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An Investigation of Host Variation in the Host-Parasite Interaction of *Sciurus carolinensis* and *Cuterebra emasculator.*

by
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College

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ABSTRACT

MILES DEBARDELEBEN: An Investigation of Host Variation in the Host-Parasite Interaction of *Sciurus carolinensis* and *Cuterebra emasculator.*
(Under the direction of Dr. Richard Buchholz)

Within a population, susceptibility to parasitism may vary. Individuals possessing certain intrinsic or extrinsic traits are often more likely to be parasitized than individuals within that population that do not possess those certain traits. As a result, parasites can have profound effects of the population dynamics of theirs hosts. Much research has been done on the host-parasite interaction of the eastern grey squirrel (*Sciurus carolinensis*) and the tree squirrel bot fly (*Cuterebra emasculator*). However, relatively little research has been done on host susceptibility to this parasite. Knowing that bot flies can lower reproductive success, reduce fitness, and cause death of their hosts, it is important to determine whether or not a certain characteristic may increase a squirrel's likelihood of becoming infested by bot flies. Along with number of bot flies larvae and bot fly scars present on the host, physical characteristics were measured from 35 squirrels including sex, second to fourth digit ratio, testes mass, body surface area, and spleen mass. There was a statistically significant relationship between the number of bots present and the sex of the host (unpaired t-test, t = -2.528, DF = 33, P = 0.0164). Males were more heavily parasitized than females. All other results were not statistically significant. Based on these findings, bot fly parasitism may affect the dynamics of squirrel populations by decreasing the fitness and reproductive success of males. Future research should focus on determining what trait makes individual males more susceptible than other males.

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INTRODUCTION

Parasites are important ecological factors that can limit host population size and can cause evolutionary change. Parasites can have detrimental effects on their hosts including causing death, altering behavior, and decreasing reproductive success. Some parasites kill their hosts by stealing nutrients. For instance, Jaenike et al. (1995) found that flies (*Drosophila putrida* and *D. neotestacea*) infected by the nematode *Howardula aoronymphium* have a significantly higher mortality rate than those that are not infected. Other parasites alter the hosts' behavior, making them more likely to become prey. Killifish (*Fundulus parvipinnis*) infected by the trematode *Euhaphorchis californiensis*, were 31 times more likely to be eaten due to a significant increase in sporadic movement (Lafferty and Morris, 1996). Parasites can also cause males to be less attractive to females. Worden et al. (2000) found that female grain beetles (*Tenebrio molitor*) are less likely to mate with males infected with tapeworms (*Hymenolepis diminuta*) because they produce a less attractive odor than those that are uninfected. Parasites may reduce a female's ability to conceive, bear, and rear offspring by stealing nutrients and forcing the female to allocate more energy to her immune response (Nordling et al., 1998). A blood parasite, *Haemoproteus prognei*, significantly reduces the clutch size and offspring survival of house martins (*Delichon urbica*) (Marzal et al., 2004). Hosts that can withstand these negative effects successfully pass on their genes to their offspring, while the genes of less fit hosts are lost. Over time there should be an evolutionary change within the host population (Clay, 2003).

Variation of a host organism's susceptibility to parasites can have evolutionary consequences (May and Anderson, 1983). Unfortunately it is not known in many cases whether the differences in susceptibility are caused by traits intrinsic to the host individuals, such as their genotype, or due to extrinsic factors, such as seasonal or climate effects (Gingrich, 1979, Jacobson et al., 1981).

Intrinsic Factors

In a host-parasite relationship, an intrinsic factor is a variable that is characteristic of the host and is not affected by outside circumstances. There are many intrinsic factors that can cause an increase or decrease in a host's susceptibility to parasites. I will review the evidence for the effects on host infection of five factors: genotype, testosterone levels, sex, age, and body size. One of the primary intrinsic factors is the host's genome. In many populations, some individuals possess genes that enable them to resist certain parasites more efficiently than other individuals. Glass and Jensen (2007) found that within a population of cattle (*Bos indicus*) some individuals express certain genes that enable them to resist the protozoan *Theileria annulata*, an otherwise deadly parasite. Variation in testosterone levels can cause variation in parasitism by suppressing the immune system, making it more difficult for the host to defend itself physiologically (Grossman, 1989). Lizards (*Psammodromus algirus*) that have higher testosterone levels also have a higher abundance of ectoparasites (Salvador et al., 1996). Aside from producing different hormones in differing ammounts, sex is also an intrinsic factor because males and females often exhibit different behavioral patterns. Certain behaviors, such as being social or territorial, could increase the likelihood of becoming infested with parasites. Territorial male gazelles (*Gazella granti* and *G. thomsoni*) are much more heavily parasitized by nematodes (*Strongyloides* spp.) than females, juveniles, and non-territorial males (Ezenwa, 2004). Age is a factor that can alter a host's susceptibility. The older an individual is, the longer it has had to acquire parasites, but it has also had longer to develop resistance. Older dusky farmerfish (*Stegastes nigricans*), a coral reef fish, have significantly higher endo- and ectoparasites than younger fish (Lo et al., 1998), while field crickets (*Gryllus veletis* and *G. pennsylvanicus*) show a decrease in parasitism by gregarines (class Sporozoa) as they age (Zuk, 1988). Body size has shown been to be a common intrinsic factor for host-ectoparasite systems. The larger a potential host is, the more likely it is to come in contact with a parasite. Yellow-necked mice (*Apodemus flavicollis*) and bank voles (*Myodes glareolus*) with greater body size were more heavily infested with ticks (*Ixodes* spp. and *Dermacentor* spp.) (Kiffner et al., 2011). These intrinsic factors are seldom the only cause of variation in parasitism, which can be affected by extrinsic factors as well.

Extrinsic Factors

Extrinsic factors are variables other than the characteristics of a host. Extrinsic factors can have an effect on parasite variation equally as profound as intrinsic factors. The main extrinsic factor is seasonal variation. The climatic conditions of the different seasons affect the development of many parasites and can cause regular patterns in parasite infestation. Bennett (1972) found that the metamorphosis of pupal bot flies

(*Cuterebra emasculator*) can be quickened by exposing the pupae to lower temperatures. Other extrinsic factors that may lead to variation in parasitism are habitat quality, host density, and host diet. If hosts are able to inhabit a variety of habitats, in areas that are not ideal for parasite development, there is a lower rate of infestation. In yellow-necked mice (*A. flavicollis*), infestation intensity of ticks (*Ixodes* spp.) decreases as the amount of vegetation in their home range decreases (Kiffner et al., 2011). In areas that would be considered ideal for a parasite, parasite density is often directly proportional to host density. A high host density provides parasites with a greater probability of infesting a host, thus raising their fitness. By examining the density of 19 mammal populations and the abundance of strongylid nematodes within each population, Arneberg et al. (1995) found that the parasite density increases as the host density increases. A host's diet is very important for determining its parasite load. The more food that a host consumes, the more likely it will be exposed to parasites (Arneberg et al., 1995) and the more likely parasites will be able to survive. Beckage and Riddiford (1983) found that reducing the amount of food taken in by the tobacco hornworm (*Manduca sexta*) also reduced the survival rate of an endoparasitic wasp (*Apanteles congregatus*).

Knowing that both intrinsic and extrinsic factors often determine a host's susceptibility to certain parasites, I will investigate whether a single factor or a combination of factors that may affect an individual host's likelihood of being parasitized by a certain parasite within a certain population.

Hypotheses

The objective of my thesis is to test three hypotheses that may explain the differences in parasite intensity between individuals within a population by examining primarily intrinsic factors.

Testosterone Hypothesis

Some authors have suggested that males of host species are more susceptible to parasitism than females. Male raccoons (*Procyon lotor*) are more highly parasitized by the hemoparasite *Dirofilaria tenuis* than females (Telford and Forrester, 1991). Similarly, *Giardia muris* occurs more often in male golden hamsters, *Mesocricetus auratus*, than in females (Kunstyr et al., 1992). Accordingly, the Testosterone Hypothesis for a sexual difference in parasitism states that because testosterone suppresses the immune system (Grossman, 1989; Folstad and Karter, 1992), male hosts are unable to resist ectoparasites (Saino et al., 1995; Perez-Orella and Schulte-Hostedde, 2005) as effectively as females. Three predictions are made from this hypothesis.

1) The Testosterone Hypothesis predicts that males will be infected more often than females due to the naturally higher testosterone levels in males. Although the exact mechanism is not known, testosterone and other androgens have been shown to inhibit the maturation of B-cells and T-cells, which are instrumental in an individual's immune response (Schuurs and Verheul, 1990).

2) Males and females with lower second digit length to fourth digit length ratios (2D:4D) will have more ectoparasites than individuals of the same sex with high 2D:4D ratios. In humans, males have a smaller 2D:4D ratio (Manning and Taylor, 2001). This

phenomenon has been seen in other mammals including mice (Brown et al., 2002) and Guinea baboons (*Papio papio*) (Roney et al., 2004). Exposure to high levels of pre-natal testosterone has been shown to reduce the 2D:4D ratio (Manning and Taylore, 2001). Pre-natal testosterone can predispose an individual to disease (Geschwind and Behan, 1982).

3) Because of the negative effects that testosterone can have on the immune system, it can also be predicted that individuals with larger testes will have more ectoparasites. Testis size has been positively correlated with testosterone level in mammals (Dixson and Anderson, 2004). In humans, testis size has been shown to be positively correlated with testosterone levels as well as other testicular functions (Takihara et al., 1987). Whitten (2000) found that testis size was correlated with testosterone levels in Old World monkeys.

Body Size Hypothesis

The Body Size Hypothesis for explaining variation in parasite burden states that the surface area of males and females determines how often they come in contact with parasite eggs (Catts, 1982). In species that are not sexually dimorphic, it can be predicted that larger individuals, independent of sex, will be more susceptible to parasitism than smaller individuals. Cone and Anderson (1977) found that as the size of pumpkinseed fish (*Lepomis gibbosus*) increased, so did the infestation intensity by ectoparasitic *Urocleidus* species. While studying several small mammal populations in Slovakia, Stanko et al. (2002) determined that host body size was positively correlated with infestation prevalence (percentage of population infested) by fleas. In regard to sexually dimorphic species, Moore and Wilson (2002) suggest that the larger sex would be more heavily parasitized because it is a larger target for ectoparasites.

Host Condition Hypothesis

The Host Condition Hypothesis states that individuals with weakened immune systems will attract more parasites (Beldomenico and Begon, 2009). Beldomenico at al. (2009) found that field voles (*Microtus agrestis*) that showed evidence of having a "poor immunological investment" were more heavily parasitized by the protozoan *Trypanosoma microti*. With the spleen mass increasing as the host's immune response increases, I predict that individual squirrels with large spleens will be more heavily parasitized by bot flies. Studies have shown that increased spleen mass in mammals is an indication of increased immune system activity (Corbin et al., 2008). Vincent and Ash (1978) found that spleen size was larger in gerbils (*Meriones unguiculatus*) that were infested by roundworm (*Brugia malayi*). Similarly, Garside et al. (1989) also observed an increase in spleen size in hamsters infected by hookworm (*Ancylostoma ceylanium*).

Hypothesis Testing

To test these hypotheses, I studied the host parasite relationship of the eastern grey squirrel, *Sciurus carolinensis,* and the tree squirrel bot fly, *Cuterebra emasculator.* The eastern grey squirrel is a rodent that is native to the eastern and midwestern United States but has been introduced and thrives in the western United States, Britain, and Ireland. This mammal is ideal to study due to its abundance, relatively rapid reproduction, and its susceptibility to parasitism by the tree squirrel bot fly. *Cuterebra* *emasculator* is an obligate ectoparasite that parasitizes *S. carolinensis*, *S. niger* (fox squirrel), and *Tamias striatus* (eastern chipmunk). Current or recent bot fly parasitism is obvious, making determining the intensity of infestation a simple process. A review of the life cycle of the bot fly will clarify the ways in which this parasite may affect its host.

Bot fly Life Cycle

The larvae of *C. emasculator* go through three larval instars, before exiting their host and pupating in soil. Larvae, of all three instars, are made up of 12 segments and contain cuticular spines that facilitate movement by anchoring the larvae during segmental expansion. First instar larvae are between 2 and 3 mm in length, weigh less than 0.01 gram, and are usually white in color. Second instar larvae can be up to 11 mm in length, weigh between 0.01 and 0.10 gram, and are a grayish-white color. Third instar larvae are approximately 25 mm in length, weigh 0.10 to 1.20 grams, and change colors from a grayish-white to a dark brown (Figure 1). Adult tree squirrel bot flies are between 16 and 20 mm in length, and resemble bumblebees with a black and yellow coloration (Bennett, 1955).

Adult female *Cuterebra* lay their eggs in clusters on structure (twigs, leaves, branches) in areas that are frequently visited or traveled by the target host species, such as nests and burrows. Within the egg, embryonic development generally takes 4-10 days in an environment of sufficient temperature and humidity. After development is complete, the larvae may remain viable within the egg for prolonged periods (Catts, 1982).

Rapid hatching of multiple eggs is caused by the abrupt absorption of heat given off by the warm-blooded host. Upon hatching, the larva (or bot) is damp from fluids inside the egg and will easily transfer from the egg onto the host if contact between the two is made. If contact is not made, the larva will flail back and fourth in the presence of a heat source attempting to transfer onto a possible host. A hatched larva may survive up to seven days, allowing the opportunity for a host, other than the one that caused hatching, to be infested (Catts, 1967). Once on a host, the larva will migrate along the host's body searching for a point of entry. Bots will enter through any wet orifice (mouth, nose, anus), the eyes, or a recent laceration somewhere on the body. The bot will travel to and dwell in the nasopharyngeal, tracheal, or esophageal regions for a couple of days before spending an additional seven days migrating through the thoracic and abdominal cavities or beneath the fascia of muscles (Slansky, 2007). This migration across multiple tissues is made possible by enzymes released by the bot that deteriorate tissue walls (Otranto, 2001).

The bot eventually reaches a subcutaneous site where a very localized swelling takes place while the warble is formed. A warble is simply the hollow area that the bot occupies while developing. At this time, the larva creates a warble pore with its two hook-like teeth. The warble pore is a hole in the host's skin that allows oxygen to reach the bot and for waste excretion from the warble. Once the warble is completely formed, the larva begins to molt to the second instar and remains at this location until its exodus one to three weeks later (Slansky, 2007).

As a second instar, the larva continues to grow by ingesting fluids that enter the warble, most likely interstitial fluid and possibly blood. Approximately 14-19 days after infecting the host, the larva will molt to the third instar. During this time it stores lipids to prepare itself for a lengthy pupation underground. *Cuterebra emasculator* has a minimum puparial development period of 225 days (Bennett, 1972). After the bot has exited, the warble closes within a few days and is healed within two weeks, leaving only the hair to grow back (Slansky and Kenyon, 2003) (Figure 2 and 3).

After the subterranean developmental period, the bot emerges as an adult fly. Each morning, males will head to an aggregation site to claim territory before the females arrive. Once the females arrive, the males pursue them. After mating, females deposit their eggs without delay. Females will lay approximately 770 eggs in clusters of about fifteen eggs. Most *Cuterebra* flies will die within fourteen days after mating (Catts, 1982).

Figure 1. Third-instar *C. emasculator* bot (dorsal view, head to the left) recently removed from a *S. carolinensis*.

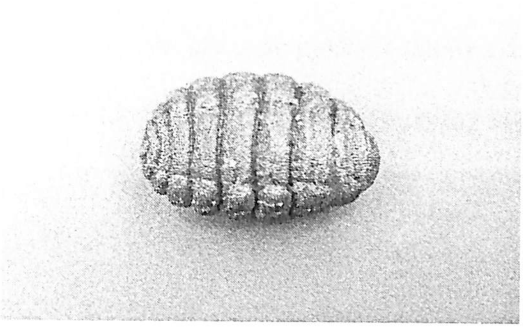
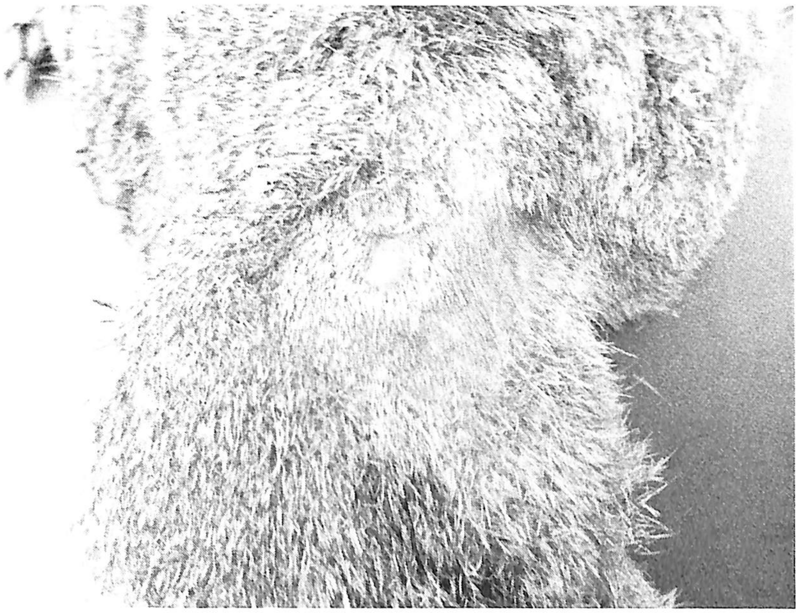


Figure 2. *C. emasculator* bot next to empty warble. As Slansky et al (2003) described, a bot cannot fully develop unless the host is alive for the entire duration of the infestation. This bot exited the warble shortly after the squirrel was shot.

Figure 3. Warble scar on the back of a *S. carolinensis*. Hair has begun to grow where the warble had caused balding and the warble pore has closed.



Effects of Bot Flies on Their Hosts

Research has been done on the effects of cuterebra parasitism on their hosts for over 150 years (Slansky, 2007). Although they can cause harm, bot fly larvae rarely have deleterious effects on the health of their hosts. Unique from many other parasitizing flies, *Cuterebra* cannot reach the third instar, or most mature myiasis larval stage, unless the host is alive for the entire duration of the infestation (Slansky and Kenyon, 2003). This distinctive attribute suggests that the *Cuterebra* are adapted to not kill their host by minimizing negative effects on their hosts' physiology and behavior that may jeopardize their own development. Evidence of this co-evolution can be seen in the lack of detrimental effects that a *Cuterebra* larvae has on atypical hosts (Baird, 1971). Bennett (1955), using a proven method of intraspecies transfer, performed 18 interspecies transfers of second and third instar larvae to atypical hosts. Of the 18 bots transferred, only two developed to maturity, two left the host within 12 hours, and 14 died *in situ*. Of the 18 hosts, three died, 15 showed a decrease in activity, and all transfer sites became purulent. Regardless, a *Cuterebra* larva may still have a negative impact on its typical hosts. The primary ways that the bot fly larvaee affect their host is by stealing nutrients and causing a change in behavior.

When a bot infests a host, its diet consists of interstitial fluids and cellular and tissue debris (Payne et al., 1965). A bot may ingest blood that seeps into the warble (Slansky, 2007). As mentioned above, on this modest diet, the bot's body mass increases 120 times during its infestation; 86% of this growth occurs during the last week of the infestation (Smith, 1975). This sudden growth can be the cause of a major decrease in the host's resource budget. To meet the deficit caused by the bot, a host must adapt in some way to make up for this potentially fatal decrease in available energy. A host can compensate for this decrease in nutrients by reducing activity levels which reduces the amount the host needs to eat (Smith, 1978) or by increasing the amount of nutrient intake (Hunter et al., 1972).

Reproduction uses a large portion of an individual's resource budget. When a squirrel, or other host, is infested with a high intensity of bots, a significant resource drain takes place. This drain could potentially reduce the ability of a female to ovulate, sustain fetuses, and/or support nursing young. A parasitic nutrient drain by bot flies (*Cuterebra fontinella*) has been shown to reduce the weight of reproductive organs including the ovaries and testes in white-footed mice (*Peromyscus leucopus*) (Timm and Cook, 1979). Aside from the indirect effects of nutrient drain, the presence of the warble could also have direct, negative effects on the reproduction of the host.

Cuterebra emasculator acquired its name because Fitch (1857) originally believed that the parasite castrated its host. This is not the case, and researchers eventually determined that when a bot is located in the groin region, the warble merely prevents the testes from descending into the scrotum (Timm and Lee, 1981). Scientists have noted warbles have been mistaken by the young as the teat, which could lead to the ingestion of harmful bacteria and toxins, as well as reduced nutrient acquisition by the offspring (Slansky and Kenyon, 2003).

Although there has been ample research on the effects of *Cuterebra* larvae on their hosts, scientists generally agree that, due to the co-evolution of the host and parasite, there would have to be an abnormally high intensity of infestation or an additional factor for *Cuterebra* parasitism to have a devastating effect on its host. This additional factor could include a previous predisposition to mortality, including poor body condition, and an extreme scarcity of food.

METHODS

Sample Collection

Squirrels were collected as a part of the legal hunting season for *Sciurus carolinensis* in Zone 1 of Mississippi under a valid hunting permit. Squirrels were shot between October 2, 2010 and October 17, 2010. All squirrels were taken from land belonging to the U.S. Army Corps of Engineers in Lafayette County, MS. The collection area was contained within the following boundaries: North- 34°26'07''N, South- 34°25'13''N, East- 89°40'40''W, West- 89°41'28''W.

Data Collection

Collection date, sex, and number of *Cuterebra emasculator* larvae or emergence scars were recorded upon collection. Specimens were frozen until dissection. Age, second digit (2D) and fourth digit (4D) lengths, body length, body mass, spleen mass, and testes masses were determined as follows. After defrosting in the laboratory, age was determined by the pelage technique described by Sharp (1958). Squirrels were assigned age classifications of juvenile (< 6 months), subadult (6 to 16 months), and adult (> 16 months). The second and fourth digits were measured on the ventral side of the paw from the basal crease where the digit joins the paw to the tip of the digit on both the front and back feet of the squirrels (Manning et al., 1998). The ratio of the 2D to 4D was calculated after measuring the digit lengths. Body length was determined with a ruler by laying the specimen on its stomach and measuring dorsally from the tip of the nose to the base of the tail. Body mass was determined using a top loading triple beam balance. Spleens and testes were extracted by dissection. The mass of the spleen and testes were measured separately with a digital balance after they were cleaned and surface dried.

A subset of squirrels was used to construct an equation to estimate the body surface area of a squirrel. To measure surface area, ten squirrels of various sizes, sexes, and ages were laid flat with ventral side down on a piece of aluminum foil and the body outline was traced. Without moving the squirrel, its other surfaces (sides) were covered with another piece of aluminum foil, and the contour was traced. The traced area of aluminum foil was cut along the lines with a scalpel, rinsed to remove hair and blood, and dried. The masses of separate pieces of aluminum foil of known areas were averaged to be $0.006 g/cm^2$. This average was used to determine the area of foil used to cover a squirrel. A multiple regression equation was developed that explained foil mass as a function of squirrel mass and length. The result of this relationship was an equation from which body surface area could be estimated from the mass and length of the squirrel.

Data Analysis

All data were entered into Statview (Abacus Concepts, 1986) statistical software and an un-paired t-test, simple regressions, and ANOVA were run to test the hypotheses.

RESULTS

Testosterone Hypothesis

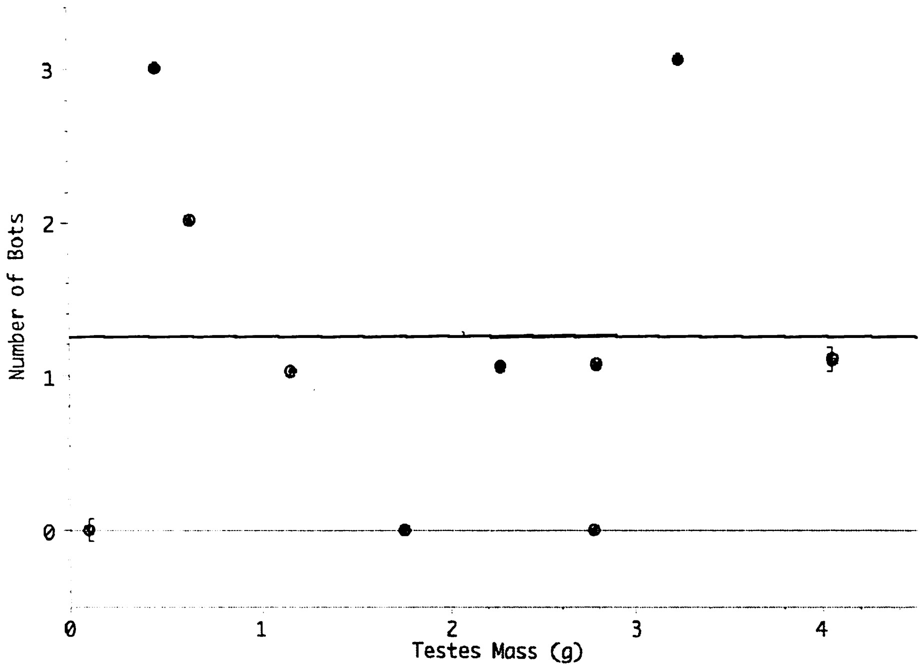
There was a significant statistical difference in number of hosts infested between male and female squirrels (unpaired t-test, t = -2.528, DF = 33, P = 0.0164). Of the 25 females, 8 (32%) had either *Cuterebra* larvae or warble scars present. Of the 10 males, 7 (70%) of the squirrels were either infested or had warble scars present. The mean number of bot flies per squirrel for females was considerably less than the average infestation number for males (Table 2).

Table 1. The infestation intensity for each sex, showing the average infestation intensity of males is three-fold that of females.

	Count	Mean	Variance	Std. Err.
Female	25	0.400	0.500	0.141
Male	10	1.200	1.289	0.359

There was no relationship between the mass of an individual's testes and the prevalence of *Cuterebra* parasitism (Figure 4). The combined weight of both testes ($\bar{x} = 1.919 \pm 0.304$ g) was not correlated with the bot fly infestation intensity ($R^2 = 0.001$, n = 10, P=0.9261).

Figure 4. There is no relationship between testes mass and the prevalence of bots in *S. carolinensis*.



Separate statistical analyses were run for each foot to determine the significance of the relationship between the 2D:4D ratio and infestation by *Cuterebra* larvae (Table 3). There was no significant relationship found between the 2D:4D ratio and infestation prevalence for either male or female squirrels. For males, the front left paw, front right paw, and back left paw all resulted in nonsignificant statistical relationships and no obvious trends (Figures 5-7). Even though the back right paw of males did not produce significant results, it did show a trend towards a significant relationship with parasite infestation (Figure 8). For females, the front left paw, the front right paw, and the back right paw had nonsignificant relationships with infestation with no obvious trends (Figures 9-10, 12). The back left paw also did not result in a significant relationship but did show a trend towards a relationship (Figure 11).

Table 2. There is no relationship between 2D/4D digit ratio and bot prevalence. The back right of males and back left of females show trends towards a positive correlation. Males show a larger average ratio for three paws.

	Count	R^2	P-Value	Mean
Male				
Front Left	10	0.011	0.7714	0.750
Front Right	10	0.003	0.8870	0.750
Back Left	10	0.002	0.9017	0.924
Back Right	10	0.311	0.0936	0.918
Female				
Front Left	20	0.000	0.9974	0.738
Front Right	20	0.055	0.3199	0.749
Back Left	21	0.121	0.1229	0.922
Back Right	21	0.056	0.3039	0.931

Figure 5. There is no significant association between male front left 2D/4D ratio and number of bots.

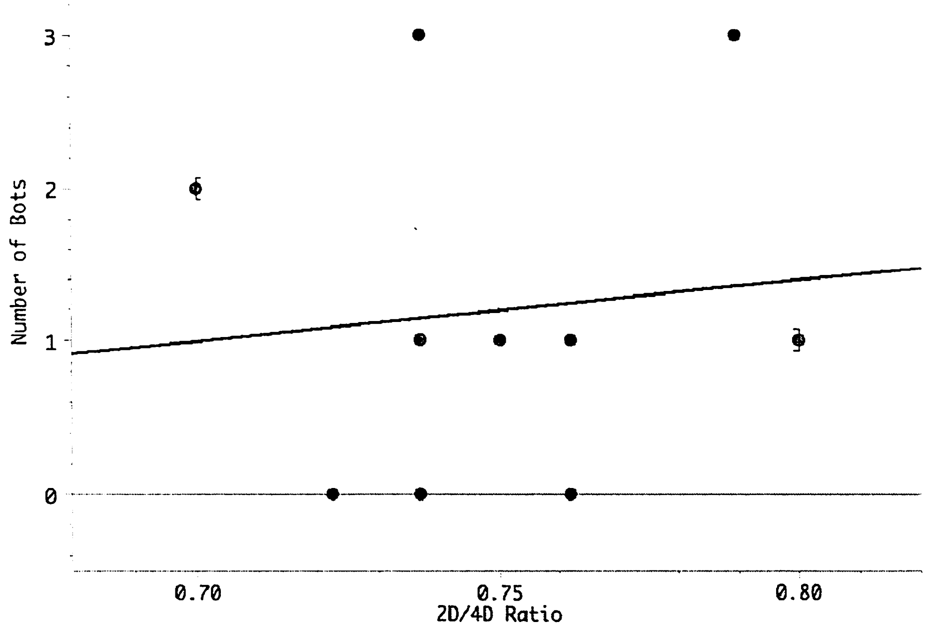


Figure 6. There is no significant association between male front right 2D/4D ratio and number of bots

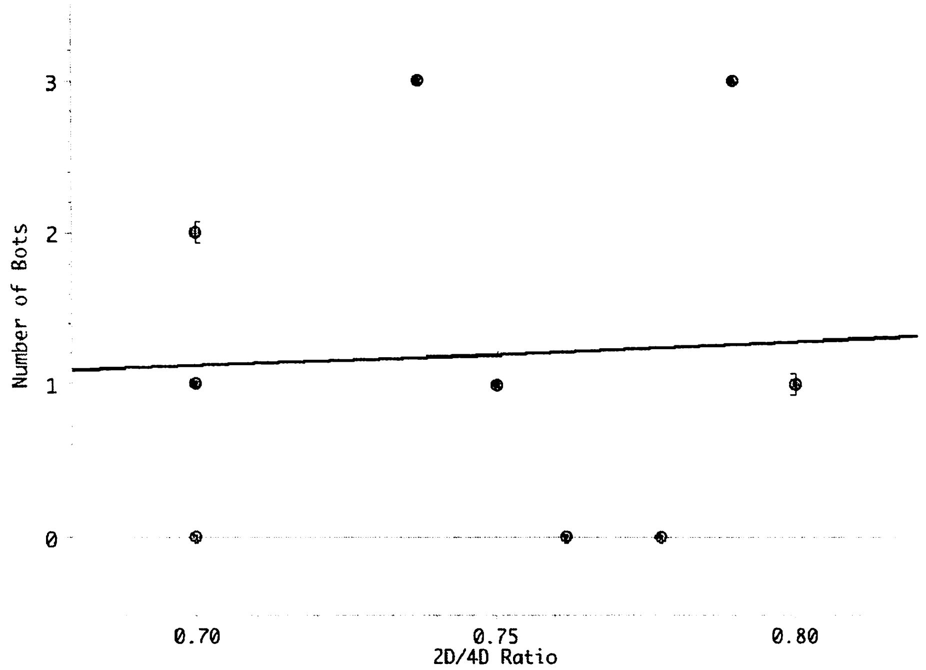


Figure 7. There is no significant association between male back left 2D/4D ratio and number of bots.

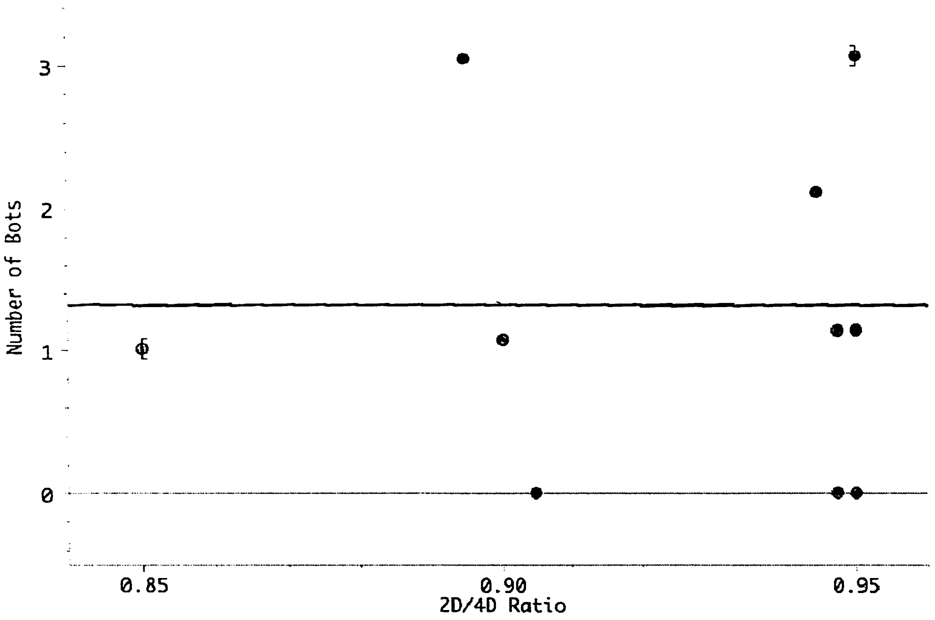


Figure 8. There is no significant association between male back right 2D/4D ratio and and number of bots.

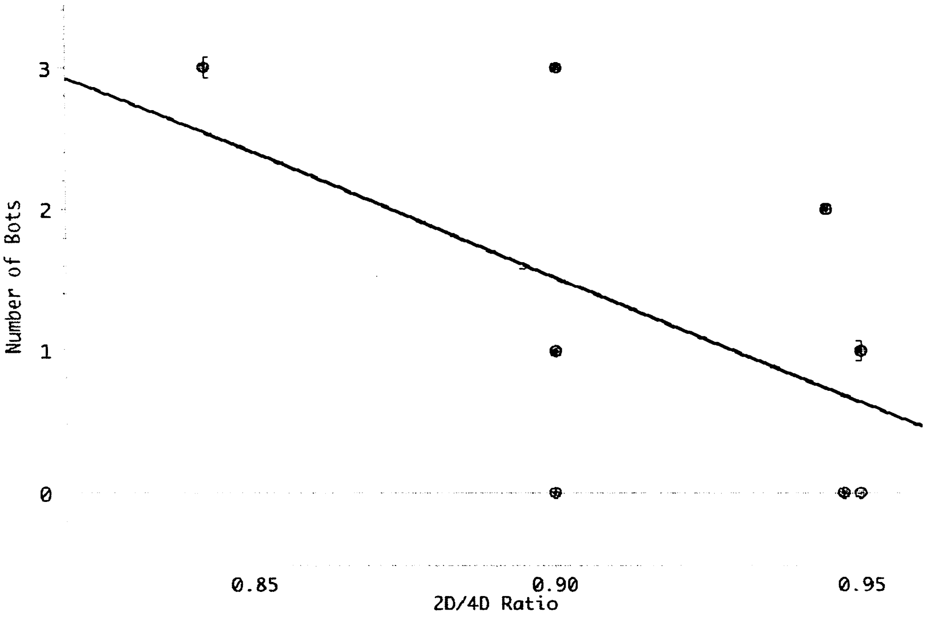


Figure 9. There is no significant association between female front left 2D/4D ratio and number of bots.

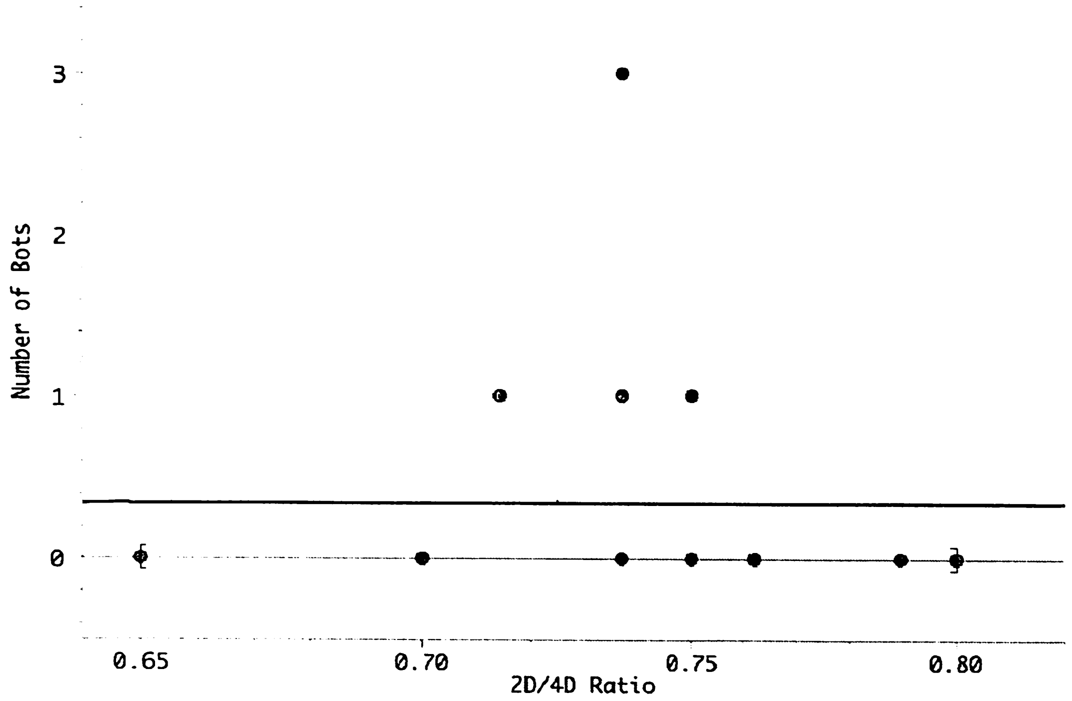


Figure 10. There is no significant association between female front right 2D/4D ratio and number of bots.

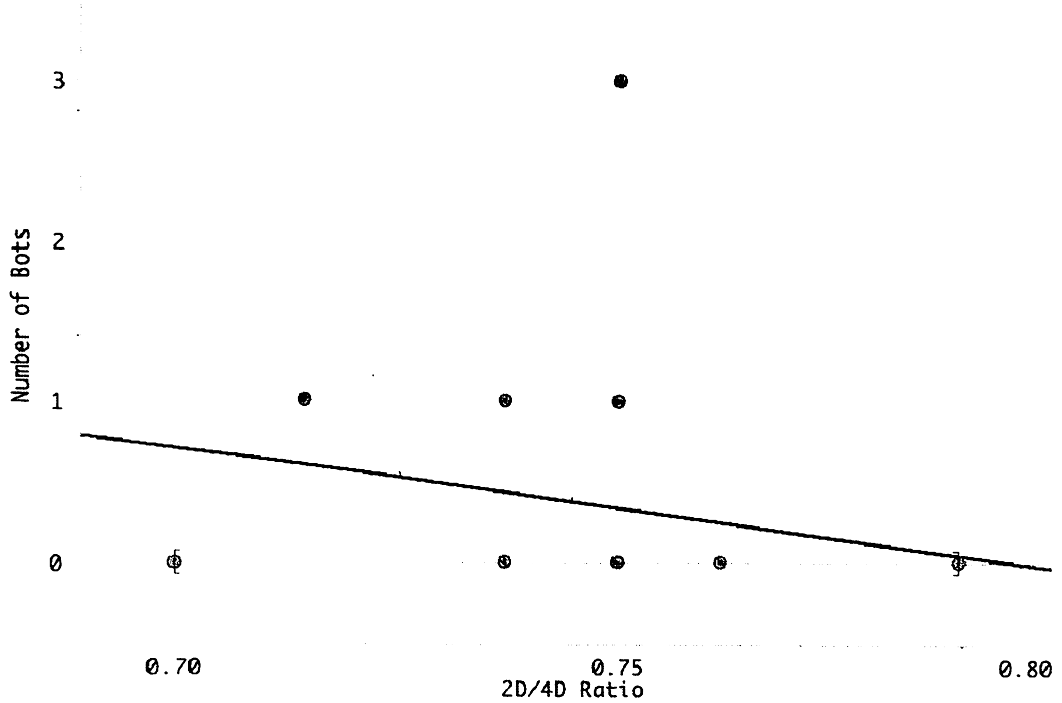


Figure 11. There is no significant association between female back left 2D/4D ratio and number of bots.

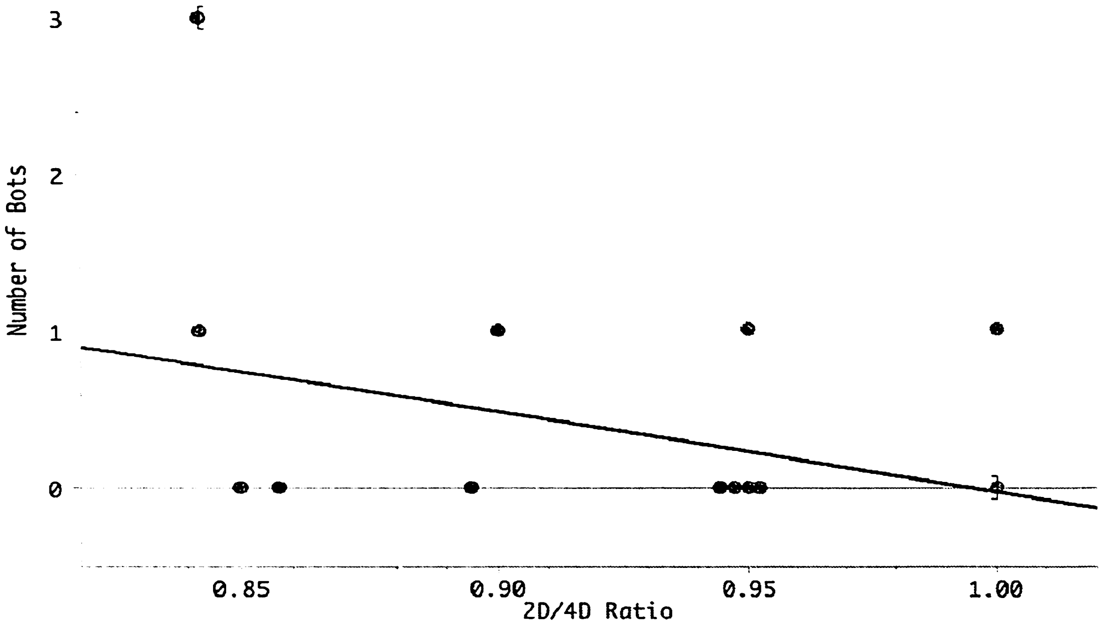
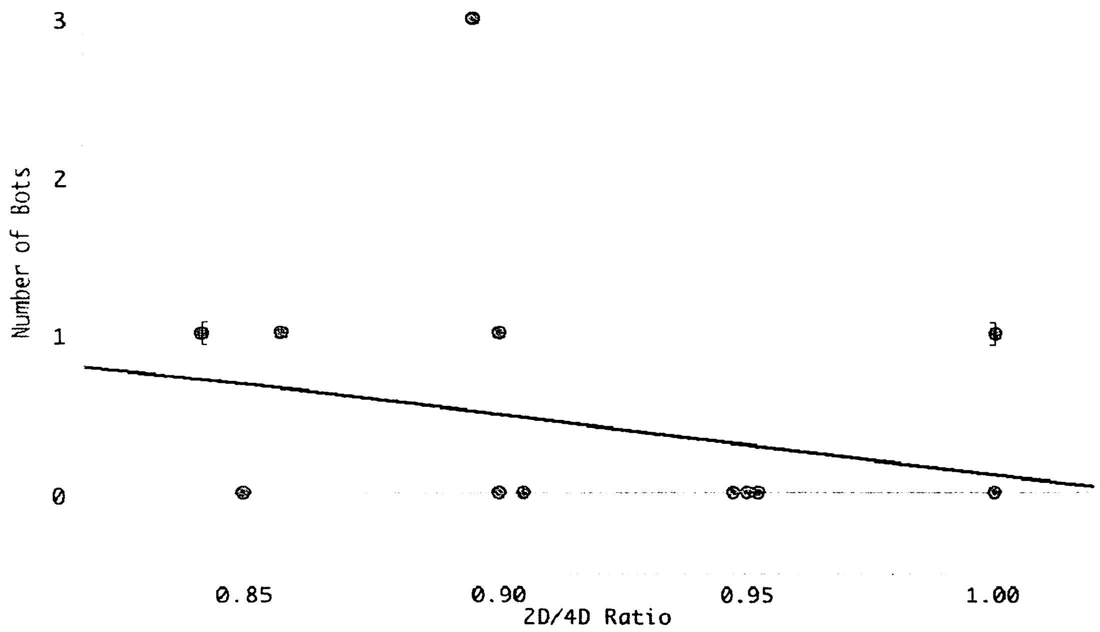


Figure 12. There is no significant association between female back right 2D/4D ratio and number of bots.



Body Size Hypothesis

The body surface area (BSA) of a squirrel was estimated using the following equation:

$$BSA = 28.32 \text{ x } (\text{mass}/\text{length})$$

This equation was statistically significant ($t = 59.2$, $p<0.001$) and explained 99.6% of the variation in BSA. When examining the relationship between the BSA of a squirrel and the prevalence of bot fly parasitism, the association between the two was not significant. The BSA of the squirrels ($\bar{x} = 273.775 \pm 0.478$ cm^2) was not related to the number of larvae and warble scars found on an individual ($P = 0.079$, $n = 35$). Male squirrels' BSA ($\bar{x} = 273.788 \pm 0.359$ cm^2) was not significantly related to the number of bot flies or warble scars present ($R^2 = 0.389$, $n = 10$, $P = 0.0539$) (Figure 13). The BSA for female squirrels ($\bar{x} = 273.775 \pm 0.141$ cm^2) also resulted in a nonsignificant relationship ($R^2 = 0.013$, $n = 35$, $P = 0.5831$) (Figure 14). ANOVA tests on male and female squirrels concluded that variation in infestation intensity could not by explained by BSA ($F_{male} = 5.096$ and $F_{female} = 0.310$).

Figure 13. There is no significant association between male BSA and number of bots.

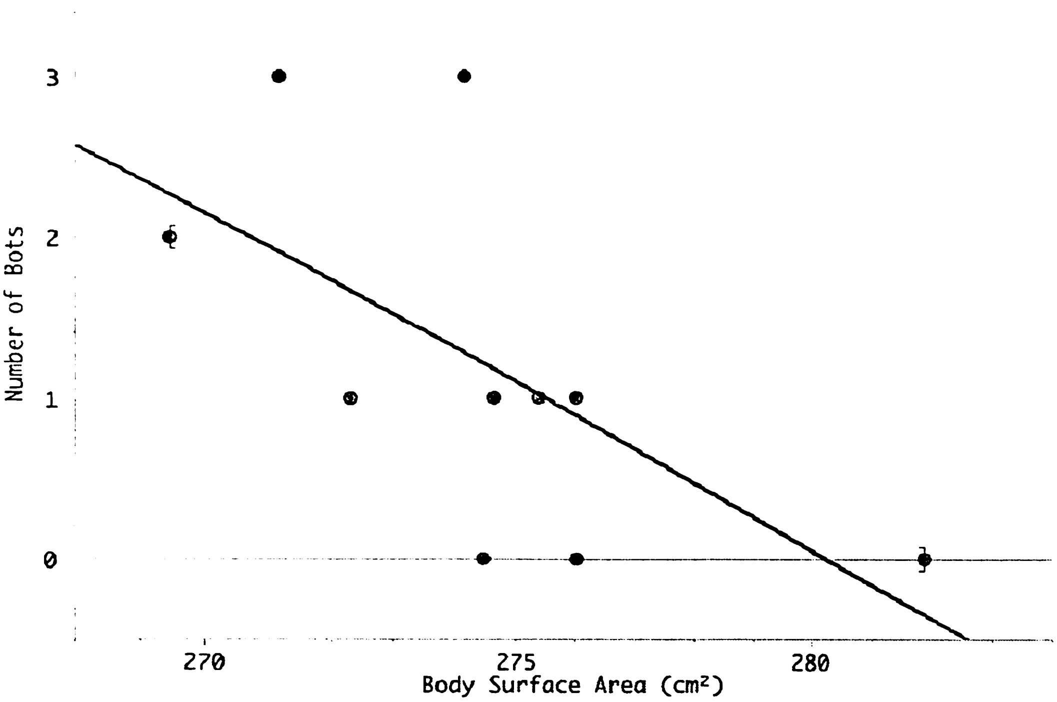
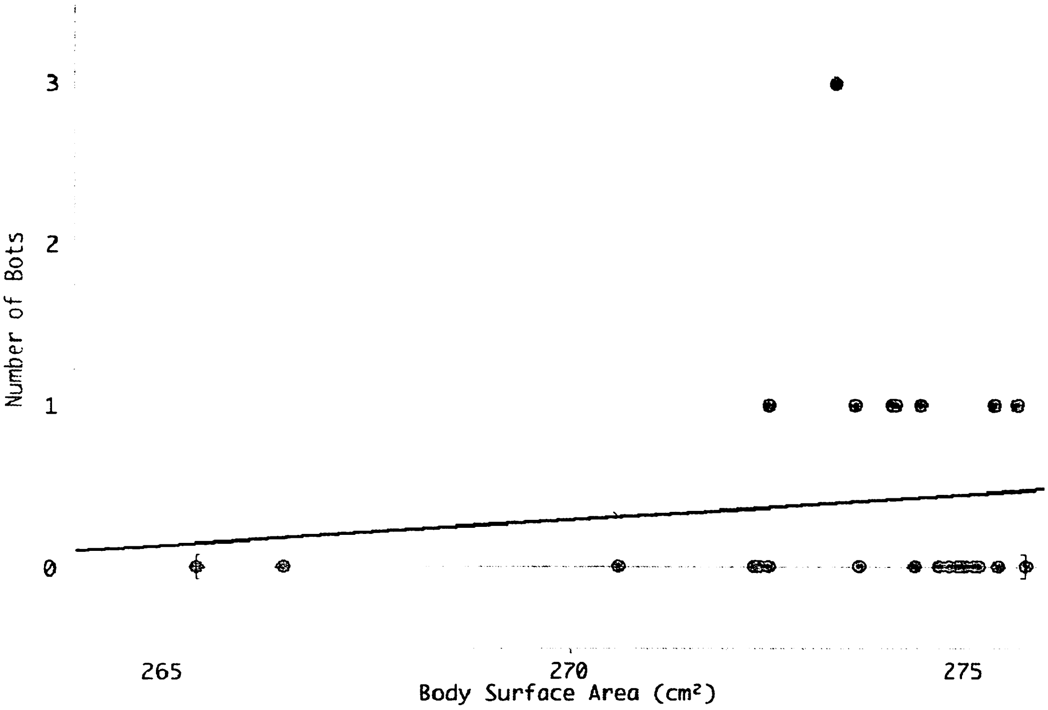


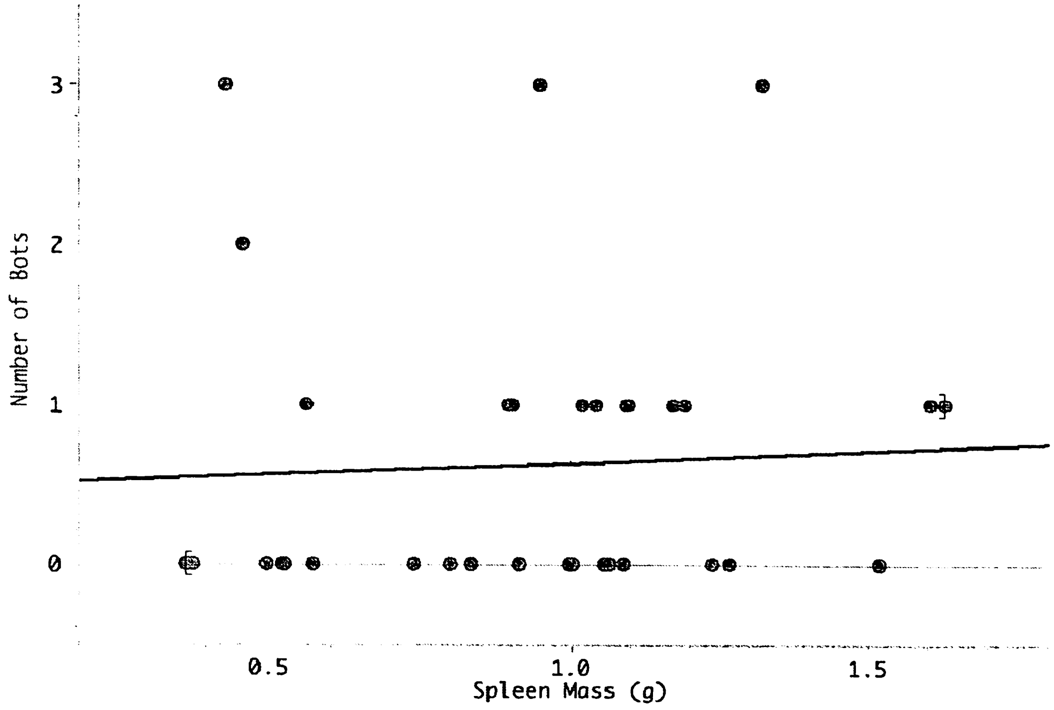
Figure 14: There is no significant association between female BSA and number of bots.



Host Condition Hypothesis

Finally, there was no relationship between parasitism and spleen mass (R^2 = 0.003, n = 35, P = 0.7493) (Figure 15). The spleen mass ($\bar{x}$ = 0.9356 ± 0.071 g) is not significantly related to the number of *Cuterebra* larvae or warble scars present. Male spleen mass ($\bar{x}$ = 0.952 ± 0.120 g) was not significantly greater than the spleen mass of females ($\bar{x}$ = 0.929 ± 0.065 g). An ANOVA indicated that infestation intensity cannot be explained by spleen mass (F = 0.104).

Figure 15. There is no significant association between spleen mass and number of bots.



DISCUSSION

Undoubtedly, parasite intensity is affected by both intrinsic and extrinsic factors. Examining intrinsic factors is more important when investigating parasite intensity within a population because the individuals mostly experience the same extrinsic factors. I started this work with three hypotheses that could potentially explain what makes an individual squirrel more likely to be infested by bot fly larvae.

Testosterone Hypothesis

Because testosterone suppresses the immune system, individuals with high levels of testosterone should be less likely to resist parasites than individuals with low levels. My first prediction for the Testosterone Hypothesis was met. My results show that males have a higher prevalence of infestation as well as a higher average intensity of infestation than females (Table 2). These results agree with the "male bias" described by Schalk and Forbes (1997), which states that males are more often parasitized than females due to testosterone levels. However, my results do not agree with the results of Jacobson et al. (1981). In a state-wide study, Jacobson et al. found that prevalence (P) and intensity (I) of infestation were very similar for both male (n = 277, P = 18.6%, I = 1.8) and female (n = 224, P = 19.2%, I = 1.8) grey squirrels in Mississippi. My results showed a greater difference in prevalence and intensity between males (n = 10, P = 70%, I = 1.2) and females (n = 25, P = 32%, I = 0.4).

My second prediction for the Testosterone Hypothesis was not met. Testes size in mammals has been shown to be associated with testosterone levels (Dixson and Anderson, 2004), so it would be expected that individuals with larger testes would be more heavily parasitized. My results did not meet such expectations (Figure 4). Kirkpatrick and Hoffman (1960) described a three-stage cycle of testicular development in squirrels that include functional, quiescent, and redevelopment stages. With all three stages occurring throughout the year, there is no regular, annual pattern to the cycle (Pudney, 1976). Even though Pudney (1976) found that the mass of the testes in the functional stage is nearly twice as much as testes in the quiescent and redevelopment stages, Zielinski (1996) reported that development stage and testosterone level are not significantly correlated. Having fluctuating testes mass while maintaining constant testosterone levels, squirrels would not exhibit a correlation between testes mass and testosterone level, thus making it unlikely to predict parasite susceptibly based on testes size.

My third prediction for the Testosterone Hypothesis was not met. There was no significant relationship between 2D:4D ratio and parasitism intensity for both male and female samples (Figures 5-12). If a lower 2D:4D ratio is an indicator of increased exposure to prenatal testosterone (Manning and Taylor, 2001), which can predispose an individual to have a weakened immune system (Geschwind and Behan, 1982), squirrels with a lower 2D:4D ratio should be more heavily parasitized. Manning and Taylor (2001) showed that human males have a smaller 2D:4D ratio than females. Contrary to this finding, my results show that males had the higher ratio for three paws (Table 3). This difference could mean that the 2D:4D ratio is not an adequate indicator of prenatal testosterone exposure in squirrels.

Body Size Hypothesis

The prediction for the Body Size Hypothesis was not met. There was no relationship between host size and infestation intensity (Figures 13 and 14). Low variation in host body size and effects of ectoparasites on host growth rates may be two explanations for this prediction not being met. Of my sample set, 91.4% were between 269.4 and 276.0 cm^2. Except for three outliers, all the samples varied no more than 6.6 cm^2. Ectoparasites have also been shown to reduce the growth rates of their host, causing more heavily infested individuals to be smaller than those less parasitized. Yellow-bellied marmots (*Marmota flaviventris*) that had a higher intensity of fleas, lice, and/or mites were shown to have significantly lower growth rates than those less heavily parasitized (van Vuren, 1996). With regard to my study, this would suggest that there would an inverse relationship between infestation intensity and BSA. My results showed neither a direct nor inverse relationship between infestation intensity and BSA.

Host Condition Hypothesis

The prediction for the Host Condition Hypothesis was not met. Using spleen mass as an indication of immune system activity, no significant association was found between spleen mass and infestation intensity (Figure 15). Beldomenico and Begon (2009) discuss a "vicious circle" which describes how parasites can take advantage of a host's deteriorated condition, thus weakening the host's immune system even further. Because of this "vicious circle," I thought that squirrels with larger spleens would be more heavily parasitized because they may be in a weakened state. Even though my results did not show this association, male samples averaged a slightly higher spleen mass than females. By documenting an increase in circulating white blood cells when infestation intensity increases, Bennett (1955) demonstrated that there is a substantial immune response by squirrels to bot parasitism. Even though the difference in male and female spleen was not significant, the larger spleen in males could be caused by the spleen's role in filtering circulating white blood cells.

Conclusions

From my results, I have determined that the Testosterone, Body Size, and Host Condition Hypotheses should not be accepted. For the Testosterone Hypothesis, the data yielded a statistically significant result for one prediction. The significant results I obtained indicate that male eastern gray squirrels are more highly parasitized by tree squirrel bot flies than are females (Table 1). Because the other two predictions of the Testosterone Hypothesis did not yield significant results, it cannot be concluded that differing testosterone levels cause differences in parasite prevalence, thus the hypothesis must be rejected.

Even though differing testosterone levels do not seem to influence the susceptibility of eastern gray squirrels to tree squirrel bot flies, there may still be an explanation for why such results were obtained. One possible explanation for the increased susceptibility in males could be due to the increased distance traveled by males during the summer and fall months (Cordes and Barkalow, 1972). Traveling longer distances would increase the likelihood of a squirrel coming into contact with bot larvae. Contrary to this idea, however, Thompson (1977) has reported that females are substantially more active than males in the summer, when bot flies deposit their eggs.

Another explanation may be that one small sample was taken from a single population. With such a small sample (n = 35), determining whether that sample is an accurate depiction of the entire population is very difficult. Also, to make conclusions about a host parasite relationship after examining only one population, a researcher would have to assume that the studied population is a good representation of all other populations. In a study similar to mine, Jacobson et al. (1981) collected a large sample (n = 501) from many populations and determined that there was no significant difference in bot prevalence between male and female eastern gray squirrels.

Within a population that experiences the same weather patterns, resource availability, density, habitat, and other extrinsic factors, one would think that physical and physiological variation between individuals should affect parasite susceptibility. From my results, it cannot be concluded that intrinsic characteristics have a significant effect on parasite susceptibility. For my study to be more statistically sound and to further investigate host susceptibility within this population, this experiment would need to be repeated several times with more sophisticated forms of data collection. Instead of collecting specimens by shooting, trapping and tagging squirrels would allow the researcher to track the change in parasitism of an individual over time while not removing individuals from the population. Measuring testosterone concentration from blood samples would allow the Testosterone Hypothesis to be reexamined directly instead of being estimated. After such a study is conducted, more confident assertions on the effect of intrinsic characteristics on host susceptibility and the effect of parasites on a population can be made.

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