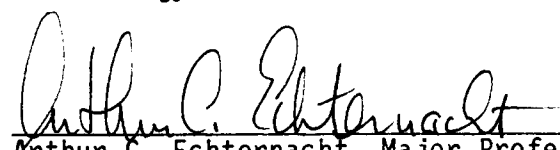
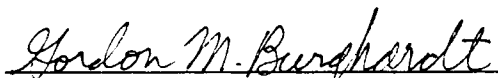


To the Graduate Council:

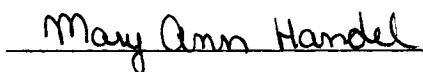
I am submitting herewith a dissertation written by Julia Karen Wade entitled "A Comparative Study of Reproduction in Two Populations of the Lizard, Anolis carolinensis." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Zoology.

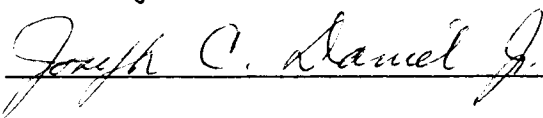

Arthur C. Echternacht, Major Professor

We have read this dissertation
and recommend its acceptance:

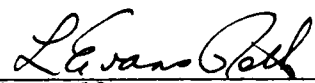








Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

A COMPARATIVE STUDY OF REPRODUCTION IN TWO POPULATIONS
OF THE LIZARD, ANOLIS CAROLINENSIS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Julia Karen Wade
August 1981

3053740

ACKNOWLEDGMENTS

First, I would like to acknowledge the financial support that I have received during this study. I received a teaching assistantship in the Department of Zoology and a fellowship from the American Association of University Women (1980-1981). The latter award is especially appreciated because it reduced my workload considerably and enabled me to complete my degree much earlier than I could have done otherwise. This award and a grant from Sigma Xi also provided research funds which were greatly appreciated.

A very special thanks goes to my graduate committee which has to be one of the best ever. Sandy Echternacht provided time, support, and encouragement throughout the study and allowed me the freedom to conduct research in some areas that were remote from his area of expertise. It was his enthusiasm and excellence as a classroom teacher that stimulated my interest in herpetology. I am indebted to him for broadening my interests by converting this mammalogist into a quasi-herpetologist. Harrison Ambrose has had an extremely positive and beneficial influence in shaping my career. He has repeatedly stimulated me to think critically and creatively, both in friendly discussions over beer and in formal seminars. His contributions, both as a teacher and especially as a friend, have been invaluable. I am indebted to Joe Daniel for providing laboratory space and equipment for my research, for introducing me to aspects of cell biology that I never knew existed, and especially for that enthusiastic personality

and winning smile that have brightened many otherwise dreary days. Mary Ann Handel is one of the few professors who can be an excellent teacher, a dedicated professional, and at the same time retain the "human" aspect of her personality. She has been a friend and confidante, and definitely is a role model for women in science. I thank Gordon Burghardt for instilling in me a deep interest in behavior that, although not reflected in this dissertation, will become part of my future research plans. I would especially like to thank all of the above people for their support and help during the final stages of this dissertation. They were extremely fast and efficient in providing feedback for revisions, and one of them (JCD) even read a draft of the dissertation while he was a patient in the hospital.

Three other zoology professors, although not on my committee, deserve special thanks for their help in certain aspects of this research. They provided advice, equipment and reagents, and were never too busy to answer the thousands of questions that I must have asked each of them. A very special thanks to Gary McCracken, Tom Chen, and Neil Greenberg.

The friendship and camaraderie of the people I have known at UT have made my years here some that I will always remember. I would like to thank the following people for their support, their advice on academic matters, and the many other ways that knowing them has enriched my life: Sue Bunch, Marie Turner, Peggy Girard, Jim Girard, Kate Ambrose, Ray Stone, Tony Dimatteo, Roy Scudder, Paul Weldon, Kitty Johansen, Bob Jones, Judy Stern, Dale Hansen, Bonnie Dunbar, Patsy Boyce, Maureen Allison, Tom Juntti, Steve Gay, George Middendorf,

John Wojowitz, Steve Seagle, Mike Sloan, Rob Wainberg, Nick Cheper, Mark Alston, Joel Harp, and Mike Schrimsher.

Those people who assisted in the collection of animals from other states deserve special mention. A very special thanks to Ray Stone for risking life and limb in helping not only with local collections but also in obtaining anoles from Birmingham, Auburn, New Orleans, and San Marcos. I also would like to thank the following people for providing and/or helping obtain anoles for this study: Marty Crump, Jon Ambrose, Ken Marion, Robert Mount, and George Folkerts. Herbert Dessauer kindly provided unpublished data for the New Orleans population.

Cornell Gilmore and Marilyn Caponetti have my thanks for aid in typing my dissertation. I would especially like to thank Sandy (-she) Echternacht for photographic assistance and Wynne Brown for doing most of the illustrations. These ladies all did exceptionally fast and accurate work.

Finally, I would like to thank my parents who have contributed more to this degree than anyone. They have provided endless moral support throughout the many years that I have worked toward this goal. It could not have been attained without their love and prayers. They deserve more thanks than I can express here.

ABSTRACT

This study of Anolis carolinensis assessed genetic variation between a Tennessee population and other mainland populations, and compared reproductive variability between the Tennessee population and the extensively studied Louisiana population. A comparison of reproductive strategies between these populations revealed adaptations this species has made while invading increasingly seasonal, temperate environments.

-- The genetic similarity and genic heterozygosity of seven mainland Anolis populations (Tellico, TN; Birmingham, AL; Auburn, AL; New Orleans, LA; San Marcos, TX; Gainesville, FL; Naples, FL) were compared. Results for overall genetic similarity were within the range found in other studies on interpopulation heterogeneity in lizards. Four populations (Birmingham, Auburn, Tellico, Gainesville) were similar, suggesting recent common derivation; least similar populations were New Orleans and Naples with Naples the most distinct. The peripheral populations, San Marcos and Tellico, had the lowest proportion of heterozygous loci.

Geographic variation in seasonal reproductive cycles was found between Louisiana and Tennessee populations. Both sexes of Tennessee anoles had larger gonads throughout most of the annual cycle. Tennessee males showed advanced spermatogenesis for five of twelve months. Hormone levels in Tennessee anoles peaked during February and March prior to maximal spermiogenesis in males and prior to the onset

vitellogenesis in females. In Tennessee and Louisiana, testicular recrudescence was correlated with temperature and testicular regression with photoperiod; however, photoperiod also was correlated with recrudescence in Tennessee anoles. Large females from both populations had a higher incidence of two oviductal eggs; however, in Tennessee the presence of two oviductal eggs also was related to the amount of precipitation. Oviposited egg weights and reproductive effort estimates differ significantly between populations.

The Tennessee population may be responding to local climatic conditions by showing more K-selected traits than Louisiana anoles. Tennessee anoles were larger as adults and as hatchlings, had larger eggs, and higher reproductive effort.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. GENETIC VARIATION AND SIMILARITY IN MAINLAND <u>ANOLIS</u>	
LIZARDS.	4
Introduction	4
Methods and Materials.	4
Results.	8
Discussion	13
III. A COMPARATIVE STUDY OF REPRODUCTION IN TWO POPULATIONS OF <u>ANOLIS CAROLINENSIS</u>	19
Introduction	19
Methods and Materials.	22
Results.	24
I. Male Reproductive Cycle	24
A. Morphology	24
1. Testes weight	24
2. Fat body.	30
3. Body size	35
B. Histology.	36
C. Hormonal Analysis.	40
II. Female Reproductive Cycle	42
A. Morphology	42
1. Ovary weight.	42
2. Fat body.	51
3. Body size	51
B. Hormonal Analysis.	54
C. Egg Data	54
Discussion	63
IV. OVERVIEW	78
LITERATURE CITED	82
VITA	90

LIST OF TABLES

TABLE	PAGE
1. Protein Loci Scored, Tissues Used, and Electrophoretic Conditions for a Study of Allozyme Variation Using Starch Gel Electrophoresis.	7
2. Allele Frequencies at Eight Polymorphic Loci in Seven Populations of <u>Anolis carolinensis</u>	9
3. Genetic Similarity Coefficients (I) Above the Diagonal and Genetic Distance (D) Below the Diagonal Calculated for All Pairs of Populations (Nei, 1972).	11
4. Levels of Heterozygosity and Proportions of Polymorphic Loci in Seven Populations of <u>Anolis carolinensis</u>	12
5. Summary Statistics for Three Studies of Mainland Populations of <u>Anolis carolinensis</u>	14
6. Relationship of Testes Weight to Stage of Spermatogenesis, Hormonal Level, Fat Body Weight, Body Weight, and Snout-vent Length at Different Times of the Male Reproductive Cycle in Tennessee Anoles	29
7. Relationship of Testes Weight to Photoperiod, High Temperature, and Low Temperature at Different Times of the Male Reproductive Cycle for Tennessee Anoles.	32
8. Statistical Analyses of Snout-vent Lengths (mm) and Body Weights (g) for Tennessee and Louisiana Populations of <u>Anolis carolinensis</u>	53
9. The Relationship of Female Size (Snout-vent Length, mm) to Number of Oviductal Eggs for Tennessee and Louisiana Populations of <u>Anolis carolinensis</u> for the Months May, June, July, and August.	58
10. Percentage of Tennessee and Louisiana Females Having Zero, One, and Two Oviductal Eggs During May, June, and July.	60
11. Environmental Data for Three Breeding Seasons (1979-1981) for a Tennessee Population of <u>Anolis carolinensis</u>	60
12. A Comparison of Oviductal and Recently Deposited Egg Weights for Tennessee and Louisiana Anoles.	61

TABLE

PAGE

13. Comparison of Reproductive Effort ($\bar{X} \pm \text{S.E.}$) Based on
Clutch Weight/Body Weight (CW/BW) and Clutch Calories/
Body Calories (CC/BC) Ratios for Oviductal and
Deposited Eggs for Louisiana and Tennessee Anoles 64

LIST OF FIGURES

FIGURE	PAGE
1. Localities Sampled in the Study of Intraspecific Genetic Variation in Mainland Populations of <u>Anolis carolinensis</u> .	5
2. Three Dimensional Principal Components Analysis Plot of Allozyme Frequencies for Seven Populations of <u>Anolis carolinensis</u>	10
3. Allele Frequencies at Six Loci for Seven Populations of <u>Anolis carolinensis</u>	15
4. Monthly Changes in Mean Paired Testes Weight for Tennessee and Louisiana Populations of <u>Anolis carolinensis</u>	25
5. Monthly Changes in Gonadosomatic Index for Tennessee and Louisiana Anoles.	27
6. The Relationship of Climatic Data (1979-80) to the Annual Testicular Cycle in a Tennessee Population of <u>Anolis carolinensis</u>	31
7. Seasonal Changes in Testes and Fat Body Weights in a Tennessee Population of <u>Anolis carolinensis</u>	33
8. Relationship of Testes Weight to Fat Body Weight in a Tennessee Population of Anoles.	34
9. Photomicrographs of Stages 1-7 of Spermatogenesis in <u>Anolis carolinensis</u>	37
10. Seasonal Changes in Testes Weight and Stage of Spermatogenesis for Louisiana and Tennessee Anoles.	39
11. Seasonal Changes in Testes Weight and Testosterone Level for a Tennessee Population of <u>Anolis carolinensis</u>	41
12. Seasonal Changes in Ovarian Weight for Tennessee and Louisiana Anoles.	43
13. Seasonal and Annual Changes in Ovarian Weight for a Tennessee Population of <u>Anolis carolinensis</u>	44
14. Monthly Changes in GSI for Tennessee and Louisiana Populations of <u>Anolis carolinensis</u>	46

FIGURE	PAGE
15. Relationship of Ovarian Weight and Fat Body Weight for a Tennessee Population of Anoles.	47
16. Annual Changes in Ovarian Weight, Precipitation, Light, Maximum Temperature, and Mean Temperature for a Tennessee Population of <u>Anolis carolinensis</u>	49
17. Relationship of Ovarian Weight and Photoperiod for a Tennessee Population of <u>Anolis carolinensis</u>	50
18. Seasonal Changes in Fat Body and Ovarian Weight for a Tennessee Population of <u>Anolis carolinensis</u>	52
19. Seasonal Changes in Ovarian Weight and Progesterone for Tennessee Anoles.	55
20. Relationship of Ovarian Weight to Progesterone Levels for a Tennessee Population of <u>Anolis carolinensis</u>	56
21. Relationship of Female Body Size (Snout-vent Length) to Percent of Oviductal Eggs Produced for Tennessee Anoles .	59
22. Summary of the Annual Reproductive Cycle of Tennessee <u>Anolis carolinensis</u>	69

CHAPTER I

INTRODUCTION

The iguanid lizard genus Anolis consists of approximately 300 species and is one of the largest of vertebrate genera (Williams, 1969). Many of these species have been studied extensively, both ecologically and taxonomically, and have been the basis for studies that have made major contributions to ecological theory. Although these studies are important, it is not known how representative or applicable some of the results and conclusions are to temperate populations of Anolis and other lizards.

Only one nominate species, Anolis carolinensis, has been successful in colonizing the more temperate regions of the mainland United States. It is believed that the ancestor of this species arrived in Florida during the Pleistocene following an overwater voyage from Cuba (Williams, 1969). The geographic range of A. carolinensis now covers the southeastern United States and extends peripherally as far west as Texas and as far north as Tennessee and North Carolina (Conant, 1975). Anoles are more common along the Coastal Plain than in the Mountain and Piedmont regions (Gordon, 1956). Outside of the Coastal Plain, populations of Anolis are often quite scattered and in some cases apparently isolated from each other.

Mainland anoles are highly variable (Williams, 1969), probably due to specific adaptations to local environmental and habitat conditions. For some populations, variability has been reported in external

morphology (Duellman and Schwartz, 1958; Gordon and Gratz, unpublished data) and in dewlap coloration (Crews, 1980). Recent studies, using electrophoretic techniques based on allozyme variation, have examined genetic variability and differentiation in insular and continental populations of Anolis carolinensis (Webster et al., 1973; Buth et al., 1980). These studies report a high degree of genetic uniformity among mainland populations. However, the Texas populations are the only populations examined near the edge of the range for this species; there have been no studies of populations from the northern and southern limits of the range. An examination of populations near the periphery of the range of this species may be crucial since selection may be more intense in regions where environmental conditions are more variable.

The discovery of a reasonably large population of Anolis carolinensis in east Tennessee, near the northern edge of the range for both the species and the genus, offered an unusual opportunity to gain insight into the means by which an essentially tropical vertebrate has adapted to decidedly temperate and seasonal conditions. Studies of this population, and others like it, provide a test of the generality of findings derived from the many studies of tropical and subtropical anoles as to how these may relate to the evolution of specific reproductive strategies. The purpose of the present study was two-fold. First, it was deemed necessary to assess the degree of genetic variability in the Tennessee population and the degree of genetic relationship between this population and others in the southeastern United States. Given the considerable geographic variation evident over the extensive range of A. carolinensis, this was considered

necessary in order to reduce the likelihood that the Tennessee population belongs to a species other than A. carolinensis. Second, a thorough study of the reproductive biology of the Tennessee population was undertaken in order that results of this study might be compared with those of the Louisiana population of A. carolinensis, the only other population of this species so studied. Although virtually all such studies of the Louisiana population have been confined to the laboratory, a comparison of the reproductive strategies of the two populations promised to provide some insight into the sorts of adaptations and possibly compromises this species has made as it has invaded increasingly seasonal, temperate environments.

CHAPTER II

GENETIC VARIATION AND SIMILARITY IN MAINLAND ANOLIS LIZARDS

I. INTRODUCTION

The green anole, Anolis carolinensis, is an iguanid lizard found commonly throughout many portions of the southeastern United States. Due to its high variability, the taxonomic status of mainland Anolis remains uncertain (Williams, 1969). Recent studies have used electrophoretic techniques to examine genetic similarities between insular and continental populations (Webster et al., 1973; Buth et al., 1980). These studies have examined southern populations in Texas, Louisiana, Georgia, and north Florida and found these mainland populations to be very similar to the insular populations. No studies have examined populations from south Florida, Alabama, or as far north as Tennessee. In this study, estimates of genic heterozygosity and similarity are presented for seven populations of A. carolinensis, including populations from near the northern, southern, and western limits of its known distribution in North America.

II. METHODS AND MATERIALS

Anoles were obtained between May 1980 and May 1981 from populations in seven localities shown in Figure 1. These sites and the number of lizards collected at each are: (1) Tellico Lake cliffs, Monroe Co., Tennessee, N = 12; (2) Birmingham, Blount Co., Alabama, 12;

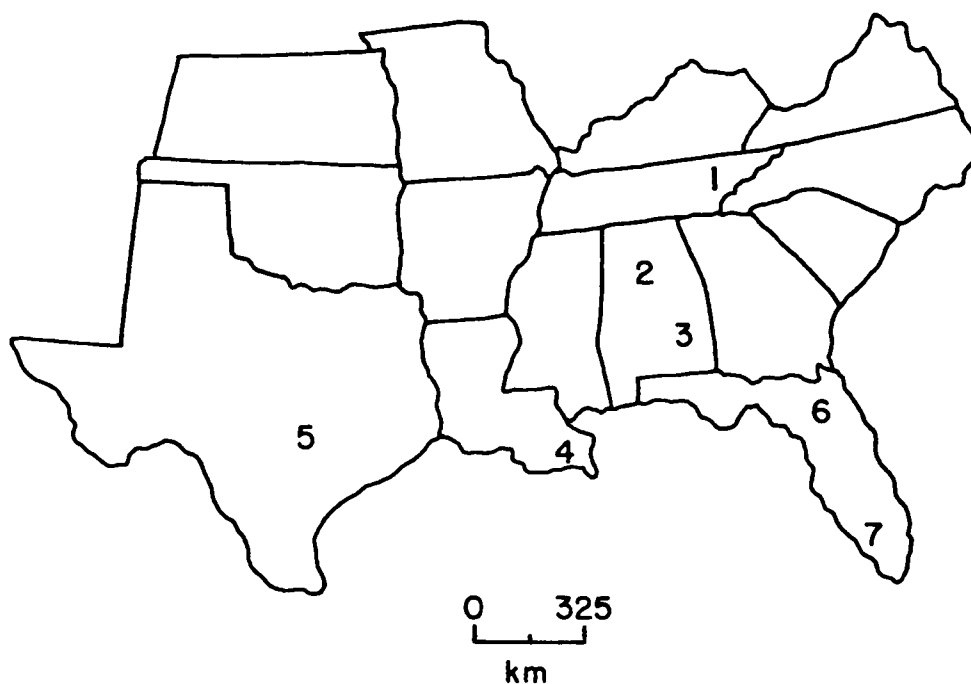


Figure 1. Localities sampled in the study of intraspecific genetic variation in mainland populations of *Anolis carolinensis*. Numbers 1 through 7 refer to populations in Tennessee (1), Alabama (2,3), Louisiana (4), Texas (5), and Florida (6,7).

(3) Auburn, Lee Co., Alabama, 15; (4) New Orleans, Orleans Parish, Louisiana, 15; (5) San Marcos, Hays Co., Texas, 12; (6) Gainesville, Alachua Co., Florida, 7; and (7) Rookery Bay near Naples, Collier Co., Florida, 7. It has been demonstrated that small samples do not significantly affect genetic distance estimates when many loci are examined (Gorman and Renzi, 1979).

All specimens were transported to the laboratory alive and processed within 3-10 days. Animals were killed by cervical dislocation, and muscle (whole hind legs) and liver were obtained from each lizard. Separate muscle and liver extracts were prepared by mixing each in a 2:1 ratio of grinding buffer to tissue (Selander et al., 1971). Tissues were minced, and centrifuged at 11,000 g for 20 min at 4°C. The supernatant fractions were collected and stored at -80°C.

Horizontal starch gel electrophoresis techniques were as described by Selander et al. (1971). Table 1 summarizes the tissues used, the electrophoretic conditions, and allozyme loci scored. Stain recipes were from Brewer (1970), Selander et al. (1971), and Harris and Hopkinson (1977). Alleles were lettered according to the relative anodal mobility of their protein products.

Genetic distance (D) and genetic similarity (I) between each pair of populations were estimated using the formulae of Nei (1972). The observed heterozygosity (H_o) per individual in a population was calculated by direct count of heterozygotes. Expected individual heterozygosity (H_e) was calculated from observed allele frequencies assuming Hardy-Weinberg equilibrium. The latter estimates were used to calculate heterozygote deviation D [$D = (H_o - H_e)/H_e$]. The proportion of polymorphic loci in each population is given as p .

Table 1. Protein loci scored, tissues used, and electrophoretic conditions for a study of allozyme variation using starch gel electrophoresis.

Protein Locus	Tissue Extract	Electrophoretic Conditions
Alcohol dehydrogenase (ADH)	Muscle	B
Creatine kinase (CK)	Muscle	B
Esterase (EST-1)	Muscle	B
Esterase (EST-2)	Muscle	B
General protein (GP-1)	Muscle	D
General protein (GP-2)	Muscle	D
General protein (GP-3)	Muscle	D
General protein (GP-4)	Muscle	D
Glutamate dehydrogenase (GDH)	Muscle	A
Glutamate oxaloacetate transaminase (GOT)	Muscle	D
α -Glycerophosphate dehydrogenase (α -GPD)	Liver	G
Isocitrate dehydrogenase (IDH)	Liver	A
Lactate dehydrogenase (LDH)	Muscle	D
Leucine aminopeptidase (LAP)	Liver	B
Malate dehydrogenase (MDH)	Muscle	F
"Malic enzyme" (ME)	Muscle	C
Mannosephosphate isomerase (MPI)	Muscle	C
Octanol dehydrogenase (ODH)	Liver	G
Peptidase (PEP-1)	Liver	C
Peptidase (PEP-2)	Liver	C
Peptidase (PEP-3)	Liver	C
Phosphoglucomutase (PGM-1)	Liver	D
Phosphoglucomutase (PGM-2)	Liver	D
6-Phosphogluconate dehydrogenase (6-PGD)	Muscle	F
Phosphoglucose isomerase (PGI)	Liver	E

A = Tris-citrate pH 8.0; B = Poulik; C = Lithium hydroxide;
D = Tris-maleate; E = Phosphate pH 6.7; F = Tris-citrate pH 6.7;
G = Tris-versene-borate (Selander et al., 1971).

III. RESULTS

Of the 25 loci examined, 18 were monogenic in all populations. Allelic frequencies for the eight polymorphic loci are presented in Table 2. The genetic relationships among all populations examined are summarized and shown graphically using a principal components analysis plot derived from the observed allele frequencies (Figure 2). The two most distinct populations are New Orleans and Naples ($I = .878$); the most similar are the Birmingham and Auburn populations ($I = .995$; Table 3). It can be seen from the principal components plot (Figure 2) that four populations (Birmingham, Auburn, Gainesville, and Tellico) are genetically very similar to one another (mean $I = .983$; range of $I = .995-.969$). The San Marcos population is intermediate between this group and the New Orleans population. New Orleans and Naples are the most distinct populations, with the Naples population the most distinctive.

The average proportion of loci heterozygous per individual (H_0) is shown in Table 4. The average H_0 for the seven populations examined was 0.0521 with a low value of 0.0183 for the Tellico population and a maximum of 0.068 for the Naples population. The Auburn population and the highest proportion of heterozygous loci ($p = 0.24$) while the Tellico and San Marcos populations had the lowest ($p = 0.08$). Observed heterozygosity (H_0) estimates did not differ significantly from those expected under Hardy-Weinberg equilibrium.

Table 2. Allele frequencies at eight polymorphic loci in seven populations of Anolis carolinensis.

Locus	Allele	Populations						
		Tellico	Birmingham	Auburn	New Orleans	San Marcos	Gainesville	Naples
ME	a	---	---	---	---	---	---	.64
	b	.85	1.0	.92	1.0	1.0	.93	.36
	c	.15	---	.08	---	---	.07	---
MPI	a	---	---	.21	.12	---	---	---
	b	1.0	1.0	.79	.88	1.0	1.0	1.0
PGM-1	a	---	.12	---	.80	---	.17	.17
	b	1.0	.88	1.0	.20	1.0	.83	.75
	c	---	---	---	---	---	---	.08
6-PGD	a	---	.04	.04	.08	---	.50	1.0
	b	1.0	.96	.96	.92	1.0	.50	---
PEP-2	a	---	.12	.07	.67	.04	---	.14
	b	1.0	.88	.93	.33	.63	1.0	.86
	c	---	---	---	---	.33	---	---
GP-1	a	---	.67	.46	---	---	---	---
	b	1.0	.33	.54	1.0	1.0	1.0	1.0
EST-1	a	.05	.21	.12	1.0	1.0	---	---
	b	---	---	---	---	---	---	1.0
	c	.95	.79	.88	---	---	1.0	---
IDH	a	---	---	---	---	.54	---	---
	b	1.0	1.0	1.0	1.0	.46	1.0	1.0

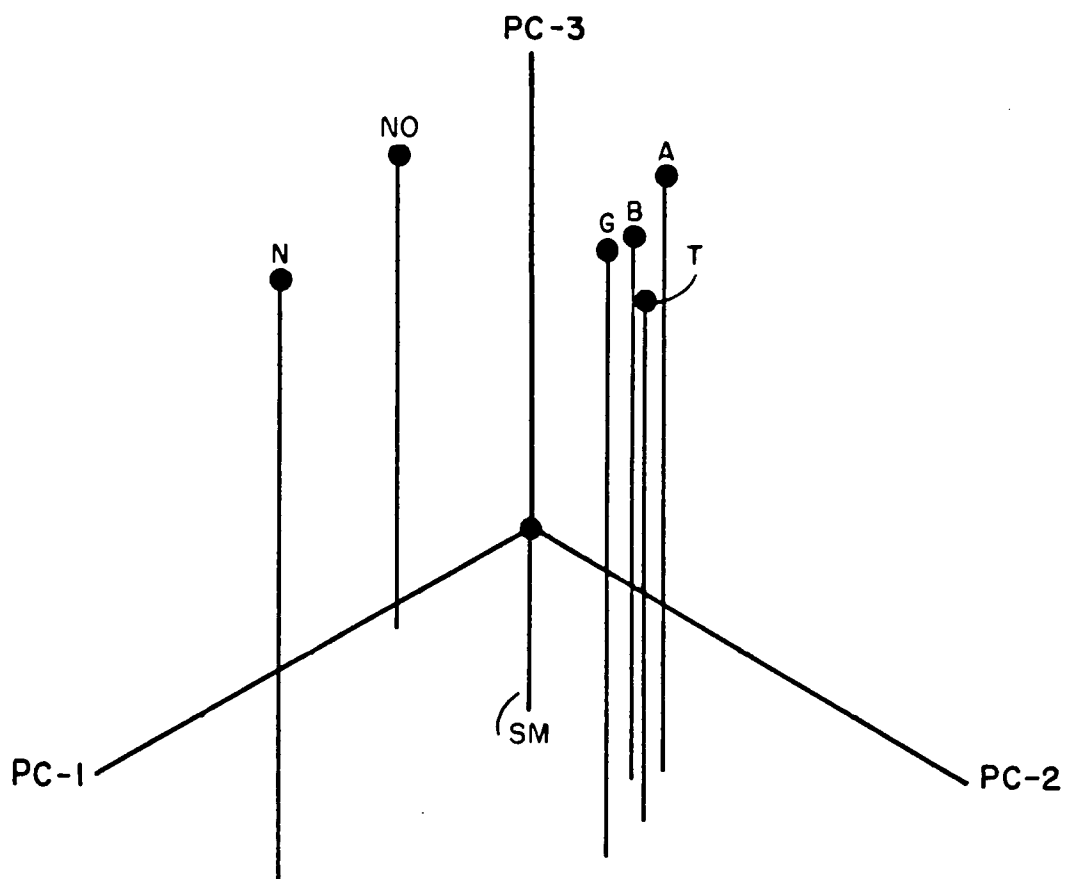


Figure 2. Three dimensional principal components analysis plot of allozyme frequencies for seven populations of Anolis carolinensis. The populations represented are Tellico (T), Birmingham (B), Auburn (A), New Orleans (NO), San Marcos (SM), Gainesville (G), and Naples (N).

Table 3. Genetic similarity coefficients (I) above the diagonal and genetic distance (D) below the diagonal calculated for all pairs of populations (Nei, 1972).*

Populations	Populations						
	Tellico	Birmingham	Auburn	New Orleans	San Marcos	Gainesville	Naples
Tellico	----	.978	.989	.916	.945	.988	.903
Birmingham	.022	----	.995	.922	.938	.969	.890
Auburn	.011	.005	----	.916	.940	.978	.895
New Orleans	.088	.081	.089	----	.948	.914	.878
San Marcos	.057	.064	.059	.053	----	.929	.882
Gainesville	.012	.031	.060	.090	.074	----	.931
Naples	.102	.117	.090	.130	.126	.072	----

*Calculations for all populations are based on allelic products of 25 loci.

Table 4. Levels of heterozygosity and proportions of polymorphic loci in seven populations of Anolis carolinensis.

Heterozygosity Estimates	Populations					
	Tellico	Birmingham	Auburn	New Orleans	San Marcos	Gainesville Naples
Observed Heterozygosity H_o	.0183	.0529	.0650	.0533	.0596	.0479 .0680
Expected Heterozygosity H_e	.0164	.0677	.0694	.0614	.0504	.0479 .3573
Heterozygote Deviation D	.1159	-.2186	-.0634	-.1319	.1825	0 .3573
Proportion of Polymorphic Loci p	0.08	0.20	0.24	0.16	0.08	0.16 0.12

IV. DISCUSSION

The major findings of this study are as follows: (1) Results for overall genetic similarity among all pairs of populations are within the range found in other studies on interpopulation heterogeneity in lizards (Hall and Selander, 1973; McKinney and Turner, 1971; McKinney et al., 1972), including other studies on anoles (Webster et al., 1973; Buth et al., 1980; Table 5). (2) The close similarity of the Birmingham, Auburn, Gainesville, and Tellico populations suggests recent common derivation; geographical locations are compatible with this explanation. (3) The New Orleans and Naples populations show the least similarity among the populations. (4) The Gainesville population is intermediate between the Naples and New Orleans populations and shows affinities with both in regard to specific alleles (for example, locus 6-PGD, Figure 3). (5) Observed heterozygotes do not differ significantly from those expected under Hardy-Weinberg equilibrium; therefore, there is no evidence for inbreeding in small populations, population subdivision (Wahlund effect), or heterosis.

The Tellico and San Marcos populations in this study were of particular interest due to their location near the limits of the range of the species. Both of these populations had the lowest proportion of heterozygous loci ($p = 0.08$) of any of the populations (Table 4). The Tellico population had an unusually low level of genic heterozygosity (.0183). Although our estimate of genic heterozygosity (.0596) for the San Marcos population was high, Webster et al. (1973) found reduced variability (.02) in Texas anoles from another locality.

Table 5. Summary statistics for three studies of mainland populations of Anolis carolinensis.

Data Summary	Wade	Buth et al.	Webster et al. (from Buth et al.)
Mean Genetic Similarity (I)	.935 $\pm$.036	.954 $\pm$.038	.992 $\pm$.006
Range	.878-.995	.932-.998	.989-.999
N/Population	7-15	2-3	15-102
No. Loci	25	35	28
% Polymorphic Loci	32	17	39
Mean Heterozygosity (H ₀)	0.0521	----	0.0490

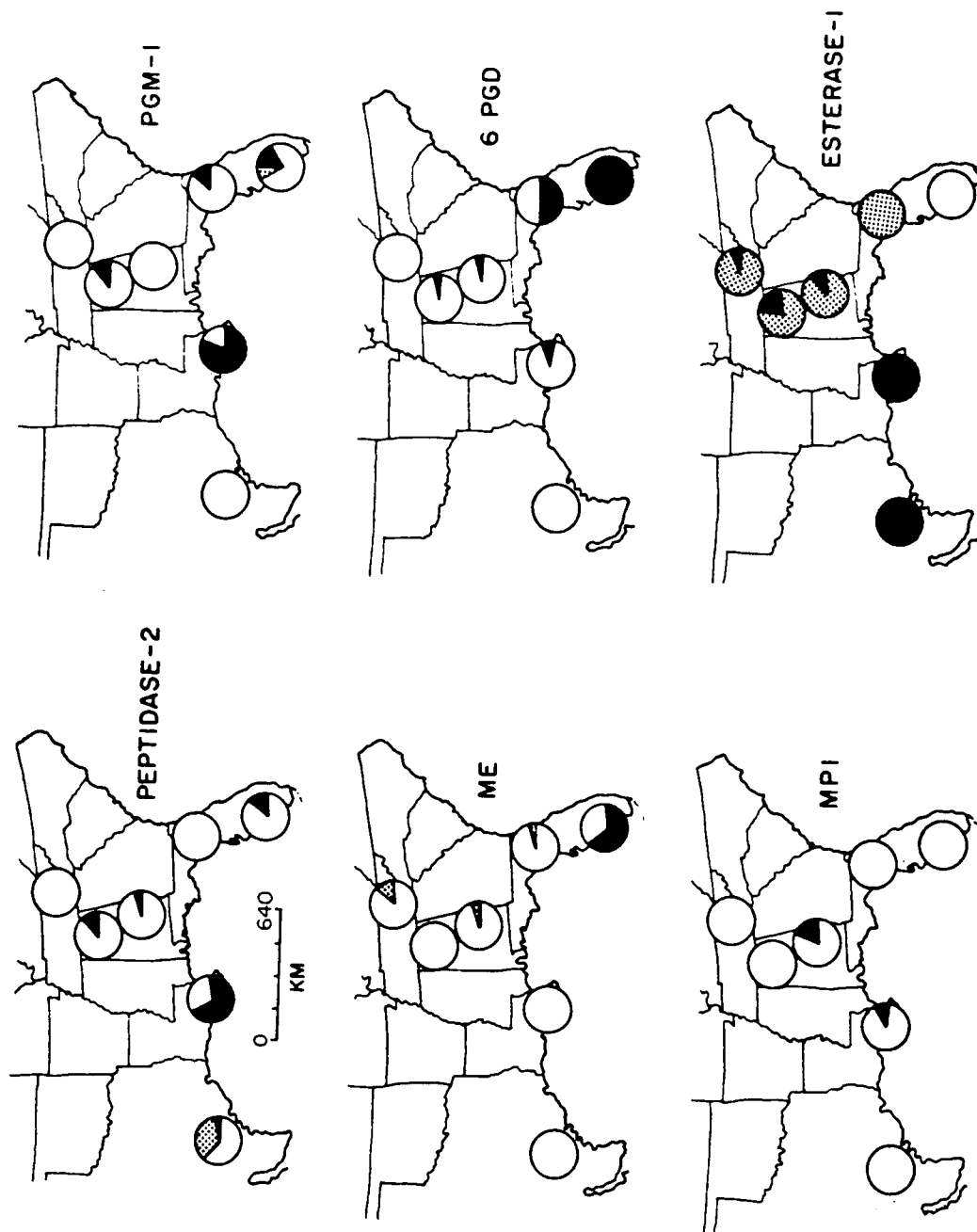


Figure 3. Allele frequencies at six loci for seven populations of *Anolis carolinensis*. The approximate location of each population is indicated by a circle with allele frequencies indicated by the proportion of the circle shaded. In the circles, dark areas represent the a allele, light areas the b allele, and stipples the c allele.

Our high heterozygosity estimate is largely the result of one robustly polymorphic locus, IDH, which is polymorphic in no other populations sampled. The San Marcos population also has another unique allele, Pep 'c,' which is found nowhere else. These results for the San Marcos and Tellico populations are likely due to genetic drift, founder effects, or decreased gene flow due to their locations near the limit of the species range. In addition, unstable climatic conditions may be exerting selection pressures on these populations.

Among anoline lizards, Anolis carolinensis is a relatively polymorphic species, and shows certain tendencies for morphological differentiation (Webster et al., 1973). Populations from south Florida have been examined previously, and morphological differences in external characters have been found (Duellman and Schwartz, 1958). Gratz and Gordon (unpublished data) found that peninsular Florida specimens showed marked differences in the number of loreal scales, subdigital lamellae, and dorsal scale rows. South Florida anoles also show variability in dewlap coloration (Crews, 1980). In this study, the Naples population was the most distinct population of the seven examined. It is not clear why this population is as distinct as it is. One possibility may be that the selection pressures of a subtropical habitat differ from those of more temperate areas.

The second most distinctive population was New Orleans. The genetic identity value between these populations (Naples and New Orleans, $I = .878$) is within but on the low end of interpopulation identifies found for conspecific lizards. Also, although not directly comparable because some different loci were used, this value is only

slightly higher than the similarity estimate of Buth et al. (1980) for A. carolinensis and its putative ancestor, A. porcatius.

Mainland populations of Anolis carolinensis are highly variable and may represent more than one species (Williams, 1969). This assumption is reasonable considering that Anolis could have readily and repeatedly colonized portions of the southeastern United States through dispersal along inland waterways. Certain populations also may represent escaped and/or released Louisiana anoles that were once sold commercially as pets. However, the sites of initial colonizations are unknown. Based on evidence from this study, there may have been two initial colonizations, one in Louisiana and one in south Florida. First, the Naples and New Orleans populations show higher genetic distances than the other populations (Table 3, page 11). Second, the Naples population is quite different from the other populations in allele frequencies at particular loci (Est-1, ME, PGM-1, and 6-PGD, Figure 3). Third, the New Orleans population shows higher frequencies than the other populations for particular alleles at certain loci (Pep-2, Est-1, and PGM-1, Figure 3).

Dispersal from a Louisiana site may have been northward, since the New Orleans population shares certain alleles with Alabama and Tennessee anoles (Est-1, PGM-1, MPI, Pep-2, and 6-PGD, Figure 3), and also westward since the New Orleans and San Marcos populations share alleles, although in different frequencies, for most of the polymorphic loci. Of particular interest is the Esterase locus because the New Orleans and San Marcos populations are the only populations

fixed for the a allele. The Gainesville population is interesting in that, though it differs from both Naples and New Orleans populations at certain loci (Est-1), it shares a high frequency of alleles with both the Naples population on the one hand (Pep-2, PGM-1, 6-PGD) and the New Orleans and other non-Florida populations on the other (MPI, ME, GP-1, IDH, Est-1, Pep-2). Thus, the Gainesville population may represent an intergrade between the other mainland populations and the Naples population (see for example, 6-PGD, Figure 3). Populations near the extreme limits of the range of the species, such as San Marcos and Tellico, may be showing, as indicated earlier, the effects of decreased gene flow or genetic drift.

In summary, it appears that the Naples and New Orleans populations represent separate colonizations of mainland anoles although the distinctiveness of the Naples population (at least) may be the result of different selection pressures in a subtropical environment. It is especially interesting to note that the vast majority of biological studies of Anolis carolinensis have been conducted on the New Orleans population, which the present study has demonstrated is not genetically representative of the species. It is apparent that comparative studies over the entire range of this species are necessary before generalities can be made.

CHAPTER III

A COMPARATIVE STUDY OF REPRODUCTION IN TWO POPULATIONS OF ANOLIS CAROLINENSIS

I. INTRODUCTION

Since it has been demonstrated that genetic variation occurs among mainland populations of Anolis (Chapter II), it would seem reasonable that geographic variation may be demonstrated in other aspects of their biology. Reproduction is highly adaptive, readily molded by natural selection, and should reflect any intraspecific variation that may be present among populations. Intraspecific variation in reproduction has been shown in lizard species such as Cnemidophorus tigris and Sceloporus undulatus (McCoy and Hoddenbach, 1966; Vinegar, 1975; Tinkle and Ballinger, 1972; Pianka, 1970) and illustrates the different ecological and genetic adaptations of a species to a particular environment (Mayhew, 1965; Licht and Gorman, 1975).

Because intraspecific differences in reproduction often are far more subtle than interspecific differences, a more detailed and extensive study of reproduction is necessary. The reproductive biology of the lizard, Anolis carolinensis, has been studied extensively with the reproductive cycle for the Louisiana population being especially well known (Gordon, 1956; Dessauer, 1955; Hamlett, 1952; Licht, 1971, 1973). This research has resulted in an extensive

literature concerning such subjects as the effects of controlled environmental influences, such as light and temperature, on reproduction (Licht, 1967a, b, 1971, 1972, 1973), the effects of social behavior on reproduction (Crews, 1973a, b, 1974b, 1975a, b, 1980; Crews and Greenberg, 1981; Crews et al., 1974), the effects of experimental manipulation of the reproductive cycle (Crews et al., 1974, 1978; Noble and Greenberg, 1941; Licht and Pearson, 1969; Pearson et al., 1976), and the effects of seasonal changes on the reproductive cycle (Dessauer, 1955; Fox, 1958). Although hormonal conditions, especially in relation to behavior and clutch size, have been determined through castration and/or hormone replacement experiments (Licht, 1970; Jones, 1975; Jones et al., 1973a, b, 1974, 1975; Crews, 1974a, Crews et al., 1978), no attempts have been made to examine both seasonal and hormonal changes in any populations of Anolis under natural conditions. Most of the above studies have been laboratory studies based on the Louisiana population and consequently, little is known concerning any intraspecific variation in reproduction that may be present in other populations of Anolis.

In addition to describing intraspecific variability in reproduction for a particular species, it is important to relate this variability to other species to provide insight into any emergent evolutionary patterns. Recent studies have demonstrated considerable interspecific variability in lizard reproduction and have related this variation to specific reproductive strategies (Tinkle, 1969; Tinkle et al., 1970; Tinkle and Hadley, 1975; Andrews and Rand, 1974). Based on studies of temperate and tropical lizards, two major strategies are

evident: early maturing, multiple brooded species and late maturing, single brooded species (Tinkle, 1969; Tinkle et al., 1970; Tinkle and Hadley, 1975). The early maturing, multiple brooded species (r-selected) are generally tropical in distribution and exert a greater reproductive effort per brood since they have fewer opportunities to reproduce. In contrast, multiple brooded, late maturing species (K-selected) are temperate in distribution, and because they live longer, they can apportion their reproductive effort over a longer reproductive life (Tinkle et al., 1970). Such trends are apparent, not only in comparisons between tropical and temperate species, but also intraspecifically where northern and southern populations have been compared (Tinkle and Ballinger, 1972; Pianka, 1970; McCoy and Hoddenbach, 1966; Vinegar, 1975). Although these strategies vary intraspecifically and have been discussed in relation to anoline lizards (Andrews and Rand, 1974), they have not been examined in mainland populations of Anolis.

This aspect of the study was designed to determine whether or not intraspecific variation in reproduction occurs between two geographically separated populations of Anolis carolinensis. Seasonal changes in the reproductive cycle of a Tennessee population were described and quantified, and this information was compared with published data for the Louisiana population. Differences between the two populations were discussed in terms of reproductive strategies that each population may be exhibiting.

II. METHODS AND MATERIALS

Anoles were collected for this study from an area (Tellico) located 60 km south of Knoxville in Monroe Co., Tennessee. The animals from the Tellico area will be referred to as the Tennessee population, and anoles from New Orleans will be called the Louisiana population. From three to seven lizards were captured by hand from the Tennessee population at two week intervals from May 1979 until July 1980 and from May through July 1981. In the field, animals were toe-clipped, and the time and location of capture were recorded. Lizards were transported to the laboratory in a cloth bag and were processed within 2-11 h after capture.

After measuring total length and snout-vent length, animals were killed by cervical dislocation, and the time of death was recorded. A midventral incision was made, and as much blood as possible (10-200 μ l) was collected directly from the heart using a heparinized syringe fitted with a 27G needle. The blood was centrifuged, and plasma frozen at -80°C for future analyses.

After blood was collected, the following measurements and/or weights were made prior to preserving the tissue: (1) both sexes--body weight, fat body weight; (2) males--testis weights, testis length and width, paired epididymis and kidney weight, and (3) females--ovary weight, oviduct weight, largest follicle size, number of eggs/oviduct, egg length and width, and egg weight. All tissues were fixed in AFA [50% water, 30% ethanol (95%), 10% formaldehyde, and 10% acetic acid for at least one week and were then stored in 70% alcohol. All carcasses were preserved in AFA and retained.

One testis, epididymis, and kidney from each male were dehydrated 1-2 h in methyl cellosolve, cleared in benzene for 1/2-1 h, and placed in 56-58°C liquid paraffin for 1-1/2 h under a 20 pound vacuum. Paraffin blocks were sectioned at 10 μ , and the sections mounted and stained with Ehrlich's hematoxylin and eosin. The stage of spermatogenesis for each male was determined based on the seven stages described by Licht (1967a), and seasonal histological changes in the gonads were described.

Plasma testosterone and progesterone were measured in males and females, respectively, using previously validated radioimmunoassays (Gay and Kerlan, 1978; Gibori et al., 1977). The extraction of corticosterone and deoxycorticosterone from the plasma samples was performed using anhydrous ether for the testosterone extraction and petroleum ether for the progesterone extraction. No column separation of either steroid was necessary due to the purity and low cross reactivity of the antibodies. The antibodies used, #250 anti-testosterone 11-BSA serum and GDN #337 anti-progesterone 11-BSA serum, were obtained from G. D. Niswender (Colorado State University, Fort Collins). The sensitivities of the assays were 32 pg/assay tube for testosterone and 63 pg/assay tube for progesterone. The mean intra- and interassay variation was 9.6% and 16.2%, respectively, for progesterone, and 8.1% and 12.4% for testosterone.

From 15 to 20 pairs (male and female) of anoles from each population (Tennessee and Louisiana) were kept in an environmental chamber for four months. Each pair was housed in a one gallon glass jar with a wire mesh lid. The jars contained sand and moss, sticks

for perches, and water dishes. Animals received crickets and water ad libitum. The parameters controlled inside the environmental chamber included temperature (30-32°C day, 23°C night), light (14 h light, 10 h dark) and humidity (approximately 20%). These conditions (except for humidity which we could not maintain at the desired 60%) have been shown to maintain spermatogenesis and vitellogenesis in captive Louisiana anoles (Licht, 1971, 1973).

Jars were checked daily for eggs. When eggs were present, the egg and female both were weighed, and their conditions recorded. After weighing, eggs were placed in circular (11.5 cm diameter) finger bowls filled with damp vermiculite. The bowls were enclosed in plastic bags and were left on a shelf at ambient temperature (which approximated that of the Tennessee study area) until the eggs either hatched or perished. Duration of incubation and hatchling size were recorded for each population.

Statistics were performed according to procedures in Sokal and Rohlf (1969). The assumption of homogeneity of variances was met before any data were pooled. Unless otherwise indicated, data are expressed as means $\pm$ 1 standard error (S.E.).

III. RESULTS

I. Male Reproductive Cycle

A. Morphology.

1. Testes weight--The overall shape of the curves for seasonal changes in mean testes weight (Figure 4) is basically the same

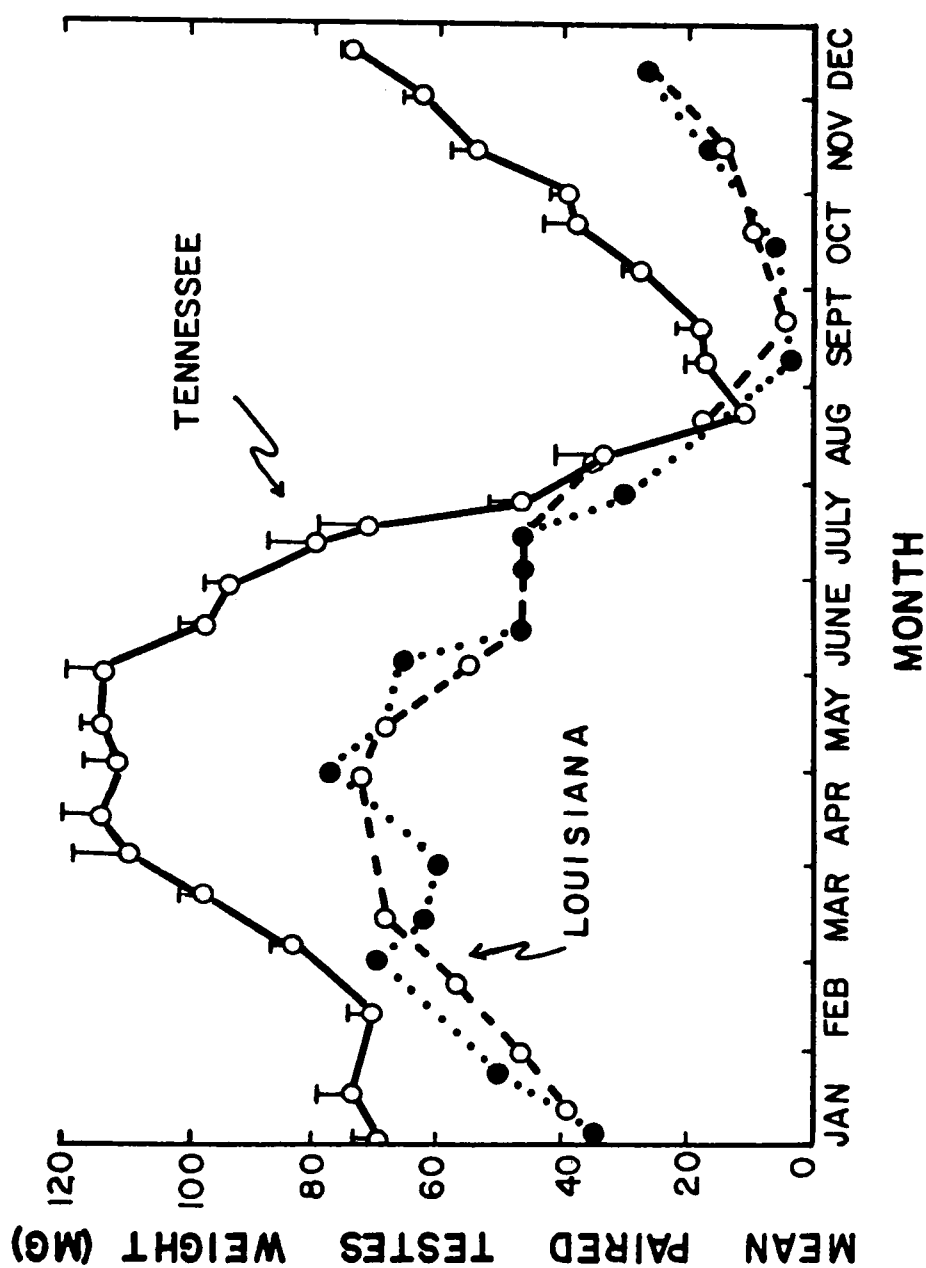


Figure 4. Monthly changes in mean paired testes weight for Tennessee (—) and Louisiana (...., Dessauer, 1955; ----, Fox, 1958) populations of *Anolis carolinensis*. Points represent mean weights and vertical lines indicate one standard error of the mean.

for the Tennessee and Louisiana populations and agrees with previous studies (Dessauer, 1955; Fox, 1958). The Tennessee data are based on 3-10 individuals (mode = 6) per point for a total of 143 males. The Louisiana data from Fox (1958) are based on 3-12 (mode = 6) animals per point for a total of 198 animals. Dessauer's data were estimated from Fig. 6 of his study (1955) and from data obtained through personal communication. His values range from 4-27 animals (usually ≤ 10) per point for a total of 169 animals.

The peak in gonad weight occurs from 1 April to 1 June for the Tennessee population and from 1 March to 1 June for the Louisiana population. The major difference between the two populations is in maximum gonad size attained during this peak. For the Tennessee population the largest mean paired testes weight for any one date occurs on 16 April and is 114 mg. Mean paired testes weights differ significantly (t-tests, $p < 0.001$) for the Tennessee and Louisiana populations at all points except August (Figure 4). These differences are still significant when testes weight is corrected for body weight to obtain a gonadosomatic index (mg testis weight/g body weight; Figure 5). Data on body weights for Louisiana males were obtained from Dessauer (personal communication) and were not available for the entire year (Figure 5). The largest paired testes weight recorded for the Tennessee population is 145 mg which is larger than the values of 130 mg and 110 mg reported by Dessauer (1955) and Fox (1958), respectively.

The Louisiana population shows a gradual recrudescence and regression in testicular size in marked contrast to the Tennessee

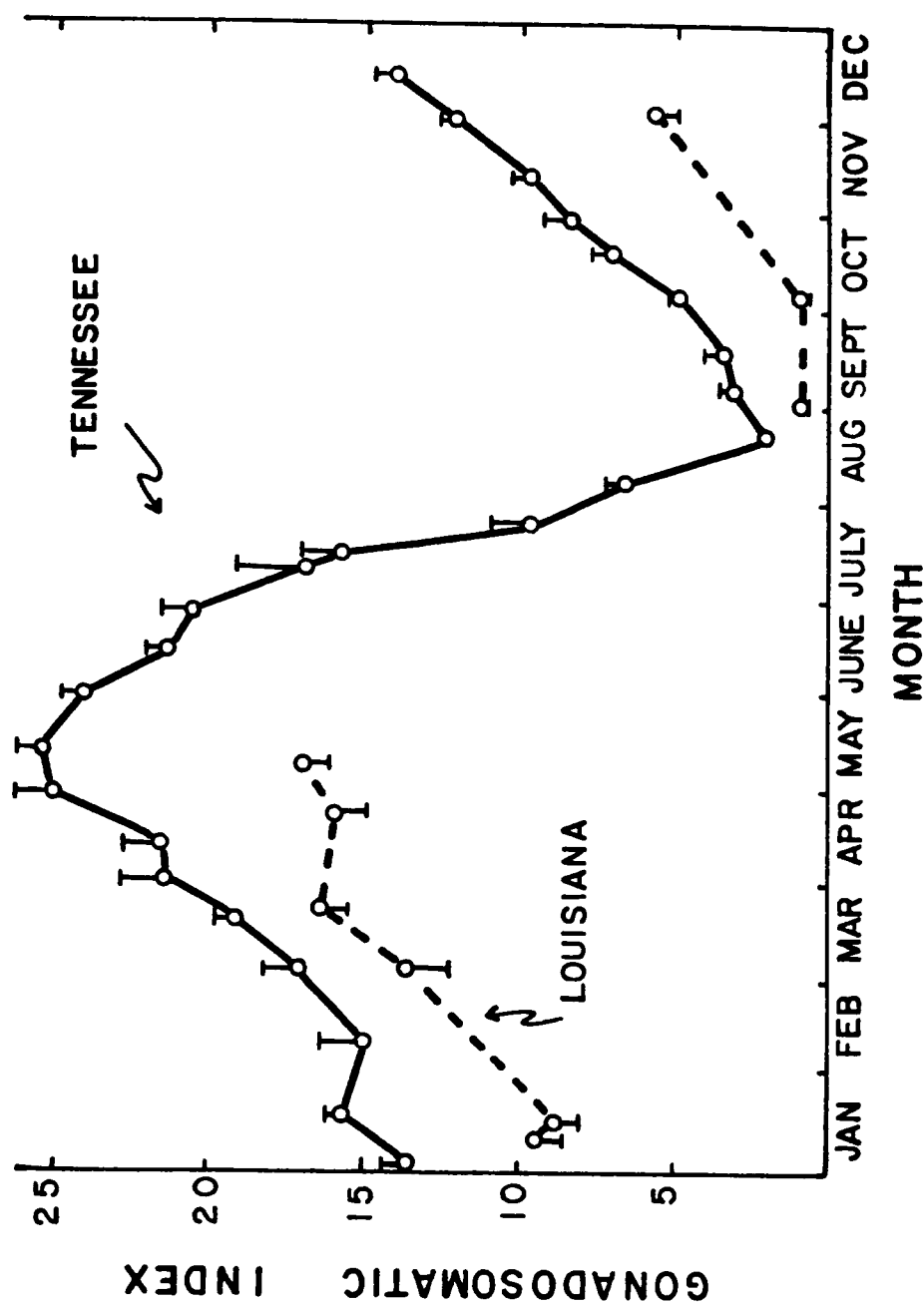


Figure 5. Monthly changes in gonadosomatic index (mg testes weight/g body weight) for Tennessee and Louisiana anoles. Points represent mean values, and vertical lines show one standard error of the mean.

population in which these phases have a steeper ascent and descent, respectively (Figure 4). Testicular regression is complete by mid-August in Tennessee but does not occur until mid-September for the Louisiana population. In addition, recrudescence begins almost immediately after testicular collapse in the Tennessee population with testicular weight beginning to increase by early September. In contrast, recrudescence in the Louisiana anoles does not begin until mid- to late-October. The smallest paired testes weights reported during the collapse are 8 mg for Tennessee and 1.5 mg (Fox, 1958) and 2 mg (Dessauer, 1958) for Louisiana.

A stepwise regression analysis indicates that the factors which significantly ($p < 0.0001$) explain 70% (R^2) of the variation in testes weight are stage of spermatogenesis and fat body size. Stage of spermatogenesis is most highly correlated with the period of early gonadal recrudescence (6 September-18 December). Fat body size is highly inversely correlated with the periods of testicular collapse (29 June-23 August) and testicular recrudescence (6 September-18 December, Table 6). Other parameters examined (body weight, snout-vent length, and testosterone level) do not contribute significantly to the R^2 value. The only time that body size (body weight and snout-vent) has a significant correlation with testes weight is during the peak in testes size (4 April-19 June). Testosterone level and testes weight are not significantly correlated except during the period of gonadal recrudescence (6 September-18 December, $p < 0.04$).

Climatic data (obtained for the Knoxville area from the National Climatic Center) examined in relation to testes weight include

Table 6. Relationship of testes weight to stage of spermatogenesis, hormonal level, fat body weight, body weight, and snout-vent length at different times of the male reproductive cycle in Tennessee anoles.*

Months	Testes Weight Versus Stage	Testes Weight Versus Testosterone	Testes Weight Versus Fat Body Weight	Testes Weight Versus Body Weight	Testes Weight Versus Snout-Vent Length
3 January-22 March	$r = 0.63$ $p < 0.0002$ $N = 29$	$r = 0.21$ $p < 0.29$ $N = 28$	$r = -0.32$ $p < 0.09$ $N = 29$	$r = 0.33$ $p < 0.08$ $N = 29$	$r = 0.29$ $p < 0.13$ $N = 29$
4 April-19 June	$r = 0.01$ $p < 0.93$ $N = 44$	$r = 0.11$ $p < 0.53$ $N = 34$	$r = -0.11$ $p < 0.45$ $N = 44$	$r = 0.53$ $p < 0.0002$ $N = 44$	$r = 0.37$ $p < 0.01$ $N = 44$
29 June-23 August	$r = 0.49$ $p < 0.01$ $N = 25$	$r = 0.31$ $p < 0.25$ $N = 16$	$r = -0.80$ $p < 0.0001$ $N = 25$	$r = -0.44$ $p < 0.03$ $N = 25$	$r = -0.32$ $p < 0.11$ $N = 25$
6 September- 18 December	$r = 0.85$ $p < 0.0001$ $N = 45$	$r = 0.32$ $p < 0.04$ $N = 43$	$r = -0.60$ $p < 0.0001$ $N = 45$	$r = 0.02$ $p < 0.90$ $N = 45$	$r = 0.23$ $p < 0.13$ $N = 45$
Overall	$r = 0.70$ $p < 0.0001$ $N = 143$	$r = 0.15$ $p < 0.11$ $N = 121$	$r = -0.80$ $p < 0.0001$ $N = 143$	$r = -0.25$ $p < 0.003$ $N = 143$	$r = 0.001$ $p < 0.99$ $N = 143$

*Significant correlations are underlined.

photoperiod, and monthly high and low temperatures (Figure 6, Table 7). In correlations over the entire year, photoperiod is significantly correlated with testes weight while high and low temperatures are not. However, when these factors are examined during different phases of the reproductive cycle, results differ. Photoperiod is important during recrudescence and regression while temperature exerts significant effects on testes weight only during recrudescence (Table 7). As photoperiod and testes weight decline from 29 June-23 August, the r value is high (0.93). When testes weight begins to increase in the autumn as photoperiod decreases, a high inverse correlation is found ($r = -0.90$). As recrudescence continues from January to March, a high positive correlation ($r = 0.71$) is found when photoperiod and testes weight are increasing.

2. Fat body--Fat bodies are located in the posterior, lateral region of the peritoneal cavity and vary in size depending on season and reproductive condition (Figure 7). For the Tennessee population there is a highly significant ($F = 246.71$, $p < 0.0001$, $r = -0.80$) inverse relationship between fat body size and gonad size (Figure 8) shown by the regression equation $Y = 99.34 - 0.36 X$. It also was found that animals with larger body weights had larger fat bodies. Fat bodies first are discernible in mid-June and increase in size attaining a mean maximum weight of 205 ± 27 mg (S.D.) by 20 September. The largest Tennessee fat body weighed 288 mg while the largest Louisiana fat body was 250 mg (Dessauer, 1955). Licht (1971) reports an average fat body size of 153 ± 17 mg (S.D.) for Louisiana

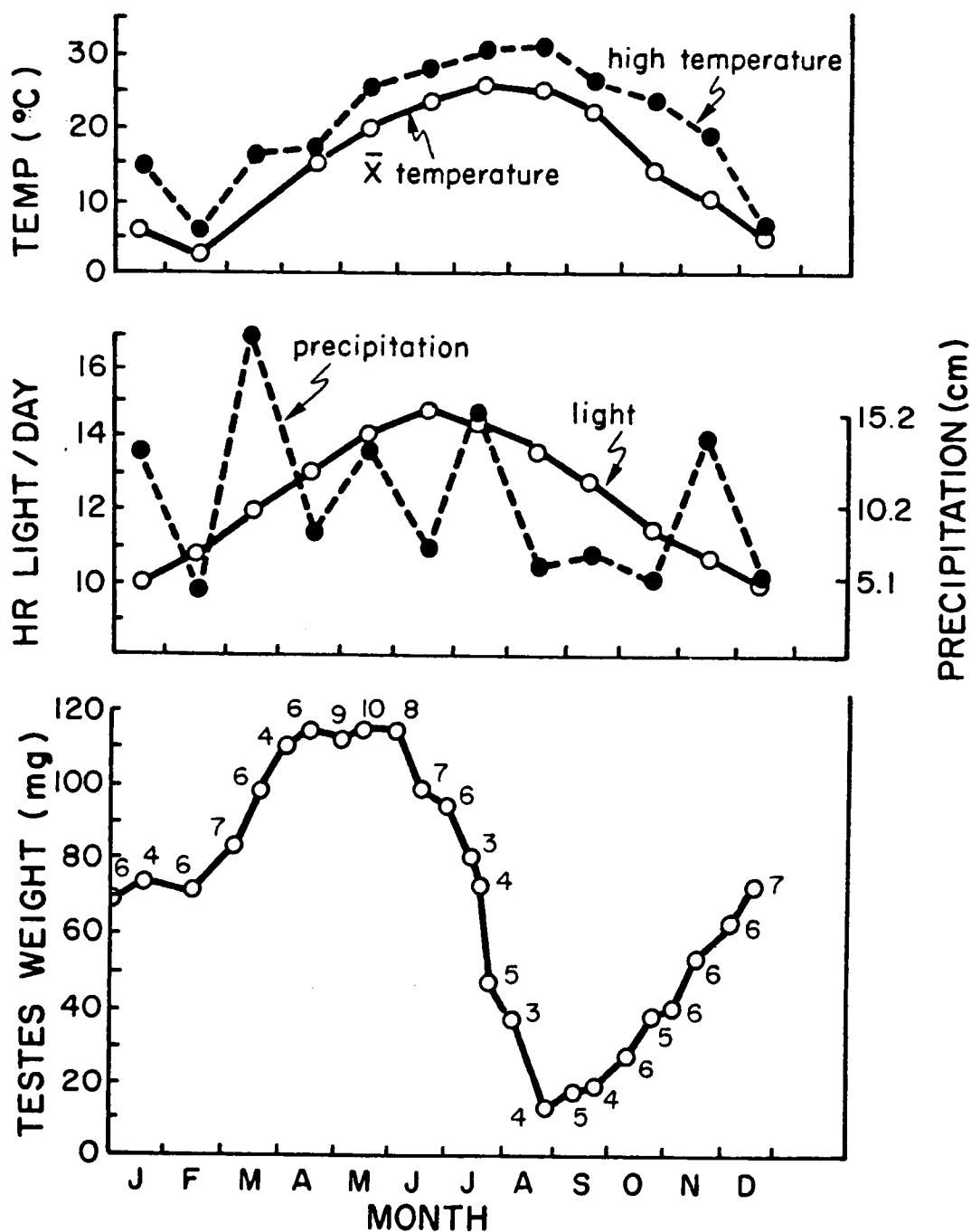


Figure 6. The relationship of climatic data (1979-80) to the annual testicular cycle in a Tennessee population of Anolis carolinensis. Points represent mean values, and numbers represent sample sizes. (Format modified from Licht, 1967a.)

Table 7. Relationship of testes weight to photoperiod, high temperature, and low temperature at different times of the male reproductive cycle for Tennessee anoles.*

Months	N	Testes Weight Versus Photoperiod	Testes Weight Versus High Temperature	Testes Weight Versus Low Temperature
3 January- 22 March	29	$r = 0.71$ <u>$p < 0.0001$</u>	$r = 0.41$ <u>$p < 0.03$</u>	$r = 0.37$ <u>$p < 0.05$</u>
4 April- 19 June	44	$r = -0.15$ $p < 0.34$	$r = -0.13$ $p < 0.38$	$r = -0.16$ $p < 0.30$
29 June- 23 August	25	$r = 0.93$ <u>$p < 0.0001$</u>	$r = -0.25$ $p < 0.22$	$r = -0.33$ $p < 0.10$
6 September- 18 December	45	$r = -0.90$ <u>$p < 0.0001$</u>	$r = -0.82$ <u>$p < 0.0001$</u>	$r = -0.87$ <u>$p < 0.0001$</u>
Overall (3 January- 18 December)	143	$r = 0.38$ <u>$p < 0.0001$</u>	$r = -0.06$ $p < 0.47$	$r = -0.10$ $p < 0.22$

*Significant correlations are underlined.

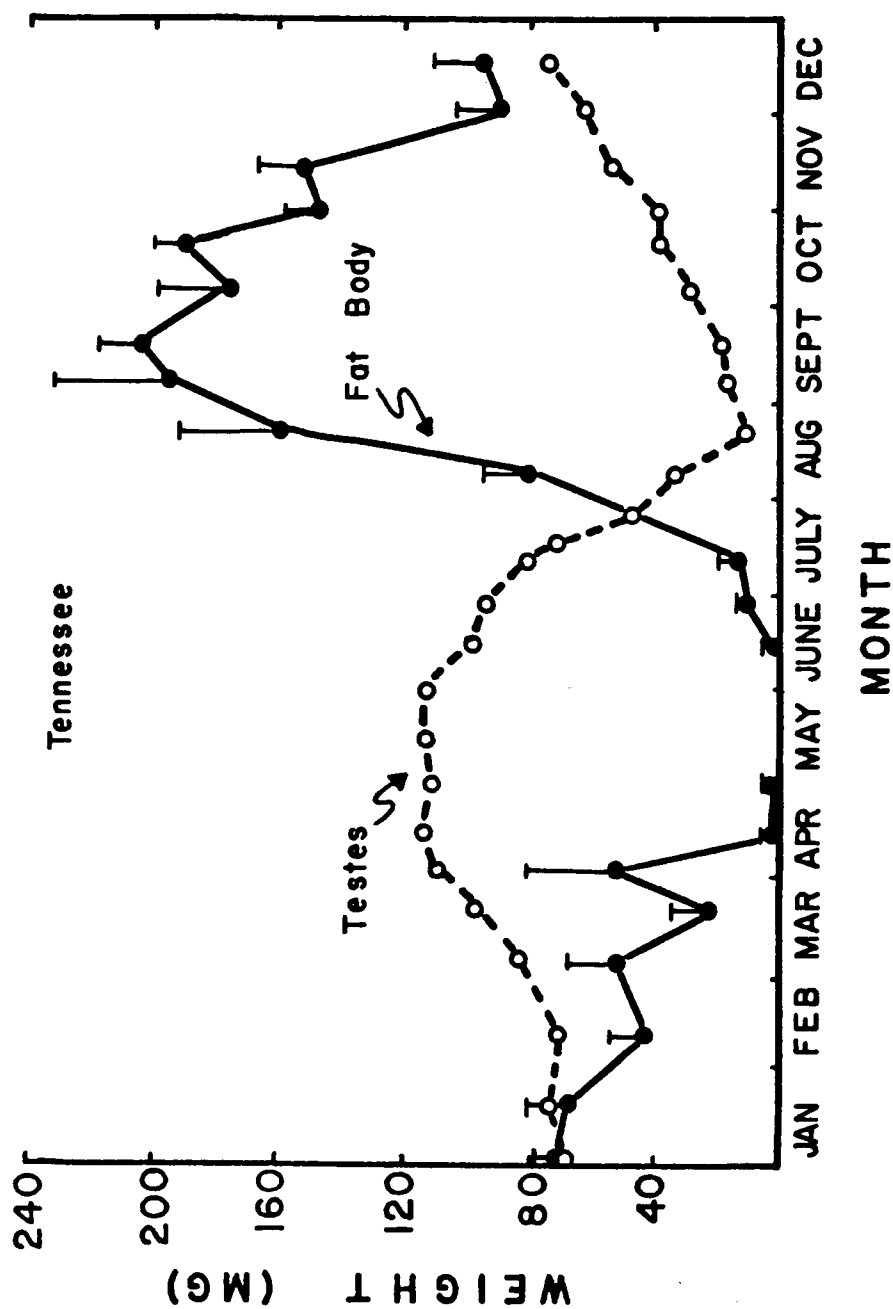


Figure 7. Seasonal changes in testes and fat body weights in a Tennessee population of Anolis carolinensis. Points represent mean values, and vertical lines indicate one standard error of the mean.

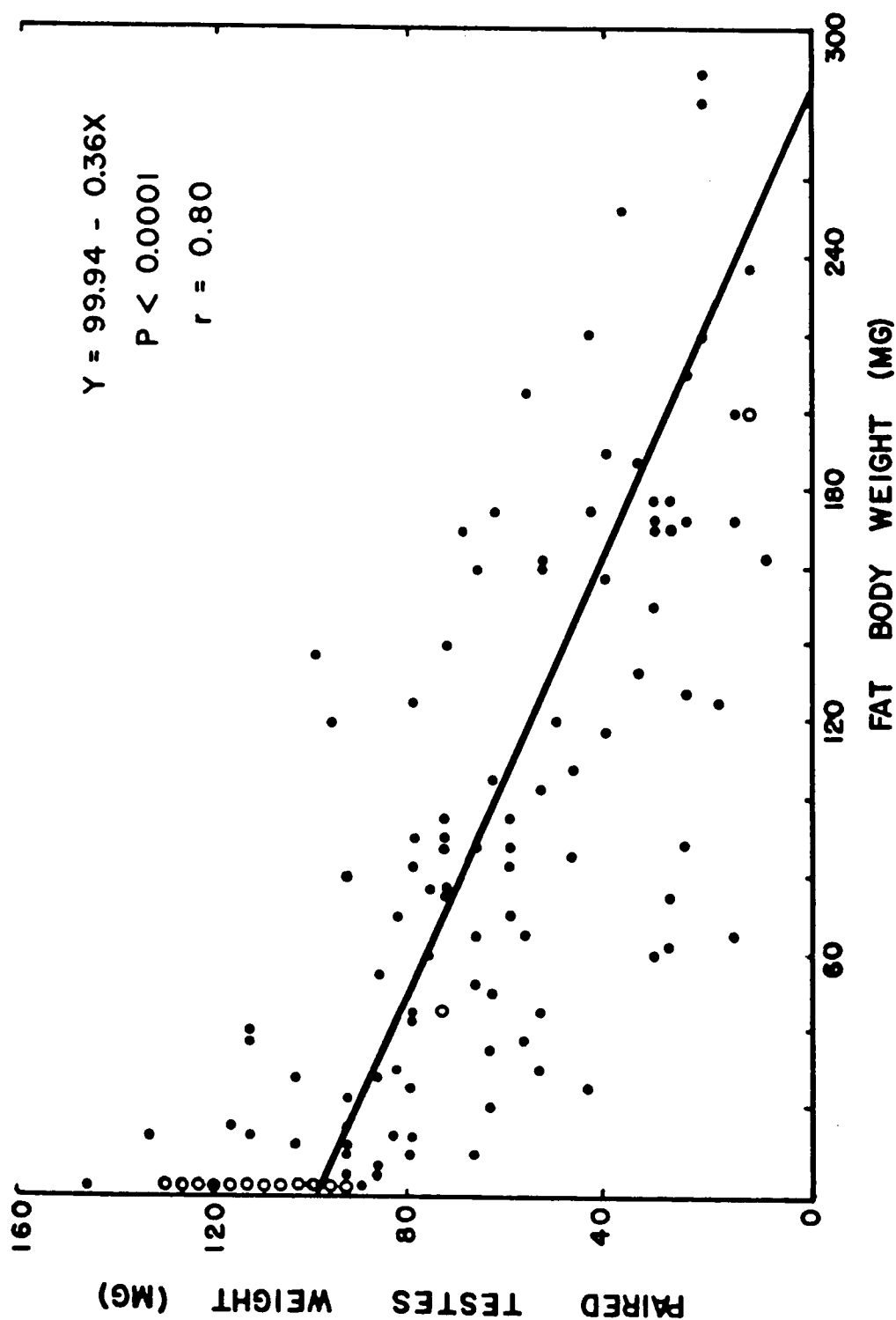


Figure 8. Relationship of testes weight to fat body weight in a Tennessee population of anoles. Solid dots represent one individual, and open dots represent 2-5 individuals per point.

anoles captured around 20 August; maximum weight for fat bodies in Louisiana occurs in October (Dessauer, 1955). Either no or very small fat bodies are found in Tennessee males from 16 April until 19 June. Fat bodies are first observed in Louisiana anoles in mid-July (Licht, 1971).

3. Body size--Differences in body size also were found between the two populations. The average snout-vent length for 143 Tennessee males is 61.46 ± 0.22 mm with an average body weight of 5.04 ± 0.06 g. A significant ($p < 0.0001$, $F = 245.11$) relationship was found between snout-vent length and body weight ($Y = 46.45 + 2.98 X$). The largest Tennessee male included in this analysis was 67 mm snout-vent length although at least one male was captured and released that was 70 mm.

Reports differ as to the body size of anoles from the Louisiana population. Gordon (1956), who has the largest sample size ($N = 600$) and includes animals that have just attained sexual maturity, reports an average snout-vent length of 56.3 mm (range = 45-70 mm). Fox (1958) used adult males that were from 60-70 mm snout-vent length, and Dessauer (1955) found males as large as 71-73 mm snout-vent length. Using data obtained from a portion of Dessauer's study (1955), the sizes of 48 Louisiana anoles were compared with 85 Tennessee anoles that were captured in the same months. The Louisiana anoles were significantly ($t = 2.85$, $p < 0.005$) larger (63.77 mm snout-vent) than the Tennessee anoles (61.62 mm); however, body weights of Tennessee anoles (5.12 g) were significantly ($t = 4.02$, $p < 0.0001$) greater

than Louisiana weights (4.52 g). This latter difference may be due to different times of emergence from winter refugia for the two populations.

B. Histology

Seasonal changes in testicular and epididymal histology were evaluated on the basis of previous descriptions by Fox (1958) and Licht (1967a). Seven stages of spermatogenesis are recognized in Anolis and are based on cell types in the seminiferous tubules, the amount of atrophy or hypertrophy in the epididymis, and the presence or absence of sperm in the epididymis. Representative photographs of each stage are shown in Figure 9.

Spermatogenesis is almost continuous in this species with only a brief decline in sperm production occurring in late August. The cycle for Tennessee anoles may be described briefly as follows. In late August, the seminiferous tubules are involuted and only one to two layers of spermatogonia are present (stage 1, Figure 9). Occasionally a few sperm are found that persist from the preceding breeding season. In September, primary spermatocytes are abundant and actively dividing (stage 2, Figure 9), but by October, spermatids become the predominant cell type in the tubules (stage 3, Figure 9). Occasional secondary spermatocytes are found but usually these cells are quite rare. Spermatids are gradually transformed into spermatozoa during stage 4 (Figure 9) which is one of the longest stages, persisting from mid-November through January. During stage 5 which lasts from late February until early April, both spermatids and spermatozoa

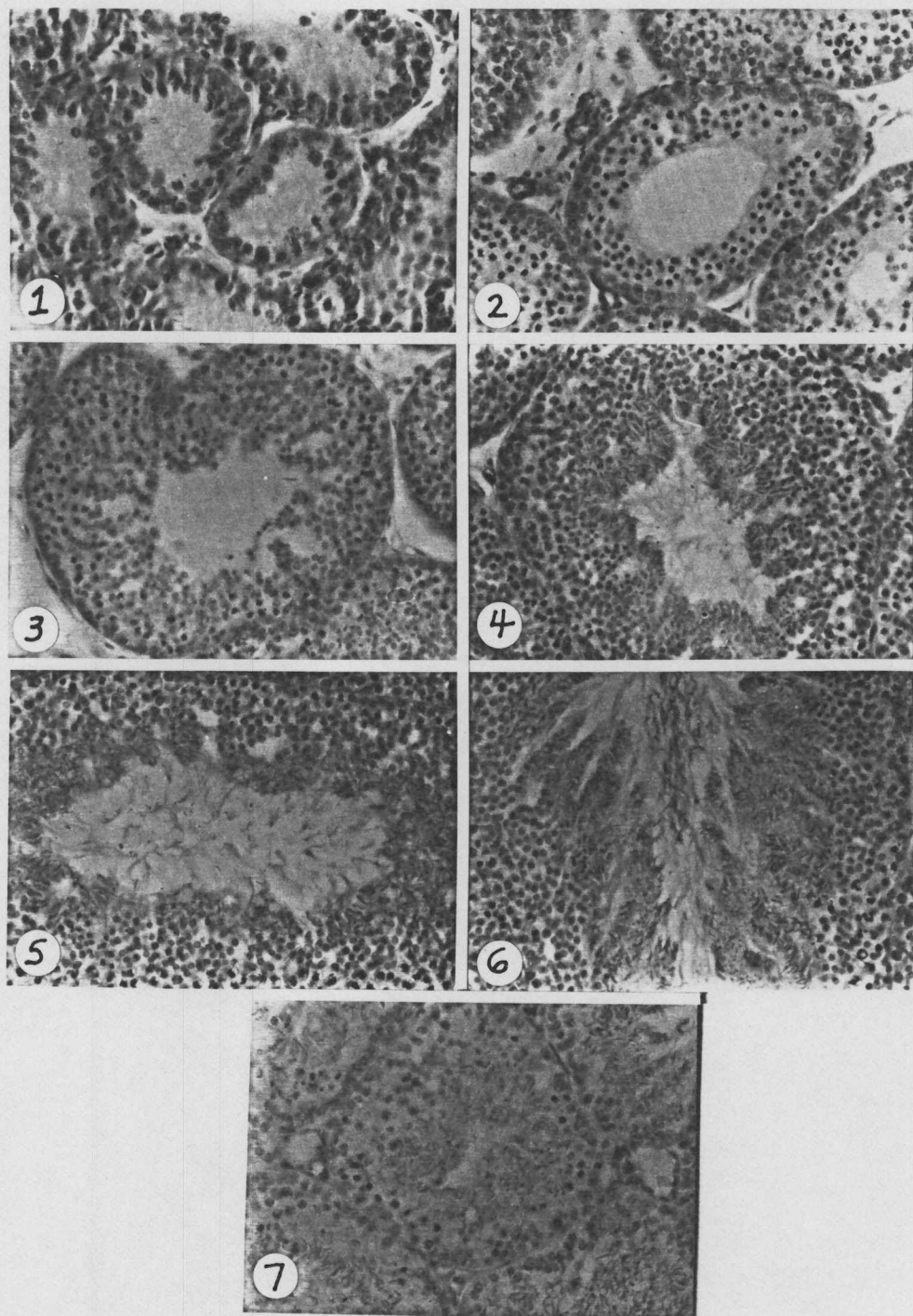


Figure 9. Photomicrographs of stages 1-7 of spermatogenesis in Anolis carolinensis. 100X.

are quite numerous (Figure 9). The period of maximal spermiogenesis occurs from mid-April until mid-July and is characterized by the presence of abundant spermatozoa (stage 6, Figure 9). Starting in late July and extending into mid-August, there is a marked reduction in the number of spermatids present in the tubules. However, spermatozoa still are quite abundant during this stage, and spermatogonia and occasionally dividing primary spermatocytes are visible. Toward the end of this stage, spermatogenesis is almost completed as tubular contents (except for spermatogonia) are eliminated (Figure 9). This termination is quite brief, however, since the whole process begins again within a few weeks.

An examination of the condition of the epididymis is necessary to accurately assign stages 4-6. Differences were found between the Louisiana and Tennessee populations in the degree of hypertrophy and the presence or absence of spermatozoa in this structure for stages 4 and 5. For the Louisiana population, the epididymis is still atrophic during stage 4. By stage 5, however, it has hypertrophied and contains few or no sperm. In Tennessee, spermatozoa were beginning to appear in the lumina of slightly hypertrophied epididymides during stage 4. By stage 5, the epididymides were quite hypertrophied and contained many sperm. During the other stages, epididymal condition was comparable for the two populations and agrees with descriptions given by Licht (1967a).

Seasonal changes in testicular weight were compared with changes in the stage of spermatogenesis for both populations (Figure 10). It was found that for five of the 12 months (February, August, September,

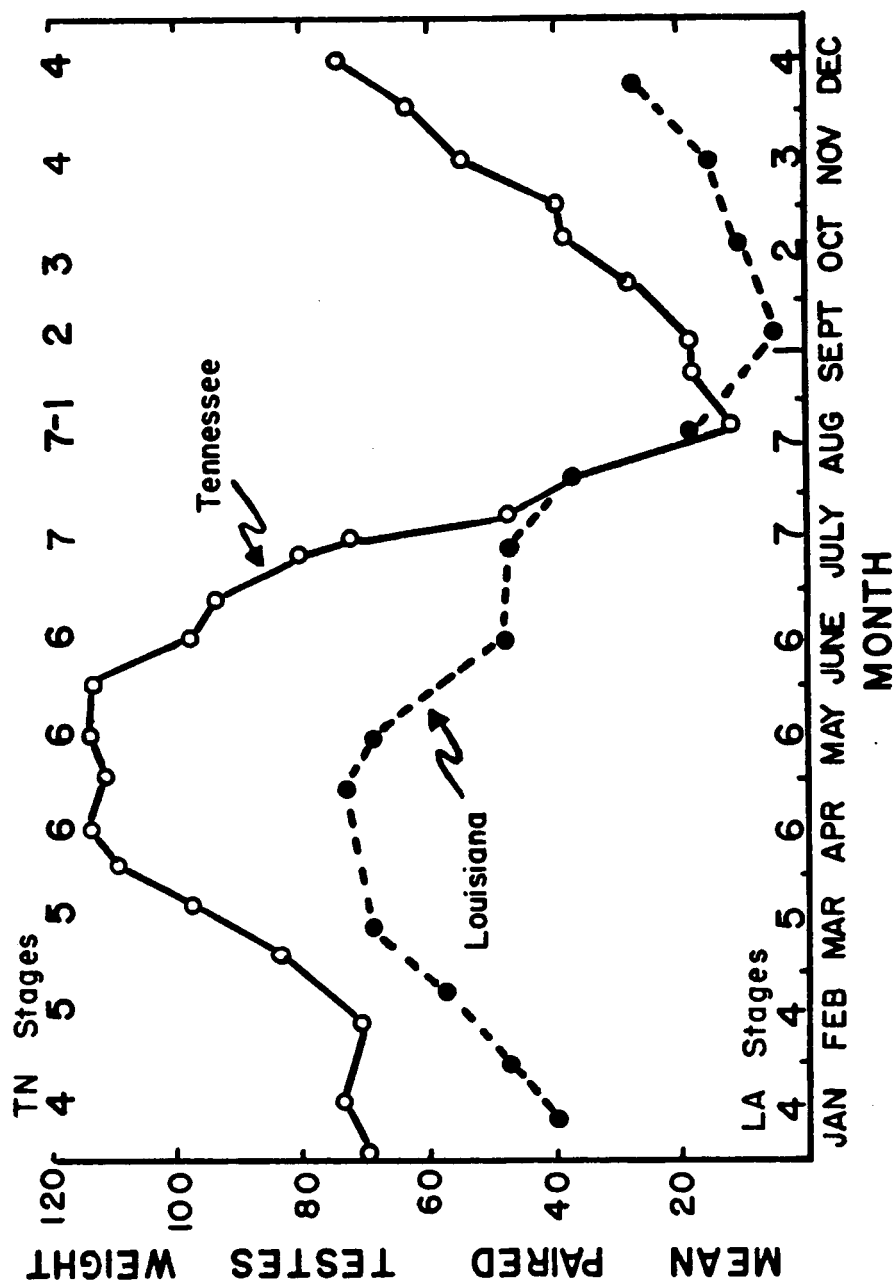


Figure 10. Seasonal changes in testes weight and stage of spermatogenesis for Louisiana and Tennessee anoles. Points indicate mean values, vertical lines represent one standard error of the mean, and numbers indicate the stage of spermatogenesis.

October, and November) Tennessee anoles showed a more advanced stage of spermatogenesis than their Louisiana counterparts. Most of these advanced stages were associated with the period of recrudescence in the autumn. The correlation coefficient for stage of spermatogenesis and testes weight for the Tennessee population was 0.70 ($N = 143$, $p < 0.0001$). The polynomial regression analysis (cubic) was highly significant ($F = 192.48$, $p < 0.0001$, $R^2 = 0.806$) and is represented by the equation $Y = 82.37 - 94.91 X + 34.94 X^2 - 3.10 X^3$.

C. Hormonal Analysis

Seasonal changes in levels of testosterone are shown in Figure 11. The highest levels of testosterone preceded the peak in testes weight and were found from 19 January until 22 March with the peak on 11 February (4.02 ng/ml). The lowest levels of testosterone coincided with the period of testicular collapse reaching 0.06 ng/ml plasma on 23 August when gonads also were smallest. The only time when hormone level and testes weight were significantly correlated ($r = 0.32$, $p < 0.04$, $N = 43$) was during the period of gonadal recrudescence (Table 6). The highest level of testosterone found in this analysis was 9.01 ng/ml plasma with the lowest value being nondetectable. More individual variation, as shown by the relatively large standard errors (Figure 11), was found in this parameter than in other parameters that were examined in relation to testes weight.

The time of day that animals were sacrificed showed little correlation ($r = -.13$) with testosterone levels. Hormone levels showed some correlation with the number of hours that the anoles were in

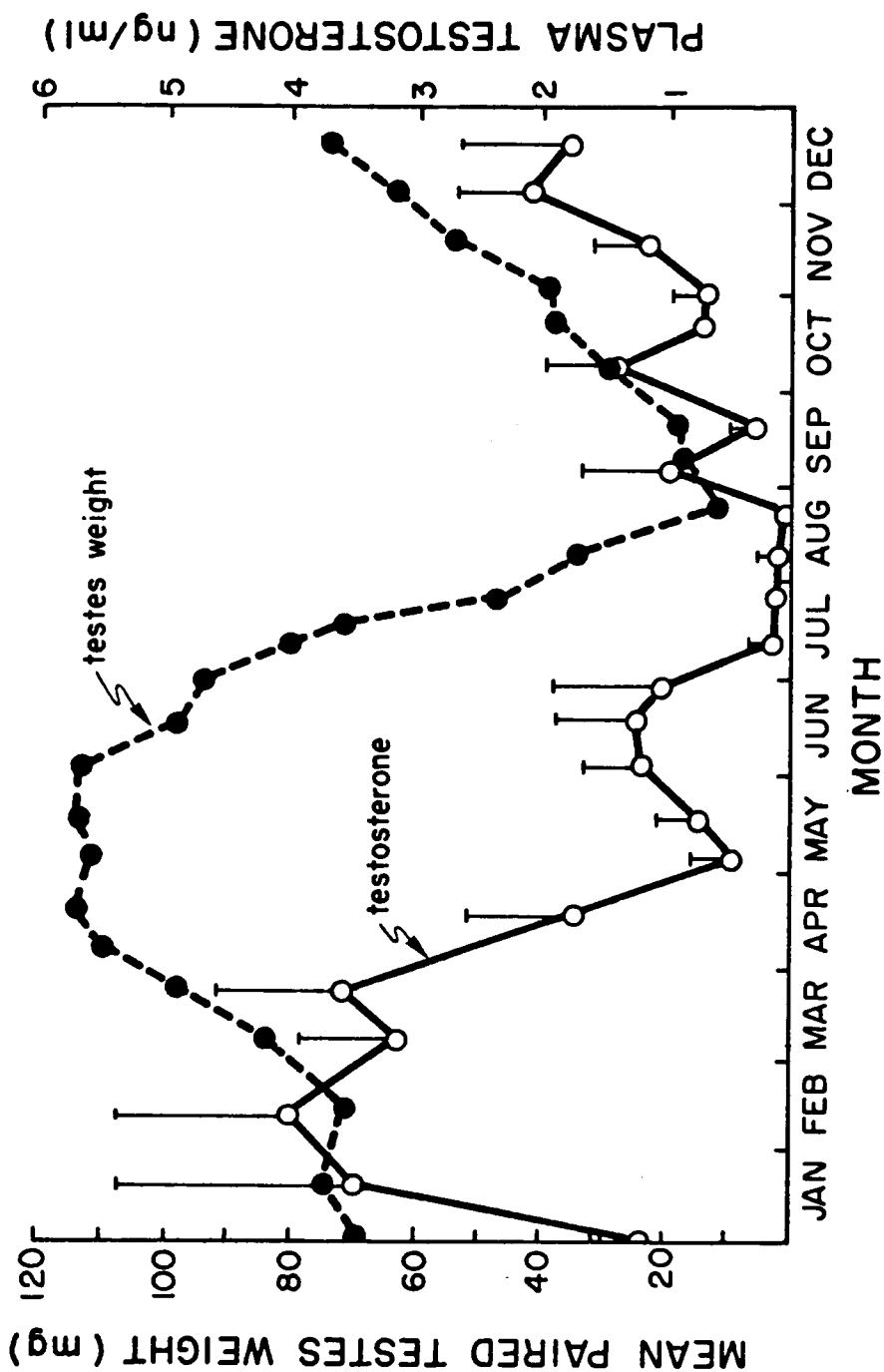


Figure 11. Seasonal changes in testes weight and testosterone level for a Tennessee population of *Anolis carolinensis*. Points represent mean values, and vertical lines indicate one standard error of the mean.

captivity prior to sacrificing ($r = -.32$). Levels were usually higher when animals were killed within the first 7 h of capture; however, after 12 h in captivity, it is not known what happens to hormone titers. In one instance, four adult males were housed in a one gallon jar for 24 h prior to sacrificing. Testosterone levels in these animals attained the highest values (11.21-32.67 ng/ml) found in the entire study. Since no other males were retained this long prior to sacrificing, these data were not used in the above analysis.

II. Female Reproductive Cycle

A. Morphology

1. Ovary weight--Seasonal changes in ovarian weights for the Tennessee and Louisiana populations are shown in Figure 12. Mean ovarian weights for the Tennessee population are based on 1-13 animals (mode = 6) per point while LA data points represent 1-10 animals (mode = 3). The Tennessee data are based on one year of sampling except for the peak period (1 May-15 July) which is based on three years of sampling.

There is little difference between the populations in terms of onset and termination of increases in ovarian weight (which is indicative of vitellogenesis). The duration of the peak of the breeding season is somewhat shorter for the Tennessee population, lasting from mid-May until mid-July. The Louisiana season lasts from late April until late July.

Yearly variation in ovarian weights for the Tennessee population is illustrated in Figure 13. Since these differences were not

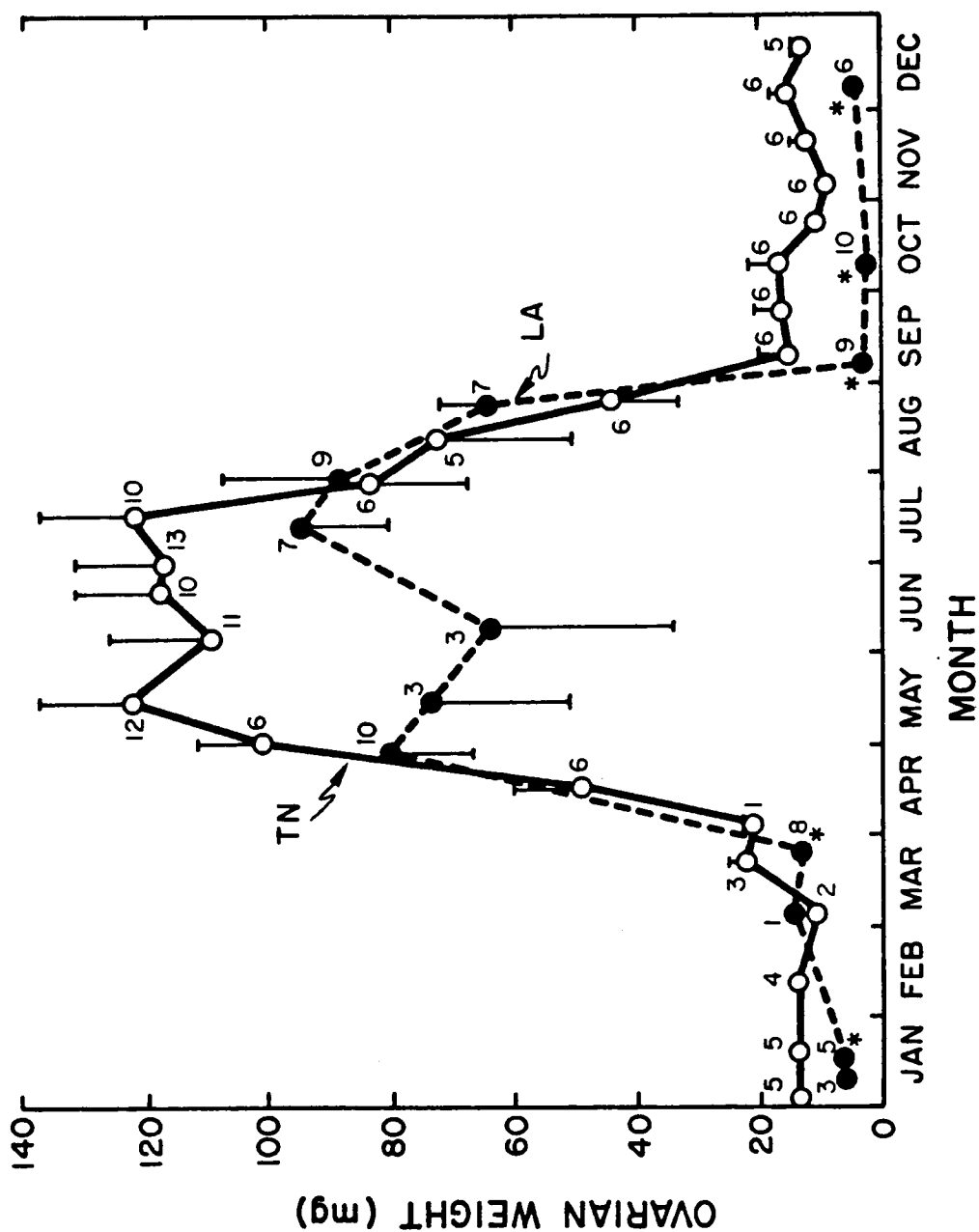


Figure 12. Seasonal changes in ovarian weight for Tennessee and Louisiana anoles. Points indicate mean weights, vertical lines are 1 S.E., and numbers represent sample sizes. Asterisks represent points where significant differences were found between the two populations.

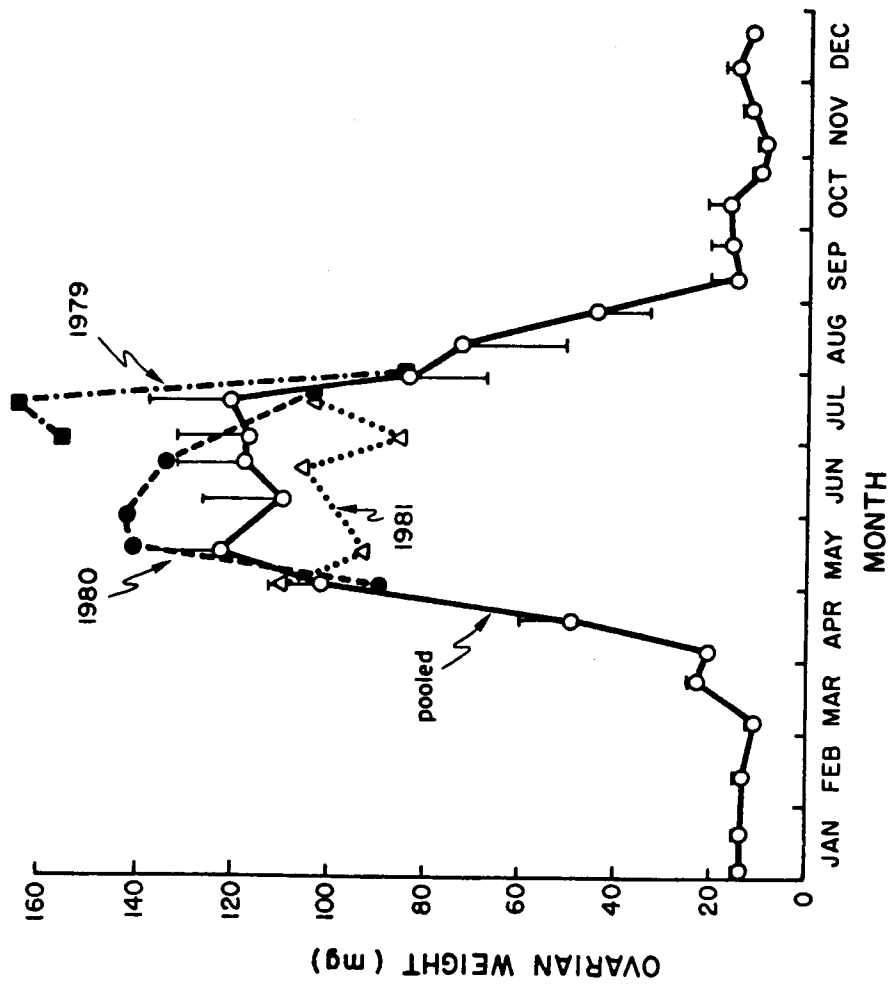


Figure 13. Seasonal and annual changes in ovarian weight for a Tennessee population of *Anolis carolinensis*. Solid line indicates pooled weights (mean $\pm$ 1 S.E.), and dotted (1981), dashed (1980), and hatched (1979) lines represent data for individual years.

significantly different, ovarian weights were pooled for each date in the 1 May-15 July interval; these pooled data are used in all other figures and analyses. When pooled data were compared with Louisiana data (Dessauer, 1955) that were within four days of the same collection date, no significant differences were found between the two populations for the peak period. This fact and the fact that standard errors were large for this peak period indicate a large degree of variation in ovarian weights during this portion of the breeding season. Highly significant differences ($p < 0.01$) in ovarian weight were found between the two populations during the nonreproductive season for January, March, September, October, and December. When ovarian weight was corrected for body weight (gonadosomatic index = mg ovarian weight/g body weight), significant differences ($p < 0.05$) were still present during the nonreproductive season for each of these months except March (Figure 14). Body and ovarian weights were not available for the Louisiana population during the peak of the breeding season, and no direct comparisons could be made for this portion of the seasonal cycle (Figure 15).

The variability in ovarian weight can be explained by several factors. The most obvious explanation is that females are captured at different times in the ovulatory cycle, resulting in weights ranging from quite low, following a recent ovulation when few yolked follicles are developing, to quite high, when follicles may be 0.7-0.8 mm in diameter with ovulation pending. Thus, the size of the largest follicle (range 15-78 mm) on the ovary and the number of follicles per animal varies with reproductive condition, with the latter ranging

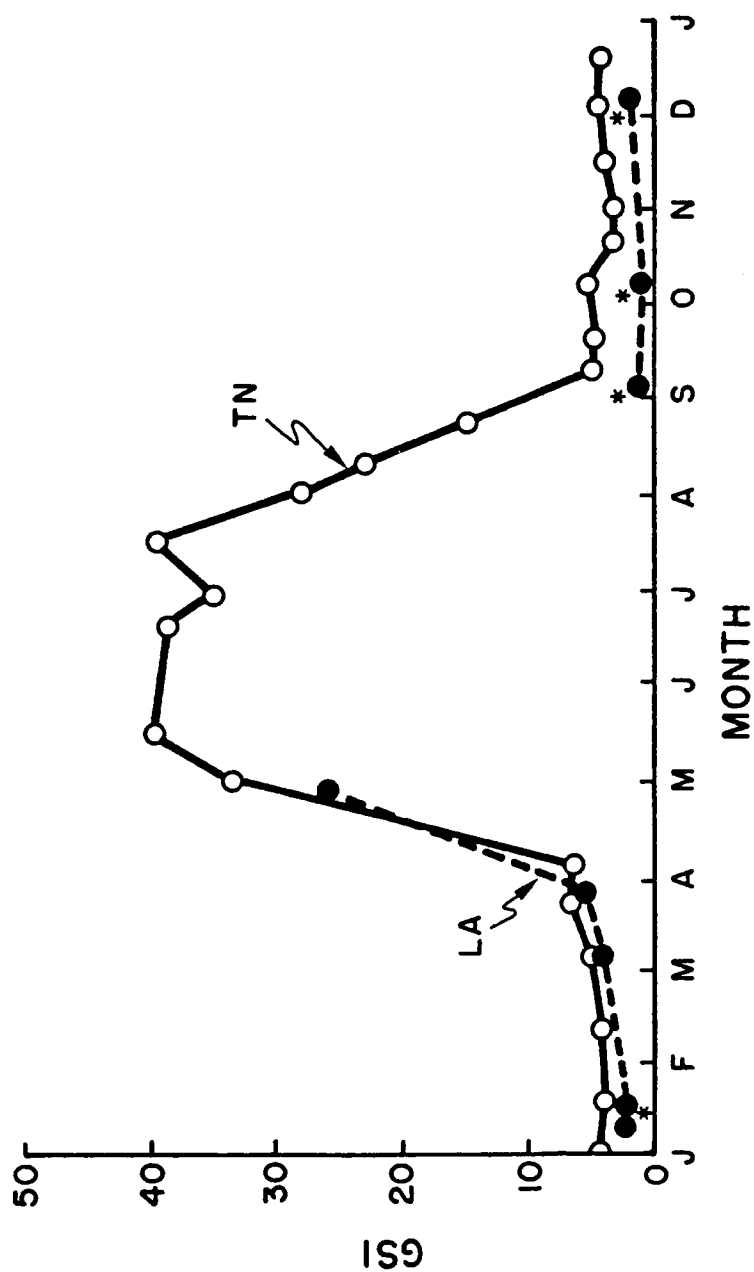


Figure 14. Monthly changes in GSI (gonadosomatic index = mg ovarian weight/g body weight) for Tennessee and Louisiana populations of *Anolis carolinensis*. Points represent mean values and asterisks represent points where significant differences were found between the two populations.

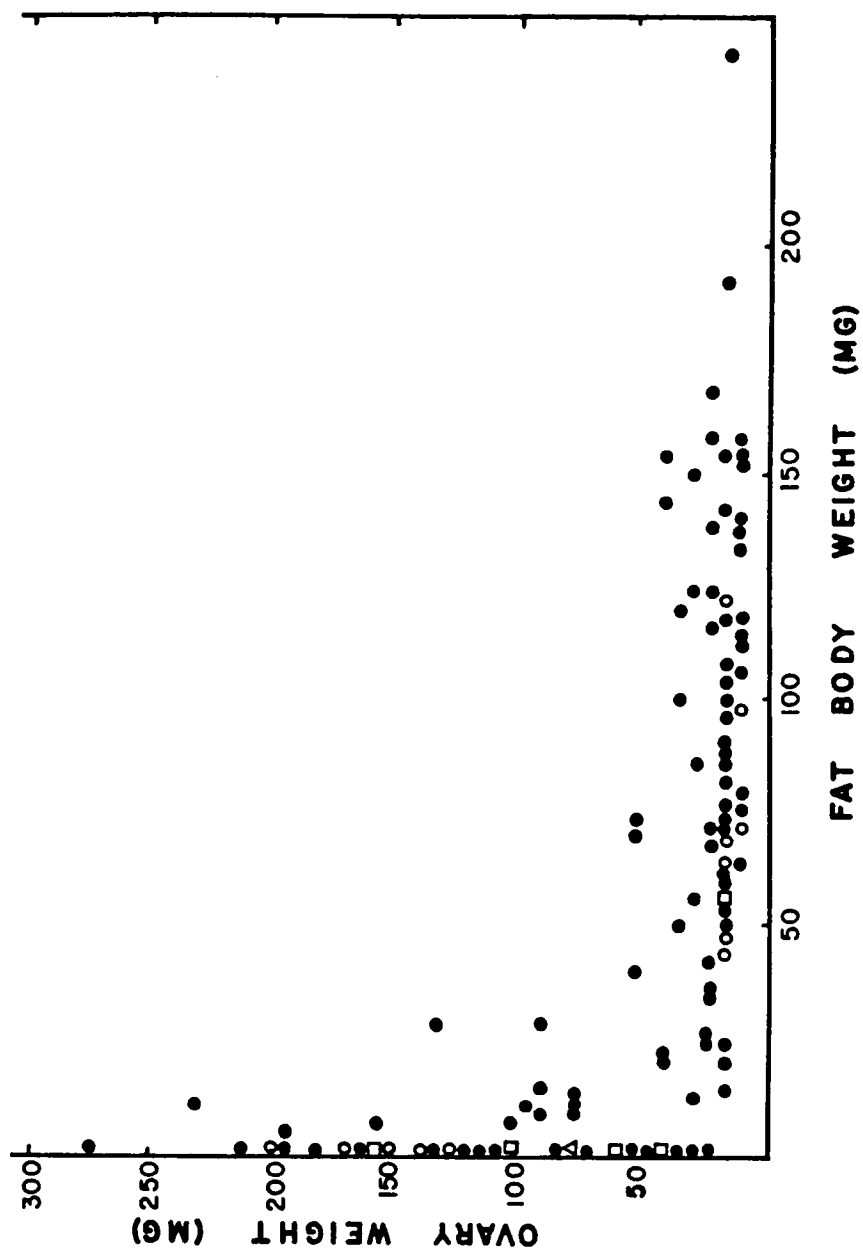


Figure 15. Relationship of ovarian weight and fat body weight for a Tennessee population of anoles. Solid symbols represent one individual per point and open symbols indicate two or more individuals per point.

from an average of 11.6 ± 0.18 ($N = 63$) during the nonreproductive season to 14.5 ± 0.20 ($N = 106$) during the breeding season. Increases in ovarian weight were paralleled by increases in oviduct weight ($r = 0.80$) which concurs with Noble and Greenberg (1941); the latter also was correlated ($r = 0.88$) with the number of oviductal eggs present (oviduct weights do not include eggs).

Fat body size was highly inversely correlated with ovarian weight; females with larger ovaries had smaller fat bodies ($r = -0.61$, $p < 0.0001$, $N = 135$). Since the relationship was curvilinear (Figure 15), a quadratic regression was performed which explained 51% (R^2) of the variability in ovarian weight ($F = 67.35$, $p < 0.0001$, $N = 134$).

Environmental parameters examined in relation to seasonal ovarian weight changes are illustrated in Figure 16. The female cycle more closely follows environmental changes than the male cycle. The peak in the breeding season corresponds closely with and slightly precedes peaks in mean temperature and photoperiod. Photoperiod appears to exert a greater effect on ovarian weight than does mean temperature. Both of these parameters were curvilinear when graphed against ovarian weight; however, a quadratic regression analysis indicated that photoperiod explained 57% (R^2) of the variation in ovarian weight as opposed to only 30% being explained by mean temperature changes (Figure 17). Whether or not the decline in reproductive activity is related to declining temperature, light, or possibly reduced rainfall (Figure 17) is unknown.

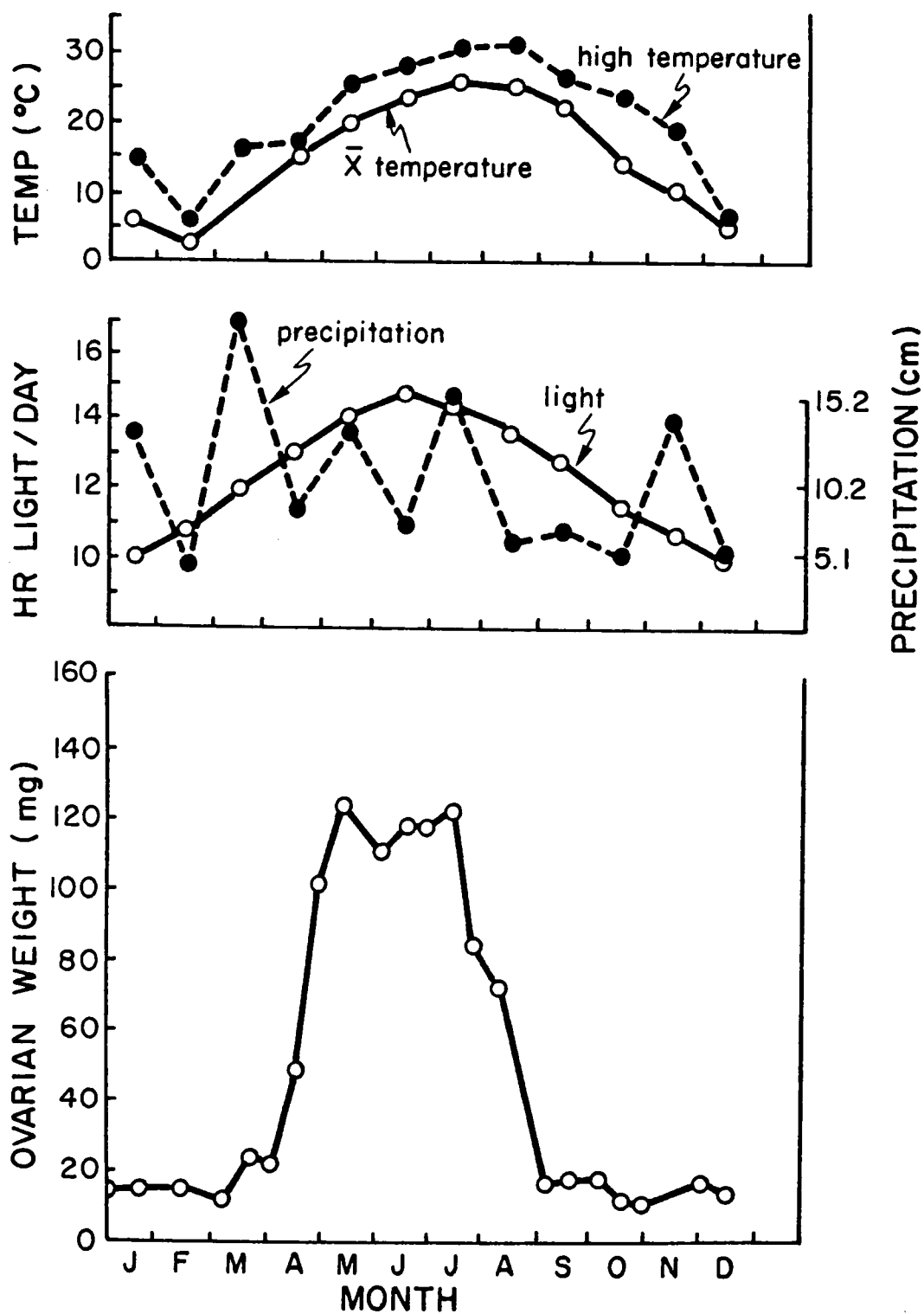


Figure 16. Annual changes in ovarian weight, precipitation, light, maximum temperature, and mean temperature for a Tennessee population of *Anolis carolinensis*. (Format modified from Licht, 1967a.)

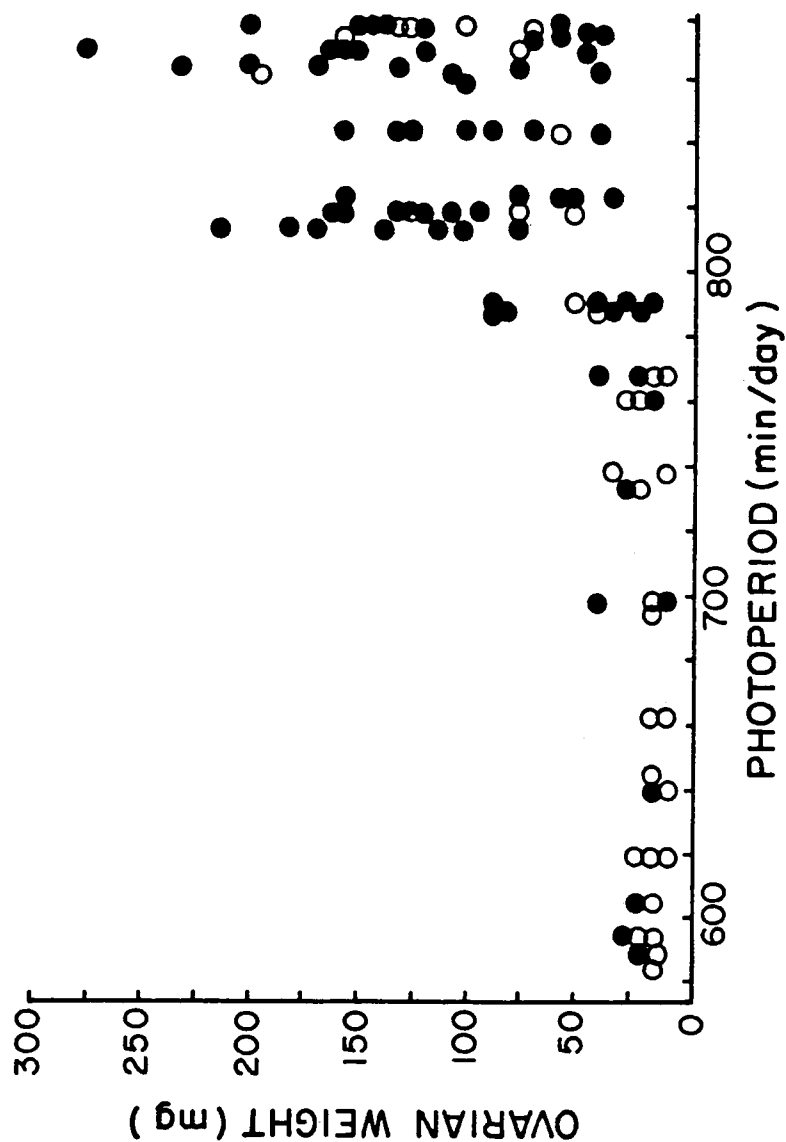


Figure 17. Relationship of ovarian weight and photoperiod for a Tennessee population of *Anolis carolinensis*. The relationship is described by the quadratic equation $Y = 909.62 - 2.82X + 0.002X^2$. Solid symbols represent one individual per point and open symbols indicate two or more individuals per point.

2. Fat body--Fat body weights in female Anolis show seasonal changes (Figure 18) comparable to those found in male Anolis. The maximum fat body size attained by the Tennessee population was 241 mg while the maximum found in some Louisiana females (Dessauer, personal communication) was 301 mg. In Tennessee anoles, fat bodies attained peak size in September and were not detectable from mid-May until the end of June.

3. Body size--A comparison of body sizes for the two populations is shown in Table 8. Estimates of body size for the Louisiana population vary depending on the author consulted. Gordon's data (1956) represent an adequate sample size, but no standard deviations or standard errors were given, and therefore, no statistical comparisons were possible. Data used in a portion of Dessauer's study (1955) were obtained for the months January, March, April, October, and December (Dessauer, personal communication) and the Tennessee and Louisiana populations compared. No significant differences ($t = 0.44$) were found between the two populations in snout-vent length. Body weights were not directly compared for the two populations because the Louisiana animals were starved four days prior to sacrificing (Dessauer, 1955). For the Tennessee population a significant ($p < 0.0001$) relationship was found between snout-vent length and body weight ($F = 240.57$, $R^2 = 0.59$) and is described by the regression equation $\hat{Y} = 40.626 + 3.770 X$.

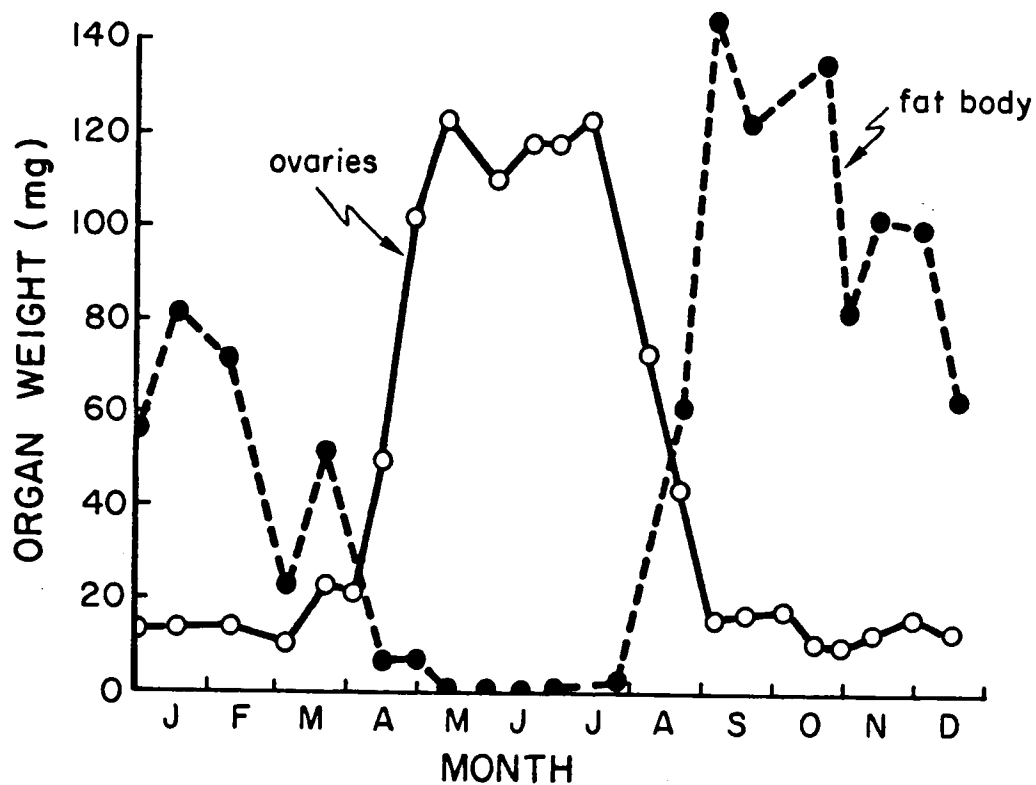


Figure 18. Seasonal changes in fat body and ovarian weight for a Tennessee population of *Anolis carolinensis*.

Table 8. Statistical analyses of snout-vent lengths (mm) and body weights (g) for Tennessee and Louisiana populations of Anolis carolinensis.

Populations	N	Mean	S.E.	Range
TENNESSEE				
Snout-Vent	176	52.70	0.19	46.5-58.0
Body Weight	176	3.20	0.04	2.14-5.10
LOUISIANA				
Gordon (1956)				
Snout-Vent	224	50.70	----	45.1-57.8
Dessauer (1955)				
Snout-Vent	34	52.94	0.45	44.0-58.0
Body Weight	51	2.65	0.07	1.65-3.55

B. Hormonal Analysis

Seasonal changes in progesterone levels for the Tennessee population of Anolis carolinensis are shown in Figure 19. No comparable data are available for the Louisiana population. The peak in progesterone secretion occurs in March when progesterone attains a mean level of 29.2 ng/ml. Individual variation in progesterone levels is quite high during this time as indicated by the large standard error. The decline in progesterone secretion coincides with the increase in ovarian weight. Progesterone values were lowest in July with an average of 2.6 ng/ml. The increase in progesterone levels started in late July when ovarian weight began decreasing (Figure 19).

Progesterone was significantly inversely related to ovarian weight ($F = 6.57$, $p < 0.002$). A quadratic model ($Y = 95.877 - 5.210X + 0.078X^2$) indicated that progesterone level accounted for 16% (R^2) of the variability in ovarian weight (Figure 20).

A slight inverse correlation ($r = -.35$) was found between progesterone level and the number of hours after capture that the animal was killed; no correlation ($r = 0.03$) was found between progesterone level and the time of day that lizards were sacrificed.

C. Egg Data

Eggs were found in Tennessee females from 1 May through 23 August. Only eight females were found with no eggs during this time period. Of those eight, two were found on 1 May, and both had large follicles (65 and 67 mm). The remaining six females were captured in August and had follicles ranging from 24-57 mm. These latter animals probably were going out of reproductive condition.

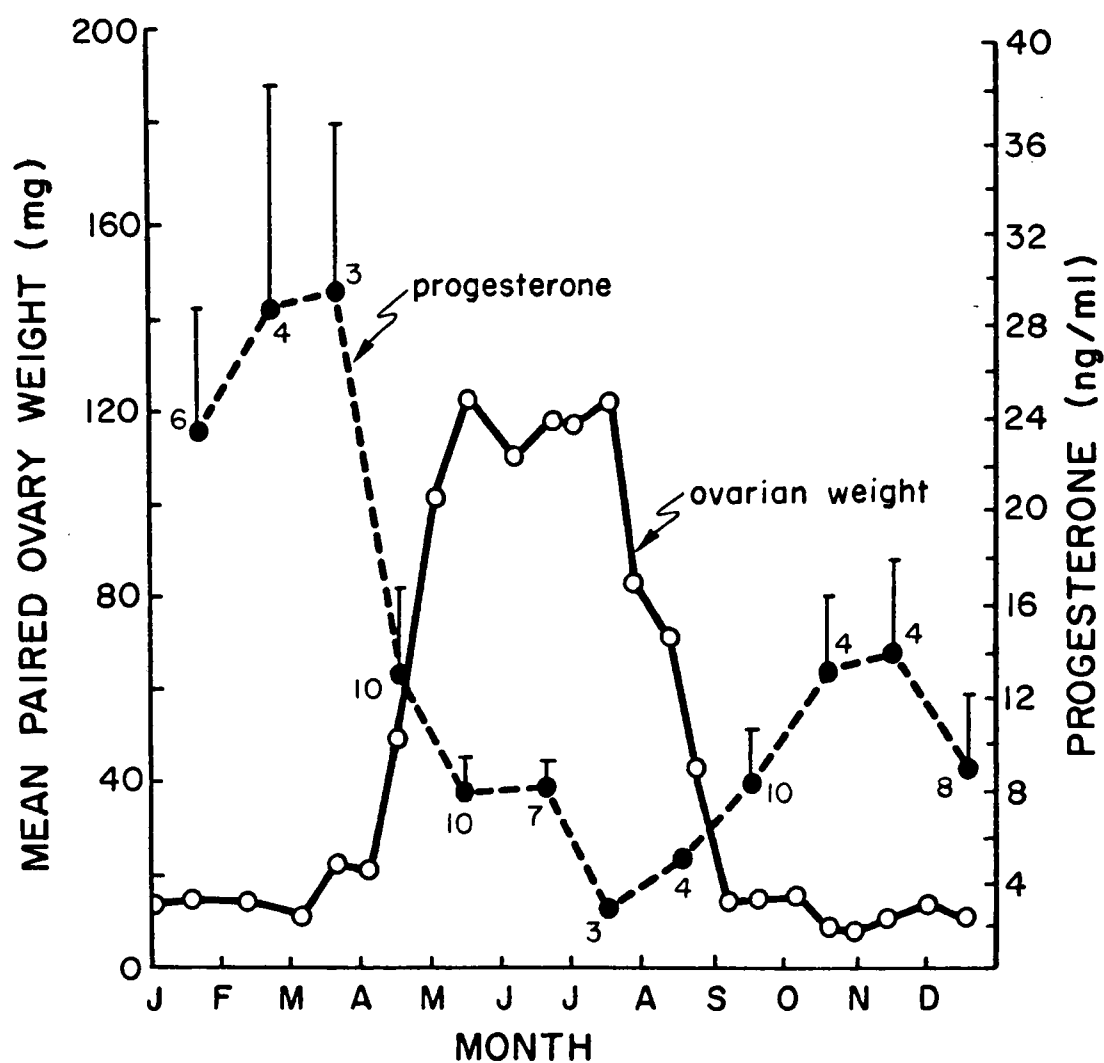


Figure 19. Seasonal changes in ovarian weight and progesterone for Tennessee anoles. Points represent means, vertical lines indicate 1 S.E., and numbers are sample sizes.

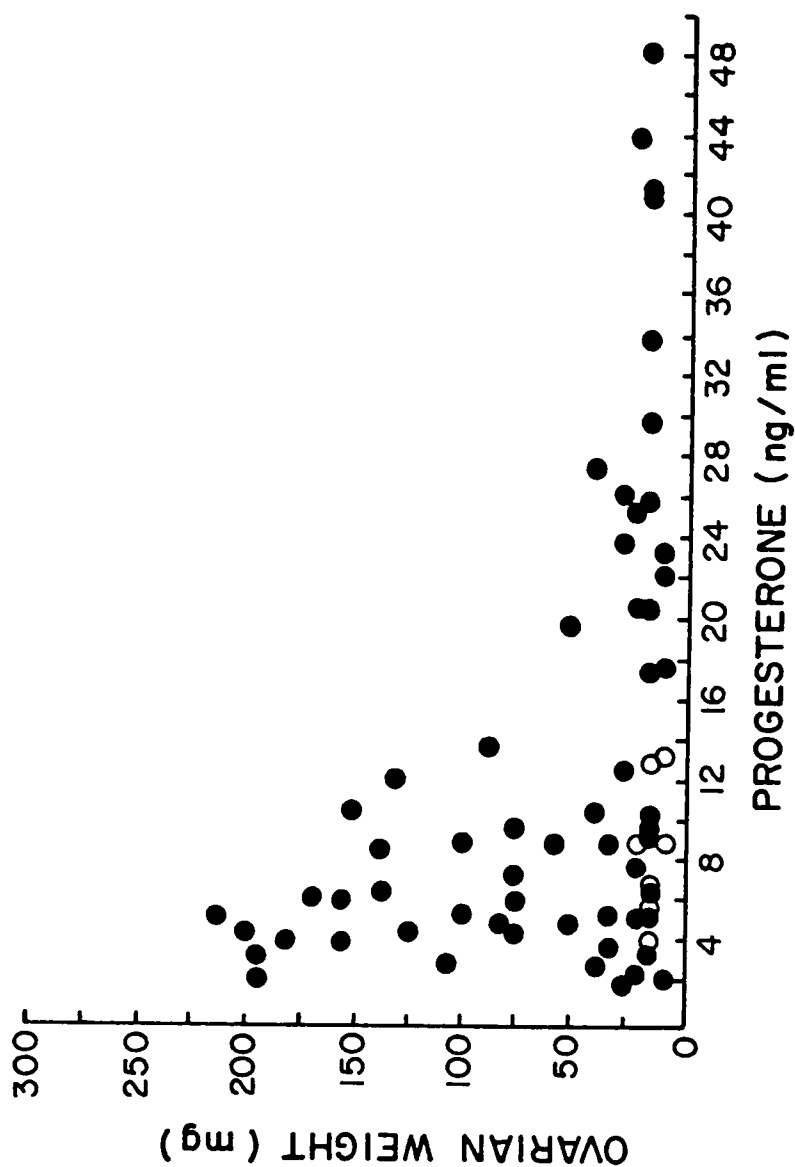


Figure 20. Relationship of ovarian weight to progesterone levels for a Tennessee population of *Anolis carolinensis*. The relationship is described by the quadratic regression equation $Y = 95.88 - 5.21X + 0.08X^2$. Solid symbols represent one individual per point and open symbols indicate two individuals.

Louisiana females usually have oviductal eggs present from April through August (Gordon, 1956; Hamlett, 1952; Dessauer, 1955) although occasional eggs are found as early as March (Gordon, 1956) and as late as September (Hamlett, 1952) and October (Cagle, 1948).

The number of oviductal eggs appears to be a function of female size for both populations; that is, the larger the female, the greater is the chance of finding two oviductal eggs (Table 9). Tennessee females larger than or equal to 51 mm in snout-vent length were more likely to have two oviductal eggs (Figure 21). This relationship between number of oviductal eggs and snout-vent length was not significant ($\chi^2 = 7.21$) for Tennessee anoles. When the number of individuals having zero, one, or two oviductal eggs was compared for the Tennessee and Louisiana populations (Louisiana data from Gordon, 1956) using a χ^2 test of independence between two or more samples, no significant differences were found between the populations ($\chi^2 = 1.52$).

Tennessee females have a higher percentage of two oviductal eggs than their Louisiana counterparts (Table 10). However, there is considerable variability in this percentage among years. When environmental differences are examined among years, the major difference appears to be the amount of precipitation received during the peak of the breeding season, May-July (Table 11). Nearly twice as much rain fell in 1979 than in 1980 and 1981, and may be one explanation for the difference in reproductive output.

A comparison of sizes of oviductal and freshly deposited eggs for the Louisiana and Tennessee populations is shown in Table 12. The size of oviductal eggs does not differ significantly ($t = 1.63$)

Table 9. The relationship of female size (snout-vent length, mm) to number of oviductal eggs for Tennessee and Louisiana populations of Anolis carolinensis for the months May, June, July, and August.

Populations		0 Eggs	1 Egg	2 Eggs
TENNESSEE	Mean	51.06	51.45	52.52
	S.E.	0.73	0.39	0.34
	N	8	44	45
	Range	48-54	48-58	49-57
LOUISIANA (Gordon, 1956)	Mean	47.40	49.90	50.30
	S.E.	---	---	---
	N	14	124	100
	Range	45-50	45-56	46-58

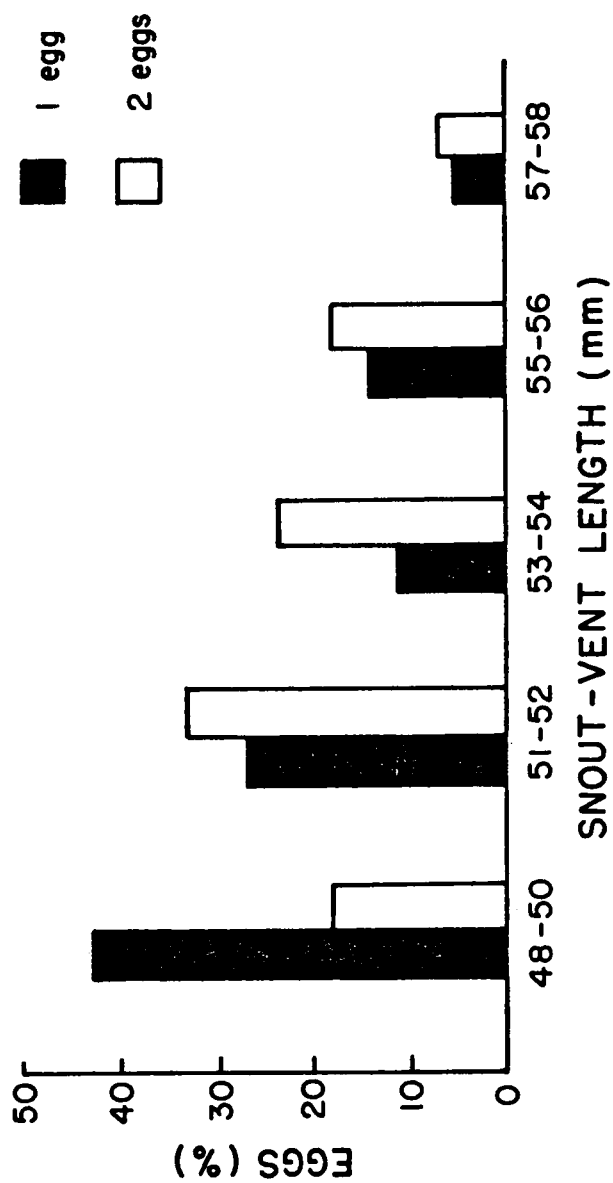


Figure 21. Relationship of female body size (snout-vent length) to percent of oviductal eggs produced for Tennessee anoles.

Table 10. Percentage of Tennessee and Louisiana females having zero, one, and two oviductal eggs during May, June, and July.

Populations	N	0 Eggs	1 Egg	2 Eggs
LOUISIANA Gordon (1956)	160	2	50.5	47.5
Hamlett (1952)	205	0	63	37
TENNESSEE Overall	86	2	48	50
1979	24	0	29	71
1980	33	3	55	42
1981	29	3	55	41

Table 11. Environmental data for three breeding seasons (1979-1981) for a Tennessee population of Anolis carolinensis.

Environmental Data	1979			1980			1981		
	May	June	July	May	June	July	May	June	July*
Mean Temperature (°C)	19.4	22.8	23.9	20.0	23.9	27.8	17.8	25.0	28.9
Maximum (°C)	30.0	31.7	31.1	31.1	33.3	38.3	29.4	33.3	35.0
Minimum (°C)	3.3	12.2	17.2	5.0	10.6	15.6	5.0	16.1	22.8
Mean Light (h)	14.0	14.5	14.2	13.9	14.6	14.3	13.8	14.6	14.4
Mean Rain (cm)	18.3	9.7	24.1	9.7	4.8	9.1	7.6	14.0	2.9

*Data for first 14 days only.

Table 12. A comparison of oviductal and recently deposited egg weights for Tennessee and Louisiana anoles.*

Egg Weights (mg)	TENNESSEE	LOUISIANA	t
Oviductal	228 $\pm$ 5	209 $\pm$ 7 ^a	1.63
	(95-383)	(131-266)	NS
	133	26	
Deposition	280 $\pm$ 12	238 $\pm$ 6	3.04
	(196-380)	(195-318)	p < 0.01
	19	19	
		220 $\pm$ 3 ^b	4.84
		(170-290)	p < 0.001
		54	

*The values reported are mean $\pm$ 1 S.E., (range), and sample size; t-tests are based on comparisons between populations.

^aData from Dessauer (1955) and personal communication.

^bData from Gordon (1956).

for the two populations; however, two interpopulation comparisons were made for data on recently deposited eggs and both were significant (Table 12). In the first comparison, the weights of eggs collected from Louisiana and Tennessee females housed in an environmental chamber were significantly different ($t = 3.04$, $p < 0.01$). When Tennessee egg weights were compared with Louisiana egg weights reported in another study (Gordon, 1956), significant differences also were found ($t = 4.84$, $p < 0.001$).

Gordon (1956) found that 75 freshly deposited eggs averaged 1.01 cm in length and 0.61 cm in width which agrees with measurements reported by Hamlett (1952). Although measurements for freshly deposited eggs are not available for the Tennessee population, the above measurements for Louisiana eggs are smaller than measurements for oviductal eggs from Tennessee females. Tennessee oviductal eggs averaged 1.14 ± 0.04 cm in length, 0.66 ± 0.01 cm in width, and had an average volume of 2.12 ± 0.03 cm ($N = 124$).

Estimates of reproductive effort were obtained both by the conversion of wet weight data to caloric data (Vitt, 1978) and by the ratio of wet egg weight to wet body weight. The latter method has been shown to be an accurate estimate of reproductive effort without converting to calories because caloric content of egg yolk does not differ between species (Ballinger and Clark, 1973). Using the caloric conversion factors, there is a perfect ($r = 1.00$, $p < 0.0001$, $N = 133$) correlation between clutch weight to body weight ratios and clutch calories to body calories ratios.

Reproductive effort estimates are smaller for oviductal eggs than for deposited eggs because the former were at different stages of development. Estimates for deposited eggs reveal significant differences ($p < 0.05$) between the two populations for both estimates of reproductive effort (Table 13).

Eggs from Tennessee females housed in an environmental chamber were collected at deposition and incubated at ambient temperature. The average incubation period for 11 Tennessee eggs was 39 ± 2 days (range = 36-43) as compared to 30 days (range = 28-41) for Louisiana eggs (Gordon, 1956). The mean snout-vent length for Tennessee hatchlings was 25 mm (range = 23-27 mm) while the hatchling size of Louisiana anoles varies from 22-25 mm (Hamlett, 1952) to 20-22 mm ($N = 72$; Gordon, 1956). Although our sample size is admittedly small, this preliminary evidence suggests that Tennessee hatchlings may be larger than Louisiana hatchlings.

IV. DISCUSSION

The cyclic nature of reproduction in many species of anoline lizards is well documented (Licht and Gorman, 1970; Sexton et al., 1971; Gorman and Licht, 1974; Stamps and Crews, 1976). Some species cue on rainfall (Licht and Gorman, 1970; Gorman and Licht, 1974; Stamps and Crews, 1976; Stamps, 1976) while high latitude or high elevation species appear to respond to temperature (Licht and Gorman, 1975; Gorman and Licht, 1974; Marion and Sexton, 1971). Licht (1973) demonstrated rather definitively that both temperature and photoperiod are important in regulating seasonal reproductive activity in Louisiana

Table 13. Comparison of reproductive effort ($\bar{X} \pm \text{S.E.}$) based on clutch weight/body weight (CW/BW) and clutch calories/body calories (CC/BC) ratios for oviductal and deposited eggs for Louisiana and Tennessee anoles.

Reproductive Effort Ratios	N	TENNESSEE	LOUISIANA	t
Oviductal Eggs				
CW/BW	133	70.57 ± 1.48	----	---
CC/BC	133	91.10 ± 1.91	----	---
Deposited Eggs				
CW/BW	19	99.78 ± 4.76	87.89 ± 3.34	2.04 (p < 0.05)
CC/BC	19	128.79 ± 6.14	113.45 ± 4.31	2.04 (p < 0.05)

Anolis carolinensis maintained in the laboratory. The major difference that he found between the sexes was in the regulation of gonadal regression which, in the female, is independent of photo-thermal conditions, and appears to be due to an "endogenous" rhythmicity. This refractory period also has been found in the iguanids, Uta stansburiana and Sceloporus undulatus (Tinkle and Irwin, 1965; Marion, 1969). In Louisiana male anoles, photoperiod was important during regression and temperature during recrudescence (Licht, 1971). Licht (1973) concluded that other factors, such as moisture and social stimulation, must be considered before any conclusions can be drawn concerning the exogenous factors affecting reproduction in this species.

In this study of the most northern population of Anolis carolinensis that has been investigated, it was found that the Tennessee population differs from the Louisiana population in some aspects of its response to environmental factors. In Tennessee males, as in those from Louisiana, photoperiod was highly correlated with testicular regression. During recrudescence in Tennessee males, however, both photoperiod and temperature were significantly correlated with testes weight. In Tennessee females, no correlation was found between ovarian weight and temperature or photoperiod during the nonreproductive phase of the cycle which agrees with the refractory period described for Louisiana females (Licht, 1973). During the period of vitellogenesis in Tennessee females, both light and temperature were correlated with ovarian weight, but photoperiod explained nearly twice as much of the variation in ovarian weight as

temperature. Thus, the Louisiana and Tennessee reproductive cycles differ primarily in the relationship of photoperiod to gonad weight. In Tennessee, photoperiod rather than temperature showed a higher correlation with gonad weight during recrudescence in males and during the period of vitellogenesis in females.

The difference in the results from this study and Licht's studies (1971, 1973) may be due to several factors. This study was based on Tennessee anoles that were sacrificed within 12 h of capture. These anoles had been exposed to completely natural surroundings where environmental conditions fluctuate daily. They also received ample stimulation from conspecifics, a factor shown to be important in regulating reproductive activity (Crews, 1980). Their diets and microhabitats were selected by the animals themselves and not imposed upon them by the investigator. Thus, these results should be more representative of what is actually occurring in natural populations of Anolis than studies based on anoles which have been maintained in the laboratory for long periods of time. This study demonstrates that laboratory results are not necessarily indicative of what is occurring under natural conditions.

Another climatic variable that seems important in the reproductive cycle of the Tennessee population is precipitation. The percentage of Tennessee females with two oviductal eggs varies depending on the amount of precipitation during the peak breeding period. These individuals simply may be retaining eggs until moisture conditions are appropriate for oviposition, as has been demonstrated in captive Louisiana anoles (Gordon, 1956). Such retention has been

demonstrated in other anoline lizards that are exposed to periodic drought conditions (Stamps, 1976) and may operate at high altitudes (Huey, 1977) or, as in this study, at higher latitudes. Stamps (1976) found that retained eggs of A. aeneus were larger than those not retained. Thus, one explanation for the large egg size found in the Tennessee population may be retention. An increase in egg size may reflect other physiological and morphological adaptations that enhance egg survival under certain environmental conditions. In certain other anoline lizards, egg physiology varies according to the environmental constraints of the species (Andrews and Sexton, 1981).

Temperate populations of Anolis show physiological adaptations for breeding that distinguish them from their tropical counterparts (Licht, 1971). Tropical anoles on Caribbean islands show only moderate declines in testes regression that occurs in October and November, and spermatogenesis rarely ceases completely. Consequently, recrudescence may be completed faster and thus earlier in the year (Licht, 1971; Licht and Gorman, 1970). As a result, these tropical anoles can be almost continually reproductive. This is in marked contrast to the abbreviated reproductive cycle in the Louisiana and Tennessee populations which is shorter than that of any other Anolis that has been studied (Licht, 1971; present study). By comparison, this reproductive cycle is more accentuated in Tennessee anoles, which are located near the northern limit of the range of the species, than in the Louisiana populations. Such a pronounced cycle has been found in high altitude anoles (Licht and Gorman, 1975) and would be predicted for species found at higher latitudes. Although tropical populations

of lizards may respond to only slight changes in photoperiod (Baker, 1947), temperate populations of Anolis show more marked responses to photo-thermal cues (Licht, 1971). These responses apparently are sufficient to distinguish them from other anoles introduced on the mainland since A. sagrei and A. distichus, which are sympatric with A. carolinensis in Florida, have testes cycles that resemble those of tropical populations (Licht, 1971; Licht and Gorman, 1970).

A summary of the events in the annual reproductive cycle of Tennessee anoles is shown in Figure 22. The males come into reproductive condition earlier than the females and begin establishing territories. Females then emerge from winter refuges around the time that vitellogenesis begins. Both sexes are at peak gonad size simultaneously for six weeks. The males start showing a decline in testes weight in June but the females continue producing eggs until mid-August. Whether or not the males continue to breed during this interval or whether females are retaining sperm is unknown. Sperm retention of up to a few months has been reported for Louisiana females (Licht, 1973; Fox, 1963). By August, the occurrence of oviductal eggs in Tennessee females has started to decline; only 45% of the females captured during this month had oviductal eggs. The period of gonadal regression is less than two weeks in the male but lasts until March in the female.

Specific differences between the Tennessee and Louisiana reproductive cycles are discussed in the results. One major difference between the populations that was apparent in both sexes was in the mean gonad size attained during the peak of the breeding season.

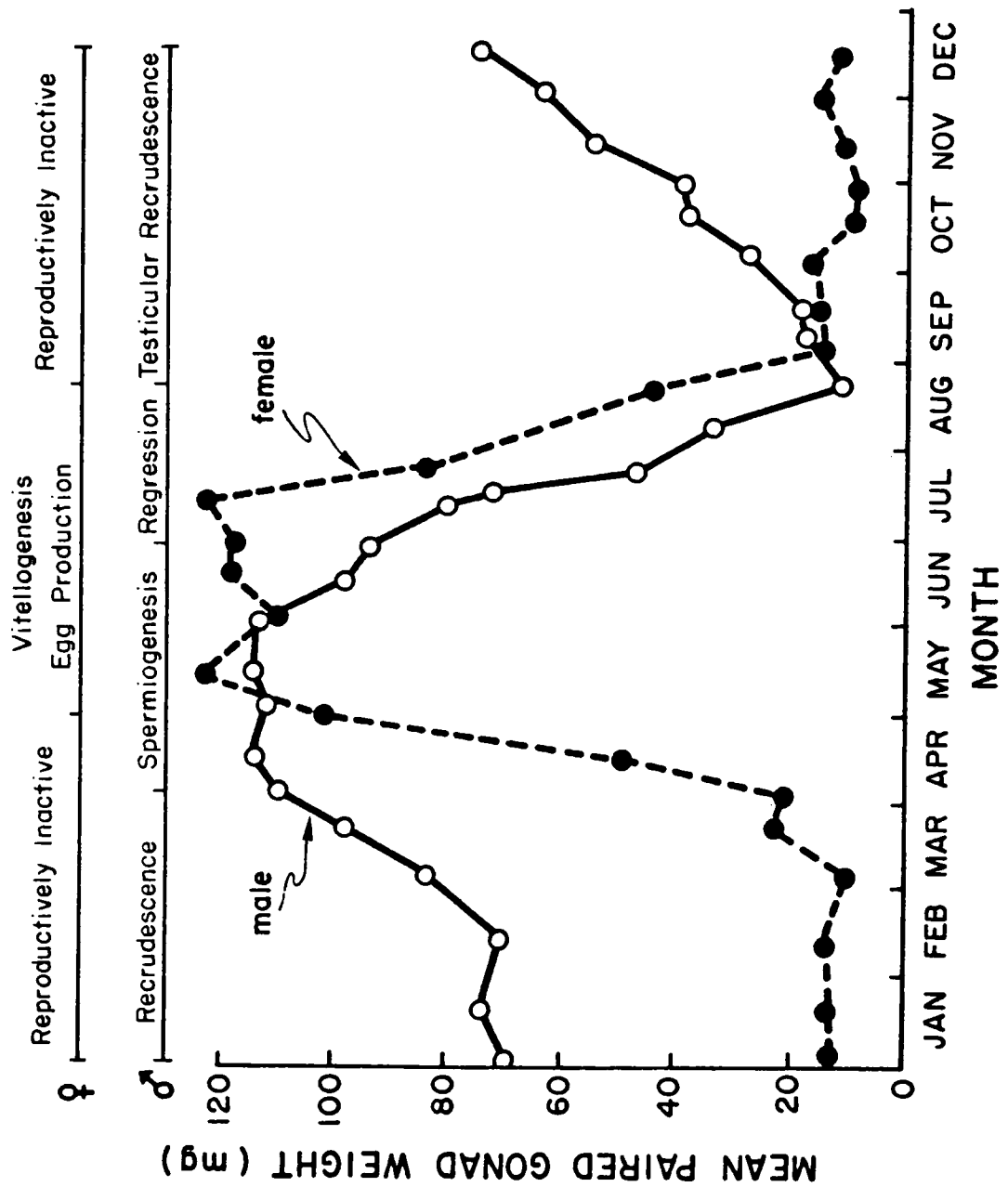


Figure 22. Summary of the annual reproductive cycle of Tennessee *Anolis carolinensis*.

One explanation for this difference among females might be related to the higher incidence of two oviductal eggs in Tennessee females, resulting in more advanced follicular development and, therefore, larger weights. In females with two oviductal eggs, one egg is more advanced than the other. The ovaries also have two yolked follicles with the largest yolked follicle on the same side as the largest egg. Thus follicular development differs from the Louisiana population where usually only one follicle matures at a time because females usually carry only one oviductal egg (Gordon, 1956; Hamlett, 1952; Smith et al., 1973).

This difference between populations in mean gonad size also occurred in males where an explanation is not readily apparent. Greater gonad size would seem not to be adaptive to an arboreal species since it would entail an additional weight burden. However, if it leads to greater reproductive success, i.e., more females inseminated over a shorter time period, then its contribution toward increasing the male's fitness would outweigh any potential disadvantages. This hypothesis would seem reasonable in a northern population whose reproductive activities may be curtailed due to climatic extremes. However, the behavioral observations necessary to confirm or refute this hypothesis have not been made.

Differences were found between the two populations in testosterone production during the annual cycle. Testosterone production was low during the peak in testes size and during regression; however, testosterone levels increased during recrudescence and attained a peak prior to the onset of the breeding season.

In Louisiana anoles, increasing testosterone levels were present during the initial period of recrudescence; however, these hormone levels decline in January and remain low throughout the cycle except for one spike occurring in July (Crews, 1980). In the latter study, samples were taken for 8 months ($N = 6-17$ anoles/month) of the year whereas in this study, results represent 23 samples ($N = 2-8$ anoles/sample) from 12 months ($N = 5-18$ anoles/month). The Tennessee data are based on freshly captured animals sacrificed within 12 h of capture and are not based on animals maintained in the laboratory or shipped across the country before they are sacrificed. Four Tennessee males that were left together 24 h before sacrificing showed the highest levels of testosterone of any animals examined in this study. These levels (mean = 20 ng/ml) were comparable to the spike shown by Louisiana males in July (Crews, 1980). Finally, our results are more compatible with the findings of Pearson et al. (1976) who found that androgen production was higher at lower temperatures (which would correspond to the winter months in this study) and that testicular growth occurred during this phase.

Testosterone levels are low during the phase of the reproductive cycle when territorial defense and courtship behavior are actively occurring. It may be that as testosterone levels decrease, testosterone is converted to another hormone responsible for maintaining reproductive behavior. According to the androgen aromatization hypothesis (Naftolin et al., 1975), brain testosterone is a precursor that is converted by aromatase enzymes to estrogen which activates male sexual behavior (McEwen et al., 1979). Aromatase enzymes are

present in some reptiles (turtles and snakes) and can convert androgen to estrone and estradiol (Callard et al., 1977, 1978). When castrated Anolis are given testosterone, dihydrotestosterone, and estradiol, testosterone and dihydrotestosterone stimulated development of the renal sex segment but only testosterone reinstated male behavior (Crews et al., 1978). However, when estradiol and dihydrotestosterone were administered simultaneously to castrates, an immediate restoration of male behavior was found in half of the animals tested (Crews, 1980). Thus, it appears that the capability of such a response is present in this species, and that estradiol and dihydrotestosterone act synergistically to elicit male territorial and reproductive behavior during the peak of the breeding season. Since one of the major functions of testosterone is anabolic, its primary role may be to prepare the gonads for the approaching breeding season by causing an increase in the size and responsiveness of the target organs and by stimulating the early stages of spermatogenesis. It appears, then, that any explanation of potential interactions between testes weight, stage of spermatogenesis, and hormone level must consider the phase of the seasonal cycle since this can determine the types of hormones present and the environmental conditions to which the organism may be responding.

The role of progesterone in the ovarian cycle of Anolis carolinensis is still uncertain. Current evidence suggests that the major effect of progesterone, especially in relation to sexual behavior and ovarian growth, may be inhibitory. Progesterone appears to be important in regulating female sexual receptivity if it is

administered after estrogen priming (McNicol and Crews, 1979); however, progesterone alone will not induce female receptivity (Crews, 1975a, 1978). It has been suggested that because of its inhibitory effect on estrus, progesterone may have a long term inhibitory effect on female sexual behavior (Crews, 1980). In another lizard, Xantusia vigilis (Xantusiidae), progesterone has an inhibitory effect on ovarian growth by reducing the uptake of vitelloprotein by oocytes (Yaron and Widzer, 1978). A similar mechanism may be operating in Anolis carolinensis since it has been shown that removal of the largest follicles from the ovaries removes an inhibitory substance (probably progesterone) that increases the sensitivity of smaller follicles to gonadotrophic stimulation (Jones et al., 1973b). In this study, evidence was found to support the hypothesis that progesterone has an inhibitory effect on ovarian growth since ovarian weight and progesterone were significantly inversely related ($p < 0.002$). Thus, the lowest levels of progesterone were present when ovarian weight was largest and when vitellogenesis was actively occurring.

This is in contrast to the model presented for captive Louisiana anoles where progesterone levels rise as follicles reach maturity and remain high following ovulation (McNicol and Crews, 1979). This difference in results may be due to the fact that Tennessee females were obtained in different phases of their follicular cycle or that the effects of progesterone vary depending on the other hormones that are present. We did not find high levels of progesterone present during vitellogenesis, but perhaps high levels are not necessary to regulate female receptivity as long as estrogen is present. It may be that,

as suggested in the male, another form of progesterone is present during the peak of the breeding season, and that it is this form that rises as follicles mature and ovulate.

As demonstrated in this study, intraspecific variation in reproduction occurs in Anolis carolinensis. In order to examine intraspecific variation more closely, it is helpful to relate this variation to specific reproductive strategies. Most studies invoking reproductive strategies have made interspecific comparisons where differences are quite obvious (Tinkle, 1969; Tinkle et al., 1970; Andrews and Rand, 1974). However, the basic principles involved also are applicable to intraspecific comparisons although the differences between populations may not be as easily discernible as those between species.

One of the major problems encountered in a study of reproductive strategies is the scarcity of available life history information. Very few studies have provided the type of detailed information that is necessary to make intra- and interspecific comparisons. The information necessary for such comparisons is often difficult to obtain and has been neglected because its importance has not been appreciated (Tinkle et al., 1970). Specific examples of the information needed include size at first reproduction, number of clutches or broods produced per season, clutch or brood intervals, egg size, female size, hatchling or neonate size and survivorship, and longevity (Tinkle et al., 1970). One of the purposes of this study was to obtain some of this information (especially estimates of reproductive effort) for a Tennessee population of Anolis which could be used for intra- and interspecific comparisons in this and other studies. Some preliminary

evidence suggests that Tennessee anoles may be showing a slightly more K-selected strategy than Louisiana anoles:

(1) In terms of snout-vent length, Tennessee females are larger than Louisiana females (Gordon, 1956), and Louisiana anoles are in turn larger than those from south Florida (King, 1966). King's (1966) mean values for females fall between approximately 39 and 44 mm with the largest female being 49 mm. Thus, increased body size may be one of the major parameters on which selection is acting in more northern habitats.

(2) There is some preliminary evidence that Tennessee hatchlings are larger than Louisiana hatchlings. This result is predictable since hatchling size is a linear function of female size in anoline lizards (Andrews and Rand, 1974).

(3) Tennessee eggs are significantly larger than Louisiana eggs, contradicting the prediction that tropical lizards (in this case, more southerly populations) should have larger eggs with a higher survivorship due to high intraspecific competition (Tinkle et al., 1970). It may be that the increased size and potentially increased survivorship of Tennessee eggs are important, not because of intra-specific competition, but as an adaptation to a temperate environment.

(4) The maximum annual reproductive output for the two populations varies. Since anoline lizards produce one egg/clutch (Smith et al., 1973), female Anolis carolinensis theoretically can produce one egg every 10-14 days during the five month breeding season in Louisiana and the three and a half month breeding season in

Tennessee, resulting in a potential annual egg production of 10-15 and 7-10 eggs/year, respectively. It should be emphasized that these estimates are based on optimal conditions and that the realized potential probably is near the lower estimate for each population. Although the Tennessee anoles have a lower reproductive output, they have a significantly larger reproductive effort than those from Louisiana. This estimate of reproductive effort for Tennessee anoles contradicts the hypothesis mentioned earlier that more temperate lizards should have a smaller annual reproductive effort since this effort is spread out over a longer reproductive life. One explanation for the higher reproductive effort and lower reproductive output for the Tennessee population may be that fewer but larger eggs are more adaptive for northern temperate environments, and that increased egg size rather than increased numbers of eggs results in a higher reproductive effort.

In conclusion, the climatic regime of an area no doubt exerts strong selection pressures that are responsible for intra- and inter-specific variation in reproduction. Fitness is increased in those females whose reproductive activity is restricted to environmentally favorable seasons (Andrews and Rand, 1974). Such seasonal restrictions could be detrimental for populations, such as the Tennessee population, located near the northern edge of the range where temperatures fluctuate not only seasonally but daily. However, preliminary evidence from this study suggests that the Tennessee females may be opportunistic and produce more eggs during wetter summers or perhaps retain eggs when conditions for oviposition are

not appropriate. It is this ability to be opportunistic and to respond to a changing environment that has no doubt been instrumental in the success of this wide ranging and highly adaptable species.

CHAPTER IV

OVERVIEW

Among the numerous species of tropical lizards that have been examined, it is somewhat unusual to find a species whose range extends from tropical to temperate areas and whose biology has been studied extensively. This study focuses on Anolis carolinensis, a member of the genus Anolis that originated in the tropics but has expanded its range into temperate and seasonal regions. Because Anolis carolinensis occurs in a wide variety of habitats over a broad geographic range, the intraspecific variation, which is predicted, is a reflection of the adaptations and comparisons that this species has made in successfully expanding its range.

One major adaptation found in the current study appears to be selection for larger body sizes in the more seasonal environment of Tennessee. This larger size also is reflected in larger gonad weight, which may or may not improve reproductive output, and in larger egg size. It appears, then, that the Tennessee population has compromised in that individuals have larger body sizes, which may be a disadvantage for an arboreal species, but which may improve fitness. This hypothesis needs to be tested by comparing adult survivorship for Tennessee and Louisiana populations and also by comparing egg survivorship under different environmental regimes. Preliminary data suggest that another adaptation of Tennessee females is that they are opportunistic in responding to environmental conditions by laying more eggs during

wetter summers and by retaining eggs during dryer summers when conditions are not appropriate for oviposition.

Findings of this study suggest several avenues for future research. This study demonstrated that the Louisiana population of Anolis carolinensis is not genetically representative of the species. This is especially important because the majority of the studies on this species have been conducted on the Louisiana population. It would be advantageous to know how comparable the Louisiana population is to other mainland populations of Anolis. The Louisiana population differs from the Tennessee population in aspects of its reproductive biology, in the genetics of some enzymes, and perhaps in other aspects of its biology as well.

The extent of the genetic variation found among mainland populations of Anolis is unknown. This study has sampled only seven populations and three of them (San Marcos, New Orleans, and Naples) diverge from the genetic similarity shown by the other four populations. Other populations need to be examined, especially populations from throughout the state of Florida where morphological (Duellman and Schwartz, 1958; Crews, 1980) and genetic divergence is most obvious. In addition, peripheral populations need to be sampled to determine whether the low levels of heterozygosity found in the Texas (Webster et al., 1973) and Tennessee populations occur in other outlying populations.

The effects of environmental parameters on gonad weight vary depending on whether the research was performed in the laboratory or in the field. More field studies of Anolis are needed to verify how applicable the results of laboratory studies are to natural populations.

Although the laboratory is suitable for partitioning out various factors that may be affecting the organism, the results must be analyzed cautiously. An animal in the field simultaneously receives multiple stimuli, all of which interact to one degree or another to integrate all aspects of its biology.

The results of the endocrinological study on Tennessee anoles conflict with those found for Louisiana anoles (Crews, 1980; McNicol and Crews, 1979). Additional studies are needed on other mainland anoles to determine whether such differences are due to regional environmental variation between populations, variation in field versus laboratory studies, or genetic variation between the populations. Research also is needed on the female endocrinological cycle, especially during the period of vitellogenesis and egg laying, and on the male to determine which androgens and/or estrogens are predominant in certain phases of the annual cycle. In fact, a thorough study of the endocrinological changes occurring during both the nonreproductive and reproductive portions of the annual cycle is needed.

One result that is difficult to explain is that the Tennessee population has larger gonads than the Louisiana population. It is not known what advantage large gonads provide for this primarily arboreal species. Is there an increased reproductive output? Are larger ovaries necessary to produce the larger eggs which may enhance hatchling survivorship? Are these larger gonads merely indicative of dominant status within a territory? Are larger gonads capable of increased gametogenesis and increased hormone production?

Perhaps future investigations will provide the necessary data required to make definitive statements concerning the reproductive biology of Anolis carolinensis.

LITERATURE CITED

LITERATURE CITED

- Andrews, R. and A. S. Rand. 1974. Reproductive effort in anoline lizards. *Ecology* 55: 1317-1327.
- Andrews, R. and O. J. Sexton. 1981. Water relations of the eggs of Anolis auratus and Anolis limnifrons. *Ecol.* 62: 556-562.
- Baker, J. R. 1947. The seasons in a tropical rain-forest. *J. Linn. Soc.* 41: 243-258.
- Ballinger, R. E. and D. R. Clark, Jr. 1973. Energy content of lizard eggs and the measurement of reproductive effort. *J. Herp.* 7: 129-132.
- Brewer, G. J. 1970. An Introduction of Isozyme Techniques. Academic Press, N.Y. 186 pp.
- Buth, D. G., G. C. Gorman, and C. S. Lieb. 1980. Genetic divergence between Anolis carolinensis and its Cuban progenitor, Anolis porcatus. *J. Herp.* 14: 279-284.
- Cagle, F. R. 1948. A population of the carolina anole. *Nat. Hist. Misc.* 15: 1-15.
- Callard, G. V., Z. Petro, and K. J. Ryan. 1977. Identification of aromatase in the reptilian brain. *Endocrin.* 100: 1214-1218.
- Callard, G. V., Z. Petro, and K. J. Ryan. 1978. Conversion of androgen to estrogen and other steroids in the vertebrate brain. *Amer. Zool.* 18: 511-523.
- Conant, R. 1975. Field Guide to Reptiles and Amphibians. 2nd ed. Houghton Mifflin, Boston. 429 pp.
- Crews, D. 1973a. Behavioral correlates to gonadal state in the lizard, Anolis carolinensis. *Horm. and Behav.* 11: 463-468.
- Crews, D. 1973b. Coition-induced inhibition of sexual receptivity in female lizards. *Physiol. and Behav.* 11: 463-468.
- Crews, D. 1974a. Castration and androgen replacement on male facilitation of ovarian activity in the lizard, Anolis carolinensis. *J. Comp. Physiol. Psych.* 87: 963-969.

- Crews, D. 1974b. Effects of group stability, male-male aggression, and male courtship behaviour on environmentally-induced ovarian recrudescence in the lizard Anolis carolinensis. J. Zool. 172: 419-441.
- Crews, D. 1975a. Effects of different components of courtship behavior on environmentally induced ovarian recrudescence and mating preferences in the lizard Anolis carolinensis. J. Comp. Physiol. Psychol. 87: 963-969.
- Crews, D. 1975b. Psychobiology of reptilian reproduction. Science 189: 1059-1065.
- Crews, D. 1978. Integration of internal and external factors in the regulation of lizard reproduction. In The Behavior and Neurobiology of Lizards. (N. Greenberg and P. MacLean, eds.) National Institute of Mental Health, Rockville, Maryland. pp. 149-171.
- Crews, D. 1980. Interrelationships among ecological, behavioral, and neuroendocrine processes in the reproductive cycle of Anolis carolinensis and other reptiles. In Advances in the Study of Behavior. Vol. 11. J. S. Rosenblatt, R. A. Hinde, C. Beer, and M. Bushnel (eds.), Academic Press, N.Y. pp. 1-74.
- Crews, D. and N. Greenberg. 1981. Function and causation of social signals in lizards. Amer. Zool. 21: 273-294.
- Crews, D., J. S. Rosenblatt, and D. S. Lehrman. 1974. Effects of unseasonal environmental regime, group presence, group composition and males' physiological state on ovarian recrudescence in the lizard, Anolis carolinensis. Endocrin. 94: 541-547.
- Crews, D., V. Traina, F. T. Wetzel, and C. Muller. 1978. Hormonal control of male reproductive behavior in the lizard, Anolis carolinensis: role of testosterone, dihydrotestosterone, and estradiol. Endocrin. 103: 1814-1821.
- Dessauer, H. 1955. Seasonal changes in the gross organ composition of the lizard, Anolis carolinensis. J. Exp. Zool. 128: 1-12.
- Duellman, W. E. and A. Schwartz. 1958. Amphibians and reptiles of southern Florida. Bull. Florida State Mus. 3: 181-324.
- Fox, W. 1958. Sexual cycle of the male lizard, Anolis carolinensis. Copeia 1958 (1): 22-29.
- Fox, W. 1963. Special tubules for sperm storage in female lizards. Nature 198: 500-501.

- Gay, V. L. and J. T. Kerlan. 1978. Serum LH and FSH following passive immunization against circulating testosterone in the intact male rat and in orchietomized rats bearing subcutaneous silastic implants of testosterone. *Arch. Androl.* 1 (3): 257-266.
- Gibori, G., E. Antczak, and I. Rothchild. 1977. The role of estrogen in the regulation of luteal progesterone secretion in the rat after day 12 of pregnancy. *Endocrin.* 100: 1483-1495.
- Gordon, R. E. 1956. The biology and biodemography of Anolis carolinensis Voigt. Ph.D. Dissertation, Tulane University.
- Gorman, G. C. and P. Licht. 1974. Seasonality in ovarian cycles among tropical Anolis lizards. *Ecology* 55: 360-369.
- Gorman, G. C. and J. Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. *Copeia* 1979: 242-249.
- Hall, W. P. and R. K. Selander. 1973. Hybridization of karyotypically differentiated populations in the Sceloporus grammicus complex (Iguanidae). *Evolution* 27: 226-242.
- Hamlett, W. D. 1952. Notes on breeding and reproduction in the lizard Anolis carolinensis. *Copeia*, 1952: 183-185.
- Harris, H. and D. A. Hopkinson. 1977. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publ. Co., Amsterdam.
- Huey, R. B. 1977. Egg retention in some high-altitude Anolis lizards. *Copeia* 1977: 373-375.
- Jones, R. E. 1975. Endocrine control of clutch size in reptiles. IV. Estrogen-induced hyperemia and growth of ovarian follicles in the lizard Anolis carolinensis. *Gen. Comp. Endocr.* 25: 211-222.
- Jones, R. E., J. J. Roth, A. M. Gerrard, and R. G. Kiely. 1973a. Endocrine control of clutch size in reptiles. I. Effects of FSH on ovarian follicular size-gradation in Leiopisma laterale and Anolis carolinensis. *Gen. Comp. Endocr.* 20: 190-198.
- Jones, R. E., A. M. Gerrard, and J. J. Roth. 1973b. Endocrine control of clutch size in reptiles. II. Compensatory follicular hypertrophy following partial ovariectomy in the lizard Anolis carolinensis. *Gen. Comp. Endocr.* 20: 550-555.

- Jones, R. E., N. B. Sedgley, A. M. Gerrard, and J. J. Roth. 1974. Endocrine control of clutch size in reptiles. III. 5-3 β -hydroxysteroid dehydrogenase activity in different-sized ovarian follicles of Anolis carolinensis. Gen. Comp. Endocr. 22: 448-453.
- Jones, R. E., R. R. Tokarz, and F. T. LaGree. 1975. Endocrine control of clutch size in reptiles. V. FSH-induced follicular formation and growth in immature ovaries of Anolis carolinensis. Gen. Comp. Endocr. 26: 354-367.
- King, F. W. 1966. Competition between two south Florida lizards of the genus Anolis. Ph.D. Dissertation, University of Miami.
- Licht, P. 1967a. Environmental control of annual testicular cycles in the lizard Anolis carolinensis. I. Interaction of light and temperature in the initiation of testicular recrudescence. J. Exp. Zool. 165: 505-516.
- Licht, P. 1967b. Environmental control of annual testicular cycles in the lizard Anolis carolinensis. II. Seasonal variations in the effects of photoperiod and temperature on testicular recrudescence. J. Exp. Zool. 166: 243-253.
- Licht, P. 1970. Effects of mammalian gonadotropins (ovine FSH and LH) in female lizards. Gen. Comp. Endocr. 14: 98-106.
- Licht, P. 1971. Regulation of the annual testis cycle by photoperiod and temperature in the lizard Anolis carolinensis. Ecology 52: 240-252.
- Licht, P. 1972. Environmental physiology of reptilian breeding cycles: role of temperature. Gen. Comp. Endocrin. Suppl. 3: 477-488.
- Licht, P. 1973. Influence of temperature and photoperiod on the annual ovarian cycle in the lizard Anolis carolinensis. Copeia, 1973: 465-472.
- Licht, P. and G. C. Gorman. 1970. Reproductive and fat cycles in Caribbean Anolis lizards. Univ. Calif. Publ. Zoology 95: 1-52.
- Licht, P. and G. C. Gorman. 1975. Altitudinal effects on the seasonal testis cycles of tropical Anolis lizards. Copeia 1975 (3): 496-504.
- Licht, P. and A. K. Pearson. 1969. Effects of mammalian gonadotropins (FSH and LH) on the testes of the lizard Anolis carolinensis. Gen. Comp. Endocrin. 13: 367-381.

- Marion, K. R. 1969. The reproductive cycle of the fence lizards, Sceloporus undulatus. Amer. Zool. 9: 1136.
- Marion, K. R. and O. J. Sexton. 1971. The reproductive cycle of the lizard Sceloporus malachiticus in Costa Rica. Copeia 1971: 517-526.
- Mayhew, W. 1967. Comparative reproduction in three species of the genus Uma. In W. W. Milstead (ed.), Lizard Ecology, a Symposium. Univ. Missouri Press, Columbia.
- McCoy, C. J. and G. A. Hoddenbach. 1966. Geographic variation in ovarian cycles and clutch size in Cnemidophorus tigris (Teiidae). Science 154: 1671-1672.
- McEwen, V. S., P. G. Davis, B. Parsons, and D. W. Pfaff. 1979. The brain as a target for steroid hormone action. Annu. Rev. Neurosci. 2: 65-112.
- McKinney, C. O. and F. B. Turner. 1971. Genetic variation in irradiated and nonirradiated populations of the lizard Uta stansburiana. Radiat. Res. 47: 530-536.
- McKinney, C. O., R. K. Selander, W. E. Johnson, and S. Y. Yang. 1972. Genetic variation in the side-blotched lizard (Uta stansburiana). Studies in Genetics VII (Univ. Texas Publ. 7213): 307-318.
- McNicol, D. and D. Crews. 1979. Estrogen/progesterone synergy in the control of female sexual receptivity in the lizard, Anolis carolinensis. Gen. Comp. Endocr. 38: 68-74.
- Naftolin, F., K. J. Ryan, I. J. Davies, V. V. Reddy, F. Flores, Z. Petro, M. Zuhn, R. J. White, Y. Takaoda, and L. Wolin. 1975. The formation of estrogens by central neuroendocrine tissues. Rec. Progr. Horm. Res. 31: 295-319.
- Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106: 283-292.
- Noble, G. K. and B. Greenberg. 1941. Effects of seasons, castration and crystalline sex hormones upon the urogenital system and sexual behavior of the lizard (Anolis carolinensis). J. Exp. Zool. 88: 451-479.
- Pearson, A. K., H. W. Tsui, and P. Licht. 1976. Effect of temperature on spermatogenesis, on the production and action of androgens and on the ultrastructure of gonadotropic cells in the lizard Anolis carolinensis.

- Pianka, E. R. 1970. Comparative autecology of the lizard Cnemidophorus tigris in different parts of its geographic range. *Ecology* 51: 703-720.
- Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphisms and systematics in the genus Peromyscus I. Variation in the old field mouse. *Studies in Genetics* VI. Tex. Univ. Publ. 7103: 49-90.
- Sexton, O. J., E. P. Ortleb, L. M. Hathaway, R. E. Ballinger, and P. Licht. 1971. Reproductive cycles of three species of anoline lizards from Isthmus of Panama. *Ecology* 52: 201-215.
- Sokal, R. R. and F. J. Rohlf. 1969. *Biometry*. W. H. Freeman and Co., San Francisco. 776 pp.
- Smith, H., J. Fawcett, G. Sinelnik, and R. E. Jones. 1973. The occurrence and significance of a protracted reproductive season with alternation of ovulation in lizards. *Bull. Can. Amphib. Rept. Cons. Soc.* 11: 1-4.
- Stamps, J. A. 1976. Egg retention, rainfall and egg-laying in a tropical lizard, Anolis aeneus. *Copeia* 1976: 759-764.
- Stamps, J. A. and D. Crews. 1976. Seasonal changes in reproduction and social behavior in the lizard Anolis aeneus. *Copeia* 1976: 467-476.
- Tinkle, D. W. 1969. The concept of reproductive effort and its relation to the evolution of life histories of lizards. *Amer. Nat.* 103: 501-516.
- Tinkle, D. W. and R. E. Ballinger. 1972. Sceloporus undulatus: a study of the intraspecific comparative demography of a lizard. *Ecology* 53: 570-584.
- Tinkle, D. W. and N. F. Hadley. 1975. Lizard reproductive effort: caloric estimates and comments on its evolution. *Ecol.* 56: 427-434.
- Tinkle, D. W. and L. N. Irwin. 1965. Lizard reproduction: refractory period and response to warmth in Uta stansburiana females. *Science* 148: 1613-1614.
- Tinkle, D. W., H. M. Wilbur, and S. G. Tilley. 1970. Evolutionary strategies in lizard reproduction. *Evol.* 24: 55-74.
- Vinegar, M. B. 1975. Life history phenomena in two populations of the lizard Sceloporus undulatus in southwestern New Mexico. *Amer. Midl. Nat.* 93: 388-402.

- Vitt, L. J. 1978. Caloric content of lizard and snake (Reptilia) eggs and bodies and the conversion of weight to caloric data. J. Herp. 12: 65-72.
- Webster, T. P., R. K. Selander, and S. Y. Yang. 1973. Genetic variability and similarity in the Anolis lizards of Bimini. Evolution 26: 523-535.
- Williams, E. E. 1969. The ecology of colonization as seen in the zoogeography of anoline lizards on small islands. Quart. Rev. Biol. 44: 345-389.
- Yaron, Z. and L. Widzner. 1978. The control of vitellogenesis by ovarian hormones in the lizard Xantusia vigilis. Comp. Biochem. Physiol. 60: 279-284.

VITA

Julia Karen Wade was born in Johnson City, Tennessee. She attended East Tennessee State University and graduated cum laude with a B.S. in biology in 1968. She holds two Master of Science degrees: one from the University of Kentucky in zoology (1970) and the other from Cornell University in wildlife science (1974). After receiving her degree from Cornell, she worked for two years as a research technician in the New York Cooperative Wildlife Research Unit at Cornell. She entered the doctoral program at The University of Tennessee, Knoxville, in 1976 and received her Doctor of Philosophy degree in zoology in 1981.

She is a member of Sigma Xi, American Society of Mammalogy, American Society of Ichthyologists and Herpetologists, and American Association for the Advancement of Science. Her recreational activities include sports (especially volleyball, paddleball, and raquetball), bridge for beginners, jogging, dancing, travel, and partying.