COVER SHEET

**TITLE:** The role of vab-1/Eph receptor during epidermal intercalation in C. elegans

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ABSTRACT

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During *C. elegans* embryogenesis, dorsal intercalation occurs when epidermal cells exchange places with one another. In this study, we describe a role for Eph receptor—a receptor tyrosine kinase, known as VAB-1 in *C. elegans*—during the process of intercalation. The literature suggests that *vab-1* is crucial for proper embryogenesis; however, no one has described *vab-1*'s role in intercalation specifically. Here, I show that VAB-1 is necessary for intercalation and determine which regions of the VAB-1 protein are required for intercalation using a variety of characterized alleles within the *vab-1* gene. Because Eph receptors are highly conserved, this research has implications for vertebrate processes that depend on cell intercalation, such as neural tube closure.

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# The role of vab-1/Eph receptor during epidermal intercalation in *C. elegans*

**By Hunter Cochran**

Senior Honors Thesis

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**Abstract**

During *C. elegans* embryogenesis, dorsal intercalation occurs when epidermal cells exchange places with one another. In this study, we describe a role for Eph receptor—a receptor tyrosine kinase, known as VAB-1 in *C. elegans*—during the process of intercalation. The literature suggests that *vab-1* is crucial for proper embryogenesis; however, no one has described *vab-1*'s role in intercalation specifically. Here, I show that VAB-1 is necessary for intercalation and determine which regions of the VAB-1 protein are required for intercalation using a variety of characterized alleles within the *vab-1* gene. Because Eph receptors are highly conserved, this research has implications for vertebrate processes that depend on cell intercalation, such as neural tube closure.

Elise Walck-Shannon

Hunter Cochran

## Introduction

Embryogenesis is an important process in all animals. During embryogenesis, morphogenesis describes how an organism develops it shape. In this study, we look at how the small roundworm, *Caenorhabditis elegans,* develops its shape during embryogenesis. Specifically, we study a process called dorsal intercalation, which changes the shape of the worm epidermis. During dorsal intercalation, epidermal cells exchange places with one another along the medial axis [Williams-Masson *et al.* 1998]. Intercalation is required for *C. elegans* to form their tubular-like shape. Additionally, in vertebrates, intercalation is necessary for proper formation of various organs with a tubular shape, such as the cochlea and kidney tubules (reviewed in [Walck-Shannon and Hardin 2014]).

There are many potential problems that can occur during intercalation. For example, cells can fail to polarize leading to a delay or failure of rearrangement [Walck-Shannon *et al.* 2015]. This can lead to an increase in intercalation time when compared to wild-type worms. Alternatively, if there are problems with the pattern of intercalation, we could see a phenomenon where two cells migrate together rather than one-by-one as seen in intercalation (suggested in [Walck-Shannon and Hardin 2016]), which we call "co-migration". It is important to note that intercalation difficulties—both increased intercalation time and co-migration—are easily quantified through microscopy, given the *C. elegans* embryo's transparent eggshell.

One of the proteins that is important in understanding this research is Cdc42, a **C**ell **D**ivision **C**ontrol protein. Cdc42 is crucial for proper embryonic development

[Miller *et al.,* 2012], as well as intercalation. For example, in *C. elegans* embryos that do not have a functioning CDC-42 protein, co-migration is observed (Figure 1; E. Walck-Shannon, unpublished observations). This demonstrates that CDC-42 loss leads to co-migration and therefore complications in the patterning of intercalation.

The VAB-1 protein is an Eph receptor, which is a receptor tyrosine kinase. Eph receptors are one of the earliest known receptors evolutionarily and are therefore highly conserved in many species. This allows the results of this study to have implications for many different organisms. Eph receptors are a large protein family in vertebrates and are present in almost every kind of cell in the human body. In *C. elegans,* however, there is only one of the Eph receptor, VAB-1, which makes its study simple [Miller *et al.,* 2012]. Our interests in the Eph receptor, VAB-1, stem from two important observations: 1) a novel expression pattern on right-hand intercalating cells [Ikegami *et al.*] and 2) a genetic interaction between *cdc-42* and *vab-1* in a large-scale screen [Lehner *et al.* 2006]. Together, this data led us to believe that VAB-1 was important for dorsal intercalation. After this realization, we decided to conduct an experiment to better understand the role VAB-1 plays in intercalation.

This experiment, as noted above, was conducted using the small roundworm, *Caenorhabditis elegans* that can be seen in Figure 2. *C. elegans* is a microscopic organism that is highly valuable in many ways. It is eukaryotic, has a transparent eggshell that allows the contents of the embryo to be easily observed, has a lack of redundancy, a fully sequenced genome, and many reagents available in order to study specific allele strains. These traits, along with the fact that *C. elegans* worms

are easily manipulated make it an ideal organism when studying biological mechanisms. Because *C. elegans* worms are so easily manipulated, many genetic screens have been performed and many mutations have been isolated. Therefore, it is not uncommon to have many alleles available for a specific gene, such as *vab-1* [Utrecht University, 2016].

**Biological Rationale**

CDC-42 and VAB-1 have a known interaction and CDC-42 is required for intercalation; therefore, a mutation rendering VAB-1 inactive should lead to intercalation difficulties. The mutant strain that renders VAB-1 null is *vab-1(dx31)*. VAB-1 has two specific domains that we feel could be important in properly functioning intercalation. The kinase domain of VAB-1 mediates many downstream processes and therefore could be involved in intercalation. The two mutant strains that affect the kinase domain are *vab-1(e2)* and *vab-1(e118)*. The ligand-binding domain in VAB-1 is responsible for binding well-studied ephrin ligands and could also function during intercalation. The mutant strain that shows effects in this domain is *vab-1(e699)*. See mutant strains and their locations on VAB-1 in Figure 3.

We hypothesize that the Eph receptor, VAB-1, is involved in dorsal intercalation and that the kinase and ligand-binding domains may be required.

## Methods

*Daily Worm Maintenance*

*C. elegans* worms were maintained on agar plates with an *E. coli,* OP50, strain thinly spread across for food. All worm strains were held in a "refrigerator" kept at 20 degrees Celsius. Twice per week (about every 3-4 days), worms were maintained by "picking" 20 worms from the old plate and putting them on a new plate using a platinum wire and a dissecting microscope (Figure 4).

*Experimental Protocol*

In this experiment we worked with four different mutant strains of *C. elegans* worms. The strains used in this experiment were *vab-1(dx31), vab-1(e699), vab-1(e2),* and *vab-1(e118)* [George *et al.,* 1998]. In order to observe dorsal intercalation, and potential defects (the number of embryos that exhibited co-migration and to gather data on the intercalation times), it is necessary to film the embryos.

*Making* C. elegans *embryo mounts*

Making the embryo mounts requires that we first "picked" the worms with visible embryos from the agar plates. After picking, we placed the worms in a watch glass with M9 buffer solution. We then used the needle-end of two small syringes to slice the *C. elegans* in half so that their embryos spill out. It is important that there are at least 10 embryos in order to make a successful mount. Next, we used an eyelash brush to sweep the embryos together. Then, we used a mouth pipette to pick up the embryos and place them on agar gel on a glass slide. Next we used M9 buffer and a glass cover slide to cover the embryos and sealed the slide with wax. Finally, we took pictures of 30 cross sections, 1 micrometer apart, every three

minutes and compiled the photos into a movie (Figure 5) [Walston and Hardin, 2010].

*Dorsal Intercalation Scoring*

We took the time points of the last division and the nuclear meeting for each embryo in the movie. To calculate the intercalation time we use the equation:

> "Nuclear meeting – Last division*3 = Intercalation time" (see Walck-Shannon *et al.* 2015)

Then we made a count of all the embryos where co-migration was observed.

*Statistical Analysis*

Excel was used to make the co-migration frequency graphs. Percent co-migration data was analyzed using Fisher's Exact Test, which is a form of Chi-Square testing that uses a 2x2 contingency table. All strains were analyzed against the wild type strain to detect any statistical significance (using a p-value $\leq 0.05$). For all genotypes, ten or more movies were analyzed (number of embryos >30).

We used the program JMP to make the box plots of the intercalation time data. Intercalation time data was analyzed using an ANOVA test with subsequent t-tests. The ANOVA test is run to first see if there is a statistical significance between any of the groups. The t-tests tell us exactly which two groups are statistically different from one another. If the p-value was less than or equal to 0.05, we are able to declare that the two strains being compared have a statistically significant intercalation time.

## Results

In the *C. elegans* worm strains, we observed *vab-1(dx31)* to have the highest percent of co-migration at 19.6%, *vab-1(e699)* exhibited 11% co-migratory embryos, *vab-1(e2)* showed 3.45% co-migration, while *vab-1(e118)* and Wild Type strains had no co-migration observed (Figure 6). The difference between *vab-1(dx31)* and the Wild Type strains is statistically significant (Fisher exact test p= 0.0128). We observed that most of the strains had at least one embryo that exhibited co-migration patterns during intercalation.

We also gathered data on all strains for the time of intercalation seen in Figure 7. We observed that *vab-1(dx31)* had an average intercalation time of 111.33 seconds (standard deviation=2.1, n=46), *vab-1(e699)* had an average of 103.62 seconds (std. dev=2.8, n=30), *vab-1(e2)* had an average of 101.17 seconds (std. dev=2.6, n=31), and *vab-1(e118)* had an average of 107.83 seconds (std. dev=2.4, n=35). These were compared to a wild type strain with the average of 94.33 seconds (std. dev=2.0, n=51). All strains were first compared using an ANOVA and the analysis was significant, $F(4, 186) = 9.72$, $p < 0.0001$ ($r = 0.248$). Subsequent t-tests were then used to compare each strain against the Wild Type Strain (*vab-1(dx31)* p<0.0001, *vab-1(e699)* p=0.0076, *vab-1(e2)* p=0.0407, *vab-1(e118)* p<0.0001).

## Discussion

We found that the *vab-1(dx31)* strain exhibited significantly more co-migration than the wild type strain (Figure 6). Because this mutation prevents the entire VAB-1 protein from being made, we can conclude that VAB-1 is involved in

intercalation. We did not see a statistical significance between the other mutant strains, but we did see co-migration in two of the mutant specific domain strains. Even though there might not be a statistical significance, it could be biologically relevant that mutations in the ligand-binding domain and the kinase domain lead to the co-migration phenotype. However, it is important to note that we did not see co-migration in all of the embryos and in fact, we only observed it in a small fraction of the total. It is possible that domains in VAB-1 other than the ligand-binding or kinase domains are essential for their function. If the kinase or ligand-binding domain is mutated then, in some cases, a different protein could be activated to continue the process, which could lead to the lower percentages of co-migration observed. It is also possible that the mutations do not fully knockout the domain, but only make them weaker.

When looking at the intercalation time data, the significance of this study becomes much more evident. We saw a statistically significant increase in all *vab-1* strains studied compared to wild-type intercalation times (Figure 7). An increase in intercalation time suggests intercalation difficulties. One possible explanation is that there are other proteins that can also activate the proper route, but that route takes extra time to activate. Another possibility is that when the ligand-binding domain is mutated the ligand finds a different receptor that has a similar function; it just takes a longer amount of time. Another possibility is that the mutations cause polarization problems within the embryos. Polarization problems cause issues with the actin polymerization, which could cause blunt tips in the migrating cells and ultimately this does not allow for efficient nuclear migration. Because of this, we are able to

conclude that the kinase domain as well as the ligand-binding domain are both important for proper intercalation.

The combination of the percent co-migration and intercalation time data gives us the best picture at how VAB-1 functions during intercalation. As one can see from Figure 6, not all embryos are experiencing co-migration. This suggests that the co-migration phenotype is not fully penetrant and, therefore, that VAB-1 could be interacting with other receptors to activate CDC-42. However, because we have data on the time of intercalation, it is obvious that each mutant strain, on average, did experience a significantly higher intercalation time compared to the wild type strain.

The increased frequency of co-migration along with the increased intercalation time data allows us to accept our hypothesis that the Eph receptor, VAB-1, is involved in dorsal intercalation and that the kinase and ligand-binding domains are required.

This is the first research that focuses on CDC-42 as well as Eph receptors during an epithelial intercalation event, which makes it valuable to the scientific community. The fact that loss of both VAB-1 and CDC-42 (Figure 1) display similar phenotypes suggests that these two proteins function together during intercalation. Specifically, this research suggests that CDC-42's interaction with VAB-1 is very important for orienting cell migration during intercalation. We also have concluded that in some way, the ligand-binding domain as well as the kinase domain are crucial to VAB-1 functioning in cell migration.

*Future Studies*

There are many possible studies that could emerge from this novel research. One option is to study other alleles in the kinase and ligand-binding domains that could be involved in dorsal intercalation. We looked at two possible mutations located in the kinase domain, which we found were both involved in the proper functioning of the VAB-1 protein. This research could help us to tease out downstream effectors that the kinase domain interacts with to orient the migration of cells during intercalation. Another possible future path is to direct our research efforts into looking at ligand mutations. In our current research, we look at what the eprhin-ligand attaches to, the ligand-binding domain; however, it would be interesting to see if cell-migration is affected when the ligands themselves are disrupted. A final option would be to research the proteins and processes that are located in between the VAB-1 and CDC-42 proteins. While we know that the kinase domain in VAB-1 is required for downstream interactions, it is currently unclear precisely which molecules lie between VAB-1 and CDC-42. Further research would help to define what the proteins are, which are crucial for intercalation, and which have a direct interaction with CDC-42 and VAB-1.

This research has implications in other organisms as well because *C. elegans* worms have conserved biological mechanisms. The intercalation process occurs in many organisms and in many different forms. This research, being the first of its kind to look at eph receptors in epithelial cells, might be the answer to certain intercalation problems in other organisms.

In conclusion, the Eph receptor, VAB-1, is involved in dorsal intercalation. The kinase domain as well as the ligand-binding domain are important to VAB-1's proper function; therefore, these domains are involved in dorsal intercalation and are essential in preventing intercalation issues.

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First and foremost I would like to thank my mentor, Elise Walck-Shannon, Ph.D. for teaching me everything I needed to know in only one year. She was not only patient and kind, she was willing to help me at all times. I would also like to thank Jeff Hardin, Ph.D. for allowing me to be a part of this zoology lab to complete my Senior Honors Thesis project. I would also like to thank Bethany Lucas for helping me learn and fix all the issues I had with the microscopes. Finally, I would like to thank Jared Strauch for making all of the agar plates to allow this research to be possible. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

**Figures**

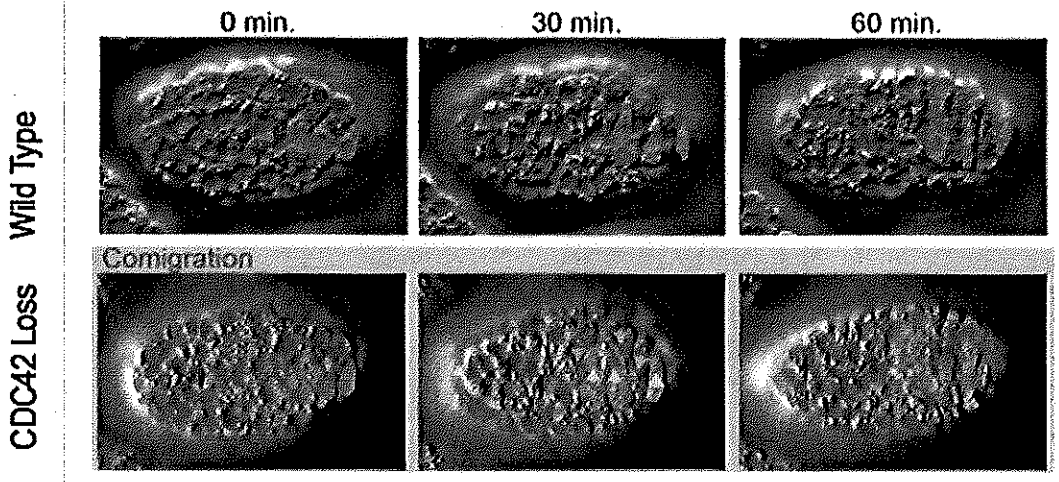


**Figure 1.** Shows intercalation in *C. elegans* Wild Type and CDC-42 loss embryos. [Figure by Elise Walck-Shannon].

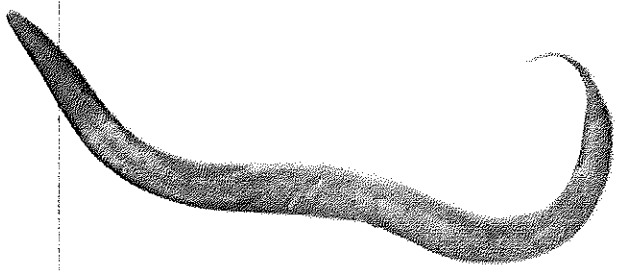

**Figure 2.** Photo of *C. elegans* worm [Utrecht University, 2016].

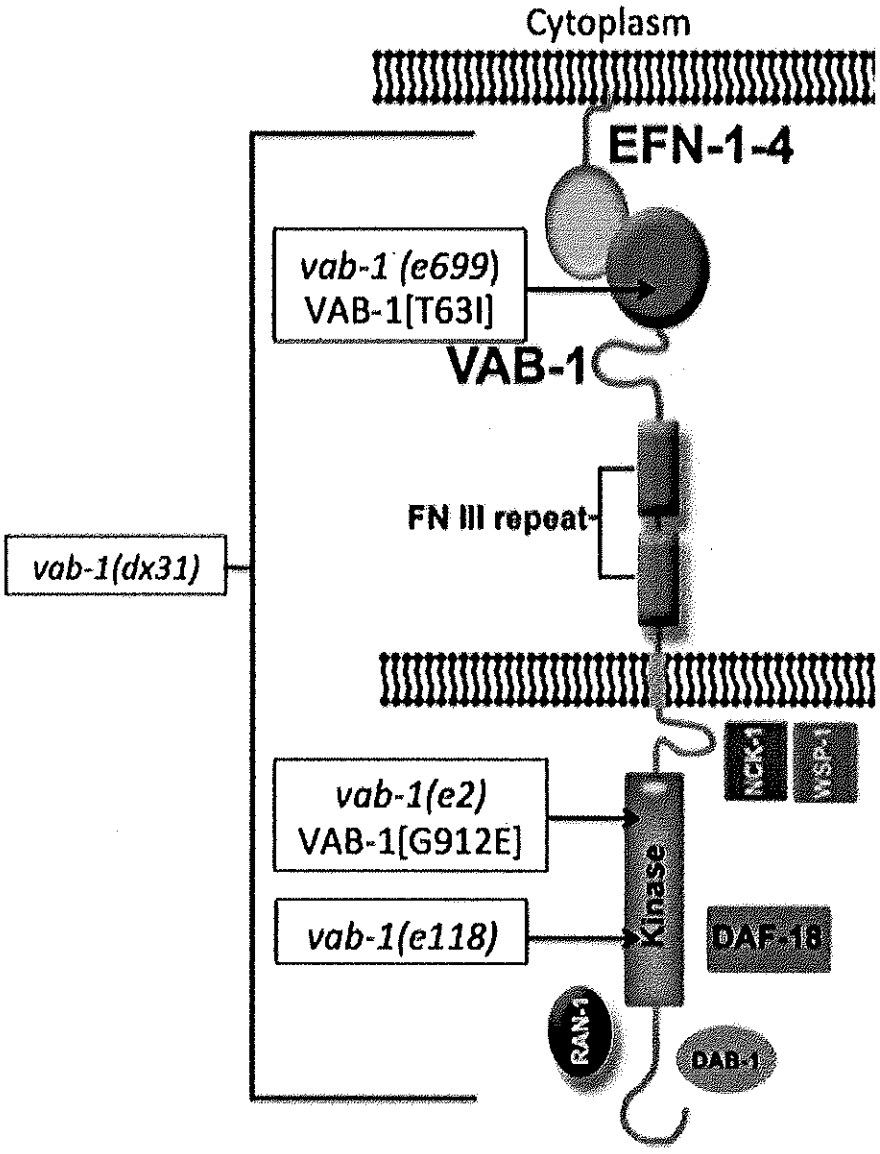


**Figure 3.** VAB-1 protein with gene mutations and their locations within the protein.

[Miller *et al.,* 2012]

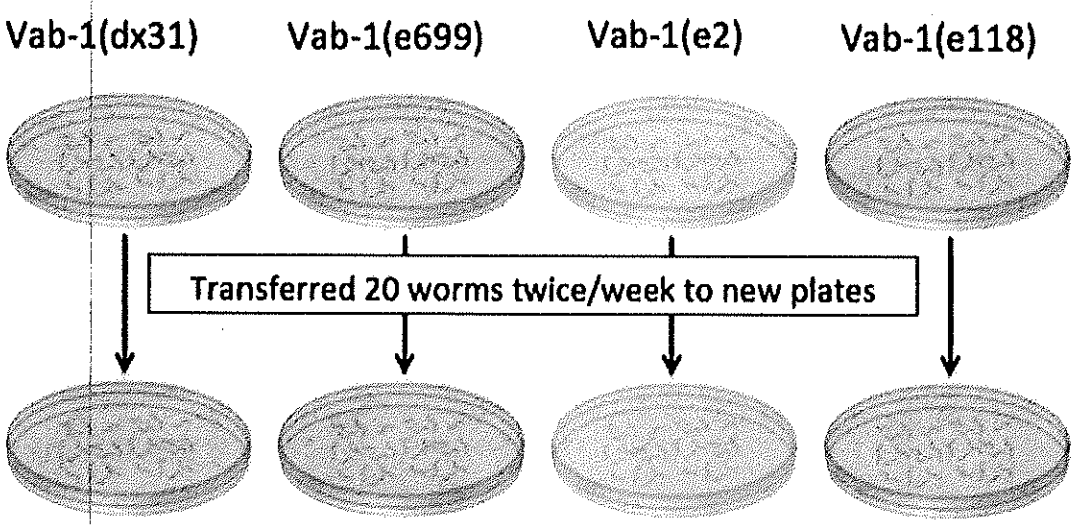


**Figure 4.** Describes the maintenance of all strains throughout the experiment.

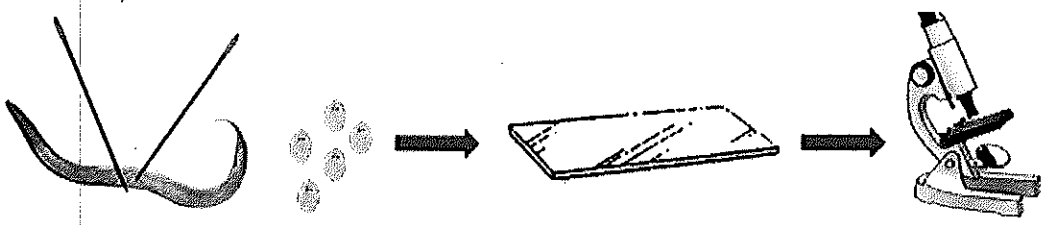

**Figure 5.** The process of making mounts and movies of embryos.

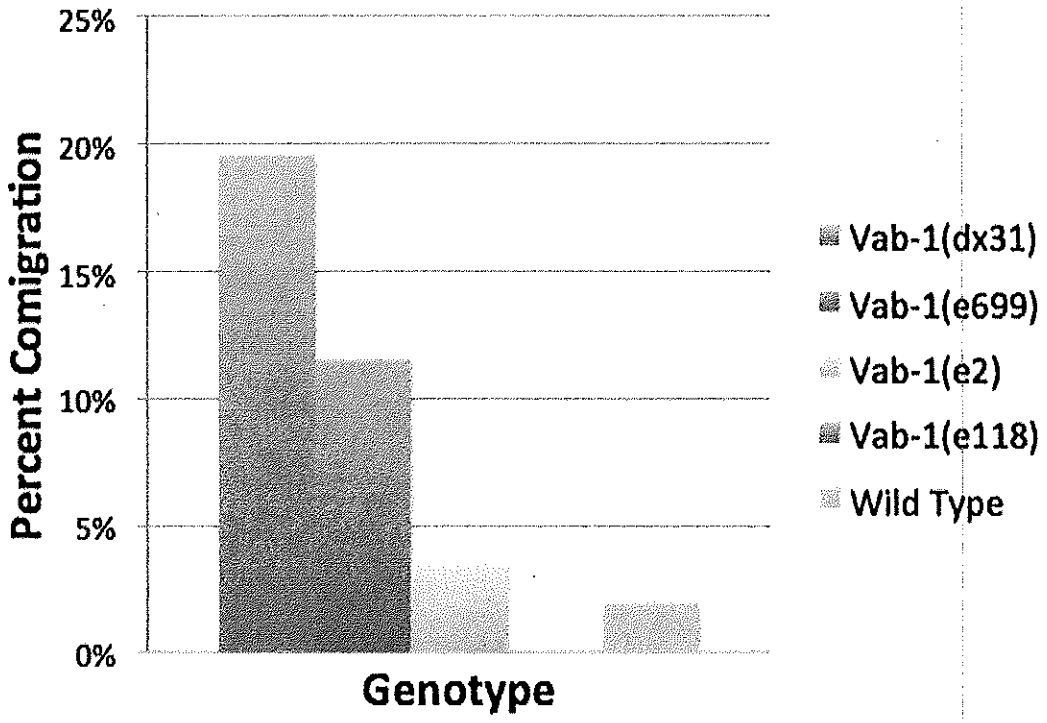


**Figure 6.** Percent of worms exhibiting co-migration in four mutated strains and the Wild Type strain. Null mutation *vab-1(dx31)* showed highest percentage of co-migration, while *vab-1(e118)* mutation exhibited no co-migration. *vab-1(dx31)* strain was only statistically significant result (0.0058, $p<0.05$). [*vab-1(dx31):* n=46, *vab-1(e699):* n=30, *vab-1(e2):* n=31, *vab-1(e118):* n=35, *WT:* n=51)].

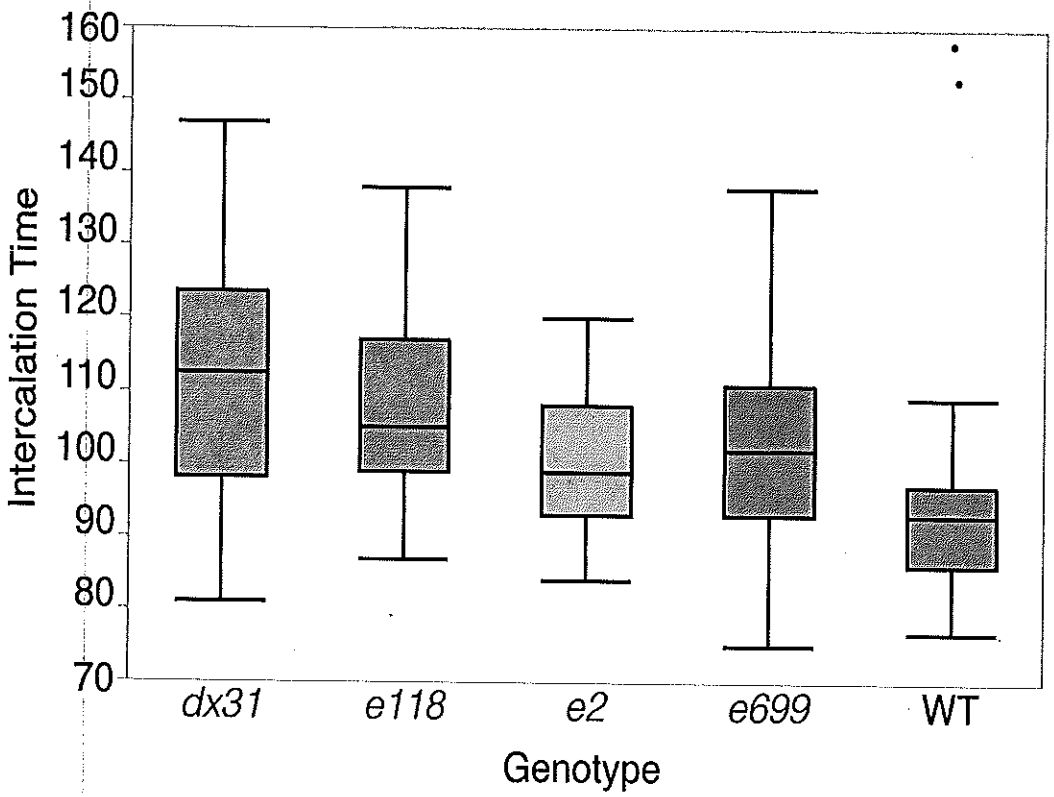


**Figure 7.** Intercalation times of wild type and four mutated strains of *C. elegans*. Null mutation *vab-1(dx31)*. Showed longest intercalation time while the wild type strain had the shortest time. All statistically significant compared to WT ($p<0.05$). [*vab-1(dx31):* n=46, *vab-1(e699):* n=30, *vab-1(e2):* n=31, *vab-1(e118):* n=35, *WT:* n=51)].

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