ABSTRACT

Seasonal Variation in the Shape of *Hetaerina Americana*

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The ability to fly strongly contributes to the success of insects. The adaptive nature of wing size and shape dictates much of the organism's success flying, both short-term (food and mate acquisition) and long-term (persistence of the species in the environment). Members of Order Odonata (dragonflies and damselflies) are among the most efficient and iconic fliers in the animal kingdom, and wing shape is among many factors contributing to their flight success. The quantitative science of morphometrics is the study and analysis of shape. My study takes a morphometric approach to investigate variation in wing shape for *Hetaerina americana*, a common species of damselfly in North America. Specifically, I focus on variation in wing shape between damselflies emerging in mid-spring after a winter-long larval development versus those emerging in late summer after a warmer, summer-long larval development. Analyses revealed that for both fore wings and hind wings winter developer wing shapes differ significantly from summer developer wing shapes. Fore wings vary in shape more distinctly by season than do hind wings. Summer developer fore wings are broader than those of winter developers, and summer developer hind wings are narrower. This variation in wing

shape may be a consequence of seasonal circumstances (shorter, warmer development with a higher larval metabolic rate), or reveal an adaptive strategy for flight in air of varying temperatures. This latter strategy would indicate a genetic plasticity capable of producing wing shapes adaptive to seasonal variation.

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SEASONAL VARIATION IN SHAPE OF *HETAERINA AMERICANA*

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

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CHAPTER ONE

Introduction

Most Texas species of damselflies emerge as temperatures warm in April. Adult emergence stops in November. Previous research has found that damselflies that emerge after egg and larval development over the winter months have more mass than those that develop more quickly over the summer (Unpublished Research, Vodopich, 2011). In light of this observation, could other morphological traits vary with development season? Adult, male *Hetaerina americana* were the subject of this study. My project aimed to determine if the shape of the wings also differed between the winter developers and the summer developers. Additionally, if shape varies with season of emergence, I wanted to determine in exactly what specific dimensions the shape varied between the two groups. Variation in shape may be interpreted in the contexts of developmental times and temperature. Ultimately seasonal variation in shape impacts the behavior of adult damselflies.

Questions:

What is the nature of variation among damselfly wing shapes?

Are fore wings shaped differently than hind wings for a pooled collection of all specimens?

Are fore wings shaped differently than hind wings among winter developers?

Are fore wings shaped differently than hind wings among summer developers?

Does fore wing shape vary between winter developers and summer developers? If so, in what dimensions?

Does hind wing shape vary between winter developers and summer developers? If so, in what dimensions?

CHAPTER TWO

Background

Damselflies

Damselflies, closely related to dragonflies, are in the order Odonata, which refers to “teeth on the mandible” (Beaton, 2). Their larval forms have a unique appendage that function as a mandible for efficient feeding. However, only the largest adult dragonflies and damselflies can bite with enough force for a person to feel it. Despite what some people may think odonates have no stingers and therefore cannot sting (Beaton, 2). They are quite harmless.

Odonates are divided into two suborders, Zygoptera for damselflies and Anisoptera for dragonflies. Zygoptera translates roughly as equal wings because, unlike dragonflies, damselfly hind wings assume the similar shape and size as their fore wings (Beaton, 3).

All odonate species have a similar structure and similar body parts (Beaton, 3). However, damselflies are smaller and less robust than dragonflies. Casual observers commonly mistake damselflies to be juvenile dragonflies. Furthermore, dragonflies keep their wings perpendicular to their bodies and flat while at rest. Conversely, most species of damselflies rest with their wings together and above their abdomen (Beaton, 7-8). Moreover, dragonflies have larger heads in relation to their bodies than damselflies. Damselfly eyes are very separated, compared to dragonflies with some species’ eyes touching dorsally (Beaton, 8).

Adult damselflies spend most of their time around vegetation and typically fly slower than dragonflies. Foraging, dragonflies fly quickly and capture their prey in flight; damselflies take their prey on vegetation from a hover and not a speedy flight (Beaton, 8).

Anatomy

Damselfly compound eyes allow for acute vision to better see and identify prey, potential challengers, and potential mates. Furthermore, these compound eyes provide sharp vision for up to 12 meters (Beaton, 3). Similarly, odonate eyes are sensitive to polarized light, i.e. light waves with vibrations in a single plane. This theoretically promotes identification of potential mates and prey (Beaton, 3). A colored spot of the head of damselflies behind their eyes aids in species identification. Other adult insects such as antlions and owlflies appear similar to odonates; however, these species have long antennae and odonates only have diminutive antennae (Beaton, 4). At the base of the head is a neck-like structure called the prothorax that connects the thorax and the head. The large thorax contains wing muscles for flight. These muscles constitute about 40 percent of the weight of odonates (Beaton, 4). Damselflies have six legs. The first pair connects to the prothorax and function to clean the head while perched. The other four legs join the underside of the thorax, and two pairs of wings join the top of the thorax. Commonly odonates perch with less than all of the legs for faster takeoffs (Beaton, 4). A long thin portion of the body known as the abdomen extends behind the thorax. The abdomen has ten sections; the first two are larger than the rest. They are commonly mistaken to be a part of the thorax. Terminal appendages called claspers in males extend from the tenth segment (Beaton, 4). These separate into the cerci above and the epiproct below. Claspers hold the females during mating and vary in shape depending on the species. In fact, these differentiate between similar species (Beaton, 5). Females have undeveloped cerci. Accessory genitalia on male odonates occur on the underside of the second segment but are less obvious than in dragonflies. Female odonates also have either ovipositors or subgenital plates under the ninth segment of the abdomen (Beaton, 6). These often distinguish species. However, similar species of damselflies females often cannot be separated based on the appearance of their appendages to the naked eye. They need to be examined under a microscope (Beaton, 6).

Life Cycle

Egg

Female damselflies commonly lay their eggs into water. Eggs typically hatch in three to four weeks (Beaton, 9). Damselflies, with one generation per year, commonly lay their eggs toward the end of the summer and the eggs are partially dormant during winter, development proceeds during late winter and early spring. Adults emerge during late spring and early summer (Beaton, 9).

Larva

Hatched, the damselflies are known as larvae, nymphs, or naiads. Larval development is underwater. Larval development ranges from one month to several years depending on the species (Beaton, 10). Larvae will molt and shed exoskeletons multiple times until its development underwater is completed (Beaton, 10). Dragonfly larvae have gills inside the rectum; they bring water over the gills by enlarging and deflating the abdomen (Beaton, 11). The age of the larvae can be determined by the size of the wing buds on their backs. Before the larvae leave the water, the wing buds will become swollen and longer than after they had just hatched (Beaton, 11). The larvae have an elongated lower lip that has two hooks at the tip. This helps them quickly ensnare prey. The larvae are excellent hunters because they are able to jut out their jaw quickly to trap prey (Beaton, 10). Additionally, the hunting style of larvae is determined by looking at the shape of the larva. Larvae that have long legs with a wide and flat body wait on the bottom, hiding in the debris, and ambush once prey comes near (Beaton, 11). Those with short, strong legs burrow and wait for prey to pass. Most damselflies are long and thin, which allows them to hide in vegetation underwater (Beaton, 10). Larval odonates are skilled predators in hunting organisms that are smaller than the larvae. Larvae have gills to breathe while they are underwater. The location of the gills easily distinguishes larval dragonfly and damselfly larvae apart. Damselflies have external gills extending from the rear of the larva and form three blades (Beaton, 11). These can be used as fins to move in a weak lateral movement. This motion allows them to move quickly away from predators when they eject water quickly. Just before the larvae come out of the water they stop eating because the mouth structures that are seen in the adult form are beginning to develop (Beaton, 11).

Emergence

The emergence of many species occurs throughout the summer (Beaton, 12). For most odonate species, emergence begins when the larvae climb vertically out of the water. This can be up a shoot of a plant, the trunk of a tree, or even a man-made structure. Unlike dragonflies, damselflies typically make this climb during the day (Beaton, 11). Within the hour that follows the crawl out of the water, the exoskeleton splits down the back and the head and thorax of the emerging adult begin to push through. The head and the thorax to harden and push fully through the old skin (Beaton, 12). With the head and the thorax free of the exoskeleton the odonate then pulls the abdomen out. The wings and abdomen soon expand as air is swallowed to inflate the appendages of the new adult form (Beaton, 12). This new adult form can be identified by a lack of coloration and shiny wings. Emergence is the most dangerous time for odonates because they are essentially defenseless. The odonate is weak, its body is still delicate, and it is unable to use its wings very well (Beaton, 13). Moreover, if the new adult happens to fall into the water, from either a gust of wind or a splash of water, it would be unable to survive. However, once the new adult is able to make its first flight from the stalk it emerged on to the safety that vegetation provides, it will be able to sufficiently harden its body. Once this has occurred, the odonate can then fly and feed as the adults of the species normally would (Beaton, 13).

Maturation

Maturation of the adult follows emergence. Here odonates spend most of their time away from their breeding habitat and spend their time eating and maturing (Beaton, 14). Most damselflies hunt their prey by flying close to vegetation and snatching the prey. Other odonates hunt and catch their prey while they are still in flight (Beaton, 14). Some odonates are even known to spend most of their time in flight and catch their prey along their path. Others carry their food back to a perch to consume it (Beaton, 14). Prey can be caught either in the mouth of the odonate or larger prey can be caught in the odonate's legs as they form a kind of funnel apparatus, aided by the spines on the legs. If the prey is caught in this funnel, it is then brought to the mouth. The head of the prey is usually consumed first (Beaton, 14). Anything that the odonates can catch will be considered prey; this includes other odonates, mosquitoes, and flies. The farther north odonates are found, then the shorter the flight season. This is due to their environmental requirements; odonates need warmer temperatures to survive (Beaton, 15). The flight muscles in the thorax need to be warm enough for them to fly. Most damselflies need it to be about 16 ˚C for them to be active (Beaton, 15). If it is too hot outside, then odonates find areas that are in the shade or will put their abdomen facing the sun to minimize the surface area of the body that is in sunlight (Beaton, 15).

Breeding

After odonates mature the next stage they enter is breeding. To find mates and breed, odonates return to the breeding habitat (Beaton, 16). Males typically claim and defend a section of the habitat to attract females. Once they have a site they have to patrol and defend it against other males that come into their territory (Beaton, 16). Males that threaten the territory can be driven away by choreographed displays, near conflicts, or confrontations. Most of the time there is never physical contact between males. This is due to the chance of debilitating injuries (Beaton, 16). Most of the time only wings will clash with each other. In order to prepare for mating with females, the males produce sperm at the end of their abdomen and then transfer it to the accessory genitalia, where it will be delivered to the female during mating. Some species can do this while in flight (Beaton, 17). Females that curl and uncurl their abdomens are rejecting the advances of the males (Beaton, 16). Once the female has chosen to mate with the male, the male's legs grab the female's thorax. Then they shift the female so that his terminal appendages at the end of the abdomen are around her prothorax (Beaton, 17). The sperm on the accessory genitalia of the male is captured by the end of the female's thorax when she curls her abdomen up. This position can resemble a heart in flexible damselflies (Beaton, 17). The female gets enough sperm from the male to fertilize every one of her eggs; however, if she is paired with another male the entire process will begin again. Other males will try to capture the female to try and breed with her even while she is connected to the first male (Beaton, 19). After fertilization, the females oviposit their eggs either in specific locations or scattered around the habitat area. Damselflies cut a stem or a log and lay their eggs within the opening. Typically, the male is still attached while the female deposits her eggs (Beaton, 19). This is done to prevent other males from capturing the female and ensures that the first male's genes are passed on (Beaton, 19).

Hetaerina americana

Hetaerina americana or the American Rubyspot is a large species of damselfly distinguished by a sizeable red spot at the base of their wings. The red thorax, bright red spot on the wings, and a green abdomen identify males (Beaton, 62). Females have similar coloring but are much duller in comparison. The thorax and abdomen are a dull green and brown (Beaton, 62). The habitat of the American Rubyspot is streams and rivers. These tend to have a medium to fast flow rate (Beaton, 62). The males and females of this species are typically found close together and may in fact perch on the same vegetation. They perch at low or medium height above the stream or river (Beaton, 62).

Wings

The flight of odonates is based on morphologically primitive thoracic and wing structures (May, 325). Unlike dragonflies, damselflies have the most surface area in the region of the wings away from the center, increasing the surface that bears the wing beat and increasing the efficiency of the stroke (Grabow and Ruppell, 184). Additionally, the wings increase in thickness from the base in order to deal with the vertical forces on the wings while odonates are flapping their wings (Sudo et al., 724). Dragonflies and damselflies have the unique ability to move each wing apart from the other three wings (Sudo et al., 726). Similarly, it was previously thought that all insects beat their wings at a constant frequency determined by their thorax muscles, like mosquitos. However, odonates can alter the frequency and the amplitude that their wings beat at during flight (Sudo et al., 726).

Veins

There are multiple forces that act upon the wings while the damselfly is in flight. The veins on the wings are adapted to handle both aerodynamic stress and inertial loads while in flight due to their shape and location on the wing (Sun and Bhushan, 4). In addition, the configuration of the veins aids odonates in the reduction of drag while in flight (Sudo et al., 724). The posterior of the wings has a system of veins that serve to stabilize the wings in flight (Kesel et al., 434).

Membranes

The wings are given additional stability through the membranes on the wings. Moreover the membranes have a wax layer that serves to decrease the ability of the wings to get wet and prevent any contamination (Sun and Bhushan, 6). While the veins along the rear section do provide some stiffening for the wing, it is not enough for the entire structure of the wing. However, together with the membrane they form a configuration that minimizes the possibility of the wing collapsing (Kesel et al., 434). The veins are able to tolerate torsion and being bent due to chitin fibrils that diagonally brace the membranes (Kesel et al., 434).

Stigma

Furthermore, the point where the wing has the greatest impact with the air is the stigma, which is sometimes referred to as the pterostigma (Sun and Bhushan, 7). This is also where all of the veins are united and subsequently increases the effectiveness of the downward and upward movement of the wing in flight. It has also been suggested, that the stigma is able to shift the center of gravity of the wing to the front of the wing (Sudo et al., 724). In moving the center of mass towards the axis of the wing, the stigma controls the wing pitch (Sun and Bhushan, 7).

Nodus

The nodus provides the wing with a flexible, elastic joint without losing any strength in areas of the wing that need rigidity (Sun, Bhushan, 7-8). The nodus also gives the wing reinforcement and absorbs shock. However, the most important function of the nodus is to establish the degree to which the wings are able to twist in flight (Sun and Bhushan, 8). Correspondingly, the nodus aids in the prevention of the collapse of the wing by dissolving stress on the longitudinal axis (Kesel et al., 435).

Corrugation

Damselfly wings are also corrugated to increase the strength and stiffness of the wings, while allowing the wing to also be lightweight. Also, the corrugation absorbs the stresses of flight and allows bending of the membranes of the wings (Sudo, Tsuyuki, and Tani, 899). Moreover, the wings have the ability to stiffen under the pressure from the forces of flight and in turn bring the wing more stability (Kesel et al., 434).

Aerodynamics

Any animal capable of flight has to be able to trust only the flapping of its wings to generate air with enough force to compensate for the mass of the animal (Marden, 235). Insect wings in general have a large amount of inward and outward rotation around the axis of their wingspan. This gives them a positive angle between the oncoming air and the line of the thorax and abdomen. Subsequently, insects can create lift through their wing strokes (Sane, 4202). Odonates have more versatile flight than other insects. They can flight forward at great speeds, hover over prey, fly backwards, takeoff from a perch vertically, and land on perches vertically (Sudo et al., 723). Similarly, odonates typically have broader wings than other insects because of they glide and soar in flight (Wooton, 134). Moreover, the area of the wings increases as the weight of the organism increases (Grabow and Ruppell, 179). The flight patterns of dragonflies and damselflies can be placed into one of two groups, powered flight and gliding (Sudo et al., 899). When odonates hover, the efficiency can be markedly improved when the beats of the fore wings and hind wings are out of sync (Wang, 189). Taking off from a perch, odonates beat both the hind wings and the fore wings together but after a couple of seconds, the hind wings move about half of a wing beat ahead of the fore wings for the rest of the flight (Alexander, 379). The motion that they use during this type of flight can be compared to rowing a boat (Wang 189). The two sets of wings work together similarly to the ways that helicopters work to fly (Lancaster and Downes, 144). The fore wings create extra energy in their strokes that can be recovered by the hind wings. This action reduces how much power odonates need to generate to be able to fly by about 22 percent (Lancaster and Downes, 144). There are asymmetric wing beats along an incline and the primary force is created from the downward wing stroke (Wang, 199-201). The aerodynamic lift during flight is largely generated by the thickest part of the wing. This is typically 0.15 mm from the base of the wing to about 0.55 mm from the base (Sudo et al., 899). The fore wing acts as the "rolling stable wing . . . during gliding flight (Sudo et al., 899)." Odonates have flexible bases for their wings due to elastic elements. These allow odonates to be able to have a variety of different flight maneuvers and to change the pattern in which they beat their wings (Sudo et al., 900). Odonate wings are relatively thin because thin wings have a reduced coefficient for drag than thicker wings (Okamoto et al., 284).

Since damselflies are relatively small compared to dragonflies and have a sizeable surface with which to generate drag, their downward force is weak (Grabow and Ruppell, 183). Even if the wings are weakened by stress there is very little effect on the overall stability of the wings of dragonflies and damselflies (Kesel et al., 433). Additionally, the corrugation of the wing gives stability in flight and helps with the aerodynamics of the flight. Folds and ridges prevent the wing from tearing from the pressure and subsequently improve lift during the downward stroke (Kesel et al., 434). Damselflies have been found to have lower frequencies in their wing beat patterns and a higher amplitude of the stroke while in flight (Wakeling and Ellington, 558). Damselflies use the "clap and fling" method while in flight. The clap is when the wings touch each other over the abdomen just before the damselfly begins the down stroke (Sane, 4199). The leading edges of the wing then fling apart generating a region of low pressure between the leading and the lagging edge of the wing (Sane, 4199). This mechanism of flight gives the damselfly a slight enhancement in their lift during flight (Sane, 4199). The "clap and fling" fliers in general have a lower amount of marginal muscles that they use to fly because they have muscles efficient enough to create a higher lift force (Marden, 244).

Morphology

The shape of the wing mirrors the environment of the animal and the way the wing functions in flight. Morphology is the study of the form and structure of animals. Morphological analysis uses statistics to quantify the variation in shape. Morphology also reflects the evolution of the damselfly.

CHAPTER THREE

Methods

Site Selection

The Brazos River flows from Llano Estacado in eastern New Mexico and northwestern Texas to the Gulf of Mexico. My collection site on the Brazos River was Falls on the Brazos Park in Marlin, Texas. This park has a low-level dam that serves to stop water flow only when the water level in the river is low. This site has a damselfly-friendly, well-vegetated shoreline (Figure 1).

Specimens were collected from Falls on the Brazos Park between April and October of 2013. About 15 specimens were collected every two weeks. Only males were taken from the site, so that the population would not be irreparably devastated.

Figure 1: Site at Falls on the Brazos where specimen were collected

Preparing Specimens for Analysis

Once the specimens were back in the lab, they were put into a jar with ethyl acetate for ten minutes to kill them. After this time the damselflies, were weighed and preserved in labeled tubes with 70% ethanol.

The wings were prepared for scanning. After all of the specimens were collected the right fore and hind wings of the damselflies were removed and placed onto petri dishes with the fore wing above the hind wing. The wings are corrugated, which would made them difficult to scan and mark the landmarks accurately. To mitigate this, six microscope slides were tapped together to press the wings flat.

Before scanning any of the wings, scanner picture distortion was tested to locate any localized areas of unwanted distortion on the surface of the scanner. Small Post-it notes were distributed on all areas of the surface and scanned into Adobe Photoshop. The height and width of each Post-it note was measured. The scanner did have a slight error that skewed much of the left side of the image from some areas of the surface of the scanner. To compensate for this, I used the area of the scanner with the least amount of distortion, and this area was marked to ensure that all wings were placed in the same location. If there were any change due to the scanner it would be the same for all of the wings scanned.

After scanning all wings, the pictures were labeled with specimen number, sex of the specimen, and Julian date of collection. Then the files were accepted by the tpsUtil program to create a file receives the coordinates for all subsequently landmarked specimens. TpsDig is the software that records the location of landmarks on the image of the scanned wings.

Landmarking the Specimens

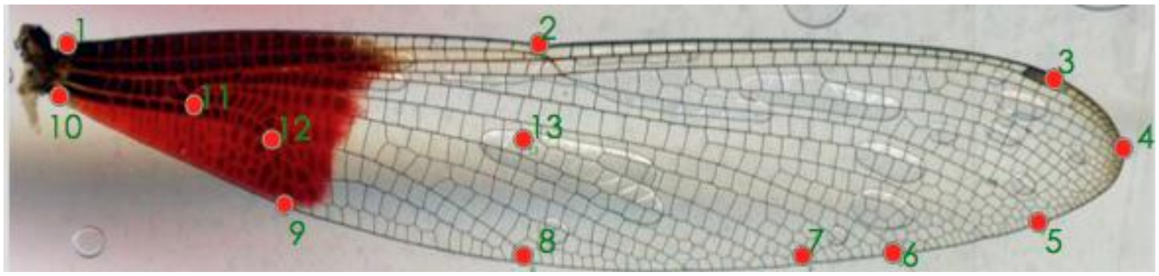

Figure 2: example of a landmarked hindwing

The first landmark was placed where the leading edge of the wing is attaches to the thorax. The second landmark is at the nodus. The third is at the anterior distal corner of the pterostigma. The fourth is where the second vein below the leading edge after the nodus meets the end of the wing. The vein that connects the nodus to the lagging edge of the wing is landmark 5. There is a conserved junction of veins at landmark 11. The point where the uppermost of the veins that comes from this junction meets the lagging edge is landmark 6. At the point where the uppermost of these veins and the next vein are separated by a third vein is landmark 13. If this vein is followed to the lagging edge then that is where landmark 7 is placed. The lowermost of those veins meets the lagging edge at landmark 8. The point where this vein joins other veins is where landmark 12 is placed. The ninth landmark is directly beneath the dip in veins. Finally, the tenth landmark is where the lagging edge of the wing is attached to the thorax. The fore wings were all landmarked and then in a separate file the hind wings were landmarked. This is because the software does not allow the repetition of numbers (file names) within the same image.

Some specimens had damaged wings and were missing landmarks. These specimens were excluded from the data set to limit the effect of outliers in the data. Additionally, if the results of the statistical tests showed outliers, the original landmarked pictures were examined to ensure that the landmarks were placed properly.

Data Analysis

After landmarking the wings, the data file of x-y coordinates of each specimen and each landmark can be opened in MorphoJ. MorphoJ calculates the shapes of all the wings and provides statistical analysis of the variation in shape. The fore wings were first compared to the hind wings as a control test of the software. Classifier variables were imported to separate the data into comparable groups. For example, the specimens were grouped by collection date to compare early collection dates (winter developers) with later dates (summer developers). Groups were separated into winter and summer developers based on adult life span. Even though development and lifespan depend on the temperature of the water and air, most references state that adults live on average 6 weeks. From this I determined that the guaranteed last winter developer would be those that were collected on June 4^{th}. Adults collected after this date could have been offspring of the earliest spring emergence. The summer developers start on August 4^{th} because these could only have been from eggs laid during the summer.

Principle Component Analysis (PCA)

Principle component analysis is the most widely used method in morphometrics. This displays the variation among specimens and displays the prominent features of the variation in the sample (Klingenberg, 1). Eigenvalues are an important part of the principle components analysis. These represent the percentage of the total shape difference and the "cumulative percentage of total variance (Klingenberg, 3)."

Canonical Variate Analysis (CVA)

Canonical variate analysis displays ordination data to distinguish between *apriori* groups. Additionally, it takes the coordinates from the landmark data from the samples and represents variation in this data along an x and y-axis, canonical variate 1 and 2, representing a synthesis of linear variation (Klingenberg, 9). These points amplify the distance between the means of the groups of data relative to the change within the group. CVA is more useful than discriminant function analysis when distinguishing among *apriori* groups (Klingenberg, 9).

Discriminant Function Analysis (DFA)

Discriminant function analysis displays the distance between two groups of data. Each specimen's landmark data are sorted into one of two groups depending on how closely it resembles a mean of a group. The scores are based on how many observations are misclassified. The cross-validation discriminant function is more "computationally intensive" (Klingenberg, 10) and is considered more reliable.

Procrustes ANOVA

The Procrustes ANOVA test provides a statistical output on the variation in the data. This test is implemented in cases when it is necessary to determine the amount of error that is associated with biological sources (Klingenberg, 3). The output from the procrustes ANOVA contains the sums of squares (SS), mean squares (MS), degrees of freedom (df), F statistics and parametric p-values for individual and residual sets of data. The individual set is determined by the winter and summer developer classifier variables

(Klingenberg, 3). The residual set of data explains any variation that may be "left over (Klingenberg, 3)" after the analysis. This could be due to specimen data that could not be clearly classified into one of the two groups in the classifier variables because of the date that they were collected. The procrustes ANOVA test compares the centroid sizes with the automatic null hypothesis that there are not significantly different centroid sizes (Klingenberg, 3). A p-value of less than 0.05 indicates that thus null hypothesis can be rejected because there is strong evidence that suggests that the centroids are statistically different. Thus the p-value is the most important output from this test (Klingenberg, 3).

CHAPTER FOUR

Results

Damselfly Collection

Adult *Hetaerina americana* were collected from April 28, 2013 to October 4, 2013 (n=182). Interpretation of those collections (Figure 3) shows a maximum of 28 specimens and a minimum of 4 specimens per collection. Collections from April 28th (day 118) to June 4th (day 155) were classified as winter developers. Collections from August 4th (day 216) to October 4th (day 268) were classified as summer developers.

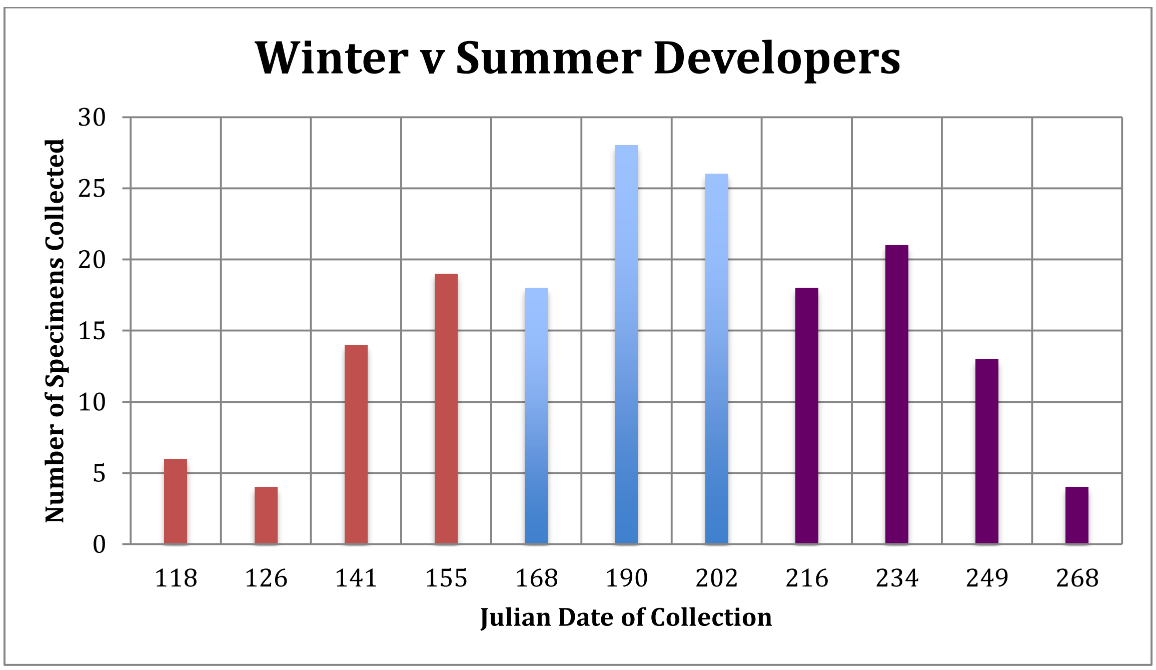

Figure 3: Number of specimens collected at each collection date. Colors indicate whether the specimen is a winter (red) or summer (purple) developer.

Wing Shape Variation Including Fore Wings and Hind Wings from All Collections

Landmark data for all wings (n=339) were pooled and analyzed using morphoJ. Procrustes adjustment was applied to this combined data set to remove wing size as a variable, while retaining the location of the landmarks relative to shape. A covariance matrix was generated to support the principle components analysis. PCA then visualized the variation in the data sets.

Principle Components Analysis

PCA provided five principle components explaining 85% of the variation (Figure 4). A scatterplot of the first principle component versus the second principle component showed the cluster of individual shapes along both principle components (Figure 5). These two principle components accounted for 55% of the variation in shape. Much overlap in fore wing and hind wing shape reflects the expected diagnostic similarities of wing characteristics of damselflies. Damselfly Suborder Zygoptera is characterized by fore wings and hind wings having "similar shapes." However, clusters of fore wing and hind wing shapes were offset and not exact duplicates (Figure 5). The lollipop graph further illustrates the variation in shape (Figure 6). Landmarks 3, 4, 7, 8, 9, and 13 showed the greatest variation. The nature of variation is the tips of the wings move proximally and distally and the lower border changes. CONCLUSIONS: Shape variation occurs along multiple principle components. Fore wings and hind wings are similarly shaped but not exactly. Landmarks at wing tips and lagging border vary the most.

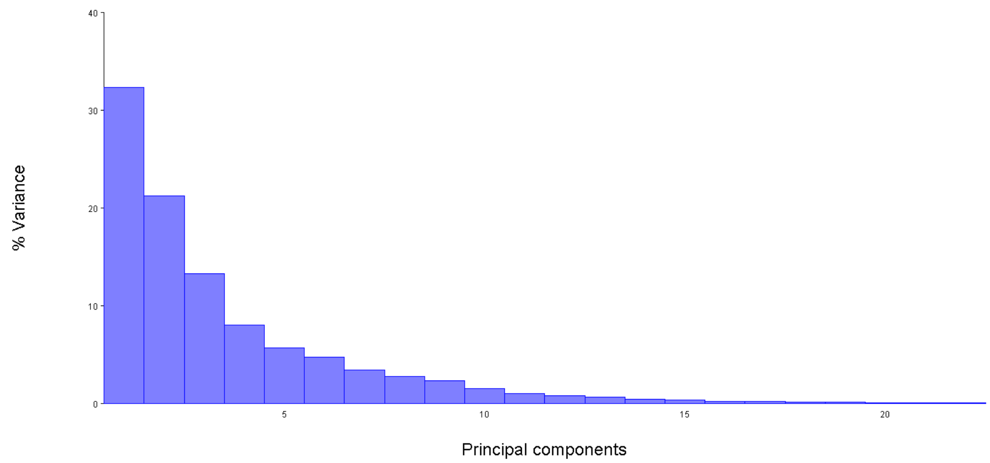

Figure 4: Percent variance accounted for by each principle component. The first principle component constitutes the most variation in the data.

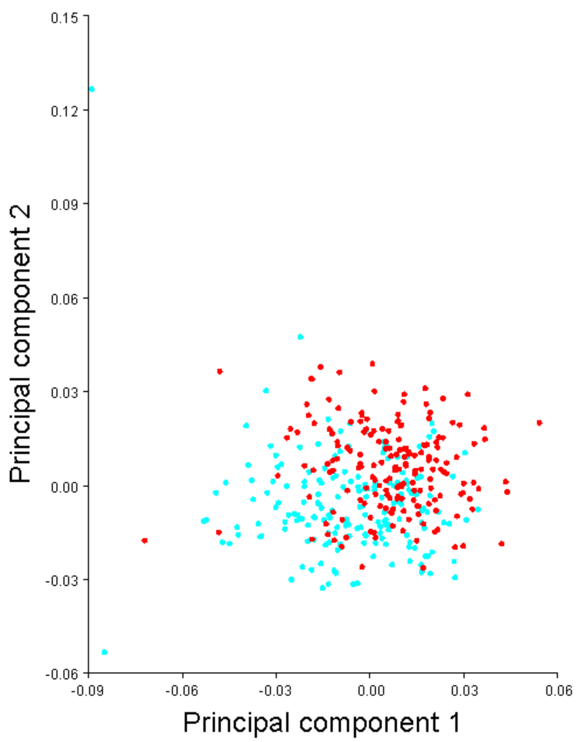

Figure 5: A visualization of variation shape of combined fore wings and hind wings (n=339). The graph of the first (accounting for the most variance in the data) and second principle component (accounting for the second most variation in the data) for the combined fore wing (red) and hind wing (blue).

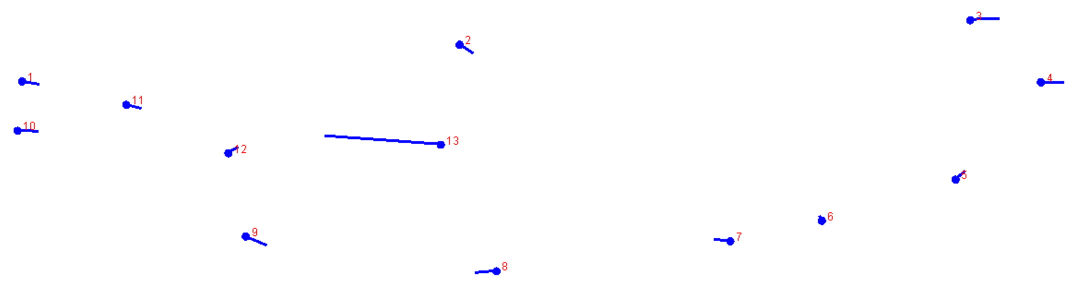

Figure 6: A lollipop graph for the PCA of the combined forewing and hind wing. This graph has a scale factor of 0.1, which expands the differences. A comparison of shape of all fore wings (n=172) versus all hind wings (n=167). The circles of each lollipop represent the mean position of each designated landmark for the fore wings. The end of the lollipop "stick" represents the mean position of each distinguished landmark for the hind wings.

Procrustes ANOVA

Procrustes ANOVA was used to detect any statistical difference between the size and shape of fore wings and the hind wings (Table 1). Procrustes ANOVA provided the sum of squares, mean squares, degrees of freedom, F statistic, and p-value of the shape variation. The critical value of F is 1.54 (Zar, 415) and the treatment value of F is 43.95 for the shape. CONCLUSION: Fore wings and hind wings are different shapes.

Procrustes ANOVA: Procrustes ANOVA ...
Dataset: Combined dataset ...

Classifiers used for the Procrustes ANOVA:
Individuals: From dataset

Centroid size:

Effect	SS	MS	df	F	P (param.)
Individual	2353032.030421	2353032.030421	1	72.49	<.0001
Residual	11749823.327534	32458.075490	362		

Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)
Individual	0.04946452	0.0022483872	22	43.95	<.0001	0.92	<.0001
Residual	0.40741924	0.0000511576	7964				

Table 1: Procrustes ANOVA table showing the statistical significance of the variation in shape and centroid size of fore wings versus hind wings.

Wing Shape Variation Between Fore Wings and Hind Wings of Winter Developers

Landmark data for winter developer fore wings (n=42) were compared to landmark data for winter developer hind wings (n=41). Procrustes adjustment was applied to both data sets to remove wing size as a variable, while retaining the location of the landmarks relative to shape. A covariance matrix was generated to support the principle components analysis. PCA then visualized the variation in the data sets. Procrustes ANOVA defined the statistical difference between the fore wings and the hind wings of winter developers.

Principle Components Analysis

PCA provided five principle components explaining 83% of the variation (Figure 7). A scatterplot of the first principle component versus the second principle component showed the cluster of individual shapes along both principle components (Figure 8). These two principle components accounted for 52% of the variation in shape. Much overlap in fore wing and hind wing shape reflects the expected diagnostic similarities of wing characteristics of damselflies. Zygoptera is characterized by fore wings and hind wings having similar shapes. However, clusters of fore wing and hind wing shapes were less offset than all of the points combined (Figure 8). The lollipop graph further illustrates the variation in shape (Figure 9). Landmarks 1, 3, 4, 6, 7, 8, 10, and 13 show the most variation. CONCLUSIONS: Shape varies along multiple principle components. The scatterplot showed a lot of overlap in shape of fore wings and hind wings, but a slight distinction was apparent. The landmarks move proximally and distally both at the tip and at the base. Variation also occurs along the lagging border of the wing.

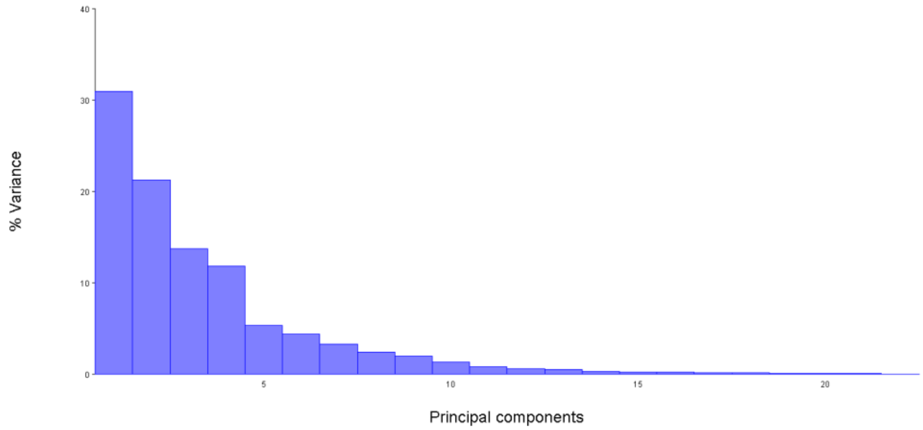

Figure 7: Percent variance accounted for by each principle component. The first principle component constitutes the most variation in the data.

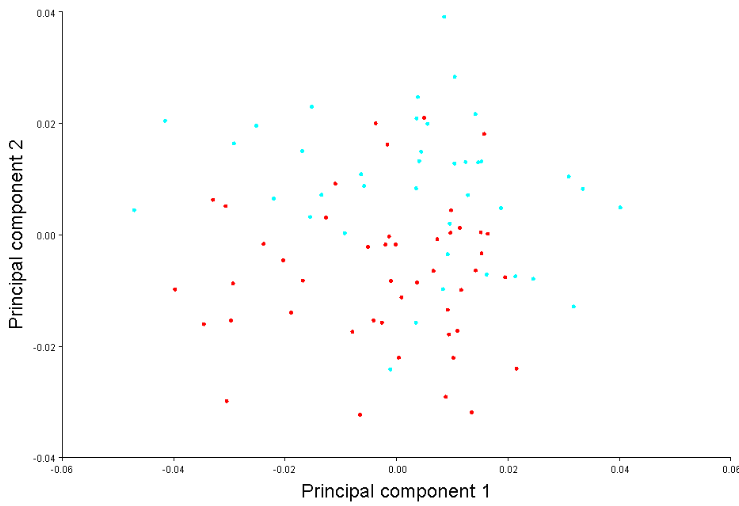

Figure 8: A visualization of variation shape of combined fore wings and hind wings (n=83). The graph of the first (accounting for the most variance in the data) and second principle component (accounting for the second most variation in the data) for the combined fore wing (red) and hind wing (blue).

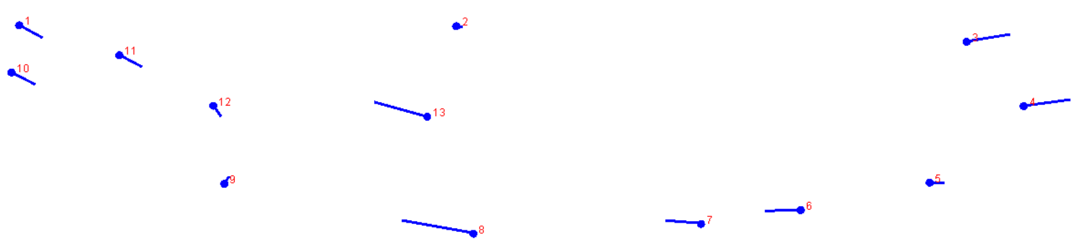

Figure 9: A lollipop graph for the PCA of the combined forewing and hind wing. This graph has a scale factor of 0.1, which expands the differences. A comparison of shape of all fore wings (n=42) versus all hind wings (n=41). The circles of each lollipop represent the mean position of each designated landmark for the fore wings. The end of the lollipop “stick” represents the mean position of each distinguished landmark for the hind wings.

Procrustes ANOVA

Procrustes ANOVA defined the statistical difference between the shape of the fore wings and the hind wings of winter developers (Table 2). Procrustes ANOVA provided the sum of squares, mean squares, degrees of freedom, F statistic, and p-value of the shape variation. The critical value of F is 1.55 (Zar, 415) and the treatment value is 12.84 for the shape. CONCLUSION: There was a significant difference in the shapes of the fore wings and the hind wings among winter developers.

Procrustes ANOVA: Procrustes ANOVA ...
Dataset: winter developers

Classifiers used for the Procrustes ANOVA:
Individuals: From dataset

Centroid size:

Effect	SS	MS	df	F	P (param.)
Individual	278681.473667	278681.473667	1	55.31	<.0001
Residual	423243.700426	5038.615481	84		

Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)
Individual	0.01191388	0.0005415400	22	12.84	<.0001	0.96	<.0001
Residual	0.07793194	0.0000421710	1848				

Table 2: Procrustes ANOVA table showing the statistical significance of the variation in shape for only the winter developers. Both the fore wings (n=42) and the hind wings (n=41) are shown.

Wing Shape Variation Between Fore Wings and Hind Wings of Summer Developers

Landmark data for summer developer fore wings (n=50) were compared to landmark data for summer developer hind wings (n=49). Procrustes adjustment was applied to both data sets to remove wing size as a variable, while retaining the location of the landmarks relative to shape. A covariance matrix was generated to support the principle components analysis. PCA then visualized the variation in the data sets.

Procrustes ANOVA defined the statistical difference between the shapes of fore wings and the hind wings of summer developers.

Principle Components Analysis

PCA provided five principle components explaining 84% of the variation (Figure 10). A scatterplot of the first principle component versus the second principle component showed the cluster of individual shapes along both principle components (Figure 11). These two principle components accounted for 60% of the variation in shape. Much overlap in fore wing and hind wing shape reflects the expected diagnostic similarities of wing characteristics of damselflies. However, clusters of fore wing and hind wing shapes were offset and not exact duplicates (Figure 11). This offset was tested for significance. See Procrustes ANOVA below. The lollipop graph further illustrates the variation in shape (Figure 12). Landmarks 1, 5, 6, 9, 10, and 13 showed the most variation. CONCLUSIONS: The majority of the variation of the fore wing and the hind wing of the summer developers can be explained by the first two principle components. The fore wings and hind wings do overlap. However, the base of the wing moves proximally and distally while the remaining landmarks do not vary much. Additionally, the lagging border varies as well.

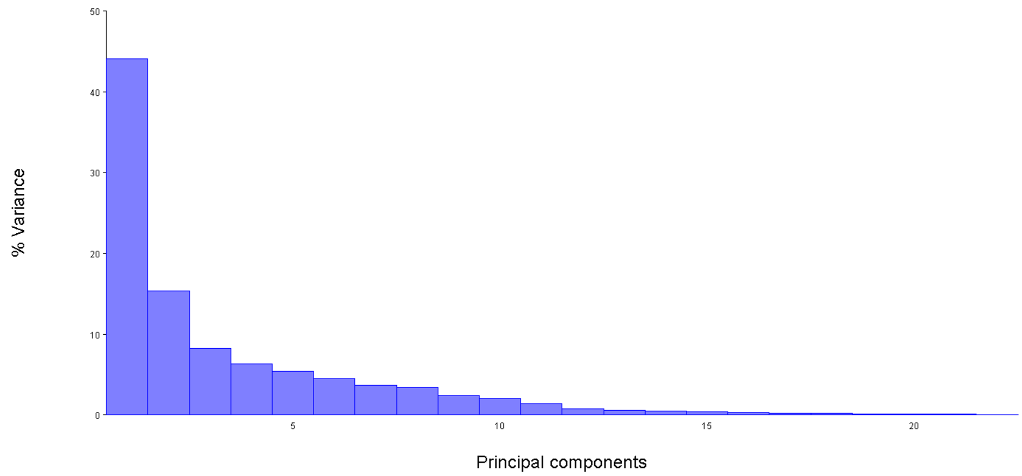

Figure 10: Percent variance accounted for by each principle component. The first principle component constitutes the most variation in the data.

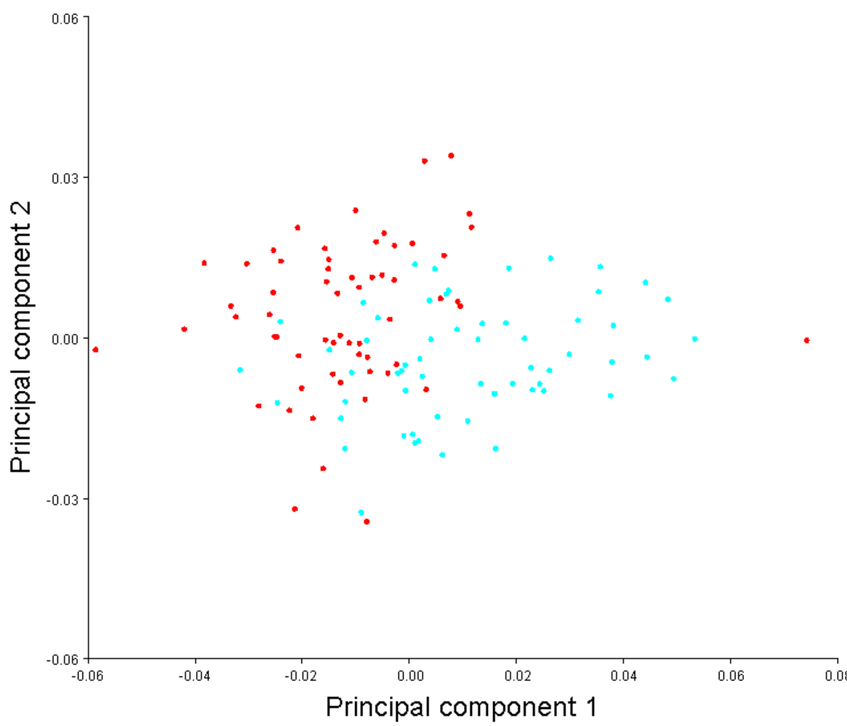

Figure 11: A visualization of variation shape of combined fore wings and hind wings (n=99). The graph of the first (accounting for the most variance in the data) and second principle component (accounting for the second most variation in the data) for the combined fore wing (red) and hind wing (blue).

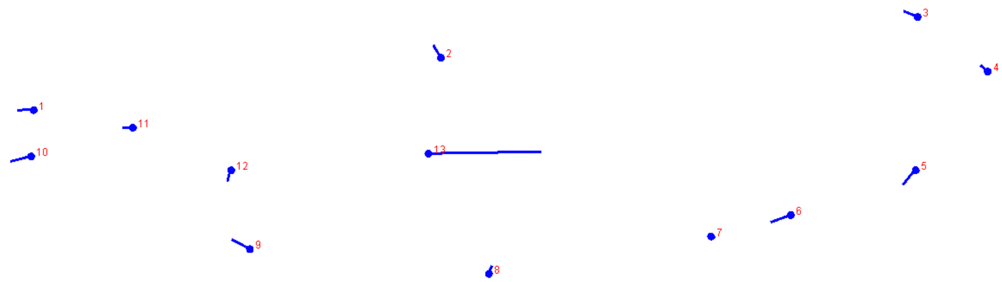

Figure 12: A lollipop graph for the PCA of the combined forewing and hind wing. This graph has a scale factor of 0.1, which expands the differences. A comparison of shape of all fore wings (n=50) versus all hind wings (n=49). The circles of each lollipop represent the mean position of each designated landmark for the fore wings. The end of the lollipop "stick" represents the mean position of each distinguished landmark for the hind wings.

Procrustes ANOVA

Procrustes ANOVA defined the statistical difference between shapes of the fore wings and the hind wings (Table 3). Procrustes ANOVA provided the sum of squares, mean squares, degrees of freedom, F statistic, and p-value of the shape variation. The critical value of F is 1.55 (Zar, 415) and the treatment value is 23.47 for the shape. CONCLUSION: Fore wings and hind wings are different shapes in the summer developers.

Procrustes ANOVA: Procrustes ANOVA ...
Dataset: summer developers

Classifiers used for the Procrustes ANOVA:
Individuals: From dataset

Centroid size:

Effect	SS	MS	df	F	P (param.)
Individual	2052791.418239	2052791.418239	1	32.36	<.0001
Residual	7485953.572646	63440.284514	118		

Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)
Individual	0.02140244	0.0009728382	22	23.47	<.0001	0.93	<.0001
Residual	0.10760124	0.0000414489	2596				

Table 3: Procrustes ANOVA table showing the statistical significance of the variation in shape in the summer developers. Both the fore wings (n=50) and the hind wings (n=49) are shown.

Fore Wing Shape Variation Between Winter and Summer Developers

Damselfly collections and their landmark data for the fore wings were divided into three groups: the winter developers (n=42), the summer developers (n=50), and those without clear separation into either category (n=81). See Data Analysis for this classification. Procrustes adjustment was applied to the first and third data sets to remove wing size as a variable. A covariance matrix was generated. CVA distinguished between summer and winter developers (Figure 13 and 14). Finally, a Procrustes ANOVA was used to find a statistical difference in the two groups of data.

Canonical Variate Analysis

The CVA provided canonical variants that explained the majority of the variation in the data within and between *apriori* groups. CVA then visualized the variation in the specimen along an axis of the first and second canonical variants (Figure 13). The wireframe shows how the individual landmarks vary in relation to the treatment (Figure 14). CONCLUSIONS: The mean of the winter developer and summer developer forewings are different. In the summer developers, the fore wings are broader than the winter developers.

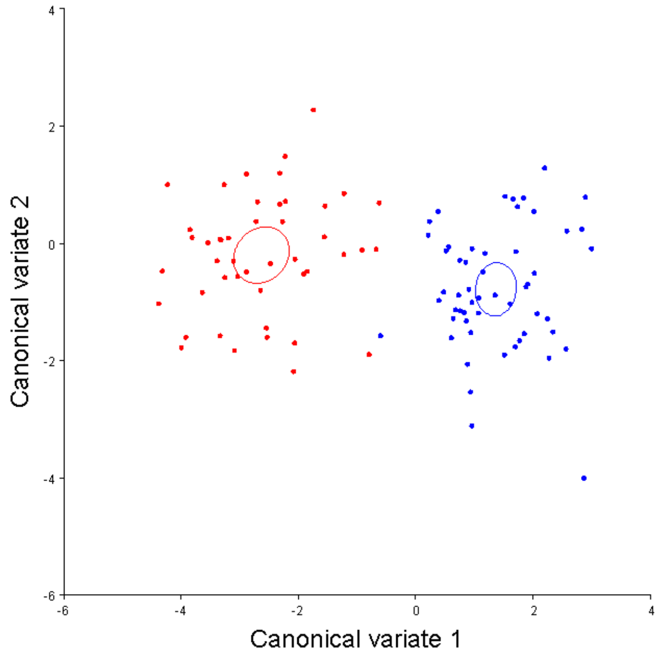

Figure 13: CV scores on the first two canonical variants for the fore wings based on the winter (red) and summer (blue) developer classifier variables. Confidence limits of 99% for the mean are enclosed in ellipses.

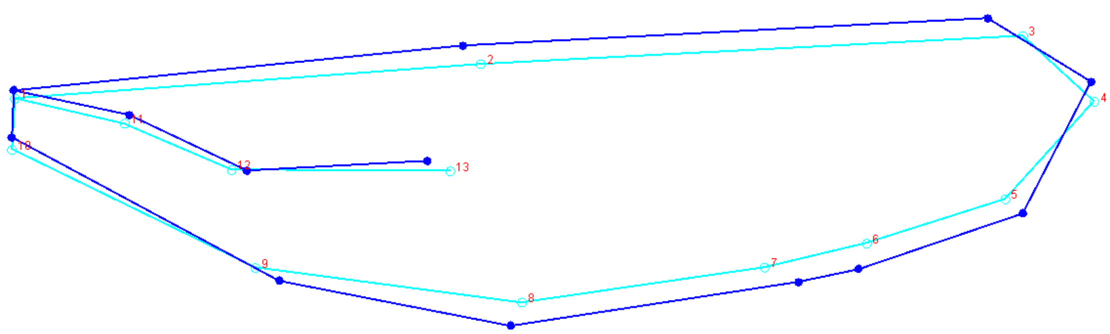

Figure 14: Wireframe diagram showing changes in relative position of landmarks as analyzed by CVA. Scale factor is 10. The light blue dots of the wireframe represent the mean position of each designated landmark for the winter developers (n=42). The dark blue dots of the wireframe represent the mean position of each designated landmark for the summer developers (n=50).

Discriminant Function

Discriminant function analysis then placed the wings into groups based on how their wing shape compared to the mean wing shapes for either the winter or the summer developer group (Figure 15 and Table 4). CONCLUSION: DFA cross-validation showed that the mean wing shape for the winter developers fore wings is different than the mean wing shape for the summer developers.

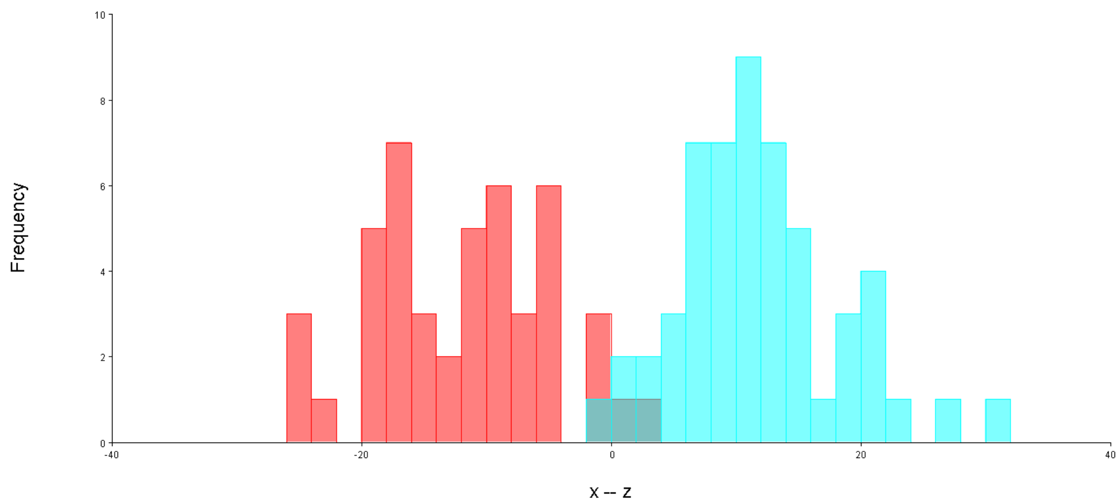

Figure 15: Cross validation scores for the fore wing shape based on winter (red) and summer (blue) developer classifiers

```
Discriminant Function Analysis 'Discriminant function ...'
Comparison: x -- z

Difference between means:
Procrustes distance:  0.02637959
Mahalanobis distance:      4.9102
T-square:  598.9000,   P-value (parametric): <.0001

Classification/misclassification tables
Group 1: x
Group 2: z
From discriminant function:
True               Allocated to
Group         Group 1        Group 2         Total
Group 1         46              0             46
Group 2          0             54             54
From cross-validation:
True               Allocated to
Group         Group 1        Group 2         Total
Group 1         44              2             46
Group 2          1             53             54
```

Table 4: Discriminate function and cross validation calculations for the fore wings classified as winter (group 1) and summer (group 2) developers.

Procrustes ANOVA

Procrustes ANOVA was used to find the statistical difference between the fore wing shapes of winter developers and summer developers (Table 5). Procrustes ANOVA provided the sum of squares, mean squares, degrees of freedom, F statistic, and p-value of the centroid size and the shape variation. The critical value of F is 1.38 (Zar, 415) and the treatment value of F is 12.43 for the shape. CONCLUSION: The shapes of fore wings of winter developers and summer developers are different.

Procrustes ANOVA: Procrustes ANOVA ...
Dataset: without missing points

Classifiers used for the Procrustes ANOVA:
Individuals: emergence

Centroid size:

Effect	SS	MS	df	F	P (param.)
Individual	415945.745153	207972.872576	2	66.77	<.0001
Residual	526388.187322	3114.723002	169		

Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)
Individual	0.02306691	0.0005242480	44	12.43	<.0001	1.02	<.0001
Residual	0.15679222	0.0000421711	3718				

Table 5: Procrustes ANOVA comparing the shapes of winter developer (n=42) versus the summer developer (n=50) fore wings.

Hind Wing Shape Variation Between Winter and Summer Developers

Damselfly collections and their landmark data for the hind wings were divided into three groups: the winter developers (n=41), the summer developers (n=49), and without clear separation into either category (n=77). Procrustes adjustment was applied to the first and third data sets to remove wing size as a variable. A covariance matrix was generated. CVA distinguished between summer and winter developers (Figure 17 and 18). The discriminant function was then used to place the wings into groups based on how their centroid shape compared to the mean centroid shapes for either the winter or the summer developer group. Finally, a Procrustes ANOVA was used to find a statistical difference in the two groups of data.

Canonical Variate Analysis

The CVA provided canonical variants that explained the majority of the variation in the data within and between *apriori* groups. CVA then visualized the variation in the specimen along an axis of the first and second canonical variants (Figure 13). The wireframe shows how the individual landmarks vary in relation to the treatment (Figure 14). CONCLUSIONS: There was a slight overlap in the specimen collected but the two groups were distinct. The wireframe showed that the hind wings of summer developers are narrower than those of winter developers.

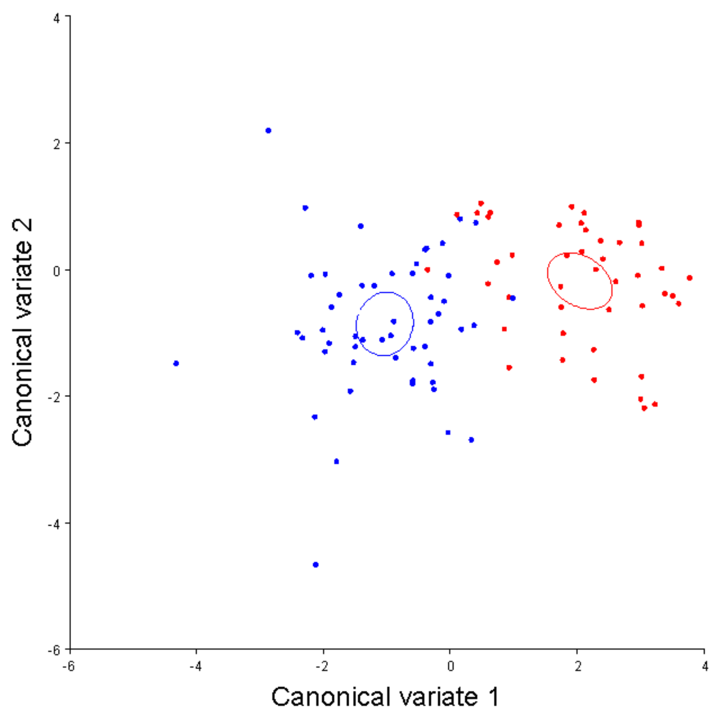

Figure 16: CV scores on the first two canonical variants for the hind wings based on the winter (red) and summer (blue) developer classifier variables. Confidence limits of 99% for the mean are enclosed in ellipses.

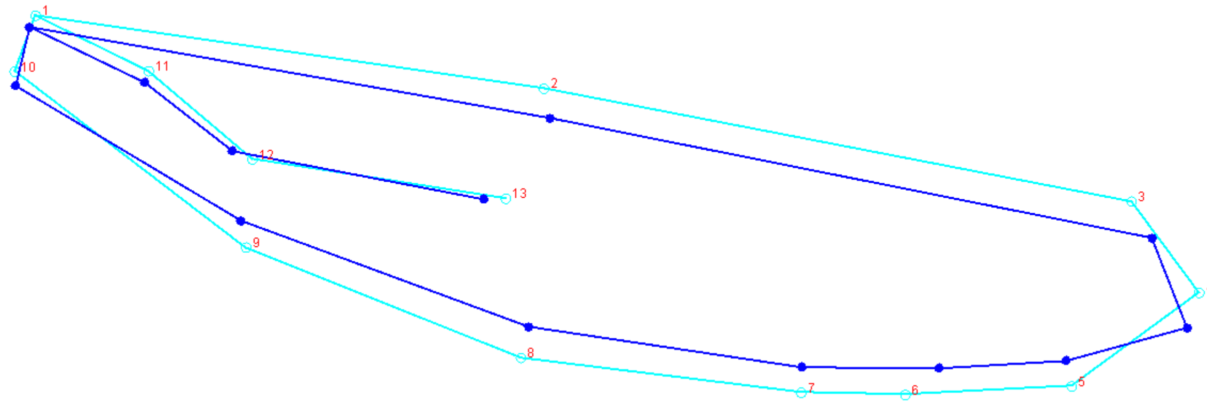

Figure 17: Wireframe diagram showing changes in relative position of landmarks as analyzed by CVA. Scale factor is 10. The light blue dots of the wireframe represent the mean position of each designated landmark for the winter developers (n=41). The dark blue dots of the wireframe represent the mean position of each designated landmark for the summer developers (n=49).

Discriminant Function

Discriminant function analysis placed the wings into groups based on how their wing shape compared to the mean wing shapes for either the winter or the summer developer group (Figure 18 and Table 6). CONCLUSION: The mean wing size of the hind wings of winter developers and summer developers were not as distinct as the fore wings but still varied in shape.

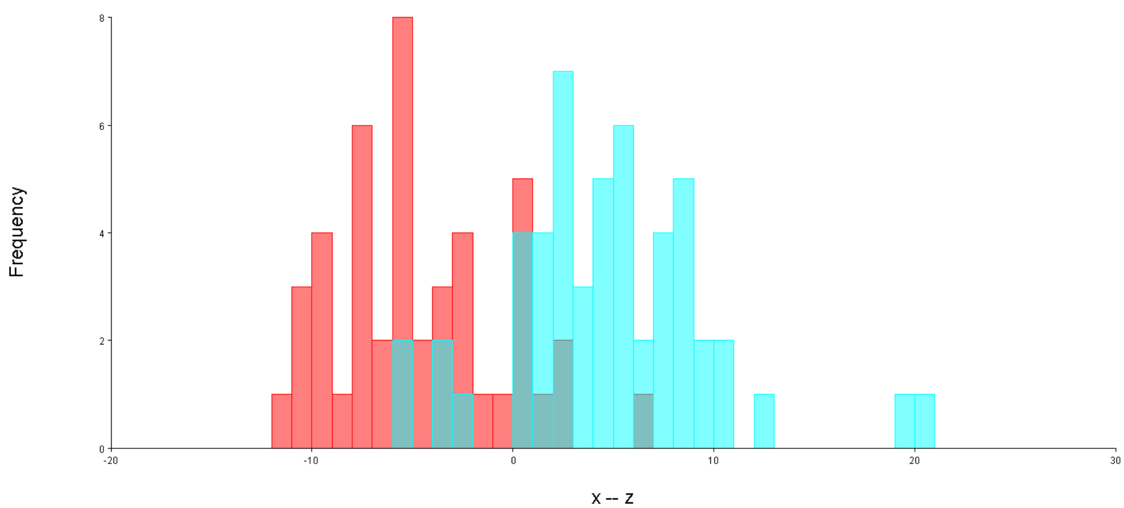

Figure 18: Cross validation scores for the hind wing shape based on winter (red) and summer (blue) developer classifiers.

```
Discriminant Function Analysis 'Discriminant function ...'
Comparison: x -- z

Difference between means:
Procrustes distance:  0.02095279
Mahalanobis distance:      3.2659
T-square:  257.3093,   P-value (parametric): <.0001

Classification/misclassification tables
Group 1: x
Group 2: z
From discriminant function:
True                Allocated to
Group          Group 1         Group 2          Total
Group 1          43              2               45
Group 2           2             50               52
From cross-validation:
True                Allocated to
Group          Group 1         Group 2          Total
Group 1          36              9               45
Group 2           5             47               52


Procrustes ANOVA: Procrustes ANOVA ...
Dataset: Without missing points

Classifiers used for the Procrustes ANOVA:
```

Table 6: Discriminate function and cross validation calculations for the hind wings classified as winter (group 1) and summer (group 2) developers.

Procrustes ANOVA

Procrustes ANOVA was used to find the statistical difference between the hind wings of winter developers and summer developers (Table 7). Procrustes ANOVA provided the sum of squares, mean squares, degrees of freedom, F statistic, and p-value of the shape variation. The critical value of F is 1.38 (Zar, 415) and the treatment value of F is 7.95 for the shape. CONCLUSION: Hind wings of winter developers and summer developers are different shapes.

```
Procrustes ANOVA: Procrustes ANOVA ...
Dataset: newDataset

Classifiers used for the Procrustes ANOVA:
Individuals: emergence

Centroid size:
Effect              SS               MS         df        F      P (param.)
Individual 2471728.281367  1235864.140684       2      26.85      <.0001
Residual   8239417.190447    46030.263634     179

Shape, Procrustes ANOVA:
Effect              SS             MS            df         F      P (param.)   Pillai tr.   P (param.)
Individual     0.01787757     0.0004063083       44       7.95      <.0001        0.91       <.0001
Residual       0.20124826     0.0000511042     3938
```

Table 7: Procrustes ANOVA comparing the shapes of winter developer (n=41) versus the summer developer (n=49) hind wings.

CHAPTER FIVE

Discussion

Despite damselflies being classified according to the similar shape and size of their fore wings and hind wings, the analysis of variation in shape showed that the fore wings and hind wings are in fact two different shapes and sizes.

Wings of adult damselflies whose larvae developed during cool, winter months are shaped differently than those of summer developers. Fore wings are broader in damselflies that developed more quickly over the warmer, summer months. Hind wings however are narrower in the summer developers. Additionally, the two groups were more distinct in the fore wings than the hind wings. More research is needed to determine why wings change depending on the season. Wing shape could either result from the circumstances (temperature, etc.) in which eggs and larvae develop or be a genetic adaption to the change in environmental conditions.

Environmental Influences on Development

One plausible explanation for the observed seasonal differences in the shapes of both the fore wings and the hind wings is that water temperature where the eggs and larvae develop is warmer for summer developers. Their development is shorter than the time that the winter developers have. These differences in the conditions of development could impact the processes of damselfly development. Warmer temperatures could accelerate larval metabolism, which would cause them to burn more fat stores, resulting in smaller body sizes and significant variation in their allometry. Wing size and shape could be affected by mechanisms similar to those affecting body siz. In other words, warm larval development may dictate variation in wing shape. Similarly, hatching times are temperature dependent. Once the water reaches a threshold temperature larvae hatch and continue through the larval stage of development. Warmer temperatures shorten the time for egg development. Less time as eggs could produce organisms with less mass, shorter abdomens, and greater variation in wing shape.

Adaptive Strategy

The observed variation in wing shape of the winter developers in contrast to summer developers could also be due to evolutionarily adaptive genetic switches that are turned on and off by environmental cues. Warmer weather could trigger "instructions" for development of broader fore wings and narrower hind wings than those of the winter developers.

Warm air is less dense than cold air and the variations in shape could be adaptations to cope. Blow flies were found to have a higher number wing beats and higher wing speeds in higher temperatures (Yurkiewicz, 25). Blow flies adaptively compensate for changes in air density. Perhaps odonate populations vary their wing shape in a similarly adaptive manner. Specifically, they develop wings more efficiently shaped to conserve energy, instead of increasing the number of times that they beat their wings to conserve energy. Odonates have broader wings than other insects because they frequently glide in flight (Wooton, 134). Fore wings could become broader to increase flight efficiency in the warmer air of summer. Moreover, when odonates hover they move their fore wings and their hind wings out of sync with each other (Wang, 189). Additionally, hind wings function to recover excess energy created by the beats of the fore wing (Lancaster and Downes, 144). The broader fore wing could be an adaptation to address the density of the air and the narrower hind wing could be an adaptive response to better recover energy from the changed fore wing.

This explanation for variation in wing shape would require the genetic code of the organisms to be able to sense changes in ambient temperatures and alter gene expression during development. The ability to express broader fore wings and narrower hind wings after summer development would require a gene pool in damselflies that has the robustness and the plasticity for both phenotypes.

Conclusions

Wing shapes vary based on seasonal development. Fore wings and hind wings are similarly shaped but not exactly. Landmarks at wing tips and lagging border vary the most. Fore wings and hind wings are different shapes as shown by the Procrustes ANOVA.

Among only the winter developers shape varies along several principle components. The scatterplot showed much overlap in shape, but there was still a slight distinction. In the winter developers, the wings move proximally and distally both at the tip and at the base. Shape also varies along the lagging border of the wing. The Procrustes ANOVA showed a significant difference in the shapes of the fore wings and the hind wings among winter developers.

The majority of the variation of the fore wing and the hind wing of the summer developers can be explained by the first two principle components. The scatter of fore wings and of hind wings does overlap. However, the base of the wing moves proximally and distally while the remaining landmarks do not vary much. Additionally, the lagging border varies in the summer developers. Fore wings and hind wings are different shapes in the summer developers.

The mean of the winter developer and summer developer forewings are significantly different on the graph of the first two canonical variates. In the summer developers, the fore wings are broader than the winter developers. DFA cross-validation showed that the mean wing shape for the winter developers fore wings differs significantly from the mean wing shape for the summer developers. Procrustes ANOVA showed shapes of fore wings of winter developers significantly differed from those of summer developers.

The shape of hind wings of winter and summer developers slightly overlapped, but the two groups were distinct. Wireframe diagrams showed that hind wings of summer developers are narrower than those of winter developers. The mean wing size of the hind wings of winter developers and summer developers were not as distinct as the fore wings but still varied in shape. Hind wings of winter developers and summer developers are different shapes.

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