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Do male physiological condition and territory quality affect female choice in the Brown Anole, Anolis sagrei?

Sarah P. Flanagan
Colby College

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Do male physiological condition and territory quality affect female choice in the Brown Anole, *Anolis sagrei*?

An Honors Thesis

Presented to

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in partial fulfillment of the requirements for the

Degree of Bachelor of Arts with Honors

by

Sarah Flanagan

Waterville, ME

Advisor: Catherine R. Bevier ____

Reader: W. Herbert Wilson ____

Reader: Andrea Tilden ____

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ABSTRACT

Female mate choice is an important component of sexual selection because traits that influence male mate preference, such as physiology, and proficiency in acquiring resources, are favored. In lizards, the importance of mate choice remains unclear as reported results from experiments are contradictory. In this study, I investigated whether male physiology and territory quality are important to female mate choice for male Brown Anoles, *Anolis sagrei*. I tested the hypotheses that female *A. sagrei* prefer males with greater physiological capacities, and prefer higher quality territories, regardless of male phenotype. To test these, male *A. sagrei* were first rated for endurance and then used in mismatched-pair female mate choice trials. Preference was scored as the amount of time the female spent actively engaging a male. Male activity level was also scored. Blood glucose levels were measured before and after the endurance tests, and before and after the mate choice trials. Finally, levels of stored glycogen were measured in leg muscle and liver samples collected after a mate choice trial. In 11 of 15 trials, the female spent more time with the male with the higher endurance score, and females preferred males with lower liver glycogen levels. Second, females were given a choice between a male in a territory supplemented with plants, and a size-matched male in a bare territory. Females spent more time with one of the two males, regardless of territory quality. These results suggest that female *A. sagrei* exhibit preferences for male traits regardless of territory quality.

INTRODUCTION

Mate choice is an important aspect of sexual selection. In many animal species, females exhibit preference for male mates based on certain traits. The nature of these traits varies from species to species and can often take the form of direct benefits, such as nuptial gifts. For example, male hangingflies (*Bittacus apicalis*) provide females with a nuptial gift that may supply enough protein to fuel egg development and female maintenance. Females of this species seem to discriminate between males based on the size of the nuptial gift as males choose larger prey when possible and females end copulations before maximal sperm transfer when the gift is small (Thornhill 1976). In animals where male mates do not provide direct benefits to females, such as food or parental care, female preferences could be based on the male's resources (Andrews 1985, Tokarz 1995) or on indirect benefits gained from male condition (Tokarz 1995, Sullivan and Kwiatkowski 2007). Male physiological condition can be reflected in courtship displays, which are advertisements to conspecifics, and are often energetically costly (Sullivan and Kwiatkowski 2007). Many lizard species, including anoline lizards exhibit such displays, and females exhibit choice for males (e.g., Greenberg and Noble 1944, Tokarz 1998, Tokarz 2007). Some of these results provide evidence of female preferences for traits such as size (e.g. Greenberg and Noble 1944), but others did not provide support that females preferred the targeted male traits (e.g. Andrews 1985, Lailvaux and Irschick 2006). This suggests that female mate choice in lizards is less understood than in other vertebrate groups, and that the specific male traits females prefer are still ambiguous.

Brown anoles (*Anolis sagrei*) are a model species to use for female choice studies. This lizard is sexually dimorphic with a polygynous mating system (Losos 2009). These characters provide a robust opportunity for mate choice, compared to monogamous species because some males will be chosen more often than others in a polygynous species. In monogamy, females have fewer opportunities to choose a male since each male will generally only be chosen once per season (Bateman 1948, Trivers 1972, Cronin 1991, Tokarz 1995). During the breeding season, which lasts roughly from April through July (Lovern et al. 2004), male *A. sagrei* approach females while head-bobbing and extending their dewlap. Anoline lizards, generally, use these two displays, in addition to push-ups, in slightly different manners for a variety of social situations, including territory defense and courtship (Greenberg and Noble 1944, McMann 2000, Tokarz 2007). Female *A. sagrei* can influence whether or not copulation occurs by responding with a display that reflects different degrees of sexual receptivity; only highly receptive females will arch their neck and lower their snouts as a signal to males.

The stereotyped displays in anoline lizards have been studied as indicators for female mate choice. The frequency of courtship displays, especially dewlap extensions, has been shown to be more important than the species-specific qualities of the male, such as the color of the dewlap (Greenberg and Noble 1944), although the presence of the dewlap itself does not play a role in mate choice (Tokarz et al. 2005). Body size, bite force, and jumping capacity have also been investigated as possible factors in female mate choice; however no preferences were found (Andrews 1985, Lailvaux and Irschick 2006). Because of these disparate results, female mate choice in anoles has been suggested to occur only rarely (Tokarz 1995). However, males spend a large portion of their time and energy displaying to females, and such displays have been likened to displays of songs and plumage in birds, which are important traits in female mate choice in birds (West-Eberhard 1983, Alcock 2009, Losos 2009). Therefore, it is likely that female choice does occur in *Anolis sagrei* (Sullivan and Kwiatkowski 2007), and that males exhibit differences in traits that are important to females. Indeed, Losos (2009) suggests that female mate choice is an aspect of anoles that is ripe for future study as there are few robust results for most species.

Physiology

The capacity to exhibit energetically costly displays may play an important role in mate choice, and this capacity stems largely from metabolic support. Lizards in the genus *Anolis* rely primarily on anaerobic metabolism to fuel high levels of activity (Rose et al. 1965, Bennett et al. 1981, Gleeson and Dalessio 1989, Gleeson 1991, Gleeson 1996, Hitchcox 2009). Anaerobic metabolism leads to the buildup of lactate. In lizards, gluconeogenesis often occurs, which allows the animals to eliminate the lactate and re-synthesize glucose (Gleeson and Dalessio 1989, Gleeson 1991, Wickler and Gleeson 1993, Gleeson 1996, Hitchcox 2009). Blood glucose levels are also regulated through glycogenolysis, which breaks glycogen into glucose-6-phosphate, which can be converted to glucose (Shephard 1984). Thus, activities such as courtship displays in lizards commonly lead to depleted glycogen stores, increased lactate, and potentially increased glucose mobilization to the blood.

Indeed, performance ability has often been associated with reproductive success in several lizard species. For example, in Collared Lizards (*Crotaphytus collaris*), males with higher sprint speeds, measured as the time it took lizards to traverse 1m intervals along a racetrack, had larger territories and sired more offspring than males with lower sprint speed performance (Husak et al. 2006). Reproductive success in the Eastern Fence Lizard (*Sceloporus undulates*) is correlated with endurance, measured by running the lizards on a treadmill; home-range size and endurance both peak during the reproductive season (John-Alder et al. 2009). Finally, dominant Orange-throated Side-blotched Lizard (*Uta stansburiana*) males, who are typically more successful at gaining mates, have significantly greater endurance on a treadmill than subordinate males (Sinervo et al. 2000). In contrast, some performance abilities, such as bite force and jumping capacity in *Anolis* lizards, are important in predicting outcomes of male contests (Lailvaux et al. 2004, Lailvaux and Irschick 2007), but do not seem to have an effect on reproductive success (Lailvaux and Irschick 2006). This suggests that females may prefer traits that do not assist males in antagonistic encounters, but rather may prefer other performance abilities or traits.

Behaviors targeted to conspecifics and subsequent interactions also require energy. However, the degree to which they are energetically costly to the performers is unclear. After antagonistic posturing to competitors, male *Uta stansburiana* show decreased endurance on the treadmill and increased blood lactate levels (Brandt 2003). Male *Sceloporus jarrovi* expend significantly more energy, measured as CO_2 expenditure with increased aggression in the defense of a territory (Marler et al. 1995). However, male *A. carolinensis* did not have significantly elevated lactate levels after agonistic encounters, indicating that their agonistic behaviors are not particularly energetically costly (Wilson et al. 1990). Reproductive behaviors, including courtship displays, may also be costly but energetic outputs have not been measured directly. If displays convey important information about a male's fitness to females (Sullivan and Kwiatkowski 2007), one would assume energy is expended and therefore the behaviors would be subject to sexual selection.

Endurance, or the length of time it takes an individual to be unable to continuing running on a treadmill, has been shown to be a predictor of reproductive success in the lizard species *U. stansburiana* and *Sceloporus undulatus* (Sinervo et al. 2000, John-Alder et al. 2009). Endurance also influences posturing behaviors such as push-up displays in *Uta stansburiana* because the posturing males were still recovering from the exhausting exercise (Brandt 2003, Sullivan and Kwiatkowski 2007). However, these relationships have not been studied in a similar way in *A. sagrei*. In addition, endurance combined with measures of metabolic pathways have not been explored with respect to their role in female mate choice. In this study, I investigated the relationships among endurance, metabolism, and female choice, using blood glucose and stored glycogen in liver and muscle tissue to understand metabolic capacity and endurance for locomotor performance. I predicted that females would prefer males with higher endurance scores, higher blood glucose levels, and higher muscle glycogen levels because these males should have the capacity for high levels of activity. Under natural conditions, such males would be able to court females more rigorously and maintain larger and better territories than other males (Tokarz 1995).

Environment

Another important factor in the natural history of *A. sagrei* is the quality of the area in which resources are acquired. Male anoline lizards are highly territorial and rigorously defend their territories against neighboring males. Body size is important in territory acquisition, and larger males of most polygynous lizard species have bigger or better territories (Tokarz 1995). Larger green anoles (*Anolis carolinensis*) frequently defeat smaller males in dominance contests (Jenssen et al. 2005). Furthermore, dominant male *Uta stansburiana* with larger total body size acquire better territories than subordinate males (Fox et al. 1981), although exhibiting a particular color morph may become more important in determining territoriality during the breeding season (Calsbeek and Sinervo 2002a). Territories serve a number of purposes, but a major component of territory value for both brown and green anoles is access to females, since several females will establish their own smaller territories within the territory of one male (Schoener and Schoener 1980, Tokarz 1998, Losos 2009).

Although female brown anoles reside within the territory of one male, they may choose their territory based on attractive qualities of the occupying male. However, since male dominance interactions have the potential to result in a new male taking over the territory, female mate choice may also be based on the resources a male's territory can provide, such as food, shelter, egg-laying sites, and protection, rather than the male's distinct phenotypic or physiological traits (Andrews 1985, Tokarz 1995). Female *Uta stansburiana*, for example, show a preference for enhanced territories with such resources (Calsbeek and Sinervo 2002b).

Although resource-based female choice has been suggested as a likely form of female choice in *Anolis sagrei* (Andrews 1985, Tokarz 1995), no studies have been published that investigate the merit of the suggestion. Therefore, I addressed this deficiency by testing if females exhibit a preference for males based on male territory quality. I expected females to prefer superior territories, regardless of male phenotype, because they would provide more refuges and potential egg-laying sites.

METHODS

Assessing the role of physiological traits

To test the relationships among female choice, endurance, liver glycogen, muscle glycogen, and blood glucose, 50 male and 15 female *Anolis sagrei* were acquired from Glades Herps Farm in Florida in October 2011. Males were stored in individual containers, each identified by and individual number. The males were assigned to the experimental group or one of four control groups using a stratified random numbering system. The lizards were given five days to acclimatize to the laboratory conditions before any tests were conducted. Four groups of control males were then placed in choice tests and each group had six individuals. Males in the first control group were used in the female preference tests, but no blood was drawn or endurance tests conducted. Males in the second control group had blood drawn and were subjected to endurance tests but were not used in female preference tests. Males in the third control group were euthanized immediately upon arrival at the lab and blood and tissue samples were dissected and frozen for later analysis. Males in the final control group were held in the laboratory for the duration of the experimental trials to control for captivity effects, then euthanized and sampled for blood and tissues at the end of female choice trials.

Endurance Trials

The endurance tests were conducted by running each lizard on a treadmill until the lizard would no longer run, even when prodded. Each lizard was given an endurance index, which is the elapsed time (in seconds) spent running on the treadmill (after Sinervo et al. 2000). Blood was drawn two hours before and immediately after each endurance test to measure initial and post-exercise blood glucose levels. All blood samples were obtained by nicking the skin on the dorsal side of the body at the base of the tail. The blood was collected using a capillary tube and the glucose levels were measured using an AlphaTrak meter (Abbot Labs). After blood was drawn following the endurance tests, the wound was covered with VetBond to facilitate healing and prevent infection. Body mass, snount-vent length (SVL) and total length of every male were also measured using a portable scale (Ohaus CS200) and a ruler. Males were rested for two weeks after the endurance tests, then used in female choice trials.

Mate Choice Trials

Males in the experimental group and the first control group were size-matched based on body length and weight, but with endurance indices that were different by at least 30 s. The experimental arena had two identical territories for males, separated by a board so that the males could not see or interact with each other, but the female could see and access either male (Fig. 1).

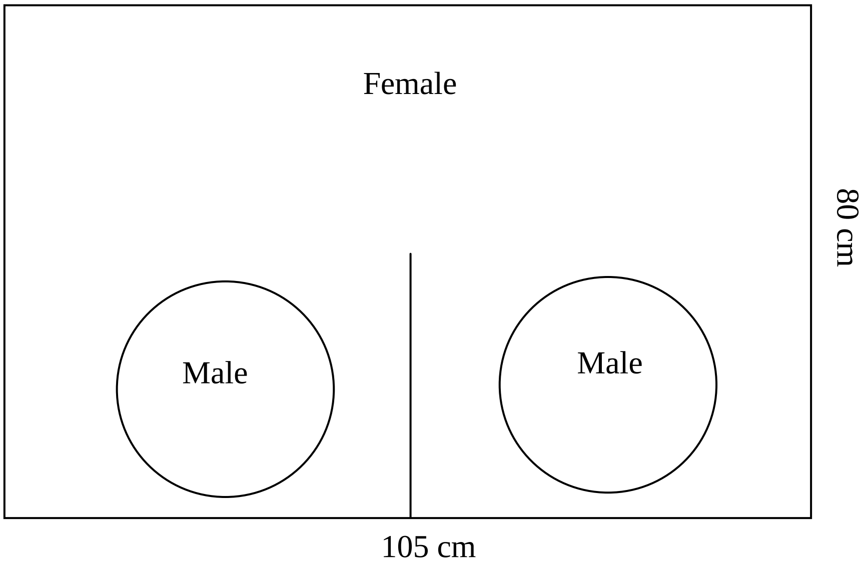

Figure 1. Schematic of the experimental arena, with an opaque divider between males 1 and 2, both of which were tethered.

Twenty-four hours before a preference test, blood from each male was sampled to measure initial blood glucose level. For each trial, both males were tethered anterior to the hind legs to posts so that they had a circle of about 30cm in radius (after LeBas and Marshall 2001, Hamilton and Sullivan 2005). Males were then acclimated to the arena without the presence of a female for 30 min. Once the female was introduced and allowed to interact with the males; her behavior was videotaped for 1.5 h. During subsequent video analysis, each male was given a score reflecting the amount of time the female spent with him (after Lailvaux and Irschick 2006). Male activity was also scored and placed in a category of 0-3, attempting to have equal numbers of . After the choice tests, the males were euthanized, and blood glucose was measured immediately. Liver and leg muscle tissue were dissected, frozen in liquid nitrogen, and stored at -80°C for subsequent glycogen assays.

Glycogen Assays

Liver and leg muscle tissue samples were analyzed for glycogen using a modified protocol from Keppler and Decker (1974) (Wells and Bevier 1997). Each tissue sample was weighed using an analytical balance, then homogenized in 10 times tissue volume of 0.6N perchloric acid solution using a glass-glass homogenizer, on ice. 200μL of each homogenate was removed and neutralized with 100μL of 1.0M $KHCO_3$ for total glucose analysis. The remaining homogenate was centrifuged at 4 ° C at 4500 rpm for 20 min using an Eppendorf Centrifuge 5804R. The homogenate was removed, neutralized with 0.05 g solid $KHCO_3$ per mL homogenate that was centrifuged, and frozen for use in free glucose analysis.

Using this protocol, glycogen must be digested to make glucose, which can then be measured. To do this, the neutralized 200μL of homogenate, for total glucose analysis, was treated with 1.0 mL of 10mg amyloglucosidase (Sigma, # A1602) per 10mL in 0.2M acetate buffer (pH = 4.8) solution. This mixture was incubated with shaking at 38 ° C for three hours. The reaction was stopped by adding 0.5 mL 0.6N perchloric acid, neutralized with 0.75 mL of 1.0 M $KHCO_3$, and centrifuged at 4 ° C at 10,000 rpm for 10 minutes.

Glucose concentrations were analyzed using a Glucose Diagnostic Kit (Sigma, # GAG020). The glucose oxidase/peroxidase reagent (PGO reagent) was resuspended in 100mL deionized water, and the o-dianisidine reagent in 2.0ml deionized water. 1.6ml of the resuspended o-dianisidine was added to the PGO reagent to create the assay reagent. 1.0 ml of this assay reagent was added to 100μl of the free glucose samples and to 150μl of the total glucose samples. The reaction of the assay reagent and the glucose in the samples turns shades of amber as a colorimeter test for glucose. After 30 min on a shaking incubator at 37 ° C, the absorbance values of each sample were measured with a Hitachi U-3010 spectrophotometer at 450nm. Glucose concentrations were calculated as the difference between total glucose and free glucose, then converted to glycosol units (μmol).

Blood Glucose Levels

Blood was collected from the base of the tail using a capillary tube; blood was transferred to a reading strip and the glucose levels were measured using an AlphaTrak meter (Abbot Labs). The differences in blood glucose level before and after the endurance and mate choice tests were calculated.

Video Analysis

Video recordings were analyzed using JWatcher 1.0 © (Blumstein, Daniel & Evans). I scored a set of behaviors that were determined to be important and consistent in the behavior trials. These included headbobs, pushups, dewlap extensions, male escape attempts, climbing, jumping, approach, and disinterest. All behaviors were given modifiers to indicate which animal did each behavior, and durations were recorded. The data gleaned from JWatcher © were placed in two groups for subsequent analysis, and included male energetic efforts and female active engagements with males. Males were determined to be exerting energy when performing headbobs, pushups, escape attempts, climbing, and jumping. For each male, the total proportion of time he spent doing all of these activities was summed. Each male was then given a rank based on the total proportion of time he spent moving. All of the males' scores were ordered and then divided as equally as possible into categories, ranging from 0-3, to describe his overall energetic effort relative to other males. Female active engagement was the total proportion of time the female spent approaching, courting (headbobs, pushups, dewlap extensions), and climbing and/or jumping onto the male's perch.

Assessing the role of territory quality

Twenty-six male and six female brown anoles were acquired from Carolina Biological Supply to supplement the remaining 15 female anoles from the previous physiological tests. Upon arrival, the snout-vent length (SVL) and body mass of each male were measured, and each was placed in an individual plastic box. Lizards were fed crickets and provided with water regularly. To avoid pseudoreplication, each female was used in only one trial.

The test arena for this experiment had two equally sized territories for males, one enriched with plants and the other with only one perch. Males were size-matched using SVL and weight and tethered in one or the other territory as determined by random assignment. The arena was constructed in such a way that the males could not see or interact with each other, but the female could see and access either male (Figure 1).

Males acclimated to their territories without the presence of a female for 15 min before the female was introduced. Once the female was introduced, her behavior was monitored and recorded using JWatcher for 45 min, following LeBas and Marshall (2001). Halfway through the trial, the males were removed from their original territory and placed in the other to control for preference for sides, and the female returned to the center of the compartment (after Andrews 1985, Smith and Zucker 1997).

Statistical Analyses

Two primary sets of data were generated, one on preferences based on physiological traits and the other on preferences for territory quality. All statistical tests were conducted using Stata11 © (StataCorp LP). All data were tested for normality using the Shapiro-Wilk test for normality. Data from males from the procedural control groups in the physiology tests were compared to those from males in the experimental group and other control groups when applicable using t-tests and Mann-Whitney U tests, depending on whether the data were normal or non-normal. Each variable measured for males in the control groups was tested separately. Data from males in the experimental group were paired to compare the males that had competed in female choice tests, and the difference between each of those variables was compared using a

Spearman Rank test. A Spearman Rank test was also used to test for a correlation between each of the physiological variables (energetic category, endurance, change in blood glucose after endurance tests, change in blood glucose after mate choice tests, liver glycogen, and muscle glycogen). Finally, Wilcoxon signed-rank tests were used to test for a difference between the blood glucose levels measured and the changes in blood glucose calculated.

Data from the territory preference trials were in the form of female choice scores and the male's environmental category (i.e. whether he had a high-quality territory or not). Wilcoxon signed-rank tests were used to test for a difference between the proportion of time females spent on each side and for a difference between the proportions of time females spent actively engaging males. A binomial test was also run to determine if females spent significantly more time than expected actively engaging one male over the other.

RESULTS

Physiology

Males exhibited a great range of variation in physiological characteristics. Mean body mass was 3.06g ± 0.14 (range 1.7 - 6.5g) and mean SVL was 4.73g ± 0.07 (range 3.8 - 5.6cm). The mean mass and mean SVL for males that were preferred were not significantly different from non-preferred males (Wilcoxon signed-rank, p>0.05) (Figure 2). The mean endurance index was 276.94s ± 18.99 (range 108.92 - 473.67s) (Figure 3). Glucose levels ranged from 141 to 488 mg/dL and the change in glucose after either the endurance or choice tests ranged from -227 to 347 mg/dL. Mean muscle glycogen was 4.49μmol ± 1.10 (range 0 – 47.94μmol), which was significantly lower than mean liver glycogen, 52.40μmol ± 13.55 (range 0.96 to 637.25 μmol)

(Wilcoxon signed-rank, z = 5.799, p<0.0001) (Figure 4). Physiological variables in males from the different control groups were not significantly different from each other or from those of the experimental group (Spearman-rank correlation, p>0.05). Blood glucose levels were not significantly different at the end of the endurance test or the mate choice trials than they were initially (Wilcoxon signed-rank, p>0.05). There was also no significant difference between the change in glucose during endurance or during mate choice trials (Wilcoxon signed-rank, p>0.05). Males with greater endurance indices males did not have a larger change in blood glucose levels than low-endurance males (Wilcoxon signed-rank, p>0.05) (Figure 5).

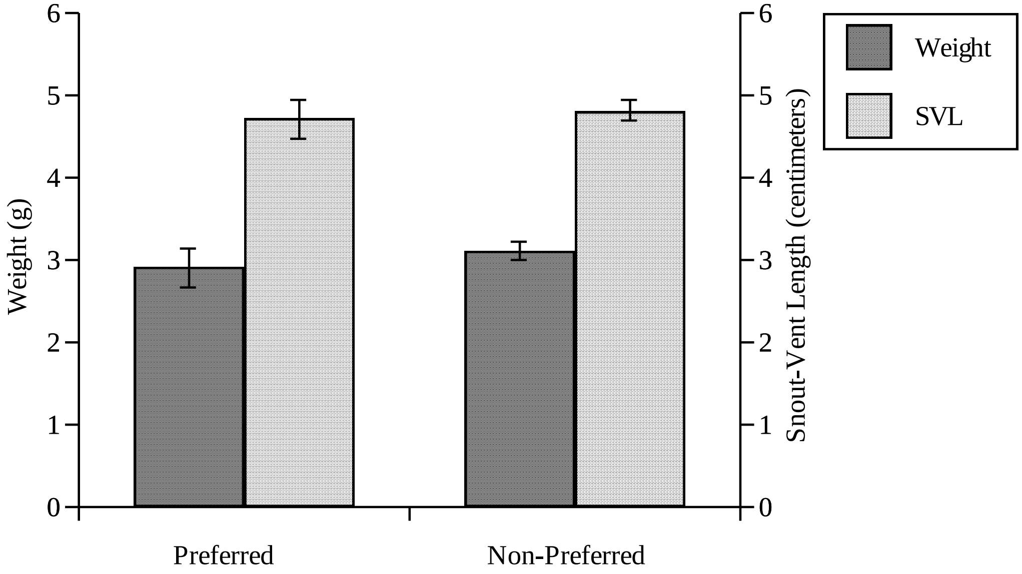

Figure 2. Mean mass and snout-vent length (SVL) of preferred and non-preferred males. Error bars indicate ±1SE. A Wilcoxon signed-rank test showed no significant difference in either mass or SVL.

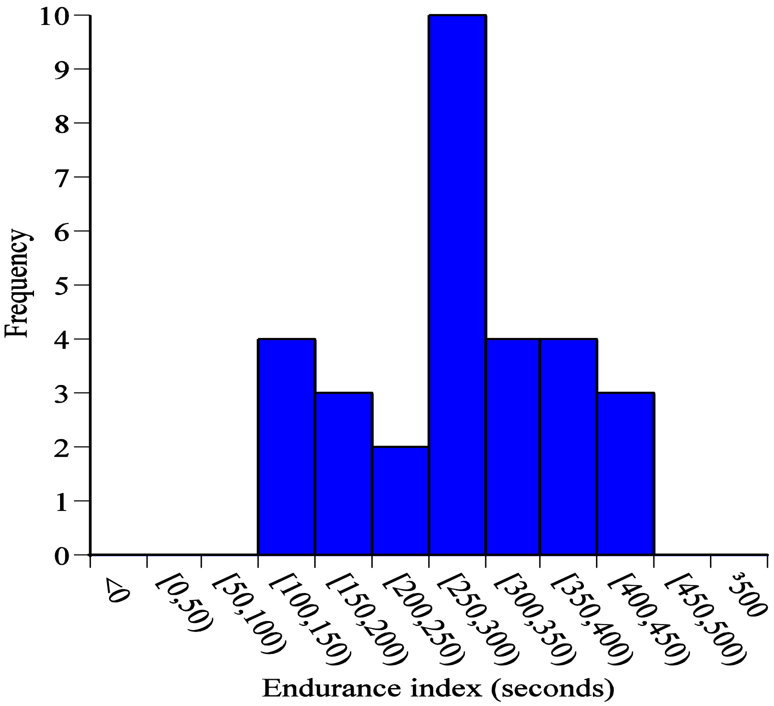

Figure 3. Distribution of male endurance indices. High-endurance males had endurance indices ranging from 210.10 seconds to 438.52s and low-endurance males had endurance indices ranging from 108.92s to 347.29s.

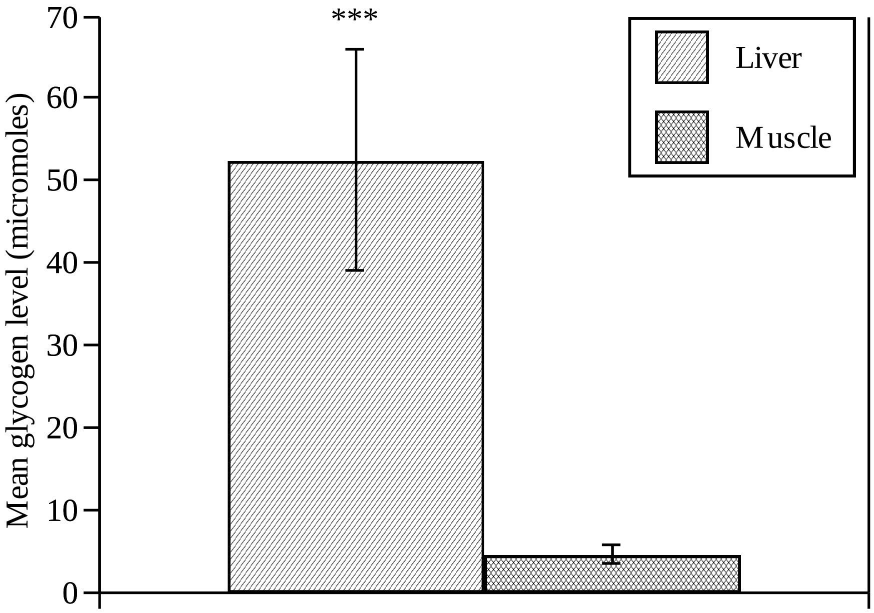

Figure 4. Mean liver and muscle glycogen levels in male *A. sagrei*. Error bars represent ±1SE. (*** indicates p<0.0001, Wilcoxon signed-rank test).

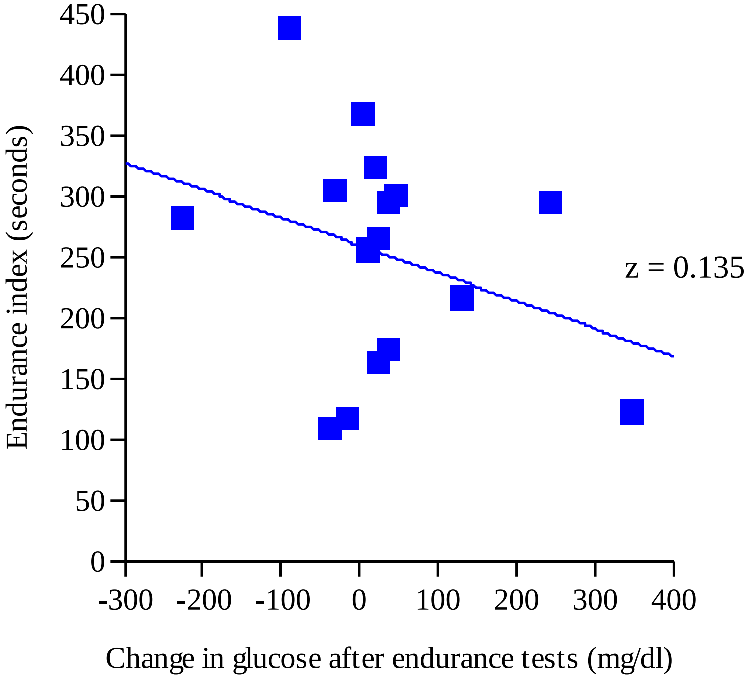

Figure 5. Relationship between endurance indices and change in blood glucose levels after endurance tests.

Of the 15 trials, females made a choice in 14. In 11 of those 14 trials, females showed a preference for the male that had a higher endurance index, which was a significant preference for high-endurance males (one-tailed binomial test, $p=0.029$). However, no significant correlation was found between the difference between female choice scores for high-endurance and low-endurance males (Spearman-rank correlation, $r_S = -0.4476$, $p=0.1446$) (Figure 6). There was a negative correlation between high endurance and low endurance males in female choice scores and liver glycogen (Spearman-rank correlation, $r_S = -0.5954$, $p=0.0411$) (Figure 7). Only three pairs of males in the experimental group were not missing a blood glucose reading so those data were excluded from correlation analysis. There was no significant correlation between any of the physiological variables when not paired ($p>0.05$).

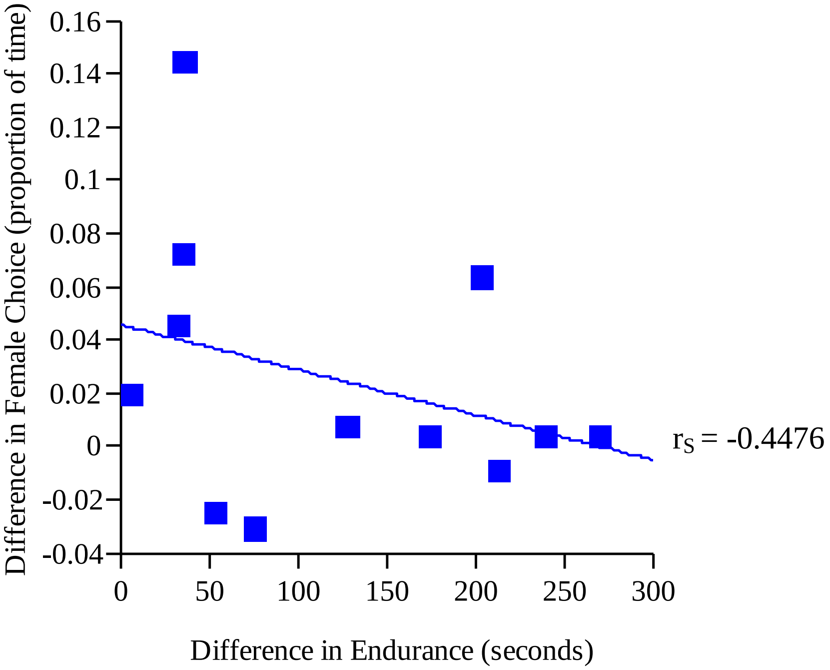

Figure 6. Relationship between difference in endurance scores of the high and low endurance males and the difference in female choice scores.

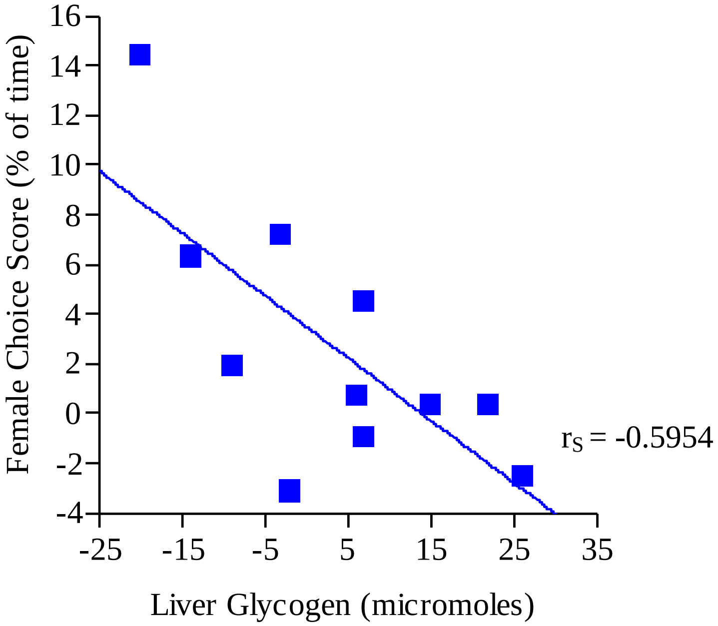

Figure 7. Relationship between the difference in female choice scores of high-endurance vs. low endurance males and liver glycogen levels.

Environment

In all 18 trials that tested female preference for the male's environment, the female remained in the center of the choice arena initially for at least 30 s, and usually for 1 min or more. Since the males were switched halfway through each trial, females had two new opportunities to choose a side per trial. In every trial half except one, the female went to one side and stayed on that side for the remainder of the period. Often she went to one of the corners in the back or behind a plant, perhaps to, unsuccessfully, look for an escape from the arena. In 8 of 18 trials (44.44%) the female returned to the same side of the arena after she was removed and returned to the center for the second half of the trial (Figure 8). Of those eight trials in which the female returned to the same side, only three of those returned to the side of the arena with the supplemented environment. Females did not spend a significantly greater proportion of time in one side than in the other (Wilcoxon signed-rank, z = 0.152, p = 0.8789) (Figure 9).

In the 10 trials (55.56%) where the female changed the side of the arena she preferred when given a choice, she chose the side with same male she had chosen in the first half of the trial. This is not significantly more often than would be expected by chance (binomial test, p =0.17) (Figure 10). However, females spent a significantly greater proportion of their time actively engaging one male over the other (Wilcoxon signed-rank, z = 2.069, p = 0.0386) (Figure 11).

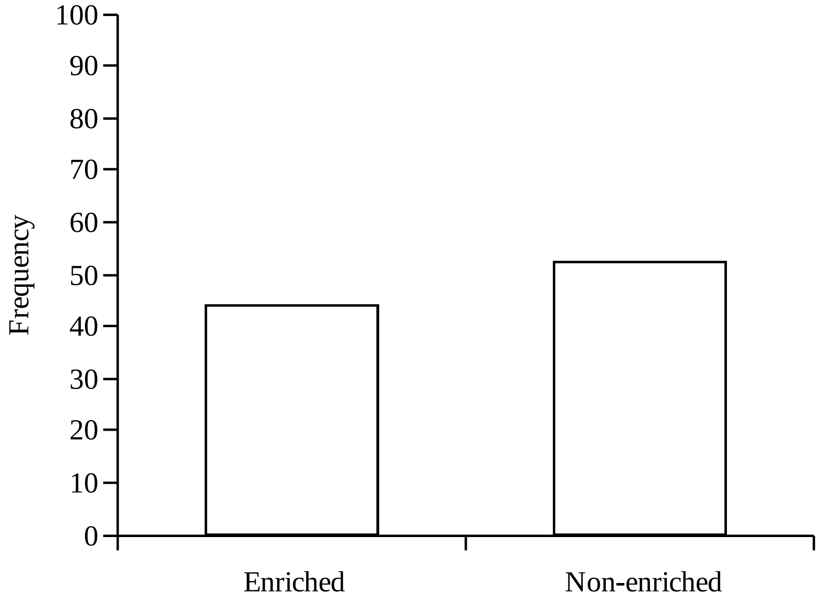

Figure 8. The percentage of times females chose the enriched side of the mate choice arena over the non-enriched side.

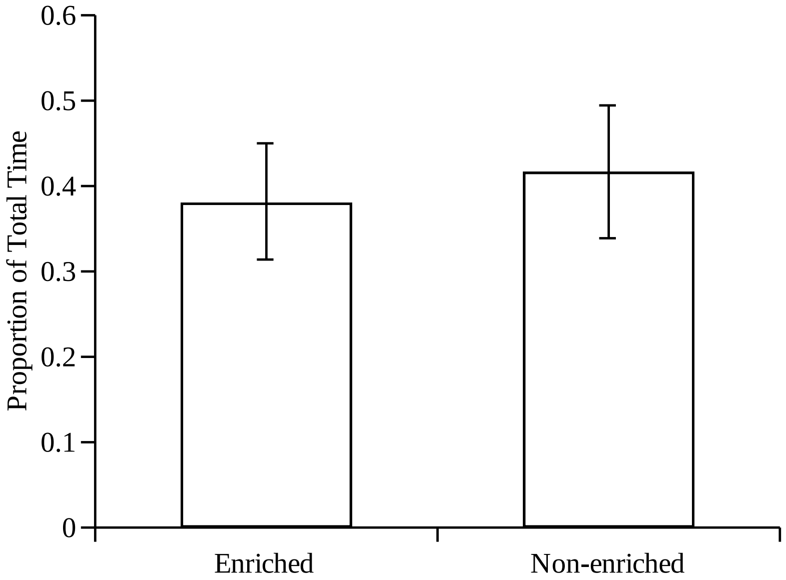

Figure 9. The mean proportion of total time the female spent in the enriched side of the arena vs. the non-enriched side.

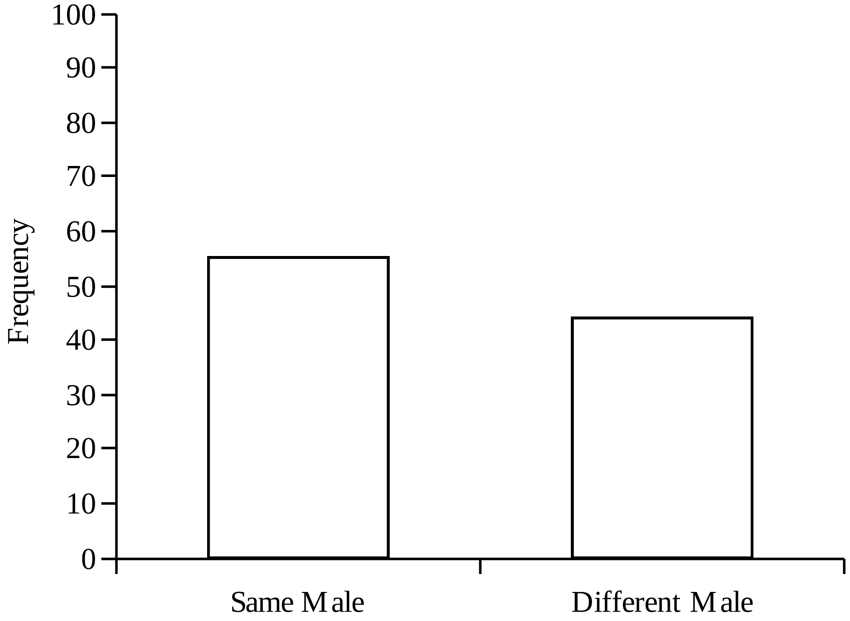

Figure 10. The percentage of trials in which the female went to the side with the male she had chosen in the first half vs. the trials in which she went to the side with the different male.

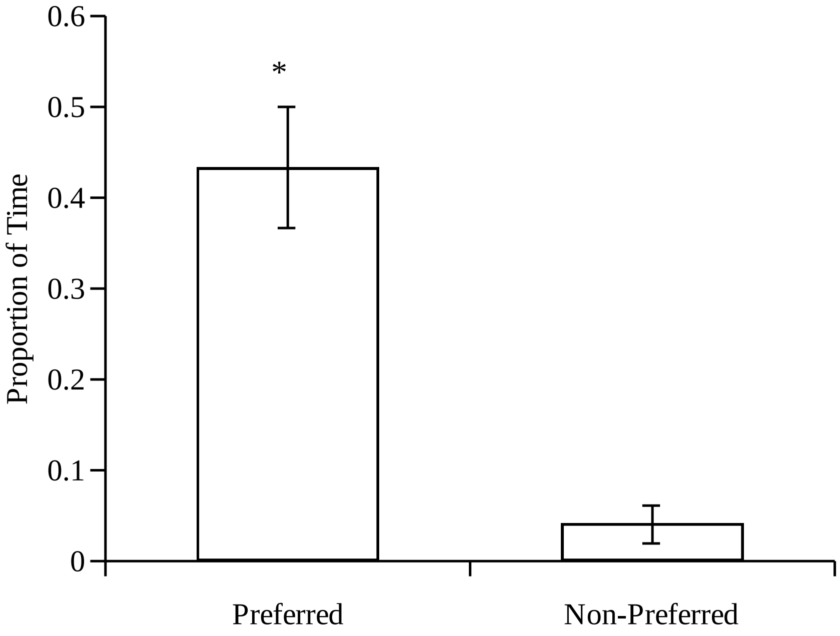

Figure 11. The proportion of time the female spent actively engaging one male versus the other. (* indicates $p<0.05$, Wilcoxon signed-rank test).

DISCUSSION

Physiology

Data from the mate choice trials reveal that females went to the side of the arena with the high-endurance male significantly more frequently than would be expected by random chance (Fig. 4), suggesting that females preferred some trait the high-endurance males possessed, which supports my hypothesis. However, since female choice scores were not correlated with endurance scores, the females may have preferred a less conspicuous trait that high-endurance males are more likely to express. I am relatively confident that the effect of captivity and undergoing the various treatments did not significantly influence the preferred males' targeted traits, since the variables measured in males from the procedural control groups were not significantly different from each other or from those of the experimental group.

There was relatively strong female preference for the males with lower liver glycogen levels (Fig. 7). This reflects that females preferred males that depleted their liver glycogen stores in the course of the choice tests. Females cannot measure hepatic glycogen levels, so they must judge an indicator of some sort in order to prefer the male. Mate choice trials generally consisted of some activity, and any prolonged activity would likely have been anaerobic, since anaerobic glycolysis serves a more predominant function in respiration in reptiles and amphibians than mammals (Rose et al. 1965, Gleeson and Dalessio 1989). In leopard frogs (*Rana pipiens*) and the American toad (*Bufo americanus*), much of the lactate produced (up to 39% in the American toad) was removed through gluconeogenesis (Gleeson 1996), which produces glucose from lactate, and primarily occurs in the liver tissues. Additionally, glucose can also be produced by glycogenolysis in liver and muscle tissues (Shephard 1984). If gluconeogenesis and glycogenolysis were occurring in the male *Anolis sagrei*, then this could explain the depleted hepatic glycogen. If the glycogen in the liver was depleted through heavy exercise, it seems likely that females were exhibiting a preference for males who were more active. However, there was no correlation between choice scores and energetic scores. This could be due to the way energetic scores were categorized, so that important differences between individuals were overlooked. The sample size was also fairly small, so effects may become more obvious with additional trials. Females also may assess males based on actions that take up a smaller proportion of time but are still energetically costly.

Results showing that females chose males that are more active is consistent with the findings of Greenberg and Noble (1944), who report that females chose the male that courted more frequently regardless of his dewlap color. Males who court more frequently or intensely would likely exert more energy in courtship than males who court less frequently or with less intensity. Thus, it is not surprising that females preferred males that depleted their liver glycogen, since this means that they were active and able to convert glycogen stores into glucose available for use during anoxic conditions caused by exercise (Shephard 1984). If frequency or intensity of courtship behaviors are the indicator of condition that females use to judge males, it could explain why female choice scores were not related to energy expenditure scores. Energy scores reported a sum of the proportion of time during each trial that males were active at all, including active behaviors that were not related to courting the female (e.g. escape attempts). If females only focused on the frequency, intensity, or duration of courtship behaviors, then the energetic scores would not accurately represent what the female evaluated.

Females may also be able to discern the difference between males that simply exert energy in one burst and males that use smaller "warm up" activities beforehand. Males that were given such "warm up" exercises before five minutes of exhaustive exercise on a treadmill did have higher blood lactate but were better able to metabolize that lactate and so had shorter recovery periods (Scholnick and Gleeson 2000). Although it is unknown if males exhibit a variety of such "warm up" behaviors naturally, it is likely that there is some variation. Perhaps females can discern any variation and prefer males that are active even before forced exercise. These males would have a decrease in liver glycogen but would also have faster recovery times, which I did not test for in my experiments.

Endurance may not be the best indicator of male physiological condition although it does provide an indicator of a male's capacity to sustain a highly taxing activity. However, high endurance comes with trade-offs in other aspects of physiological condition, such as immune function. Male *Anolis sagrei* with higher endurance had low immune function, and thus a lower probability of survival (Calsbeek and Bonneaud 2008). If females prefer males that have higher probability of survival so that their offspring may have higher chances of survival, females would likely choose males with lower or intermediate endurance scores since they would have higher survival rates. Indeed, the highest female choice score in my study was for a male with a fairly low endurance score.

Environment

In contrast to results of the physiology experiments, my hypothesis for the environment preference experiments was not supported in any way. I expected that females would exhibit a preference for the half of the arena that was supplemented with plants and perches. Results from the experiments show that there was no significant difference between the amount of time the females spent on either the supplemented environment side or on the non-supplemented side. Resource-based choice has been suggested as a likely mode of female choice in anoline lizards (Schoener and Schoener 1980, Andrews 1985, Tokarz 1995), but this study suggests that females do not preferentially choose better male territories in which to temporarily reside.

Females did exhibit a preference for one of the two males in the trial. Males were matched for size and tethered to reduce potential display behavior. Although females did not choose one male twice as frequently as would be expected by chance, females did spend a greater proportion of the total trial time actively approaching one of the males than the other. This suggests that one of the males may have exhibited one or more preferred traits. Unlike in

Uta palmeri, it seems that selection for male morphological (or physiological) traits in *Anolis sagrei* may not be through differential mating success because of an ability to hold superior territories (Hews 1990): female *A. sagrei* do not apparently choose males based on their ability to hold a superior territory.

Although these results clearly show that female preference is stronger for males compared to environmental quality, field studies investigating the importance of male territories are warranted. The artificial setting of these lab experiments could lead to unrealistic results, but the presence of an observer and temporary captivity did not significantly alter the display behaviors of *A. sagrei* in the field (McMann and Paterson 2003).

More research is necessary to determine if the male characters driving female mate choice in anoline lizards are to be understood. My study suggests that females do exhibit choice for male traits, and not for male resources, but the specific traits females prefer are still unknown. Based on the female preference for males that deplete their liver glycogen stores during exercise during the physiology tests, it seems likely that females evaluate males based on indicators of physiological traits. However, there are other possibilities for female preferences. It could be that other indicators of physiological condition, such as sprint speed, jumping capacity, or frequency of courtship displays, play a role in female choice. Although the presence of the dewlap and its color have been shown to be unimportant in female choice (Greenberg and Noble 1944, Tokarz 2002, Tokarz et al. 2003, Tokarz et al. 2005), ultraviolet reflection on the dewlap or on the body could act to enhance intraspecific signals, as it does in the Augrabies flat lizard (*Platysaurus broadleyi*) (Whiting et al. 2006). Other non-visual cues could also play a role, such as olfactory cues during courtship in *P. broadleyi* (Whiting et al. 2009). Olfaction has not been investigated in anoline lizards, and could be an important mode of intraspecific communication.

CONCLUSION

The results of this study show that female brown anoles (*Anolis sagrei*) exhibit non-resource based mate choice and have a preference for specific male traits. Females likely judge males on their physiological condition, since they showed a preference for males that depleted their liver glycogen, which most likely occurred through activity. Males likely possess a trait or suite of traits that acts as an indicator of their physiological condition that females can discern and on which they may judge males. Further study into the specific traits females prefer is necessary for a greater understanding of intraspecific communication and intersexual selection in *A. sagrei*.

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