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Experimental Analysis of Supercoiling in Twisted Polymer Line

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Abstract

It is well known that DNA has a double helix structure due to chemical interactions between its base paired molecules. These interactions, along with mechanical forces, cause DNA to bend and twist, which controls gene expression and other processes such as transcription and translation. Additionally, due to its extreme length, DNA must supercoil in order to achieve the compact state necessary to fit on histones within a nucleus [1]. Current nano-torsional tests and mathematical models on DNA have hypothesized that at certain critical forces during twisting, DNA forms supercoils due to a torsional buckling instability of the structure; however, there is little experimental research to verify the proposed models of this process [1]. A typical test would have to measure the twist on the DNA molecule while applying a measured force and observing the onset of buckling. A supercoil is defined in this paper as a loop structure created as a mechanism to release torsional energy. For the experiments described in this paper, an isotropic polymer line was twisted and stretched until it exhibited supercoiling when observed in a mechanical load frame. Supercoiling was achieved by looping the polymer line through a weight and then knotting the ends around a stable hook. This doubled feature allowed the line to twist in a manner which mimics that of DNA: two strands crossing over each other. The weight was twisted until the first supercoil occurred. The radius of the formed loop was also measured. The data measured supports the hypothesis that the first supercoil occurs when a critical torque, or twist, is reached. Additionally, the results indicate that supercoiling is primarily a strain driven process, as the formation of the supercoils is dependent on shortening of the line caused by applied torsion. Furthermore, the line undergoes plastic deformation even after the supercoils are allowed to relax and the line returns to a state of zero applied torsion.

Keywords: Supercoiling, DNA, Twist

1. Introduction

It is well known that DNA is a double helix structure composed of four base pairs: adenine, guanine, cytosine, and thymine which are linked to ribose phosphate [1]. DNA is an immensely long molecule with tens of millions of base pairs; however, it is required to compact itself to a micron-sized scale in order to wrap around histones, creating chromatin, and fit within a nucleus [1]. These structures typically contain around three meters of DNA [2]. Most of this compaction is achieved through supercoiling, a series of loops created by excessive torsion either mechanically or chemically applied as a means of relieving torsional energy [1]. The DNA molecule must also untwist from its supercoiled structure without tangling so replication can occur [2].

Due to its applications in targeted drug therapies, gene expression is currently a highly researched topic. It has been determined that gene expression can, in some cases, be mediated by supercoiling in specific regions of DNA [3]. Supercoiling can cause these regions to destabilize locally, thus allowing specific promoters to become active, resulting in varied protein synthesis [3-6]. Currently, research is being conducted to explore torsional properties of DNA and subsequent supercoiling using micromanipulation techniques such as magnetic or optical tweezing [1, 2, 6]. In these

experiments, DNA is constrained at one end, and torsion is applied with a specific force to the other to induce supercoiling [1, 6]. The major consideration with this method of experimentation lies in that it considers the entire length of the DNA strand being tested, while actual DNA is supercoiled only in specific regions [5]. Because of that restriction, the torque is not uniform throughout the molecule. Consequently, there is still a lack of understanding and direct evidence of how twisting directly affects gene expression.

In order to more fully understand the biological purpose of supercoiling, supercoil buckling has been experimentally examined from a mechanical standpoint; the theoretical understandings based mostly on mechanical beam theory are also mechanical and mathematical expressions. Currently, several mathematical models exist to explain the relationship between supercoils and the energy they relieve; however, there is limited experimental data to verify them. Many of these models propose a correlation between torsion applied to the DNA molecule and the physical dimensions of the resultant supercoil, but fail to back up their propositions with extensive experimental, repetitive, hard data [1]. As such, the primary goal of our experimentation was to investigate the mechanics of supercoiling using a well-defined isotropic polymer line as a simulation for DNA. Defining the relationship between the radius of the first supercoil and the twisted and applied forces will help increase the understanding of the mechanism of supercoiling in terms of energy release and provide a more theoretical explanation of its role in DNA mechanics.

2. Experimental Methods

Several hundred yards of 50 pound test polypropylene fishing line with a 0.025 inch diameter was purchased from Danielson Company [7]. The line was cut to selected lengths that were then double looped and knotted in order to simulate DNA as an isotropic polymer line. A separate single strand of the line was cut to determine Young's modulus, E, in a uniaxial tensile test. This was done using a screw-driven Instron test frame with cross-head rate controls of 0.025in/s with a calibrated load cell. The diameter of the tensile line was measured in the stress free condition to determine the elastic modulus of the polymer line. Four sets of data were taken using weights of 0.06 lb, 0.16 lb, 0.26 lb, and 0.36 lb hanging from the double looped line. The line was looped through the weights so that the fishing line would overlap itself when a rotation was applied, mocking the double helix structure of DNA. The same procedure was followed with all four weights. All measurements of the supercoils formed were taken using a Vernier caliper. First, the original length (L_0) of the fishing line from the hook to the weight was measured. The weights were then turned 360 degrees for *n* number of times until the first supercoil of buckling occurred. The new length (L_1) from the hook to the weight and the diameter of the first loop were then recorded. Lastly, the weight was released of its torque, allowing the torsional energy to decrease and a number of turns to unwind. The length (L_2) from the hook to the unwound weight was then recorded. Graphs were created to show the relationships between L_0 and the number of turns when supercoiling occurred, the radius of the supercoil created, L_1, and L_2. See Figure 1 where L_0, L_1, and L_2 are shown.

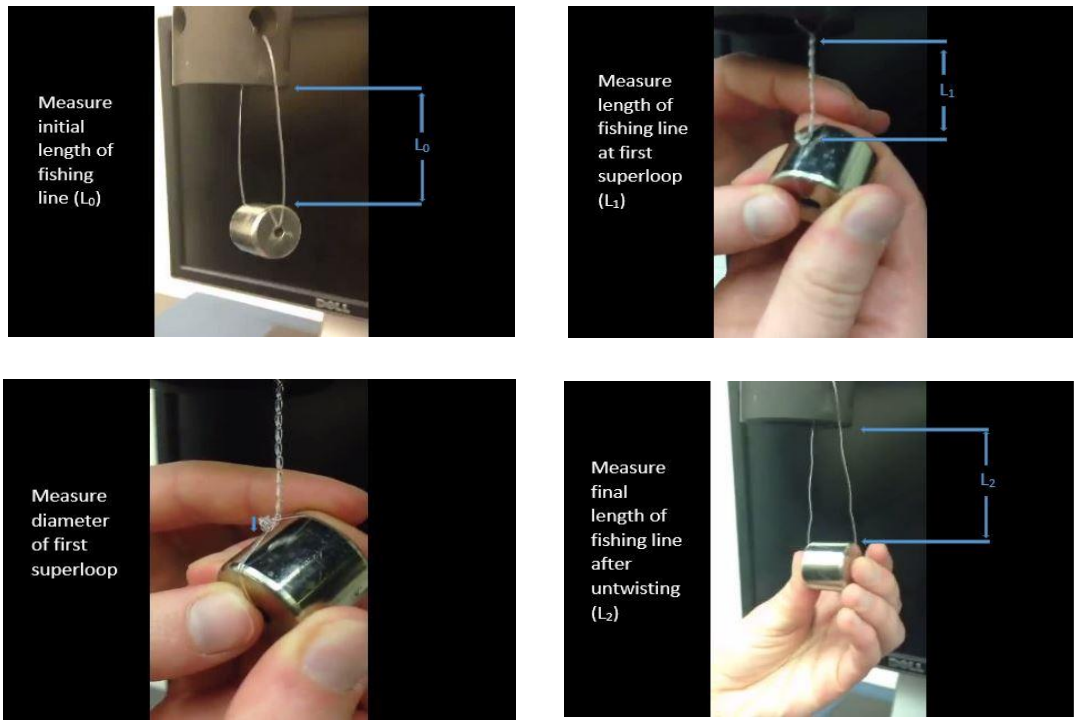

Figure 1: Experimental Procedure.

3. Results

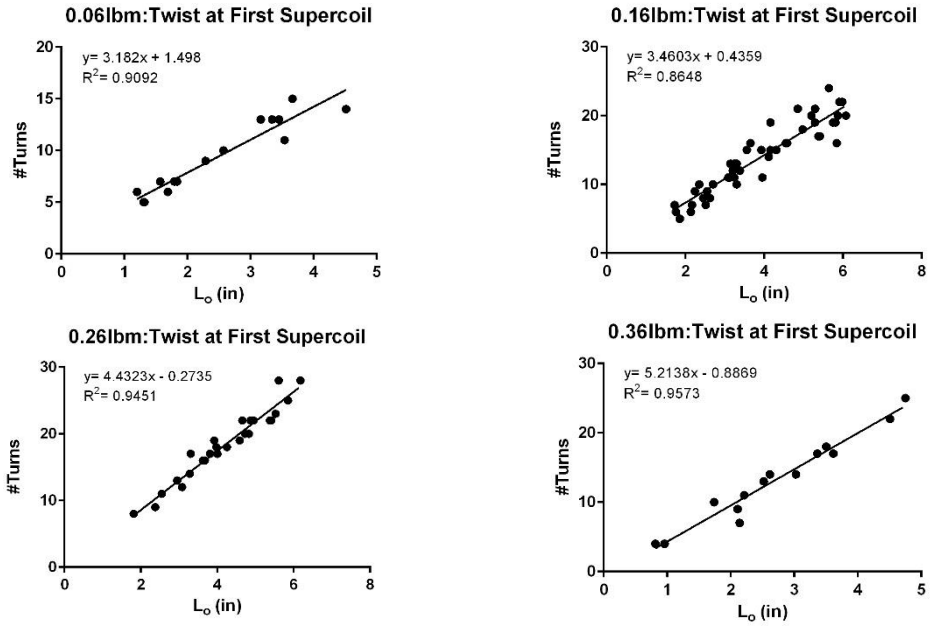

Figure 2: Experimental measurements of the onset of supercoil formation versus the twist (turns/length) of the line. This is the number of turns in the weight versus the original length for all four weights (n=13-50).

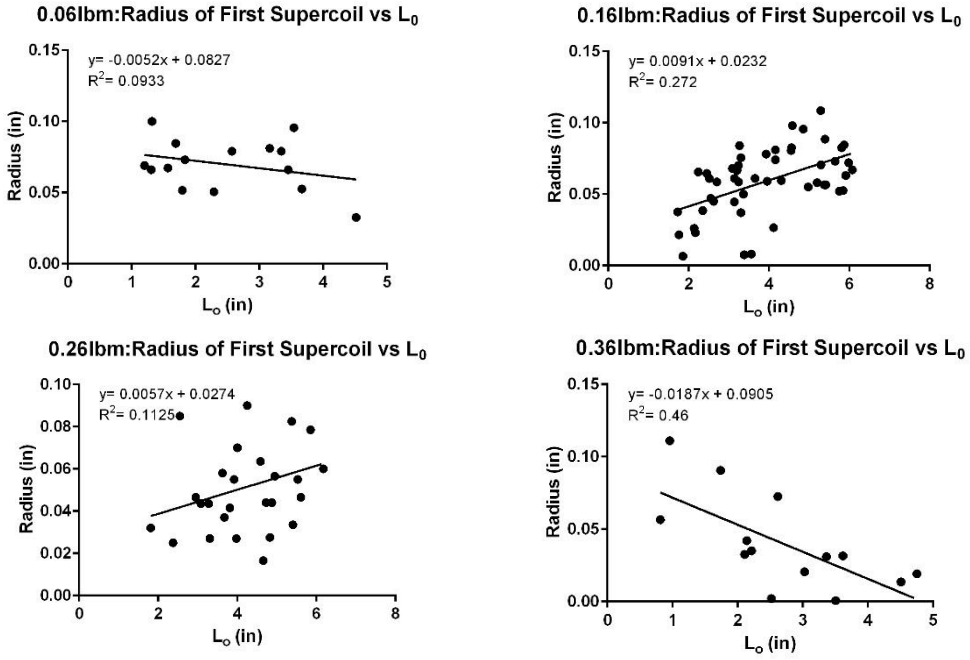

Figure 3: Plots displaying the radius of the first supercoil versus the original length (L_0) for all four weights (n=13-50).

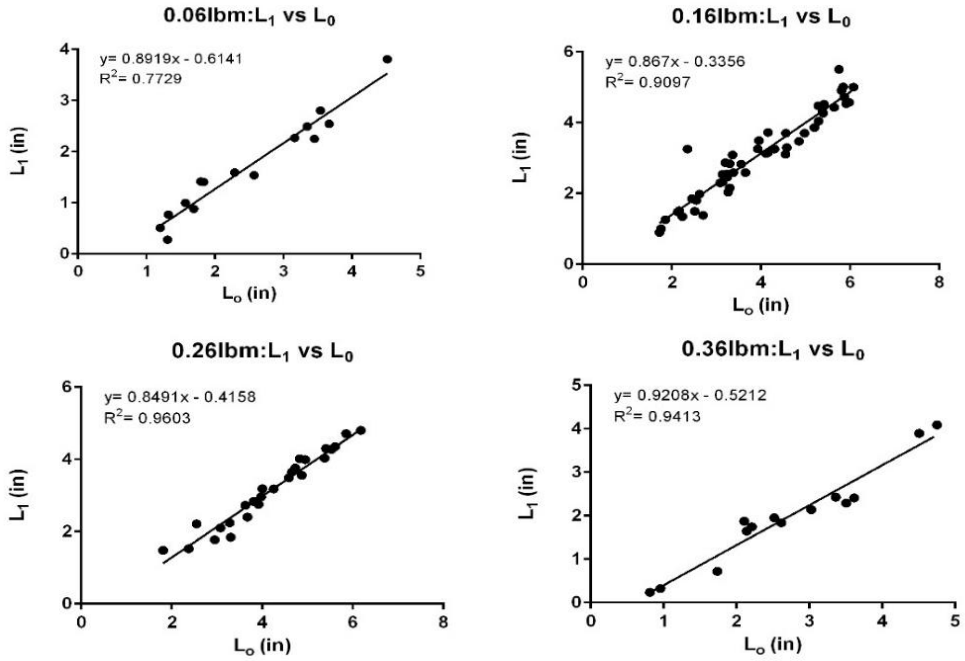

Figure 4: Plots displaying the relationship between the length after twisting to first supercoil (L_1) vs the original length (L_0) for all four weights (n=13-50).

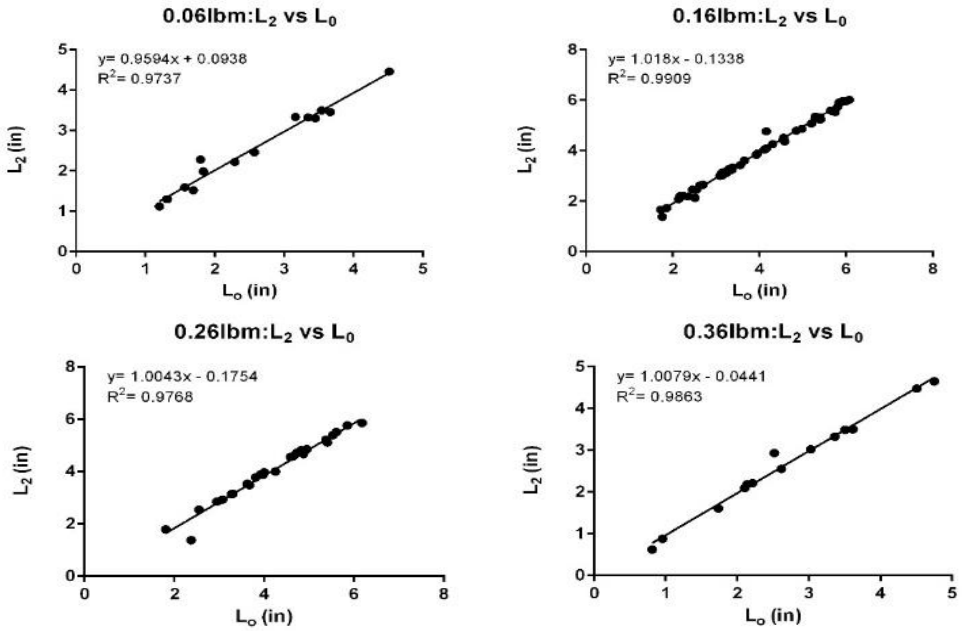

Figure 5: Plots displaying the length after untwisting (L_2) versus the original length (L_0) for all four weights (n=13-50).

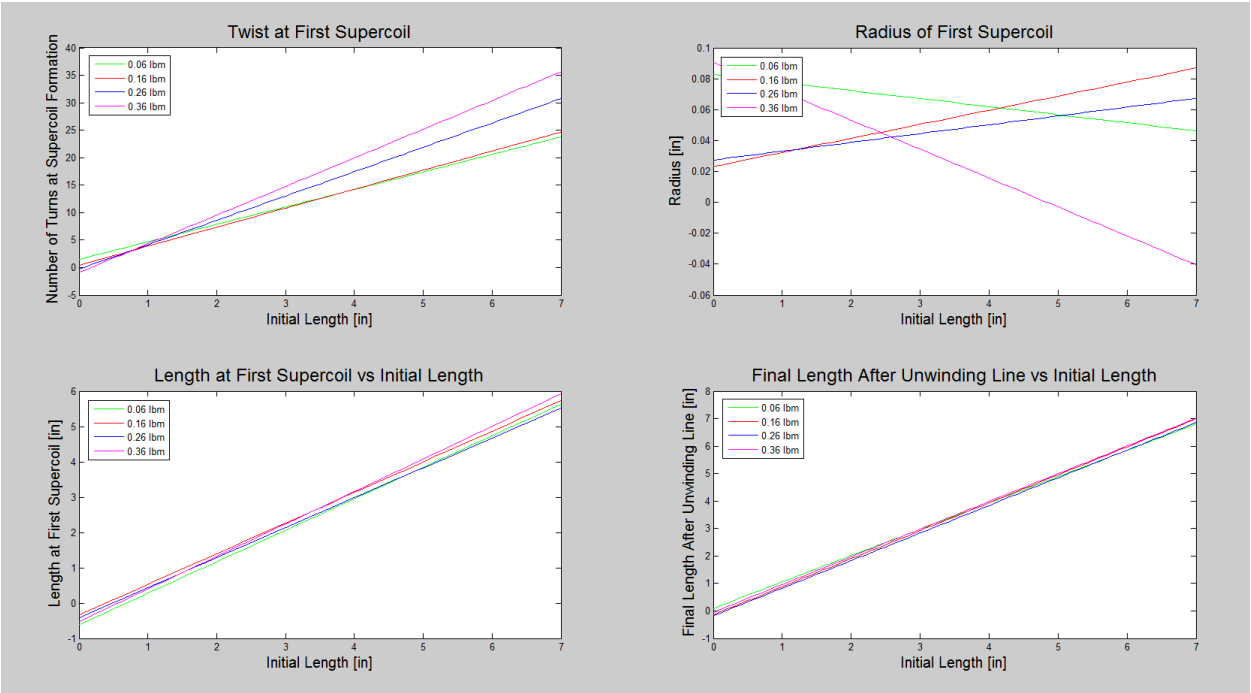

Figure 6. Graphs comparing each of the four tests at each weight.

Table 1: Radius of first supercoil i.e., the y-intercept from Figure 3 lines, slope at supercoil formation (the slope values found in Figure 4), and slope after unloading from the slope values found in Figure 5.

Comparisons of Critical Radii and Slopes at Each Mass			
Mass (lbm)	**R_c (in)**	**Slope at Supercoil Formation**	**Slope After Unloading**
0.06	0.0827	0.8919	0.9594
0.16	0.0232	0.8670	1.0180
0.26	0.0274	0.8491	1.0043
0.36	0.0905	0.9208	1.0079

4. Discussion

The data presented in Figures 2 and 6 led to the conclusion that there is a significant correlation between the numbers of turns until the first supercoil occurred and the original length of the fishing line (L_0). By using a linear regression in the general form:

$$y=mx+b \quad (1)$$

Where *y* is the number of turns, *x* is L_0, and *b* is the y-intercept, a correlation is clearly observed. The m and b values of all four equations appear to be very close in value. By observing Figures 2 and 6, it is noticed that the four curves follow a pattern very similar to each other. This experimental data proves that there is a linear relationship between the number of turns and the original length.

The data presented in Figures 3 and 6 show that there is no obvious correlation between the radius of the supercoil and the original length. Therefore, it is concluded that radius of the first supercoil and the original length must depend on other variables.

The data presented in Figures 4 and 6 lead to the conclusion that there is a strong correlation between the length after turning (L_1) and the original length of the fishing line (L_0). By again using a linear regression where *y* is L_1, *m* is a constant slope, *x* is L_0, and *b* is the y-intercept, it is clearly observed that a strong correlation exists. The *m* and *b* values of all four equations appear to be very close in value. Therefore it is concluded that a general form of the relationship between L_1 and L_0 can be found.

The data presented in Figures 5 and 6 led to the conclusion that there is a significant correlation between the final length (L_2) and the original length of the fishing line (L_0). By using a linear regression to find a line of best fit for the data, it is clearly observed that a correlation exists. Again, the m and b values were similar for this test. Therefore it is concluded that a general form of the relationship between L_2 and L_0 can be found.

Having this understanding of the relationships between L_0, number of turns until the first supercoil, L_1, and L_2 would allow for strain based understanding of these variables to be established. Future biologists could predict these values without taking the immense amounts of time to do the experimental analysis. Furthermore, establishing that these relationships describe supercoiling allows for more experiments on gene expression to occur, eliminating the unknown of how DNA reacts in different conditions.

5. Conclusion

Mathematical modeling of DNA has been attempted many times, however no macroscopic experimental data has been published until now. As stated earlier, though DNA is a complex, biological molecule, it must be observed from a mechanical standpoint. In order to understand how supercoiling affects gene expression in DNA, determining accurate mathematical modelling is crucial to further developing the field. The relationships found in figures 2, 4, and 5 show excellent correlations that highlight which parameters of DNA have good connections. The lack of correlation seen in Figure 3, which displays the relationship between L_0 and supercoil radii, was due to its dependence on some other

parameter. Future experimentation should be geared toward further data acquisition to find better descriptive mathematical models that establish the relationships modeled in Figures 2-6. The most important conclusion gained from these experiments is that supercoiling is a strain, not a stress, driven phenomena.

6. Acknowledgements

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7. References

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