Does Selection by Sheet Webs Maintain Polymorphism in Midge (Diptera: Chironomidae) Body Size at the University of Michigan Biological Station?

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Abstract

Natural selection should favor the most fit form of a trait and result in the loss of all other forms, yet polymorphism, the existence of two or more forms of a trait in a population, is quite common. There are a few ways polymorphism can be maintained, including disruptive and directional selection. When two or more predators favor different forms of the same trait in a prey species, polymorphism can be maintained. It has been observed that orb webs preferentially capture midges with smaller body sizes at the University of Michigan Biological Station (UMBS) (Johnson et al. 2002). Despite this directional selection, body size among midges at UMBS remains polymorphic. We investigated the possibility that sheet webs, a different type of web, are preferentially capturing larger midges i.e. that sheet webs select against smaller midges. To test this hypothesis, we compared means and variances for thorax length, thorax width, and the ratio of thorax length to width for midges caught by sheet webs vs. uncaught (free) midges. We did this separately for two midge species, tentatively called A and B, in two sites on the UMBS campus: Blissville and Manville. We found that evidence of directional selection acting on Species B midges in Manville (length: p=0.005, width: p=0.005, ratio: p=0.28). However, like orb webs, sheet webs favored larger midges. We also found evidence of disruptive selection acting on Species B midges in both Blissville (length: p=0.020, width: p=0.008, ratio: p=0.007) and Manville (length: p=0.004, width: p=0.000016, ratio: p=0.48). We conclude that disruptive selection, but not directional selection, may explain the persistence of polymorphism in midge body size at UMBS, particularly if the total mortality caused by sheet webs exceeds that caused by orb webs. Alternatively, as yet unidentified factors may play a role in the maintenance of this polymorphism.
Introduction

Natural selection is a mechanism of microevolution that causes adaptive evolution by increasing the proportion of high fitness phenotypes. In order for natural selection to occur, phenotypic variation must be in part genetically based, and varying versions of the trait must differentially affect the fitness of the individuals in a population (Karlin & Lieberman 1974). Although natural selection is a very powerful driving force in eliminating less fit phenotypes, multiple forms of traits typically exist instead of fixed, or monomorphic, traits. The existence of different phenotypes of a trait within a single population is known as polymorphism. In fact, polymorphism is much more common in nature than monomorphism.

Polymorphism remains prevalent in all organisms despite the differential fitness the alternate morphs may bring the individuals. One of the most notable polymorphisms in vertebrates is body color (Galeotti et al. 2003). In the Gouldian Finch (Erythrura gouldiae), polymorphic head color traits (black and red-headed) correlated with polymorphic personality traits (Mettke-Hofmann 2012). Black-headed birds tended to be more confident when approaching novel objects while red-headed birds were more reluctant. The study suggested that red heads may make the birds more conspicuous to predators, but the more confident behavior shown in black birds may expose them to more dangerous situations. Consequently, conflicting selective pressures cause multiple morphs to remain in the finch population. Additionally, Vanhoenacker et al. (2010) suggested that polymorphism for scape (the leafless stem arising from the root) length in the perennial rosette herb (Primula farinose) exists due to opposite selective forces favoring the different lengths of scape morphs, thus not eliminating either morph. This widespread coexistence of natural selection and polymorphism exemplified by the previous examples suggests that natural selection plays a role in the maintenance of polymorphic traits.

Galeotti et al. (2003) suggested that polymorphism is maintained when different morphs of a trait each have some selective advantage and disadvantage that result in a “selective balance” in the population. Two forms of natural selection (disruptive and directional) are able to preserve polymorphic traits. Disruptive selection, when there is selection for two or more alternate morphs in a population, can retain polymorphic traits when two or more extreme morphs have a selective advantage over intermediate morphs. Feder et al. (2003) showed that two different forms of the trait, timing of diapause cessation in fruit flies (Rhagoletis pomonella),
are maintained in the population because each coincides with the phenology of a different host tree species. Because the fruit flies lay eggs in the fruit of the trees, they need to emerge from diapause when the fruit from their host species is ripe. Therefore, morphs that cease diapause when either tree species is ripening have higher fitness than the morphs with intermediate timing. In this way, disruptive selection causes the maintenance of polymorphism for diapause cessation in the fruit fly population. Directional selection maintains polymorphism when two or more forms of a trait are favored by different selective pressures. Rausher et al. (2000) studied color polymorphism in the common morning glory (\textit{Ipomoea purpurea}) and found that the heterozygous morph, the intermediate lightly pigmented color, was most prevalent in populations. The homozygous morphs increased in frequency when in low density, resulting in the subsequent increase in heterozygous offspring. This example of inverse frequency-dependent selection also occurred in a study by Galeotti et al. (2003). The study suggested that color polymorphism in hawks (\textit{Falconiformes}) exists due to this type of directional selection, in which the less common color morphs have higher fitness because they are less recognizable to the prey. However, when a morph becomes more common, its fitness decreases due to increased predator recognition.

Polymorphism can also be maintained in traits of prey species (such as body color) by selective pressures in the form of predation (Maan et al. 2008). Optimal Foraging Theory proposes that predators impose selective mortality on their prey in order to maximize the energy gain from their kill (Schumann et al. 2012). This suggests that predators likely choose their prey based on physical characteristics that identify more rewarding prey. For example, adult giant water-bugs (\textit{Lethocerus americanus}) and larval giant scavenger beetles (\textit{Hydrophilis triangularis}) preferentially ate larger “adult” mosquitofish (\textit{Gambusia affinis}) over the smaller “juvenile” mosquitofish, presumably to maximize their energy intake and minimize their energy output (Schumann et al. 2012).

Size-selective predation, when predators choose prey based primarily on their size class (Calow 1998), can have a major impact on the prey population. If a predator were to impose size-selective mortality on body size (assuming body size is partially genetically based), then individuals that were chosen as prey would clearly have lower fitness. Consequently, predators would impose natural selection for the favored body size, and the prey population would evolve to have primarily or, in the extreme case, exclusively individuals of the favored size.
Polymorphism of a trait in a population can be maintained when two or more versions of the trait are favored with roughly equal strength (Losey et al. 1997). As a result, neither version of the trait would be eliminated by natural selection. For instance, if one predator were to favor larger prey body size while another predator were to favor smaller body size in the same population, then theoretically neither phenotype would be “weeded out”. As a result, polymorphism would be maintained by means of predation. In contrast, if both predators imposed selection favoring the same body size, polymorphism for size should disappear from the prey population.

As major predators in the insect world, spiders and their webs -- a phenotypic extension of the spiders themselves -- have the ability to either eliminate or maintain polymorphism in traits of their prey species (Henaut et al. 2006). A previous study at the University of Michigan Biological Station (UMBS) revealed that orb webs (Figure 1), spiral-shaped vertical webs, appeared to disproportionately capture smaller sized midges (Diptera: Chironomidae) over larger sized midges (Johnson et al. 2002). This would result in a higher fitness for the larger midges, and natural selection should increase their phenotypic frequency in the population and, in the absence of an opposing selective force, eventually result in the elimination of small sized midges.

However, body size (which we assume to be a partially genetically determined trait) in midge populations at UMBS remains a polymorphic trait in spite of this directional selection. Therefore, there is likely another force that selects against larger sized midges. Both sheet webs (Figure 2), horizontally oriented pyramidal webs, and orb webs capture midges, but it has yet to be determined whether sheet webs preferentially capture midges of a particular size. Orb webs tend to capture stronger-flying insects compared to sheet webs, so it is possible that the two types of webs would impose different forms of selective
mortality on flying insects (Henaut et al. 2006). If polymorphism of body size is being maintained through predation, then there is a possibility that the sheet web-building *Linyphiid* spiders are exerting different size-selective pressures on the prey population than are orb-weaving spiders (Welch et al. 2009). If *Linyphiid* spiders impose selective mortality on large sized midges, then they would impose directional selection in opposition to that imposed by orb webs, and thereby contribute to the maintenance of polymorphism in midge body size at UMBS.

The goal of our study is to determine whether sheet webs impose selection favoring smaller midge body size, and therefore help to maintain polymorphism in midge body size. Accordingly, we first ask if sheet webs exert selective pressure on midge body size by capturing a non-random subset of midges at UMBS. If so, we will then ask if they impose a different type of directional selection than orb webs. We hypothesize that, unlike orb webs, sheet webs disproportionately capture larger midges.

**Materials and Methods**

To determine whether sheet webs and orb webs exert differing selective pressures on midge body size, we modeled our study after the Johnson et al. (2002) study done on orb webs by previous EEB 381 students, so that the results of the two studies can be compared.

*Study Site and Organisms*

We conducted our study from late May to early June 2013 at UMBS in Pellston, Michigan. Johnson et al. (2002) observed orb webs on cabins in two study sites on the UMBS campus. However, sheet webs generally do not occur on cabins, so we observed webs in nearby vegetated areas. Study Site 1 was “Blissville” which was comprised of two rows of cabins lining the shore of Douglas Lake. Study Site 2 called “Manville”, was uphill from Blissville and was also comprised of two rows of cabins.
We consistently observed two midge species, which we called “A” and “B”, at UMBS. We could not identify the specific species taxonomically, so instead we assumed that phenotype could be used as an indicator of midge species. An easily identifiable trait of midges is body color, so we identified Species A by its green abdomen (Figure 3) and Species B by its black abdomen (Figure 4).

**Web Marking and Measurement**

In our study, we focused on sheet webs present in Blissville and Manville. We surveyed our two study sites for sheet webs from May 22, 2013 to June 9, 2013, approximately three times a week and upon finding a sheet web, placed a numbered flag as a marker. We marked all sheet webs (n=50) we found, whether they initially had a midge in them or not. Over the next 17 days, we periodically marked an additional 50 webs, resulting in 100 webs in each site. We believe the 100 webs included in this study comprise a representative sample. For each web, we measured length and width (the longer side and the shorter side, respectively, in millimeters) of the base, the large platform of the web parallel to the ground. We also measured the elevation of the web, defined as the distance from the ground to the base of the web. The height was defined as the distance from the base of the web to the top of the pyramid. We used a thermocouple to measure the temperature in the web. In addition, we recorded the location of the web, what the web was attached to such as branches or leaves, and whether the web was occupied by a spider. Since many spiders hide while waiting for prey to arrive, it was difficult to establish the occupancy status of the web with certainty. We also measured the temperature within a 6-inch radius around
the web and recorded weather on the day of collection and the previous day.

Collecting Midges

We collected samples of captured midges approximately every three days from May 22, 2013 to June 9, 2013. During daylight hours, we used forceps to extract all captured midges, which we placed into a separate vial for each web. Occasionally, webs were broken while extracting a midge, but we assumed broken webs continued to capture midges of the same body size as intact webs. Under a dissecting scope, we measured thorax length and width of each captured midge, to the nearest 0.01 millimeter. Midges that were damaged during extraction and could not be measured were excluded from our analyses. This could have introduced bias into our data if the distribution of body size of the damaged midges was not representative of the sample size as a whole. However, we are confident that these midges did not have a significantly different body size than the non-damaged sample. Therefore, the exclusion of these midges in our measurements did not cause us to only measure differentially smaller, larger, or more medium sized midges than was representative of the UMBS population.

In order to determine the body sizes of the whole midge population present at UMBS, we used a UV light bug trap to catch “free” midges. The trap includes a bucket and a light on top to attract the midges. When they enter, evaporated ethanol asphyxiates the midges. We placed one trap in each of our two sites at night approximately every three days from May 27, 2013 to June 9, 2013. We assumed that midges caught in the UV traps were representative of the UMBS population as a whole. In addition to the UV traps, we collected free samples during daylight hours using aspirators. An aspirator is a vial with a special stopper that includes two tubes and is used to collect free flying midges with suction. This was used sparingly on occasions when the UV traps did not perform well.

Data Analysis

To determine if sheet webs exert selective pressure on midge body size, we compared average thorax length, thorax width, and the thorax ratio (defined as length divided by width) of the captured midges to that of free midges. For each species, we first compared body size between captured midges in Blissville vs. Manville to see if there was a significant difference between captured midges at the two sites; we then did the same for free midges. When we saw
no difference between the two sites, we pooled the data. To determine whether directional selection is occurring, the mean of the caught sample was compared to the mean of the free sample by a t-test or Mann-Whitney U test, depending on the normality of the data. We ran the tests separately for each species.

It is also possible that sheet webs impose stabilizing or disruptive selection, rather than directional selection, on midge body size. If stabilizing selection is acting on midge body size, variation will be lower among free midges than among caught midges, because selection is favoring an intermediate size in the live midges and therefore selecting against small and large sizes midges. The caught sample (the dead midges) shows the forms of the trait that are being selected against. Alternatively, if disruptive selection is acting on midge body size, there will be higher variation among free midges than among caught midges, because selection is favoring small and large midges and selecting against intermediate sizes. To determine if either stabilizing or disruptive selection is acting on midge body size, we performed F-tests to compare variances of caught vs. free midges for each species at each site.

**Results**

*Differences between Sites for Species A*

From Species A (the green midge), we collected a total of 121 free midges (30 in Blissville and 91 in Manville) and a total of 33 caught midges (12 in Blissville and 21 in Manville).

We first determined if the thorax length, width, and ratio were the same between Blissville and Manville for each species. To compare the body sizes, we checked our data for normality and found that some data were normal while others were not normal. For our normal data, we ran the t-test and for our non-normal data, we used the Mann-Whitney U test. Using the Mann-Whitney U test, we found that thorax length of caught midges of Species A was not significantly different between Blissville and Manville (0.846±0.163 versus 0.931±0.272, Mann-Whitney U=145.0, p=0.49). In contrast, for free midges, thorax length was significantly different between the two sites (0.799±0.208 versus 0.875±0.195, Mann-Whitney U=1,713.0, p=0.036). Thus, we could not pool the data.
Thorax width was not significantly different for caught midges between Blissville and Manville (0.467±0.142 versus 0.507±0.143, Mann-Whitney U=159.5, p=0.21). However, for free midges, thorax width was significantly different between the two sites (0.430±0.147 versus 0.490±0.100, Mann-Whitney U=1,913.0, p=0.001), so the data could not be pooled.

The thorax ratio was not significantly different between Blissville and Manville for caught midges (1.874±0.283 versus 1.840±0.192, Mann-Whitney U=98.5, p=0.30). However, for free midges, the thorax ratio was significantly different between the two sites and thus, the data could not be pooled (1.910±0.319 versus 1.808±0.329, Mann-Whitney U=1,019.0, p=0.038).

Differences between Sites for Species B

For Species B, we collected a total of 90 free midges and a total of 88 caught midges. Specifically, we collected 39 free midges and 24 caught midges in Blissville, whereas in Manville, we collected 51 free midges and 64 caught midges. We asked whether or not the thorax length was significantly different for caught midges in Blissville and Manville, but since thorax length was not significantly different in both sites, the data could be pooled (0.792±0.242 versus 0.716±0.185, t=1.572, p=0.12). The results were similar for free samples in that the thorax length was not significantly different so again, the data could be pooled (0.937±0.371 versus 1.018±0.290, t=-1.165, p=0.25).

Next, we looked at thorax width as a measure of body size between Blissville and Manville. The thorax width was not found to be significantly different between Blissville and Manville for all caught midges (0.460±0.130 versus 0.430±0.118, t=1.057, p=0.29). In contrast, thorax width was significantly different between Blissville and Manville for all free samples (1.000±0.543 versus 0.637±0.207, Mann-Whitney U=1,237.5, p=0.047). Thus, the data could not be pooled for thorax width.

Finally, we compared the thorax ratio between Blissville and Manville. We found that the thorax ratio was not significantly different between the two sites, so the data could be pooled (1.732±0.245 versus 1.700±0.328, Mann-Whitney U=690.5, p=0.47). The results were similar for our free samples in that the thorax ratio was not significantly different (1.759±0.402 versus 1.635±0.326, Mann-Whitney U=805.5, p=0.12).

Differences between Free and Caught Midges of Species A (green midges)
In Blissville, there was no significant difference in the mean thorax length between free and caught midges (0.799±0.208 versus 0.846±0.163, Mann-Whitney U=152.5, p=0.45; Figure 5). When we compared the variances of thorax length between free and caught midges, we also found no significant difference in variance of thorax length between free and caught midges (free=0.043, caught=0.027, F=1.593, p=0.21; Figure 5). Thus, our results suggest there is no directional or disruptive selection acting on thorax length in Blissville. In Manville, we found no significant difference in mean thorax length between free and caught midges (0.875±0.195 versus 0.931±0.272, Mann-Whitney U=899.5, p=0.68; Figure 6). However, variance of thorax length was significantly greater for caught than free midges (free=0.038, caught=0.074, F=1.947, p=0.02; Figure 6), suggesting that stabilizing selection may be occurring. To summarize, we found that there is no selection acting on thorax length in Blissville, but our results suggest that stabilizing selection is acting on thorax length in Manville (Figure 6).

In Blissville, we found no significant difference in mean thorax width between free and caught midges (0.430±0.147 versus 0.467±0.142, Mann-Whitney U=156.500, p=0.52). The variance of thorax length among midges found in Blissville also had no significant difference (free=0.022, caught=0.020, F=1.100, p=0.46), suggesting that no selection is acting on thorax width. For the midges in Manville, we found no significant difference in the mean thorax width (0.490±0.100 versus 0.507±0.143, Mann-Whitney U=932, p=0.86). However, we saw a significant difference in the variance of thorax length between free and caught midges (free=0.010, caught=0.020, F=2.000, p=0.01). The caught sample had a higher variance meaning stabilizing selection is likely acting on thorax width for midges in Manville. In summary, our analyses suggest that there is no selection acting on thorax width for midges in Blissville (Figure 7), but in Manville, we found that stabilizing selection may be acting on the thorax width (Figure 8).

We also compared the thorax ratio between free and caught midges for each site. In Blissville, we found that the mean thorax ratio of free midges did not significantly differ from the mean thorax ratio of caught midges (1.910±0.319 versus 1.874±0.283, Mann-Whitney U=192.0, p=0.75). When we compared the variation in thorax ratio among free and caught midges in Blissville, we found that the variation in thorax ratio between midges did not significantly differ (free=0.102, caught=0.080, F=1.275, p=0.35). Neither directional nor disruptive selection appears to be acting on thorax width in Blissville. In contrast, we found no
significant difference in the mean thorax ratio between free and caught midges in Manville (1.808±0.329 versus 1.840±0.192, Mann-Whitney U=861.5, p=0.48). However, we did find that free and caught midges in Manville showed a significant difference in the variation of thorax ratio (free=0.108, caught=0.037, F=2.919, p=0.004). The variance was higher in the free sample meaning disruptive selection is likely occurring. Based on our statistical analyses, we saw no directional or disruptive selection in Species A midges in Blissville (Figure 9), but disruptive selection in Manville (Figure 10).

**Differences between Free and Caught Midges of Species B**

We computed the same types of statistical analyses for Species B, black midges, in Blissville and Manville. We found that among all free verses caught midges from Blissville, the mean thorax length did not significantly differ (0.937±0.371 versus 0.792±0.242, t=1.708, p=0.09). Therefore, directional selection is not likely to be occurring. However, the p-value was under 0.10 so the sample size should be increased to confirm the results of this test. To determine if disruptive or stabilizing selection is acting on the measures of body size we looked at variance. First we compared the variance of thorax length between free and caught midges in Blissville and found a significant difference (free=0.137, caught=0.059, F=2.322, p=0.02). The free sample had a higher variance meaning disruptive selection is likely to be acting on thorax length. In contrast, we found a significant difference in the mean thorax length between free and caught midges in Manville. Our data suggests that directional selection is acting on thorax length among the midges (1.018±0.290 versus 0.716±0.185, t=6.471, p=0.0005), preferentially capturing smaller midges. We also found a significant difference in the variance of thorax length between free and caught midges in Manville (free=0.084, caught=0.034, F=2.471, p=0.0004). Because the free sample had a larger variance, it is likely that disruptive selection is acting on thorax length. To summarize, we saw disruptive selection in Species B in Blissville (Figure 11) and evidence of both directional and disruptive selection in Manville acting on thorax length (Figure 12).

We also looked at the differences in the mean thorax width between free and caught midges in each site. We found that in Blissville, the mean thorax width did not differ significantly between free and caught midges (0.143±2.12 versus 0.460±0.130, t=1.916, p=0.06), but we found a significant difference in the variance of thorax width (free=0.045, caught=0.017, F=2.647, p=0.008). The free sample had a higher variance meaning disruptive selection is likely
occurring. This is in contrast to the midges from Blissville, because we found a significant difference in the mean thorax width between free and caught midges (0.637±0.207 versus 0.430±0.118, Mann-Whitney U=2,728.0, p=0.0005) and also a significant difference in the variance of thorax width in Manville (free=0.043, caught=0.014, F=3.071, p=0.00002) where the free sample had a higher variance. Our data suggests that disruptive selection is acting on the midges. Overall, we saw disruptive selection in Blissville (Figure 13) and both disruptive and directional selection in Manville acting on thorax width in Species B (Figure 14).

We also ran the statistical analysis on the thorax ratio. We found that in Blissville, the thorax ratio did not differ significantly between free and caught midges (1.759±0.402 versus 1.732±0.245, t=0.333, p=0.74). Then, we looked at the variance in the thorax ratio of free and caught midges in Blissville and found a significant difference in the two (free=0.162, caught=0.060, F=2.70, p=0.01). The free sample had a higher variance suggesting disruptive selection. On the other hand, the midges in Manville showed no difference in the mean thorax ratio between free and caught midges (1.635±0.326 versus 1.700±0.328, Mann-Whitney U=1,440.0, p=0.28). There was also no significant difference in the variation of the thorax ratio between free and caught midges from Manville (free=0.106, caught=0.108, F=1.019, p=0.48). Therefore, thorax ratio is not experiencing selection. All in all, Species B midges in Blissville are experiencing disruptive selection (Figure 15) while those in Manville are experiencing no selection (Figure 16).

**Differences in Free and Caught Species B Midges at UMBS**

As stated earlier, our statistical analyses suggest that the data from Blissville and Manville for Species B midges could be pooled in regards to thorax length and the thorax ratio. In order to determine if sheet webs are exerting selective pressures on Species B midges in all of UMBS, we compared body sizes, specifically thorax length, thorax width, and the thorax ratio of free and caught midges. We found that there was a significant difference in the mean thorax length between free and caught midges (0.983±0.328 versus 0.736±0.204, t=6.042, p=0.0005). This suggests directional selection on thorax length is favoring larger midges. We also found a significant difference in the variance of thorax length between free and caught midges (free=0.107, caught=0.042, F=2.548, p=0.000009). Since the free sample had a higher variance, it is possible that disruptive selection is occurring. Interestingly, we found no significant
difference in the mean thorax ratio between free and caught midges (1.670±0.364 versus 1.708±0.307, Mann-Whitney U=3,712.5, p=0.47) and also found no significant difference in the variation of the thorax ratio (free=0.137, caught=0.094, F=1.404, p=0.06). Therefore, no selection is acting on thorax ratio in Species B. In summary, our data suggests that Species B midges are experiencing either directional or disruptive selection on thorax length but no selection on thorax ratio.

Discussion

In our study, we compared body size between free and caught midge species in Blissville and Manville in order to see if there were any selective pressures that cause polymorphism of body size to be maintained. Our data suggests that among Species A in Blissville, no selection is occurring in any body measurement due to predation by sheet webs. However in Manville, sheet webs appeared to be exhibiting differential predation on the midges. Both thorax length and width of Species A are likely under stabilizing selection while thorax ratio is undergoing disruptive selection.

In Species B, our data suggests that much of the midge body size is experiencing selective pressure from sheet webs. In Blissville, it is possible that thorax length, width, and ratio are undergoing disruptive selection. In Manville, the sheet webs preferentially caught smaller Species B midges, i.e. those with lower thorax length and width. This suggests that thorax length and width are experiencing directional selection favoring larger body size. Our data also suggest that there are no selective pressures from sheet webs acting on the thorax ratio of Species B midges in Manville.

When we pooled the data for length and ratio of Species B, we could analyze the selective forces acting on the UMBS population of Species B midges as a whole. Our results suggest that sheet webs are exerting disruptive selection on thorax length, but no selective pressure on the ratio.

Two opposing forces of directional selection on a trait can cause multiple forms of a trait to remain in a population (Galeotti et al. 2003). Since Johnson et al. (2002) found that orb webs exerted directional selection favoring larger midges, and since midge body size clearly is a polymorphic trait at UMBS, we expected that sheet webs impose directional selection favoring
smaller midges. Thus, neither extreme would be eliminated and polymorphism would be maintained. However, our results suggested that directional selection by sheet webs on thorax length and width of Species B in Manville favored large midges, as did selection by orb webs. Therefore, polymorphism in midge body size cannot be explained by opposite directional forces from different spider predators.

Although stabilizing selection does not typically maintain polymorphism, we still analyzed our data for its presence in UMBS midges. We found that stabilizing selection was present in the thorax length and width of Species A in Manville. This again does not contribute to the maintenance of polymorphism in midges. Additionally, no selective forces were found to be acting on any body measurement of Species A in Blissville, nor on the thorax ratio of Species B in Manville and the ratio in the pooled Species B sample.

Disruptive selection can inherently maintain polymorphism in the traits upon which it acts (e.g. Feder et al. 2003). Our results suggest the existence of disruptive selection acting on thorax ratio of Species A in Manville, length, width, and ratio of Species B in Blissville, and length in the pooled Species B sample. It is possible that the disruptive selection we found could overpower the directional selection imposed on midges by the orb webs. If this were true, disruptive selection imposed by sheet webs could be the primary force in maintaining polymorphism in midge body size. However, increased variance and a similar means in the free sample compared to the caught sample alone do not necessarily confirm the existence of disruptive selection. After visually comparing the distribution of body measurements between caught versus free midges in those that appear to be undergoing disruptive selection, we determined that the bimodal distributions were distinct enough in the free samples to confirm the existence of disruptive selection. Furthermore, when collecting our samples, we noticed that there were more large Species B midges in our free samples compared to our caught samples. This observation also affirms the presence of disruptive selection in many of the Species B body sizes. If these disruptive forces are the driving forces in maintaining polymorphism in midge body size, then there must be a reason why the directional selection imposed by orb webs is weak in comparison. Casual observation suggests that, although orb webs appear to catch more prey per web than sheet webs, sheet webs are substantially more common at UMBS. Thus, sheet webs may have a greater effect on the midge population than do orb webs and also have the possibility to impose a strong enough disruptive force to maintain polymorphism in midges.
During this study, it was necessary to make certain assumptions. We assumed that the webs’ abilities to catch midges remained the same whether the webs were abandoned or occupied. Additionally, we assumed the webs’ abilities to catch midges did not change if the webs were broken while extracting midges. Not only did we assume that the webs were still able to catch midges if damaged or abandoned, but also that they continued to catch the same distributions of body sizes as they did when undamaged and occupied. It is unclear whether this assumption is justified because, according to Nentwig (2013), spiders tend to consume prey whose length is 50-80% of their own body size. Therefore, the presence of a spider in a web would likely result in unconsumed, smaller prey left in the web while larger prey would be absent. This would influence the distribution of body sizes left in the webs, thus skewing the distribution of body sizes for caught midges.

In comparing the effects of sheet webs and orb webs on midge body size, we assumed the environmental conditions at UMBS had not changed in the past 10 years. However, this assumption may not be justified because the forests around UMBS are still undergoing succession. This would influence the types of fauna present in our study sites, affecting spiders and their web-making. Additionally, it was an abnormally cold spring this year causing a difference in composition of spider and midge populations as well as the availability of resources compared to the environmental conditions 10 years ago. The differing availability of resources affects the types of niches present at UMBS. According to Skulason (1996), when there are open niches, species adapt to fill them. Therefore, it is possible that a different better-adapted portion of the spider population is now present and may impose different selective pressures on midges than in 2002.

Furthermore, we assumed that midge mortality caused by sheet webs was high enough to be able to affect the frequency of traits in midges need to explain a bit more. Thus, we suggest a future study investigating the impact that sheet webs have on the mortality of midges to determine if our results are relevant as selective pressures on the midge population.

Additionally, in order to compare selective pressures on midges by orb webs and sheet webs, we assumed that both webs capture midges from the same population. This could be unjustified, however, due to the suggestion of Rypstra (1983) that orb web-weaving spiders are able to produce new orb webs more frequently to correlate with increased density of prey than are sheet web-weavers. That is, if a large population of midges arrives in parts of UMBS, the orb
web-weaving spiders may move in and start capturing more of the new midges before the sheet web-weaving spiders are able to leave their old webs and arrive. However, when they do arrive, they may not be able to feed in the same place due to competition for substrate space with the early arriving orb-weaving spiders. Therefore, the two types of spiders may not be feeding on the same midge population, so the selective forces they impose may not be affecting the same population.

Given that we could not determine with confidence if sheet webs are the driving force in maintaining polymorphism in midge body size, we must consider a number of other explanations. Polymorphism could be maintained in spite of the directional selection imposed by orb webs if another force besides spiders exerts directional selection in the opposite way; there could be another advantage to being small that could balance the selective pressure imposed by orb webs. McLachlan et al. (2003) found that predatory male dance flies (Diptera: Empididae) exert a large selective pressure on swarming midge populations, their prey. Their study found that both the dance fly and the midge were extremely good at flying, both in speed and aerobatics. It was found that both predator and prey were equal in speed, but the midge had a distinct advantage in aerobatic flight. The larger the midge was, though the less aerobatic it could be, therefore making it easier for the dance fly to catch it. This gives smaller midges a higher fitness and could be a reason why there is still polymorphism in body size of midges.

In addition, there might be gene flow from another population that continues to contribute variation to the body size trait in the UMBS population. Also, if natural selection were favoring one morph over another, causing directional selection to occur, we may only be seeing a snapshot of a long-term process. In this way, we may not be able to see a significant difference in distributions at this time. Finally, we may have been mistaken in thinking that body size is a primarily genetically-based trait. Anecdotal evidence suggests that body size may be determined instead mainly on environmental conditions during the growth stage (larval stage) of the midge.

Our study could be improved in a few ways, the first of which being the addition of replicate experiments. Our relatively small sample sizes, especially for specific species in specific habitats (i.e. Species A caught in Manville) could confound or add noise to our results, making us see or overlook selective forces that were or were not actually there. Additionally, in order to see which forms of selection could also play a role in maintaining polymorphism of body size, we suggest examining and collecting data on other possible forces of selective
mortality that are acting on midge body size. If any forces are found that oppose those exerted by sheet webs and orb webs, then they could contribute to the maintenance of polymorphism.

Lastly, our study could have been improved with more stringent restrictions on species identification of the midges we measured. We suspect that some of the individuals we included in our species may have been from other midge species that are differentiated by size, rather than abdomen color. If this proves true, then the exceptionally large midges in our sample size may be skewing evidence for selection.

In conclusion, our data did not support our hypothesis that polymorphism is maintained by opposing directional selective pressures favoring both extremes of body size. However, we did find evidence that sheet webs, through disruptive selection, could be maintaining polymorphism by overpowering the directional selection imposed by orb webs. It is unlikely though, that this is the sole force causing polymorphism. It is more likely that many selective pressures are acting together and therefore, we suggest that future studies look into the aforementioned pressures. All in all, polymorphism in midge body size remains a partial mystery and we suggest future studies on additional forces causing its maintenance.
Acknowledgements

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Appendix

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>Free/Caught</th>
<th>n</th>
<th>Mean Thorax Length (mm)</th>
<th>Mean Thorax Width (mm)</th>
<th>Mean Thorax Ratio (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blissville</td>
<td>Free</td>
<td>30</td>
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<td>0.430</td>
<td>1.910</td>
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<td>0.467</td>
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<td>Free</td>
<td>91</td>
<td>0.875</td>
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<td>1.840</td>
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<td>Caught</td>
<td>64</td>
<td>0.716</td>
<td>0.430</td>
<td>1.699</td>
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Table 1. Descriptive Statistics of Species A and Species B.

<table>
<thead>
<tr>
<th>Species</th>
<th>Body Size Measurements</th>
<th>Blissville Free</th>
<th>Blissville Caught</th>
<th>Manville Free</th>
<th>Manville Caught</th>
<th>p-value</th>
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<tbody>
<tr>
<td>A</td>
<td>Thorax Length (mm)</td>
<td>0.799±0.208</td>
<td>0.846±0.163</td>
<td>0.449</td>
<td>0.875±0.195</td>
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<td>Thorax Width (mm)</td>
<td>0.430±0.147</td>
<td>0.467±0.142</td>
<td>0.518</td>
<td>0.490±0.100</td>
<td>0.507±0.143</td>
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<tr>
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<td>Thorax Ratio (mm)</td>
<td>1.910±0.319</td>
<td>1.874±0.283</td>
<td>0.752</td>
<td>1.808±0.329</td>
<td>1.840±0.192</td>
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<tr>
<td>B</td>
<td>Thorax Length (mm)</td>
<td>0.937±0.371</td>
<td>0.792±0.242</td>
<td>0.093</td>
<td>1.018±0.290</td>
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<td>0.143±2.12</td>
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¹indicates significance of difference between free and caught within site

Table 2. Summary of Statistical Analyses: Comparison of Means
indicates significance of difference between free and caught within site

Table 3. Summary of Statistical Analyses: Comparison of Variances

<table>
<thead>
<tr>
<th>Body Size Measurements</th>
<th>Free</th>
<th>Caught</th>
<th>p-value</th>
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<td>n</td>
<td>mean</td>
<td>n</td>
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<tr>
<td>Thorax Length (mm)</td>
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<td>0.983±0.328</td>
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</tr>
<tr>
<td>Thorax Ratio (mm)</td>
<td>90</td>
<td>1.689±0.364</td>
<td>88</td>
</tr>
</tbody>
</table>

¹indicates significance of difference between free and caught within site

Table 4. Summary of Statistical Analyses: Comparison of Means between Free and Caught Species B at UMBS
We found no difference in the mean (p = 0.45) or variance (p = 0.21) of thorax length between free and caught midges meaning no selection is occurring.

We found no significant difference in mean thorax length between free and caught midges (p = 0.68) but a significant difference in the variance of thorax length (p = 0.02), thus stabilizing selection is occurring.
Figure 8. Comparison of the Distributions of Thorax Width of Species A in Blissville
We found no significant difference in the mean (p = 0.52) or variance (p = 1.1) of thorax width between free and caught midges, suggesting that no selection is occurring.

Figure 9. Comparison of the Distributions of Thorax Width of Species A in Manville
We found no significant difference in the mean thorax width between free and caught midges (p = 0.86) but a significant difference in the variance of thorax width (p = 0.01) that suggests stabilizing selection is occurring.
We found no significant difference in the mean (p= 0.75) or variance (p= 0.35) of thorax ratio between free and caught midges, suggesting there is no selection occurring.

We found no significant difference in the mean thorax ratio (p= 0.48) but a significant difference in the variance of thorax ratio (p= 0.004) between free and caught midges, suggesting there is disruptive selection occurring.
Figure 12. Comparison of the Distributions of Thorax Length of Species B in Blissville
We found no significant difference in the mean thorax length between free and caught midges (p= 0.09) but a significant difference in the variance of thorax length (p= 0.02) thus, disruptive selection is occurring.

Figure 13. Comparison of the Distributions of Thorax Length of Species B in Manville
We found a significant difference in the mean thorax length between free and caught midges (p= 0.0005), suggesting directional selection against small midges. We also found a significant difference in the variance of thorax length between free and caught midges (p= 0.0004), suggesting disruptive selection.
Figure 14. Comparison of the Distributions of Thorax Width of Species B in Blissville
We found no significant difference in the mean thorax width between free and caught midges (p=0.06) but a significant difference in the variance of thorax width (p=0.008) that suggests disruptive selection is occurring.

Figure 15. Comparison of the Distributions of Thorax Width of Species B in Manville
We found a significant difference in the mean thorax width between free and caught midges (p=0.0005) and a significant difference in the variance of thorax width (p=0.00002) suggesting there is evidence for both directional and disruptive selection.
We found no significant difference in the mean thorax ratio between free and caught midges ($p = 0.74$) but a significant difference in the variance of the thorax ratio ($p = 0.01$) suggesting that disruptive selection is occurring.

We found no significant difference in the mean thorax ratio (p= 0.28) or variance of the thorax ratio (p= 0.48) between free and caught midges suggesting that no selection is occurring.