Developing and Optimizing Micro-resolution Mosquito Bite Blocking Textiles

by

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Abstract

Textiles have been applied in the context of entomological applications since the primitive inventions of clothing and shelter. In many cases insects are injurious to humans. Mosquitos kill over a million people each year and it all stems from the problem that hematophagous insects bite humans. We have attempted to control populations since the Sumerians first used sulfur to kill pests. However, environmental and societal health risks pose a strong threat to the efficacy of primary control methods. Traditional bed nets were instrumental in the fight against mosquito borne diseases and still are. Unfortunately, they become less efficient as some species of mosquitos are adapting to day feeding. It is always pertinent to revisit old technologies in light of new advances in order to engineer better products. Wearing "normal" clothing is not sufficient to reduce the ability of insects, particularly mosquitos, to bite humans. Engineering clothing to mechanistically block mosquito bites without the use of insecticides is a challenging problem. This research explores a series of parameters and knitting structures that can enhance the bite blocking efficacy of clothing. It was discovered that a single knit structure, interlock, after washing, is capable of blocking mosquito bites. Other prototype sleeves were created using different knit structures while varying parameters such as yarn diameter, stitch length, spandex content, and post-manufactured shrinking. Here we define variables and treatments proven to directly contribute to the bite blocking ability of knitted textiles.

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AU	Auburn University
CDC	Center for Disease Control
EPA	Environmental Protection Agency
CI	Cytoplasmic Incompatibility
DDT	Dichloro-diphenyl-trichloroethane
IPM	Integrated Pest Management
ITNs	Insecticide-treated Nets
ITMs	Insecticide-treated Materials
SIT	Sterile Insect Technique
WT	Wild Type

Chapter 1: Introduction

1.1 Mosquitos

Mosquitos are known vectors of many medically significant diseases such as malaria, dengue, West Nile, yellow fever, Zika, chikungunya, and lymphatic filariasis (CDC 2019). Through the spread and transmission of these viruses, mosquitos kill over one million people per year, making them the world's deadliest animal (AMCA n.d.). Malaria, which is spread by *Anopheles sp.*, is the most notable and significant disease spread by mosquitos. In 2020, it accounted for an estimated 627,000 deaths (WHO 2021). Additionally, nearly half of the world's population was at risk of contracting malaria (WHO 2021). There were 241 million estimated cases of malaria in 2020, an increase of 14 million cases from the year prior (WHO 2021). This increase in disease prevalence and spread has made it undoubtedly necessary to increase mosquito control and decrease the spread of mosquito transmitted viruses.

Perhaps one of the most prevalent species of mosquito is *Aedes aegypti*, more commonly referred to as the yellow fever mosquito. It is well known for transmitting yellow fever, dengue, and Zika (CDC 2020). While this species is not known to transmit malaria, it is still one of the most problematic species of mosquito. One of the primary reasons behind this is that they aggressively feed on humans. They prefer to live near humans and would rather feed on humans over animals (CDC 2020). They are naturally a tropical insect but have been expanding their range in the United States for some time. They are now commonly found across the southern United States (CDC 2020). This species, like all mosquitos, is holometabolous. This means that they have four distinct life stages: egg, larvae, pupae, and adult. *Aedes aegypti* mosquitos have specifically adapted to live in close proximity with humans. This has inherently made it difficult to eradicate them as well. While it takes 7-10 days for development from egg to adult (CDC 2020), the eggs are viable and able to inhibit larval hatching for nearly nine months (Fischer et.

al. 2019). After which, they can begin development stages when conditions become more favorable (Fischer et. al. 2019). Their evolutionary ability to survive over winter and induce diapause during egg stages is a likely reason as to why eradication of this species has proven to be such a challenge (Lima et. al. 2016; Trewin et. al. 2019). This, in part with how easy they are to culture, is why this mosquito is a fantastic test model against mosquito-bite resistant textiles.

The mosquito mouthpart (proboscis) is comprised of six complex stylets, each of which aid in feeding. The labrum (Lb) is a needle-like structure that is used to pierce and draw blood. The hypopharynx (Hp) is covers of the labrum. Additionally, it exudes saliva, which is where the transmittable diseases are carried. It coats the labrum in saliva before penetrating the skin to feed. This is how diseases are transmitted from the mosquito. The maxillary (Mx) and mandibular (Md) stylets are used to saw the skin at a frequency of 30 Hz, allowing for less force to be required to fully penetrate and feed (Izumi et. al. 2008) (**Fig 1**). Perhaps, the most important trait that the mosquito proboscis has is its malleability. It can bend up to 90 degrees and is controlled by a delicate muscle complex, allowing the mosquito to intricately contort its labrum through pores as small as 25 μm (Gordon et. al. 1939). Additionally, the proboscis is 2.32 mm long, with an overall width of 60 μm (Lee et. al. 1983; Christophers 1960; Kong et. al. 2010; Clements 1992; Ramasubramanian et. al. 2008). Therefore, mechanically blocking a mosquito from feeding with a physical barrier of clothing can prove to be a difficult task.

1.2 Mosquito Management

As stated above, mosquitos pose a tremendous health risk to humans and animals. However, insect management strategies are not a new occurrence. Humans have been implementing pest management strategies since 2,500 BC when the Sumerians used sulfur to kill insects (PSU 2022). The field of Medical Entomology, and the knowledge that insects can carry and transmit diseases is now about 150 years old. It was not until 1871, that the first textbook identified insects to be vectors of diseases (Patterson 2016). But in the years following, the field of Medical Entomology would make huge strides with the discovery that insects, particularly mosquitos, were intermediate carriers and vectors for pathogens and viruses.

The first person to make this discovery was Sir Patrick Manson. Considered to be the "Father of Tropical Medicine", he was nominated for the Nobel Prize of Physiology and Medicine. Manson's research was primarily focused on filarial worms, nematodes that cause lymphatic filariasis (also known as elephantiasis) (EB n.d.). In 1872, Manson discovered that microfilariae were not always present in his patients. In fact, he discovered that they existed in the highest concentrations at night. Thus, he concluded that the host must be a night-time biting mosquito (Eldridge 1992). Manson proceeded by studying *Culex quinquefasciatus* that had fed on microfilariae infected patients. He dissected the mosquitos at different intervals and looked at the size of the filarial worm. He discovered morphological development as the time between feeding and dissection increased (Eldridge 1992). Unfortunately for Manson, his discovery and correct evaluation of the filariae life cycle stopped there. His hypothesis that mosquitos only took one bloodmeal and laid one clutch of eggs during their lifetime caused Mason to inaccurately conclude that the filarial parasite was transmitted to human hosts through water consumption. He theorized that the worms entered water sources when the mosquitos died and that humans contracted the parasite through drinking the contaminated water (SU n.d.). While his hypotheses were not entirely accurate, his discovery of mosquitos as vectors was applied to many other tropical diseases, including Malaria.

Sir Ronald Ross was a British doctor who won the Nobel Prize of Physiology and Medicine in 1902 (EB SRR n.d.). Ross is best known for his discovery of *Plasmodium* in the gastrointestinal track of *Anopheles* mosquitos. Similar to Manson's techniques, he studied *Anopheles sp.* that fed on malaria infected humans. Following dissection, he discovered malarial parasites in the gut of the now infected mosquitos (Scientist 2011). This discovery, while groundbreaking, was not his most profound. He later discovered the transmission of this parasite from infected organisms to healthy ones. Using birds as his model organism, Ross was able to demonstrate the transmission of the malarial parasite from infected birds to healthy ones through the bite of an *Anopheles* mosquito (EB SRR n.d.). This discovery aided in suggesting the transmission of malaria to humans.

After such discoveries, Ross attempted to eradicate the *Anopheles* mosquito (Dworkin et. al. 2011). Eradication is an extremely difficult task to carry out. There are many factors that can lead to the demise of such a large-scale mission. While most eradication attempts have been wildly unsuccessful, there are those that found moderate success. However, in the case of Ross' attempt to eradicate *Anopheles* mosquitos, he had nearly no success. His first attempt at eradication was in Freetown, Sierra Leone (Bockarie 1999). Ross, along with Dr. H. E. Annett, and Dr. E. E. Austen, an entomologist from the British Museum of Natural History, arrived in Freetown with the theory that if they could eliminate breeding grounds the mosquitos would die, and their eradication attempt would be nothing but successful. Unfortunately, their attempt was vastly unsuccessful. Ross grossly underestimated the number of breeding sites as the mosquitos were not only laying egg clutches in stagnant pools of water, but also in domestic containers (Bockarie 1999; Dworkin et. al. 2011). While the sanitary authorities of Freetown heeded Ross' recommendations to drain pools and treat stagnant water with tar, the mosquitos proved to be effectively adaptive at changing its breeding grounds when confronted with environmental pressure (Dworkin et. al. 2011). This is a primary reason as to why eradication of mosquitos can prove to be such a daunting task. Mosquitos are effective at adapting to their surroundings. Additionally, eliminating all potential breeding sites is an even more difficult task. It was not until the development and use of synthetic pesticides that eradication attempts could become more successful. Unfortunately, even with the use of DDT and other synthetic pesticides, eradication was, and still is, a difficult task. One of the most successful attempts at large-scale eradication came in Brazil during the late 1940's (Löwy 2017). However, even with the use of DDT and the appearance of successful eradication of *Aedes* mosquitos, they still managed to "show back up" just 30 years later. There are many attributions to the perceived success of this attempt. For example, the widespread use of DDT throughout Brazil made this eradication attempt significantly less labor intensive (Löwy 2017). However, as previously stated, eradication attempts can prove to be an extremely difficult task. Some of the issues faced during these attempts are high cost of anti-mosquito campaigns and eradication attempts, opposition to pesticide spraying and larval searching in homes, a lack of supervision and delegation of tasks, increasing mosquito resistance to DDT, and American entomologists' opposition to eradications (Löwy 2017). Fortunately, many more methods of mosquito management and control have had high success and acceptance amongst the general population.

With the increasing resistance both genetically from the mosquito and socially from humans, the widespread use of DDT was forced to come to a halt. The EPA officially issued a cancelation of DDT use in 1972 (EPA n.d.). This was largely due to its negative environmental impacts as well as health risks to humans and animals. DDT is now known to be an extremely persistent pesticide (meaning that it is not easily bio-degradable), accumulate in fatty tissues, and travel long distances into the atmosphere. Additionally, it is now classified as carcinogen by the United States and international authorities (EPA n.d.). With the increasing public interest in DDT related health risks, other synthetic pesticides are now being used to combat mosquito populations. Today, the most commonly used insecticides for mosquito control are pyrethroids. However, mass spraying of pesticides is still an understudied topic in regard to the potential health risks. This has created a necessary alternative to pesticide spraying.

Bed nets treated with insecticides have been an instrumental development in the fight against arthropod borne viruses, particularly malaria. The creation and experimental use of insecticide-treated nets (ITNs) came in 1983 in Burkina Faso (Robert 2020). ITN's are used commonly in African countries to combat malaria transmission and deaths. Currently, there are two insecticide classes approved by the CDC for use in ITN's, pyrroles and pyrethroids (CDC 2019). However, only pyrethroid-based insecticides are registered by the EPA for application and use in bed nets (Kitchen et. al. 2009). Pyrethroids are a class of synthetic insecticides commonly used both in home and commercially (Bradberry et. al. 2005). In insects, the primary target of the insecticide is the nervous system (Saillenfait et. al. 2015). While pyrethroids have been proven to be very effective against insects, the study of how these insecticides affects humans is lacking. However, recent studies have shown that the constant exposure to pyrethroids may pose considerable health risks to humans and ecosystems (Jabeen et. al. 2015). This is especially prevalent due to the ability of pyrethroids to survive for long periods of time as they are not easily biodegradable (Jabeen et. al. 2015). Additionally, the washing of garments and textiles that have been impregnated with insecticides only adds to the pollution originally created by constant application to environments.

Throughout the history of mosquito control, pesticides have consistently been thought to have negative health and environmental impacts. Chemical control is typically the last method that should be used, according to IPM (Barzman et. al. 2015). IPM certainly does not discourage the use of pesticides, there are other control methods that should be considered prior to the use of synthetic chemical agents. While, IPM generally refers to the control of pests during agricultural production of crops, its methods can be somewhat translated to vector control. The first level of control for IPM is cultural control. This method refers to the use of manual or mechanical means to change the crop and soil environment making them less conducive for pests (Ferr n.d.). Similarly for vector control, perhaps the easiest method is economic development. Economic development is the method of changing our surroundings to be less available for vectors to transmit diseases. While it is primarily applicable in lesser-developed countries it has been shown to significantly reduce bite rate (Tusting et. al. 2013; Rek et. al. 2018). Economic development typically includes things such as: adding windows to houses, using bed nets, changing roofing, etc. While it has been shown to be successful, it does come with some major limitations. For instance, a person cannot stay inside all day, every day. At some point they will have to leave. What happens when they leave the sanctuary of their home? Similar to cultural control in IPM, economic development should not be used by itself. It works significantly better when paired with another control strategy (Rek et. al. 2018).

A newer control strategy that has seen some success is genetic control. For mosquitos, there are two main forms of genetic control: releasing modified mosquitos that carry lethal symbionts to control populations and reduce vector competence and the use of refractory mosquitos to replace vector species (Wilke et. al. 2015). The primary method being researched and used is the release of genetically modified mosquitos. This methodology is based loosely on the technique developed by Dr. Edward Knipling, SIT. SIT (Sterile Insect Technique) is the concept of using insects to control themselves (Hendrichs et.al. 2009). This was done by significantly reducing the reproductive capabilities of the pest insects through radiation-induced sterilization (Hendrichs et.al. 2009). The process is rather simple. Insects are sterilized using a form of radiation in large bio-facilities. In Knippling's strategy, this was done using cobalt, whereas now, gamma-radiation is commonly used. The dosage is only enough to sterilize, allowing the insects to maintain flight and mating capabilities (Hendrichs et.al. 2009). However, some problems can and have risen from this technique. SIT focusses on sterilization through radiation (typically the sterilization and release of only males) (Alphey 2002). Then, the modified organisms are released and forced to compete with WT (Wild Type) males for mates. While, in theory, the radiation dosage is only enough to disrupt reproductive capabilities, it often makes the males less competitive for mates, as compared to the WT. Consequently, the efficacy of this technique decreases when modified males are outcompeted. Therefore, other methods of genetic modification were in need of development to ensure population reduction (Alphey 2002).

Currently, the most researched form of insect paratransgenesis, particularly mosquitos, is through the host symbiont modifications (Wang et. al. 2013). There are two highly studied modes of action for the symbiont reduce disease transmission. First is the modification of symbionts to express anti-pathogen molecules (Wang et. al. 2013). The second is to create modified organisms by trans-infecting with the endosymbiont *Wolbachia* (Walker 2011). *Wolbachia* is a genus of maternally transmitted obligate intracellular bacteria found in most insects (Hertig et. al. 1924; Saridaki et. al. 2010; Werren et. al. 2008). It can induce multiple reproductive modifications in insects. Perhaps the most studied phenotype induced is CI (Saridaki et. al. 2010). CI is gene-drive mechanism which is induced by a deubiquitylating enzyme encoded in the *Wolbachia* genome (Beckmann et. al. 2017). It is modeled under a toxin-antidote system and is regulated by two genes, CifA (antidote) and CifB (toxin) (Beckmann et. al. 2019). CifB, when expressed in modified males, cause sperm sterility. If the female is not infected, then their relative offspring will not hatch. However, if the female is also infected (i.e. carrying the antidote, CifA), the offspring can be "rescued" and will be viable. This is how the symbiont induces gene-drive. Additionally, even if the frequency of *Wolbachia* rises in WT individuals, its reduction in vector competence can still significantly reduce transmission rates (Pereira et. al. 2018).

With all these control methods in mind, the most efficient and least problematic control method has yet to really be studied. Non-insecticide treated textiles offer solutions to every downside each control method has from above. A non-impregnated textile is a net or garment that has been developed without the application of any additional chemicals or insecticides. The mechanism for bite-resistance is purely a physical barrier. Insect-proof nets have been used widely for agricultural purposes. In Mwanza Region, Lake Zone, Tanzania insect net tunnels have been implemented in the effort to reduce sweet potato seed degradation due to vector viruses (Ogero et. al. 2019). However, implementation of such nets is sometimes regarded as detrimental to crop yield due to limited air flow, especially if applied to greenhouses (Agrafioti et. al. 2020). However, nets with high porosity, particularly 3353BT Biorete 50 Mesh Air Plus, allow for increased ventilation and result in no significant yield loss as compared to a positive control of no net (Formisano et. al. 2020). This was achieved by using a low denier high density polyethylene (HDP) monofilament. The increased airflow allows for a more improved microclimate and lower humidity, resulting in more adequate conditions as compared to those created from the use of traditional insect-proof net (Formisano et. al. 2020).

A new form of bed net has been developed that has shown adequate abilities to kill mosquitos without the use of insecticides. Researchers at NC State University have recently developed an attract-trap-kill bed net (Mouhamadou et. al. 2020). This bed net utilizes a cone shaped knitting structure that is able to trap and kill mosquitos at a 4.3-fold greater rate than the Permanent 2.0, the most used bed net in Africa, when used in experimental hut (Mouhamadou et. al. 2020). Additionally, it showed at 12.7-fold greater control rate when used at the community level against insecticide-resistant *Anopheles sp* (Mouhamadou et. al. 2020).

While there has been research on insecticide-free nets for agricultural purposes, the application of such textiles for garments is a new and underdeveloped topic. Garments treated with insecticides are widely used to prevent insect bites and subsequently, the spread of arthropod-borne viruses. However, insecticide-resistance and the potential health risks of constant pyrethroid exposure raise concerns about the health impacts of wearing such textiles (Luan et. al. 2021). Developing insecticide-free textiles is a difficult task. Mechanistically speaking, blocking a 25 µm wide flexible needle is extremely challenging.

Researchers at NC State University have begun developing garments that are free of insecticides but are capable of physically blocking mosquito bites (Luan et. al. 2021). They achieved this by using an ultra-fine synthetic knit comprised of 80 percent polyamide of 20 denier count, 20 percent elastane of 20 denier count, and had a weight of 96 g/m2 (Luan et. al. 2021). Denier count is the weight in grams per 9000 meters of fiber. The denier of human hair is around 20, while microfibers are typically less than 1, usually around 0.9 (SF 2020). Its pattern is a jersey knit structure (front facing loops on each needle) with a pore size between 20 µm and 28 µm, allowing for adequate air flow (Luan et. al. 2021). Breathability is essential in developing a garment that can be worn when mosquitos are most active, during the summer.

1.3 Knitting Methods

Knitting is a large field that has many different methods and products. Typically, the chosen knitting method will depend strongly on what is being produced. For clothing, knitting and weaving are the preferred methods of creation (Adanur 1995). Knitting is a method of textile development that utilizes the interlacing of loops in either the weft or warp direction (Anbumani 2007). Knitting, in general, makes fabrics capable of stretching far more than woven fabrics (Fan 2020). Within knitting there are two methods, weft (or filling) knitting and warp knitting (Anbumani 2007). While both methods utilize the formation of loops to create malleable textiles, they are developed in very different ways. Weft knitted textiles are developed by the interlacing of loops in rows while warp knitting is done by constructing loops into columns (Mohamed 1990). Weft knitting certainly has its advantages. For instance, to create a weft knitted textile, only one yarn is required (Anbumani 2007). However, in warp knitting, similar to weaving, more than one yarn is required to construct a textile (Anbumani 2007). Weft knitting is the most used method in the textile industry. Most of the clothing developed uses a single jersey pattern, which is the most basic form of weft knitting (front facing loops on every needle). Weaving is the process of creating textiles by interlacing yarns perpendicular to one another alternating over and under. Unlike knitted fabrics, woven fabrics do not have much elasticity. In fact, they are only capable of stretching in one direction, whereas knitted fabrics can stretch in any direction (Anbumani 2007). In addition to elasticity, knitted fabrics have several advantages over woven: due to their natural elasticity they are much more comfortable and more equipped to contort to the human body, higher shrinking capability, higher moisture absorption, higher air permeability due to the natural open structure of loops, and resistant to creasing (Anbumani 2007). While some of these advantages are controllable by yarn type, knitted fabrics have them without the use of specialized yarns. Thus, for the purpose of developing textiles that are necessary to be breathable, stretchy, and comfortable, knitted fabrics would be the ideal manufacturing method.

Both knitted and woven fabrics are made of yarns. Yarns are created from tightly spun fibers. There are two types of fibers, natural and synthetic. Natural fibers are made from organic materials which include crops, animals, and mineral based materials (Clemons 2005). Some examples of common natural fibers are cotton, wool, silk, and hemp. Synthetic fibers are produced by humans through chemical processes. They are usually developed to either replicate the properties or as an enhancement of natural fibers. Some examples of synthetic fibers include polyester, rayon, acrylic, and elastane.

Both types have their own respective advantages and disadvantages. Natural fibers, or yarns, typically have a higher strength, are developed from natural and renewable resources, and have a relatively low cost (Chandramohan et. al. 2011). However, natural yarns tend to have higher insulating properties. Which, for the purposes of blocking mosquito bites, can be less than ideal. Wearing a garment developed from naturally insulating yarns during the summer can be extremely uncomfortable. Synthetic yarns tend to be more durable, stretchy, and don't shrink as much.

Fibers, once spun into yarns, will typically maintain their properties. Yarns, however, can add more variation to fabrics. There are several parameters that can be controlled in yarn production: staple yarn production, filament yarn production, yarn size, number of plies, and blending (Encyclopedia n.d.). Staple yarn is the method of spinning most natural fibers. The primary exception to this is silk. Silk, like synthetic yarns, is made from filaments, which tend to be stronger than staple yarns (Encyclopedia n.d.; Ravnitzky 1989). Staple is a term that refers to the length of the fibers being spun (Encyclopedia n.d.; Pan 1993). While synthetic fibers are typically spun into filament yarns, they can be spun into staple yarns as well. However, the caveat with this is that they must be cut into shorter staples (Hagewood 2014). Natural yarns on the other hand, except for silk, can be spun using long staples. Filament yarns are those that are spun from an endless strand. These typically come from synthetic fibers and silk (Encyclopedia n.d.). Filament yarns are spun in 2 primary fashions: monofilament and multifilament. This refers to the number of plies used when spinning the yarn. A ply refers to the strand of fiber used for spinning (EB PY n.d.). Therefore, 2-ply yarn would imply that two strands of fibers were spun together in the manufacturing process (EB PY n.d.). Increasing the number of plies directly increases the thickness of the yarn. Multi-ply yarns provide increase strength and durability, but can also be used to develop sleek, sheer fabrics (EB PY n.d.). In addition to yarn thickness, blending yarns can dramatically change the characteristics of a developed fabric. Blended yarns are typically manufactured with the goal to utilize the good characteristics of both fibers. For example, blending cotton and polyester can increase tensile strength, comfort, and ease of care (Baykal et. al. 2006). While cotton is prone to significant shrinking, blended yarns tend to be more resilient in keeping their original shape and size. It is easy to see how important yarns can be in the design of clothing and textiles for non-fashion purposes.

Fortunately, fiber and yarn creation are only half of the customization when it comes to fabric manufacturing. The actual knitting process allows for equally, if not more, customization. Knit manufacturing is a well-established field. The main form of customization (in reference to weft knitting, which is the focus of this research) is the style or structure of the knit. Some common knit structures include jersey, alternate jersey, purl, rib, and interlock (Ahmad 2017). The development of these structures is based on different iterating patters of front or back knits (Savci et. al. 2000). As stated previously, the most common structure used in the fabric industry is single jersey. Jersey is a simple knit structure created by repeating “front” or “face” loops (Savci et. al. 2000). Front or face loops are those that are created by the passing of the new loop from back to front of the loops below it in the column. On flatbed knitting machines these are made automatically on the front needle bed (Savci et. al. 2000). Back loops are those made by passing from front to back. These are made on the back needle bed (Savci et. al. 2000). Interlock is a slightly more complex structure. It is made by alternating front and back loops on row one. Then alternating back and front loops on row two (Choi et. al. 2000). The different combinations of front and back loops allow for abundant customization. As discovered in this research, changing the knit structure can have a direct impact on the fabric’s capabilities regarding both comfort and blocking ability.

It is important to understand the basics of knitting in order to develop fabrics with such a purpose as proposed in this thesis. Knitting technologies have come a long way. Machines are now very customizable in the sense of fabric design and creation. Flatbed knitting machines, in particular, have many parameters that can change physical characteristics of the fabrics being developed. Stitch length is one of the primary parameters that is easily controlled on the machine (Ichetaonye et. al. 2014). Stitch length, which is a common variable in knitting technologies, is the length of the yarn used to create an individual loop. Additionally, it is one of the most important variables in changing the overall stitch density of a fabric. It has a direct affect on course per unit length (CPU), wale per unit length (WPU), and tightness factor (Ichetaonye et. al. 2014). As the stitch length decreases the density of the fabric and values of WPU and CPU increase (Ichetaonye et. al. 2014). Understandably, this will be an important factor in developing fabrics that are intended to block mosquito bites. The ability to directly control the theoretical tightness and density of loops in a fabric will have a direct correlation on how well a mosquito will be able to probe through the material.

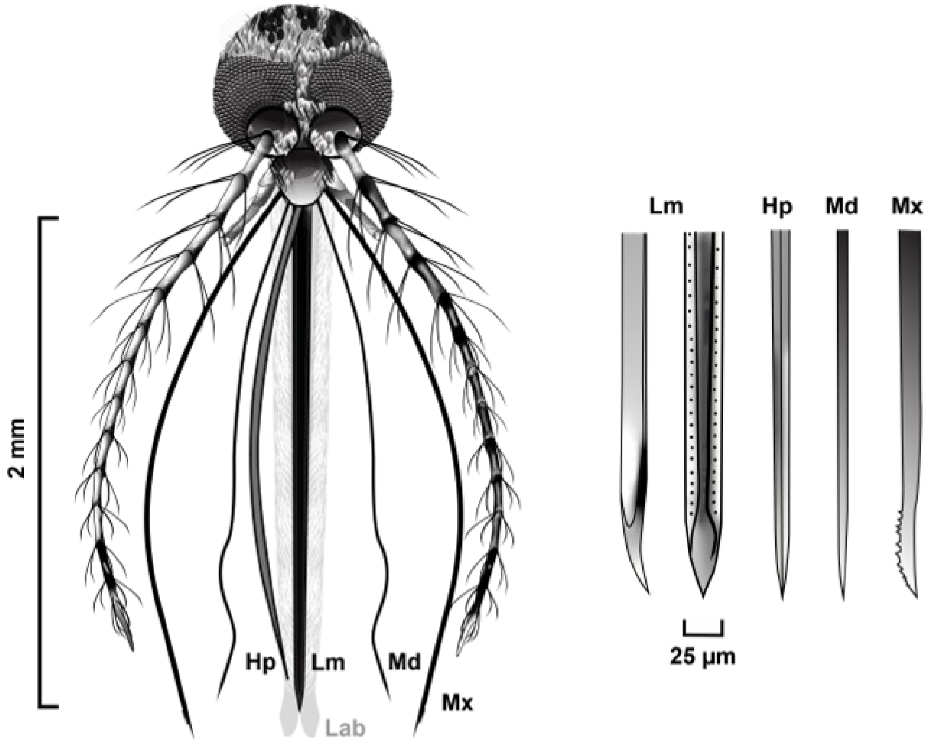

Figure 1. Diagram of a female *Aedes aegypti* mosquito head and mouthparts. Mosquito probiscis can bend up to 90 degrees and is controlled by a complex of delicate muscles. This allows the mosquito to contort is labrum through pores as small as 25 µm (Gordon et. al. 1939). Additionally, the proboscis has an overall length and width of 2.32 mm and 60 µm, respectively (Lee et. al. 1983; Christophers 1960; Kong et. al. 2010; Clements 1992; Ramasubramanian et. al. 2008). The mouthpart consists of four necessary stylets. The Labrum (Lm) is the feeding tube. It draws up blood and is covered by a fascicle, the hypopharynx (Hp). The hypopharynx exudes saliva. Pairs of mandibular (Md) and maxillary (Mx) stylets saw at a frequency of 30 Hz decreasing the amount of force required to penetrate skin. (Beckmann ADECA Grant)

Chapter 2: Research Findings

2.1 Introduction

Engineering clothing to block mosquito bites is a challenging task. The fascicle of a female mosquito is an intricate system. It has a unique muscular complex that allows it to bend up to 90 degrees and efficiently probe through the small pores found in normal, everyday, clothing. Therefore, there is a market for development of textiles that are capable of blocking mosquito bites. The following research contains the methodology and mechanisms discovered that could aid in the ongoing fight against mosquitos and their pathogens.

2.2 Preliminary Experiments and Data

Preliminary experiments and data collection for this research was conducted by our lab at Auburn University. These experiments laid the groundwork for the research conducted and contained in this thesis. Dr. Beckmann and his students conducted multiple assays that were instrumental in the progression of this research and allowed me to join with a conclusive direction on how to proceed.

Modeling Exposure to Mosquito Bites in Alabama

Citizens of Auburn were surveyed to determine what proportion of skin people chose to expose to mosquito bites. Fashion choices were surveyed during random walks on Auburn University's campus while the weather was 80 degrees Fahrenheit. 324 individuals were surveyed over three days with an even split of males and females. For the upper body males chose to wear short sleeve t-shirts over long sleeves at 80%. Similarly, females chose to wear short sleeves over long sleeves or dresses at 80%. For the lower body, males and females favored shorts over pants at 54% and 61%, respectively (**Fig 2a 2b**). However, modeling the amount of skin susceptible to mosquito bites is not as simple as the amount of skin exposed by wearing short sleeves or shorts. In fact, if mosquitos can bite through everyday clothing, then the relative exposure could be much higher.

Thus, if mosquitos can bite through common clothes, then the amount of skin susceptible to mosquito bites would need to include surface areas where the clothing clings to skin. Three standard individuals modeled each clothing item studied during the previous survey. Based on each individual clothing item surveyed, these calculations were made by counting the number of red pixels, which were marked red at points of contact between skin and clothing in contrast to folds or draping cloth. These values were calculated in triplicates for the front and back of both males and females. Given the hypothesis that mosquitos cannot bite through common clothing, the average percentage of skin susceptible to mosquito bites is 55.9%. Furthermore, even while wearing full long sleeves and pants, the amount of skin exposed is still 35% and 32% for males and females, respectively (**Fig 3d**). This data demonstrates that the concept of "wearing long sleeves to protect yourself from mosquitos" might be flawed. In fact, wearing long sleeves, when compared to short sleeves, only increases protection from mosquitos by around 14%, which is not statistically significant for either males or females.

Quantifying Mosquito Bites Through Textiles

Next, we wanted to test the hypothesis of whether common clothes were capable of blocking mosquito bites. Thus, a series of textiles were screened to see if any available clothing possessed this ability. This process included two points of data, the number of bites received and the number of blood fed females. Both quantifications are useful to assess the full capability of these textiles. Number of bites measures how likely one is to receive transmission of a pathogen whereas the percent bloodfed females measures the mosquito's ability to acquire a full bloodmeal and hence reproduce; importantly, bites and bloodfed percent are not equivalent and often can show stark differences. For example, a mosquito struggling to acquire a bloodmeal might bite continually and increase bite rate but never acquire a full bloodmeal. Thus, we observed both numbers. To conduct the experiments, the experimenter exposed their arm to a cage of twenty female *Aedes aegypti* for 15 minutes and subsequently counted the number of bites received on their arm and the number of females that were able to receive a blood meal (see **Methods: Mosquito Experiments**).

The textiles tested included a set of five white 100% cotton weaves (Poplin, Twill, Oxford, Royal Oxford, and Pinpoint Oxford), four knits (Under Armour^{TM}, Leggings, Jeggings, and Rynoskin), and one "other" (Horse Mesh). Each tested sleeve was compared to the negative control of a bare arm (**Fig 3a 3b**). It was discovered that only one textile, Jeggings, possessed any amount of bite blocking capabilities. The jegging (a cotton, polyester, and spandex blended weft knit) was the only textile to have any statistical significance from the bare arm control. This finding led to the conclusion that at least for the weaves we tested, they were incapable of blocking bites. There was little to no variation in the blocking ability of any weave tested. However, the knits showed more variation, and one possessed an advanced ability to block (**Fig 3a 3b**). While this data does not include every weave or knit possible, it does give insight into the properties of a blocking textile. This led to the hypothesis that knitted textiles could block and that blocking knits can be optimized through weft knitting technologies.

Furthermore, a test was conducted to see if it was possible to replicate the findings found during this series of experiments. A single jersey weft knit was designed by our collaborator, Dr.

Jim McCann from Carnegie Mellon University, and subjected to the same series of tests. This experiment was validated and the data for this single jersey knit resembled that of the jeggings.

Detectability of Mosquito Landing Events

After observing that common clothes do not block mosquito bites, we wondered whether wearing long sleeves might actually be worse than exposed skin; this condition might arise if the long sleeves reduced a human's ability to sense and perceive mosquito landing events. . Therefore, an experiment was conducted with three individuals to quantify their ability to detect when a mosquito landed on their bare arm versus wearing a long sleeve Under Armour™ compression shirt. On average, each individual was able to detect a mosquito landing event on their bare arm 41.19% of the time (**Fig 3c**). Conversely, while wearing the Under Armour™ compression shirt, they were largely unable to detect mosquito landings (**Fig 3c**). This further confirms the need for mosquito bite blocking textiles. If long sleeve shirts do significantly decrease the wearers susceptibility to mosquito bites, it would arguably be better to wear short sleeves. Thus, there is at least a 40% chance of killing the mosquito prior to being bit.

2.3 Objectives and Aims

Based on the preliminary data presented above, I will proceed with the following objectives and specific aims:

1. Develop weft knitted textiles that are capable of physically blocking mosquitos from biting and receiving a blood meal.
2. Determine precisely which mechanisms can be controlled that will increase the blocking ability of said textiles.

3. Re-engineer blocking textiles to maximize comfort while being worn in hot climates.

2.4 Materials and Methods

M1 Plus

M1 Plus is a knitting program designed by Stoll and is used to create knitting files that are loaded directedly into the machine via a flash drive. It is a comprehensive program that is easy to use and understand. Every knit that was developed at Auburn was first designed in M1 Plus. The files are created by using different iterations of front and back loops to develop intricate patterns and fabric structures. M1 Plus allows for high variability as there are encoded parameters that may directly contribute to blocking ability.

Stoll ADF 530-16 Ki BcW Flatbed Knitting Machine

The Stoll ADF 530-16 Ki BcW is a flatbed weft-knitting machine. This means that the fabric produced is a flat sheet with seams along the sides. Some machines only have one bed of needles; however, this machine has two, one on the front and one on the back. This is how the machine can develop more intricate weft-knits. All fabrics developed from Auburn University were created on this machine. This machine, like M1 Plus, allows for the variation of some parameters. It is slightly more limited than the program, but it does allow for additional variation when developing fabrics.

Brother International CS7000X Sewing and 1034D Serger Machines

A Brother International CS7000X Sewing Machine was used to generate test sleeves for mosquito bite experiments. Each sleeve is created by taking a flat sheet of fabric from the Stoll

Flatbed Knitting Machine that is sewn together using measurements for Dr. Beckmann's arm. Once each sleeve is sewn, a Brother International 1034D Serger Machine is used to create hems and finish raw edges.

Yarns

The primary control yarn used to produce each knit is a 100% polyester yarn of size 2/150/96 (number of plies/denier of each ply/number of filaments in each ply) with diameter 282 microns. This yarn was acquired from Unifi, inc. Unifi is a fiber and yarn production company headquartered in Greensboro, NC. Other yarns used in the development of textiles throughout the course of this research were also acquired from Unifi.

Mosquito Rearing

Aedes aegypti mosquitos were reared in a clean lab in the absence of disease pathogens. Mosquitos were kept in an incubator at 28°C with a rotating 12-hour light/dark cycle. Mosquito eggs are hatched by submerging egg papers in medium sized shoebox tubs until pupation. Larvae and pupae are fed approximately 5 mL of a yeast and water mixture. Pupae are transferred by hand into a mesh cage for eclosion. As a control for age, pupae are allowed to eclose for 72 hours, then removed and placed into a new cage. Mosquito biting experiments were performed with females aged 4-7 days old.

Mosquito Experiments

Every mosquito experiment was conducted under the same controlled parameters. The mosquitos were first anesthetized on ice and sorted using a cold block 24 hours prior to the experiment. The females were then placed in a cage with only water, starving them for the 24 hours leading up to the experiment. To maintain normal mosquito circadian rhythm, all experiments were conducted in the afternoon. The experimenter then puts on a knitted sleeve and covered their hand with a latex glove which does not allow for mosquito penetration. The covered arm was placed in a cage of 20 female mosquitos for 15 minutes.

For full body tests, 40 females are sorted in the same manner. The experimenter dressed in long white sleeves and pants and stood in a full body cage for 15 minutes. The landing events were recorded by two observers, one in the front and one in the back. Each time a mosquito landed and attempted to probe; a mark was recorded on a human body outline corresponding to that area. This experiment was done in three separate replications on three separate cages of 40 mosquitos. The replicates were digitalized and overlayed in Adobe Photoshop to produce a comprehensive heat map of mosquito probing behavior and relative body area attraction.

Mosquito Videos

To acquire video captures of mosquito feeding and biting, twenty females *Aedes aegypti* mosquitos were transferred into a cage with two arm openings. The experimenter puts on a textile prototype and latex gloves. A Moment Macro Lens V2 iPhone camera attachment is used to acquire high quality microscopic videos of mosquito probing behavior. Each video is recorded, tracking the behavior of a single female for 1 minute. The videos are analyzed, and two data sets are generated: time to fly away and number of probes.

Statistics

GraphPad Prism 9 was used to generate figures and run statistical analyses for significance. Every set of data collected for each sleeve was first entered into GraphPad and an Ordinary One-way ANOVA and Tukey's multiple comparisons were run to test significance.

2.5 Results

The results found during the preliminary studies of this project are truly shocking. Citizens of Auburn surveyed during the clothing choice assays showed significant preference for comfort over risk of exposure. Additionally, it was found that even if students chose to wear long sleeves and pants, they would still be susceptible to mosquito bites. This proves that there is a market for clothing that not only blocks mosquito bites but is also comfortable to wear during hotter seasons. Additionally, while most clothing studied did not block mosquito bites, there was one knit that exhibited enhanced blocking capabilities. This led to the hypotheses that certain mechanical properties must control the blocking ability of textiles. The goal of my research was to determine precisely what these mechanisms are and if we can control them to enhance the entomological applications of these fabrics.

Knit Structure Contributes to Bite Blocking Capabilities

With the hypothesis that knits are a viable form of mosquito bite blocking textile, I proceeded by producing as many different knit structures as possible. First, we wanted to see if varying knit structure contributed to the blocking ability of knitted textiles. This was done by researching the capabilities of weft knitting. Weft knitting is the simplest and most common method of constructing knits. It is used in the textile industry to generate multiple types of knit structures that can stretch in multiple directions. It utilizes one yarn to construct rows of loops, whereas warp knitting requires multiple yarns and creates columns of loops (Anbumani 2007). Some common weft knitted structures include single jersey, purl, interlock, rib, tubular, and cardigan (**Fig 4**).

These structures were programmed in Stoll's software M1 Plus and ran on the Stoll ADF 530-16 Ki BcW Flatbed Knitting Machine. Once the knitted textiles are made, they are sewn and serged into prototype sleeves. Each sleeve was tested a minimum of three times to determine the efficacy against mosquito bites (see **Methods: Mosquito Experiments**). Of the knit structures that we developed, only one demonstrated any bite blocking capabilities, interlock. However, it was not until after the sleeve was washed that it became a true blocking textile.

Interlock, as stated in Chapter 1, is a slightly more complex structure consisting of alternating front and back loop patterns, followed by alternating back and front loops patterns in the next row (**Fig 4c**). Immediately after the textile was created it was tested for bite blocking. The initial series of tests showed moderate blocking but with high variability. It received 19, 17, 11, and 8 bites in its four iterations of testing (**Fig 5**). While this data was highly variable it did show promise. After careful consideration, it was determined that the textile should be washed and tested again, as that is common practice in the fashion industry. Once the sleeve had been washed, its bite blocking capabilities were significantly enhanced. It received an average of 2.6 bites over 5 tests (5, 0, 0, 4, 4), a 76% increase from before washing (**Fig 5a**). Additionally, an average of only 1.6 mosquitos were able to receive a full blood meal over the five experiments (3, 0, 0, 3, 2) (**Fig 5a**). Every textile from then on was washed prior to testing and previous textiles were washed and retested. After which, the interlock sleeve remained the only bite blocking textile developed. This experiment proved two things. First, certain knit structures are inherently capable of blocking mosquito bites and secondly, other mechanisms may contribute to bite blocking capabilities, like washing or shrinking.

Shrinking Fabric Contributes to Bite Blocking Capabilities

Post-knit fabric shrinking is a major component of bite blocking textiles. Knitted fabrics have a tendency to shrink more than woven fabrics because of their developmental structure. Additionally, it is a common practice in the fabric industry to pre-wash all garments prior to marketing. We wanted to test whether a simple wash and dry cycle, which is known to shrink knitted textiles, could physically manipulate the structure and enhance its entomological applications. An interlock textile was made and assessed before and after a wash and dry cycle. No chemicals or soap were used during the wash. After washing, the structure of the knit changed dramatically. The fabric went from about 8.5 wales per centimeter to about 10.3 wales per centimeter (**Fig 6a/b**). Additionally, the amount of space between wales went from about 150 microns to zero. This means that the washing and drying physically manipulated the fabric to have less space for the mosquito proboscis to probe through. The number of bites received during mosquito experiments reflects this phenomenon. The interlock fabric post-wash had a 76% increase in blocking ability. When compared to the negative control of a bare arm, the unwashed fabric only decreased the number of bites received by 13.5% whereas the washed interlock reduced the number of bites by 83.6% (**Fig 5a** (compare “Interlock Washed” to “Arm”)). This proves that washing and drying developed fabrics is an integral part of the manufacturing process and that it can enhance the bite blocking capabilities of previously non-blocking textiles (**Fig 6c/d**).

Spandex Content Contributes to Bite Blocking Capabilities

Spandex is a type of elastic synthetic fiber made from 85% polyurethane (by weight) (EB PE n.d.). It is commonly used to develop tight contorting fabrics that cling tightly to the skin and can naturally stretch in all directions. It is rather durable and is resistant to deformation and degradation. It is easily washed and quick drying. These characteristics make it an ideal candidate for textile development. It is commonly used by blending it with other fibers like cotton or polyester. The only blocking textile that was discovered during preliminary testing uses a unique blend of cotton, polyester, and spandex. We have hypothesized that spandex content might be an integral parameter in developing textiles for this specific entomological purpose. While we have attempted to acquire spandex, purchasing this yarn has proven to be exceedingly difficult. Thus, we comprised a specific experiment that was able to test this theory. We attempted to replicate the jegging knit structure with our 100% polyester control yarn. A sample of the jeggings was sent to Stoll. They deconstructed the sample to discover the knit structure that was used to manufacture this fabric. We received a knit diagram that showed it was comprised of repeating patterns of two rows (**Fig 4e**). After replicating this diagram in M1 Plus a prototype test sleeve was constructed and tested. Our sleeve received an average of 19.33 bites over three separate iterations of testing, a 78.2% increase from the jeggings (**Fig 7c/d**). This leads us to believe that natural elasticity of spandex can physically manipulate the structure of a knit post-development. After researching the literature on this subject, a study published in 2003 was found that measured the dimensional variations of knits made with and without spandex (Marmarali 2003). It was discovered that fabrics developed with spandex yarns have higher loop densities and that the spacing between wales and courses is reduced when spandex is introduced. This further aids in the conclusion that textiles developed with spandex blended yarns will be measurably denser and tighter than those without. Hence, the jeggings were capable of blocking and our replicated prototype was not.

It is important to note that this experiment is not yet completed. We are waiting for our own blend of spandex yarn. The order has been placed but there has been some opposition to AU's vendor requirements. Thus, the yarns have yet to be received. However, one the yarns have been acquired, we plan to knit a single jersey prototype with the implementation of spandex. The yarns ordered are of the same size and dimensions as our control yarn. Thus, this test will give us a more accurate representation of spandex contributing to bite blocking.

Yarn Diameter Contributes to Bite Blocking Capabilities

Yarns diameter is a primary variable in textile development. The diameter of yarns is typically determined by the ply of each yarn (i.e. the number of fibers used during construction). For example, the control yarn used in this research is a 2-ply yarn of 100% polyester. This means that there are two fibers of polyester twisted together. To test this hypothesis, we constructed two sleeves of the same knit structure (single jersey) with different sized yarns. The first, is our control yarn (2/150/96) of 282.17 microns. The second is another 100% polyester yarn with a diameter of 328.3 microns (3/150/96). This means that the yarn is twisted at a 3-ply count. Additionally, we compared this to the single jersey sleeve developed by Dr. Jim McCann comprised of 100% Acrylic with a yarn diameter of 433.3 microns (microscopy images **Fig 8a 8b 8c**). We hypothesized that the control yarn would not block, the intermediate diameter yarn would provide partial blocking, and the thicker acrylic yarn would provide full blocking. All three were subjected to at least three iterations of mosquito testing. The thinnest control yarn received an average of 21.6 bites over five experiments (**Fig 8d**). The intermediate yarn received an average of 13.6 bites over five experiments (**Fig 8d**). Finally, the acrylic yarn received an average of 0.67 bites over six experiments (**Fig 8d**). Each of the prototypes were statistically significant from one another. Thus, increasing yarn diameter contributes directly to increasing bite blocking capabilities (**Fig 8**).

Stitch Length Contributes to Bite Blocking Capabilities

Stitch length is the primary controllable parameter when developing knit files in M1 Plus. It is defined as the amount of yarn used to create a single loop. Therefore, as the stitch length decreases, the tighter and denser a loop will be. This correlates directly with the overall density of the fabric. Stitch length has a direct positive impact on CPU and WPU. To assess this hypothesis, an experiment was designed where five different sleeves at decreasing stitch lengths, starting at a stitch length of 11 and decreasing in increments of 0.5 (11, 10.5, 10, 9.5, 9), would be tested for bite blocking ability. Unfortunately, this data has not yet been gathered. However, microscopy images were taken of interlock textiles as the stitch length decreases. The fabrics visibly tighten which lead me to believe that the bite blocking ability of textiles developed with smaller stitch lengths will increase (**Fig 9**).

High Resolution Video Screening of Knitted Fabrics

High resolution videos were taken of mosquitos probing on three different fabrics: Under Armour™, jeggings, and 100% acrylic single jersey, to test if mosquitos would exhibit behavioral differences when probing on textiles that do not block versus ones that do. Videos are taken, following one female mosquito, for one minute. The videos are then analyzed, and two sets of data are generated: time to fly away and number of probes. My hypothesis was that mosquitos would spend less time and probe more on textiles that are capable of blocking. It was theorized that they would spend less time on a fabric that does not block since a majority of that time would be spent feeding. Furthermore, it was hypothesized that mosquitos would, on average, probe more on blocking textile because they would not be able to penetrate the fabric as easily.

On average, mosquitos spent 48.2, 21.3, and 12.1 seconds on the Under Armour^{TM}, jeggings, and acrylic single jersey, respectively (**Fig 10a**). Additionally, the mosquitos, on average, probed 21.7, 14.9, and 9.9 times on the Under Armour^{TM}, jeggings, and acrylic single jersey, respectively (**Fig 10b**). Recall that Under Armour^{TM} does not block, whereas the jeggings and acrylic jersey do. Therefore, the hypotheses were partially correct. I was correct in theorizing that they would spend less time on a blocking textile. This is more than likely an evolutionary defense mechanism where they choose to spend less time probing if initial penetration is unsuccessful. They are eager to fly away before they get slapped. Conversely, they probed significantly fewer times on bite blocking fabrics. More than likely, this is due to the same reason. A mosquito is not going to continue probing if penetration is unsuccessful. The longer they spend probing the more likely they are to be killed.

Mosquito Probing Preference Heat Map

To maximize the comfort potential of full body prototypes, we wanted to explore if mosquitos were attracted towards certain areas of the body. We hypothesized that mosquitos would prefer to probe on certain regions of the human body over others and thus bite blocking knits need only be applied in those areas. Full body testing was conducted, and a heat map was generated using the data collected (See **Methods: Mosquito Experiments**). It was discovered that *Aedes aegypti* chose to land and probe predominately on the lower body near the ankles. However, they also probed on the upper and mid back (**Fig 11a**). Interestingly, I believe that this is also an evolutionary behavior. Mosquitos are adapting to probe on harder to reach areas where they are less likely to be killed. This data can be used to construct clothing that utilize tighter bite blocking knits in the more frequented areas and loose more breathable knits in the less probed regions. Furthermore, this would not reduce the efficacy of our prototypes as we would design the looser knit areas to not cling to the skin. Therefore, regardless of the knit's ability to block, mosquitos would not be able to reach the skin underneath.

Mosquitos Prefer to Probe on Darker Fabrics

Mosquitos are attracted to heat and carbon dioxide. Due to darker colors naturally holding on to heat at a higher rate, I wanted to test if this could be applicable to our textiles. As mosquitos are attracted to heat, I hypothesized that they would prefer to probe more on a textile made from black yarn than that of a white yarn. To test this theory, our collaborator Dr. Jim McCann designed a sleeve that was split half white and half black. Twenty female *Aedes aegypti* mosquitos were tested to see where they chose to land and probe. Five iterations of this experiment were conducted, rotating the sleeve incrementally to control for lighting and behavioral predispositions. The mosquitos landed and probed on the black side at a 4.61-fold higher rate (**Fig 11b**). This confirmed our hypothesis that mosquitos will be attracted to darker clothing.

2.6 Discussion

Following the findings discovered during preliminary experiments, the goal of this research was to determine precisely which mechanisms contribute to the bite blocking efficacy of knitted textiles. In comparison to weaves, knits are the ideal candidate for bite blocking textiles due to the structural ability to contort and stretch around the skin. Additionally, knits have higher shrinking capability, moisture absorption, air permeability, and crease resistance (Anbumani 2007). While not all knits are capable of blocking, one was discovered to possess an innate ability to physically hinder mosquitos from reaching the skin: interlock. It should be noted that immediately after the interlock prototype was produced, it exhibited limited blocking capabilities. However, after a detergent-free wash and dry cycle, the WPU and CPU increased. This means that the fabric's physical density was enhanced. This enabled a previously intermediate blocking knit to become fully blocking.

While a simple wash-dry cycle enabled physical changes, additional parameters were discovered to manipulate the bite blocking ability of manufactured knits. Stitch length is a parameter controlled during the design of knit programs. As previously described, it is the amount of yarn used to create a single loop. Thus, decreasing the stitch length significantly increases the density of the completed prototype. Furthermore, it increases the number of wales and courses per unit length and can decrease the inter-wale space.

Yarn diameter also has a direct impact on the efficacy of these knits. Thicker yarns have been proven to enhance bite blocking capabilities as compared to thinner yarns of the same fiber content. A 100% polyester yarn of size 3/150/96 (328.33 microns) has been shown to reduce the number of bites received by 37%, when compared to the control polyester yarn of size 2/150/96 (282.17 microns). These sleeves were also compared to an acrylic yarn of size 433.33 microns.

Both sleeves paled in comparison to the much thicker acrylic yarn prototype, which was capable of reducing bites by 96.9%, from the control.

The final parameter found to contribute to bite blocking is spandex content. It was discovered that spandex is capable of physically manipulating the fabric's smoothness, overall density, and the space between wales and courses. All these qualities contribute to the bite-blocking ability of these textiles (Marmarali 2003). Without the acquisition of spandex yarn, an experiment was designed to compare a textile developed without the implementation of spandex to a market textile, jeggings. The jeggings, which contain 3% spandex, are capable of blocking mosquito bites, while the lab replicated prototype that did not contain any spandex was not able to block at all. It is hypothesized that, once spandex yarn is acquired, a single jersey sleeve developed with spandex blended yarns will be able to block mosquito bites when compared to its non-spandex counterpart.

In addition to the discovery of the pervious contributing parameters, a series of experiments were conducted to test whether comfort could theoretically be increased by exploiting mosquito probing behavior. Through full body testing, a heat map was developed that shows the highest probed area on a human body. *Aedes aegypti* mosquitos were subjected to a human in a full body cage and allowed to probe. A heat map was generated that correlated directly to the areas where an individual mosquito chose to land and feed. It was discovered that *Aedes aegypit* predominantly chose to land on the lower body (particularly ankles) and the mid to upper back. Exploiting this discovery, a prototype knit could be developed that utilizes slightly less comfortable, tight, bite blocking knits in the high frequency areas, and loose, non-form fitting fabrics in the less frequented areas. This would theoretically make the developed prototype more breathable without reducing its efficacy. Additionally, considering mosquitos land and probe on darker materials at a 4.61-fold greater rate, it is proposed that dark yarns be used to construct the tight, bite blocking knits. Through the exploitation of color attraction, and evolutionary attraction to certain body areas, mosquitos can further be deterred from probing on the loose breathable areas. Thus, enhancing the efficacy of the proposed comfortable knit even more.

This research can be applied directly for entomological purposes. It provides a solution to a problem that many other control methods, like insecticide spraying and sterile insect technique, generate: individuals have little control over mosquito management. The steps that can be taken by an individual to control disease transmission are limited. However, with the production of bite-resistant clothing, that problem is negated. Persons may have little control of mass spraying and the release of genetically modified mosquitos; however, they can easily protect themselves with the use of bite resistant clothing. Which is now made possible through the discoveries found in this thesis.

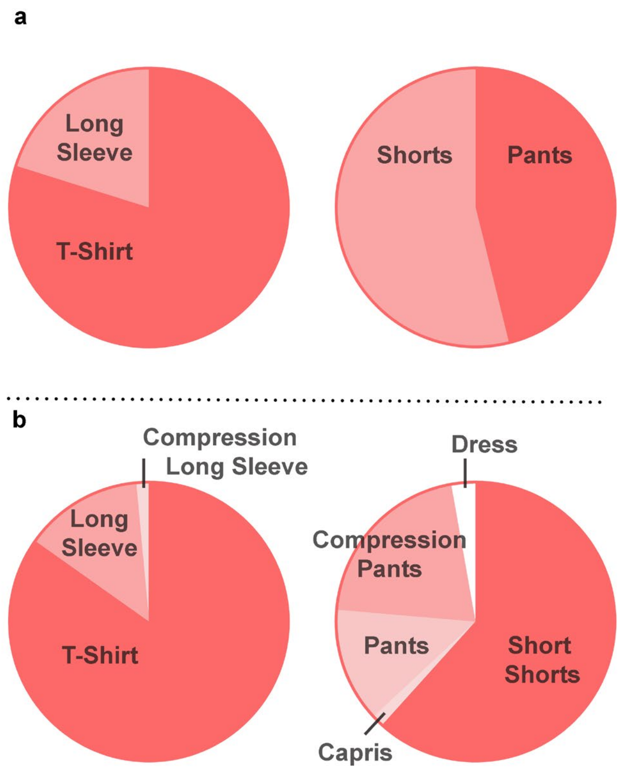

Figure 2. Modeling fashion choices of 324 individuals on Auburn Universities campus during 80-degree weather. This data reflects a sex ratio of 50:50. **a.** Male fashion choices for upper body (left) and lower body (right). **b.** Female fashion choices for upper body (left) and lower body (right). (Beckmann ADECA Grant)

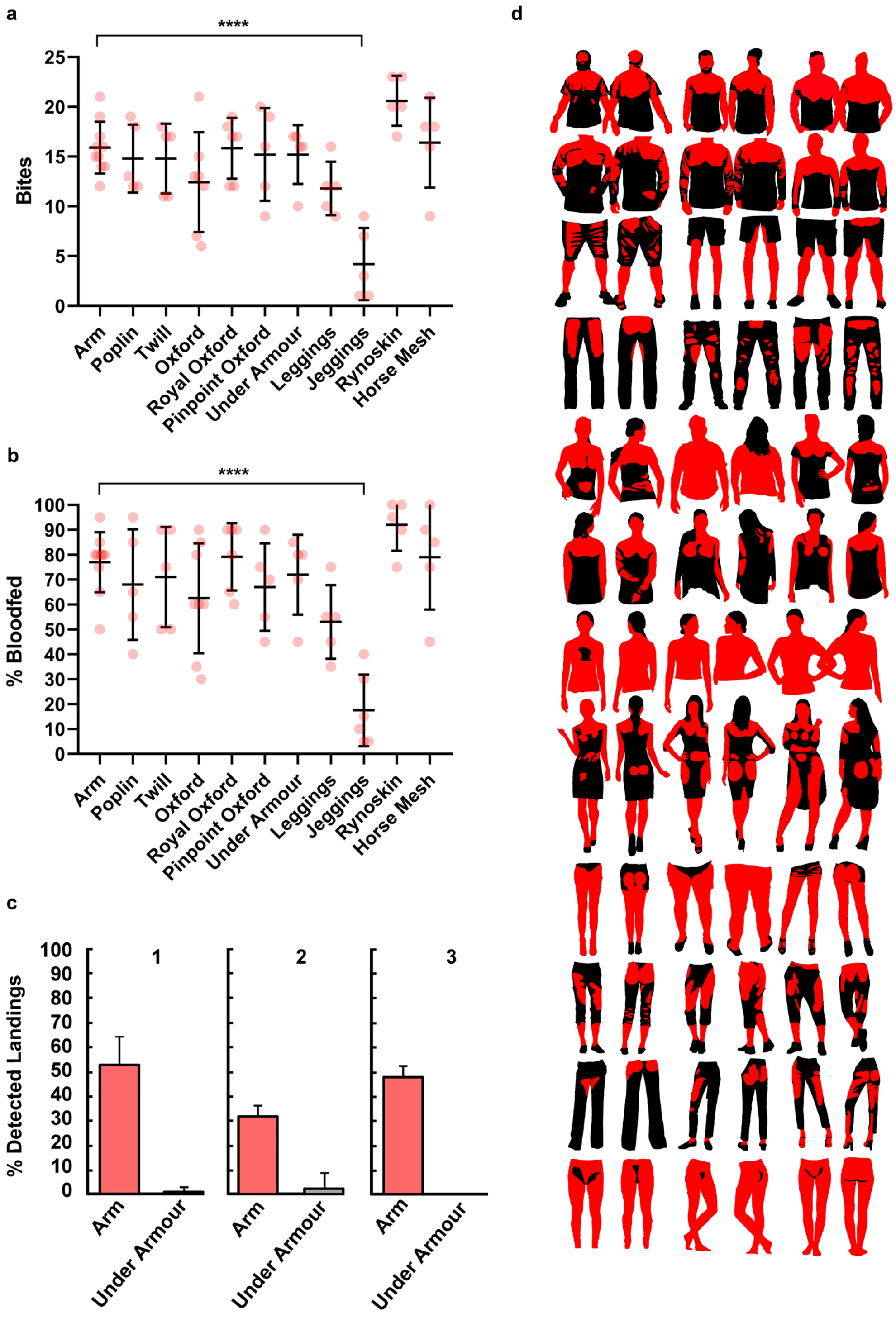

Figure 3. Data collected during preliminary studies. **a.** Number of bites received when wearing standard weaved and knitted clothing. Discovery of a single bite blocking textile known as jeggings. **b.** Percent of females, out of twenty, who received a blood meal when feeding through

standard clothing. **c.** Percent of detected mosquito landing events of three separate individuals. Wearing Under Armour^{TM} significantly decreases a human's ability to detect mosquito landings. **d.** Red indicates body regions of susceptibility through the lack of clothing or where clothing clings to the skin. The diagram shows each commonly worn clothing item in triplicates. In descending order starting at the first row: male short sleeve t-shirts, male long sleeve t-shirts, male shorts, male pants, female short sleeve t-shirts, female long sleeve t-shirts, female dresses, female shorts, female capris, female pants, and female leggings. (Beckmann ADECA Grant)

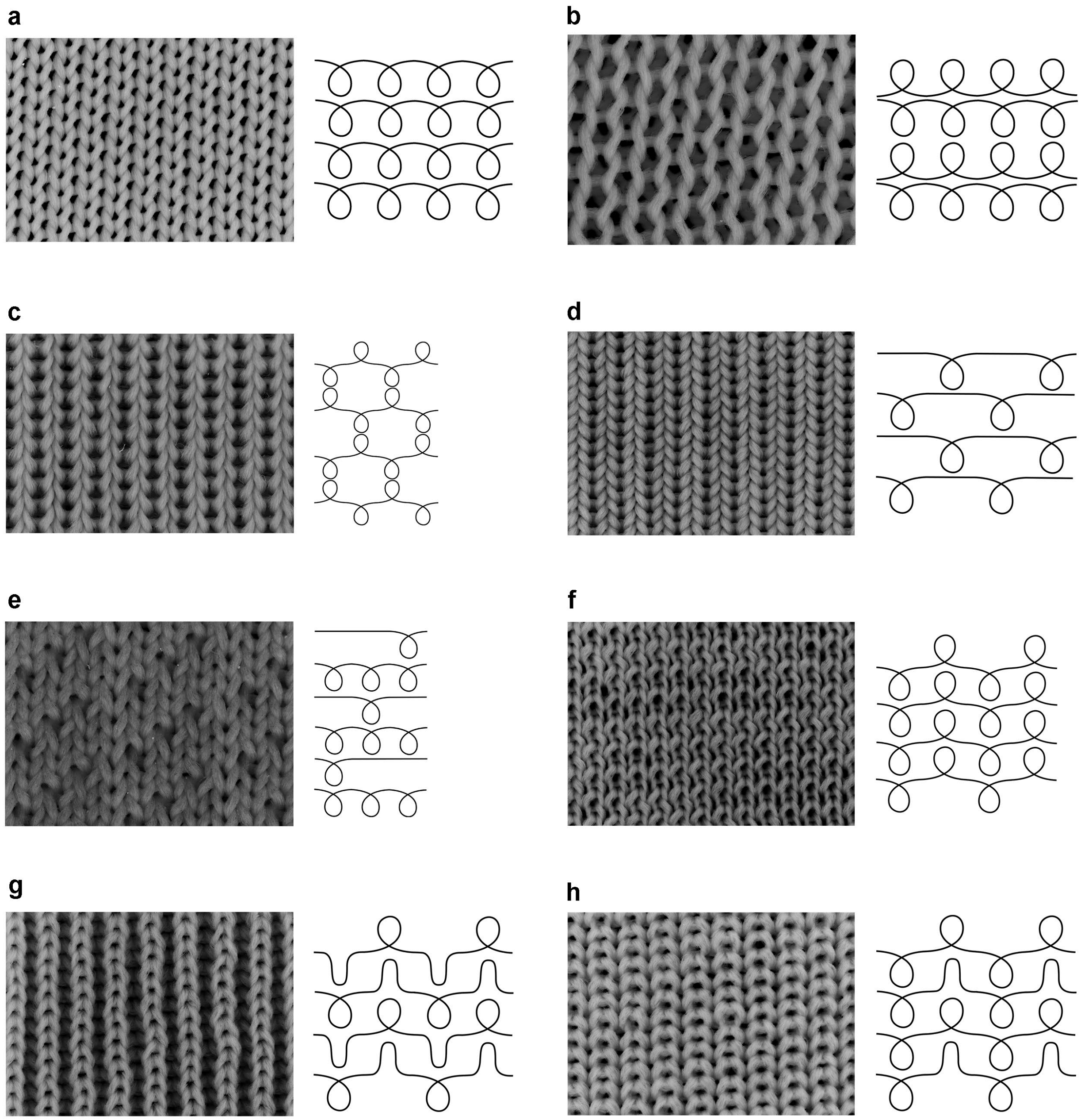

Figure 4. Microscopy images and corresponding knit diagrams of different structures tested for bite blocking abilities. **a.** Single jersey **b.** Tubular **c.** Interlock **d.** Alternate jersey **e.** Jersey skip (jegging replication) **f.** Full rib **g.** Full cardigan **h.** Half cardigan

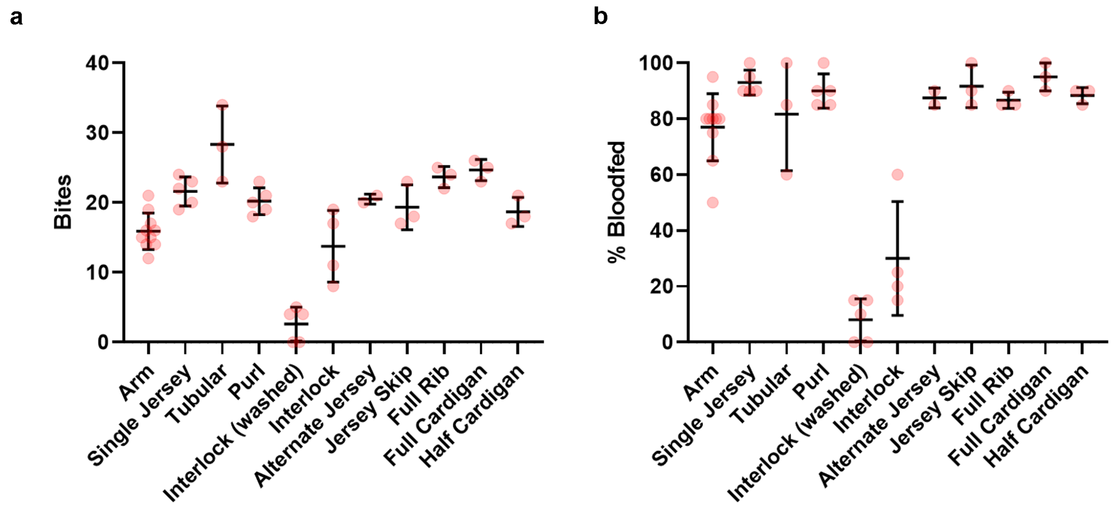

Figure 5. Mosquito bite blocking experiments to test if knit structure contributes to bite blocking ability. Each red dot corresponds (**a**) to the number of bites received (interlock (washed) is statistically significant from the control of a bare arm (p-value: <0.0001)) or (**b**) percent of bloodfed females from a single experiment (both interlock and interlock (washed) are statistically significant from the bare arm control (p-value: <0.0001)). Each knit was tested a minimum of three times.

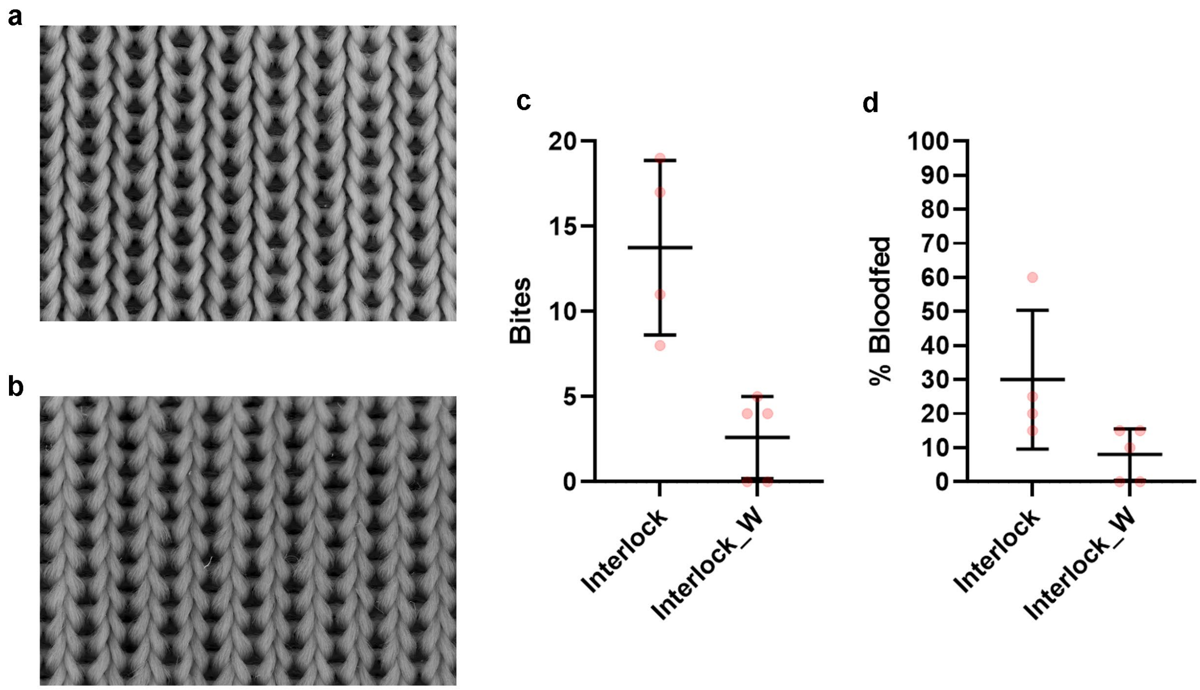

Figure 6. Fabric shrinking contributes to bite blocking ability. The interlock knit prior to a simple wash a dry cycle did not exhibit bite block abilities. However, once the textile was washed and dried the density of the fabric increased and consequently the bite blocking capabilities also increased. **a.** Microscopy image of an interlock knit prior to being washed and dried. **b.** Microscopy image of an interlock knit after being washed and dried. **c.** Number of bites received after a series of mosquito experiments for both the unwashed (Interlock) and washed (Interlock_W) interlock fabrics (p-value: 0.0004). **d.** Percent of females (out of twenty) that were able to penetrate and receive a full blood meal (p-value: 0.8450).

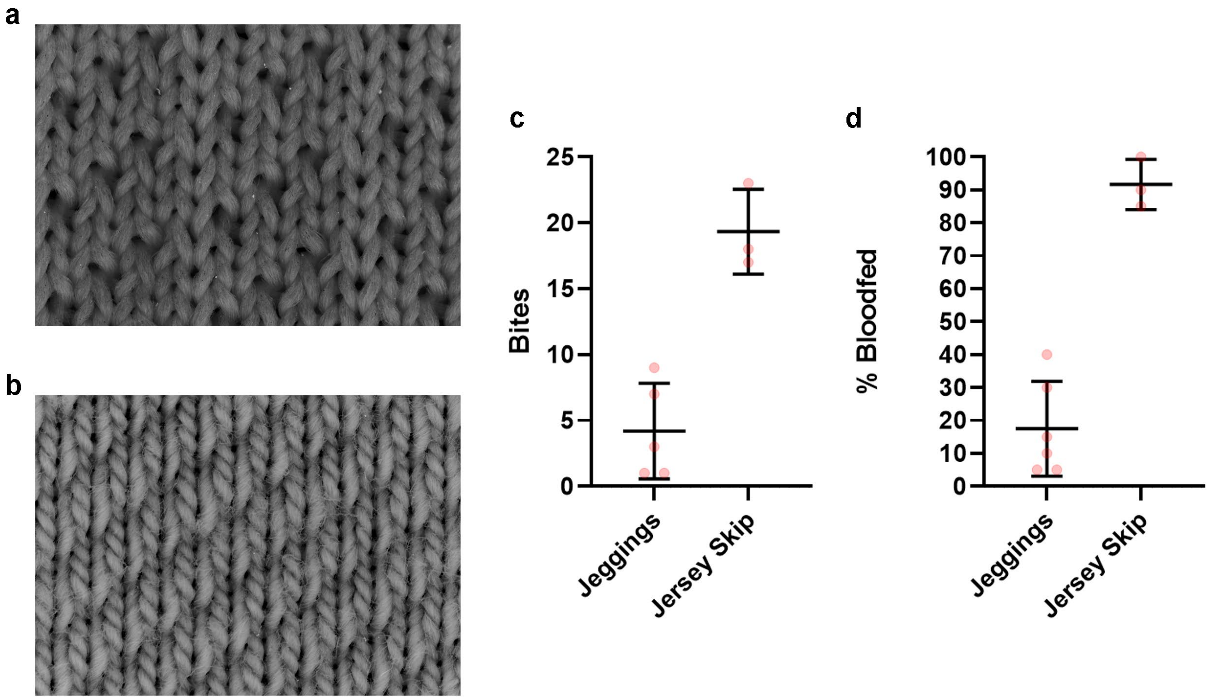

Figure 7. Spandex content contributes to bite blocking ability. During preliminary experiments, jegging was the only textile found to exhibit bite blocking ability. The jegging knit was recreated using the jersey skip knit structure which was sent to me by Stoll. It was created using the control yarn of 100% polyester. Our replication, which does not contain spandex, is unable to block mosquito bites. **a.** Microscopy image of jersey skip knit. **b.** Microscopy image of jeggings. **c.** Number of bites received during mosquito experiments (p-value: <0.0001). **d.** Percent of females (out of twenty) able to receive a full blood meal (p-value: <0.0001).

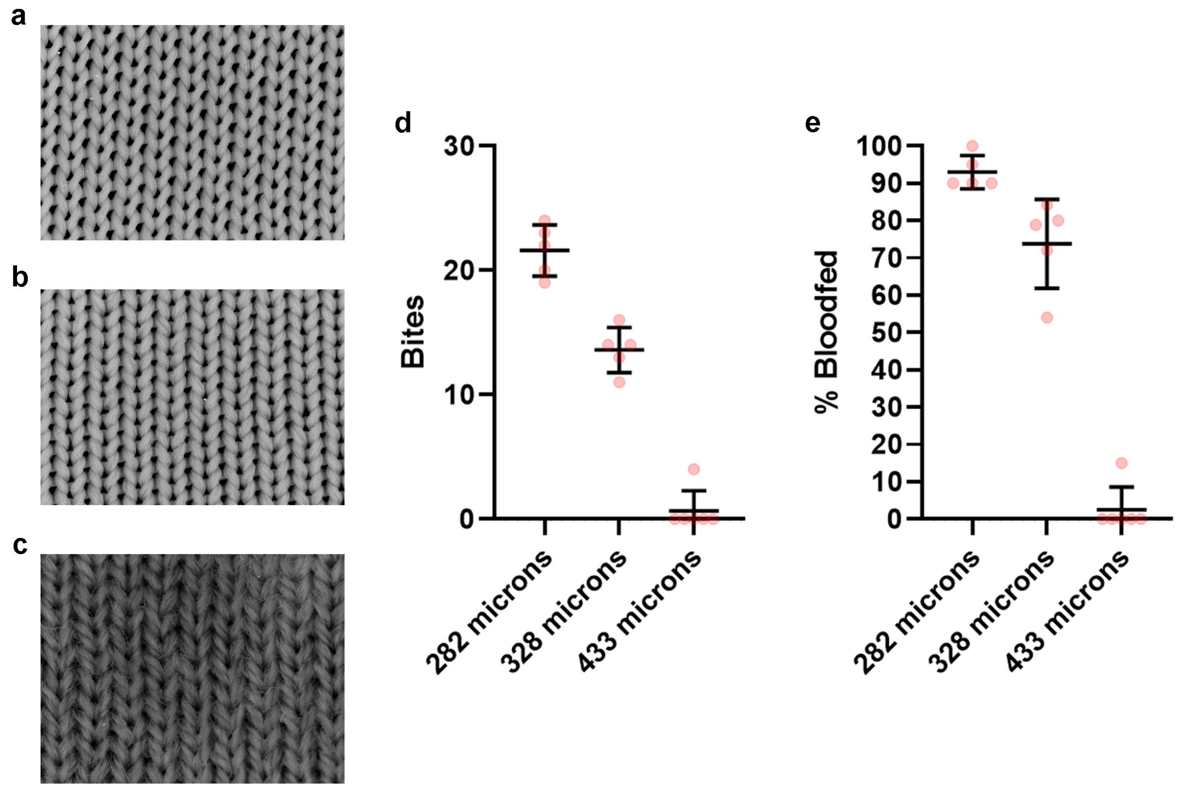

Figure 8. Yarn diameter contributes to bite blocking ability. Three single jersey knits of increasing yarn diameter were created and tested for enhanced bite blocking ability. Microscopy images of single jersey knits constructed with yarns of diameters **a.** 282 microns **b.** 328 microns **c.** 433 microns. **d.** Number of bites received during mosquito experiments (p-values: 0.0324, <0.0001). **e.** Percent of females (out of twenty) able to receive a full blood meal (282- and 328-micron knits are not significant. P-value of 282 compared to 433 micron knits: <0.0001).

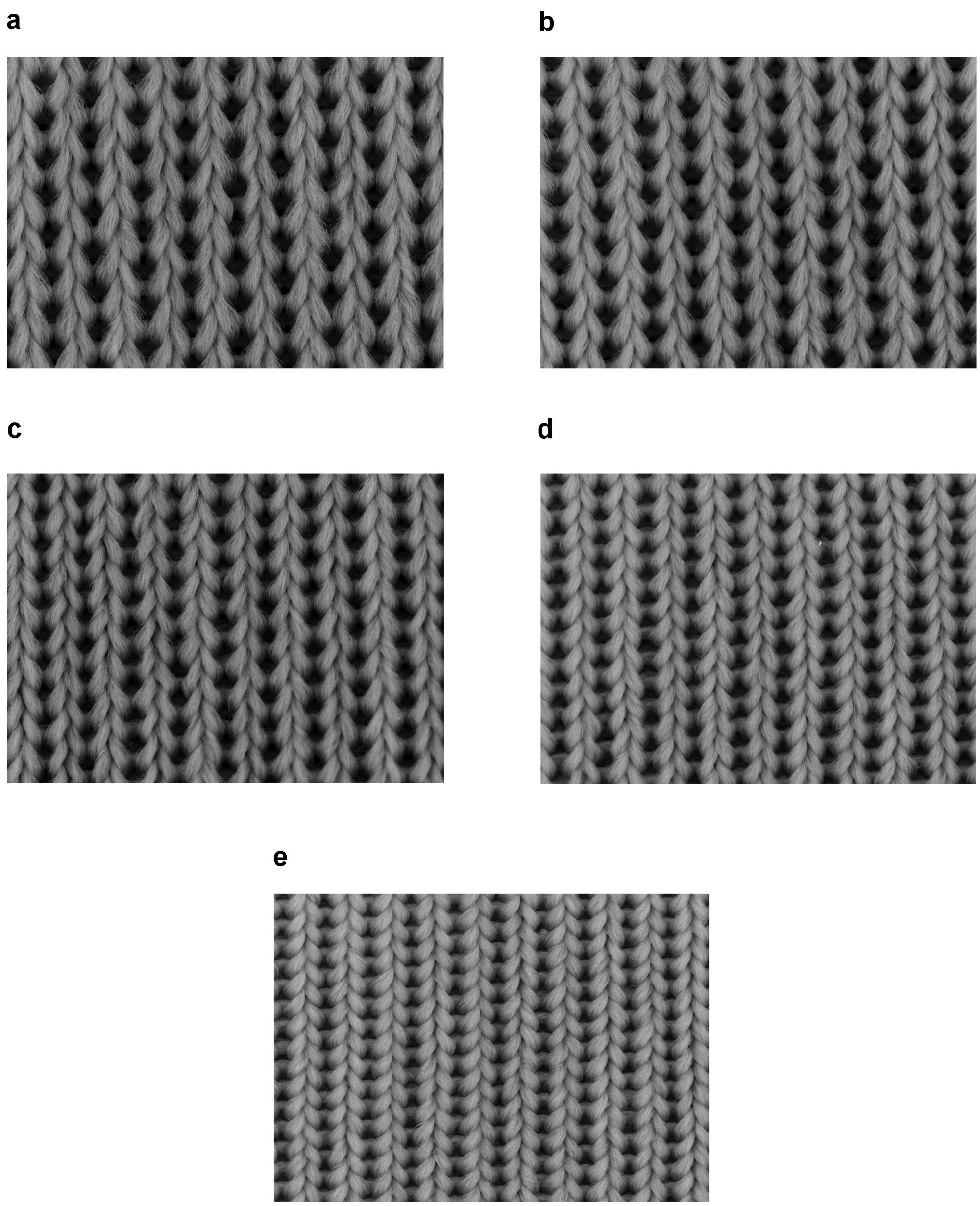

Figure 9. Stitch length contributes to bite blocking. Microscopy images of five interlock knits at stitch lengths of (**a**) 11, (**b**) 10.5, (**c**) 10, (**d**) 9.5, (**e**) 9. It is important to note that this data is not yet finished. Mosquito experiments need to be conducted to adequately test this hypothesis. However, the knits get visibly tighter as the stitch length decreases. Therefore, I hypothesize that an interlock knit at stitch length 9 will be able to block better than an interlock knit at stitch length 11.

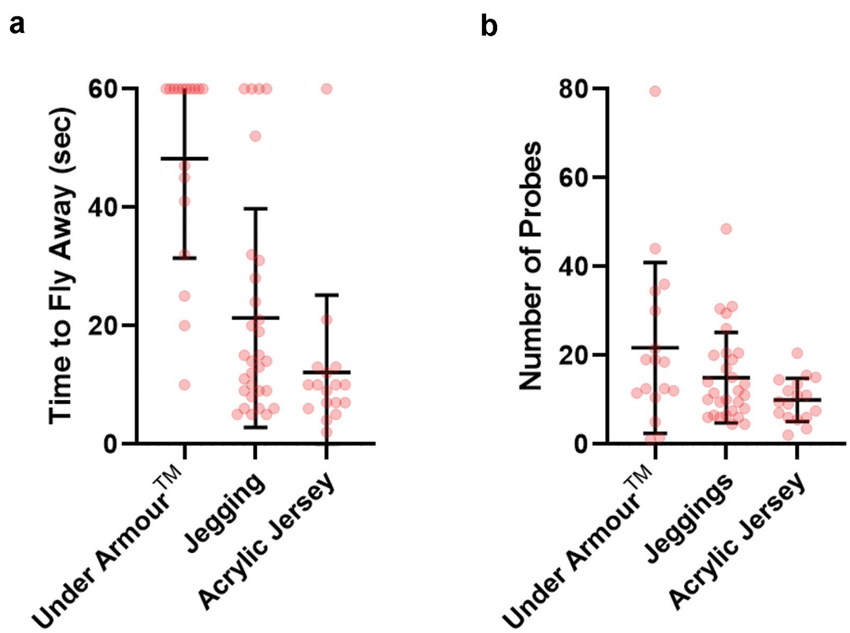

Figure 10. High-resolution video screening. Each red dot represents a single video which recorded one female *Aedes aegypti* mosquito for one minute. Under Armour™ is used as a negative control **a.** Amount of time each mosquito spent probing on the textile (p-values: <0.0001). **b.** Number of times each mosquito probed during a 60-second video (p-values: 0.1872, 0.0205).

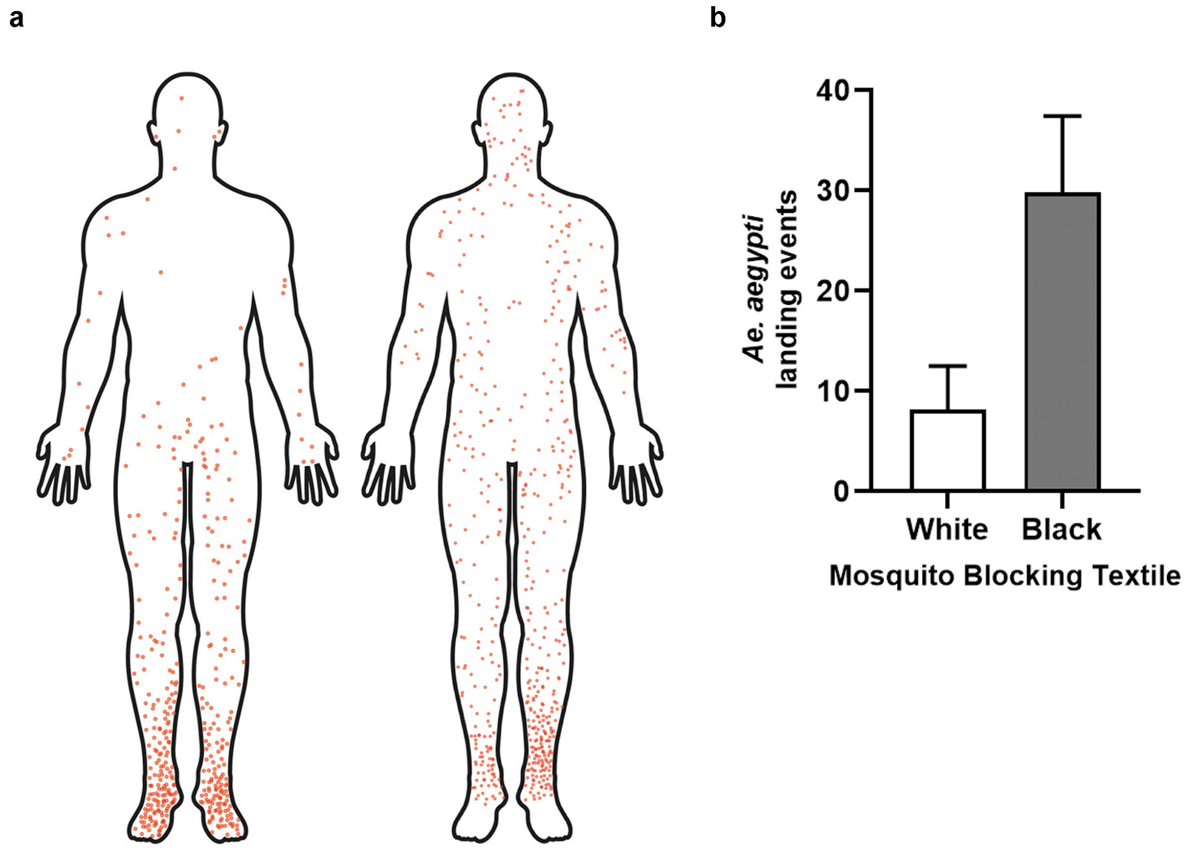

Figure 11. Mosquito preference. **a.** Mosquito landing heat map generated by quantifying where on a human body mosquitos prefer to land and probe (left: front; right: back). **b.** Graph of color choice. Mosquitos chose to land and probe on the black side of a textile at a 4.61-fold greater rate. This data can be used to design comfortable bite blocking clothing that exploits mosquito behavior to attract them towards areas with tighter knits.

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