 2.11 and 2.12) it seems that in Klar A homozygous Wolbachia is accumulated more in the posterior region than in Klar A heterozygous. However it has to be mentioned that the difference between these two values is only about 0.12 and additionally only 9 Klar A heterozygous embryos were examined which are fixed at the early cycles compared with Klar A homozygous where 37 embryos were fixed at early cycles.

2.3.6 Klar A fertilized embryos at mid cycles and late cycles

Figure 2.11 shows the quantification results of Wolbachia in fertilized embryos of Klar A homozygous and Klar A heterozygous which are fixed at the mid cycles. The big differences between both can be seen at the categories r and +++. About 55% of 29 examined Klar A homozygous embryos fixed at mid cycles show a small ring pattern at the posterior region whereas in Klar A heterozygous embryos 10% of 10 examined Klar A heterozygous embryos fixed at mid cycles show also this pattern. At the category +++ in figure 2.11 the difference between Klar A homozygous and heterozygous is about 50% whereas in the categories ++ and + no significant difference can be seen.

The medium ring pattern which was only observed in Klar A homozygous could be found only in those embryos which are fixed at the mid cycles (see figure 2.11).

Looking at table 2.13 and 2.14 it is interesting that the average values between Klar A homozygous and heterozygous are about 0.9 and 2.3 respectively. So this shows that Wolbachia is weak accumulated at the posterior region in those embryos which are fixed at mid cycles compared with Klar A heterozygous. But it has to be mentioned again that only 10 Klar A heterozygous embryos were fixed at mid cycles compared with Klar A homozygous where 29 embryos were fixed at mid cycles.

The quantification results at the late cycles of the cellularization phase are not really exciting. Looking at figure 2.12 all embryos of Klar A homozygous and Klar A heterozygous which are fixed at late cycles show that Wolbachia is accumulated strong till weak at the posterior region. Interestingly the tables 2.15 and 2.16 show that the average value of Klar A homozygous is about 2.2 and for Klar A heterozygous the average value is about 1.9. Also here like in Klar ΔS-15 Wolbachia is stronger accumulated at the posterior region in the homozygous ones than in the heterozygous ones. However it has to be mentioned that 48 Klar A homozygous embryos which are fixed at late cycles were examined and only 22 Klar A heterozygous embryos which are fixed at late cycles were examined.

2.3.7 Klar YG3 fertilized embryos

Figure 2.13 represents the overall quantification results of Wolbachia in fertilized embryos of Klar YG3 homozygous and heterozygous. As it can be seen the quantification results of Klar YG3 homozygous are more spreaded through the eight categories than of Klar YG3 heterozygous. However it has to be mentioned that 38 Klar YG3 heterozygous embryos were examined compared with 60 Klar YG3 homozygous embryos. It is obvious in figure 2.13 that for the first time a big ring pattern at the posterior region was observed only in Klar YG3 homozygous (see Figure 2.1G). 5% of 60 examined Klar YG3 homozygous embryos showed the big ring pattern. Interestingly the medium ring pattern and cortex accumulation was also only found in Klar YG 3 homozygous which is about 6% and 2% of 60 examined Klar YG3 homozygous embryos respectively. At the category small ring pattern in figure 2.13 more Klar YG3 heterozygous embryos showed this pattern than Klar YG3 homozygous. The difference between the homozygous ones and heterozygous ones is about 4% (see table 2.17 and 2.18). A big difference can be seen at the category + too. About 58% of 38 examined Klar YG3 heterozygous showed that Wolbachia is weak accumulated at the posterior region whereas 30 % of 60 examined Klar YG3 homozygous embryos showed this pattern. No big difference can be observed at the category ++ in figure 2.13. In the category +++ about 21% of 60 Klar YG3 homozygous embryos showed that Wolbachia is strong accumulated at the posterior region. Whereas in Klar YG3 heterozygous about 5% of 38 total examined embryos showed that Wolbachia is strong accumulated at the posterior region.

Looking at the average values in table 2.17 and 2.18 it is obvious that Wolbachia is more accumulated at the posterior region in Klar YG3 homozygous than in Klar YG3 heterozygous. However it has to be mentioned again that the average values do not include the small, medium and strong ring structures and also not the cortex accumulation.

All in all it seems that Wolbachia is mostly weak accumulated at the posterior region in Klar YG3 heterozygous whereas in Klar YG3 homozygous no trend is visible because of the different localization patterns of Wolbachia.

2.3.8 Klar YG3 fertilized embryos at early cycles

It has to be mentioned that for Klar YG3 heterozygous only 4 embryos were fixed at early cycles compared with 27 Klar YG3 homozygous embryos. But looking at figure 2.14 and compare it with figure 2.15 and 2.16 the strong ring structures were observed only at early cycles of the cellularization phase. The same can be said for the medium ring pattern at the posterior region. 50% of the 4 Klar YG3 heterozygous embryos which were fixed at the early cycles showed that Wolbachia is weak accumulated at the posterior region and the other 50% showed a small ring pattern. Looking at figure 2.14 in Klar YG3 homozygous where 27 embryos were fixed at early cycles no trend can be observed. The quantification results for the homozygous one are spreaded through the different categories.

Moreover the tables 2.19 and 2.20 show that Wolbachia is stronger accumulated at the posterior region in Klar YG3 homozygous with the different average values of 1.3 for Klar YG3 homozygous and 0.5 for Klar YG3 heterozygous.

2.3.9 Klar YG3 fertilized embryos at mid and late cycles

Looking at figure 2.15 which represents the quantification results of Wolbachia in fertilized embryos of Klar YG3 homozygous and Klar YG3 heterozygous at mid cycles it can be seen that more than 70% of 4 examined Klar YG3 heterozygous embryos showed a weak Wolbachia accumulation at the posterior region. The rest showed strong Wolbachia accumulation at the posterior region. Interestingly in the 8 Klar YG3 homozygous which were fixed at the mid cycles figure 2.15 shows that +++,++,r and mR have the same percent value of 25%.

The average values which can be seen in the table 2.21 and 2.22 are 1.25 for Klar YG3 homozygous and 1.5 for Klar YG3 heterozygous. Also here it can be seen that in Klar YG3 heterozygous a trend is visible whereas in Klar YG3 homozygous the quantification results are spreaded through the different categories.

The last figure of fertilized embryos (figure 2.16) shows the quantification results of Klar YG3 homozygous and heterozygous at the late cycles of the cellularization phase. It can be seen that the small ring pattern which was observed in Klar YG3 heterozygous was only observed in the late cycles (13.33% of 30 Klar YG3

heterozygous embryos). About 55% of 30 examined Klar YG 3 heterozygous embryos fixed at late cycles showed weak Wolbachia accumulation at the posterior region. Out of the 30 examined Klar YG3 heterozygous embryos fixed at the late cycles showed about 26% medium Wolbachia accumulation at the posterior region and about 3% weak Wolbachia accumulation at the posterior region. For Klar YG3 homozygous the quantification results shown in figure 2.16 are only located in the categories +++, ++, and +. 12% of 25 examined Klar YG3 homozygous embryos fixed at the late cycles showed strong Wolbachia accumulation at the posterior region. Whereas 40% and 48% of 25 examined Klar YG3 homozygous embryos fixed at the late cycles showed medium and weak Wolbachia accumulation respectively.

The table 2.23 and 2.24 show that Wolbachia is more accumulated at the posterior region in Klar YG3 homozygous embryos than in Klar YG3 heterozygous embryos with the calculated average values of 1.64 and 1.2 respectively.

2.3.10 Klar ΔS-15 unfertilized embryos

Beside the Wolbachia accumulation at the posterior region only a homogenous distribution of Wolbachia was observed in Klar ΔS-15 homozygous looking at figure 2.17. About more than 5% of 19 examined Klar ΔS-15 homozygous embryos showed a homogenous distribution of Wolbachia. The same could not be found in Klar ΔS-15 heterozygous. For Klar ΔS-15 heterozygous 21 embryos were examined and about 48% of 21 embryos showed strong Wolbachia accumulation at the posterior region. The rest of about 28% and 23% showed medium and weak Wolbachia accumulation at the posterior region respectively. Nearly the same values were gained for Klar ΔS-15 homozygous embryos at the categories +++, ++ and + in figure 2.17. About 43% of 19 examined Klar ΔS-15 homozygous embryos showed strong Wolbachia accumulation at the posterior region. The rest of about 27% and 27% showed medium and weak Wolbachia accumulation at the posterior region respectively.

The tables 2.25 and 2.26 also indicate that Wolbachia is more accumulated at the posterior region in Klar ΔS-15 heterozygous embryos than in Klar ΔS-15 homozygous embryos with average values of 2.05 and 2.23 respectively.

All in all it can be said that indeed Klar ΔS-15 homozygous has an effect concerning the Wolbachia localization.

2.3.11 Klar A unfertilized embryos

Figure 2.18 which represent the quantification results of Wolbachia in Klar A unfertilized embryos it can be seen that about 11.11% of 18 examined Klar A homozygous embryos showed a small ring pattern at the posterior region whereas in Klar A heterozygous this pattern was not observed. Interestingly about 72% of 18 examined Klar A homozygous embryos showed strong Wolbachia accumulation and 5% showed that Wolbachia is homogenous distributed and about 11% showed that Wolbachia is weak accumulated at the posterior region. The quantification results of Klar A heterozygous where 39 embryos were examined about more than 50% showed strong Wolbachia accumulation at the posterior region. About 33% of 39 examined Klar A heterozygous embryos showed medium Wolbachia accumulation at the posterior region and about 15% of 39 examined Klar A heterozygous embryos showed weak Wolbachia accumulation at the posterior region. Looking at the table 2.27 and 2.28 it can be seen that Wolbachia is stronger accumulated at the posterior region in Klar A homozygous than in Klar A heterozygous (2.7 and 2.4 respectively).

Although the the calculated average of the examined Klar A homozygous embryos is higher than in Klar A heterozygous it seems that Klar A homozygous has an impact concerning the Wolbachia localization in unfertilized embryos otherwise no small ring pattern would not occur.

2.4 Discussion

The question if Klar is needed for the localization of Wolbachia at the posterior regin in embryos of *D. melanogaster* can be answered with yes. Our results showed that nine different patterns were observed. Beside the three patterns where Wolbachia is strong till weak accumulated at the posterior region also small till big ring patterns were observed. Looking at figure 2.1 E-G it can be seen that Wolbachia can not enter the germ line which is located at the posterior region. It seems that due to the mutation of Klar the regulation of the motor proteins is impaired. In a three dimensional picture the ring structure would look like a donut. So in the middle of the donut which should be the germ cells at the posterior region Wolbachia is missing there.
Also the other pattern (see figure 2.1 H) show that Wolbachia can not go to the top of the posterior region where the germ cells are located (Jaglarz and Howard, 1995). The last localization pattern of Wolbachia showed that Wolbachia is not located in the area of the posterior region. It is located at the cortex (see figure 2.1 I). However there it can be seen that Wolbachia is strongly accumulated at the nucleus but mainly at one side. So these five Wolbachia localization patterns which are not reported in the study of Veneti *et al.* indicate that Klar must be an important regulator for the motor proteins and also that Wolbachia use motor proteins.

However three questions are still unanswered right now. The first question is why Wolbachia shows so many different localization patterns in unfertilized and fertilized embryos? One explanation might be the following. In vitro single motor proteins fall off after a certain traveled distance on the microtubule track. So in vivo like in the fixed embryos this would mean that after a certain time a single motor protein is changed by a new one. So for this switch there must be a regulation (Gross *et al*., 2000).
In case of a mutation in the Klar protein this could mean that turn on and turn off of the motor proteins do not work very well anymore. Looking at the quantification results of Klar A for example it is interesting that in the fertilized and unfertilized

embryos the small ring structure occurred. The Klar allele A belongs to the class I allele which is known that they disrupt the net apical droplet transport in phase III. The allele Klar A induces a stop codon in exon 3 (Guo *et al.,* 2005).

So this stop codon could induce a conformational change of the protein Klar which is not able to interfere with the known dynein cofactor dynactin very well anymore for example (Welte, 2009).

It could be also possible that Klar interferes with the protein LSD 2. This protein regulates the lipid droplet motion and metabolism. Interestingly it was also observed that when embryos are centrifuged the regulator Klar accumulates on one side of the embryo. The same happened with LSD 2 too. Also this protein accumulated at the same side as the regulator Klar. (Welte *et al*., 2005)

If the mutated Klar interferes with dynactin and/or LSD 2 this could mean that the cellular function of dynein is impaired and a tug of war between kinesin and dynein occurs. Therefore the switch between the different motor types is much more difficult due to this mutation and the ring pattern for example is an indicator that something is wrong with the coordination of the motor proteins.

A better understanding for these thoughts should give the quantification results of Klar YG3 in fertilized embryos. It was interesting that only in this Klar allele big ring patterns occurred. The big ring pattern indicates that Wolbachia is far away from germ line which is located at the posterior region (Jaglarz and Howard, 1995).

The Klar allele YG3 has a ~11kb deletion around exon 0. As already mentioned in the introduction studies showed that the different Klar isoforms (α, β and γ) are generated not only by alternative splicing but also by different promotors. One promoter region is located at exon 0 (Guo *et al.,* 2005).

So it could be possible that with this mutation an important amino acid sequence is missing which normally makes it easier to go to the posterior region for Wolbachia. This could mean that the mutated Klar protein induced by the Klar promoter deletion allele YG3 interacts or not for example with dynactin, LSD 2 and Wolbachia. The result would be that the molecular signaling pathway between the motor proteins, their regulators and Wolbachia is impaired. Therefore Wolbachia can not go to the posterior region because of the dysfunction of the molecular signaling pathway between motor proteins and their regulators.

The second question which has to be answered is why the biggest differences between homozygous and heterozygous of the all three Klar alleles were observed in the early cycles of the cellularization phase?
Studies with lipid droplets in Klar embryos showed that a difference of travel velocity and travel distance compared with wild type embryos can be especially recognized in phase II which is the beginning of cycle 14 until the end of cellularization phase (Welte *et al.,* 1998).
In our case the biggest differences between the homozygous and heterozygous ones of all three Klar alleles were in the first four cycles of the cellularization phase. It seems that for Wolbachia it is very important to come to the germ line in the embryos very early in order to be transmitted to the next generation.
Studies from Veneti *et al.* showed that there is positive correlation between level of cytoplasmic incompatibility (CI) and the Wolbachia accumulation in the posterior region of embryos. Additionally the study of Veneti *et al.* tells that a high Wolbachia density in the posterior region of the embryos causes a high level of cytoplasmic incompatibility. This could be one reason why Wolbachia tries to get to the germ line as early as possible in order to be maintained in a host population (Charlat *et al.,* 2004). Another and interesting explanation why the biggest difference between homozygous and heterozygous embryos of all three Klar alleles were found at early cycles of the cellularization phase could be the following. Studies showed that the vital transepithelial migration of the germ cells in the embryos is initiated by changes in the structure of the endodermal epithelium and not by the motile machinery of the germ cells (Jaglarz and Howard, 1995).
So this could be a reason why the biggest differences between the homozygous and heterozygous embryos of all three Klar alleles were found at early cycles. Therefore for Wolbachia it is important to go to the germ cells as early as possible because otherwise it would be too late and Wolbachia can not be transmitted maternally anymore.

The last question which has to be answered is why in all three Klar alleles Wolbachia is stronger accumulated at the posterior region in the homozygous

ones than in the heterozygous ones. As already mentioned above the transepithelial migration of the germ cells in the embryos is initiated by changes in the structure of the endodermal epithelium and not by the motile machinery of the germ cells (Jaglarz and Howard, 1995). So this could mean that Wolbachia moves with the germ line in order to stay there in the cytoplasm. In case of a mutation in the regulation of the motor proteins Wolbachia can not move back and forth and therefore sticks at the posterior region. However no investigations were made in the gastrulation phase because this would be the next phase after the cellularization. Although the quantification in embryos which are already in the gastrulation phase is more difficult it could be possible that at least with Klar YG3 localization differences between Klar YG3 homozygous and heterozygous would be observed.

3 OUTLOOK

Although a fecundity difference between the Wolbachia subtype 01 and 07 can be found in *D. mauritiana* the question remains if Wolbachia increases the fecundity in this host. In order to proof this a negative control has to be taken. The flies have to be treated with antibiotics. After the treatment with antibiotics it should be waited until the 5th generation is born because the side effects of the antibiotic treatment are already low in this generation. Then the non infected flies are crossed as already described in subchapter 1.2. Additionally the negative control should be tested against Wolbachia with the PCR. It would be also interesting if there are differences of the transmission rate of Wolbachia 01 and 07. For this the qPCR can be taken.

For the Klar project the next step would be to knock out the Klar gene with the GAL 4 system and to what is going on in the embryos. Moreover it would be also interesting how Wolbachia behaves in the testis if Klar is mutated. How would Wolbachia behave in the embryos if LSD 2 mutated but not Klar? Can we see the same like in the Klar project?

4 REFERENCES

Champion de Crespigny and Nina Wedell (2006). Wolbachia infection reduces sperm competitive ability in an insect. Proc. R. Soc. B 273. 1455-1458

Charlat, J. W. O. Ballard and H. Mercot (2004). What maintains noncytoplasmic incompatibility inducing Wolbachia in their hosts: a case study from a natural *Drosophila yakuba* population. J. Evol. Biol. 17. 322-330

Dobson, Eric J. Marsland, and Wanchai Rattanadechakul (2002). Mutualistic Wolbachia infection in *Aedes albopictus*: Accelerating Cytoplasmic Drive. Genetics 160. 1087-1094

Ferree, Horacio M. Frydman, Jennifer M. Li, Jian Cao, Eric Wieschaus and William Sullivan (2005). Wolbachia utilizes host microtubules and dynein for anterior localization in the Drosophila oocyte. Plos Pathogens, Volume 1, Issue 2 ,e14. 111-124

Fry, MR Palmer and DM Rand (2004). Variable fitness effects of Wolbachia infection in *Drosophila melanogaster*. Heredity 93. 379-389

Giordano, Scott L. O'Neill and Hugh M. Robertson (1995). Wolbachia infections and the expression of cytoplasmic incompatibility in D. sechellia and D. mauritiana. Genetics 140. 1307-1317

Gross, Michael A. Welte, Steven M. Block and Eric F. Wieschaus (2000). Dynein-mediated cargo transport in vivo: A switch controls travel distance. The Journal of cell biology, Volume 148, Number 5. 945-955

Guo, Sushrut Jangi and Michael A. Welte (2005). Organelle-specific Control of intracellular transport: Distinctly targeted isoforms of the regulator Klar. Molecular Biology of the Cell Vol. 16. 1406-1416

Hoerauf, Kerstin Nissen-Pähle, Christel Schmetz, Kim Henkle-Dührsen, Mark L. Blaxter, Dietrich W. Büttner, Michaela Y. Gallin, Khaled M. Al-Qaoud, Richard Lucius and Bernhard Fleischer (1999). Tetracycline therapy targets intracellular bacteria in the fiarial nematode Litomosoides sigmodontis and results in filarial infertility. The Journal of Clinical Investigation, Volume 103, Number 1. 11-17

Jaglarz and Kenneth R. Howard (1995). The active migration of Drosophila primordial germ cells. Development 121. 3495-3503

Müller, Stefan Klumpp and Reinhard Lipowsky (2008). Tug-of-war as a cooperative mechanism for bidirectional cargo transport by molecular motors. PNAS Vol. 105, No. 12. 4609-4614

Poinsot and Herve Mercot (1997). Wolbachia infection in *Drosophila simulans*: Does the female host bear a physiological cost? Evolution 51(1). 180-186

Teixeira, Alvaro Ferreira and Michael Ashburner (2008). The bacterial symbiont Wolbachia induces resistance to RNA viral infections in Drosophila melanogaster. Plos Biology Volume 6, Issue 12. 2753-2763

Welte. Fat on the move: intracellular motion of lipid droplets (2009). Biochem. Soc. Trans. 37. 991-996

Welte and S. P. Gross (2008). Molecular motors: a traffic cop within?. HFSP Journal Vol. 2, No. 4. 178-182

Welte, Silvia Cermelli, John Griner, Arturo Viera, Yi Guo, Dae-Hwan Kim, Joseph G. Gindhart and Steven P. Gross (2005). Regulation of lipid droplet transport by the perilipin homolog LSD 2. Current Biology, Vol. 15. 1266-1275

Welte, Steven P. Gross, Marya Postner, Steven M. Block and Eric F. Wieschaus (1998). Developmental regulation of vesicle transport in Drosophila embryos: Forces and Kinetics. Cell, Vol. 92. 547-557

Werren, Laura Baldo, Michael E. Clark (2008). Wolbachia: master manipulators of invertebrate biology. Nature Reviews Microbiology Volume 6. 741-751

Veneti, Michael E. Clark, Timothy L. Karr, Charalambos Savakis and Kostas Bourtzis (2004). Heads or Tails: Host-Parasite interactions in the Drosophila-Wolbachia system. Applied and environmental microbiology, Vol. 70, No.9. 5366-5372

Zabalou, Angeliki Apostolaki, Savvas Pattas, Zoe Veneti, Charalampos Paraskevopoulos, Ioannis Livadaras, George Markakis, Terry Brissac, Herve Mercot and Kostas Bourtzis (2008). Multiple rescue factors within a Wolbachia strain. Genetics 178. 2145-2160

5 APPENDIX

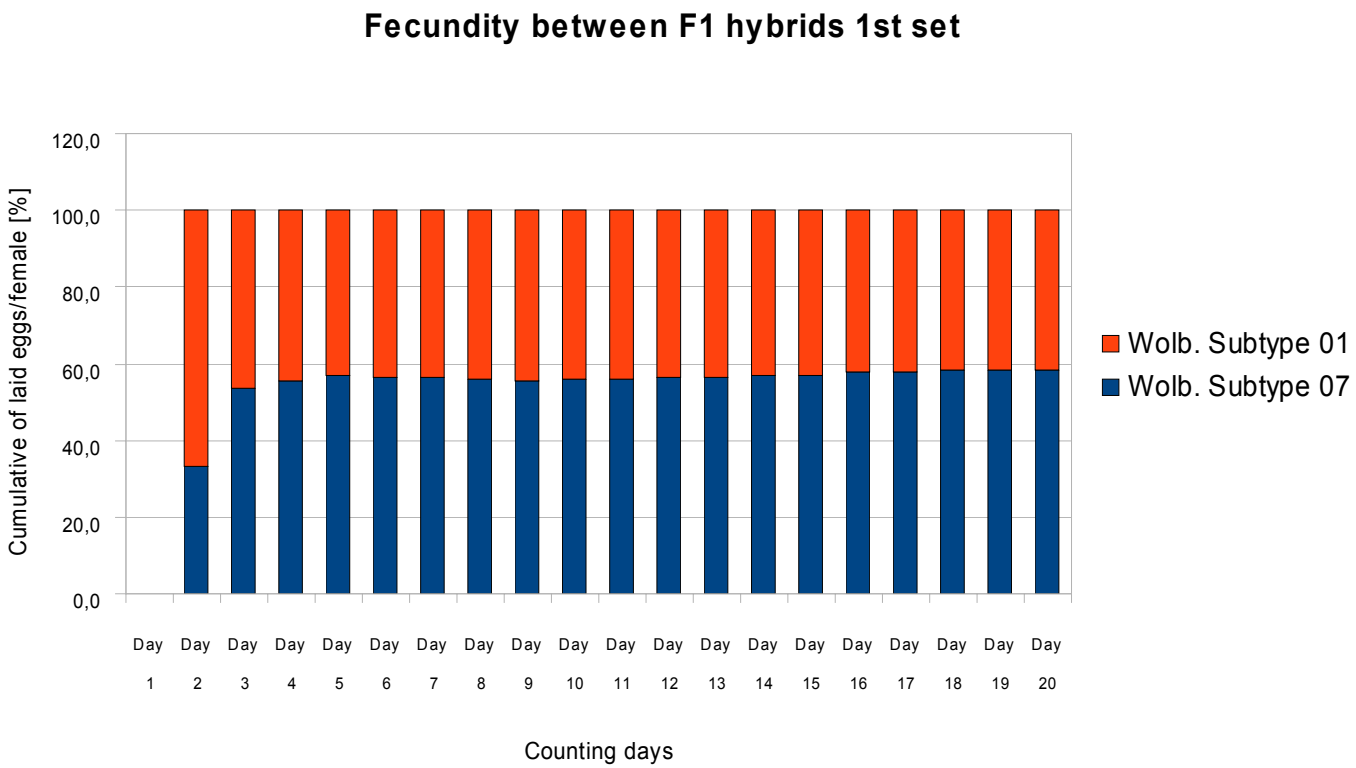


Figure 1.1: It shows the cumulative of laid eggs per female in percent of the F1 hybrids from the first set as a function of 20 counting days. The blue bars represent the F1 hybrids which are infected with the Wolbachia subtype 07. The orange bars represent the F1 hybrids which are infected with the Wolbachia subtype 01.

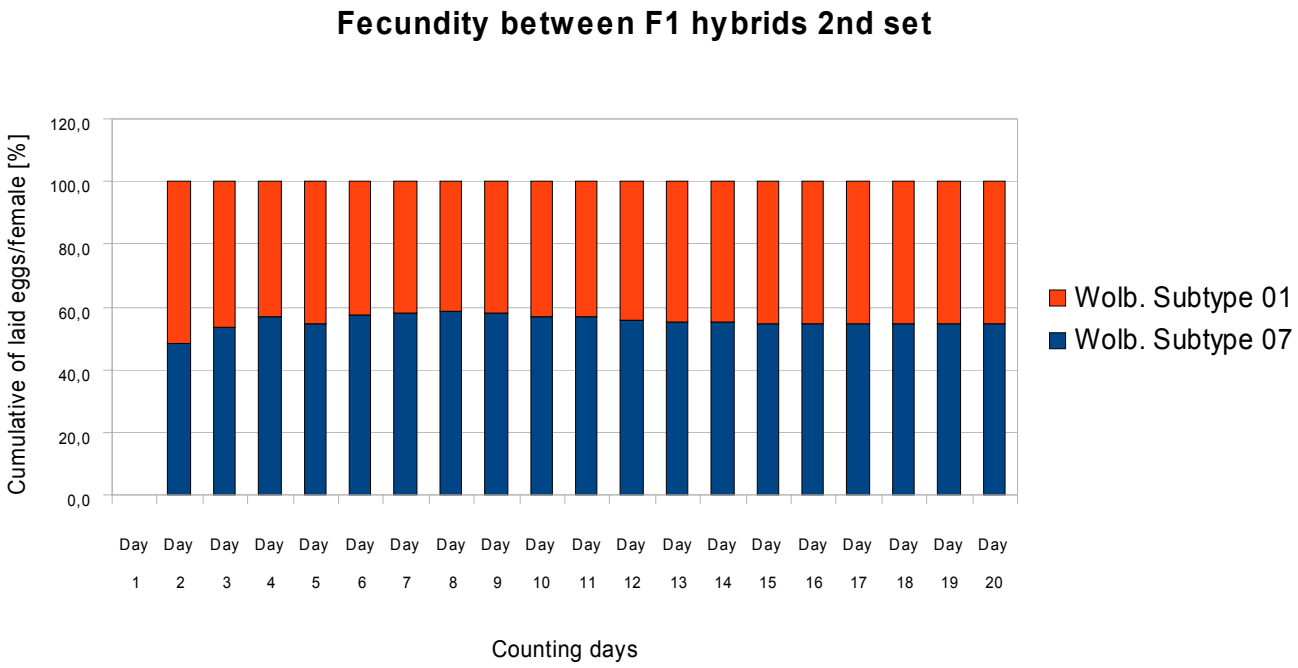


Figure 1.2: It shows the cumulative of laid eggs per female in percent of the F1 hybrids from the second set as a function of 20 counting days. The blue bars represent the F1 hybrids which are infected with the Wolbachia subtype 07. The orange bars represent the F1 hybrids which are infected with the Wolbachia subtype 01.

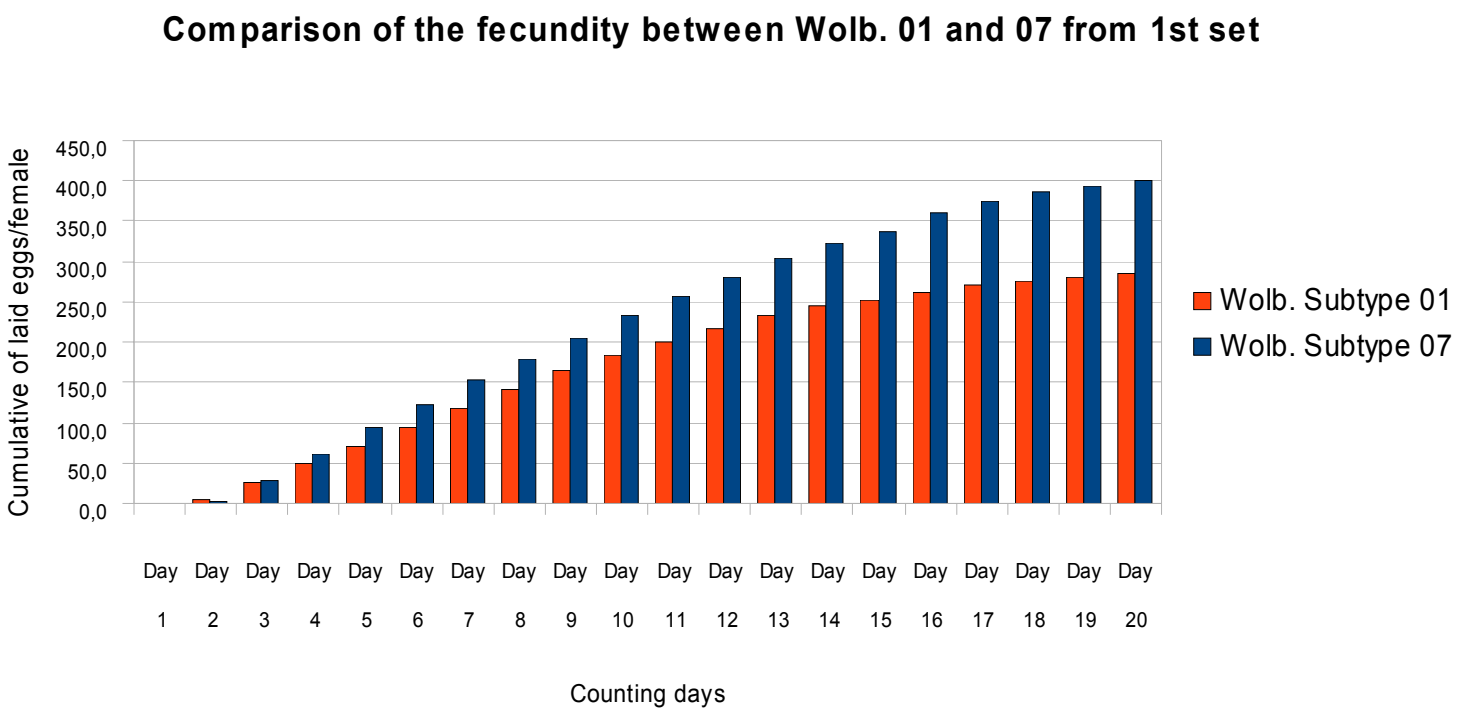


Figure 1.3: It shows the cumulative of laid eggs per female of the F1 hybrids from the first set as a function of 20 counting days. The blue bars represent the F1 hybrids which are infected with the Wolbachia subtype 07. The orange bars represent the F1 hybrids which are infected with the Wolbachia subtype 01.

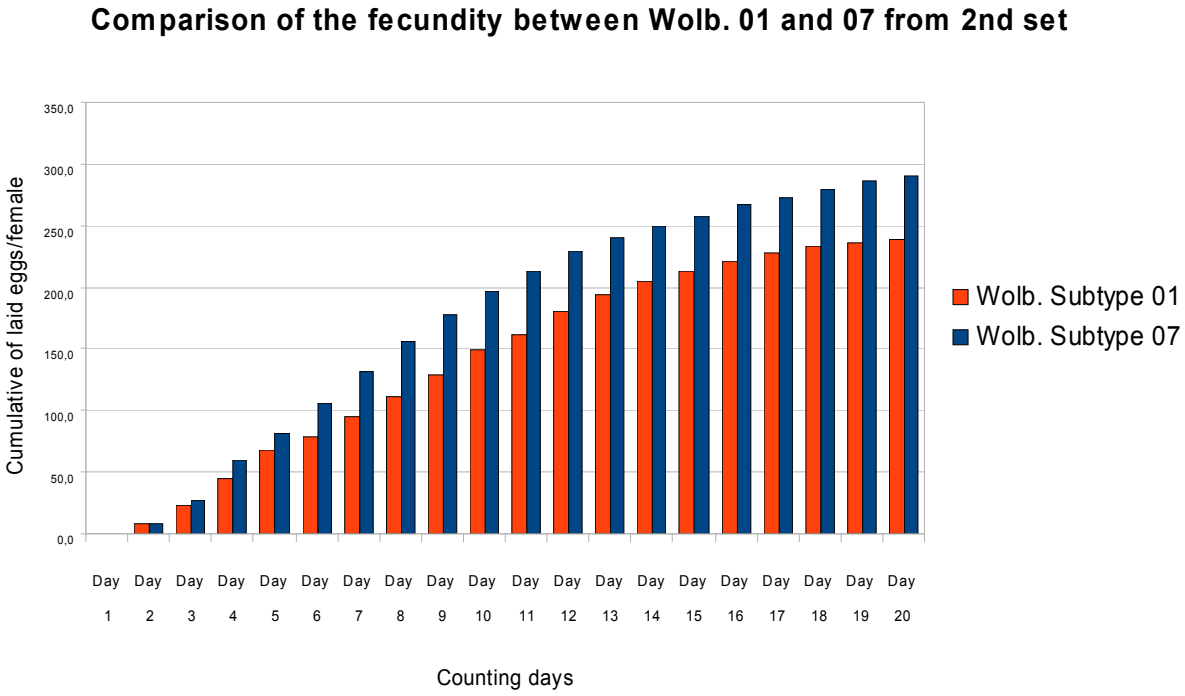


Figure 1.4: It shows the cumulative of laid eggs per female of the F1 hybrids from the second set as a function of 20 counting days. The blue bars represent the F1 hybrids which are infected with the Wolbachia subtype 07. The orange bars represent the F1 hybrids which are infected with the Wolbachia subtype 01.

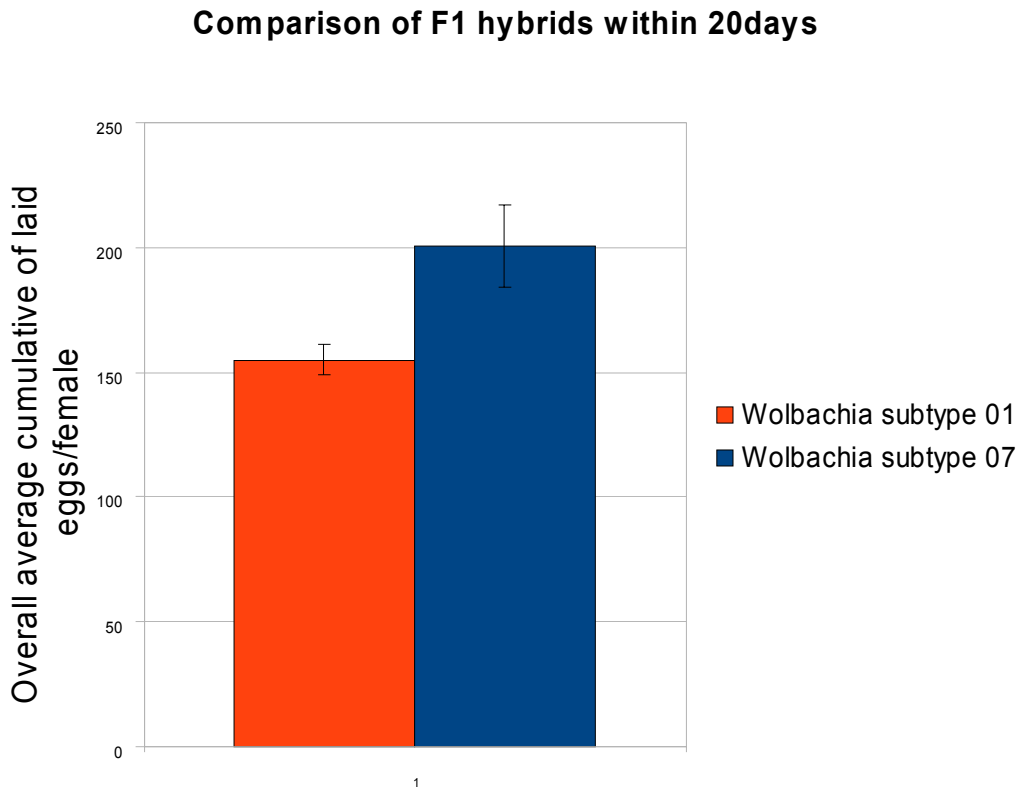


Figure 1.5: It shows the overall average cumulative of laid eggs per female as a function of 20 counting days. The blue bar represents those F1 hybrids which are infected with the Wolbachia subtype 01. The orange bar represents those F1 hybrids which are infected with the Wolbachia subtype 07.

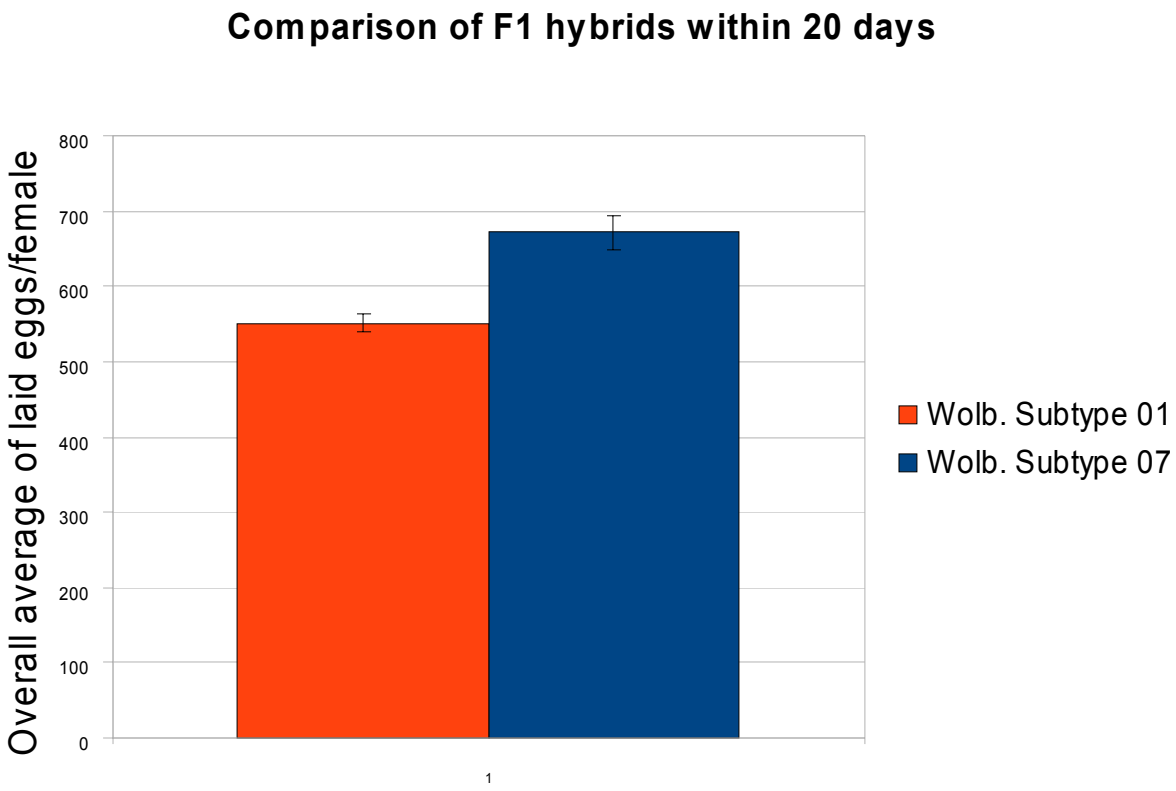


Figure 1.6: It shows the overall average of laid eggs per female as a function of 20 counting days. The blue bar represents those F1 hybrids which are infected with the Wolbachia subtype 01. The orange bar represents those F1 hybrids which are infected with the Wolbachia subtype 07.

	Cumulative of laid eggs/female at 20th day of the 1st set	**Cumulative of laid eggs/female at 20th day of the 2nd set**
Wolbachia subtype 01	284.0	239.4
Wolbachia subtype 07	401.3	289.9

Table 1.1: It shows the cumulative of laid eggs per female from the F1 hybrids which are infected with the Wolbachia subtype 01 from the first and second set at the 20th counting day. Also the cumulative of laid eggs per female from the F1 hybrids which are infected with the Wolbachia subtype 07 from the first and second set at the 20th counting day is shown.

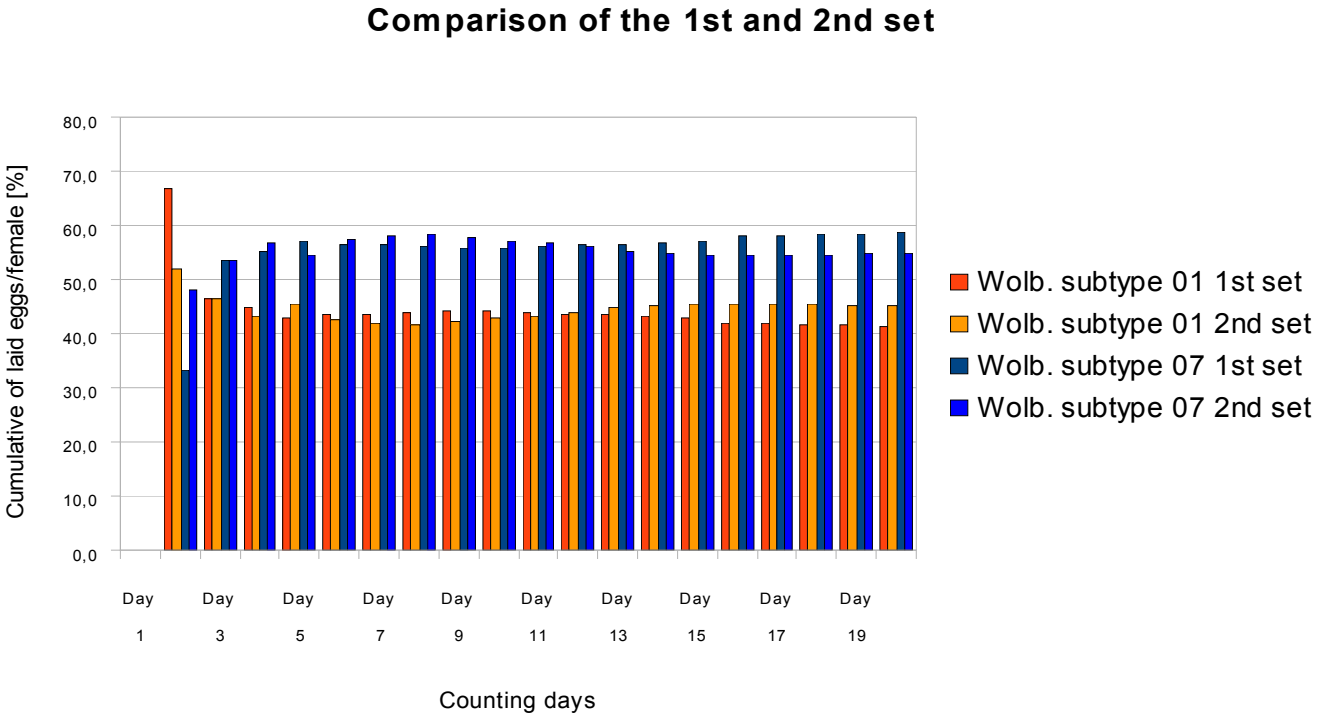
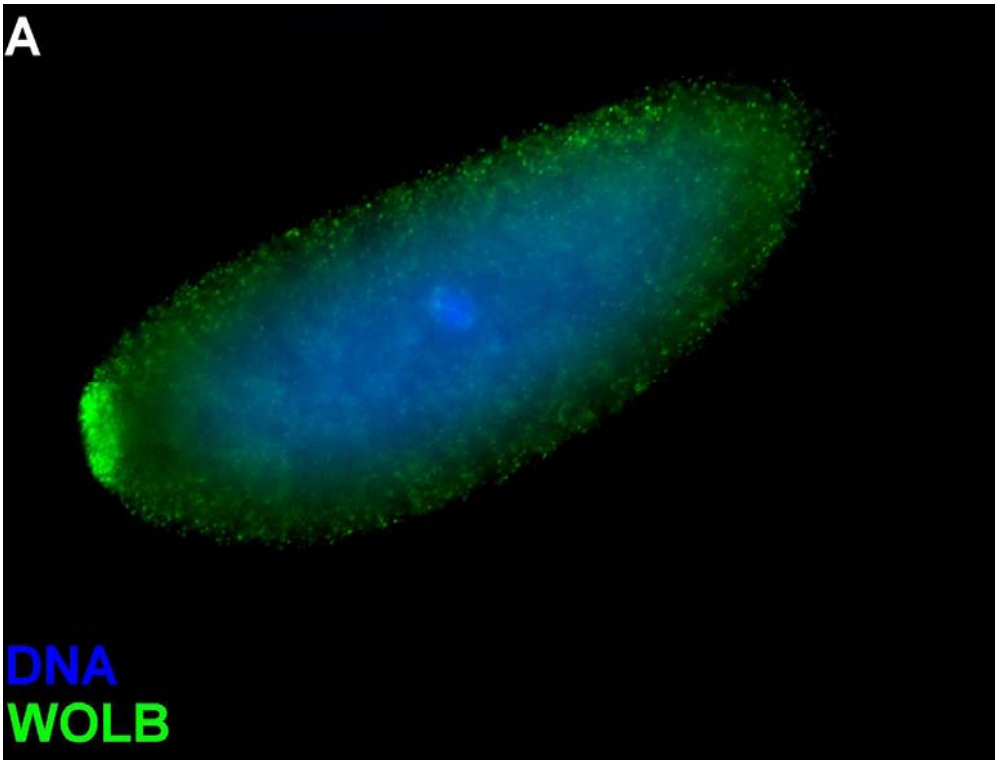


Figure 1.7: It shows the cumulative of laid eggs per female in percent as a function of 20 counting days. The blue bars represent those F1 hybrids which are infected with the Wolbachia subtype 01 from the 1st set. The orange bars represent those F1 hybrids which are infected with the Wolbachia subtype 01 from the 2nd set. The yellow bars represent those F1 hybrids which are infected with the Wolbachia subtype 07 from the 1st set. The green bars represent those F1 hybrids which are infected with the Wolbachia subtype 07 from the 2nd set.

Sample	n	U_a	critical U values (min.; max.)	z	α	MR (1st set; 2nd set)
Wolb. 01	20	257.5	127;273	-1.54	0.05	17.6; 23.4
Wolb. 07	20	142.5	127;273	1.54	0.05	23.4; 17.6

Table 1.2: The table shows the results of the U-Test. The cumulative of laid eggs per female in percent of the Wolbachia subtype 01 from the 1st and 2nd set were tested against each other within 20 days. Also the cumulative of laid eggs per female in percent of the Wolbachia subtype 07 from the 1st and 2nd set were tested against each other within 20 days. For both Wolbachia subtypes a sample number of n=20 was taken which are the 20 counting days. An U_a value of 257.7 based on the cumulative of laid eggs per female in percent of the Wolbachia subtype 01 from the 1st set was obtained. For the Wolbachia subtype 07 an U_a value of 142.5 based on the cumulative of laid eggs per female in percent from the 1st set is obtained. It is assumed that the significance level is α= 0.05 and therefore the lower critical value of U is 127 and the upper critical value of U is 273. The mean ranks of Wolbachia 01 and 07 from the 1st set and 2nd set are (17.6; 23.4) and (23.4; 17.6) respectively.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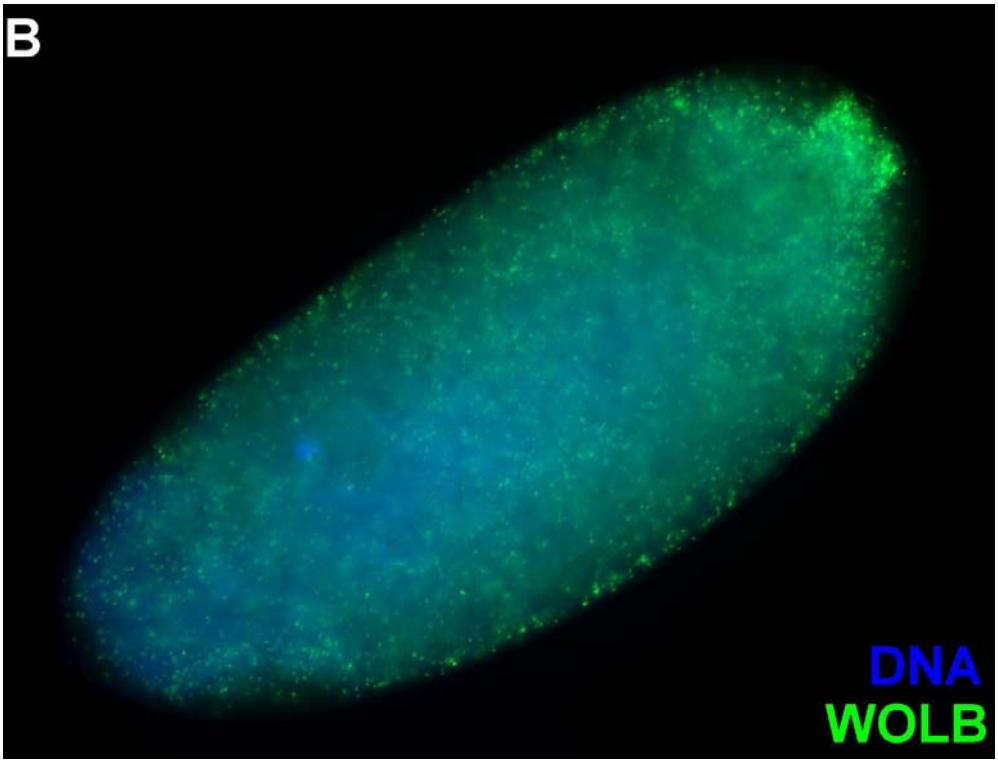
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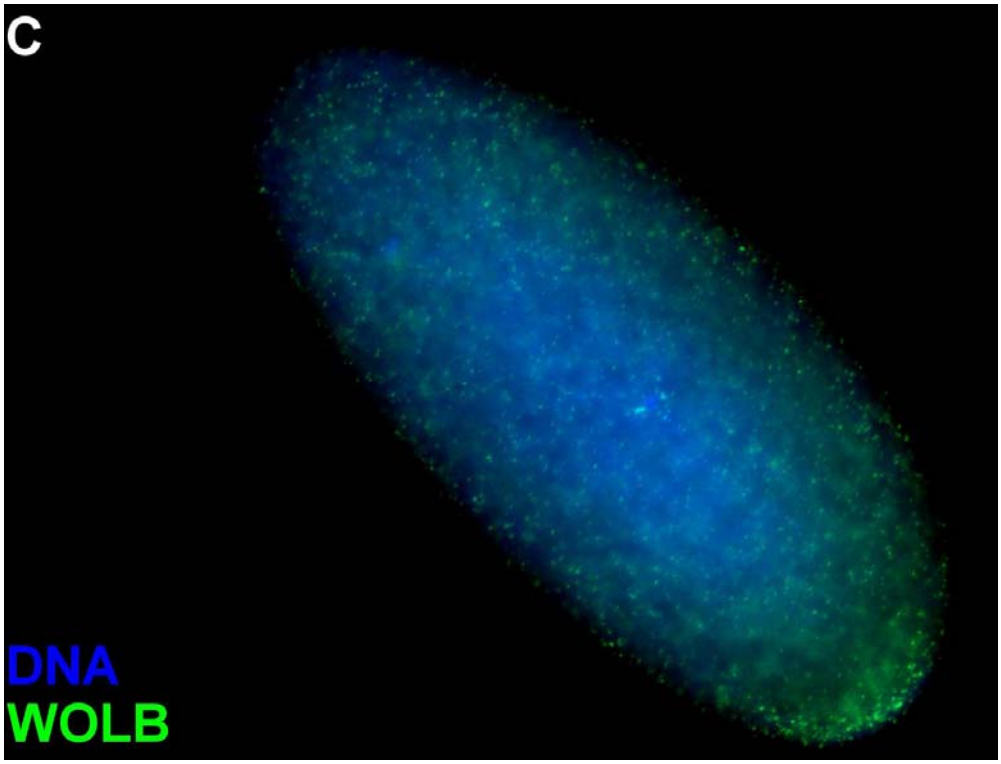
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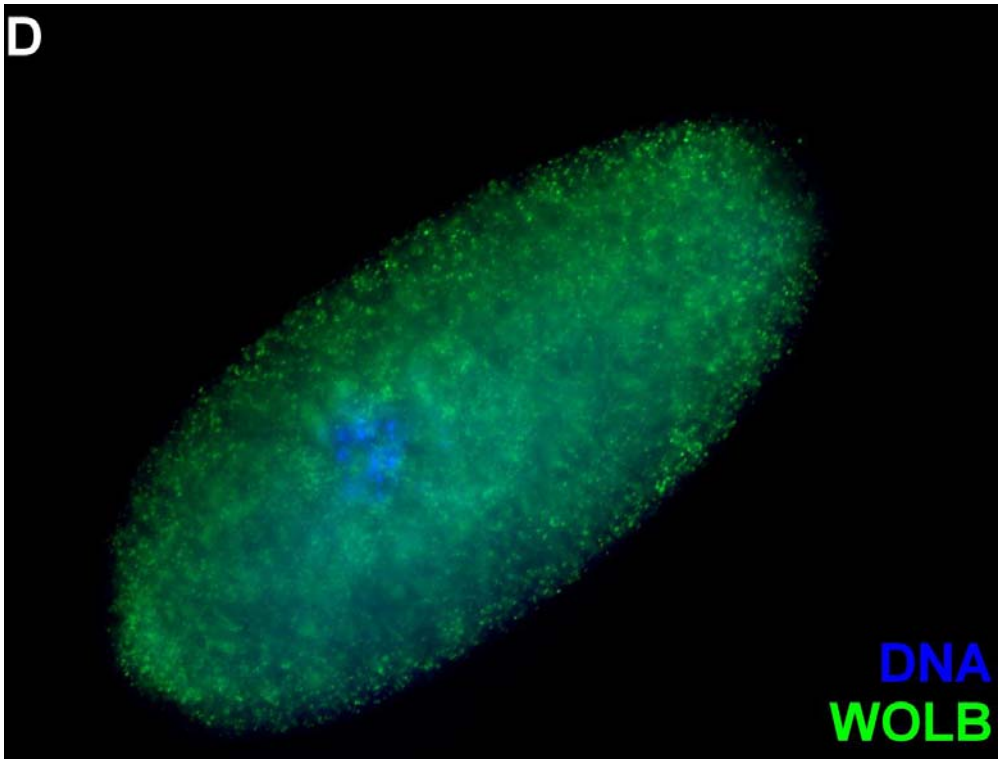
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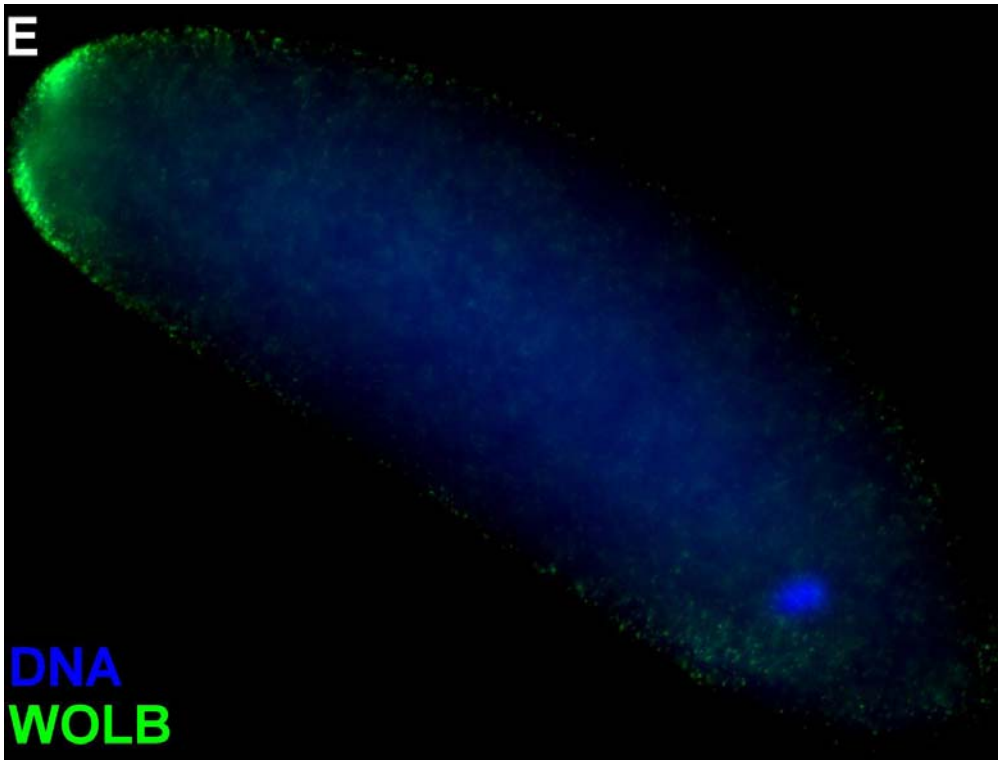
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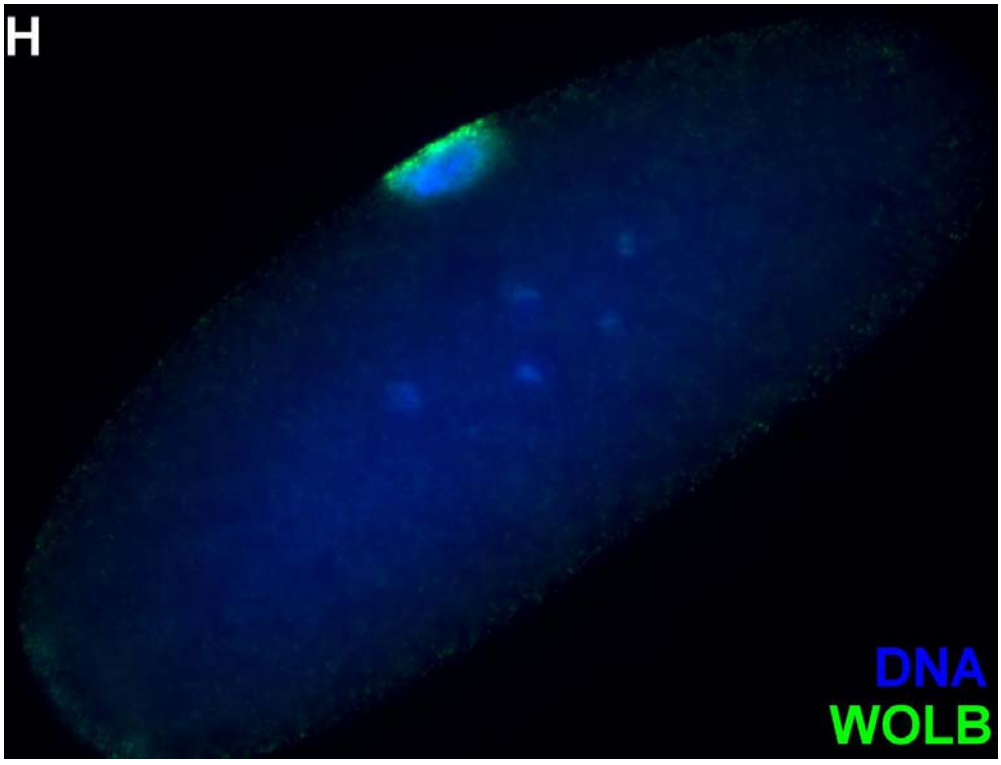
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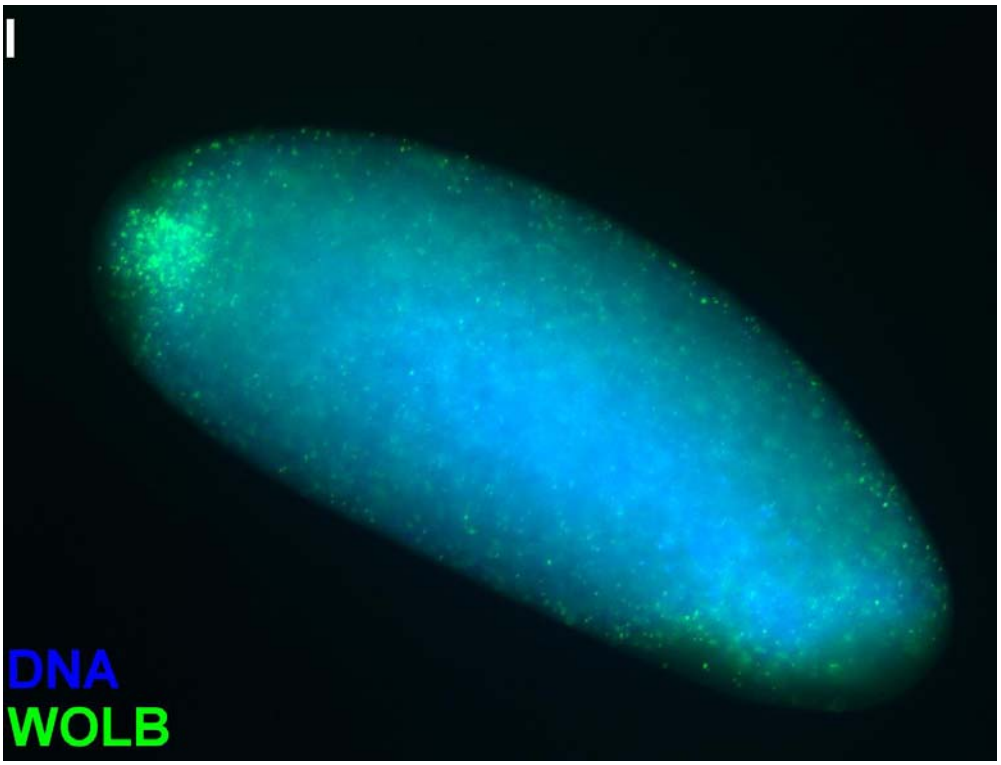
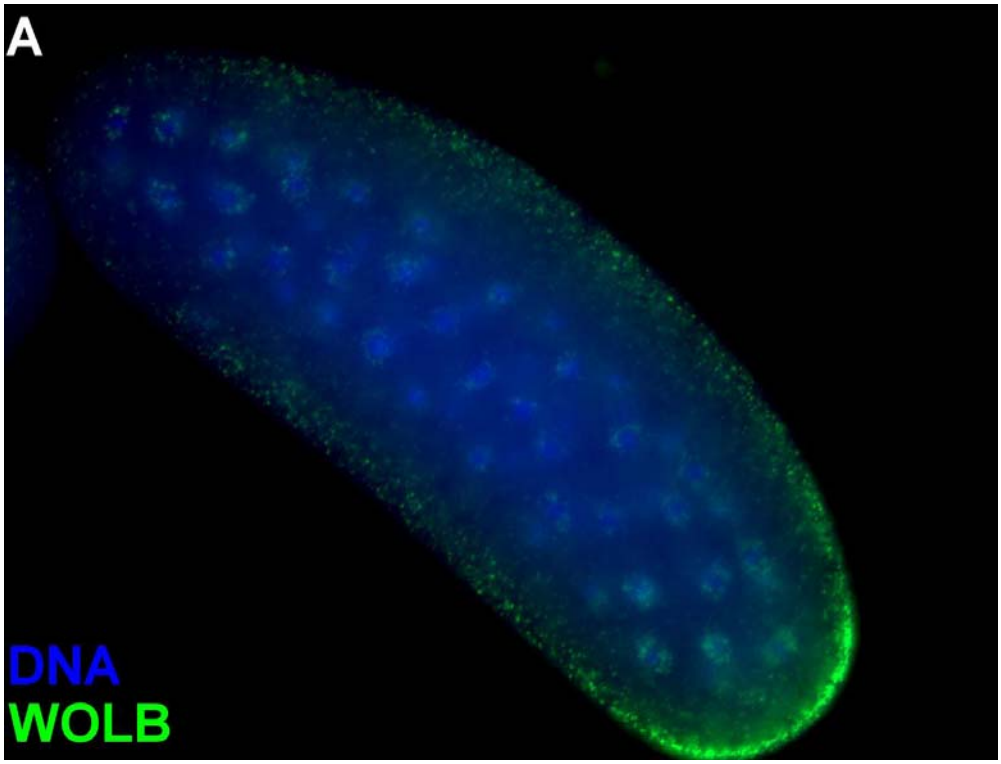


Figure 2.1: It shows the eight observed localization patterns of Wolbachia in fertilized embryos fixed at the early cycles of the cellularization phase. Green shows Wolbachia and blue shows DNA. A) Strong Wolbachia accumulation at the posterior region (+++). B) Medium Wolbachia accumulation at the posterior region (++). C) Weak Wolbachia accumulation at the posterior region (+). D) Homogenous distribution of Wolbachia (0). E) Small ring pattern at the posterior region (r). F) Medium ring pattern at the posterior region (mR). G) Big ring pattern at the posterior region (R). H) Wolbachia is accumulated at the cortex (c). I) Wolbachia is not accumulated at the posterior top (n).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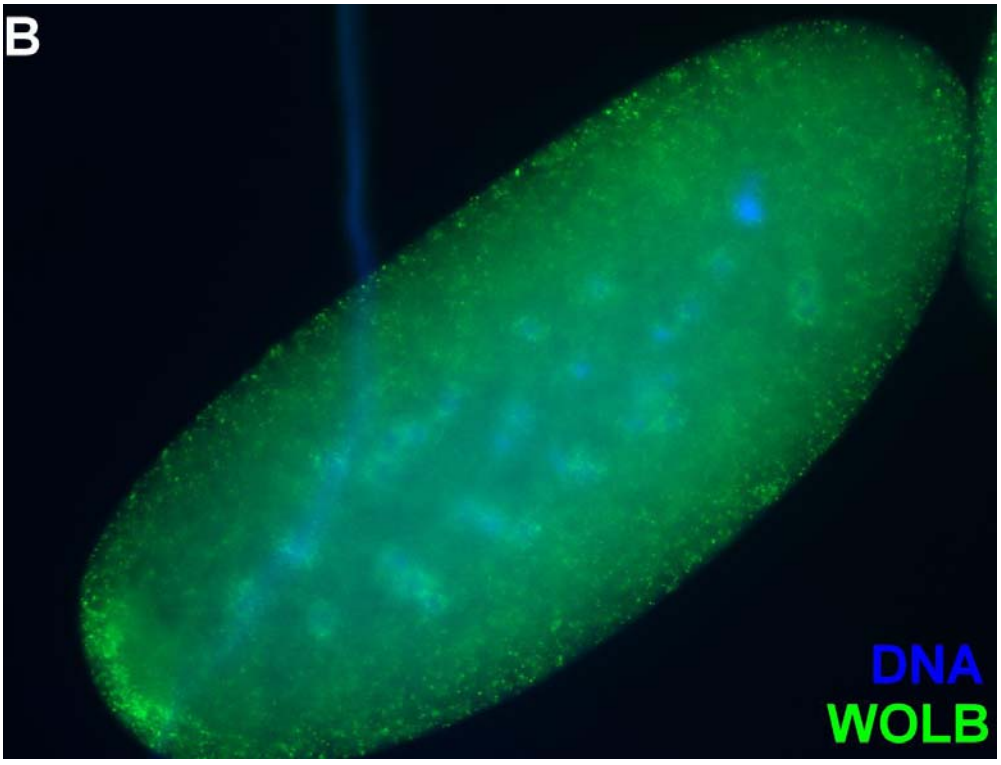
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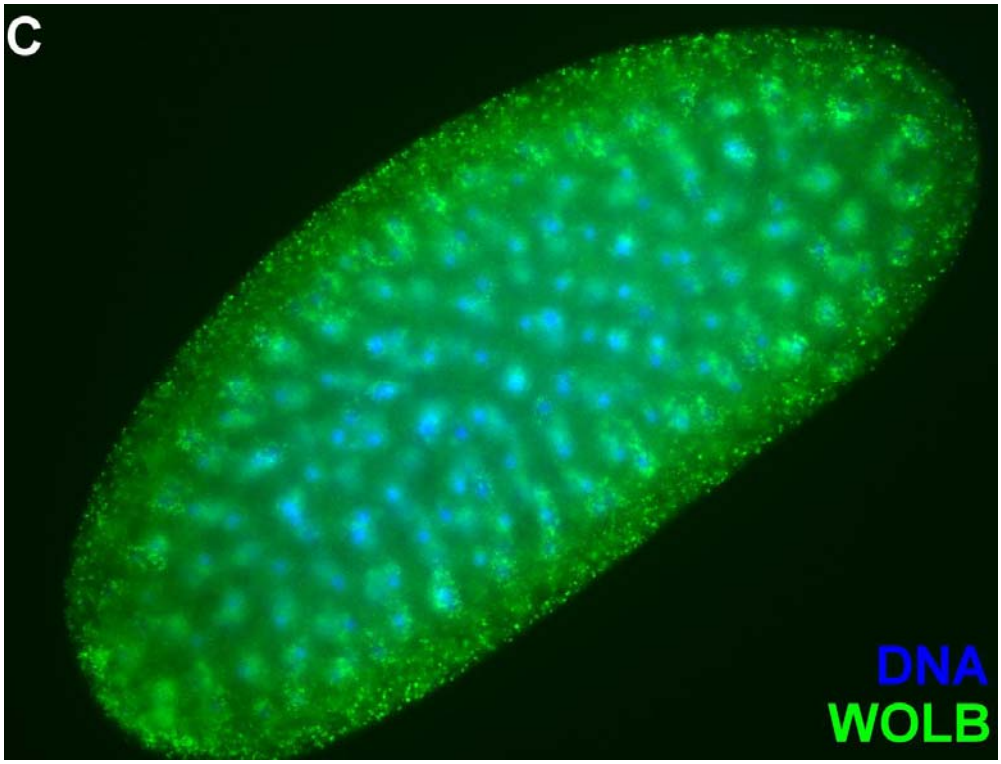
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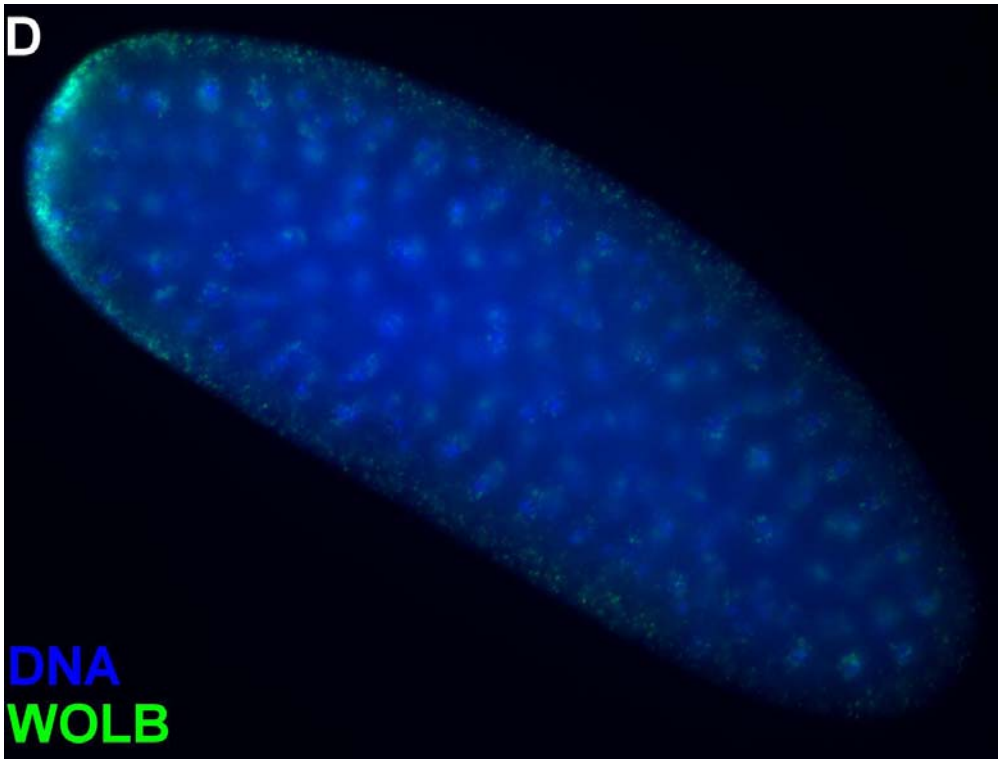
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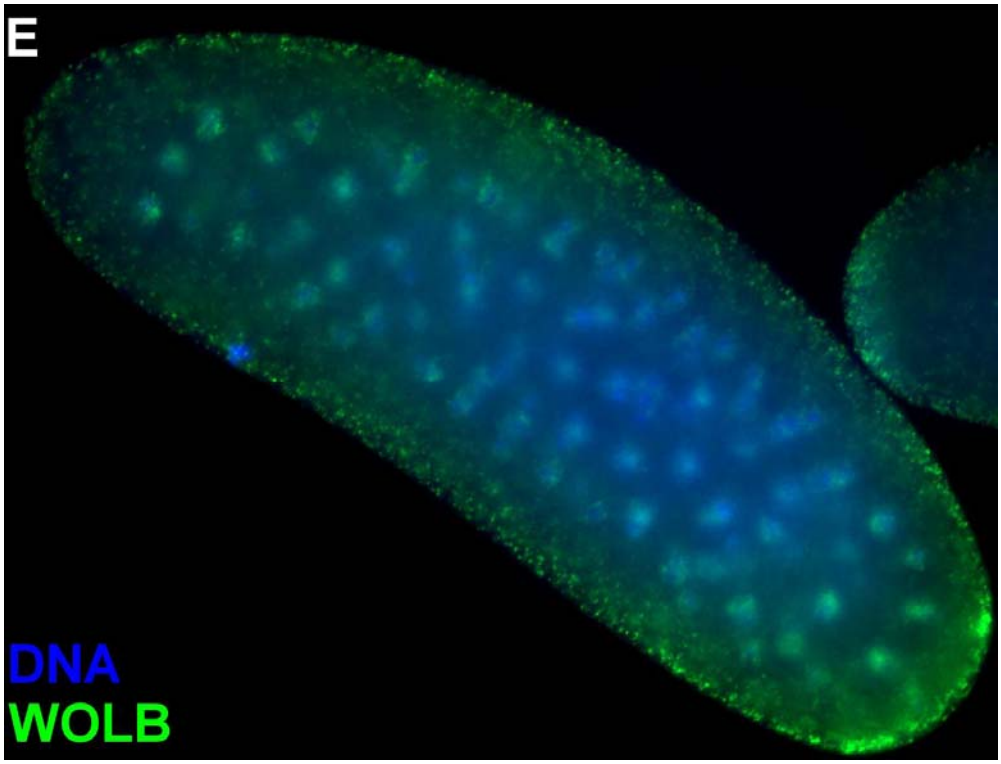
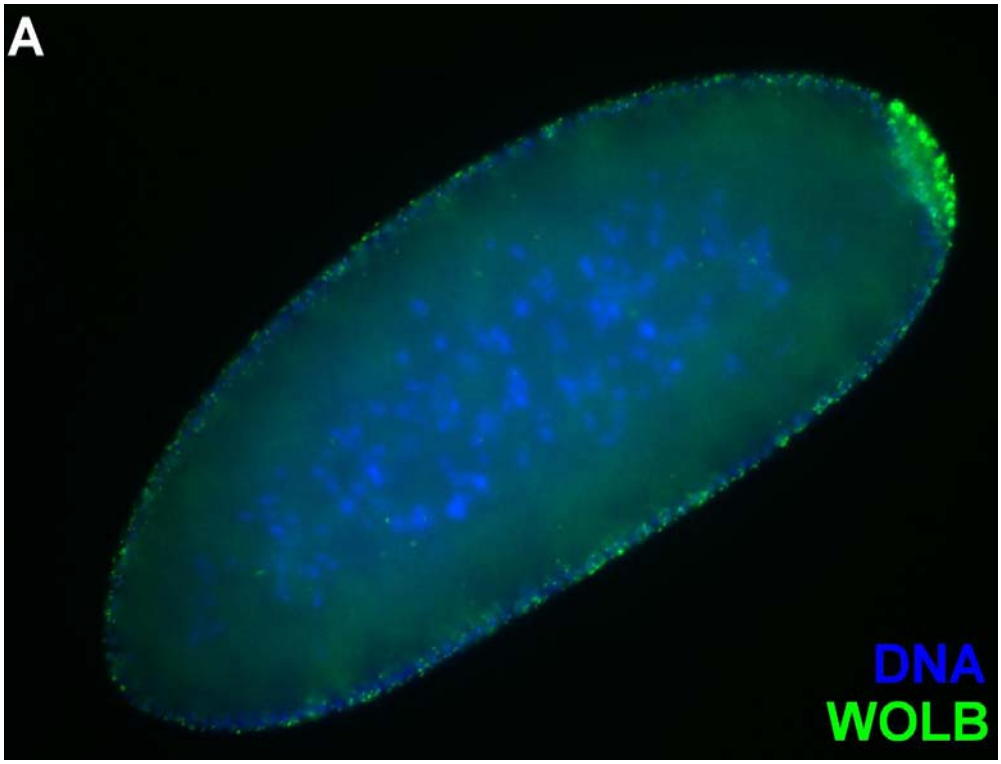


Figure 2.2: It shows the five observed localization patterns of Wolbachia in fertilized embryos fixed at the mid cycles of the cellularization phase. The color green shows Wolbachia and the color blue shows DNA. A) Strong Wolbachia accumulation at the posterior region (+++). B) Medium Wolbachia accumulation at the posterior region (++). C) Weak Wolbachia accumulation at the posterior region (+). D) Small ring pattern at the posterior region (r). E) Medium ring pattern at the posterior region (mR).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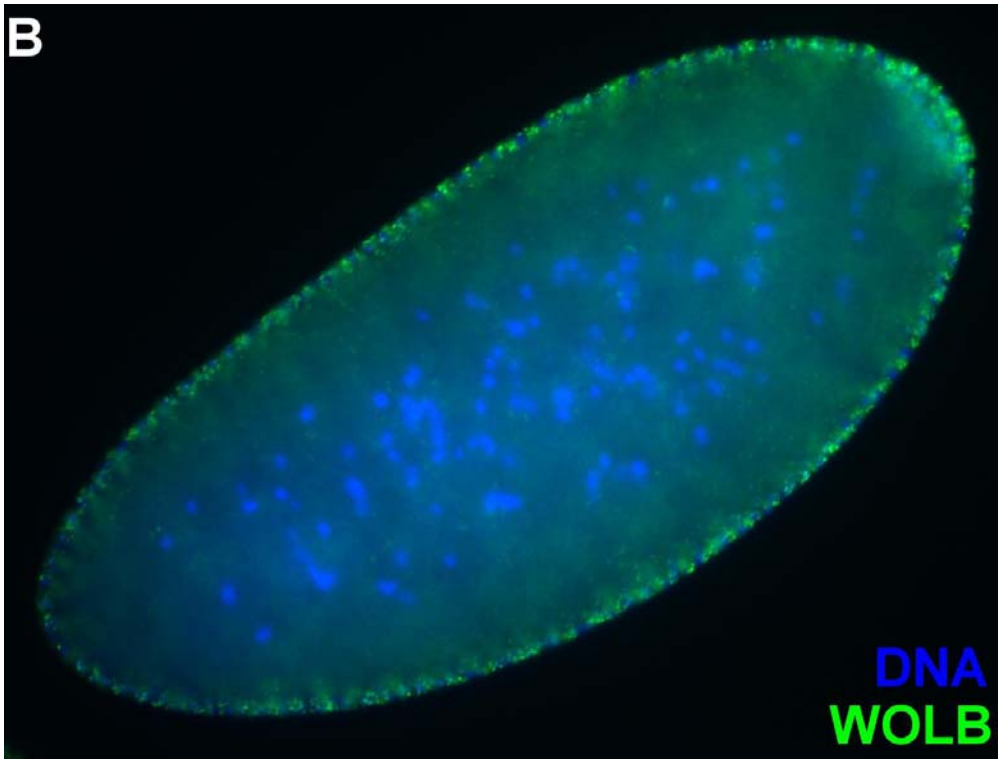
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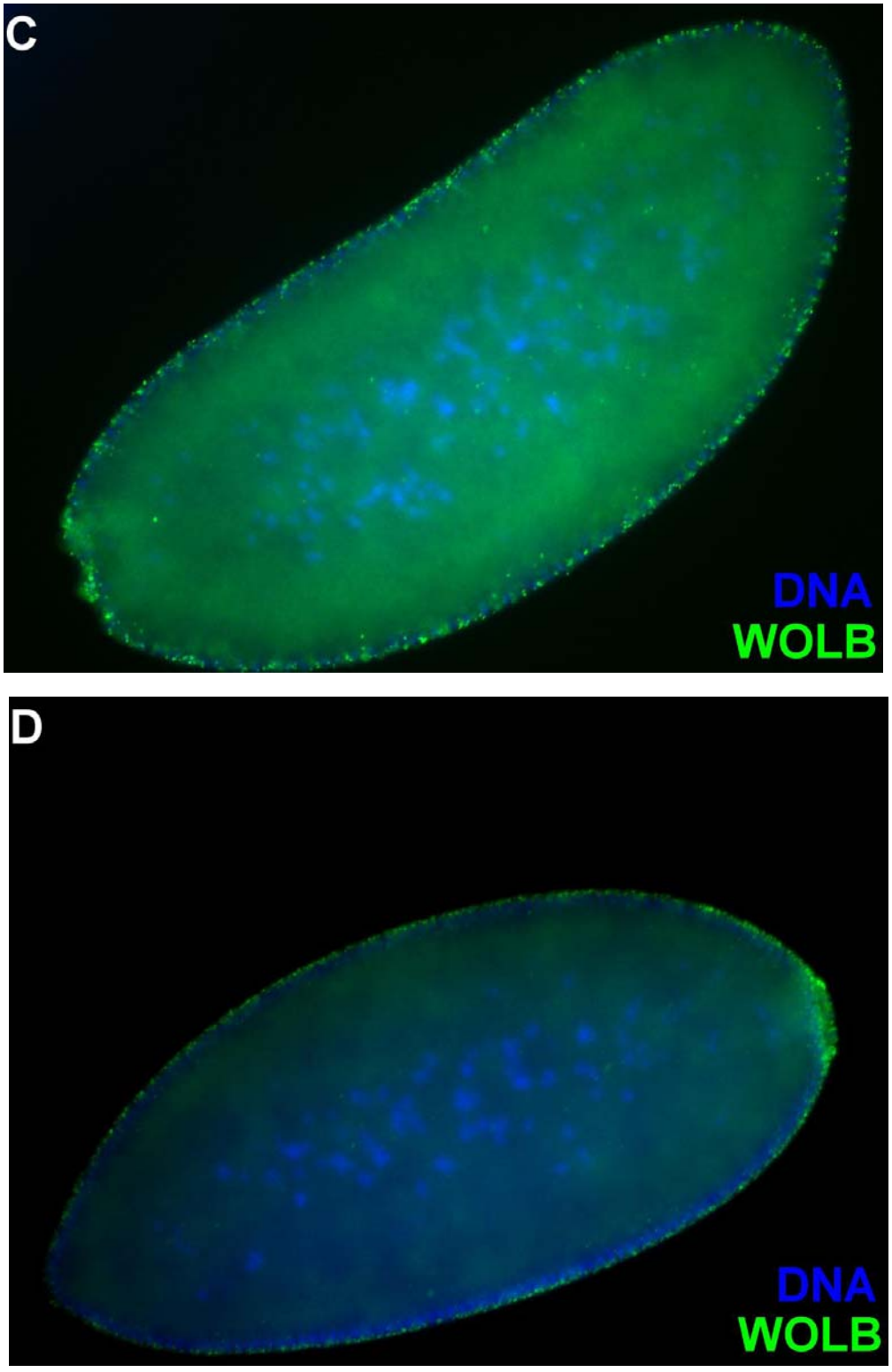
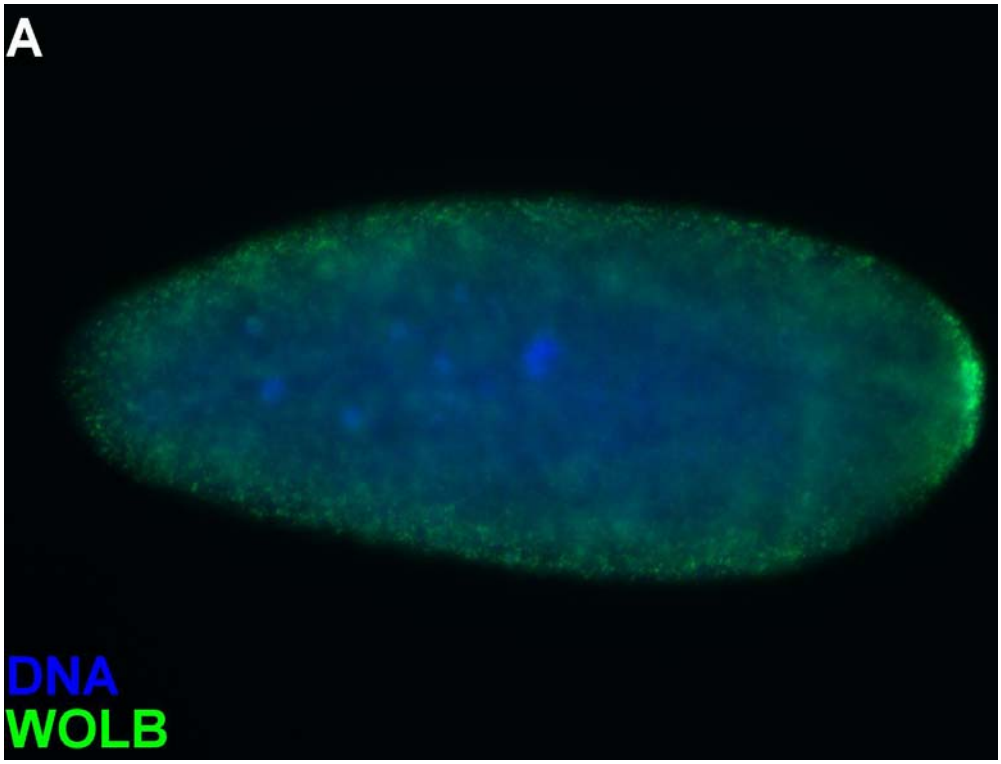
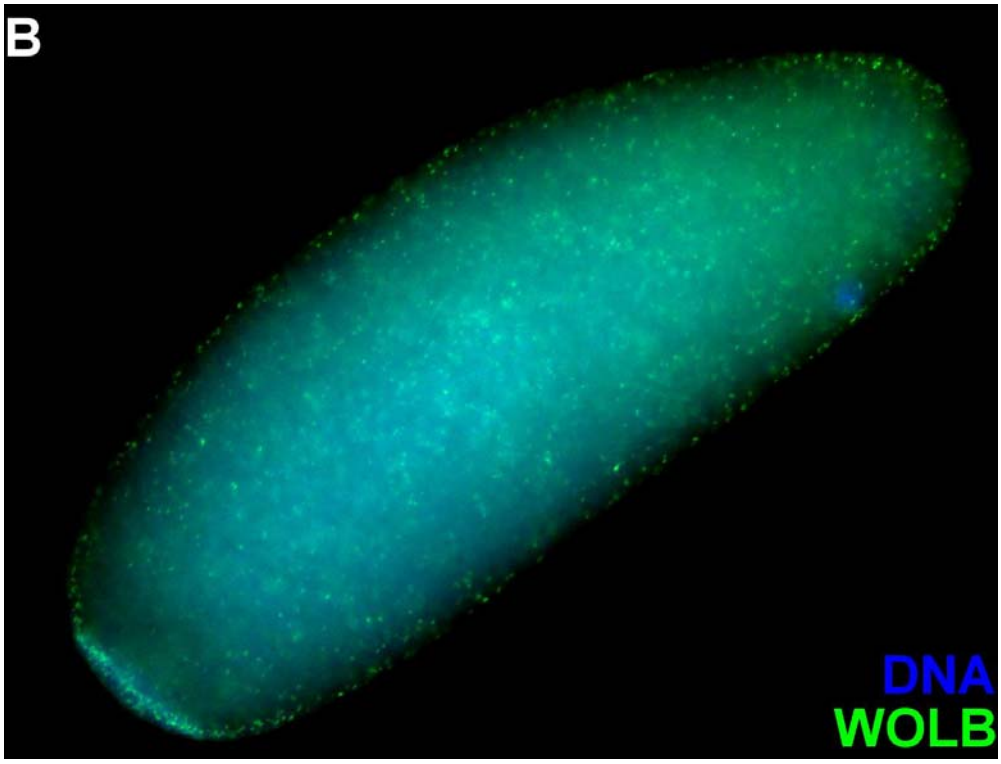
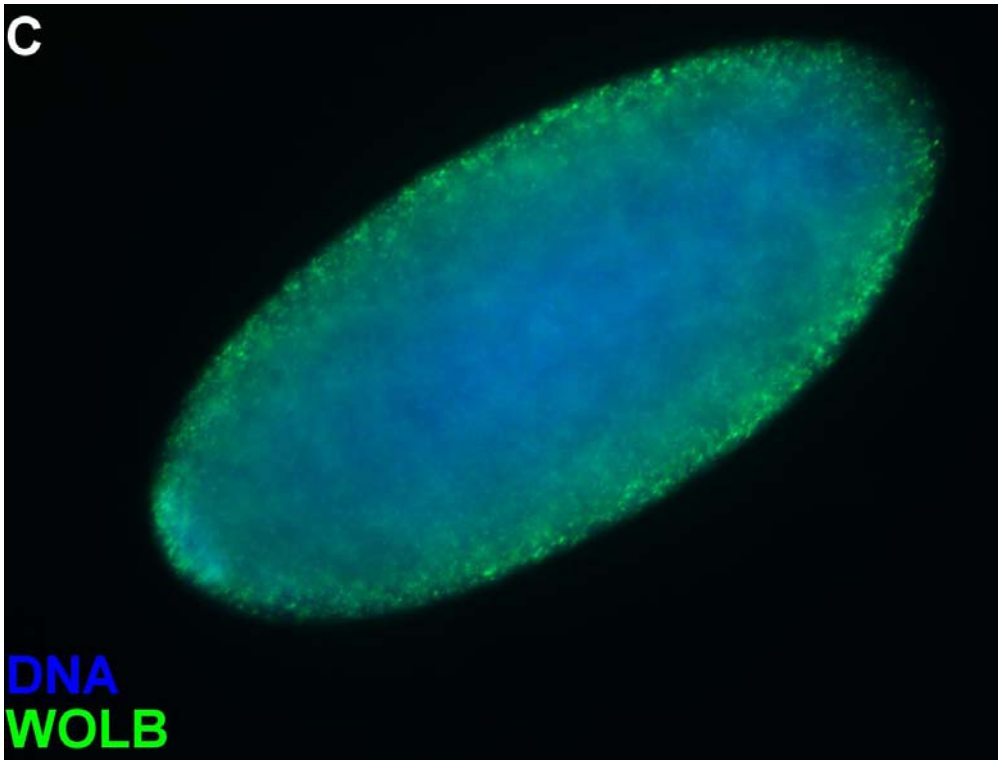
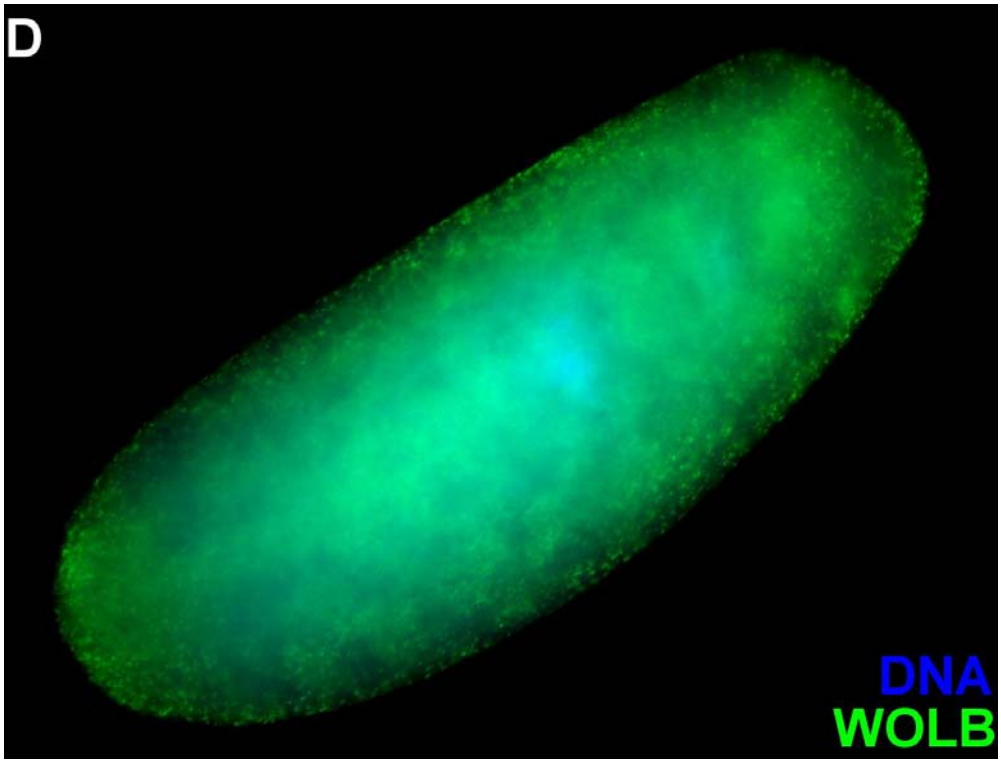


Figure 2.3: It shows the four Wolbachia localization patterns observed in fertilized embryos fixed at the late cycles of the cellularization phase. The color green shows Wolbachia and the color blue shows DNA. A) Wolbachia is strong accumulated at the posterior region. B) Wolbachia is medium accumulated at the posterior region. C) Wolbachia is weak accumulated at the posterior region. D) Small ring structure of Wolbachia at the posterior region.


A
DNA
WOLB


B
DNA
WOLB


C
DNA
WOLB


D
DNA
WOLB

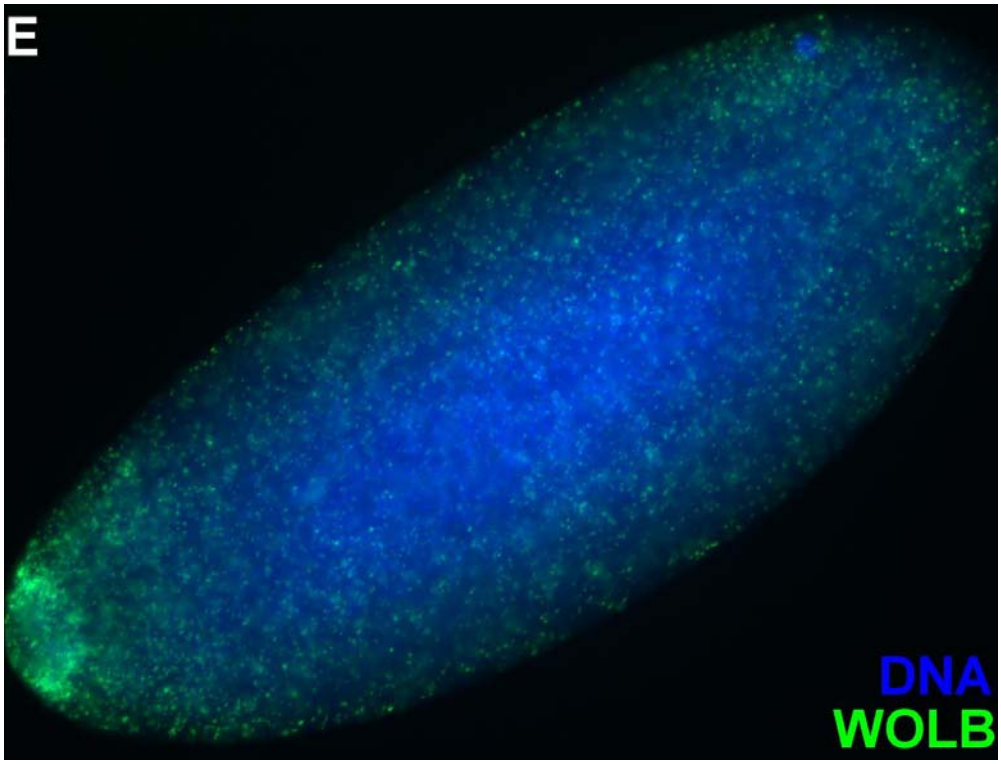


Figure 2.4: It shows the five observed localization patterns of Wolbachia in unfertilized embryos. The color green shows Wolbachia and the color blue shows DNA. A) Strong accumulation of Wolbachia at the posterior region (+++). B) Medium Wolbachia accumulation at the posterior region (++). C) Weak Wolbachia accumulation at the posterior region (+). D) Homogenous distribution of Wolbachia (0). E) Small ring pattern of Wolbachia at the posterior region (r).

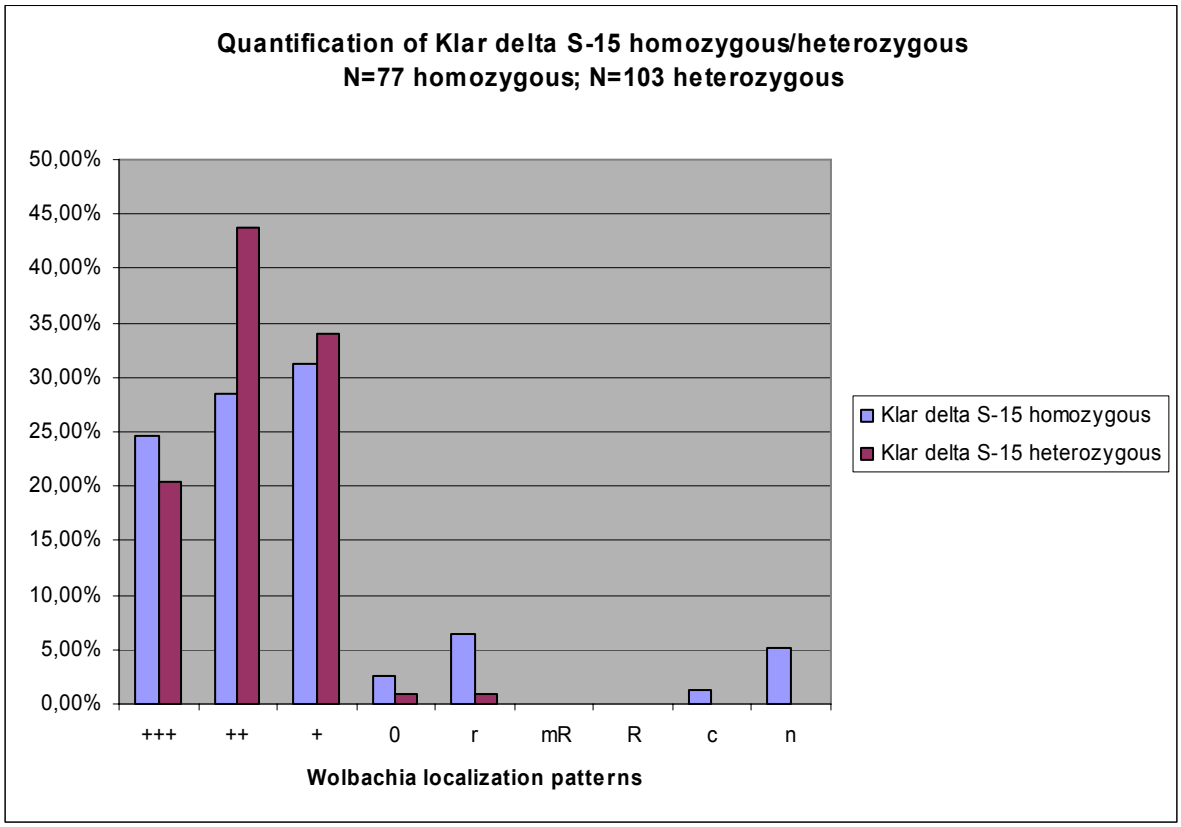


Figure 2.5: It shows the overall quantification results in percent of Klar ∆ S-15 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. Total 77 Klar ∆ S-15 homozygous and 103 Klar ∆ S-15 heterozygous fertilized embryos were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia in the fertilized embryos. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern of Wolbachia at the posterior region. c indicates cortex accumulation of Wolbachia. n indicates that Wolbachia is not accumulated at the posterior top of the fertilized embryos.

	Number	Sign	Number*Sign	%
	19	3	57	24.68%
	22	2	44	28.57%
	24	1	24	31.17%
	2	0	0	2.60%
	5	r		6.49%
	0	mR		0.00%
	0	R		0.00%
	1	c		1.30%
	4	n		5.19%
Total	77		125	100.00%
Average	**1.62**			

Table 2.1: The table shows the quantification results of Klar Δ S-15 homozygous of figure 2.5. 77 Klar Δ S-15 homozygous fertilized embryos were examined. The average value of 1.62 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 24.68% of 77 Klar Δ S-15 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 28.57% of 77 Klar Δ S-15 homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 31.17% of 77 Klar Δ S-15 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 2.60% of 77 Klar Δ S-15 homozygous fertilized embryos showed homogenous distribution of Wolbachia. 6.49% of 77 Klar Δ S-15 homozygous fertilized embryos showed a small ring pattern at the posterior region. 1.30% of 77 Klar Δ S-15 homozygous fertilized embryos showed Wolbachia accumulation at the cortex. 5.19% of 77 Klar Δ S-15 homozygous fertilized embryos showed Wolbachia accumulation not at the posterior top.

	Number	Sign	Number*Sign	%
	21	3	63	20.39%
	45	2	90	43.69%
	35	1	35	33.98%
	1	0	0	0.97%
	1	r		0.97%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	103		188	100.00%
Average	**1.83**			

Table 2.2: The table shows the quantification results of Klar Δ S-15 heterozygous of figure 2.5. 103 Klar Δ S-15 heterozygous fertilized embryos were examined. The average value of 1.83 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 20.39% of 103 Klar Δ S-15 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 43.69% of 103 Klar Δ S-15 heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 33.98% of 103 Klar Δ S-15 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 0.97% of 103 Klar Δ S-15 heterozygous fertilized embryos showed homogenous distribution of Wolbachia. 0.97% of 103 Klar Δ S-15 heterozygous fertilized embryos showed a small ring pattern at the posterior region.

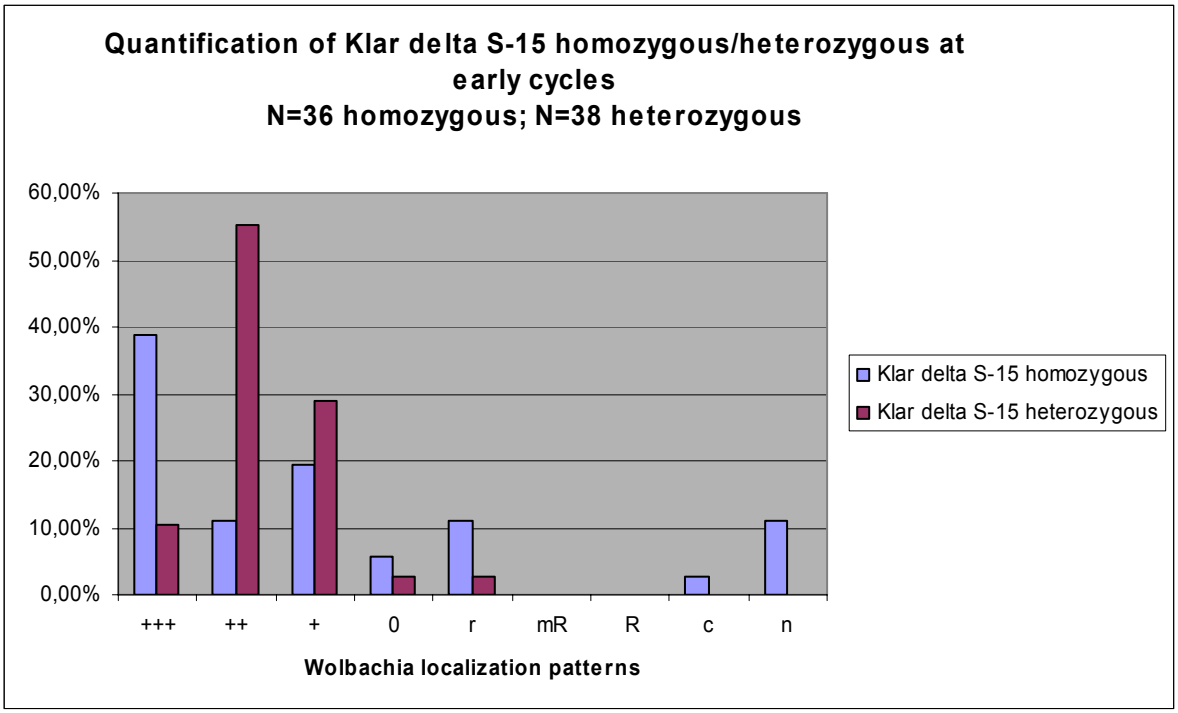


Figure 2.6: It shows the quantification results in percent of Klar ∆ S-15 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 36 Klar ∆ S-15 homozygous and 38 Klar ∆S-15 heterozygous fertilized embryos fixed at the early cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	14	3	42	38.89%
	4	2	8	11.11%
	7	1	7	19.44%
	2	0	0	5.56%
	4	r		11.11%
	0	mR		0.00%
	0	R		0.00%
	1	c		2.78%
	4	n		11.11%
Total	36		57	100.00%
Average	**1.58**			

Table 2.3: The table shows the quantification results of Klar Δ S-15 homozygous of figure 2.6. 36 Klar Δ S-15 homozygous fertilized embryos were examined at the early cycles of the cellularization phase. The average value of 1.58 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 38.89% of 36 Klar Δ S-15 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 11.11% of 36 Klar Δ S-15 homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 19.44% of 36 Klar Δ S-15 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 5.56% of 36 Klar Δ S-15 homozygous fertilized embryos showed homogenous distribution of Wolbachia. 11.11% of 36 Klar Δ S-15 homozygous fertilized embryos showed a small ring pattern at the posterior region. 2.78% of 36 Klar Δ S-15 homozygous fertilized embryos showed that Wolbachia is accumulated at the cortex. 11.11% of 36 Klar Δ S-15 homozygous fertilized embryos showed that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	4	3	12	10.53%
	21	2	42	55.26%
	11	1	11	28.95%
	1	0	0	2.63%
	1	r		2.63%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	38		65	100.00%
Average	**1.71**			

Table 2.4: The table shows the quantification results of Klar Δ S-15 heterozygous of figure 2.6. 38 Klar Δ S-15 heterozygous fertilized embryos were examined at the early cycles of the cellularization phase. The average value of 1.71 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 10.53% of 38 Klar Δ S-15 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 55.26% of 38 Klar Δ S-15 heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 28.95% of 38 Klar Δ S-15 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 2.63% of 38 Klar Δ S-15 heterozygous fertilized embryos showed homogenous distribution of Wolbachia. 2.63% of 38 Klar Δ S-15 heterozygous fertilized embryos showed a small ring pattern at the posterior region.

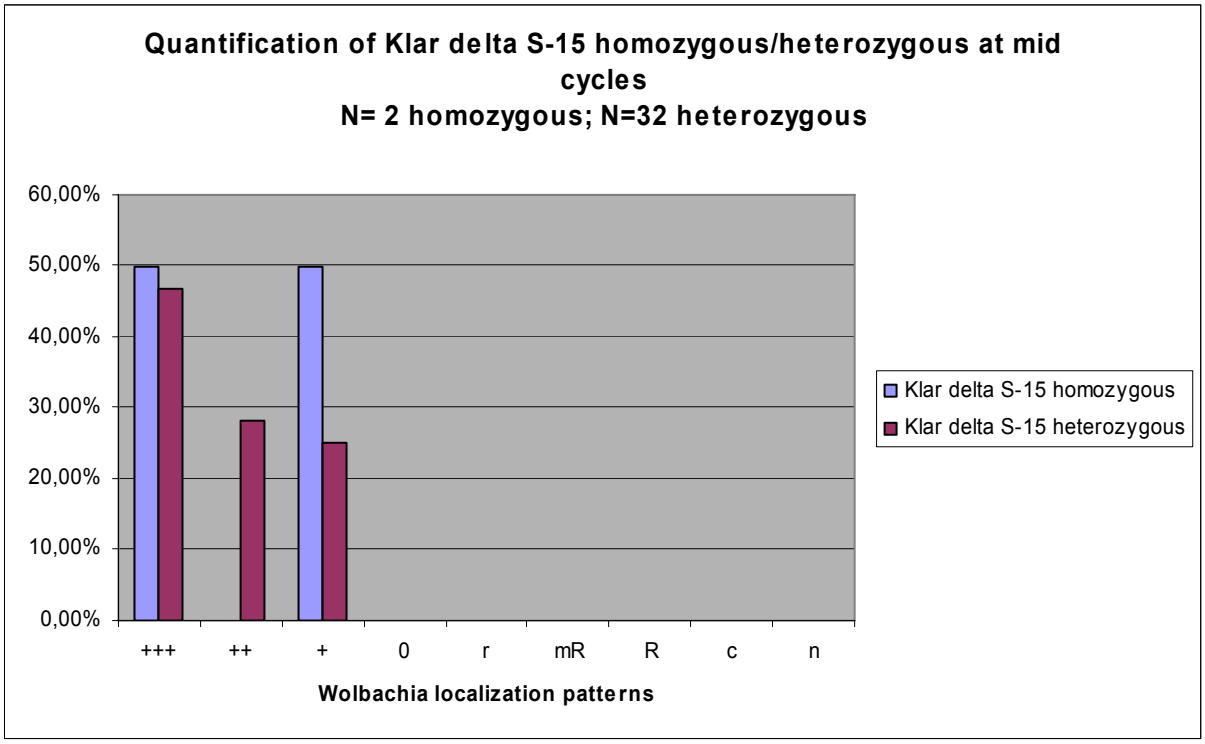


Figure 2.7: It shows the quantification results in percent of Klar ∆ S-15 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 2 Klar ∆ S-15 homozygous and 32 Klar ∆ S-15 heterozygous fertilized embryos fixed at the mid cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	1	3	3	50.00%
	0	2	0	0.00%
	1	1	1	50.00%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	2		4	100.00%
Average	**2**			

Table 2.5: The table shows the quantification results of Klar ∆ S-15 homozygous at figure 2.7. 2 Klar delta S-15 homozygous fertilized embryos were examined at the mid cycles of the cellularization phase. The average value of 2 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 50% of 2 Klar ∆ S-15 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 50% of 2 Klar ∆ S-15 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region.

	Number	Sign	Number*Sign	%
	15	3	45	46.88%
	9	2	18	28.13%
	8	1	8	25.00%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	32		71	100.00%
Average	**2.21**			

Table 2.6: The table shows the quantification results of Klar Δ S-15 heterozygous of figure 2.7. 32 Klar Δ S-15 heterozygous fertilized embryos were examined at the mid cycles of the cellularization phase. The average value of 2.21 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 46.88% of 32 Klar Δ S-15 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 28.13% of 32 Klar Δ S-15 heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 25% of 32 Klar Δ S-15 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region.

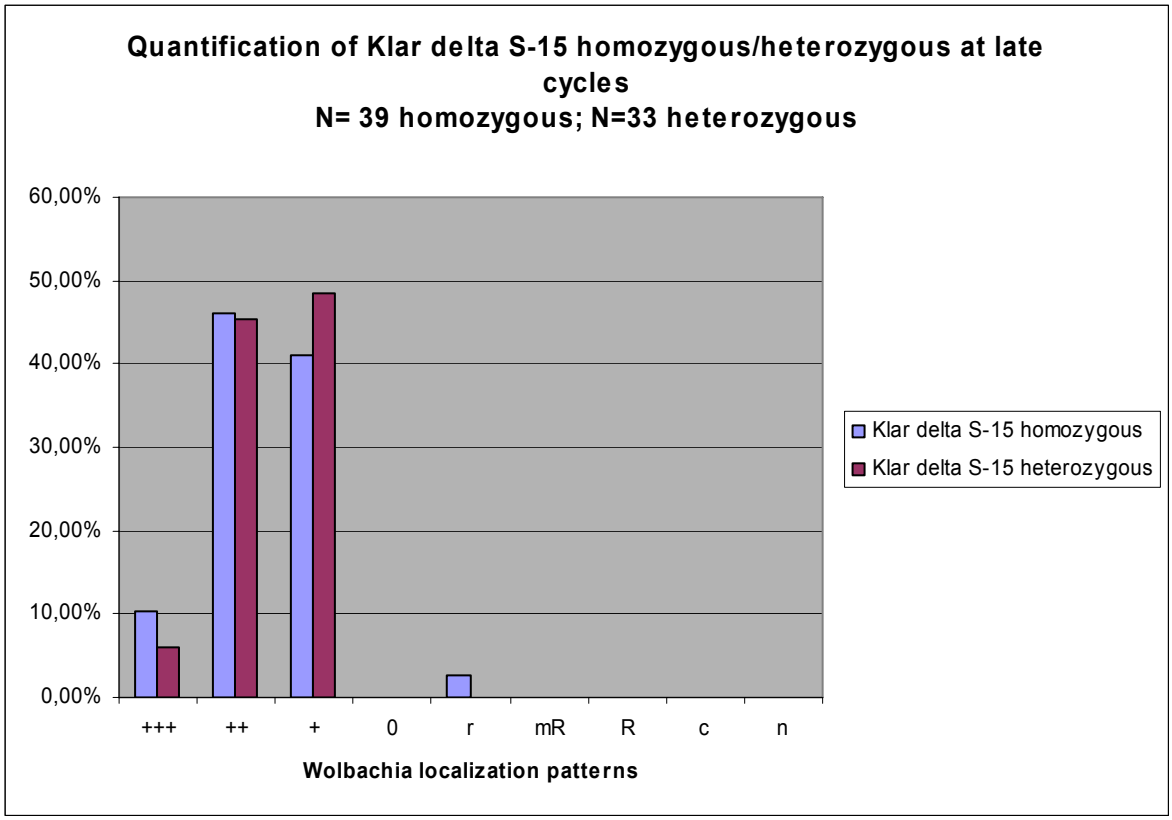


Figure 2.8: It shows the quantification results in percent of Klar ∆ S-15 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 39 Klar ∆ S-15 homozygous and 33 Klar ∆ S-15 heterozygous fertilized embryos fixed at the late cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	4	3	12	10.26%
	18	2	36	46.15%
	16	1	16	41.03%
	0	0	0	0.00%
	1	r		2.56%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	39		64	100.00%
Average	**1.64**			

Table 2.7: The table shows the quantification results of Klar Δ S-15 homozygous of figure 2.8. 39 Klar Δ S-15 homozygous fertilized embryos were examined at the late cycles of the cellularization phase. The average value of 1.64 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 10.26% of 39 Klar Δ S-15 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 46.15% of 39 Klar Δ S-15 homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 41.03% of 39 Klar Δ S-15 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 2.56 % of 39 Klar Δ S-15 homozygous fertilized embryos showed a small ring pattern at the posterior region.

	Number	Sign	Number*Sign	%
	2	3	6	6.06%
	15	2	30	45.45%
	16	1	16	48.48%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	33		52	100.00%
Average	**1.58**			

Table 2.8: The table shows the quantification results of Klar ∆ S-15 heterozygous of figure 2.8. 33 Klar delta S-15 heterozygous fertilized embryos were examined at the late cycles of the cellularization phase. The average value of 1.58 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 6.06% of 33 Klar ∆ S-15 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 45.45% of 33 Klar ∆ S-15 heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 48.48% of 33 Klar ∆ S-15 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region.

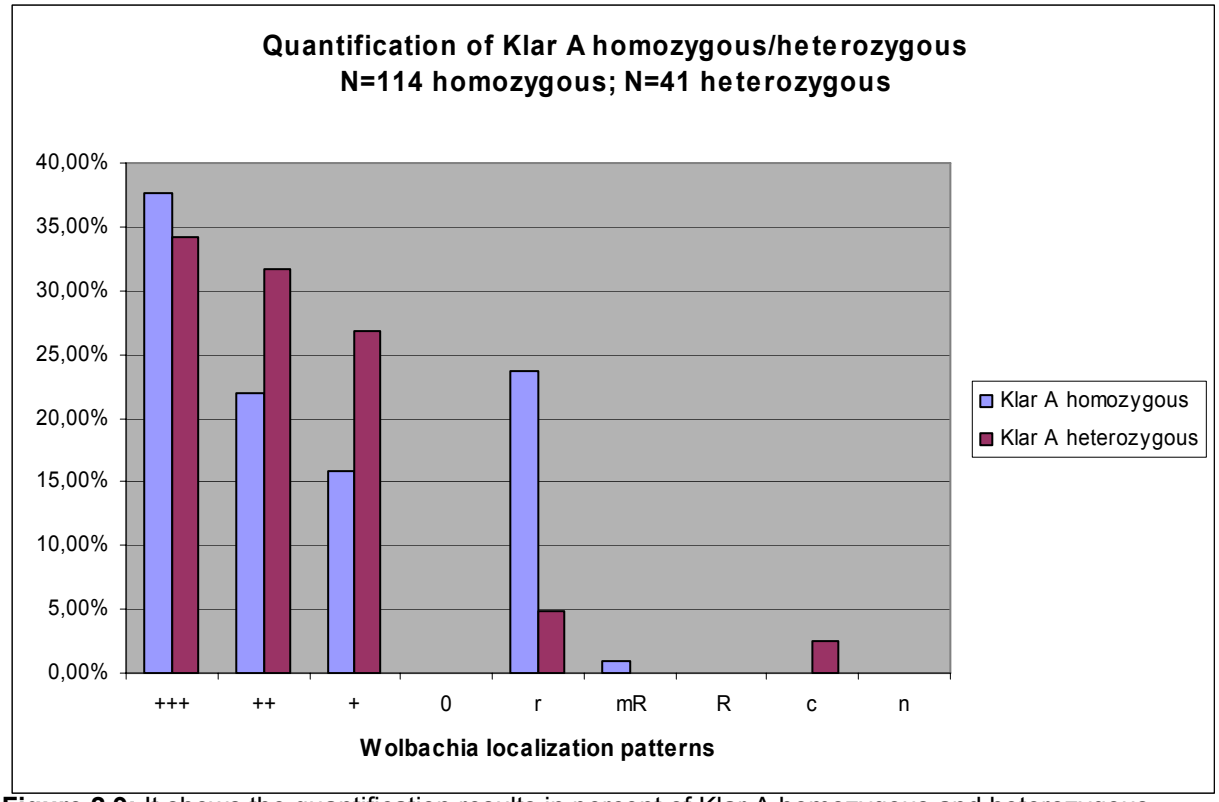


Figure 2.9: It shows the quantification results in percent of Klar A homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. Total 114 Klar A homozygous and 41 Klar A heterozygous fertilized embryos were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	43	3	129	37.72%
	25	2	50	21.93%
	18	1	18	15.79%
	0	0	0	0.00%
	27	r		23.68%
	1	mR		0.88%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	114		197	100.00%
Average	**1.73**			

Table 2.9: The table shows the quantification results of Klar A homozygous of figure 2.9. 114 Klar A homozygous fertilized embryos were examined. The average value of 1.73 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 37.72% of 114 Klar A homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 21.93% of 114 Klar A homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 15.79% of 114 Klar A homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 23.68% of 114 Klar A homozygous fertilized embryos showed a small ring pattern at the posterior region. 0.88% of 114 Klar A homozygous fertilized embryos showed a medium ring pattern at the posterior region.

	Number	Sign	Number*Sign	%
	14	3	42	34.15%
	13	2	26	31.71%
	11	1	11	26.83%
	0	0		0.00%
	2	r		4.88%
	0	mR		0.00%
	0	R		0.00%
	1	c		2.44%
	0	n		0.00%
Total	41		79	100.00%
Average	**1.93**			

Table 2.10: The table shows the quantification results of Klar A heterozygous of figure 2.9. 41 Klar A heterozygous fertilized embryos were examined. The average value of 1.93 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 34.15% of 41 Klar A heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 31.71% of 41 Klar A heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 26.83% of 41 Klar A heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 4.88% of 41 Klar A heterzygous fertilized embryos showed a small ring pattern at the posterior region. 2.44% of 41 Klar A heterzygous fertilized embryos showed that Wolbachia is accumulated at the cortex.

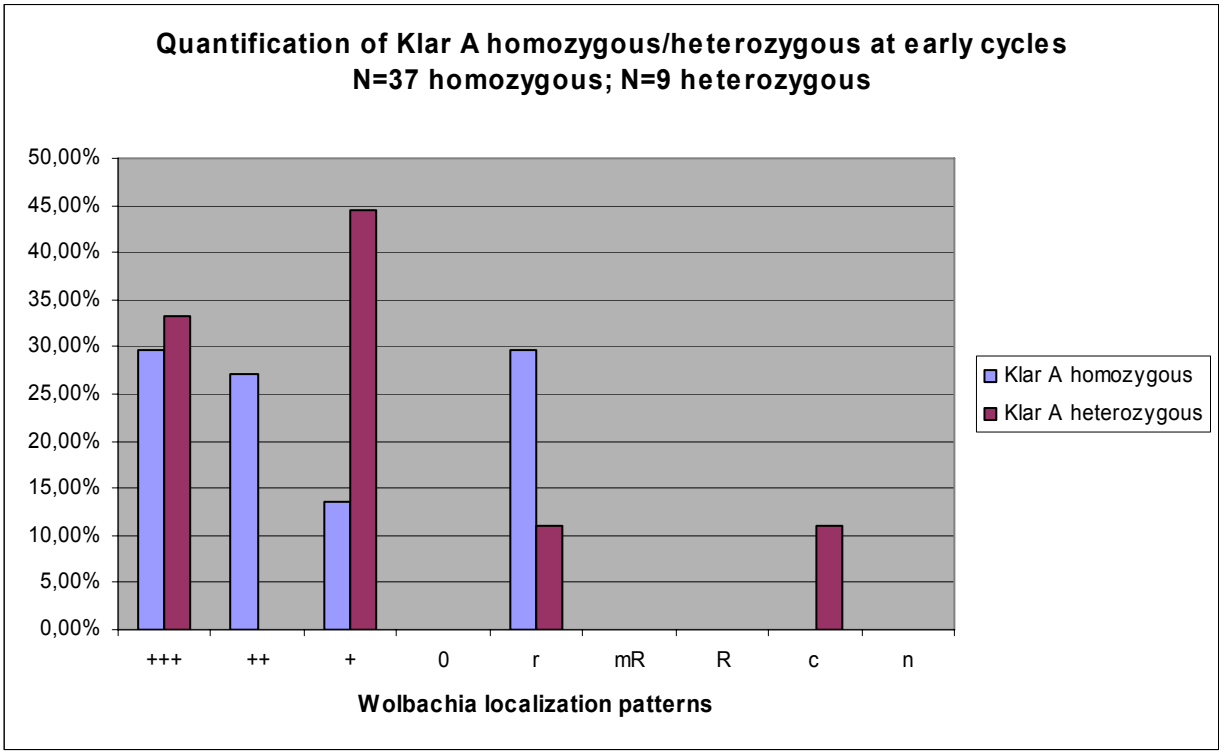


Figure 2.10: It shows the quantification results in percent of Klar A homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 37 Klar A homozygous and 9 Klar A heterozygous fertilized embryos at the early cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	11	3	33	29.73%
	10	2	20	27.03%
	5	1	5	13.51%
	0	0	0	0.00%
	11	r		29.73%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	37		58	100.00%
Average	**1.57**			

Table 2.11: The table shows the quantification results of Klar A homozygous of figure 2.10. 37 Klar A homozygous fertilized embryos were examined at the early cycles of the cellularization phase. The average value of 1.57 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 29.73% of 37 Klar A homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 27.03% of 37 Klar A homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 13.51% of 37 Klar A homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 29.73% of 37 Klar A homozygous fertilized embryos showed a small ring pattern at the posterior region.

	Number	Sign	Number*Sign	%
	3	3	9	33.33%
	0	2	0	0.00%
	4	1	4	44.44%
	0	0	0	0.00%
	1	r		11.11%
	0	mR		0.00%
	0	R		0.00%
	1	c		11.11%
	0	n		0.00%
Total	9		13	100.00%
Average	**1.44**			

Table 2.12: The table shows the quantification results of Klar A heterozygous of figure 2.10. 9 Klar A heterozygous fertilized embryos were examined at the early cycles of the cellularization phase. The average value of 1.44 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 33.33% of 9 Klar A heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 44.44% of 9 Klar A heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 11.11% of 9 Klar A heterozygous fertilized embryos showed a small ring pattern at the posterior region. 11.11% of 9 Klar A heterozygous fertilized embryos showed that Wolbachia is accumulated at the cortex.

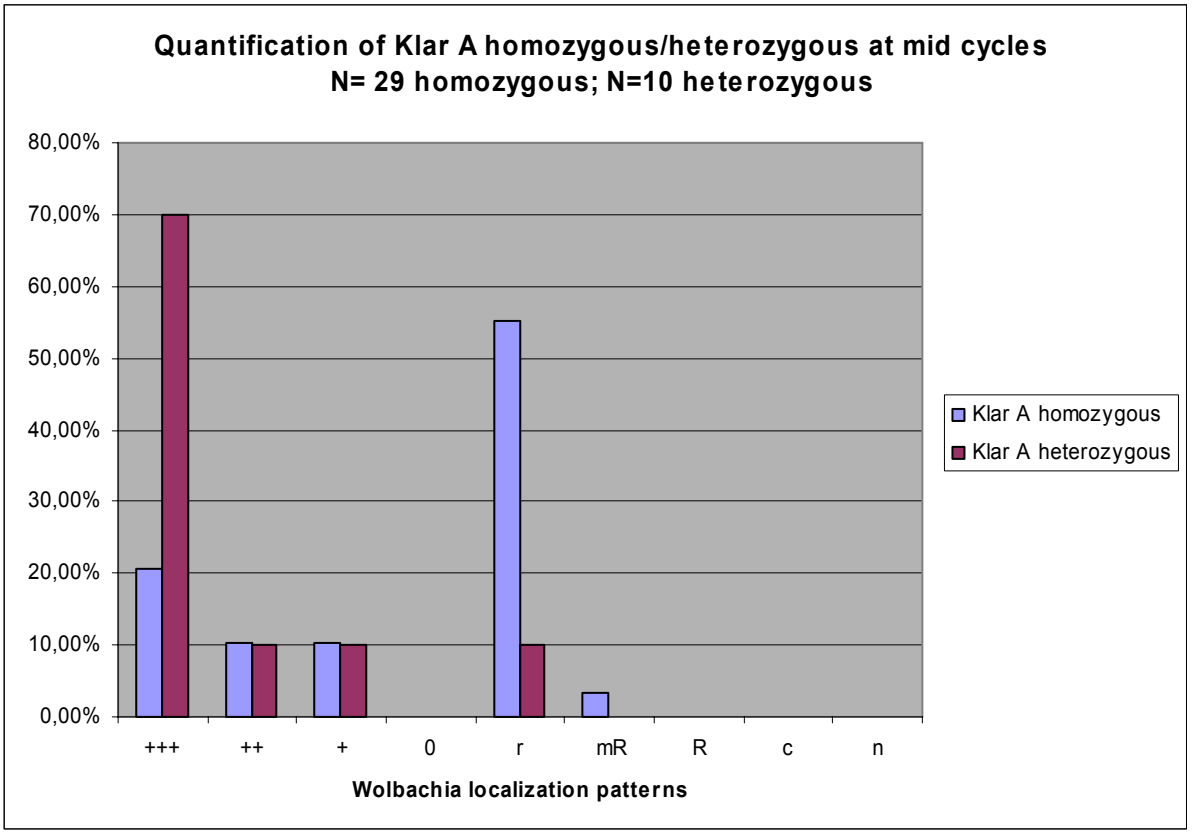


Figure 2.11: It shows the quantification results in percent of Klar A homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 29 Klar A homozygous and 10 Klar A heterozygous fertilized embryos fixed at the mid cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	6	3	18	20.69%
	3	2	6	10.34%
	3	1	3	10.34%
	0	0	0	0.00%
	16	r		55.17%
	1	mR		3.45%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	29		27	100.00%
Average	**0.93**			

Table 2.13: The table shows the quantification results of Klar A homozygous of figure 2.11. 29 Klar A homozygous fertilized embryos were examined at the mid cycles of the cellularization phase. The average value of 0.93 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 20.69% of 29 Klar A homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 10.34% of 29 Klar A homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 10.34% of 29 Klar A homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 55.17% of 29 Klar A homozygous fertilized embryos showed a small ring pattern at the posterior region. 3.45% of 29 Klar A homozygous fertilized embryos showed a medium ring pattern at the posterior region.

	Number	Sign	Number*Sign	%
	7	3	21	70.00%
	1	2	2	10.00%
	1	1	0	10.00%
	0	0	0	0.00%
	1	r		10.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	10		23	100.00%
Average	**2.3**			

Table 2.14: The table shows the quantification results of Klar A heterozygous of figure 2.11. 10 Klar A heterozygous fertilized embryos were examined at the mid cycles of the cellularization phase. The average value of 2.3 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 70% of 10 Klar A heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 10% of 10 Klar A heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 10% of 10 Klar A heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 10% of 10 Klar A heterozygous fertilized embryos showed a small ring pattern at the posterior region.

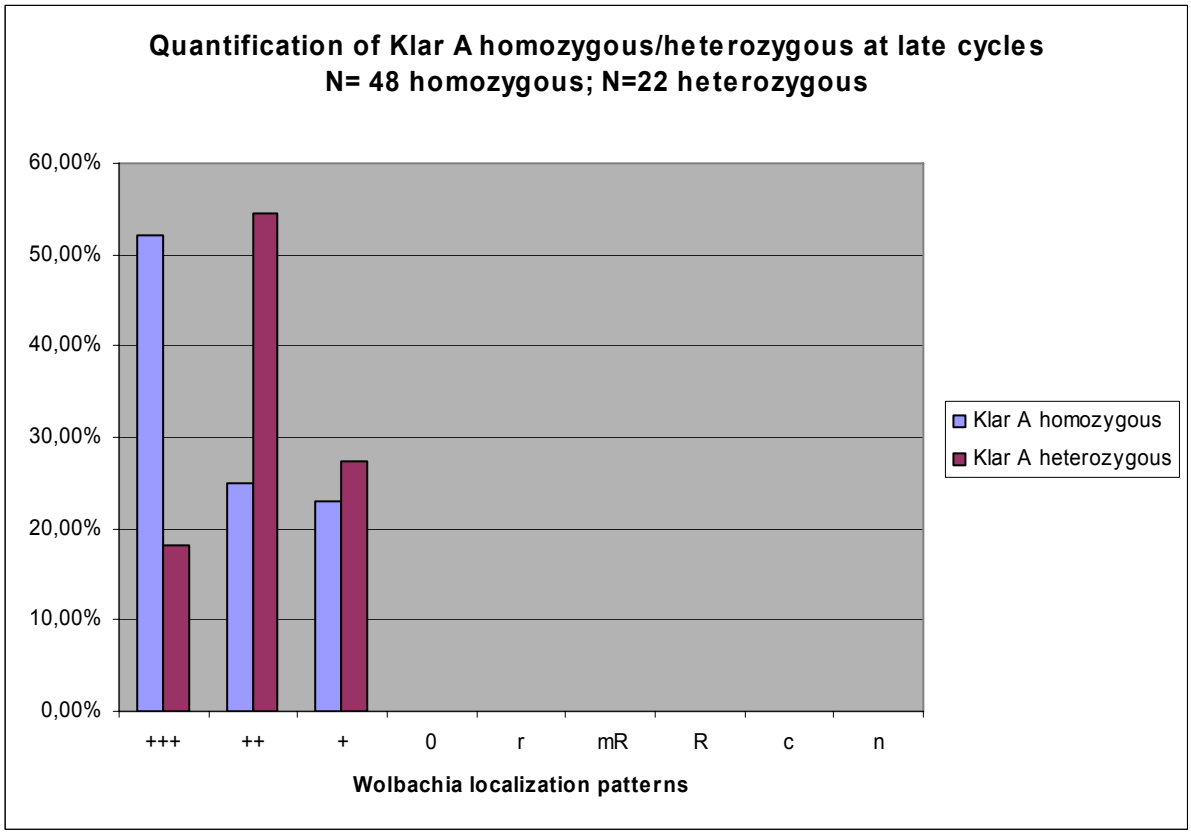


Figure 2.12: It shows the quantification results in percent of Klar A homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 48 Klar A homozygous and 22 Klar A heterozygous fertilized embryos fixed at the late cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	25	3	75	52.08%
	12	2	24	25.00%
	11	1	11	22.92%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	48		110	100.00%
Average	**2.29**			

Table 2.15: The table shows the quantification results of Klar A homozygous of figure 2.12. 48 Klar A homozygous fertilized embryos were examined at the late cycles of the cellularization phase. The average value of 2.29 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 52.08% of 48 Klar A homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 25% of 48 Klar A homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 22.92% of 48 Klar A homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region.

	Number	Sign	Number*Sign	%
	4	3	12	18.18%
	12	2	24	54.55%
	6	1	6	27.27%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	22		42	100.00%
Average	**1.91**			

Table 2.16: The table shows the quantification results of Klar A heterozygous of figure 2.12. 22 Klar A heterozygous fertilized embryos were examined at the late cycles of the cellularization phase. The average value of 1.91 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 18.18% of 22 Klar A heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 54.55% of 22 Klar A heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 27.27% of 22 Klar A heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region.

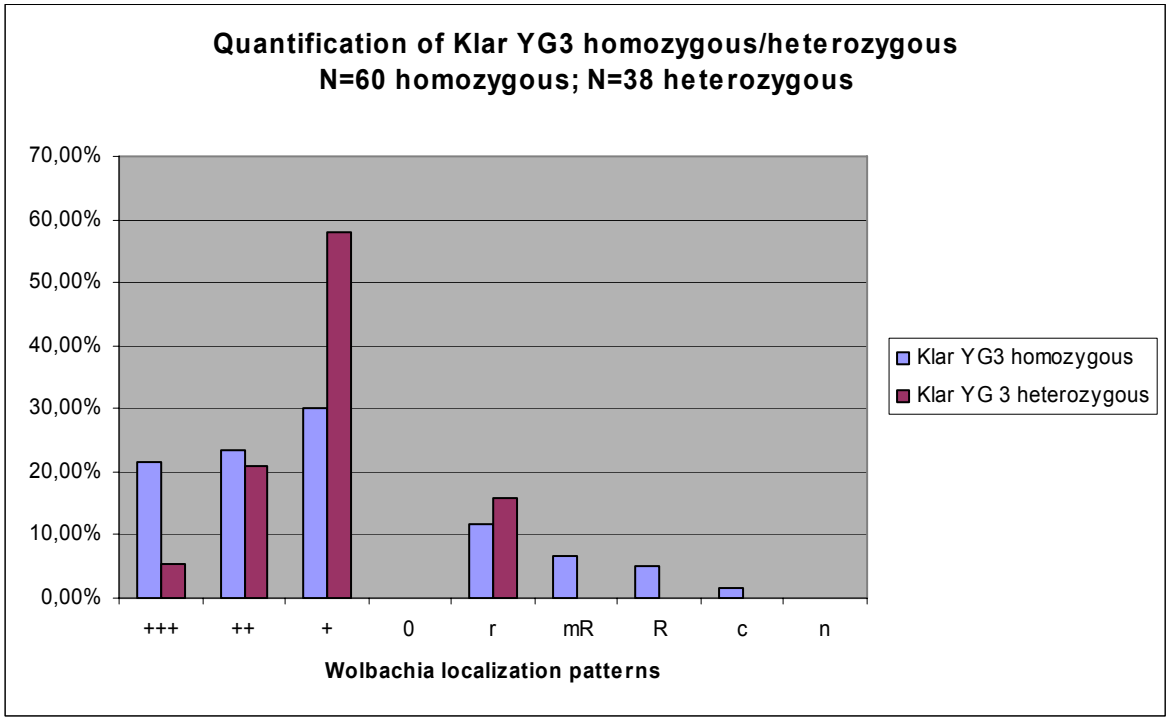


Figure 2.13: It shows the overall quantification results in percent of Klar YG3 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. Total 60 Klar YG3 homozygous and 38 Klar YG3 heterozygous fertilized embryos were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	13	3	39	21.67%
	14	2	28	23.33%
	18	1	18	30.00%
	0	0	0	0.00%
	7	r		11.67%
	4	mR		6.67%
	3	R		5.00%
	1	c		1.67%
	0	n		0.00%
Total	60		85	100.00%
Average	**1.42**			

Table 2.17: The table shows the quantification results of Klar YG3 homozygous of figure 2.13. 60 Klar YG3 homozygous fertilized embryos were examined. The average value of 1.42 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 21.67% of 60 Klar YG3 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 23.33% of 60 Klar YG3 homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 30% of 60 Klar YG3 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 11.67% of 60 Klar YG3 homozygous fertilized embryos showed a small ring pattern at the posterior region. 6.67% of 60 Klar YG3 homozygous fertilized embryos showed a medium ring pattern at the posterior region. 5% of 60 Klar YG3 homozygous fertilized embryos showed a big ring pattern at the posterior region. 1.67% of 60 Klar YG3 homozygous fertilized embryos showed that Wolbachia is accumulated at the cortex.

	Number	Sign	Number*Sign	%
	2	3	6	5.26%
	8	2	16	21.05%
	22	1	22	57.89%
	0	0		0.00%
	6	r		15.79%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	38		44	100.00%
Average	**1.16**			

Table 2.18: The table shows the quantification results of Klar YG3 heterozygous of figure 2.13. 38 Klar YG3 heterozygous fertilized embryos were examined. The average value of 1.16 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 5.26% of 38 Klar YG3 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 21.05% of 38 Klar YG3 heterozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 57.89% of 38 Klar YG3 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 15.79% of 38 Klar YG3 heterozygous fertilized embryos showed a small ring pattern at the posterior region.

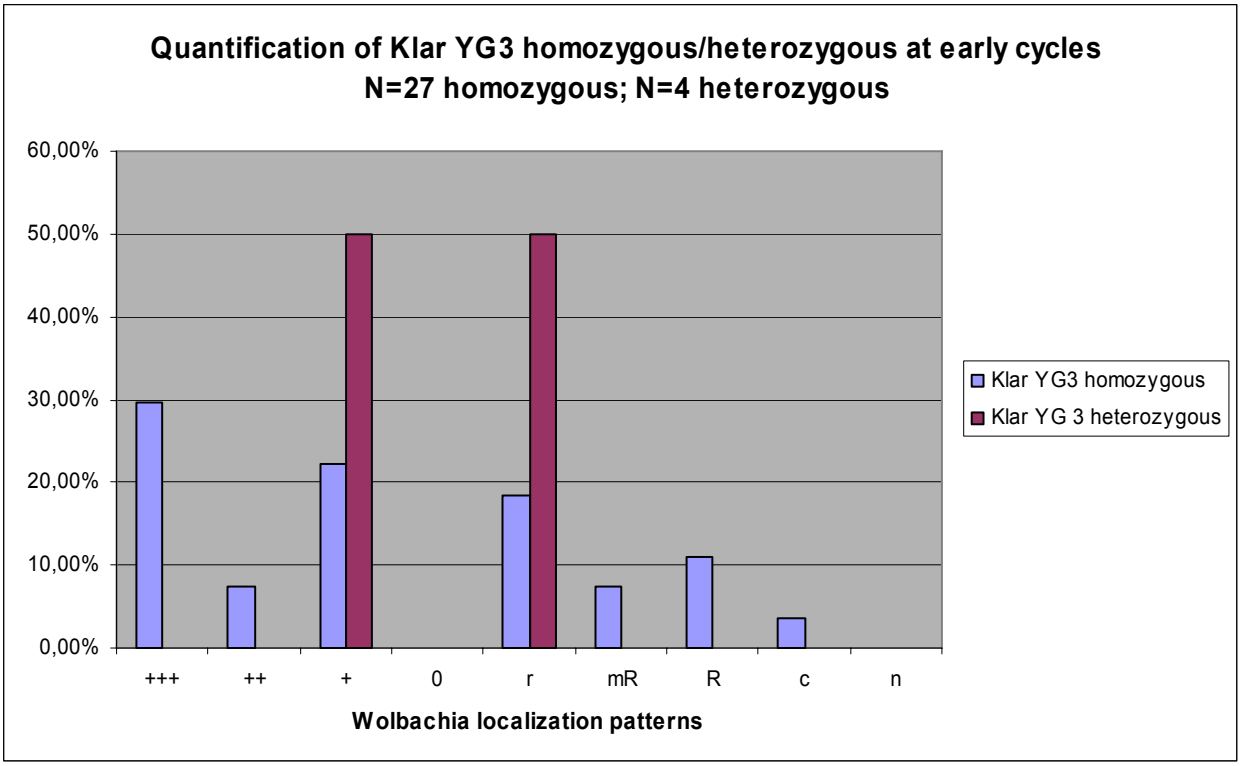


Figure 2.14: It shows the quantification results in percent of Klar YG3 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 27 Klar YG3 homozygous and 4 Klar YG3 heterozygous fertilized embryos were examined at the early cycles of the cellularization phase. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	8	3	24	29.63%
	2	2	4	7.41%
	6	1	6	22.22%
	0	0	0	0.00%
	5	r		18.52%
	2	mR		7.41%
	3	R		11.11%
	1	c		3.70%
	0	n		0.00%
Total	27		34	100.00%
Average	**1.26**			

Table 2.19: The table shows the quantification results of Klar YG3 homozygous of figure 2.14. 27 Klar YG3 homozygous fertilized embryos were examined at the early cycles of the cellularization phase. The average value of 1.26 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 29.63% of 27 Klar YG3 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 7.41% of 27 Klar YG3 homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 22.22% of 27 Klar YG3 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 18.52% of 38 Klar YG3 heterozygous fertilized embryos showed a small ring pattern at the posterior region. 7.41% of 27 Klar YG3 homozygous fertilized embryos showed a medium ring pattern at the posterior region. 11.11% of 27 Klar YG3 homozygous fertilized embryos showed a big ring pattern at the posterior region. 3.70% of 27 Klar YG3 homozygous fertilized embryos showed that Wolbachia is accumulated at the cortex.

	Number	Sign	Number*Sign	%
	0	3	0	0.00%
	0	2	0	0.00%
	2	1	2	50.00%
	0	0	0	0.00%
	2	r		50.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	4		2	100.00%
Average	**0.5**			

Table 2.20: The table shows the quantification results of Klar YG3 heterozygous of figure 2.14. 4 Klar YG3 heterozygous fertilized embryos were examined at the early cycles of the cellularization phase. The average value of 0.5 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 50% of 4 Klar YG3 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 50% of 4 Klar YG3 heterozygous fertilized embryos showed a small ring structure at the posterior region.

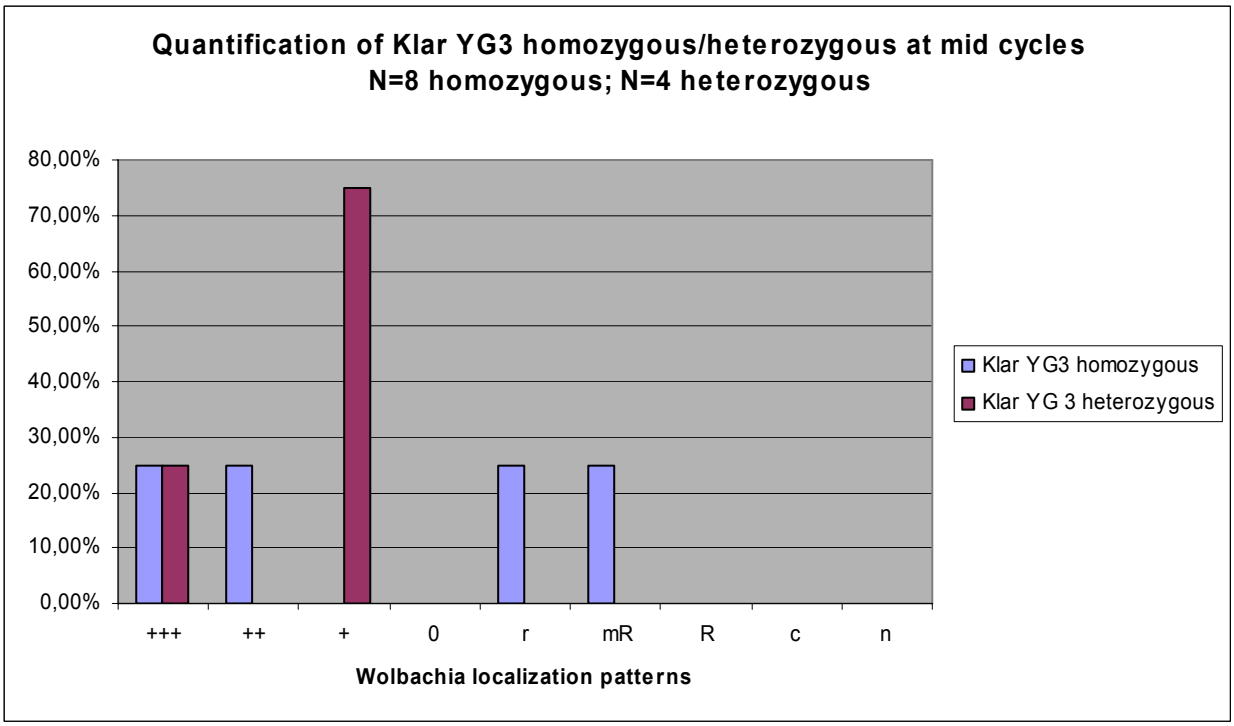


Figure 2.15: It shows the quantification results in percent of Klar YG3 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 8 Klar YG3 homozygous and 4 Klar YG3 heterozygous fertilized embryos fixed at the mid cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	2	3	6	25.00%
	2	2	4	25.00%
	0	1	0	0.00%
	0	0	0	0.00%
	2	r		25.00%
	2	mR		25.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	8		10	100.00%
Average	**1.25**			

Table 2.21: The table shows the quantification results of Klar YG3 homozygous of figure 2.15. 8 Klar YG3 homozygous fertilized embryos were examined at the mid cycles of the cellularization phase. The average value of 1.25 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 25% of 8 Klar YG3 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 25% of 8 Klar YG3 homozygous fertilized embryos showed medium Wolbachia accumulation at the posterior region. 25 % of 8 Klar YG3 homozygous fertilized embryos showed a small ring pattern at the posterior region. 25% of 8 Klar YG3 homozygous fertilized embryos showed a medium ring pattern at the posterior region.

	Number	Sign	Number*Sign	%
	1	3	3	25.00%
	0	2	0	0.00%
	3	1	3	75.00%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	4		6	100.00%
Average	**1.5**			

Table 2.22: The table shows the quantification results of Klar YG3 heterozygous of figure 2.15. 4 Klar YG3 heterozygous fertilized embryos were examined at the mid cycles of the cellularization phase. The average value of 1.5 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 25% of 4 Klar YG3 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 75% of 4 Klar YG3 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region

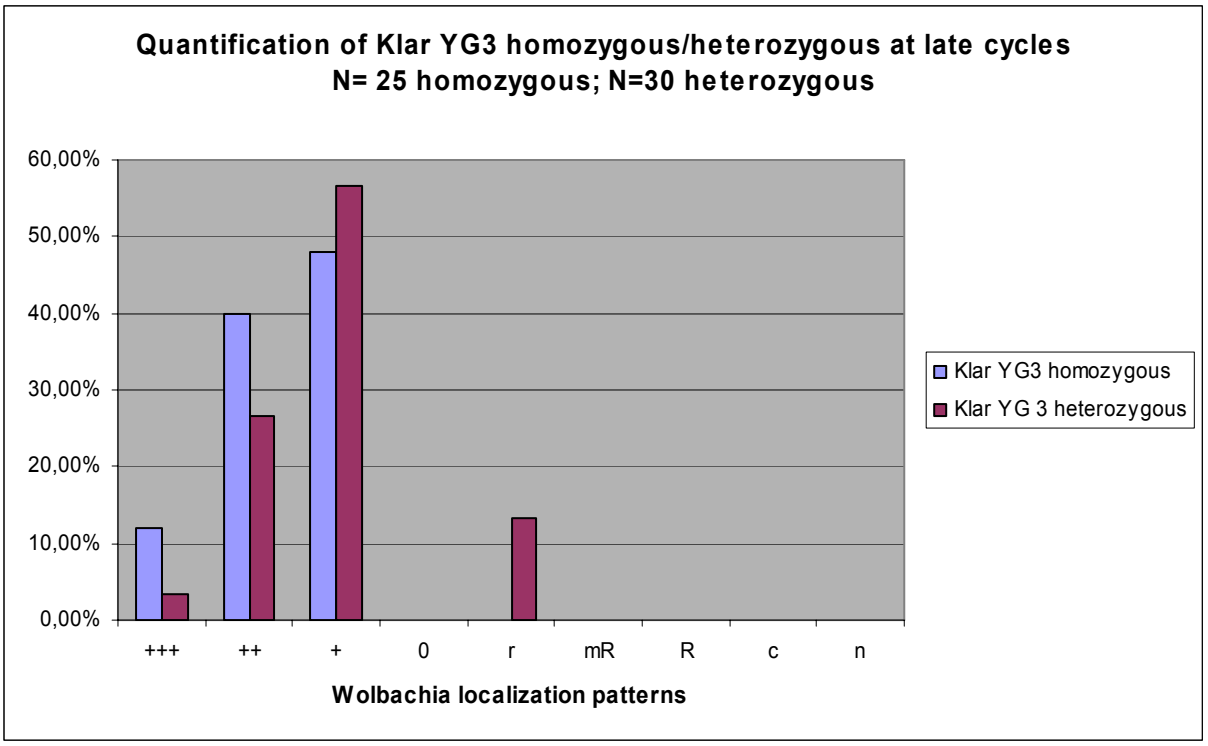


Figure 2.16: It shows the quantification results in percent of Klar YG3 homozygous and heterozygous in fertilized embryos as a function of Wolbachia localization pattern. 25 Klar YG3 homozygous and 30 Klar YG3 heterozygous fertilized embryos fixed at the late cycles of the cellularization phase were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	3	3	9	12.00%
	10	2	20	40.00%
	12	1	12	48.00%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	25		41	100.00%
Average	**1.64**			

Table 2.23: The table shows the quantification results of Klar YG3 homozygous of figure 2.16. 25 Klar YG3 homozygous fertilized embryos were examined at the late cycles of the cellularization phase. The average value of 1.64 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 12% of 25 Klar YG3 homozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 40% of 25 Klar YG3 homozygous fertilized embryos showed mid Wolbachia accumulation at the posterior region. 48% of 25 Klar YG3 homozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region.

	Number	Sign	Number*Sign	%
	1	3	3	3.33%
	8	2	16	26.67%
	17	1	17	56.67%
	0	0	0	0.00%
	4	r		13.33%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	30		36	100.00%
Average	**1.2**			

Table 2.24: The table shows the quantification results of Klar YG3 heterozygous of figure 2.16. 30 Klar YG3 heterozygous fertilized embryos were examined at the late cycles of the cellularization phase. The average value of 1.2 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 3.33% of 30 Klar YG3 heterozygous fertilized embryos showed strong Wolbachia accumulation at the posterior region. 26.67% of 30 Klar YG3 heterozygous fertilized embryos showed mid Wolbachia accumulation at the posterior region. 56.67% of 30 Klar YG3 heterozygous fertilized embryos showed weak Wolbachia accumulation at the posterior region. 13.33% of Klar YG3 heterozygous fertilized embryos showed a small ring pattern at the posterior region.

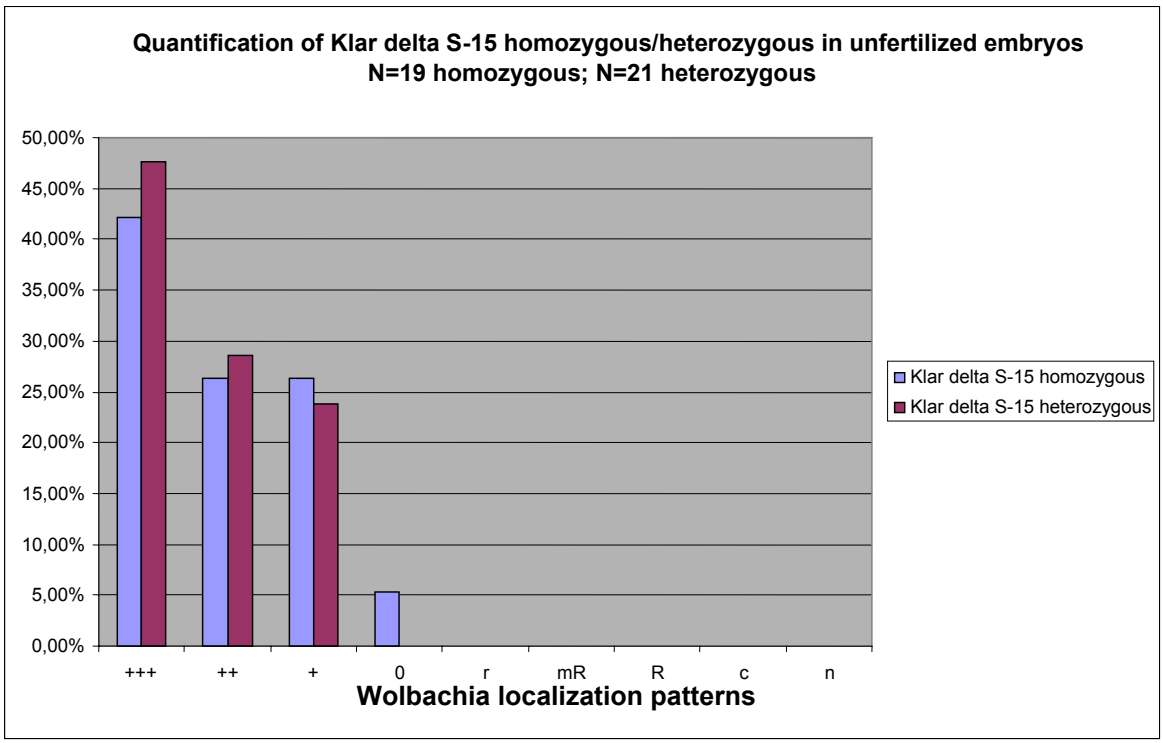


Figure 2.17: It shows the quantification results in percent of Klar ΔS-15 homozygous and heterozygous in unfertilized embryos as a function of Wolbachia localization pattern. Total 19 Klar ΔS-15 homozygous and 21 Klar ΔS-15 heterozygous unfertilized embryos were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	8	3	24	42.11%
	5	2	10	26.32%
	5	1	5	26.32%
	1	0	0	5.26%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	19		39	100.00%
Average	**2.05**			

Table 2.25: The table shows the quantification results of Klar delta S-15 homozygous of figure 2.17. 19 Klar delta S-15 homozygous unfertilized embryos were examined. The average value of 2.05 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 42.11% of 19 Klar delta S-15 homozygous unfertilized embryos showed strong Wolbachia accumulation at the posterior region. 26.32% of 19 Klar delta S-15 homozygous unfertilized embryos showed mid Wolbachia accumulation at the posterior region. 26.32% of 30 Klar delta S-15 homozygous unfertilized embryos showed weak Wolbachia accumulation at the posterior region. 5.26% of Klar delta S-15 homozygous unfertilized embryos showed a homogenous distribution of Wolbachia.

	Number	Sign	Number*Sign	%
	10	3	30	47.62%
	6	2	12	28.57%
	5	1	5	23.81%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	21		47	100.00%
Average	**2.24**			

Table 2.26: The table shows the quantification results of Klar delta S-15 heterozygous of figure 2.17. 21 Klar delta S-15 heterozygous unfertilized embryos were examined. The average value of 2.24 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 47.62% of 21 Klar delta S-15 heterozygous unfertilized embryos showed strong Wolbachia accumulation at the posterior region. 28.57% of 21 Klar delta S-15 heterozygous unfertilized embryos showed mid Wolbachia accumulation at the posterior region. 23.81% of 21 Klar delta S-15 heterozygous unfertilized embryos showed weak Wolbachia accumulation at the posterior region.

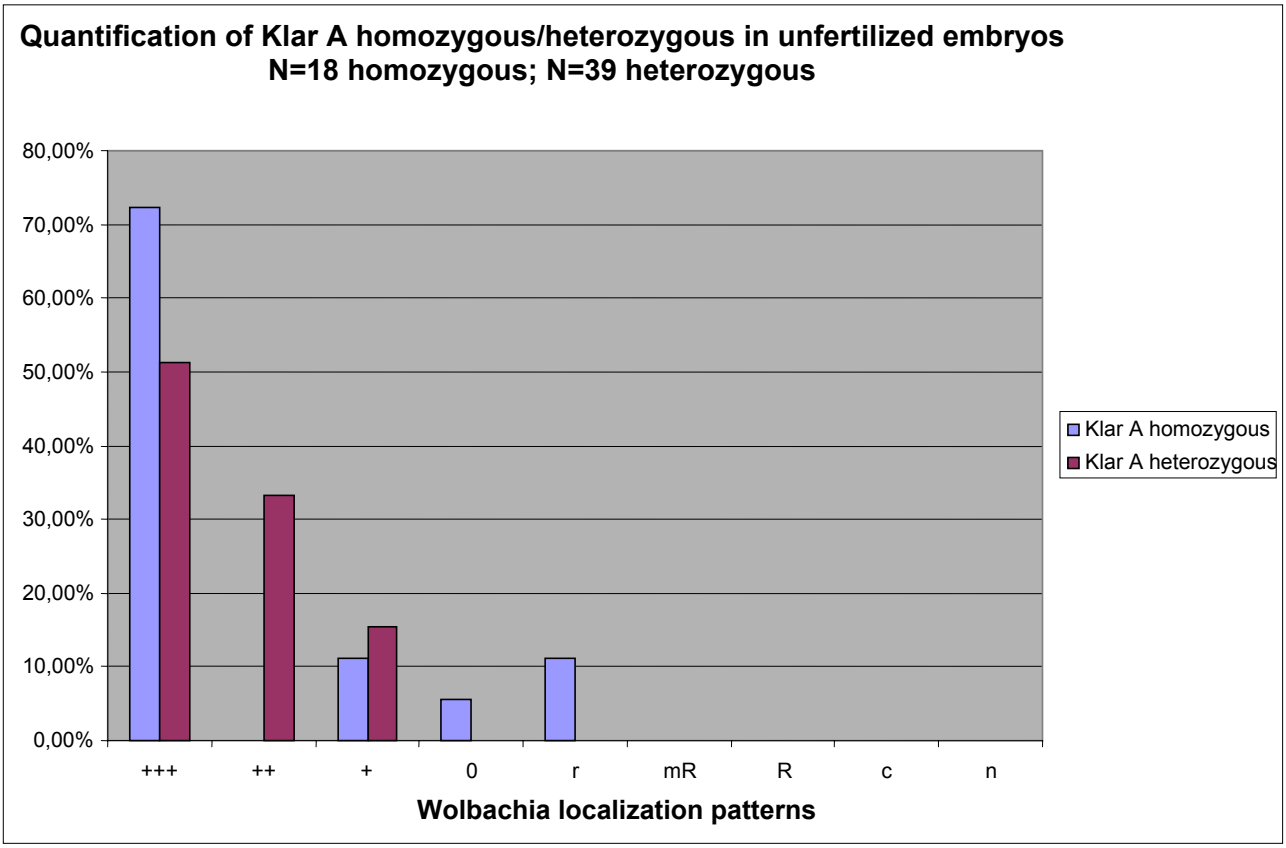


Figure 2.18: It shows the quantification results in percent of Klar A homozygous and heterozygous in unfertilized embryos as a function of Wolbachia localization pattern. Total 18 Klar A homozygous and 39 Klar A heterozygous unfertilized embryos were examined. +++ indicates strong Wolbachia accumulation at the posterior region. ++ indicates medium Wolbachia accumulation at the posterior region. + indicates weak Wolbachia accumulation at the posterior region. 0 indicates homogenous distribution of Wolbachia. r indicates a small ring pattern of Wolbachia at the posterior region. mR indicates a medium ring pattern of Wolbachia at the posterior region. R indicates a big ring pattern at the posterior region. c indicates that Wolbachia is accumulated at the cortex. n indicates that Wolbachia is not accumulated at the top of the posterior region.

	Number	Sign	Number*Sign	%
	13	3	39	72.22%
	0	2	0	0.00%
	2	1	2	11.11%
	1	0	0	5.56%
	2	r		11.11%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	18		41	100.00%
Average	**2.73**			

Table 2.27: The table shows the quantification results of Klar A homozygous of figure 2.18. 18 Klar A homozygous unfertilized embryos were examined. The average value of 2.73 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 72.22% of 18 Klar A homozygous unfertilized embryos showed strong Wolbachia accumulation at the posterior region. 11.11% of 18 Klar A homozygous unfertilized embryos showed weak Wolbachia accumulation at the posterior region. 5.56% of 18 Klar A homozygous unfertilized embryos showed that Wolbachia is homogenous distributed. 11.11% of 18 Klar A homozygous unfertilized embryos showed a small ring pattern at the posterior region.

	Number	Sign	Number* Sign	%
	20	3	60	51.28%
	13	2	26	33.33%
	6	1	6	15.38%
	0	0	0	0.00%
	0	r		0.00%
	0	mR		0.00%
	0	R		0.00%
	0	c		0.00%
	0	n		0.00%
Total	39		92	100.00%
Average	**2.36**			

Table 2.28: The table shows the quantification results of Klar A heterozygous of figure 2.18. 39 Klar A heterozygous unfertilized embryos were examined. The average value of 2.36 was calculated by total Number*Sign divided by the total Number which indicates the average strengthens of Wolbachia accumulation at the posterior region. 51.28% of 39 Klar A heterozygous unfertilized embryos showed strong Wolbachia accumulation at the posterior region. 33.33% of 39 Klar A heterozygous unfertilized embryos showed mid Wolbachia accumulation at the posterior region. 15.38% of 39 Klar A heterozygous unfertilized embryos showed weak Wolbachia accumulation at the posterior region.