ABSTRACT

Title of Dissertation: GENETIC DIFFERENTIATION OF SELECTED EASTERN BOX TURTLE (*TERRAPENE CAROLINA*) POPULATIONS IN FRAGMENTED HABITATS, AND A COMPARISON OF ROAD-BASED MORTALITY RATES TO POPULATION SIZE

Susan Hagood, Doctor of Philosophy, 2009

Dissertation Directed By: Professor Lowell Adams
Department of Environmental Science and Technology

The decline of eastern box turtle populations is associated with habitat loss and fragmentation, lack of recruitment into breeding populations, removal of individuals from the wild for pets, and road mortality. Box turtle populations in many areas of the eastern United States may effectively be isolated as high traffic volumes on roads adjacent to turtle habitats prevent successful dispersal. If so, populations surrounded by heavily used roads may be less genetically diverse than those in relatively intact habitats. I investigated whether populations in three Montgomery County, Maryland parks that were surrounded by roads were genetically differentiated relative to populations in two larger habitats in Maryland's Prince George's and Anne Arundel counties. Sampling was conducted between 2005 and 2008. I used 10 microsatellite markers to compare these populations, and sampled in an additional five sites (two in

Maryland, and one each in Pennsylvania, the District of Columbia, and Florida), to better assess population structure. I found little evidence of genetic differentiation among central Maryland populations regardless of the extent of isolation. I attribute these findings to the slow rate of change in turtle evolution; the observed similarities in genetic diversity may reflect past rather than present gene flow. I found moderate to great differentiation in populations separated by substantial distances. To determine whether road mortality exceeds additive mortality levels believed to be a threat to population persistence, I estimated population size in the three Montgomery County, Maryland, parks using mark-recapture techniques, and compared these estimates to the number of dead, injured, and live turtles in or very near roads observed during walking and driving surveys conducted in 2006. Road-based morality rates fell within the range estimated to be inconsistent with population growth in one of the parks. Road mortality appeared to affect females out of proportion to their abundance in the population. Suggestions for reducing box turtle mortality in areas associated with high mortality rates are included.

GENETIC DIFFERENTIATION OF SELECTED EASTERN BOX TURTLE (*TERRAPENE CAROLINA*) POPULATIONS IN FRAGMENTED HABITATS, AND A COMPARISON OF ROAD-BASED MORTALITY RATES TO POPULATION SIZE

By

Susan Hagood

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2009

Advisory Committee:
Professor Lowell Adams, Chair
Dr. John Hadidian
Professor Patrick Kangas
Dr. Tim King
Professor W. Ray Stricklin

DEDICATION

To Jerdo, to my parents, and to Drew, the best turtle dog who ever wore hair

ACKNOWLEDGEMENTS

Seldom has a graduate committee been asked to serve so thanklessly for so long. I am indebted to them all – Drs. Gwen Brewer, John Hadidian, Patrick Kangas, Tim King, and Ray Stricklin, and in particular to Dr. Lowell Adams.

I am indebted to Dr. King as well for having opened his Aquatic Ecology Lab to me and for having helped so much in the analysis of DNA results. I thank members of his staff, in particular Mike Eackles, Robyn Johnson, Barb Lubinski, Cheryl Morrison, John Switzer and Colleen Young for their active interest and support, and for their patience with my many questions.

Dr. Ken Dodd provided the initial suggestion that led to this project, and arranged for me to sample turtles on Egmont Key National Wildlife Refuge. I was fortunate to spend nearly a week there with Dr. Dodd and his wife Marian Griffey. Ms. Griffey provided suggestions that served me well throughout the rest of the study for most efficiently and humanely obtaining blood samples from the tails of recalcitrant turtles.

Rob Gibbs of the Maryland National Capital Park and Planning Commission approved my request to conduct research in county parks and provided invaluable assistance in suggesting study sites, establishing plots, and illustrating road survey routes. Chris Swarth, Director of Anne Arundel County's Jug Bay Wetlands Sanctuary, not only granted me access to the box turtle populations of Jug Bay, but permitted me to use a dog – normally strictly prohibited – in my quest for a sufficient number of blood samples. He was steadfast in his willingness to talk turtles and to share his observations based on years of his own box turtle research at Jug Bay.

Ken Ferebee allowed me to collect samples at Rock Creek National Park, as did Carolyn Davis at Gettysburg National Military Park; both were delightful field companions for several days. Dr. Paula Henry facilitated my work in the Stickel Site at Patuxent Wildlife Research Center, organized the search for turtles, coordinated the collection of additional samples through the master's research of Jennifer Green, and was a constant source of encouragement and support. Though I did not ultimately include the sites in the study, I very much appreciated the willingness of Neal Fitzpatrick and Stephanie Mason of the Audubon Naturalist Society of the Central Atlantic States to allow me to search at Woodend Sanctuary, and that of Betty Boyland at Fort Detrick.

Amy Brody and Heather Seebach served admirably in the often disheartening task of collecting data on box turtles that attempted road crossings, and I am grateful to them for tackling this job with dedication and professionalism. Ms. Brody also helped in compiling maps and data on dead and injured box turtles.

Dr. Gwen Brewer was an enthusiastic and supportive member of my graduate committee, but was unfortunately unable to attend the dissertation defense. Graduate School rules prevent the listing of her name among committee members, despite her participation in the committee for all but the last three hours. I wish it were otherwise, and remain grateful to her for her interest and support and to both Dr. Brewer and Mary Goldie, also of the Maryland Department of Natural Resources (DNR), for their review and issuance of state permits for this work. Glenn Therres of the Maryland DNR shepherded my request for funding under the Maryland State Wildlife Grant, and I am grateful. Fred Bank of the Federal Highway Administration's Office of Natural Environment supported this research from its initiation both in principal and with FHWA research funds. Dr. Paul Garrett of the FHWA's Office of Project Development and Environmental Review took over upon Mr. Bank's retirement, and may now finally get his report to the Administration. The Washington Biologists' Field Club also provided critical financial support, and it too may at last see a return for its support.

Jim Hines and Dr. Jim Nichols of Patuxent Wildlife Research Center provided thoughtful and invaluable suggestions in the use of MARK. I am very grateful. Justin Clarke of the Maryland National Capital Park and Planning Commission was extremely helpful in providing information on traffic volumes in the county. Scott Farnsworth of Towson University was generous with his time and knowledge of ArcGIS. Dr. Peter Paul van Dijk shared his books and his in-depth knowledge of the process by which species in jeopardy are so recognized. Sandy Barnett nursed back to health a very sick little turtle from one of the study sites, and I am grateful. Her husband Colin was a source of constant support and professional quality box turtle photographs.

Deborah Morrin-Nordlund and Christi Lockard of the MEES Program, University of Maryland, were endlessly patient and helpful in guiding, steering and pushing me through the process.

This all started when Dr. John Grandy, Senior Vice President of Wildlife and Habitat Protection at The Humane Society of the United States (HSUS), said to me in an annual employee review: "Hagood, get a Ph.D." I will always be indebted to him for that directive, and for his acceptance of school work that often interfered with my work for The HSUS. No organization could have been more supportive of my doctoral distractions.

My husband Jerry designed and constructed the turtle holder that was indispensable in each and every sample I obtained, and about which the airport security personnel in Baltimore, Atlanta, and Tampa are still marveling. Our Labrador retriever "Drew" found so many of the turtles captured for this study that I often felt my role was simply to drive her from home to park and back again. My father – a gentleman and a scholar – modeled for me the kind of Ph.D. I'd like to be. While he passed away in 2007, perhaps he knows I may at last succeed.

TABLE OF CONTENTS

List of Tables ix

List of Figures xi

CHAPTER 1: INTRODUCTION 1
  1.1 Research Objectives 1
  1.2 Box Turtle Natural History 2
  1.3 Karyotype 6
  1.4 Box Turtle Status 6
  1.5 Research Hypotheses and Application 8

CHAPTER 2: GENETICS 10
  2.1 Introduction 10
  2.2 Review of Previous Genetic Studies 10
  2.3 Conservation of DNA sequences 14

CHAPTER 3: BOX TURTLES AND ROADS 16
  3.1 Introduction 16
  3.2 Direct Mortality 16
  3.3 Importance of Adult Box Turtles in Population Persistence 17
  3.4 Influence of Road Mortality on Box Turtle Sex Ratios 18

CHAPTER 4: METHODS 19
  4.1 Study Areas 19
  4.2 Control Sites – Genetics 19
    4.2.1 Patuxent Wildlife Research Center (PWRC) 19
    4.2.2 Jug Bay Wetlands Sanctuary (JBWS) 21
  4.3 Treatment Sites - Genetics 22
    4.3.1 Blockhouse Point Park (BHP) 22
    4.3.2 Muddy Branch Park – Unit Number 3 (MB) 22
    4.3.3 Wheaton Regional Park (WRP) 23
  4.4 Additional Sampling Locations – Genetics 24
    4.4.1 Casey Property (Casey) 24
    4.4.2 Isle of Wight Wildlife Management Area (IW) 24
    4.4.3 Rock Creek Park, District of Columbia (RCP) 24
    4.4.4 Gettysburg National Military Park, Pennsylvania (GNMP) 25
    4.4.5. Egmont Key National Wildlife Refuge, Florida (EK) 25
  4.5 Study Site Groupings 26
  4.6 Study Design 26
    4.6.1 Study Plot Surveys and Blood Sample Collection 26
    4.6.2 Road Surveys 29

4.7 Study Protocol and Permits 33
4.8 DNA Analysis 34
4.9 Data Analysis 35
4.9.1 Genetics 35
4.9.2 Population Size 38
4.9.3 Roadkill Rates 39

CHAPTER 5: GENETICS RESULTS AND DISCUSSION 43
5.1 Results 43
5.2 Discussion 56
5.2.1 Genetic Diversity within Populations 58
5.2.2 Genetic Diversity among Populations and Genetic Structure 61
5.2.3 Possible Causes of Weak Genetic Differentiation among Maryland Populations 63

CHAPTER 6: MORTALITY RESULTS AND DISCUSSION 67
6.1 Results 67
6.2 Discussion 72
6.2.1. Road Mortality 72
6.2.2. Natural Mortality Rates 74
6.2.3. Gender Bias 74
6.2.4. Patterns of Road-based Mortalities and Potential Remedies 76
6.2.5 Recommendations for Future Research 76

CHAPTER 7: CONCLUSIONS 79

APPENDICES 80

LITERATURE CITED 147

LIST OF TABLES

Table 5.1. Summary of allele frequency statistics for 10 collections of *Terrapene carolina* from Maryland, Pennsylvania, and Florida. Sample size (N), mean number of alleles per locus (Â), number of private alleles, observed and expected heterozygosity and estimates of the inbreeding coefficient (F_{IS}). F_{IS} values in bold are significantly different from zero. 44

Table 5.2. Allelic richness (corrected to the smallest sample size (n = 13)) per locus and population for 10 collections of *Terrapene carolina* from Maryland, Pennsylvania and Florida. See Table 5.1 for collection abbreviations. 45

Table 5.3. Linkage disequilibrium by locus pair, after Fisher (1935). 47

Table 5.4. Results of maximum likelihood assignment tests for 10 eastern box turtle populations using multilocus genotypes derived from 10 microsatellite DNA markers. Each row assigns every sampled individual to a population. Boxes highlight the assignment of individuals to their population. The final column indicates the percentage of correct assignment of individuals to their population. See Table 5.1 for collection abbreviations. 48

Table 5.5. Matrix of F_{ST} (below the diagonal) and R_{ST} (above the diagonal) values for all pairwise comparisons among 10 box turtle populations. Measures were derived from 10 microsatellite loci. Values in bold are significant after sequential Bonferroni correction. See Table 5.1 for population abbreviations. 53

Table 5.6. F_{ST}-based AMOVA for three population groupings. Percentage of variation does not sum to 100 in all cases due to rounding. See Table 5.1 for population abbreviations. 55

Table 5.7. R_{ST}-based AMOVA for three population groupings. Percentage of variation does not sum to 100 in all cases due to rounding. See Table 5.1 for population abbreviations. 57

Table 5.8. Results of microsatellite studies of other Emydid turtles. N refers to the total number of animals sampled. Â refers to the mean number of alleles per locus. NR indicates that the statistic was not reported. 59

Table 6.1. Search schedule, by year and park. 67

Table 6.2. Estimated turtles per hectare, estimated population size (N), and annual road and natural mortality rates, by park, on the basis of 2006 road and field surveys. 67

Table 6.3. Probability of mortality for a turtle crossing the designated road at the estimated traffic volume. Probability assumes that the turtle has a carapace length of 136 mm and travels in a path perpendicular to the road and at 3.3 m/minute. 68

LIST OF FIGURES

Figure 4.1. Eastern box turtle genetic sampling and population size estimation sites. a. All sites. b. Northern sites only. c. Maryland sites only. BHP, MB and WRP were used as treatment sites for analysis of genetic diversity in habitats isolated by roads and to estimate population size. PWRC and JBWS were used as control sites for analysis of genetic diversity in contiguous habitats. Please see Table 5.1 for explanation of abbreviations. 20

Figure 4.2. Representative photos of carapace (left) and plastron of study turtle. 28

Figure 4.3. Turtle holder used to restrain turtles to obtain blood sample (left). A small amount of blood was withdrawn from the caudal vein for DNA analysis. 29

Figure 4.4. Location of Blockhouse Point Park study plot and road-mortality survey route. The survey route on the western edge was eventually eliminated from the study as turtles found along it were more likely to represent the C&O Canal population than the BHP population. 30

Figure 4.5. Location of Muddy Branch Stream Valley Unit 3 study plot and road-mortality survey route. 31

Figure 4.6. Location of Wheaton Regional Park study plot and road-mortality survey route. 32

Figure 4.7. Road densities surrounding study parks, Montgomery County, MD. 42

Figure 5.1. Log probability of allelic data as a function of *k*. Probability determined for 13 potential population clusters (the number of collections (10), plus 3, after Evanno et al. 2005). The maximum value of LnP(D) is associated with the true number of population clusters (Evanno et al. 2005). 48

Figure 5.2. Summary plot generated by the sequential cluster analysis of the program STRUCTURE performed on the multilocus (*N* = 10) genotypes of 10 populations of eastern box turtles. Each individual is represented by a single vertical line, broken into *k* colored segments, the length of which is proportional to the membership fraction in each of 2, 3, 4, and 5 clusters in a, b, c, and d, respectively. Numbers below each bar correspond to populations as follows: 1. Patuxent Wildlife Research Center; 2. Gettysburg National

Military Park; 3. Jug Bay Wetlands Sanctuary; 4. Blockhouse Point Park; 5. Muddy Branch Stream Valley Park; 6. Wheaton Regional Park; 7. Rock Creek Park; 8 Casey Development;; 9 Isle of Wight; 10. Egmont Key. 49

Figure 5.3. Principal component analysis of all populations. The Florida population is confined to the oval on the left. Circles are 95% confidence elipses. 50

Figure 5.4. Principal component analysis excluding the Florida population. Circles are 95% confidence elipses. 50

Figure 5.5. Neighbor-joining tree with 100% bootstrap support. Branch support was < 70% for each population; the scale represents chord distance units. 51

Figure 5.6. Interpolation of genetic landscape shape. Surface plot heights reflect genetic distances. a. All sites. b. All sites except for EK. c. All sites except for EK and IW. 52

Figure 5.7. Autocorrelation plot comparing pairwise geographic distance with the average genetic distance between pairs of individuals for four distance classes. The dotted line is the average pairwise genetic distance from the full data set. Its intercept with the autocorrelation line indicates the maximum distance of significant spatial autocorrelation. 53

Figure 6.1. Location of turtles encountered in roads bordering BHP. Turtles found in the western section of the map near Senaca Road were most closely associated with the Chesapeake and Ohio Canal National Historic Park and are not included in road mortality counts for BHP. Round symbols indicate male or female turtle was alive. Diamond-shaped symbols indicate male, female, or turtle of indeterminate sex was dead. 69

Figure 6.2. Location of turtles encountered in roads bordering MB. Round symbols indicate male or female turtle was alive. Diamond-shaped symbols indicate male, female, or turtle of indeterminate sex was dead. 70

Figure 6.3. Location of turtles encountered in roads bordering WRP. Round symbols indicate male or female turtle was alive. Diamond-shaped symbols indicate male, female, or turtle of indeterminate sex was dead. 71

CHAPTER 1: INTRODUCTION

1.1 Research Objectives

Populations of the eastern box turtle are declining largely as a result of habitat loss and fragmentation, commercial collection, and highway morality (Ernst and Lovich 2009; Gibbons et al. 2000). The most serious direct threat to the box turtle is road mortality (Dodd 2001). Taken together, these factors reduce the size of local populations and increase the distance between them, thereby rendering them vulnerable to demographic and environmental stochasticity and possibly reduced genetic diversity (McDougal 2000; Shepard et al. 2008a).

Genetic theory predicts that small and isolated populations of a species are susceptible to reduced genetic diversity and inbreeding (Hedrick and Kalinowski 2000; Rubin et al. 2001). To the extent that eastern box turtle populations in central Maryland effectively are isolated from other populations by roads and other anthropogenic landscape changes that render surrounding habitat inhospitable and successful dispersal unlikely, populations confined to small habitat patches may exhibit less genetic diversity than populations in relatively contiguous habitats (McDougal 2000). I investigated what effects habitat fragmentation may be having on the genetic structure of eastern box turtle populations in central Maryland.

Quantitative assessments of road-based mortality in box turtle populations have not been undertaken. I also estimated the percentage of the adult population that is lost to vehicular mortality. If annual mortality rates exceed the 5% of population size predicted by Gibbs and Shriver (2002) to be unsustainable in terrestrial turtle species, management programs must be structured to reduce roadkill as one of the few sources of mortality within our ability to influence. If road-based mortality rates are sufficiently high, genetic effects are more likely to result.

1.2 Box Turtle Natural History

The genus *Terrapene* includes four North American species: *carolina; coahuila; nelsoni; and ornata.* There are six extant *T. carolina* subspecies: *bauri; carolina, major; triunguis; mexicana; yucatana*. Box turtles are members of the family Emydidae, the largest family of extant turtles with 12 genera and 48 living species (Ernst and Lovich 2009).

The range of the eastern box turtle extends from southern Maine west to Michigan, Illinois, eastern Kansas, Oklahoma and Texas, and south to the Florida Keys (Ernst and Lovich 2009). Throughout much of eastern North America, the eastern box turtle occurs in mesic forests and bottomlands, open fields, and the edge habitats between them (Dodd 2001).

Box turtles in the mid-Atlantic region are most associated with oak-hickory and hardwood-pine forests (Dodd 2001). Forested box turtle habitat is characterized by heaps of woody debris and tangles of shrubs and vines that provide abundant resting and hiding places for turtles (Dodd 2001; Stickel 1950).

Box turtles are omnivorous. Dodd (2001) reported that the principal food of the box turtle throughout its range consists of invertebrates such as earthworms, gastropods, and insects in all developmental stages. A wide variety of plant matter is also eaten, including leaves, buds, fruits, seeds, mosses and fungi; carrion is also consumed (Ernst and Lovich 2009).

Habitat use varies with season. Turtles tend to overwinter and spend the summer months in forested areas, and venture into edges and open fields during spring and fall (Ernst and Lovich 2009). Daily habitat use varies as well, dictated by temperature and humidity/moisture levels. The most favorable daily conditions for turtle activity include warm temperatures, high humidity and frequent rains (Dolbeer 1969; Stickle 1950). During periods of hot and dry or cool weather, and at night, turtles are relatively inactive (Dodd 2001; Stickel 1950). Turtles may move some distance to exploit seasonally available foods (Dodd 2001), and in apparent forays to find mates and suitable nesting sites (Stickel 1950). Temperature also dictates the length of the box turtle's activity season (Dodd 2001). In northern latitudes (including central Maryland), the activity period of box turtles generally is from April to October (Dodd 2001), though weather conditions will influence both emergence from torpor and entry into it.

A number of individuals within many box turtle populations may be transients, in that they are marked once and never seen again, despite intensive searching (Dolbeer 1969; Schwartz and Schwartz 1974; Stickle 1950). Many adult box turtles, however, appear to maintain a home range -- an area within which they spend the majority of their time and in which food sources and resting and wintering sites are well known (Schwartz and Schwartz 1974; Schwartz and Schwartz 1991; Stickle 1950). The size of home ranges varies depending on the study and the manner in which it was calculated (summarized by Dodd 2001). Estimates of mean home range size generated during long-term studies of *T. carolina* indicate that home range size varies over time in the same location. For instance, Hallgren-Scaffidi (1986), calculated a mean home range size of 0.2 ha during her studies at Patuxent Wildlife Research Center, whereas Stickle (1989) found mean home range size to be 1.2 ha for males and 1.13 ha for females. Schwartz and Schwartz (1974) reported that mean home range size was 1.53 ha for males and 1.45 ha for females, and in a later paper noted that it had increased to 5.2 ha for males and 5.1 ha for females (Schwartz et al. 1984).

According to Dodd (2001), box turtles may mate at any time during the active season, though the mechanism by which turtles locate each other is unknown. Female box turtles are able to store sperm within their oviducts for some time, in some cases for several years (Ewing 1943). Nest site selection on the part of the female determines both the temperature at which the eggs develop (and thus the sex of the hatchlings, as higher nest temperatures produce predominately female hatchlings), and the likelihood of predation of both eggs and hatchlings (Dodd 2001). Stickle (1950), reported that female box turtles may travel up to 774 m from their home range to suitable nesting sites.

Eggs are deposited in flask-shaped excavations dug by the female using only her hind legs (Allard 1948). Four to five eggs are typically deposited; eastern box turtles typically nest two or three times per activity season (Ernst and Lovich 2009). Eggs hatch after an average of 73 days (50-110, $n = 33$; Ernst and Lovich 2009).

Predation of eggs and hatchlings is high (Dodd 2001; Ernst and Lovich 2009). Little is known about habitat use in very young box turtles, although Forsythe et al. (2004) monitored two nests in Illinois, removed hatchlings upon emergence in 2001, and overwintered them in a protected outdoor area. Transmitters were attached to three hatchlings from each nest before being returned to the nest site in the early spring of

2002. Three transmitters were subsequently found without turtles. The authors believed that the turtles were preyed upon on the basis of tooth marks on the transmitters. The remaining three turtles were tracked for one month, and demonstrated a preference for areas with less canopy closure and higher light intensity than randomly chosen areas within the study site.

Stickel and Bunck (1989) studied the growth of eastern box turtles during a long-term study in central Maryland. According to a bivariate analysis of age-related growth, males grow an average of 6.7% per year in carapace length between 8 and 13 years, whereas females grow an average of 5.3% per year. Growth slows between 14 and 19 years (2.3% per year for males; 3.4% per year for females), and slows again after age 20. Maximum carapace length in males was 159 mm, and in females 152 mm. Adult males are larger than females in every dimension except carapace height.

It is not known at what age eastern box turtles begin to reproduce, but three males in the Stickel and Bunck study acquired the red eye color typical of adults at about 20 years of age. Dodd (2001) speculated that the faster growth rate of females in their late teens relative to males occurs so as to achieve as large a size as possible prior to the onset of reproduction. If true, females begin to reproduce in their late teens to early twenties.

Reported adult sex ratios tend to be male-biased. In the long-term Maryland study at Patuxent Wildlife Research Center, ratios were variously reported as either male biased (Hall et al. 1999; Stickel 1989), or nearly equal (Stickel 1950, 1989). The population declines reported from 1945 to 1995 (Hall et al. 1999) were coincident with male bias. Male bias also has been observed at Jug Bay Wetlands Sanctuary, where turtles also are relatively remote from heavily used roads. About 60% of the population is composed of adult males, while 35% is made up of adult females (C Swarth, unpublished data). Female box turtles at Jug Bay have larger home ranges than do males, and thus may encounter more roads (C Swarth, pers comm, 2 July 2009).

Schwartz and Schwartz (1974) found nearly equal sex ratios in *T.c. triunguis.* Dodd (1997) reported that the Egmont Key population of *T.c. bauri* was consistently recorded as 1.6 males:1 female over 18 collecting trips from 1991 to 1995. Dolbeer (1969) reported a *T.c. carolina* male:female ratio of 1.61:1; this ratio had increased to 3:1 by 1972 (Yahner 1974). Ernst and Lovich (2009) reported that a northern Virginia *T.c. carolina* population was found to have a male:female ratio of 1.19:1.0 (Boucher 1999); a second Virginia population was found to have a 1:1 male:female ratio (Wilson and Ernst 2005). In contrast, Budischak et al. (2006) reported an eastern box turtle male:female ratio of 0.74:1.00.

According to Stickel (1978), box turtles do not generally survive more than 30 to 40 years, despite reports of much older individuals. Dodd (2001) noted that ages exceeding 50 years seem readily attainable by box turtles.

1.3 Karyotype

Box turtles have 50 diploid chromosomes, including 26 macrochromosomes and 24 microchromosomes (Killebrew 1977).

1.4 Box Turtle Status

Range-wide, remaining box turtle habitat increasingly is fragmented by development and the construction and use of roads (Dodd 2001; McDougal 2000). In eastern North America, many populations exist in isolated fragments of forest habitat located in urban and suburban areas (see, e.g., Brisbin et al. 2008; Ferebee and Henry 2008). Across Maryland, forest cover has been declining for decades, with an average loss of more than 2428 ha/yr between 1986 and 1999. For those counties for which forest clearing was reported, an average of 1125 ha/yr of forest has been cleared without replacement since 1992 (Maryland Department of Natural Resources 2009).

Increasing concern is being expressed about the box turtle's status throughout its range (Dodd 2001; Thorbjarnarson et al. 2000; Williams and Parker 1987), and in Maryland (Hall et al. 1999; Hallgren-Scaffidi 1986; Stickel 1978). Few long-term studies of box turtle populations have been conducted, but those that have are unanimous in their conclusion that populations are declining (Hall et al. 1999; Schwartz and Schwartz 1991; Stickel 1978).

Concern over the status of box turtles has led to the adoption of protective measures by the Conference of the Parties to the Convention on International Trade in Endangered Species of Wild Fauna and Flora. At their 1994 meeting, the Parties voted to add all *Terrapene* species to Appendix II, including the two species native to the United States – the eastern box turtle and the ornate box turtle (*T. ornata*). Appendix II includes species that, although not necessarily threatened with extinction at present, may become so unless trade is strictly controlled. The Parties' action was prompted not only by the substantial increase in box turtle exports that occurred as the availability of certain tortoise species was declining, but also by long-term populations declines of native *Terrapene* populations (U.S. Fish and Wildlife Service 1996).

In addition, the Tortoise and Freshwater Turtle Specialist Group of the International Union for the Conservation of Nature is currently re-assessing the Red List status of the eastern box turtle. Preliminary information indicates that the species, last assessed in 1996 as 'Near Threatened,' may be evaluated as 'Vulnerable' by 2010, based on the scale of habitat loss and degradation, combined with population impacts from collection and road mortality during the past two turtle generations, and the expectation that these impacts will continue for at least another box turtle generation (P P van Dijk, in Litt, 9 Sept 2009).

Demographic characteristics render turtles particularly vulnerable to human impacts (Gibbs and Amato 2000). These animals are both long-lived and slow to reach sexual maturity. The first decade of a box turtle's life largely is devoted to growth and the development of the shell that is its primary means of protection from predators. Egg and hatchling mortality is typically high (Dodd 2001; Ernst and Lovich 2009) increasing the importance of juveniles and adults to the population. Congdon et al. (1993) concluded in part that the suite of life-history traits that coevolve with longevity results in populations that are severely limited in their ability to respond to increases in neonate mortality and even less so to increased mortality of juveniles or adults.

1.5 Research Hypotheses and Application

I tested two hypotheses: 1) Genetic diversity of box turtle populations isolated by roads is the same as that of populations in contiguous habitats; and, 2) road mortality rates do not exceed those predicted to be consistent with population persistence.

The results of this research should inform box turtle management programs by determining the degree to which populations isolated by roads are less genetically diverse than those in intact habitats, and thus less able to adapt to a changing environment (Converse et al. 2005; McCallum et al. 2009; Rubin et al. 2001). Information on genetic structure should help to determine whether repatriation or relocation efforts should be incorporated in conservation programs. In addition, the determination of road-based mortality rates will indicate whether programs to protect populations in fragmented habitats must incorporate structures designed to help turtles safely navigate roads.

The broader scientific relevance of this research lies in a better understanding of the vulnerability of long-lived organisms to increased mortality, habitat destruction and the genetic consequences of small population size and habitat fragmentation. Understanding that long-lived vertebrates are limited in their ability to compensate for increases in mortality rates is important in structuring management programs (Congdon et al. 1994).

CHAPTER 2: GENETICS

2.1 Introduction

Habitat fragmentation has been identified as the primary cause of contemporary extinctions (Wilcox and Murphy 1985), although Fahrig (1997) asserted that the effects of habitat loss and fragmentation are often confounded in studies claiming to show fragmentation effects. Though the negative effects of habitat loss outweigh those of habitat fragmentation (Fahrig 1997), fragmentation also reduces population size (Mitchell and Klemens 2000; Soulé et al. 1988), and potentially increases the importance of stochastic demographic and genetic effects and the threat posed by environmental extremes (Forman et al. 2003). Roads are a primary source of habitat fragmentation (Forman and Alexander 1998; Trombulak and Frissell 2000) that may contribute to reduced genetic variation in turtles (Gray 1995).

As noted by Rubin et al. (2001), there are at least three reasons that render the preservation of genetic variation a major goal of conservation biology: 1) loss of genetic variation may increase the risk of extinction of the population through declines in fecundity and viability; 2) populations with reduced genetic variation may be less able to adapt to future environmental change; and, 3) the preservation of genetic variation may be integral to identifying genetically distinct populations for management purposes.

2.2 Review of Previous Genetic Studies

The genetic structure of *Terrapene* has received little research attention; the limited investigations that have been conducted generally are characterized by a finding of relatively little genetic differentiation among populations. For instance, Butler (2008) reported no structuring of *Terrapene* populations in the Florida panhandle as part of an investigation of the evolutionary history and genetic relationships among four *Terrapene* subspecies.

Terrapene c. carolina populations in fragmented habitat in Michigan's Lower Peninsula were not genetically differentiated at a distance of up to 60 km (Marsack and Swanson 2009). Richtsmeier et al. (2008) found little differentiation between populations of *T.o.ornata* in Iowa separated by approximately 120 km, one of which is currently isolated by anthropogenic landscape changes.

The endangered Coahuilan box turtle (*Terrapene coahulia*), the only obligate aquatic box turtle, is native to the desert springs of Cuatro Ciénegas, Mexico. An investigation into local and regional dispersal between springs using demographic and genetic estimates found that differentiation has not yet occurred, despite increasing isolation of the study populations (Howeth et al. 2008).

Terrapene populations separated even for decades evidenced little differentiation. Kuo and Janzen (2004) used microsatellite[1] DNA to examine the effects of a population bottleneck on *T. o. ornata*. They found only limited differentiation between an Illinois population that had experienced a bottleneck for the past century, and a larger and relatively undisturbed Nebraska population.

Widely separated populations that are nonetheless genetically similar may reflect historical rather than contemporary gene flow (Bossart and Prowell 1998), and some turtles must successfully disperse sufficient distances to promote gene flow between

---

[1] DNA fragments of tandemly repeated sequences consisting of one to six nucleotides repeated between approximately 5 and 100 times (Allendorf and Luikart 2007).

populations despite the challenges of movement in fragmented habitat. For instance, during long-term studies of three-toed box turtles (*Terrapene c.triunguis)* in Missouri, Kiester et al. (1982) concluded that three of seven turtles newly found despite more than a decade of intensive mark-recapture activities were indeed transients, with one traveling a straight-line distance of approximately 10.0 km during the fourteen months in which it was tracked. The three turtles were all males, two of which were seen mating with or very near females on several occasions

The results of investigations of genetic structure in other Chelonians in many cases mirror the findings in *Terrapene* for populations separated for a relatively short period of time. The wood turtle (*Glyptemys insculpta*) is believed to be one of the most endangered freshwater turtles in North America (Castellano et al. 2008). This species occupies riverine and wetland habitats affected by habitat loss and degradation. As a result, populations increasingly are isolated. An investigation of genetic structure in this species at Delaware Water Gap National Recreation Area found little differentiation between populations, suggesting that these populations are still able to sustain sufficient gene flow to offset the effects of isolation and inbreeding (Castellano et al. 2008).

Scribner et al. (1986) also found that gene flow among population clusters of yellow-bellied slider turtles (*Pseudemys scripta*) separated by distances up to 1.95 km was sufficient to prevent differentiation. Some measures of genetic differentiation, however, indicated that populations separated by aquatic habitat experienced lower gene flow relative to those separated by terrestrial habitat. Rosenbaum et al. (2007) found low levels of genetic differentiation in populations of the threatened bog turtle (*Glyptemys muhlenbergii*) widely separated in time and space.

Other investigators have found evidence of Chelonian genetic population divergence. In contrast to the findings of Castellano et al. (2008), Tessier et al. (2005) found that two wood turtle populations on the north shore of the St. Lawrence River in Quebec, Canada, were genetically differentiated from each other and from four populations sampled on the south shore. They believe that wood turtles independently colonized opposite shores of the St. Lawrence 5000-8000 years ago with the retreat of Pleistocene glaciers, with refugia southwest and east of the Great Lakes serving as the source of populations colonizing the north and south shores, respectively.

Ciofi et al. (2002) reported that populations of the giant Galápogos tortoise were in general genetically divergent, reflecting a long history of isolation and low gene flow. Schwartz and Karl (2005) found that widely separated populations of the gopher tortoise (*Gopherus polyphemus*) at 21 locations throughout Florida and southern Georgia were clearly differentiated. Likewise, populations of Blanding's turtles (*Emydoidea blandingii*) believed to have been separated for 4000-8000 years were found to be genetically distinct (Mockford et al. 1999; Rubin et al. 2001); investigators believed that relatively small populations in urban landscapes in the Greater Chicago area, Nova Scotia and Wisconsin may be genetically depauperate (Rubin et al. 2001). Gray (1995) discovered that northern and southern populations of *Clemmys marmorata* were genetically distinct, with coastal populations, in particular, of the much smaller northern population exhibiting greatly reduced genetic variability such that unrelated individuals in these coastal populations were more similar genetically than were first-degree relatives from the larger southern population.

2.3 Conservation of DNA sequences

Genetic divergence seems to be the exception rather than the rule in Testudines. Schwartz et al. (2003) found all nine of the selected microsatellite loci in four North American *Gopherus* species. Six of these loci were found in striped mud turtles (*Kinosternon bauri*), and three in the green sea turtle (*Chelonia mydas*). King and Julian (2004) tested 27 microsatellite primer sets developed for *Glyptemys muhlenbergii* in 13 additional species, each of which represented a genus within the Emydidae family. Of 351 locus-species combinations, 305 (87%) amplified[2] one or more fragments. FitzSimmons et al. (1995) found that polymorphic microsatellite loci were conserved across two extant families of marine turtles – Cheloniidae and Dermochelyidae – and could be amplified in a freshwater turtle, *Trachemys scripta*. These authors concluded that it appears that the origin of some of these microsatellites occurred in an ancestral lineage that predated the split of marine from freshwater turtles, indicating a conservation of the flanking regions across 300 million years.

Avise et al. (1992) found that mitochondrial DNA sequence divergence in Testudines is an average of eight times slower than the commonly accepted 2% per million years reported for several mammals, gallinaceous birds and geese, salmonids, and Hawaiian *Drosophila.* Chromosome arm homologies inferred from G-band patterns have been conserved for over 200 million years among the families of the suborder Cryptodira

---

[2] Amplification exponentially increases the number of copies of the DNA fragment of interest. It relies on the binding of primers to regions that flank the microsatellite. Only if these regions are identical will the primers properly bind. Successful amplification of loci from a diverse assemblage of Chelonians is evidence that these regions are identical, despite the thousands of years that separate the species.

(Bickham 1981). Nuclear DNA restriction fragment length polymorphisms (RFLPs)[3] were shared at green turtle rookeries around the world, potentially indicative of the slow rate of evolution of nuclear DNA (Karl et al. 1992).

---

[3] RFLPs are produced by the digestion of DNA into fragments that vary in number on the basis of mutations within the restriction sites at which the DNA is digested.

CHAPTER 3: BOX TURTLES AND ROADS

3.1 Introduction

Roads can negatively affect wildlife by decreasing habitat quality and quantity, compromising access to needed resources, subdividing populations, and as a source of direct morality of individuals (Forman and Alexander 1998). In areas in which road density and/or vehicle speeds are high, road-based mortality can negatively impact populations (Glista et al. 2007; Rosen and Lowe 1994; Row et al. 2007; Shepard et al. 2008b; Szerlag and McRobert 2006). As relatively slow-moving animals, reptiles are vulnerable to direct mortality (Haxton 2000; Smith and Dodd 2003).

3.2 Direct Mortality

Concern about the impact of direct highway mortality on box turtles has increased in recent decades (see, e.g., Ernst et al. 1994; Ernst and Lovich 2009; Gibbons 1987; Gibbs and Shriver 2002; Stickel 1978). Steen and Smith (2006) noted that there is no evidence to suggest that turtles learn to avoid roads. Shepard et al. (2008a), however, concluded that eastern box turtles avoid road crossings, as crossing frequency of turtles was less than that predicted in randomizations of movement paths. Brisbin et al. (2008) described the behavior of one adult female that seemed to indicate a reluctance to cross a well-travelled suburban street.

Jaeger et al. (2005) predicted that traffic volume has a greater effect than road size, that a population sensitive to traffic mortality (or other additive mortality), will be most vulnerable if individuals do not avoid crossing roads. They concluded that species with low overall road avoidance that are highly sensitive to traffic mortality are among those highly vulnerable to the negative impact of roads. Avoidance of road crossings should offer protection to box turtle populations in the short-term. If variation in crossing likelihood is heritable and the likelihood of mortality high, then selection will favor those animals that do not cross, potentially leading to inbreeding in populations isolated by roads (Shepard et al. 2008a).

If some eastern box turtles avoid crossing roads, it is clear that others do not. Certainly many box turtles attempt road crossings and are killed (Adams and Geis 1981; Ashley and Robinson 1996; Dodd et al. 1989; Doroff and Keith 1990).

3.3 Importance of Adult Box Turtles in Population Persistence

Adult survivorship is the critical factor in the persistence of turtle populations (Brooks et al. 1991, Congdon et al. 1993, 1994, Litzgus 2006), including box turtles (Doroff and Keith 1990). Congdon et al. (1994) concluded that, for adult (>15 years) common snapping turtles (*Chelydra serpentina*), an annual increase in mortality of 1% would halve the number of adults in less than 20 years. Other studies have found that additive annual adult mortality of 2-3% is inconsistent with population growth (Brooks et al. 1991; Congdon et al. 1993,1994; Doroff and Keith 1990). Modeling studies of box turtles indicate that the annual loss of 2% of the adult females in a population to additive causes can result in a long but irreversible decline to extinction (Seigel 2004).

Gibbs and Shriver (2002) identified roads as a source of fragmentation and probable decline of terrestrial turtles. Their model included estimates of road crossings, road density, and probability of being killed, and was used to estimate annual road-associated mortality in land, large pond, and small pond turtles at scales approximately

≥10 km^2. They concluded that land areas with >1 km of roads/km^2 and with traffic volumes of >100 vehicles/lane/day are sufficient to contribute to excessive loss of adult terrestrial turtles. Road density and road crossing frequency were strongly and positively linked. Excessive annual road mortality (>5% of individuals) is likely to be occurring among land turtles in the northeast (Gibbs and Shriver 2002), including Maryland.

3.4 Influence of Road Mortality on Box Turtle Sex Ratios

Beyond reducing population size, road-based mortality may be altering sex ratios in populations close to roads. I could find no evidence that work in this regard has been conducted on box turtles, although Hall et al. (1999) reported that L.F. Stickel believed that nesting migrations in the Patuxent population may expose females to both mowing and increased vehicular traffic, perhaps accounting for at least some of the reported male bias.

A review of 157 aquatic and semi-aquatic turtle studies by Steen et al. (2006) concluded that the road-encounter rate for female aquatic and semi-aquatic turtles was higher than that of males, despite male bias in populations in areas of high road density. Several studies have reported male bias in sex ratios of aquatic (Aresco 2005; Marchand and Litvaitis 2004; Steen and Gibbs 2004) and aquatic and semi-aquatic turtles (Steen et al. 2006), a finding attributed to female nesting migration. Steen et al. (2006) concluded that adult female aquatic and semi-aquatic turtles are more likely to cross roads than males, and their destruction in such attempts is leading to male-biased sex ratios in habitats near roadways. They did not observe a skew in sex ratios in terrestrial species.

CHAPTER 4: METHODS

4.1 Study Areas

Comparisons between sample results in the two control (Jug Bay Wetlands Sanctuary and Patuxent Wildlife Research Center) and three treatment (Blockhouse Point Park, Muddy Branch Park, Wheaton Regional Park) sites were used to determine the effect of isolation by roads on box turtle genetic diversity. Samples from each of the ten sites described below were used in a broader investigation of genetic diversity and population structure. The three sites used as treatments in the genetic isolation investigation also were used in estimates of population size and road- and natural-mortality rates. The location of study sites is shown in Figure 4.1.

4.2 Control Sites – Genetics

4.2.1 Patuxent Wildlife Research Center (PWRC)

Box turtle studies have been conducted in the U.S. Department of the Interior's Patuxent Wildlife Research Center since the mid-1940s (Hall et al. 1999), primarily in the "Central Tract" in Prince George's County. Studies focused primarily on home range size (Stickel 1950) and population demographics and trends (Hall et al. 1999; Stickel 1978). I found and sampled turtles mostly in the 11.8 ha plot in the forested alluvial floodplain in which these researchers concentrated their efforts. Overstory species included American beech (*Fagus grandifolia*), black walnut (*Juglans nigra*), tuliptree (*Liriodendron tulipifera*), eastern sycamore (*Platanus occidentalis*), white oak (*Quercus*

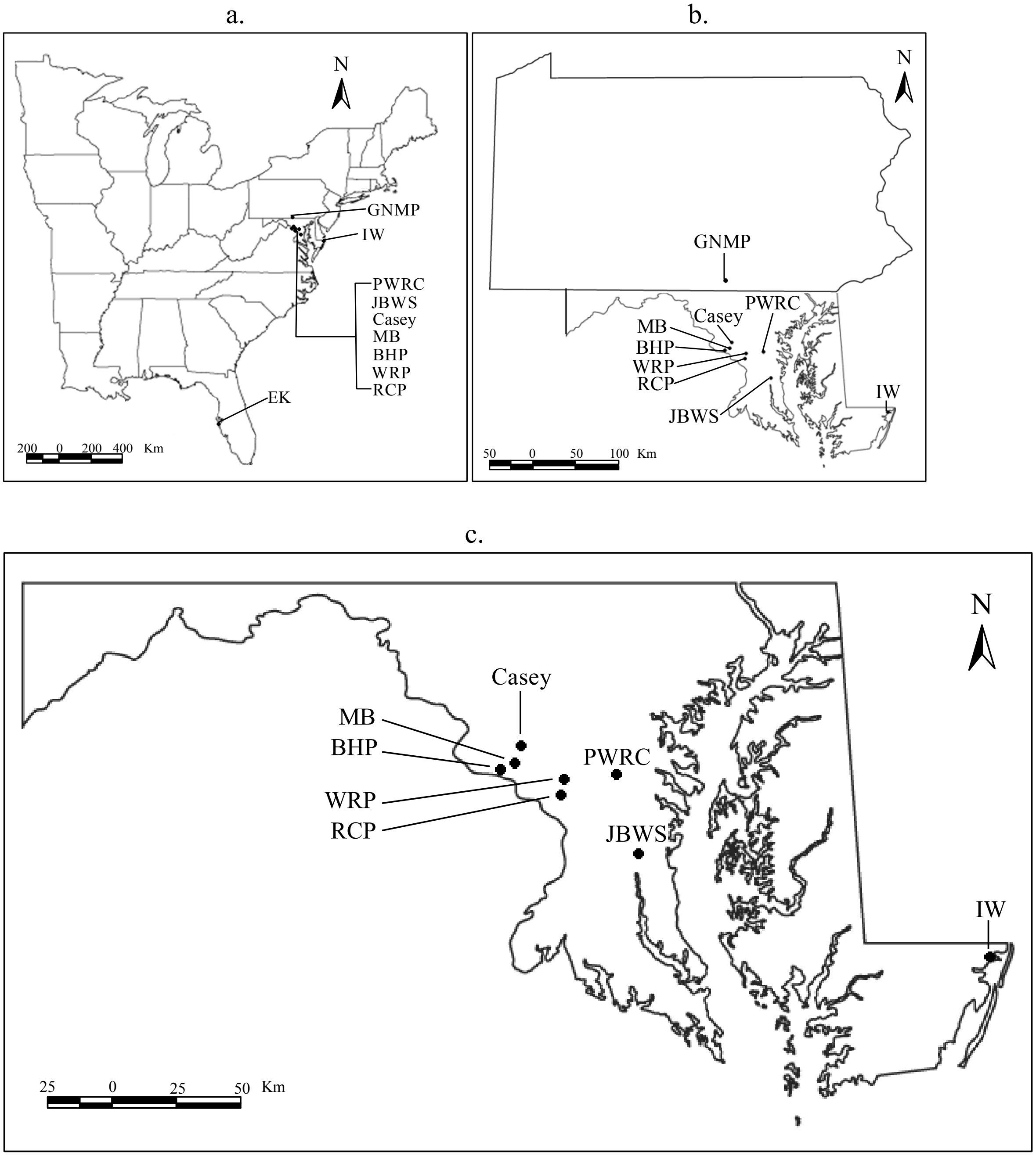


Figure 4.1. Eastern box turtle genetic sampling and population size estimation sites. a. All sites. b. Northern sites only. c. Maryland sites only. BHP, MB and WRP were used as treatment sites for analysis of genetic diversity in habitats isolated by roads and to estimate population size. PWRC and JBWS were used as control sites for analysis of genetic diversity in contiguous habitats. Please see Table 5.1 for explanation of abbreviations.

alba) and overcup oak (*Quercus lyrata*). Understory trees included pawpaw (*Asimina triloba*), American hornbeam (*Carpinus caroliniana*), persimmon (*Diospyros virginiana*), and sassafras (*Sassafras albidum*). Herbaceous vegetation included Jack-in-the-pulpit (*Arisaema triphyllum*), stilt grass (*Microstegium vimineum*), smartweed (*Polygonum* sp.), wineberry (*Rubus phoenicolasius*) and poison ivy (*Toxicodendron radicans*).

Given the large size of the Central Tract (about 1012 ha), the limited road network and use, and protected status of the area, I anticipated that genetic structure should reflect as closely as possible that of an undisturbed population.

4.2.2 Jug Bay Wetlands Sanctuary (JBWS)

Jug Bay Wetlands Sanctuary was a county preserve located in Anne Arundel County near Upper Marlboro, Maryland. Its 486 ha consisted of tidal freshwater wetlands, forests, meadows and fields along the Patuxent River. Overstory trees included American beech, American elm *(Ulmus americana*), black walnut, red maple (*Acer rubrum*), tuliptree, and sweet gum (*Liquidambar styraciflua*). Understory trees and shrubs included American holly (*Ilex opaca*), devil’s walking stick (*Aralia spinosa*), flowering dogwood (*Cornus florida*), mountain laurel (*Kalmia latifolia*), pawpaw and sassafras. The herbaceous layer included mayapple (*Podophyllum peltatum*), partridge-berry (*Mitchella repens*), spotted wintergreen (*Chimaphila maculata*) and spring beauty (*Claytonia virginica*).

A long-term study of eastern box turtles was underway at this location; 515 turtles have been marked within a 50-ha study area (Chris Swarth, pers comm., 4 Nov 09).

4.3 Treatment Sites - Genetics

4.3.1 Blockhouse Point Park (BHP)

Blockhouse Point Park in Montgomery County was characterized by dry, acidic oak/hickory uplands separated by ravines and rich, mesic slopes. Common trees included American beech, red maple and tuliptree. Shrubs included common serviceberry (*Amelanchier arborea*), lowbush blueberry (*Vaccinium vacillans*) pawpaw, mountain laurel and spicebush (*Lindera benzoin*). Ferns (*Osmunda* sp.) were common in mesic areas, along with skunk cabbage (*Symplocarpus foetidus*), marsh blue violet (*Viola cuculiata*) and spring cress (*Cardamine bulbosa*).

Blockhouse Point Park contained 254.6 ha. I worked only in the 140.5 ha region south of River Road. This section was bordered by the C&O Canal National Historic Park on the west, Pennyfield Lock Road on the east, and by the C&O Canal and the Potomac River on the south.

4.3.2 Muddy Branch Park – Unit Number 3 (MB)

Muddy Branch Stream Valley Unit Three was the northernmost of three Muddy Branch stream valley park units that occur in a roughly northeast to southwest corridor over a straight line distance of approximately 51.5 km. MB was composed of 126.8 ha located between Quince Orchard, Route 28 (Darnestown), and Dufief roads in Montgomery County. It was characterized by relatively dry, acidic oak/hickory uplands, open and forested floodplains, and old fields. Slopes were dominated by tuliptree and red maple, although eastern sycamore, black walnut, black cherry (*Prunus serotina*), silver maple (*Acer saccharinum*), Eastern cottonwood (*Populus deltoides*), and black gum (*Nyssa sylvatica*) were also common. The understory contained box-elder (*Acer* *negundo*) and spicebush. The herbaceous layer was dominated by invasive species, including ground ivy (*Glechoma hederacea*), stilt grass, garlic mustard (*Alliaria petiolata*), and multi-flora rose (*Rosa multiflora*).

4.3.3 Wheaton Regional Park (WRP)

Wheaton Regional Park was located in a densely populated section of Montgomery County. It was approximately 217 ha, and was surrounded by well-used roads; roads also passed through the park. The western half of the park supported artificial ponds, sports facilities, picnic areas, botanical gardens, playgrounds, and a miniature railroad. As a result, potential box turtle habitat was reduced to approximately 108.5 ha.

The eastern half of Wheaton Regional Park was characterized by gently rolling terrain with small seeps and streams. A maturing, mixed deciduous forest predominated. Common overstory species included tuliptree, red maple, white oak, black and red oaks (*Q. velutina, Q. rubra*, respectively), American beech, and black gum. Common understory species included spicebush, mapleleaf viburnum (*Viburnum acerifolium*) and flowering dogwood. The herbaceous layer included Jack-in-the-pulpit, bloodroot (*Sanguinaria canadensis*) and ferns in addition to extensive areas of alien species such as stilt grass, ground ivy, multi-flora rose and Japanese barberry (*Berberis thunbergii*).

This park was also home to Brookside Nature Center, on the grounds of which box turtles picked up in other locations have been released (L Hallgren-Scaffidi, pers comm, 20 June 2004). The release of turtles from other areas may affect box turtle genetics at this site. However, I focused my searches in areas relatively remote from the Nature Center, where releases are thought not to have occurred.

4.4 Additional Sampling Locations – Genetics

4.4.1 Casey Property (Casey)

The Casey property consisted of 50.7 ha of mixed forest and meadows in Gaithersburg, Maryland. Most of the property has since been cleared for development. Overstory trees included tuliptree, oaks, black gum and red maple. Understory trees included American holly, dogwood, persimmon, and spicebush. Herbaceous vegetation was a mixture of native and exotic, including Jack-in-the-pulpit, Japanese barberry, multi-flora rose, ferns and stilt grass.

4.4.2 Isle of Wight Wildlife Management Area (IW)

Isle of Wight Wildlife Management Area was located in Assawoman Bay and was sheltered from the Atlantic Ocean by the same coastal barrier island that supported Ocean City, Maryland. Its 81 ha was primarily forested. Dominant overstory trees included loblolly pine (*Pinus taeda*), red maple and sweetgum. Common understory trees included American holly, black cherry, persimmon, flameleaf sumac (*Rhus copallinum*), and waxmyrtle (*Myrica cerifera*). Common understory species included *Smilax*, ferns, and stilt grass.

4.4.3 Rock Creek Park, District of Columbia (RCP)

Rock Creek Park was managed by the National Park Service. Its 710 ha extended from the District of Columbia/MD line due south to the Potomac River. Sampling was conducted primarily in floodplain habitat at the northern end of the park. American beech, white oak, red maple, sycamore and tuliptree were common overstory trees. Common understory plants included Japanese honeysuckle (*Lonicera japonica*), spotted jewelweed (*Impatiens capensis*), ferns, and stilt grass.

4.4.4 Gettysburg National Military Park, Pennsylvania (GNMP)

Gettysburg National Military Park (GNMP) was located in a largely rural area east of the Appalachian Mountains of south central Pennsylvania's Adams County. The GNMP was 2424 ha, although sampling in the park was conducted in an area of approximately 6 ha. The landscape was characterized by mature and maturing woodlands and woodlots, agricultural fields, pasturelands and intermittent streams. The National Park Service, which manages the park, has since cleared the sampling area as part of a program to return portions of the park to the state that existed during the Battle of Gettysburg. At the time of sampling, however, overstory species included white oak, white ash (*Fraxinus americana*), and red oak. The understory included black cherry, spicebush, and white ash saplings. Herbaceous vegetation included skunk cabbage, Jack-in-the-pulpit, and cardinal flower (*Lobelia cardinalis*). Invasive plant species, including multi-flora rose, Japanese barberry, and mile-a-minute (*Persicaria perfoliata*) were common.

4.4.5. Egmont Key National Wildlife Refuge, Florida (EK)

Egmont Key National Wildlife Refuge was located in Hillsborough County at the mouth of Tampa Bay. The island was 180 ha. A detailed description of the vegetation is included in Dodd et al. (1994). Much of the island was forested; non-native trees, including Australian pine (*Casuarina equisetifolia*), Brazilian pepper (*Schinus terebinthifolius*) and cabbage palm (*Sabal palmetto*) dominated. Open areas included both lawns and sea oat (*Uniola paniculata*) meadows.

4.5 Study Site Groupings

The following sites are referred to throughout this document as the "Central MD/PA sites": PWRC, JBWS, BHP, MB, WRP, RCP, Casey, and GNMP. The Isle of Wight and Egmont Key sites are referred to as IW and EK, respectively.

4.6 Study Design

4.6.1 Study Plot Surveys and Blood Sample Collection

Using ArcView 3.2, plots of 11.8 ha were overlain on five potential treatment sites in 2004. Two sites, the Audubon Naturalist Society's Woodend Sanctuary and Fort Detrick, were dropped from the study after repeated searches yielded only 2 and 3 turtles, respectively. Two plots at Wheaton Regional Park were eliminated from potential selection for population size estimation because it was known that box turtles collected elsewhere were allowed to be released in that area, thus potentially influencing the genetics of the population apart from its isolation, if any, by roads. These plots are included, however, in population estimate extrapolation, because most of the area within each was undeveloped.

Each plot was searched for box turtles by walking at approximately the same rate of speed in a north-south or east-west pattern for the length or width of the plot. Upon reaching the edge of the plot, a 90-degree turn was made, a distance of approximately 12 m was paced off in that direction, and a parallel transect begun in the direction opposite that of the previous transect.

All searches were conducted with a Labrador retriever trained to find and deliver box turtles. She worked off leash, but was usually within sight. By observing her behavior, I could tell when she picked up a scent and could then watch to see just where she found a turtle for purposes of recording its capture location. On those occasions when I did not see the exact location of the capture, I estimated it based on the direction the dog was travelling as she brought me a turtle. I also searched for turtles as I walked transects, deviating slightly from the transect line as necessary to search likely hiding spots, such as under logs and in thick vegetation.

I stopped searching to process turtles as they were found. I recorded the GPS location and habitat characteristics of each capture site as well as the time of capture, activity of the turtle when captured (limited to those I located and captured), the temperature and relative humidity at the capture location, and the extent of cloud cover and general weather conditions. I weighed each turtle with a spring scale (Pesola), rounding to the nearest five grams. Using a digital caliper (Mitutoyo), I recorded carapace length in mm, carapace width at the hinge, carapace width at the widest point, length of the posterior portion of the plastron, plastron width at the widest point, and height at the hinge. If the length of the carapace was ≤107 mm, I considered that turtle to be a juvenile after Stickel (1950); turtles between 108-117 mm were considered subadults, and all over 118 were classified as adults (all rounded to the nearest mm).

I noted the sex of each turtle based on analysis of secondary sex characteristics – the presence or absence of plastron concavity, eye color, and tail and hind claw characteristics (Ernst and Lovich 2009). I counted the number of marginal scutes on both sides, and noted and diagrammed any shell abnormalities. I assigned to each turtle of sufficient size a unique identifying mark by filing one or more notches in the marginal scutes (omitting scutes 4-7 on each side) with a triangular file, using the method described by Cagel (1939). After marking, I photographed the carapace and plastron (Figure 4.2).

Figure 4.2. Representative photos of carapace (left) and plastron of study turtle.

The turtle then was placed in a restraining device (Figure 4.3), for blood collection. Most turtles opened and began to struggle to escape the holder after periods ranging from a few minutes to an hour. Once the hind feet began to move, I could usually grasp the tail, swab it with alcohol, and insert a 25-guage syringe in an effort to penetrate the caudal vein and withdraw two to three drops of blood (Figure 4.3). Each turtle was released at or very close to its capture location after removal from the holder.

Each treatment site was searched from two to four times during the course of the study to mark new turtles and record recaptures of previously marked turtles. Searches of each site were conducted over the course of several weeks during each field season. The location of dead turtles encountered in each site was recorded; their shells were removed from the field to avoid double counting.

Figure 4.3. Turtle holder used to restrain turtles to obtain blood sample (left). A small amount of blood was withdrawn from the caudal vein for DNA analysis.

4.6.2 Road Surveys

During the 2005 and 2006 field seasons, summer research assistants conducted surveys of public roads closest to the borders of BHP, MB and WRP to look for live or dead turtles. Assistants were asked to vary the times of their searches throughout daylight hours and to search on foot whenever possible, although vehicular searches frequently were conducted depending on road characteristics, traffic volume, weather, and time available. Roadside searches conducted on foot extended to five meters of the roads' edge in mown or bare areas, and to three meters (2005) and one meter (2006) in heavily vegetated areas; vegetation was pushed aside wherever possible in such areas to provide a clear view of the ground. Driving surveys were conducted at the slowest speed possible given traffic conditions. In general, the busiest roads were driven multiple times at 24 to 48 km/hr. Roads with less traffic were searched at speeds between 8 and 24 km/hr. Survey routes and the location of study plots within each park are shown in Figures 4.4 through 4.6.

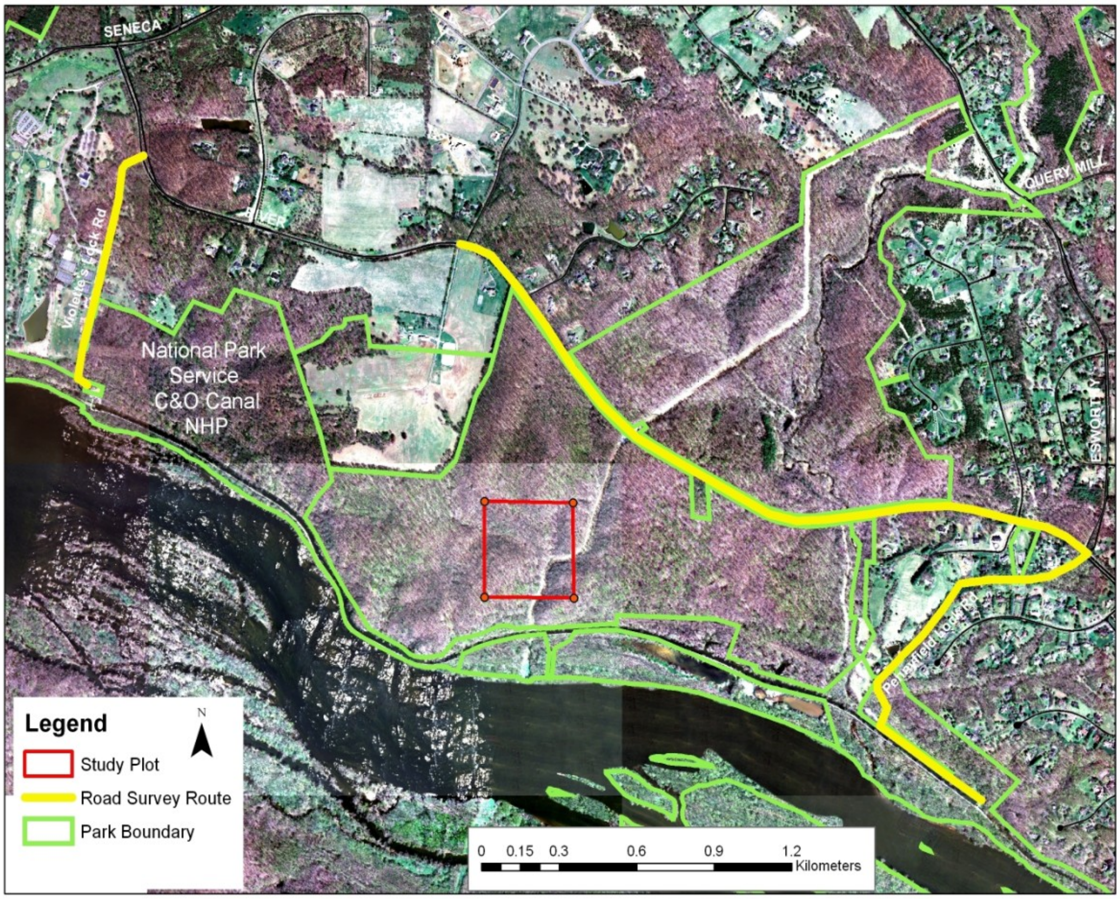


Figure 4.4. Location of Blockhouse Point Park study plot and road-mortality survey route. The survey route on the western edge was eventually eliminated from the study as turtles found along it were more likely to represent the C&O Canal population than the BHP population.

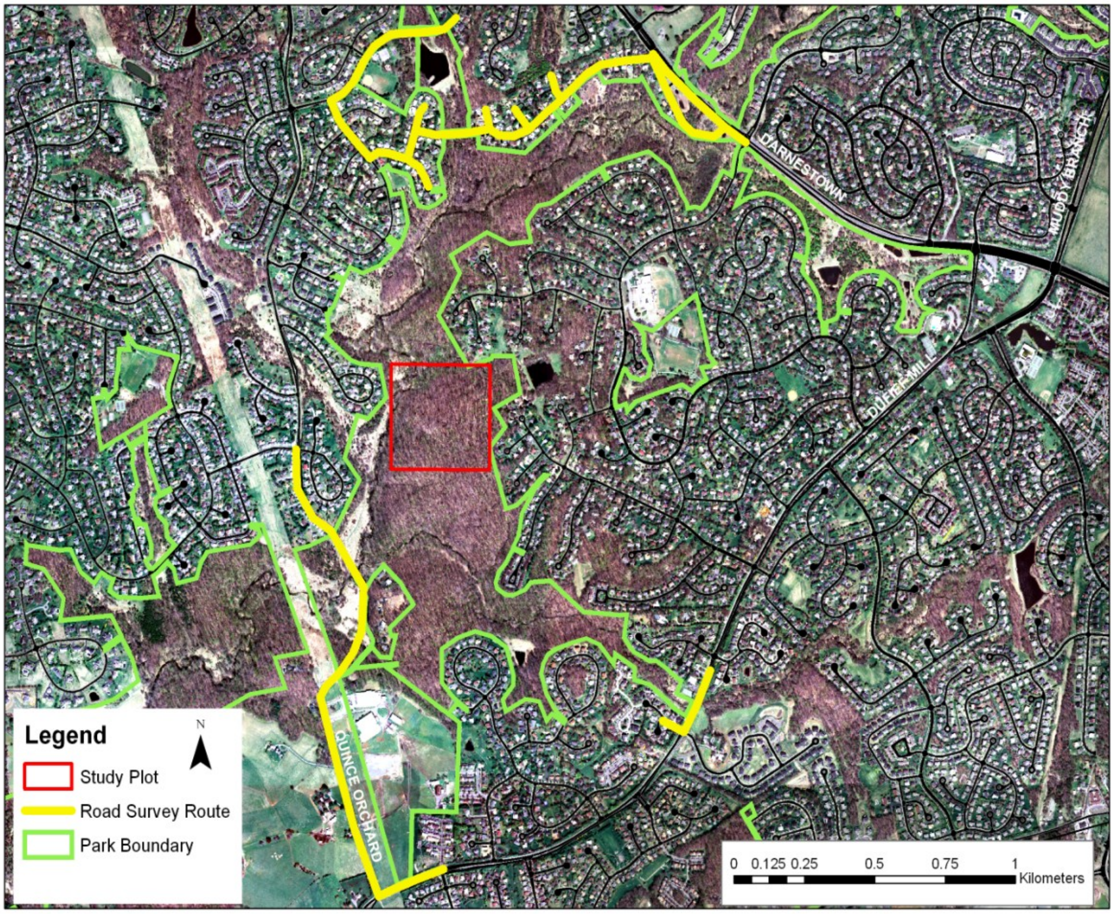


Figure 4.5. Location of Muddy Branch Stream Valley Unit 3 study plot and road-mortality survey route.

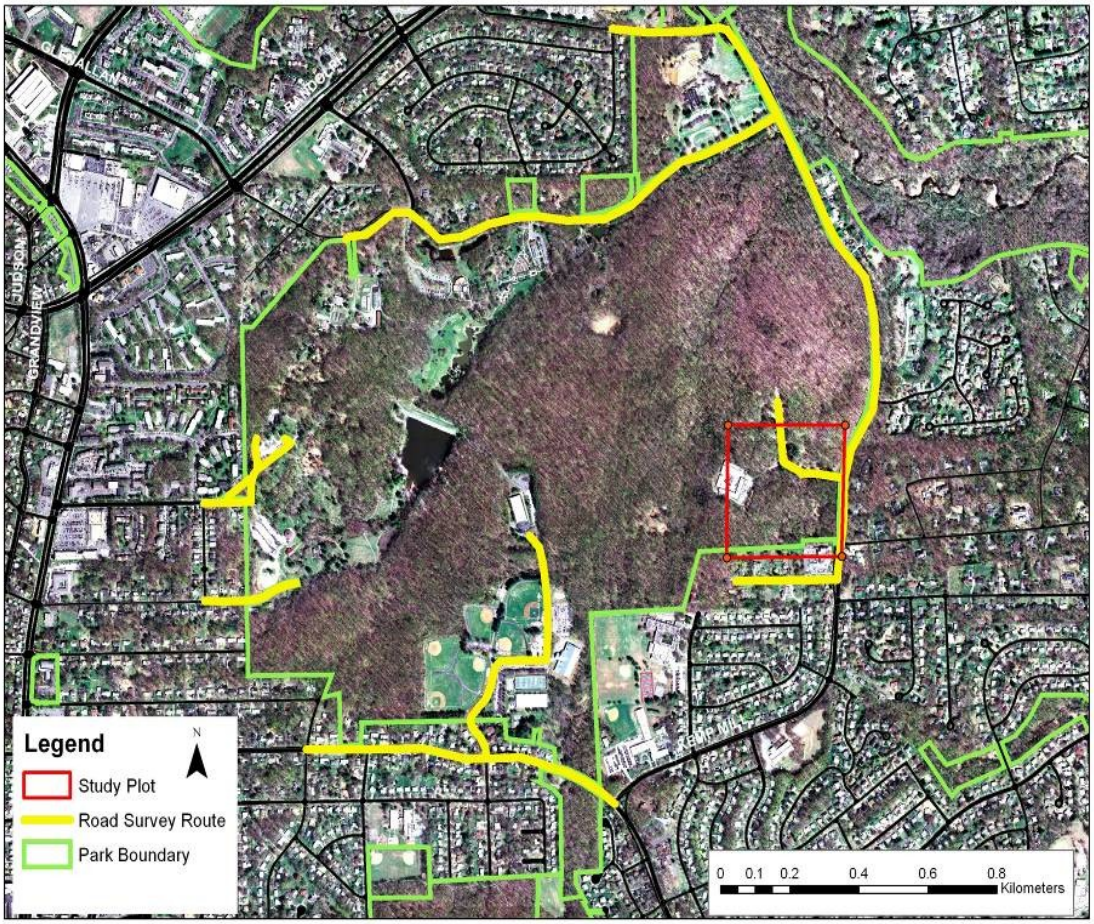


Figure 4.6. Location of Wheaton Regional Park study plot and road-mortality survey route.

Active turtles observed at or near the road's edge were watched for a time period sufficient to determine whether a crossing attempt was likely. If the turtle moved toward the road, it was released across the road after processing; the probability of mortality during a crossing attempt was calculated based on traffic volume and other factors (see section 4.9.3). Turtles showing no inclination to move closer to the road were left on the side where first observed. The location of all turtles was recorded, and each was weighed, measured, marked and photographed as described above.

Turtles injured or killed in their attempt to cross the road were examined for the presence of notches and photographed; their GPS locations were recorded. Because obtaining accurate measurements of carapace length of turtles killed by vehicles was frequently difficult, dangerous or impossible, noticeably small turtles were classified as juveniles; all others were considered to be adults. Injured turtles were taken to a wildlife rehabilitation center for treatment or euthanasia. Dead turtles were moved well off the roadside to avoid double counting.

Road surveys were conducted approximately twice each week in 2005, and almost daily in 2006. Road mortality rates as a percentage of population size were calculated on the basis of 2006 surveys only, given the far greater reliability of daily road surveys in finding the remains of animals, in particular small animals, killed by vehicles (Dodd et al. 1989; Glista et al. 2007).

4.7 Study Protocol and Permits

Field work was conducted under University of Maryland Institutional Animal Care and Use Committee Protocol Number R-04-27, Maryland Department of

Natural Resources Scientific Collection Permit 44750, National Park Service permits GETT-2007-SCI-0002 and ROCR-2005-SCI-0009, and U.S. Fish and Wildlife Service permit 05044.

4.8 DNA Analysis

I performed DNA extraction using the Puregene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN; buccal cell protocol), following manufacturer's instructions. I subjected twelve microsatellite regions to PCR[4] that were initially identified by King and Julian (2004) for *Glyptemys muhlenbergii* (*Gmu*), and which contained three or more alleles upon cross-species amplification for *T.c. carolina*. After repeated attempts failed to produce useful results, I eliminated two of the twelve loci.

PCR reactions were conducted in 10-μL volumes, each containing 100 ng template DNA, 5X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 μM forward and reverse primer (forward primer fluorescently labeled with FAM, HEX, or NED; Applied Biosystems (ABI), Foster City, CA), 0.2 mg/ml BSA, and 0.05 units/μL of GoTaq® Flexi DNA polymerase (Promega Corp., Madison, WI). Amplifications were performed in a PTC-200 Thermal Cycler (MJ Research, Watertown, MA). Amplification cycles consisted of an initial denaturing at 94 °C for 2 min; 35 cycles of 94 °C denaturing for 45 sec, 58 °C annealing for 45 sec, 72 °C extension for 1 min 30 sec; and a 5 minute extension

---

[4] Polymerase chain reaction (PCR) is used to produce millions of copies of a desired segment of DNA. During PCR, primers attach to regions flanking the desired segment when DNA strands separate with heating. Nucleotides then fill in along the single strands, thereby creating four DNA strands from two. Repeated heating and cooling exponentially increase the number of copies of the target DNA.

at 72 °C. After the addition of 0.25 µL GeneScan-500 ROX internal size standard, PCR products were subjected to capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer. Alleles were sized and scored with Genotyper™ version 3.6NT (ABI).

4.9 Data Analysis

4.9.1 Genetics

Allelic data initially were examined in Microsatellite Toolkit (Parks 2001) to identify data entry errors and detect genetically identical samples. Once the data were finalized (Appendix A), GenAlEx Version 6.2 (Peakall and Smouse 2006) was used to export data into formats required by several other software programs. Allele frequency, number and mean number of alleles per locus, and observed and mean observed heterozygosity (H_O) were derived using FSTAT (Goudet 2001). Corrected estimates of allelic richness (based upon the smallest sample size), and Wright's *F*-statistics[5] (F_{ST}; Weir and Cockerham 1984) were also estimated in FSTAT. The number of unique alleles was derived using GenAlEx, as was expected heterozygosity (H_E; unbiased). GenAlEx was also used to estimate pairwise F_{ST} and R_{ST}[6] values to assess population subdivision based on allele frequency and size, respectively, and to

---

[5] Wright (1931, 1951) developed inbreeding coefficients to describe the distribution of genetic variation among and between populations. F_{IS} is a measure of inbreeding. F_{ST} measures allele frequency divergence among subpopulations, and F_{IT} measures overall departure from Hardy-Weinberg proportions in the entire base population due to non-random mating within local subpopulations (F_{IS}) and allele frequency divergence among subpopulations ($F_{ST;}$ Allendorf and Luikart 2007).

[6] R_{ST} is an analog of F_{ST} that uses a stepwise mutation model to characterize microsatellite loci. Stepwise mutation models assume the loss or gain of a single tandem repeat unit.

determine the probability of identity (PI_{sib})[7] – the probability that the sibling of a particular individual would have the same genotype. To further detect genetic population subdivision, I used the GenAlEx analysis of molecular variance (AMOVA; Excoffier et al. 1992) to partition allelic variances among population groupings. Three AMOVAs, in which some or all sampling locations were grouped into two or three assemblages based on geographic location, were run on the basis of F_{ST} and R_{ST} values with 999 permutations. Within population variation was suppressed in each case.

The Markov chain randomization test of Guo and Thompson (1992), in Genepop 4.0 (Raymond and Rousset 1995), was used to test the significance of F_{IS} values and the conformance of genotypes at each locus to Hardy-Weinberg equilibrium (HWE). The HWE compares the observed genotype frequencies with those expected in a large, randomly mating population in which there is no mutation, natural selection or migration. Markov chain parameters for all tests included 10,000 dememorizations, 20 batches, and 5000 iterations per batch. Gametic or linkage disequilibrium (LD) also was assessed in Genepop 4.0, using 10,000 dememorizations, 100 batches, and 5000 iterations per batch. Significance levels for HEW, LD, and all other multiple comparison tests were adjusted using sequential Bonferroni methods (Rice 1989), and an initial α level of $0.05/k$ (k = number of comparisons).

---

[7] $PI_{sibs} = 0.25 + [0.5 \sum(p_i^2)] + [0.5 \sum(p_i^2)^2] - [0.25 \sum(p_i)^4]$ where p_i is the frequency of the i^{th} allele at a locus.

To transform allele frequency data into a distance matrix, genetic distance estimates were determined using the chord distance measure of Cavalli-Sforza and Edwards (1967) – a geometric view of the distances between multi-dimensional points. The program BIOSYS-1 (Swofford and Selander 1981) was used to produce a chord distance matrix, which was then exported to MEGA Version 4.0 (Tamura et al. 2007) for construction of a neighbor-joining tree.

The program STRUCTURE 2.1 (Pritchard et al. 2000) uses a Bayesian approach to identify population structure. With the input of a genotypic data set and a prediction of the number of clusters (*k*), STRUCTURE models the likelihood of the observed dataset being produced by the predicted number of clusters through an admixture model using a set number of iterations. An initial burn-in period of 10,000 iterations was followed by 10,000 Markov chain Monte Carlo (MCMC) replications and calculation of the probability of $k = 1$ to $k = C + 3$ (the number of clusters, C, plus 3 after Evanno et al. 2005) with 20 runs for each *k*. The true number of populations is often established using the maximum value among clusters of the log likelihood of the data averaged over MCMC replications, after subtracting half of their variance, resulting in a value called LnP(D) (Evanno et al. 2005). A new version of STRUCTURE (2.3.2; Hubisz et al. 2009) that allows the proportion of individuals assigned to a particular cluster to vary by location was also used. The burn-in iterations and MCMC replications remained the same, and the admixture model was used.

I used the program Alleles in Space (AIS; Miller 2005) to interpolate a genetic surface among the sampled box turtle populations so as to visualize areas of genetic differentiation, using a 100 x 100 grid and a distance weighting parameter of 0.2. I conducted a spatial autocorrelation analysis in AIS to test whether the observed genotype of an individual at one location is dependent on the genotype of an individual at a neighboring location (Manel et al. 2003). I plotted the slope of the autocorrelation statistic A_y[8] across four distance classes and tested for its significance against a 1000-replicate randomization. I also conducted a Mantel test in AIS, using 1000 replications, to investigate the degree to which genetic and geographical distances of sampled individuals are correlated.

PAST (PAleontological STatistics, Version 1.93; Hammer et al. 2001) was used to conduct principal components analysis. Eigenvalues provide a measure of the variance accounted for by the corresponding eigenvectors.

I used GeneClass Version 2.0 (Piry et al. 2004) to assess the likelihood of each individual's multilocus genotype being found in each of the other sampling locations based on maximum likelihood assignment tests (Paetkau et al. 1995) using the Bayesian method ("leave one out" procedure). A constant likelihood of 0.01 was set for null frequencies.

4.9.2 Population Size

The Cormack-Jolly-Seber (CJS) model in the program MARK (version 5.1; White and Burnham 1999) was used to estimate population size in each of the three county parks. Given the length of time over which each sampling period extended

---

[8] The average genetic distance between pairs of individuals that fall within distance class *y*. *Ay* takes on a value of 0 when all individuals within distance class *y* are genetically identical and takes on a value of 1 when all individuals within distance class *y* are completely dissimilar.

(see Table 6.1), an open population model was used. The "recaptures only" CJS model was used for encounter histories as there were encounter history data for only 2, 3, and 4 years, depending on the park. Other models, such as POPAN or Jolly-Seber, require the estimation of more parameters than this limited data set allowed. As a result, only survival (Φ) and capture (p) probabilities were estimated, with both held constant across populations.

Once capture probabilities were derived, population size was estimated by dividing the number captured in a given year by the probability of capture as estimated from encounter histories, and estimates between years averaged to obtain a single population size estimate for the study plot in each park. On this basis, turtles/ha was calculated, and a population estimate for the entire park derived.

4.9.3 Roadkill Rates

The probability that a live turtle observed during road surveys, either in the road or approaching it and within a few meters of the road surface, would have been killed had research assistants not intervened was estimated using an equation of Gibbs and Shriver (2002) that was derived from that of Hels and Buchwald (2001):

$$p_{\text{killed}} = 1\text{-}e^{-Na/v},$$

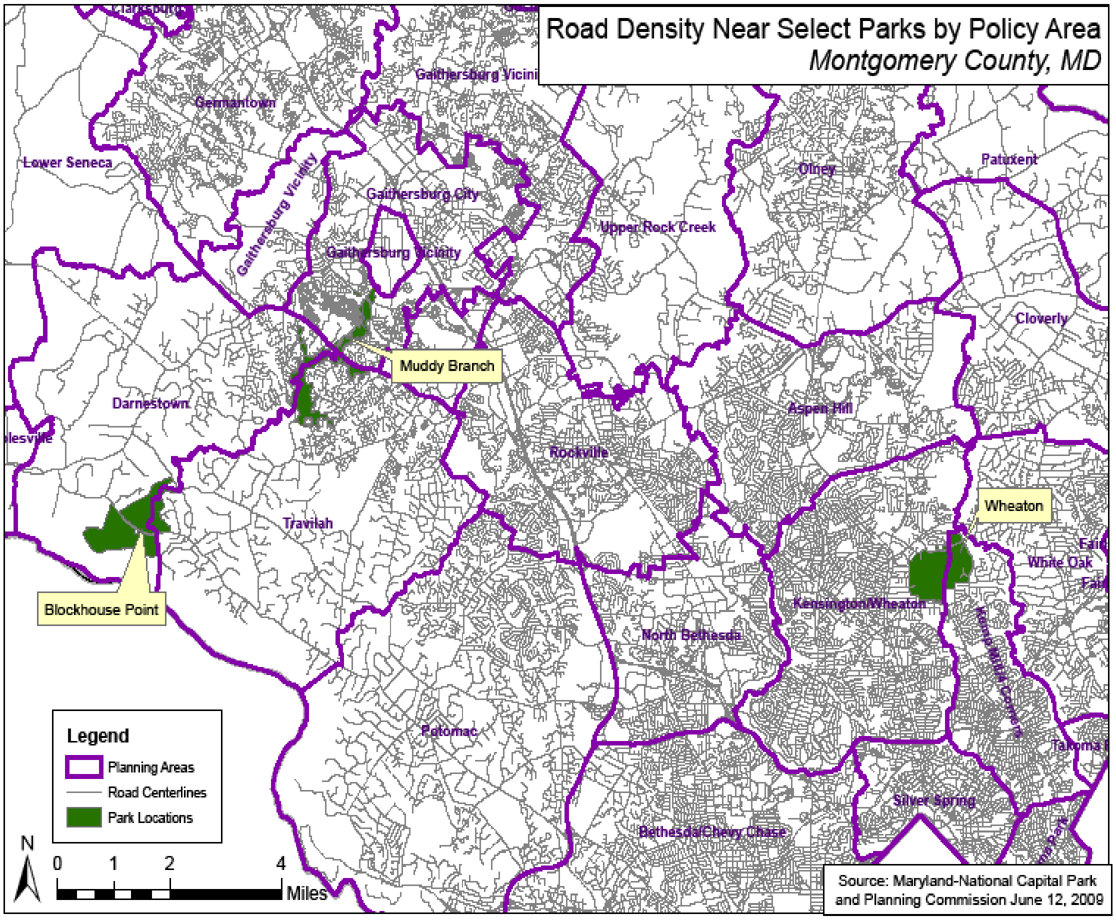
in which N is traffic volume in vehicles per minute, a is the width of the kill zone in the road, and v is the estimated velocity (m/minute) of a turtle moving through the kill zone.

Estimates of N are based on information obtained from the Maryland-National Capital Park and Planning Commission (MNCPPC) and the Maryland State Highway Administration, and largely confined to state roads near study parks. Estimates for county roads bordering some of the parks, some of which are heavily used, are limited. I used 2008 average annual daily traffic (AADT) counts for Blockhouse Point Park that were collected on River Road (MD 190) near its intersection with Seneca Road (MD 112). For Muddy Branch Park, I used 2008 AADT on Darnestown Road (MD 28) near its intersection with Quince Orchard Road. I relied on the minimum estimate of a field assistant who conducted road surveys for both 2005 and 2006 for Pennyfield Lock and Quince Orchard roads and Orebaugh Avenue.

Estimates on roads bordering Wheaton Regional Park were generated by the MNCPPC during morning and evening peak hours only and for one day in 2008. For Kemp Mill Road traffic volumes, I averaged counts recorded at the intersections of Randolph Road and Kemp Mill Road (2005 data) and Kemp Mill Road and Arcola Avenue (2004 data). I used counts of traffic turning southeast onto Glenallen Avenue from Randolph Road (2008 data). I assumed for each calculation that non-peak volumes would average one half those of peak hours (after Festin 1996). All non-peak volumes were reduced by 20% to conform to volumes occurring between 0600 and 1800 hours (Festin 1996), the period of highest turtle movement (Gibbs and Shriver 2002).

Hels and Buchwald (2001) estimated the kill zone *a* as two times tire width (0.5 m total) per lane plus two times the body length of the species in question. Shell length was estimated to be 0.1361 m, the average carapace length of 170 and 182 adult males and females, respectively, as reported by Stickel and Bunck (1989). Turtle velocity (3.3 m/minute) was estimated based on the mean of the maximum recorded for voluntary adult *T.c. carolina* locomotion based on temperature (Adams et al. 1989; 3.0 m/minute), and slope (Muegel and Claussen 1994; 3.6 m/minute at zero slope). I assumed that turtles crossed the road perpendicular to its direction, which maximizes survival probability. If p was ≥ 51%, I assumed that that individual would have been killed in its attempt to cross the road.

The county is divided into Policy Areas, on the basis of which local road densities are calculated (J Clarke, pers comm, 12 Jun 09). The Policy Areas in which study parks occur are shown in Figure 4.9. Road density estimates were provided by MNCPPC for Montgomery County generally, and for the Policy Areas containing each study park.



Road Density Near Select Parks by Policy Area
Montgomery County, MD

Source: Maryland-National Capital Park and Planning Commission June 12, 2009

Figure 4.7. Road densities surrounding study parks, Montgomery County, MD.

CHAPTER 5: GENETICS RESULTS AND DISCUSSION

5.1 Results

Genotypes at 10 microsatellite DNA loci were determined for 436 *Terrapene carolina* from 10 locations. Allele frequency by locus and population, individual locus heterozygosities, mean heterozygosities, and mean number of alleles per locus are presented in Appendix B. The microsatellites surveyed revealed high levels of allelic variation; 169 alleles were detected, ranging from 7 at *Gmu*B08 to 23 at *Gmu*D16.

Ten percent (17) of the total number of alleles were unique to populations (Table 5.1). The number of unique or "private" alleles ranged from 0 in RCP to 5 in EK. Observed heterozygosity (H_O) across the ten study sites averaged 0.791, and in six sites (60%) exceeded 0.800**.** The lowest H_O occurred at EK (0.641); the highest was found in GNMP (0.824). Expected heterozygosity (H_E) ranged from 0.684 at EK to 0.858 at GNMP.

Estimates of allelic richness, a measure of the number of alleles per locus that takes into account sample size, ranged from 3 (*Gmu*D79 at EK) to 14 (*Gmu*D16 at IW; Table 5.2). Mean allelic richness values were similar for central MD/PA populations. Richness values were lower for IW and EK than the other populations, but significantly so only for EK ($P = 0.0004$).

As indicated by a positive inbreeding coefficient (F_{IS}), a deficit of heterozygotes relative to that predicted by the Hardy-Weinberg equilibrium was evident in all sampled populations, but was statistically significant ($P \leq 0.004$) only

Table 5.1. Summary of allele frequency statistics for 10 collections of *Terrapene carolina* from Maryland, Pennsylvania, and Florida. Sample size (N), mean number of alleles per locus ($\hat{A}$), number of private alleles, observed and expected heterozygosity and estimates of the inbreeding coefficient (F_{IS}). F_{IS} values in bold are significantly different from zero.

Subspecies	County, State	Collection	Location abbrev	N	$\hat{A}$	Private alleles	Heterozygosity		F_{IS}
							H_O	H_E	
T. c. carolina	Prince George's, MD	Patuxent Wildlife Refuge Center	PWRC	51	13.3	2	0.794	0.857	**0.074**
	Adams, PA	Gettysburg National Military Park	GNMP	33	11.6	1	0.824	0.858	0.043
	Prince George's, MD	Jug Bay Wetlands Sanctuary	JBWS	51	12.7	1	0.792	0.843	**0.061**
	Montgomery, MD	Blockhouse Point Park	BHP	44	12.5	1	0.805	0.851	0.054
	Montgomery, MD	Muddy Branch Park	MB	58	13.4	3	0.796	0.847	**0.061**
	Montgomery, MD	Wheaton Regional Park	WRP	51	12.2	1	0.807	0.843	0.043
	District of Columbia	Rock Creek Park	RCP	17	9.7	0	0.816	0.823	0.009
	Montgomery, MD	Casey Development	Casey	39	12.8	1	0.817	0.848	0.038
	Worchester, MD	Isle of Wight Wildlife Mgmt Area	IW	32	10.6	2	0.818	0.846	0.033
T.c. bauri	Hillsborough, FL	Egmont Key National Wildlife Refuge	EK	60	7.7	5	0.641	0.684	**0.064**

Table 5.2. Allelic richness (corrected to the smallest sample size (*n* = 13)) per locus and population for 10 collections of *Terrapene carolina* from Maryland, Pennsylvania and Florida. See Table 5.1 for collection abbreviations.

Locus	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
A19	10.59	11.91	10.71	10.14	10.79	11.05	12.20	9.54	11.41	7.05
B08	5.17	5.45	4.66	5.15	5.19	5.21	4.53	5.25	5.66	3.86
D16	12.41	10.61	12.93	11.98	12.49	11.16	11.53	11.49	14.00	8.27
D40	12.39	11.61	10.17	10.66	10.88	11.49	10.90	11.66	9.57	8.47
D55	10.68	10.78	10.30	11.02	9.83	10.59	9.59	10.26	9.20	4.76
D79	4.35	4.38	3.97	4.05	4.59	4.46	3.95	4.04	3.40	3.00
D87	12.16	11.50	12.10	11.48	11.63	12.06	10.86	12.69	9.17	5.74
D88	8.16	7.85	7.66	7.89	8.91	7.56	5.71	8.40	8.25	8.51
D90	8.81	7.96	8.39	8.32	8.55	7.32	8.56	8.00	7.71	5.13
D121	10.80	10.71	9.87	11.49	10.49	11.03	12.63	11.08	9.71	4.01
Mean	9.55	9.28	9.08	9.22	9.33	9.19	9.04	9.24	8.81	5.88

for PWRC, JBWS, MB and EK (Table 5.1). *F*-statistics are presented by locus and allele in Appendix C. F_{ST} values, while generally low, are nonetheless indicative of weak genetic structure.

The estimated PI_{sib} based on the first five loci averaged 4.8×10^{-3} across populations, and dropped to 4.8×10^{-5} with the inclusion of all 10 loci. Marker polymorphism was sufficient to generate unique multilocus genotypes for every individual.

Nearly all of the genotypes observed in the 10 sample collections conformed to Hardy-Weinberg (HW) expectations (Appendix D). After Bonferroni correction, five out of the 100 (5%) population by locus comparisons remained out of conformance ($\alpha = 0.05$, $P < 0.0005$), a level that is consistent with that which would be expected by chance alone (.05 x 10 x 10). Three of the five departures from HW occurred at the locus *Gmu*D40 in the Patuxent Wildlife Research Center, Gettysburg National Military Park, and Jug Bay Wetlands Sanctuary populations. Another locus in the Jug Bay population, *Gmu*A19, was also significantly out of equilibrium. *Gmu*D79 was significantly out of HW only in the Egmont Key population. In each case, the failure of these loci to conform to HW was due to heterozygote deficits, the most common direction of deviation from HWE and potentially a result of violating the criteria of an ideal population (Selkoe and Toonen 2006). The observed heterozygote deficit may also be a result of the presence of null alleles – the failure of alleles at certain loci to amplify based on mutations in the flanking region or less than ideal PCR conditions (Selkoe and Toonen 2006).

Significant linkage or gametic disequilibrium was detected for 2 of 45 (4.4%) locus by locus comparisons (Table 5.3) and for 2 of 450 (0.4%) population by locus pair comparisons after correction for multiple tests (overall $\alpha = 0.05$, $P < 0.0001$; Appendix E). In both analyses, gametic disequilibrium was less than that expected by chance alone.

Table 5.3. Linkage disequilibrium by locus pair, after Fisher (1935).

Locus Pair		Chi-Sq	d.f.	*P*-value	Locus Pair		Chi-Sq	d.f.	*P*-value
A19	& B08	22.65144	20	0.30623	B08	& D88	13.74286	20	0.84329
A19	& D16	9.98055	18	0.93254	D16	& D88	1.09798	18	1.00000
B08	& D16	14.75943	18	0.67841	D40	& D88	∞	20	0.00000
A19	& D40	23.72233	20	0.25473	D55	& D88	10.54268	20	0.95723
B08	& D40	18.84502	20	0.53192	D79	& D88	17.05734	20	0.64925
D16	& D40	9.31793	18	0.95192	D87	& D88	15.16422	20	0.76694
A19	& D55	10.14813	20	0.96541	A19	& D90	11.46507	20	0.93326
B08	& D55	13.10220	20	0.87296	B08	& D90	14.82531	20	0.78632
D16	& D55	12.01898	18	0.84626	D16	& D90	12.05931	18	0.84416
D40	& D55	7.28569	20	0.99564	D40	& D90	22.50277	20	0.31386
A19	& D79	17.08297	20	0.64758	D55	& D90	13.60966	20	0.84972
B08	& D79	9.49373	20	0.97645	D79	& D90	7.62726	20	0.99406
D16	& D79	12.06754	18	0.84373	D87	& D90	20.28611	20	0.44017
D40	& D79	9.69489	20	0.97337	D88	& D90	17.23219	20	0.63785
D55	& D79	16.08185	20	0.71153	A19	& D121	7.59403	20	0.99423
A19	& D87	16.40125	20	0.69144	B08	& D121	12.73777	20	0.88835
B08	& D87	6.80158	20	0.99729	D16	& D121	5.82710	18	0.99685
D16	& D87	∞	18	0.00000	D40	& D121	7.44434	20	0.99495
D40	& D87	15.08997	20	0.77124	D55	& D121	12.53551	18	0.81840
D55	& D87	6.25062	20	0.99851	D79	& D121	34.15596	20	0.02509
D79	& D87	20.83338	20	0.40700	D87	& D121	7.06001	18	0.98961
A19	& D88	6.92548	20	0.99692	D88	& D121	21.96499	20	0.34241
					D90	& D121	13.82678	20	0.83917

Population assignment tests produced results consistent with weak genetic differentiation, as the percentage of individuals correctly assigned to their population was quite low for central MD/PA populations, and even for the IW collection (Table 5.4; Appendix F). All EK animals were correctly assigned to their population.

Table 5.4. Results of maximum likelihood assignment tests for 10 eastern box turtle populations using multilocus genotypes derived from 10 microsatellite DNA markers. Each row assigns every sampled individual to a population. Boxes highlight the assignment of individuals to their population. The final column indicates the percentage of correct assignment of individuals to their population. See Table 5.1 for collection abbreviations.

Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	%
PWRC	**16**	6	7	6	4	5	2	4	1		31.4
GNMP	4	**9**	2	8	2	3	2	2	1		27.3
JBWS	15	3	**9**	4	6	4	3	6	1		17.6
BHP	2	6	2	**12**	7	5	2	7	1		27.3
MB	6	3	5	10	**12**	6	3	10	2	1	20.7
WRP	5	6	5	4	9	**17**	1	4			33.3
RCP		1	4	2	2	2	**4**	2			23.5
Casey	4	1	4	8	7	7	1	**7**			17.9
IW	2	3	4	1	1	5	3		**13**		40.6
EK										**60**	100.0
Assign to Region	Mid-Atlantic 25%								IW 41%	EK 100%	

Results from the STRUCTURE analysis indicated that the sampled populations represent two clusters – MD/PA and EK (Figures 5.1 and 5.2a). In analyses where *k* = 3, *k* =4, and *k* = 5 (Figure 5.2b, 5.2c, and 5.2d, respectively), additional structure was indicated based on collection location.

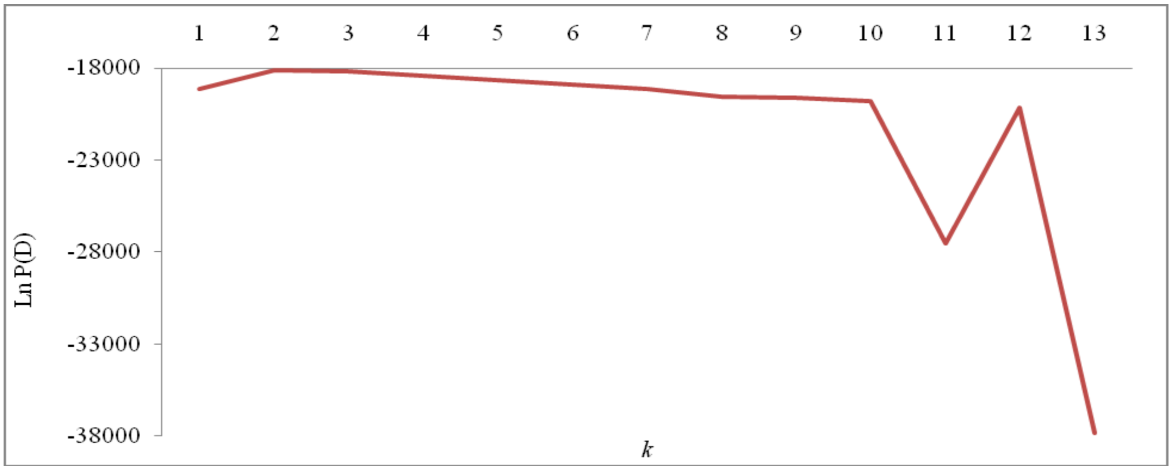


Figure 5.1. Log probability of allelic data as a function of *k*. Probability determined for 13 potential population clusters (the number of collections (10), plus 3, after Evanno et al. 2005). The maximum value of LnP(D) is associated with the true number of population clusters (Evanno et al. 2005).

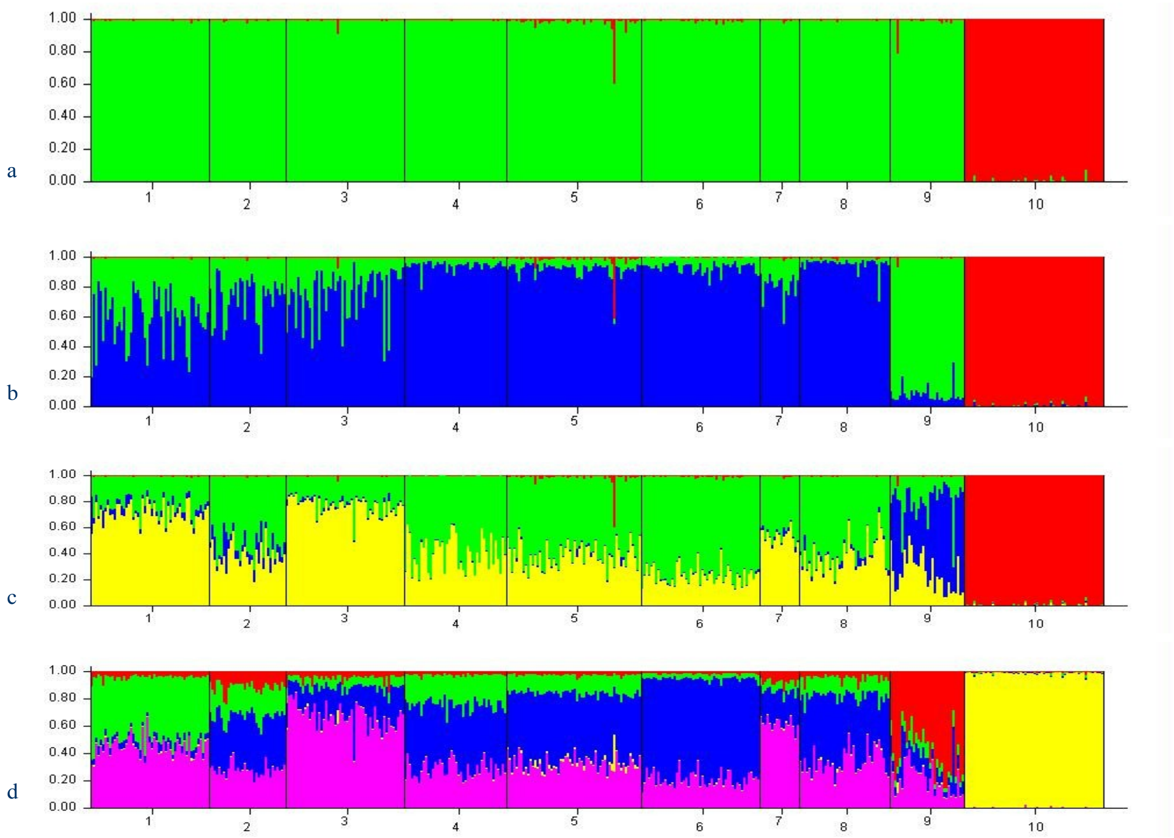


Figure 5.2. Summary plot generated by the sequential cluster analaysis of the program STRUCTURE performed on the multilocus (*N* = 10) genotypes of 10 populations of eastern box turtles. Each individual is represented by a single vertical line, broken into *k* colored segments, the length of which is proportional to the membership fraction in each of 2, 3, 4, and 5 clusters in a, b, c, and d, respectively. Numbers below each bar correspond to populations as follows: 1. Patuxent Wildlife Research Center; 2. Gettysburg National Military Park; 3. Jug Bay Wetlands Sanctuary; 4. Blockhouse Point Park; 5. Muddy Branch Stream Valley Park; 6. Wheaton Regional Park; 7. Rock Creek Park; 8 Casey Development;; 9 Isle of Wight; 10. Egmont Key.

Principal components analysis grouped Maryland and Pennsylvania populations as distinct from the Florida population (Figure 5.3), and as one overlapping population with the removal of the Florida population (Figure 5.4). A neighbor-joining tree likewise indicated little difference, particularly among northern populations (Figure 5.5).

The genetic surface interpolation among the sampled box turtle populations indicated areas of genetic differentiation that are consistent with the findings of other analyses (Figure 5.6). EK is clearly differentiated when included in the analysis (a.). Surface plot heights distinguish central Maryland populations from IW and GNMP when

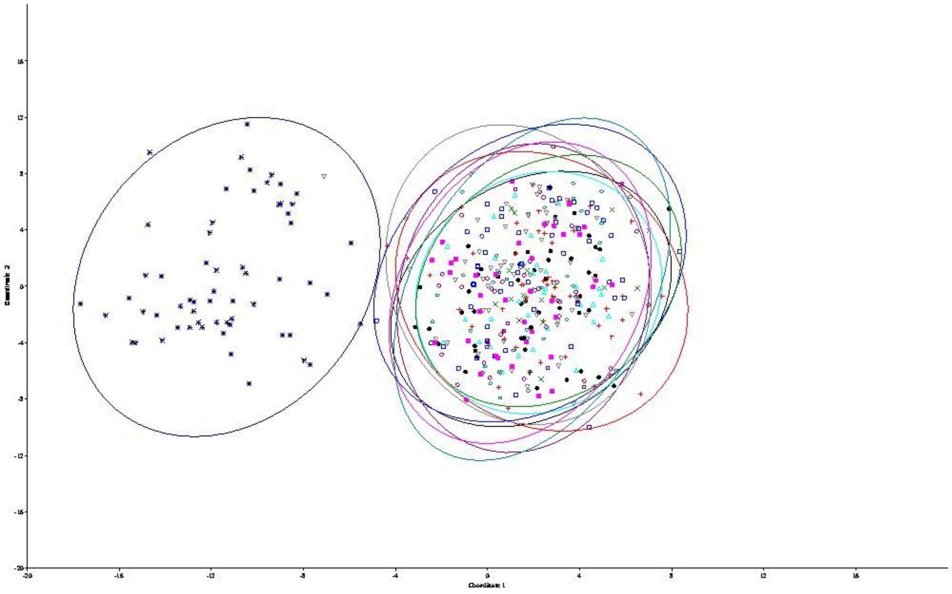


Figure 5.3. Principal component analysis of all populations. The Florida population is confined to the oval on the left. Circles are 95% confidence elipses.

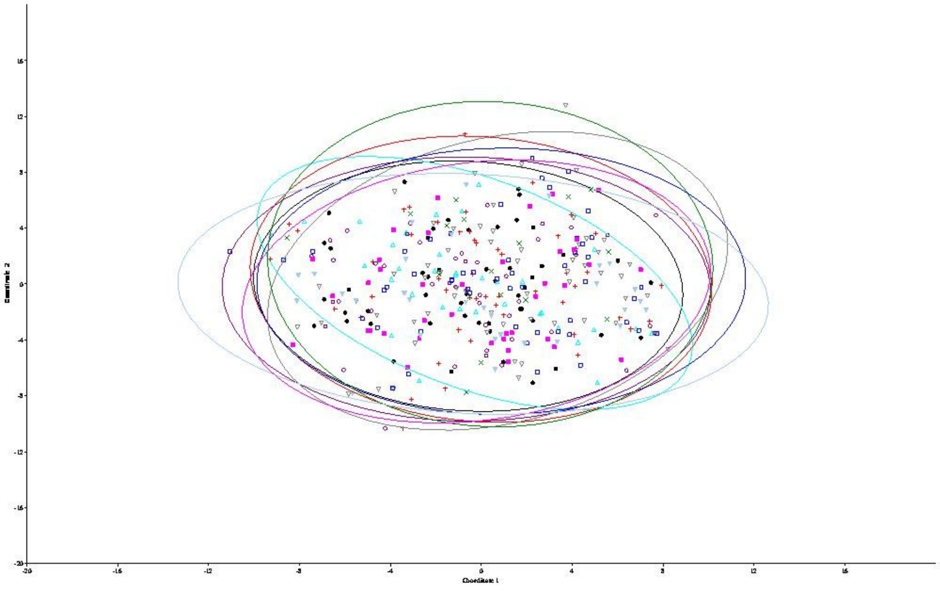


Figure 5.4. Principal component analysis excluding the Florida population. Circles are 95% confidence elipses.

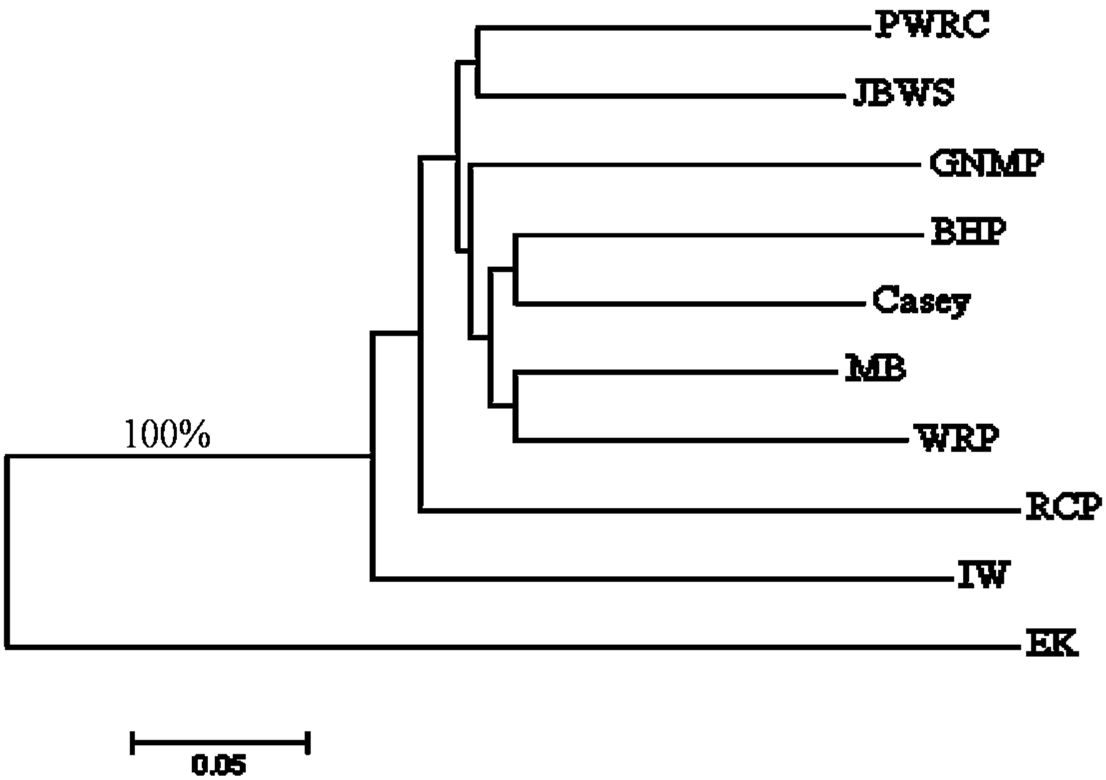


Figure 5.5. Neighbor-joining tree with 100% bootstrap support. Branch support was < 70% for each population; the scale represents chord distance units.

EK is excluded (b.). The exclusion of both EK and IW (c.) indicates relatively little differentiation among remaining populations.

Spatial autocorrelation analysis in Alleles in Space indicated that no gene exchange would occur at geographic distances of 219 to 439 km and beyond (Figure 5.7). The results of a Mantel test in AIS (not shown) produced an *r* value of 0.3637 (*P* = 0.001).

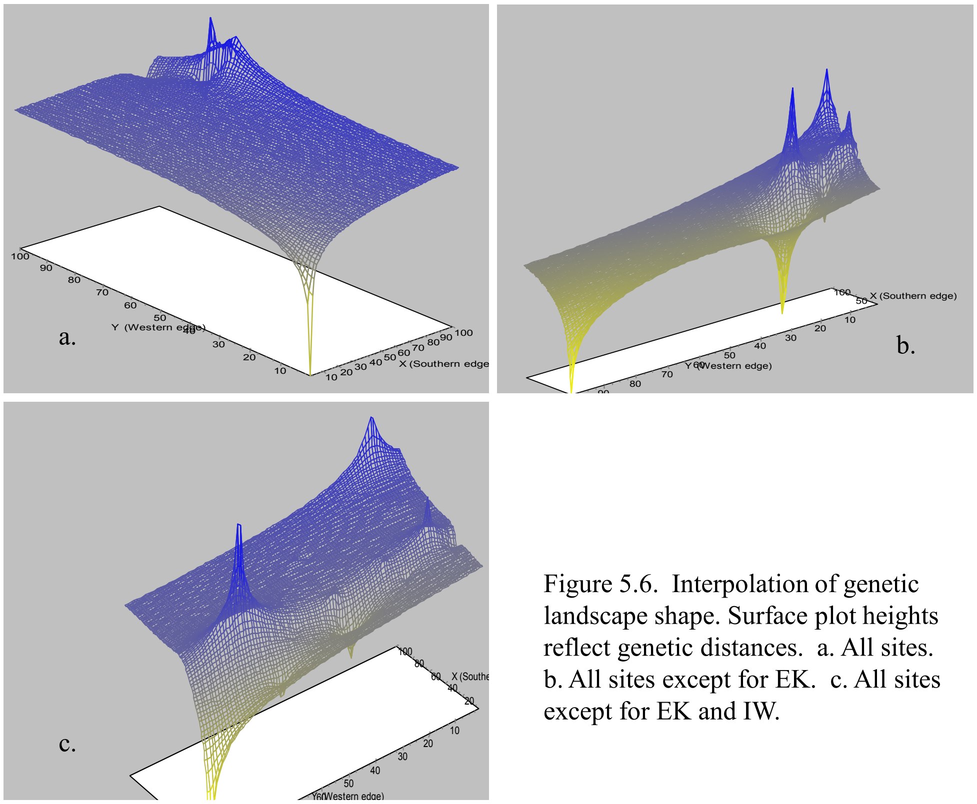


Figure 5.6. Interpolation of genetic landscape shape. Surface plot heights reflect genetic distances. a. All sites. b. All sites except for EK. c. All sites except for EK and IW.

As is expected based on previous analyses, pairwise F_{ST} and R_{ST} estimates generally indicate little genetic differentiation, particularly among central Maryland populations (Table 5.5). Significant differences in F_{ST} estimates were limited to pairwise population comparisons between IW, EK and all other populations, and between WRP and JBWS. Estimates ranged from a low of 0.001 between both PWRC and GNMP and MB and WRP to a high of 0.150 between IW and EK. Significant differences in R_{ST} estimates occurred between JBWS and each of three other sites – PWRC, MB and WRP – between IW and all other sites, and between EK and PWRC, JBWS, BHP, MB, Casey and IW. R_{ST} values ranged from a low of 0.000 at four comparisons (BHP and GNMP, WRP and MB, Casey and GNMP, and Casey and BHP) to a high of 0.446 between IW and JBWS. Interestingly, R_{ST} values were higher for IW than for EK, despite the proximity of IW to Maryland populations relative to EK.

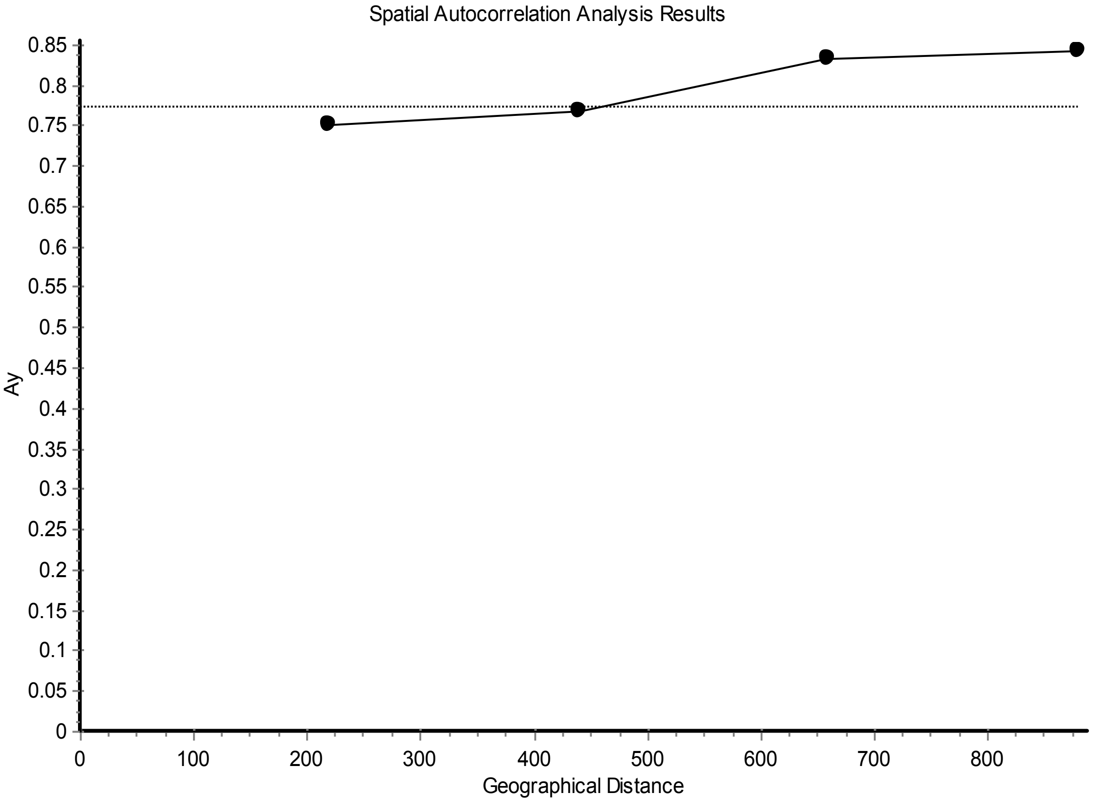


Figure 5.7. Autocorrelation plot comparing pairwise geographic distance with the average genetic distance between pairs of individuals for four distance classes. The dotted line is the average pairwise genetic distance from the full data set. Its intercept with the autocorrelation line indicates the maximum distance of significant spatial autocorrelation.

Table 5.5. Matrix of F_{ST} (below the diagonal) and R_{ST} (above the diagonal) values for all pairwise comparisons among 10 box turtle populations. Measures were derived from 10 microsatellite loci. Values in bold are significant after sequential Bonferroni correction. See Table 5.1 for population abbreviations.

Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
PWRC		0.011	**0.066**	0.011	0.006	0.013	0.032	0.020	**0.197**	**0.060**
GNMP	0.001		0.014	0.000	0.010	0.019	0.012	0.000	**0.295**	0.059
JBWS	0.003	0.005		0.015	**0.066**	**0.084**	0.078	0.018	**0.446**	**0.110**
BHP	0.005	0.004	0.006		0.013	0.019	0.012	0.000	**0.271**	**0.054**
MB	0.006	0.005	0.006	0.003		0.000	0.009	0.013	**0.162**	**0.034**
WRP	0.008	0.002	**0.009**	0.005	0.001		0.004	0.022	**0.148**	0.022
RCP	0.009	0.008	0.002	0.006	0.008	0.007		0.016	**0.246**	0.022
Casey	0.006	0.002	0.005	0.000	0.001	0.003	0.010		**0.290**	**0.063**
IW	**0.022**	**0.027**	**0.033**	**0.027**	**0.026**	**0.025**	**0.036**	**0.030**		**0.131**
EK	**0.128**	**0.134**	**0.138**	**0.125**	**0.118**	**0.127**	**0.149**	**0.124**	**0.150**	

The results of the F_{ST}-based AMOVA for various combinations of populations revealed little structure within and between populations for most comparisons (Table 5.6). Nearly 100% of the allele frequency variation in a comparison of the intact and isolated populations occurred within populations, rather than between isolated and intact population groupings. When populations were organized in three regions (Central MD/PA, IW, and EK), 9% of the variation was attributed to among region differences; 91% occurred within populations. Grouping the nine northern populations into two regions (IW and all other MD/PA populations) again indicated that among region variation was small at 2%.

Table 5.6. F_{ST}-based AMOVA for three population groupings. Percentage of variation does not sum to 100 in all cases due to rounding. See Table 5.1 for population abbreviations.

Population groupings	All pops, three groups - Central MD/PA, IW, EK				Nine pops, two groups - Central MD/PA, IW			
Variance component	Variance	Percentage of variation	Fixation indices	*P*-value	Variance	Percentage of variation	Fixation indices	*P*-value
Among regions	0.406	9	0.089	0.001	0.097	2	0.022	0.001
Among populations w/n regions	0.022	0	0.005	0.001	0.020	0	0.005	0.001
Within populations	4.145	91	0.094	0.001	4.238	97	0.027	0.001

Population groupings	Intact vs Isolated – PWRC/JBWS vs BHP, MB,WRP			
Variance component	Variance	Percentage of variation	Fixation indices	*P*-value
Among regions	0.017	0	0.004	0.002
Among populations w/n regions	0.012	0	0.003	0.005
Within populations	4.254	99	0.007	0.001

Hartl and Clark (1997) assert that *F*-statistics with values less than 0.05 are indicative of little genetic differentiation; those between 0.05 and 0.15 represent moderate genetic differentiation, and those between 0.15 and 0.25 indicate that populations are strongly differentiated. Fixation indices for among-regions analyses were consistent with the finding of little genetic structure with the exception of the comparison between central MD/PA, IW and EK populations. The AMOVA produced a moderate F_{ST} value (0.089, $P = 0.001$) for the among regions comparison.

The results of AMOVA for various combinations of populations revealed more structure on the basis of allele size, rather than frequency (Table 5.7). Though nearly all variation in allele size occurred within populations when the intact vs. isolated grouping was analyzed, variation among regions increased to 13% with the inclusion of all sites and to 25% with the exclusion of the Florida population. Fixation indices indicated moderate structuring for central MD/PA, IW and EK populations ($R_{ST} = 0.133$, $P = 0.001$) and great differentiation ($R_{ST} = 0.255$, $P = 0.001$) when the Florida population was excluded.

5.2 Discussion

Turtle populations in much of the eastern United States exist in habitats surrounded by heavily used roads that restrict movement between populations (Gibbs and Shriver 2002). Increasing concern is being expressed about the status of the eastern box turtle (Dodd 2001; U.S. Fish and Wildlife Service 1996). Among turtles, the terrestrial box turtle is particularly vulnerable to road mortality and habitat destruction, as aquatic species typically limit overland movements and statutes such

Table 5.7. R_{ST}-based AMOVA for three population groupings. Percentage of variation does not sum to 100 in all cases due to rounding. See Table 5.1 for population abbreviations.

Population groupings	All pops, three groups - Central MD/PA, IW, EK				Nine pops - two groups - Central MD/PA, IW			
Variance component	Variance	Percentage of variation	Fixation indices	*P*-value	Variance	Percentage of variation	Fixation indices	*P*-value
Among regions	1414.413	13	0.133	0.001	2680.745	25	0.255	0.001
Among populations w/n regions	132.954	1	0.014	0.001	149.274	1	0.019	0.001
Within populations	9083.512	86	0.146	0.001	7696.393	73	0.269	0.001

Population groupings	Intact vs Isolated – PWRC/JBWS vs BHP, MB,WRP			
Variance component	Variance	Percentage of variation	Fixation indices	*P*-value
Among regions	48.983	1	0.006	0.056
Among populations w/n regions	178.128	2	0.022	0.004
Within populations	7895.898	97	0.028	0.001

as the federal Water Quality Act of 1987 (P.L. 100-4; “Clean Water Act”) restrict to varying degrees the extent of destruction of aquatic habitats.

Traffic volumes in Montgomery County, Maryland, where treatment sites were located, have increased. Annual average daily traffic (ADT) measured along the same section of Interstate 270 near several of the study sites increased 683%, from 21,500 ADT in 1989 to 168,443 ADT in 2008 (Maryland State Highway Administration 2009). Traffic volumes on the roads surrounding each of the treatment sites undoubtedly have increased as development has expanded into rural sections of the county. Based on the vulnerability of box turtle populations to the loss of adults and the density of the highway system in the general study area, I believed that turtle populations in habitats surrounded by roads would be less genetically diverse than those in contiguous habitats. This was not the case, however. Moreover, I was able to employ primers developed for use in the bog turtle, a species that diverged from box turtles more than 15 million years ago (Feldman and Parham 2002; Holman 1987), providing further evidence that flanking regions in Emydid turtles are highly conserved (King and Julian 2004).

5.2.1 Genetic Diversity within Populations

Moderately high genetic diversity was found among 436 individuals sampled in 10 sites. Microsatellite-based studies of other Emydid turtles have revealed generally lower levels of genetic variation (Table 5.8).

I detected high levels of heterozygosity in the sampled box turtle populations; observed H_O in nine of the ten populations was close to or exceeded 0.80 (Table 5.1).

Table 5.8. Results of microsatellite studies of other Emydid turtles. *N* refers to the total number of animals sampled. $\hat{A}$ refers to the mean number of alleles per locus. NR indicates that the statistic was not reported.

Study	Species	No. Pop (*N*)	$\hat{A}$	Mean H_O
Kuo and Janzen (2004)	ornate box turtle	2 (74)	9.3	0.57
Hauswaldt and Glenn (2005)	diamondback terrapin	9 (320)	9.7	0.77
Tessier et al. (2005)	wood turtle (Quebec, CA)	6(136)	9.0	0.75
Castellano et al. (2008)	wood turtle (NJ/PA, USA)	1(80)	18.4	0.75
Howeth et al. (2008)	Coahuilan box turtle	7(156)	NR	0.74

Again, this is higher than that reported in several other studies (Table 5.8), though slightly lower than the 0.83 reported by Marsack and Swanson (2009) for 163 *T.c. carolina* in fragmented habitat in Michigan's Lower Peninsula. Interestingly, H_O in the remaining population – Florida's Egmont Key (0.64) – was more similar to the mean H_O of 0.63 for 13 populations of the Giant Galápogos tortoises (*Geochelone* sp.) on 6 islands in that archipelago (Ciofi et al. 2002).

Allelic richness provides another important indicator of genetic diversity, as heterozygosity may be lost at a much slower rate than the number of alleles as populations decline (Allendorf and Luikart 2007). Allelic richness was quite similar among all populations with the exception of IW and EK. IW is located near the southern end of the Eastern Shore of the Chesapeake Bay, and is connected to the peninsula only by a narrow strip of land. As a result, IW turtles are separated by distance from the central MD/PA populations, by the formation of the bay itself some 20,000 years ago, and increasingly by development and traffic on highways that likely prevent successful dispersal from the isthmus. IW turtles effectively are isolated, and the reduction of allelic richness relative to central MD/PA populations, while not significant, may be a manifestation of this isolation. The significant reduction in allelic richness, the relatively high number of private alleles, and the finding of reduced H_O may reflect the total isolation of EK turtles from mainland populations. Alternatively, MD and PA populations may be influenced by the introduction of alleles from turtles captured at some distance and released in study sites. Since measures of genetic diversity in MD and PA populations were similar for populations on public land and those closed to the public, however, this alternative is less likely.

I detected private alleles in all populations except RCP (Table 5.1), a finding that could be a result of small sample size (N=17) or the suspected release in the park of turtles from other areas (K Ferebee, pers comm, 26 Sept 05).

Inbreeding, as indicated by $F_{IS} > 0$, was detected in all populations (Table 5.1). The highest F_{IS} value occurred for the PWRC study area, one of the largest and most intact sites sampled. I collected the majority of samples for DNA analysis in the 11.8 ha floodplain forest plot that was established by Lucille F. Stickel in 1944. Box turtle surveys were conducted in that plot every 10 years through 1995. Populations surveyed in 1995 had declined by 77% from the high recorded in 1955, a decline that is believed to be a result of hydrologic changes and flooding (Hall et al. 1999). The finding of high F_{IS} values for PWRC may reflect this history. Allelic richness at PWRC, however, was relatively high. It is possible that allelic richness has, in fact, declined at PWRC along with the population, but benchmark data do not exist.

The lowest F_{IS} value occurred at RCP, a finding that is consistent with the small number of samples and/or the suspicion that turtles captured elsewhere not uncommonly are released in the park.

Five locus by population comparisons failed to conform to HWE (Appendix D). The loci were dispersed throughout four populations, although *Gmu*D40 was involved in three of the comparisons (PWRC, GNMP, JBWS), and so failed to conform to HWE in 30% of the sampled populations. This raises the possibility that populations in these sites are not panmictic or that a null allele is present (Allendorf and Luikart 2007). A second locus – *Gmu*A19 – was out of HWE at JBWS. Given the performance of the eight other loci in this population, and the findings pertaining to other populations, sampling error or the presence of a null allele remains the most likely explanation. The failure of *Gmu*D79 at EK to conform to HWE can be explained in the same way. Although this locus was not highly polymorphic, relatively small samples (e.g., RCP with 17 and IW with 32), had at least four alleles compared with the three alleles found in 53 D79 scores from EK (Appendix B).

5.2.2 Genetic Differentiation among Populations and Genetic Structure

In general, weak genetic differentiation was observed among the sampled populations in Maryland and Pennsylvania. The Florida population was moderately differentiated from those in the MD/PA group.

F_{ST} and R_{ST} values are assessed on the basis of whether they significantly differ from zero – the situation in which all subpopulations belong to one randomly breeding population (Balloux and Lugon-Moulin 2002). Pairwise F_{ST} values (Table 5.5) support the establishment of two population clusters. Values for EK are within the range associated with moderate differentiation (Hartl and Clark 1997). Those for IW, while indicative of little genetic differentiation between IW and central MD/PA populations, are nonetheless significantly different from MD/PA populations and EK.

High rates of polymorphism typical of microsatellites and observed in the study populations have the effect of reducing F_{ST} (Balloux and Lugon-Moulin 2002), resulting in the appearance of less structure than actually exists. Collection-based analyses are helpful in identifying weak structure, as shown in Figure 5.2 where k = 3,4.

Pairwise R_{ST} values for IW, with several exceeding the 0.25 associated with very great genetic differentiation (Hartl and Clark 1997), indicate that IW is less similar to the other northern sites on the basis of allele size than is EK (Table 5.5). Given the time and distance by which EK has been separated from the other populations, these results are unexpected. Under the stepwise mutation model (Slatkin 1995), on which R_{ST} is calculated, the difference in allele size is proportional to the time elapsed since divergence from a common ancestor (Lowe et al. 2004). Variation at microsatellite loci is rarely as simple, however, as that assumed by the stepwise mutation model (Peakall and Smouse 2006). The weakness of R_{ST} is its high variance (Balloux and Lugon-Moulin 2002; e.g., Table 5.7). For those data sets containing loci with widely differing variances, loci exhibiting low variance will contribute little to the final value of F_{ST} and R_{ST} (Lowe et al. 2004). Regardless, the reason why EK R_{ST}-values differ less than those of IW in pairwise comparisons with central MD/PA populations is unclear.

The findings of AMOVAs based on allele frequency (Table 5.6) and size (Table 5.7) are, as would be expected, similar to pairwise results (Table 5.5). Of particular interest is the fact that most fixation indices for the various groupings were highly significant, thereby signaling differences, though small, between population groupings.

An autocorrelation plot (Figure 5.7), estimated that gene exchange would occur between the sampled populations at distances of less than 219 km. The longest distance between two sampling locations in the central MD/PA group is between GNMP and JBWS at 122 km. On the basis of the autocorrelation plot, gene exchange should occur, therefore, among all central MD/PA populations. The autocorrelation plot estimates conform to F_{ST} values in that, for all pairwise comparisons (Table 5.5), there are no significant differences between central MD/PA populations with the exception of JBWS and WRP.

Although interrupted by the expanse of the Chesapeake Bay, IW is a straight-line distance of 145 km from JBWS, the closest central Maryland site to IW. The longest distance between IW and a central Maryland/PA site is 243 km, just over the 219 km threshold for genetic exchange. The actual distance between IW and all other northern sites is much greater than the straight-line distance, however, given the intervening expanse of the Chesapeake Bay. Pairwise F_{ST} values between IW and all other populations are significant and reflect the extent to which the IW population is separated from other sampled populations.

5.2.3 Possible Causes of Weak Genetic Differentiation among Maryland Populations

The results of the genetic analyses in this study generally are consistent with other turtle studies that likewise found few differences between populations, even those separated for long periods of time (Castellano et al; 2008; Howeth et al. 2008;

Kuo and Janzen 2004; Marsack and Swanson 2009; Richtsmeier et al. 2008; Scribner et al. 1986).

According to Rosenbaum et al. (2007), specialist species occupying isolated patch habitats, such as the federally threatened bog turtle, should be genetically divergent. The bog turtle has the most disjunct distribution of all northerly turtles at both local and regional scales, yet even this species evidenced surprisingly low levels of genetic variation.

Mühlmann-Diaz et al. (2001) found that the entire chromosome 1 of five species (pond slider (*Trachemys scripta*), painted turtle (*Chrysemys picta*), desert tortoise (*Gopherus agassizii*), loggerhead sea turtle (*Caretta caretta*), and eastern box turtle) was labeled with the probe designed from chromosome 1 of *T. scripta*, thereby demonstrating "that this chromosome has maintained a remarkable stability with regard to translocations or other interchromosomal rearrangements in species as diverse as desert tortoises and loggerhead sea turtles." Avise et al. (1992) speculated that a reason for this conservatism may lie with the relatively low metabolic rate of turtles, which in turn results in lowered production of oxidative free radicals.

Remnant populations of long-lived species may not exhibit genetic differentiation even after population bottlenecks of 100-200 years (Kuo and Janzen 2004). Indeed, changes in *Terrapene* microsatellite allele frequency and size may require many centuries to become apparent (Avise et al. 1992; FitzSimmons et al. 1995).

With only limited investigations of genetic structure in eastern box turtles and closely related species, a study of gopher tortoise population and conservation genetics provides a basis for comparison between two Chelonians facing similar anthropogenic threats. Unlike many of the aquatic or semi-aquatic species for which genetic information is available, the gopher tortoise and box turtle are predominantly terrestrial. Gopher tortoise populations also are increasingly isolated by intensive development and extensive highway systems (Ernst and Lovich 2009). Based on an analysis of 9 microsatellite loci from 300 individuals in 18 locations in Florida and 3 in Georgia, Schwartz and Karl (2005) identified genetic subpopulation differentiation in the three Georgia populations and concluded that the Florida populations should be classified as five genetic assemblages. Given the similarities in life history between gopher tortoises and box turtles and the threats faced by both species, the genetic differentiation observed for gopher tortoises may serve as a prediction of that which box turtle populations will eventually face.

Large expanses of suitable habitat were available to eastern box turtles in the study region throughout much of the 20th century. Judging by the heterozygosities observed in the sampled populations and the lack of differentiation between populations, gene flow in the form of juvenile turtles and so-called “transient” adults (Dodd 2001) was effectively unimpeded. If bog turtle populations are unexpectedly undifferentiated, habitat generalists like the box turtle will certainly remain so, absent stochastic environmental perturbations or disease (e.g., *Ranavirus* (family Iridoviridae) for decades to come.

The proximity of heavily used roads does not appear to be resulting in reduced genetic diversity in box turtle populations relative to populations that are not surrounded by roads (Tables 5.6 and 5.7). While it is evident that many individuals do not survive their attempt to cross roads, enough may do so to ensure that genetic diversity is maintained. Alternatively, the study roads, and in particular the high traffic volumes that are the norm on many of them, may not have been in existence long enough to have caused observable changes in population genetic structure.

Given the results of other studies on turtle genetics, these findings are not surprising. While the sampled box turtle populations exhibit genetic diversity consistent with large population size, increasing fragmentation and isolation of populations, combined with declining population size, may eventually result in reduced genetic diversity as seen in IW and EK.

CHAPTER 6: MORTALITY RESULTS AND DISCUSSION

6.1 Results

The study plot search schedule and encounter histories are presented in Table 6.1 and Appendix G, respectively. Based on encounter histories, the MARK program estimated the probability of recapture for all sites at 25.81%. On this basis, the box turtle population at BHP, MB and WRP was estimated to be 698, 1214 and 846, respectively (Table 6.2).

Table 6.1. Search schedule, by year and park.

	Blockhouse Point			Muddy Branch			Wheaton Regional Park		
Year	Begin	End	Days	Begin	End	Days	Begin	End	Days
2004			0[a]	20-Jul	6-Sep	13	10-Sep	10-Oct	9[b]
2005	15-May	10-Jun	9	30-May	31-Jul	13	22-Jun	23-Aug	11
	9-Aug	11-Sep	9						
2006	26-May	9-Oct	11	13-Aug	1-Oct	15	10-Jun	2-Oct	11
2007							10-Jun	28-Jul	11

[a] A study plot randomly selected in 2004 resulted in only one capture. Another plot was randomly selected in BHP for subsequent study years.

[b] WRP 2004 searches were terminated before plot completion and were finished on May 12 and 20, 2005. Turtles found on those dates are included as 2004 captures.

Table 6.2. Estimated turtles per hectare, estimated population size (*N*), and annual road and natural mortality rates, by park, on the basis of 2006 road and field surveys.

	Park		
	BHP	MB	WRP
Turtles/ha	5.4	9.6	7.8
Estimated *N*	698	1214	846
Road Mortality (percent *N*)	18 (2.58)	12 (1.00)	11 (1.30)
Natural Mortality (percent *N*)	0 (0)	0 (0)	1 (0.1)

Road survey dates, duration of surveys and findings are summarized in Appendix H. The probability of mortality in a crossing attempt of each of the roads on which turtles were found is shown in Table 6.3. Annual road-based mortality, including turtles found crossing in a location associated with a >51% probability of being killed, represent 2.58%, 1.00% and 1.30% of the estimated park population size for BHP, MB and WRP, respectively (Table 6.2). The location of turtles found in or near the road during road surveys is presented in Figures 6.1, 6.2 and 6.3 for BHP, MB and WRP, respectively.

Table 6.3. Probability of mortality for a turtle crossing the designated road at the estimated traffic volume. Probability assumes that the turtle has a carapace length of 136 mm and travels in a path perpendicular to the road and at 3.3 m/minute.

Park	Road	Traffic Volume 0600-1800 hrs	Percent probability
BHP	River Road	2817	78
	Pennyfield Lock Rd	72[a]	4
MB	Darnestown Road	21,202	100
	Quince Orchard Rd	1200[a]	48
WRP	Glenallen Avenue	4166	88
	Kempmill Road	23,578	100
	Orebaugh Avenue	720[a]	32
	Randolph Road	79,106	100

[a]estimated

The male:female ratio for study plot turtles for which gender could be determined was strongly biased in favor of males in all park plots (BHP, 25M:2F, $\chi^2 = 19.6$, $P < 0.0001$; MB, 53M:19F, $\chi^2 = 16.1$, $P < 0.0001$; WRP, 46M:25F, $\chi^2 = 6.2$, $P = 0.01$). For BHP, this is true even assuming all animals whose sex was recorded as

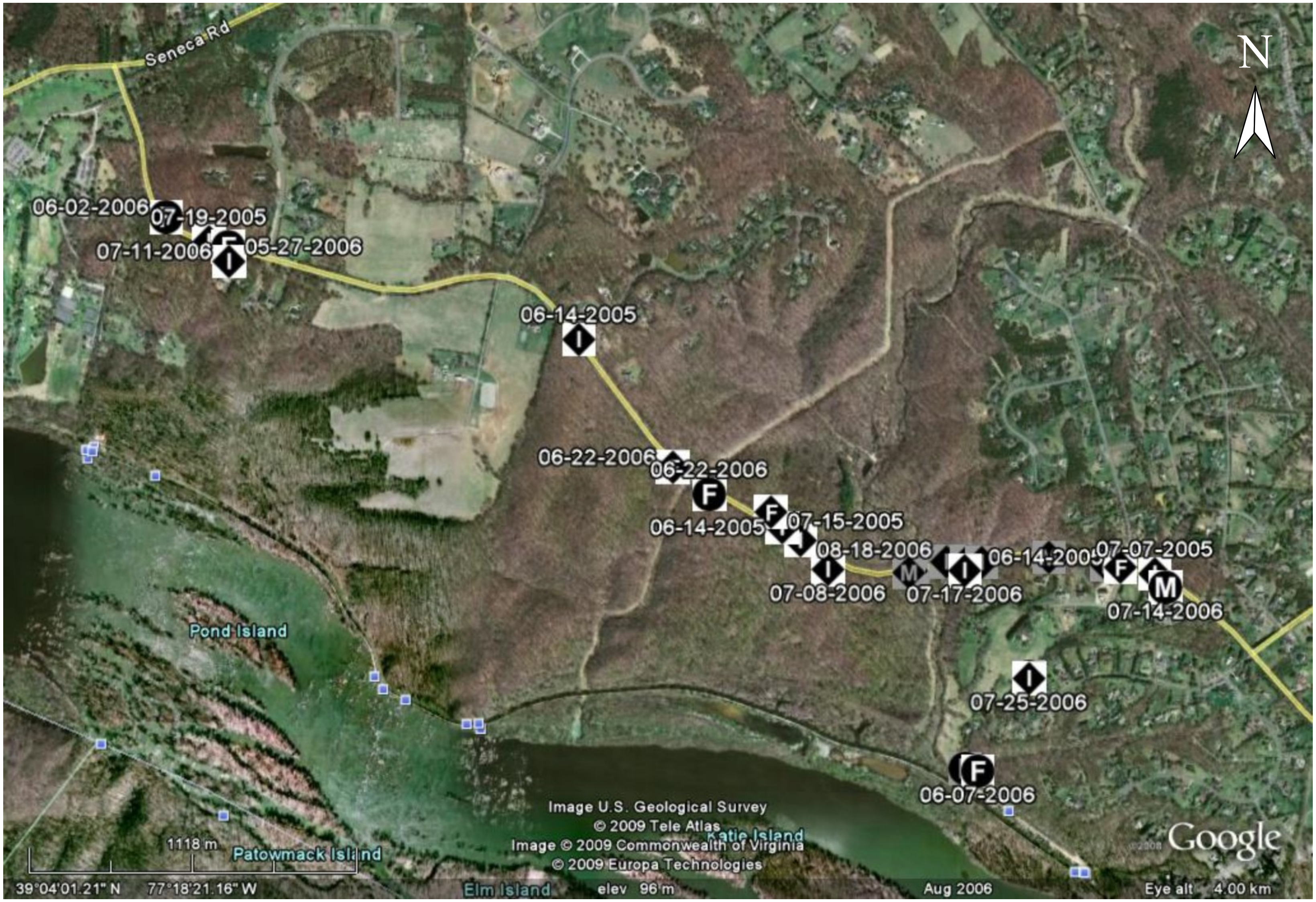


Figure 6.1. Location of turtles encountered in roads bordering BHP. Turtles found in the western section of the map near Senaca Road were most closely associated with the Chesapeake and Ohio Canal National Historic Park and are not included in road mortality counts for BHP. Round symbols indicate male or female turtle was alive. Diamond-shaped symbols indicate male, female, or turtle of indeterminate sex was dead.

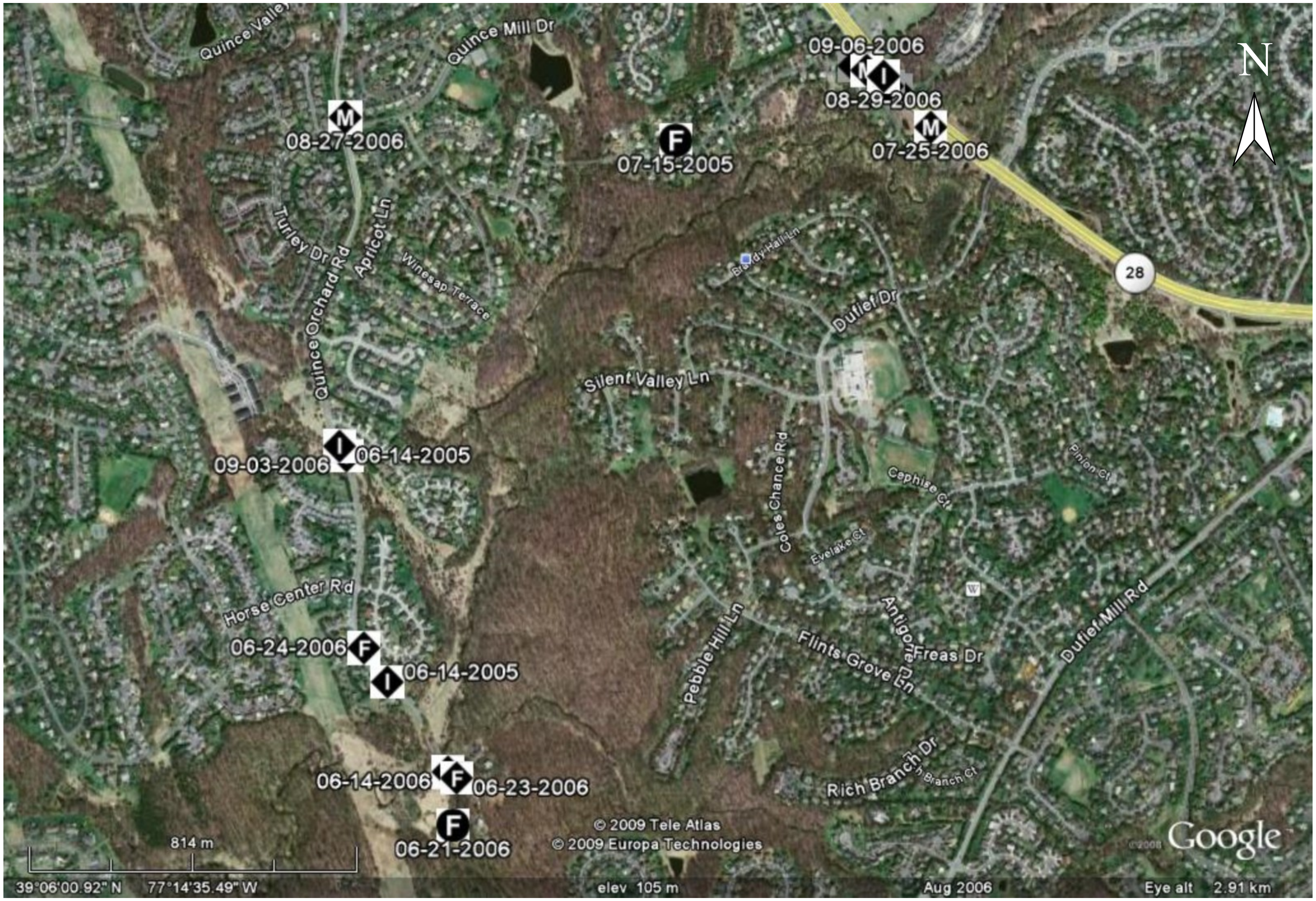


Figure 6.2. Location of turtles encountered in roads bordering MB. Round symbols indicate male or female turtle was alive. Diamond-shaped symbols indicate male, female, or turtle of indeterminate sex was dead.

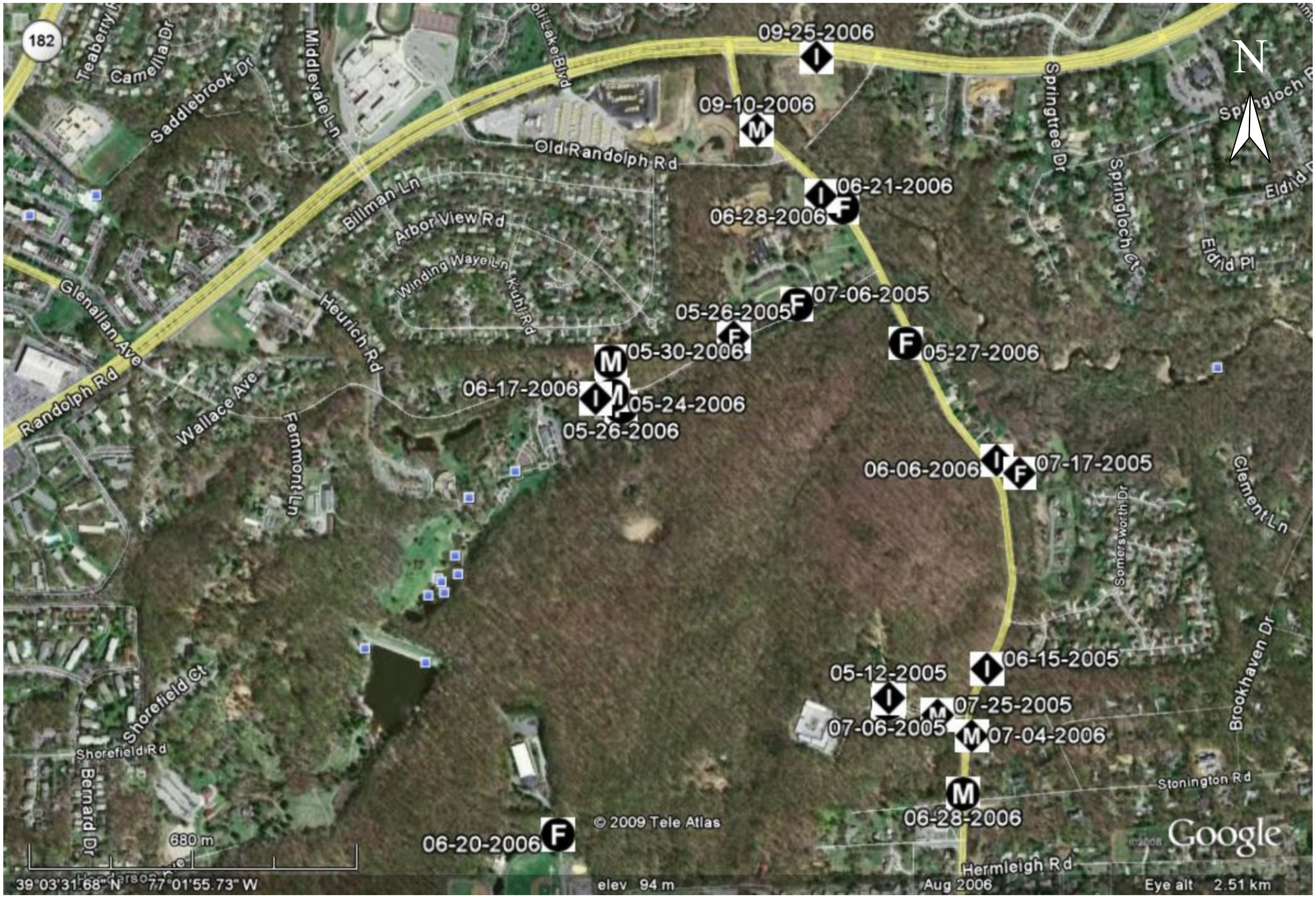


Figure 6.3. Location of turtles encountered in roads bordering WRP. Round symbols indicate male or female turtle was alive. Diamond-shaped symbols indicate male, female, or turtle of indeterminate sex was dead.

indeterminate were female, and nearly so for MB (BHP, 25M:8F, $\chi^2 = 8.8$, $P = 0.003$; MB, 53M:35F, $\chi^2 = 3.6$, $P = 0.058$).

The sex of most turtles killed in the road in 2005 and 2006 could not be determined. Of those for which gender and age determination were possible, the adult male/adult female/juvenile ratio for BHP was 5:6:2. For MB and WRP, the ratios were 3:3:1 and 5:4:1, respectively. Although juvenile turtles are less likely to be found in roadside surveys, either alive or dead, 46 of 50 (92%) turtles killed or likely to have been killed in the roads were adults.

No turtles were found to have died as a result of natural causes in 2006, with the exception of one adult at WRP (Table 6.2).

Road densities in Montgomery County average 4.5 km/km^2. Road densities for Montgomery County Policy Areas in which BHP, MB, and WRP are located are 3.4 km/km^2, 4.6 km/ km^2, and 10.4 km/km^2, respectively.

6.2 Discussion

6.2.1. Road Mortality

Losses of box turtles to road mortality in the study parks did not exceed the annual rate of 5% that has been defined as “excessive,” and a threat to population persistence (Gibbs and Shriver 2002). However, losses in BHP were within the 2-3% annual rate of loss that is believed to be inconsistent with population growth (Brooks et al. 1991; Congdon et al. 1993;1994; Doroff and Keith 1990). As the calculation of road kill probability assumed that all turtles crossed the road at an angle perpendicular to the direction of traffic and at the mean maximum speed recorded by Adams et al. (1989) and Muegel and Claussen (1994), mortality rates may be underestimated.

Confidence in mortality rates as a percent of population size is further reduced by the presence of box turtle populations in areas adjacent to the parks and possible movement of individuals between areas. Both BHP and MB are adjacent to and part of a larger network of parks. WRP is loosely connected at its northeast corner to Northwest Branch Park. Each park is surrounded and/or fragmented by local and collector roads in suburban developments, but movement between them is possible. Therefore, road mortality figures for BHP and MB, and possibly for WRP, in all likelihood reflect mortality of members of populations outside the study area. Thus the estimated losses at BHP and MB may overestimate mortality of resident turtles in the study populations. Road mortality figures for Wheaton Regional Park most accurately reflect losses from that population, as the park is virtually surrounded by intense suburban development. I also conducted four population surveys in that park, and on that basis the population size estimation to which annual road mortality is compared should be the most reliable.

Road-mortality levels at BHP and MB must reflect the substantial connection these parks have with adjacent box turtle habitats. Until recently, movement across the roads which now separate park segments may have been relatively easy. Over the course of two or three turtle generations, animals whose movements promoted gene flow among populations are now often eliminated from their populations, and the ratio of stayers vs. dispersers (Fraser et al. 2001) may increase, eventually leading to a reduction in allelic diversity.

Gibbs and Shriver (2002) reported that the relationship between road density and expected road-crossing frequency was strongly, linearly and positively related for land and large- and small-bodied pond turtles. At 3.4 km/km^2, 4.6 km/ km^2, and 10.4 km/km^2

for BHP, MB, and WRP, respectively, road densities in park areas, combined with traffic volumes on adjacent roads (Table 6.3), are predicted to produce a cumulative probability of annual road-associated mortality in land turtles > 50% (Gibbs and Shriver 2002). The annual drain of road mortality, particularly the apparent bias toward adult females that I observed, is a serious challenge to box turtle populations. Moreover, the likelihood of surviving an attempt to cross many of the surrounding roads is low, and populations may be genetically trapped within their current habitats.

6.2.2. Natural Mortality Rates

Natural mortality rates for each park were calculated on the basis of individuals found dead within the study plot in 2006. In contrast to road mortality estimates, which may include turtles from adjacent populations, natural mortality estimates better reflect turtles having home ranges within the study park. Regardless, natural mortality rates are undoubtedly greatly underestimated. Though mortality rates must be higher in hatchlings and juveniles than adults (Ernst and Lovich 2009), the chances of finding dead hatchlings and juveniles are low. Evidence of natural mortality was limited to adults, and on that basis greatly underestimates the substantial losses of young turtles assumed to occur to predators (Dodd 2001), and possibly to disease and environmental factors. In addition, the dog used to find turtles was not as reliable in finding shells only, and evidence of natural mortality even of adults undoubtedly was missed.

6.2.3. Gender Bias

The male bias observed in the study plots may exist throughout the surveyed parks. Although it is possible that the habitat in the study plots was more attractive to males than to females, I could find no reference in the literature to gender-based habitat preference with the exception of the use of meadows and other open areas by females for nesting. The large size of the study plots should have ensured that, if any such preference exists, it did not skew the result in favor of one sex.

AT PWRC, most of the turtles I encountered were in or near the Stickel plot, although, as in Hall et al. (1999), turtles encountered outside the plot and elsewhere on the property were included in gender analyses. Male bias observed in the 1995 surveys appears to continue, as 39 of 47 (83%) turtles encountered during the 2004-2007 period in which blood samples were obtained and for which gender determination could be made were males. Unlike the park populations sampled, there was relatively little opportunity for PWRC animals, particularly those in and around the Stickel plot, to encounter public roads. Meadows adjacent to the plot were mowed in strips, however, and a proximate two-track dirt road was regularly used by PWRC vehicles. Since both meadows and the bare ground of an unpaved road are known to be used by female box turtles for nesting (Dodd 2001), destruction of females during nesting movements could account for at least some of the male bias observed at PWRC.

Sex ratios in a box turtle population at any given time reflect decades of variation in climate and precipitation, land management, predation levels and habitat succession/change. A short-term study such as this is merely a snapshot, and indeed even "true" sex ratios vary over time. I believe that both sexes had an equal probability of capture by the dog, and that the male bias that I observed accurately represents the gender distribution in the study plots at the time of sampling.

If the male bias observed in the study plots exists throughout the park, it should also be reflected in road mortality rates. Excluding mortality figures for those animals for which gender could not be determined, however, females were killed at greater rates than their abundance in the population. Studies of freshwater species (Aresco 2005; Marchand and Livaitis 2004; Steen et al. 2006; Steen and Gibbs 2004) have linked increasing male bias with area road density. Male bias has been thought to affect terrestrial turtles less than aquatic species, as vulnerability to road mortality is believed to be more comparable between the sexes (Gibbs and Steen 2005; Steen et al. 2006). The findings of road and plot surveys in this study suggest that road mortality is affecting adult females out of proportion to their abundance in the population, thereby presenting a serious threat to the persistence of these populations.

6.2.4. Patterns of Road-based Mortalities and Potential Remedies

Although road-killed turtles and turtles found alive in the roads appear to be widely distributed (Figures 6.1 - 6.3), summer field assistants quickly identified areas in which they were more likely to encounter live or dead turtles. Not surprisingly, such areas were characterized by turtle habitat, usually forested, on both sides of the road.

Relatively modest and low cost alterations, such as the addition of fencing in the target area that guides animals to existing culverts (assuming that the culverts do not normally carry water or that there is dry land within the culvert and along its length), provide for safe passage under roads, and have been shown to be used by box turtles (Hagood and Bartles 2008). Given the importance of virtually every adult to its population (Seigel 2004), such fence/culvert systems may prove critical in providing for continued gene flow in the short term and ensuring that populations persist in the long term. Where appropriate culverts do not currently exist, future highway improvement projects should incorporate them. Alternatively, concrete or metal pipes can be punched under existing roads (Iuell et al. 2003), and box turtles and other relatively small animals directed to them by fencing.

6.2.5. Recommendations for Future Research

A number of factors reduce confidence in both population size estimates and mortality percentages. As mentioned previously, each park map was overlain with 11.8 ha survey plots, from which one was randomly selected for mark-recapture population size estimation. The selection of 11.8 ha plots was based on the long-term work of Stickel (1950), in anticipation of future comparisons between population densities at PWRC and other study sites. In addition, the use of large study plots, a strategy employed in other Chelonian studies (von Seckendorff Hoff and Marlow 2002), ensured that a variety of habitats were searched for turtles, with population estimates reflecting areas of turtle concentration as well as paucity.

Population size estimates were derived by calculating density/ha in the sampled plot, and multiplying that density by the size of the park. This method assumes that all hectares in the park contain the same average number of turtles as that of the sample plot, which may not be the case.

Future research should condense mark-recapture efforts to as few consecutive days as possible, and increase the number of times such efforts are conducted in each area. Surveying multiple smaller plots as many times as possible rather than remaining in one relatively large plot would also permit a better estimate of turtle population density throughout an area.

From a practical perspective, reducing uncertainties associated with mortality percentages is difficult, as it would have, in this case, required estimates of population size in the entire network of parks, and much more extensive road surveys. In hindsight, the selection of parks completely surrounded by inhospitable habitat that virtually eliminates the possibility that any turtles found on border roads could be from outside the park would have been preferable, though finding such areas that still contain sufficient numbers of turtles would be difficult. Reliability of mortality percentages could have been increased had the surveys been conducted for more than two seasons, with the conduct of daily surveys and increased frequency of walking, as opposed to driving, surveys. However, many similar surveys were conducted only by vehicle (e.g. Glista et al. 2007; Haxton 2000; Rosen and Lowe 1994; Shepard et al. 2008b). Detection rates likely were improved in this study relative to those that used only driving surveys, though reliance on vehicles on many survey days undoubtedly resulted in fewer detections than would otherwise have been the case (Langen et al. 2007).

CHAPTER 7: CONCLUSIONS

The sampled eastern box turtle populations in central Maryland and Pennsylvania are weakly genetically differentiated. Populations in habitats surrounded by roads are not, at this point in time, less genetically diverse than those in intact habitats. Sampled populations on Maryland's Eastern Shore and Florida's Egmont Key are differentiated from other sampled populations, yet remain genetically diverse despite their isolation from other box turtle populations.

"Turtle chromosomes are notoriously conservative" (Ernst and Lovich 2009). While the rate of evolutionary change suggested by such genetic conservatism predicted the general outcome of this investigation, the possibility that the findings of this study reflect past rather than present gene flow cannot be dismissed.

Weaknesses in study design identified above have caused the estimation of road-based mortality rates as a percentage of population size to be of limited use. The finding of male bias in all park populations and female bias in road mortalities, however, has not been reported for eastern box turtles, and may pose a considerable challenge to population persistence.

Areas in which turtles are known to attempt road crossings on a regular basis should be targeted for improvements, such as the addition of culvert/fence systems that provide turtles and other small wildlife with alternatives to at-grade crossings.

APPENDICES

Appendix A. Allele sizes at 10 microsatellite DNA markers for 436 eastern box turtles from 10 sample sites.

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
	Patuxent Wildlife Research Center																			
Tca1-01	143	143	187	199	168	204	126	156	191	207	156	160	209	217	120	140	122	124	145	163
Tca1-02	149	157	196	199	168	168	148	160	167	199	156	156	185	253	144	150	124	154	153	171
Tca1-03	149	149	0	0	176	180	130	134	191	227	160	160	229	233	132	136	114	114	121	129
Tca1-04	133	163	196	199	142	172	160	164	187	203	148	156	225	253	128	132	126	126	153	187
Tca1-05	133	167	193	199	158	164	152	152	203	203	148	148	213	241	124	144	130	154	145	171
Tca1-06	141	143	196	196	184	184	184	184	187	195	148	160	217	217	116	120	118	122	145	163
Tca1-07	149	167	196	199	158	164	142	142	183	191	148	160	229	241	124	128	114	122	153	157
Tca1-08	163	167	193	199	126	158	118	130	203	207	148	164	241	249	124	128	102	162	121	133
Tca1-09	163	167	193	205	154	164	118	152	191	203	148	148	213	241	124	154	114	154	125	129
Tca1-10	133	163	196	196	164	188	118	148	203	223	160	160	201	253	124	124	114	124	137	137
Tca1-11	127	167	190	199	180	180	138	152	191	223	156	156	217	217	120	124	114	114	133	137
Tca1-12	141	147	190	199	172	192	148	176	203	211	160	160	217	249	120	132	122	130	137	137
Tca1-13	133	167	196	196	168	184	152	156	195	219	156	160	177	253	136	144	114	114	137	175
Tca1-14	149	167	193	199	168	192	152	156	203	211	156	156	217	229	128	136	102	114	121	133
Tca1-15	143	161	193	196	126	184	148	184	195	231	160	160	213	217	128	140	114	124	137	167
Tca1-16	147	149	187	196	158	172	152	160	195	215	148	148	201	201	120	120	114	148	149	157
Tca1-17	133	147	193	196	172	188	160	160	207	215	148	156	229	229	124	124	114	162	157	163
Tca1-18	149	167	190	199	158	176	156	160	199	211	148	160	201	225	124	132	114	126	137	153
Tca1-19	141	167	199	199	154	164	130	148	167	187	160	160	213	241	124	144	136	136	137	171
Tca1-20	133	149	196	199	164	196	168	168	203	215	164	164	205	229	132	158	122	122	129	137
Tca1-21	133	141	199	199	150	184	148	172	195	215	160	160	221	241	128	128	114	154	157	163
Tca1-23	133	155	199	199	150	168	180	180	167	195	160	160	205	217	120	132	118	124	137	171
Tca1-24	149	167	0	0	158	176	156	176	187	203	160	160	221	229	124	124	124	124	133	171
Tca1-25	149	149	199	199	176	176	168	172	207	215	156	160	249	257	136	150	114	124	125	125
Tca1-26	141	141	190	193	158	180	130	160	191	195	148	160	225	229	120	124	124	124	129	163
Tca1-27	139	149	187	199	154	176	156	176	191	203	160	164	229	257	132	132	114	122	121	137
Tca1-28	141	155	199	199	142	176	124	134	191	211	148	156	221	237	124	124	118	124	149	157
Tca1-29	133	133	196	199	150	200	160	160	195	199	148	148	213	225	104	136	130	130	121	167
Tca1-30	133	147	193	202	158	164	148	152	195	215	156	160	201	229	124	124	122	122	129	145
Tca1-31	133	139	193	196	196	200	134	172	187	199	156	156	201	233	136	144	114	126	133	157

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca1-32	135	141	196	199	172	184	0	0	207	227	156	164	201	217	128	128	122	136	121	175
Tca1-33	163	167	196	199	146	158	156	156	167	203	156	160	213	241	132	144	124	166	157	163
Tca1-34	147	171	193	199	158	164	148	148	167	199	156	160	213	241	120	132	114	118	137	175
Tca1-36	141	149	199	199	164	180	130	130	199	203	160	188	229	241	120	128	114	126	145	171
Tca1-37	133	161	199	199	180	192	142	142	199	203	156	164	217	221	128	132	118	126	133	175
Tca1-39	163	167	193	193	176	184	176	176	203	203	156	160	241	249	132	144	124	136	125	163
Tca1-40	133	133	199	199	0	0	148	152	199	207	156	156	177	229	132	136	114	122	133	137
Tca1-41	127	139	196	199	158	184	156	156	211	215	156	160	221	241	128	132	114	114	129	137
Tca1-42	137	149	196	199	0	0	148	164	191	195	156	160	221	225	132	136	114	124	129	157
Tca1-43	139	161	187	193	0	0	156	168	199	223	148	160	249	261	120	132	124	124	133	163
Tca1-44	147	167	193	199	0	0	152	152	191	211	148	160	233	237	124	132	122	124	133	133
Tca1-45	149	157	199	199	176	188	160	164	195	203	156	160	177	257	136	144	114	126	157	163
Tca1-46	141	149	190	199	176	180	130	134	191	227	148	160	229	233	124	124	124	166	121	129
Tca1-47	147	171	199	199	142	154	148	160	183	243	148	160	201	249	132	154	124	136	137	163
Tca1-48	133	139	193	196	0	0	134	172	167	199	156	160	201	257	124	132	114	122	163	163
Tca1-49	149	169	196	199	0	0	152	180	215	215	156	160	201	201	124	140	114	122	137	163
Tca1-50	127	141	196	199	0	0	156	172	203	211	156	156	213	225	124	140	122	126	121	133
Tca1-51	141	141	196	199	0	0	118	168	203	203	156	156	201	237	124	128	122	124	129	133
Tca1-52	141	141	187	190	164	176	126	148	167	199	156	184	201	237	124	132	122	162	121	133
Tca1-53	127	133	196	196	0	0	160	168	191	191	156	160	177	225	124	124	114	136	133	167
Tca1-54	147	149	199	199	0	0	164	164	195	207	148	148	229	257	124	124	102	122	129	137
Jug Bay Wetlands Sanctuary																				
Tca2-01	141	157	199	202	172	176	134	156	211	231	156	164	225	253	120	128	114	122	137	157
Tca2-02	147	149	190	190	164	200	160	160	187	203	164	164	217	229	124	132	114	124	137	167
Tca2-03	141	167	190	199	172	200	156	160	191	207	156	156	213	213	124	124	114	154	137	137
Tca2-04	139	141	190	199	184	184	130	130	191	191	156	160	201	221	124	124	114	162	129	137
Tca2-05	141	147	190	190	180	200	148	160	187	207	164	164	229	229	124	132	124	124	129	137
Tca2-06	145	147	190	196	154	158	156	172	195	203	160	160	205	229	124	132	114	122	129	133
Tca2-07	133	139	196	199	150	204	156	156	167	203	160	160	201	217	120	124	102	124	121	157
Tca2-08	141	167	196	199	150	180	130	156	195	211	160	160	213	233	132	136	136	148	137	145
Tca2-09	155	157	193	199	154	158	156	156	191	211	156	160	201	201	120	124	122	122	137	153

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca2-10	141	167	193	199	180	192	160	160	191	203	148	160	225	233	124	132	102	126	121	129
Tca2-11	149	155	199	199	184	196	148	176	203	203	156	156	221	225	120	136	124	136	137	137
Tca2-12	141	149	196	199	196	196	130	160	191	211	160	160	201	217	124	132	114	148	129	145
Tca2-13	149	167	193	196	158	188	156	156	191	191	148	160	201	221	132	162	122	124	153	167
Tca2-14	135	147	196	199	0	0	152	164	183	187	160	160	201	237	124	124	114	118	137	157
Tca2-15	149	155	193	196	158	180	148	156	203	235	156	164	185	217	120	124	102	122	137	157
Tca2-16	133	145	199	199	158	172	134	134	187	191	156	160	225	253	120	150	102	126	121	157
Tca2-17	143	143	196	199	142	158	118	152	191	203	160	160	213	217	116	128	114	126	145	153
Tca2-18	143	147	196	196	180	180	148	156	187	187	156	164	201	241	120	124	114	124	129	157
Tca2-19	139	167	199	199	180	184	156	156	167	215	160	164	237	245	120	124	124	148	125	137
Tca2-20	131	141	196	199	172	200	160	172	167	187	156	160	201	221	124	140	122	124	133	157
Tca2-21	133	169	193	199	126	200	142	152	191	199	156	160	221	249	124	124	122	148	129	171
Tca2-22	161	167	196	199	146	154	160	160	187	211	148	160	233	261	124	128	114	136	133	145
Tca2-23	133	133	199	199	0	0	130	130	179	191	148	160	225	225	132	136	126	126	121	133
Tca2-24	133	149	199	199	164	180	148	156	187	203	148	156	201	213	124	124	114	114	125	137
Tca2-25	141	167	190	199	158	168	152	168	191	211	160	160	177	241	120	136	124	130	145	157
Tca2-26	141	167	196	199	150	188	172	176	183	187	156	160	225	241	124	132	114	122	125	129
Tca2-27	155	155	193	199	158	208	130	168	191	223	156	160	185	229	116	128	122	124	137	145
Tca2-28	141	141	196	199	154	180	148	168	187	207	148	160	177	229	128	132	114	126	137	175
Tca2-29	141	147	199	199	172	188	130	130	167	195	156	160	233	233	132	136	114	130	133	157
Tca2-30	139	139	199	199	168	192	130	164	183	199	156	160	237	237	128	136	122	134	133	167
Tca2-31	135	141	193	196	126	180	148	156	191	203	148	160	201	213	120	132	114	114	133	175
Tca2-32	141	147	196	199	158	172	152	160	191	199	156	160	201	229	124	128	124	154	133	133
Tca2-33	141	147	199	199	142	158	118	148	191	195	148	160	213	253	124	128	102	122	133	137
Tca2-34	135	149	193	196	158	200	156	160	203	219	160	160	213	257	120	120	124	148	121	157
Tca2-35	151	151	193	196	154	158	148	160	195	203	160	160	229	241	116	124	114	124	129	167
Tca2-36	161	161	190	193	172	184	152	152	183	187	160	160	229	233	124	132	122	122	125	145
Tca2-37	141	143	190	199	164	168	156	168	199	223	148	160	213	237	124	132	118	126	153	157
Tca2-38	139	145	199	199	188	192	142	142	191	215	156	160	201	225	124	132	114	122	133	137
Tca2-39	133	133	187	190	146	180	160	160	191	191	156	160	201	213	124	128	118	122	125	133
Tca2-40	139	167	199	202	164	172	130	184	183	191	160	160	205	221	104	144	124	126	125	125

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca2-41	141	155	196	199	164	180	152	172	183	187	156	156	225	237	116	124	114	124	133	167
Tca2-42	141	143	196	196	188	192	142	156	179	207	156	156	213	245	132	150	114	122	137	137
Tca2-43	141	149	190	199	126	188	152	152	187	203	148	160	229	229	120	132	114	114	145	163
Tca2-44	133	167	193	193	146	188	152	156	183	207	148	160	225	265	112	120	122	122	129	157
Tca2-45	131	167	199	199	146	146	130	148	207	211	160	160	241	249	124	144	102	126	133	163
Tca2-46	133	149	196	199	158	164	164	184	187	203	148	148	225	233	124	132	118	122	153	167
Tca2-47	141	141	199	199	126	192	130	130	167	183	156	164	213	249	120	128	124	136	141	141
Tca2-48	139	167	199	199	150	164	124	160	183	187	148	148	201	217	124	132	102	122	125	145
Tca2-49	167	167	196	199	168	200	134	142	195	203	160	164	217	253	128	136	114	122	145	157
Tca2-50	141	141	193	196	158	200	130	184	199	207	156	164	241	241	128	128	114	114	121	133
Tca2-51	133	141	199	199	154	168	130	142	167	199	156	164	201	217	104	128	124	124	129	129
Blockhouse Point Park																				
Tca3-01	0	0	196	196	154	184	152	152	183	199	148	160	225	249	112	132	114	114	121	133
Tca3-03	141	167	193	199	146	204	130	130	187	207	148	160	201	221	128	132	124	154	121	137
Tca3-04	133	141	199	199	154	168	156	184	191	199	156	156	177	241	136	144	114	122	141	171
Tca3-05	139	171	193	199	0	0	152	152	203	211	156	168	201	201	0	0	118	118	137	137
Tca3-06	145	167	193	196	142	192	130	142	215	239	160	160	217	225	132	136	114	114	125	133
Tca3-07	139	163	193	199	0	0	148	168	187	223	160	160	233	249	132	136	114	114	125	133
Tca3-08	141	163	199	199	142	154	134	172	167	187	148	156	233	237	120	140	118	122	121	163
Tca3-09	133	163	193	193	0	0	130	148	211	227	156	156	201	217	120	124	102	122	125	145
Tca3-10	167	167	193	199	142	196	130	152	199	223	148	148	213	249	124	150	114	118	137	137
Tca3-11	149	149	190	199	142	188	124	130	167	211	156	160	201	237	124	124	122	122	133	137
Tca3-12	139	167	193	199	180	188	168	172	183	183	148	156	237	245	124	124	124	134	125	171
Tca3-13	133	141	199	199	168	180	134	142	183	187	156	160	237	245	124	136	114	118	167	171
Tca3-14	139	145	196	199	164	176	130	160	203	207	148	160	221	241	120	124	118	122	137	145
Tca3-15	133	155	193	199	154	164	130	160	187	223	160	160	201	225	120	128	124	136	157	163
Tca3-16	135	141	199	199	164	188	124	152	191	195	148	160	185	233	104	124	122	130	121	171
Tca3-17	141	147	199	202	164	172	130	130	167	191	160	160	217	217	128	128	122	124	0	0
Tca3-18	133	167	196	199	168	200	118	130	199	219	160	160	201	241	120	120	122	154	133	153
Tca3-19	139	167	193	196	154	164	148	160	187	199	160	168	213	229	120	124	122	154	137	141
Tca3-20	149	157	199	199	142	164	134	134	199	211	156	184	217	241	124	124	102	114	153	183

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca3-21	133	167	193	193	154	184	130	130	187	215	148	160	225	229	128	128	114	124	157	167
Tca3-22	141	141	196	199	158	158	148	160	203	219	156	156	201	233	120	124	102	124	121	121
Tca3-23	141	167	193	193	180	188	156	168	191	195	156	160	201	241	128	132	124	158	121	125
Tca3-24	133	161	193	199	172	188	124	130	191	203	156	160	225	229	136	136	114	126	145	171
Tca3-25	133	167	187	199	184	188	148	168	187	195	148	168	201	201	116	132	122	134	133	145
Tca3-26	167	167	196	196	142	158	152	160	199	207	156	156	217	245	128	132	114	126	163	167
Tca3-27	133	175	187	196	164	168	134	148	187	203	156	156	217	237	128	158	122	124	137	153
Tca3-28	141	167	199	199	154	168	134	152	187	203	148	160	185	237	128	128	118	118	137	163
Tca3-29	0	0	0	0	0	0	130	130	199	199	160	160	221	261	0	0	114	126	133	171
Tca3-30	141	157	193	196	164	168	148	160	167	187	156	160	209	241	120	132	130	134	137	137
Tca3-31	139	163	196	196	164	188	118	168	203	207	156	160	213	225	120	128	114	154	121	149
Tca3-32	133	139	190	199	180	184	142	152	183	207	160	160	217	245	124	150	118	122	157	171
Tca3-33	147	167	190	196	142	200	164	164	167	207	156	160	201	249	128	128	114	122	125	133
Tca3-34	141	161	196	199	164	192	152	168	191	195	156	160	233	237	120	128	118	154	129	149
Tca3-36	141	149	196	199	158	164	148	176	191	219	156	160	185	201	120	136	102	102	121	133
Tca3-37	133	167	193	196	142	146	142	160	183	199	148	156	201	229	124	124	114	114	133	137
Tca3-38	161	167	193	196	196	196	142	152	187	195	156	160	217	217	124	124	114	124	121	183
Tca3-39	133	141	196	199	158	180	134	152	183	207	156	160	217	237	136	150	114	122	133	157
Tca3-40	141	143	199	199	158	176	156	156	183	199	148	148	233	257	124	136	114	114	141	163
Tca3-41	157	167	193	196	188	216	138	152	167	187	160	160	221	245	136	136	114	114	125	137
Tca3-42	143	143	199	205	168	188	130	164	191	215	156	160	201	213	112	120	114	118	129	149
Tca3-43	139	163	193	199	158	172	130	168	187	187	156	156	201	201	124	150	114	136	145	167
Tca3-44	133	171	193	202	164	168	152	156	187	203	160	168	201	213	128	150	118	124	129	133
Tca3-45	141	167	187	199	172	172	134	160	167	203	156	156	201	225	132	136	122	154	133	153
Tca3-46	139	139	193	199	0	0	148	152	223	223	156	156	225	241	124	132	118	124	145	149
									Muddy Branch Park											
Tca4-01	135	141	193	193	150	168	160	160	187	195	160	164	201	229	124	132	114	124	121	153
Tca4-02	141	167	199	199	134	158	148	148	183	187	160	164	217	253	120	124	154	162	137	145
Tca4-03	145	163	187	199	192	200	148	152	167	199	160	160	213	237	136	140	122	122	121	129
Tca4-04	135	141	193	199	126	158	148	156	191	195	160	160	237	261	116	116	122	122	133	153
Tca4-05	141	167	193	199	150	176	148	156	183	187	156	168	217	229	128	136	122	122	145	145

Appendix A. Continued

.

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca4-06	137	141	190	199	146	158	160	160	187	195	156	156	185	213	128	132	124	154	137	137
Tca4-07	167	167	193	196	158	188	130	160	199	207	148	156	225	233	120	124	124	126	137	167
Tca4-08	133	155	193	202	154	172	142	168	167	183	156	160	217	233	116	140	114	122	125	153
Tca4-09	133	143	193	196	142	154	160	172	183	195	156	156	205	245	128	128	122	130	137	153
Tca4-10	133	139	193	193	154	164	152	152	187	191	160	168	213	241	132	150	122	122	137	137
Tca4-11	0	0	193	199	0	0	160	164	187	199	156	160	201	201	124	132	0	0	0	0
Tca4-12	133	147	199	199	154	168	130	148	199	207	156	160	201	233	116	124	114	114	171	171
Tca4-13	133	163	196	199	158	176	138	172	187	199	160	160	213	229	136	154	114	122	129	129
Tca4-14	145	167	193	199	154	184	130	142	199	199	156	160	217	237	120	120	124	124	137	153
Tca4-15	149	155	193	193	154	192	142	148	167	183	156	160	217	229	124	140	102	114	129	153
Tca4-16	133	139	193	202	158	172	142	168	167	203	148	156	201	233	116	140	118	122	125	179
Tca4-17	133	145	193	199	150	168	148	164	187	199	160	192	217	257	124	140	114	122	121	133
Tca4-18	141	167	190	199	158	158	148	156	191	231	156	160	233	233	124	128	122	154	121	145
Tca4-19	141	167	196	199	158	158	148	148	187	231	160	160	225	233	124	136	122	154	121	171
Tca4-20	141	167	193	199	154	164	118	142	183	187	156	160	217	233	128	140	126	126	129	137
Tca4-21	141	161	199	202	168	184	164	164	183	215	160	164	213	225	112	120	122	124	167	175
Tca4-22	139	147	190	193	154	158	152	152	187	207	160	160	233	257	132	132	122	126	137	163
Tca4-23	141	163	196	196	184	192	134	134	203	207	156	160	201	245	132	150	122	122	129	137
Tca4-24	149	167	199	205	0	0	0	0	203	207	148	160	237	237	120	120	114	130	129	137
Tca4-25	133	167	196	199	164	200	152	152	183	203	160	160	225	229	124	128	130	134	137	145
Tca4-26	143	161	196	199	180	188	156	172	187	195	0	0	185	237	124	124	122	124	137	171
Tca4-27	133	133	196	199	172	188	148	164	191	195	160	160	225	241	124	124	102	114	121	121
Tca4-28	147	167	199	199	0	0	0	0	0	0	160	160	221	225	0	0	0	0	0	0
Tca4-29	139	149	196	199	164	192	152	160	187	191	164	168	217	225	124	128	114	124	133	171
Tca4-30	141	141	190	199	0	0	130	156	183	187	148	160	201	241	124	136	118	118	129	129
Tca4-31	131	167	196	199	154	168	152	164	191	203	160	160	217	225	124	124	126	158	141	167
Tca4-32	139	139	199	199	146	184	134	168	191	191	160	160	225	233	124	136	114	148	125	133
Tca4-33	149	151	193	196	154	176	160	164	191	199	160	160	201	241	124	136	102	118	125	163
Tca4-34	133	143	196	199	164	192	172	172	183	191	148	160	225	273	124	124	118	122	121	171
Tca4-35	135	167	196	199	158	172	130	148	203	211	156	160	221	237	128	128	114	124	137	167
Tca4-36	133	147	193	196	164	176	148	156	195	203	156	156	217	217	128	132	122	124	125	137

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca4-37	141	167	196	199	142	196	138	160	191	219	160	160	217	217	140	140	122	126	137	145
Tca4-38	149	149	193	196	164	184	160	164	187	203	156	156	217	221	132	136	114	130	137	171
Tca4-39	133	161	193	199	158	164	130	156	167	191	156	164	213	249	120	140	122	124	133	171
Tca4-40	133	133	199	202	164	200	114	176	183	211	160	160	201	233	132	136	118	122	171	171
Tca4-41	141	167	199	202	164	184	148	156	191	215	148	156	241	245	128	128	118	122	141	157
Tca4-42	135	139	196	199	142	158	152	160	167	207	156	160	213	213	116	124	118	124	133	145
Tca4-43	141	167	196	196	164	192	156	156	183	215	160	160	201	205	132	136	122	122	149	153
Tca4-44	145	145	196	196	150	168	152	180	167	191	156	160	217	237	120	150	114	118	121	133
Tca4-45	147	149	196	199	126	180	164	180	187	211	156	156	217	229	116	116	124	136	137	171
Tca4-46	139	163	190	193	158	208	148	148	183	203	148	160	185	221	124	128	114	136	145	145
Tca4-47	149	157	193	193	0	0	156	156	183	203	160	160	221	225	128	154	114	122	121	121
Tca4-48	133	133	199	199	168	188	118	130	191	203	156	160	213	249	124	140	124	124	121	171
Tca4-49	149	167	193	199	164	164	134	152	195	199	160	160	201	217	128	144	102	114	121	175
Tca4-50	143	167	187	199	0	0	138	180	167	211	156	156	217	237	128	132	114	124	133	153
Tca4-51	141	149	193	196	0	0	152	168	203	207	160	160	201	217	128	140	114	114	137	167
Tca4-52	155	171	199	199	0	0	134	164	183	183	160	160	217	249	128	140	114	130	121	121
Tca4-53	139	149	196	199	172	184	134	164	195	207	160	164	217	245	104	132	114	154	133	163
Tca4-54	133	147	199	199	0	0	156	156	187	191	160	192	221	233	144	144	114	126	121	167
Tca4-55	141	141	196	196	164	196	152	156	183	243	156	160	241	253	120	124	136	154	133	137
Tca4-56	133	163	199	202	0	0	142	160	187	191	156	160	185	201	128	132	122	136	125	157
Tca4-62	141	163	193	196	0	0	168	172	167	187	156	160	213	213	116	128	114	122	121	137
Tca4-65	141	143	193	202	0	0	156	156	203	219	160	160	213	221	136	162	114	122	171	171
									Wheaton Regional Park											
Tca5-01	127	133	199	199	142	150	134	134	187	199	156	156	233	257	128	128	118	122	167	179
Tca5-02	133	149	193	199	154	184	142	148	199	207	156	156	217	249	132	150	114	122	121	175
Tca5-03	133	145	190	199	168	168	156	176	195	199	156	160	201	253	120	128	114	122	125	167
Tca5-04	127	143	196	199	154	180	152	160	183	187	160	164	229	237	112	136	126	126	125	133
Tca5-05	139	149	196	196	0	0	0	0	191	219	0	0	0	0	0	0	0	0	0	0
Tca5-06	145	167	193	199	126	158	152	160	183	187	156	160	229	229	124	128	102	118	129	129
Tca5-07	127	139	193	196	164	172	148	168	187	199	160	160	221	225	132	132	114	114	133	145
Tca5-08	139	141	187	199	0	0	118	124	199	199	160	160	225	241	124	124	124	124	133	153

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca5-10	127	147	193	196	154	180	148	180	199	207	148	156	221	229	128	128	102	122	121	179
Tca5-11	147	163	193	196	154	188	148	152	183	199	160	160	245	253	124	128	114	114	133	157
Tca5-12	143	147	196	196	154	158	148	168	187	207	148	160	217	229	128	128	118	122	121	129
Tca5-13	141	141	196	196	154	164	152	164	199	211	160	168	217	225	124	136	118	124	0	0
Tca5-14	139	149	196	196	142	154	148	164	199	211	160	168	217	237	124	124	114	114	137	137
Tca5-15	139	167	193	199	0	0	168	172	175	187	156	160	221	225	120	132	124	124	133	171
Tca5-16	135	141	196	199	164	172	164	172	187	207	160	160	221	245	112	128	114	122	133	133
Tca5-17	133	149	199	205	142	164	118	152	191	203	160	160	225	253	132	136	122	126	137	153
Tca5-18	133	133	196	199	172	180	160	168	167	191	156	160	237	253	124	132	114	162	137	163
Tca5-19	137	141	199	199	184	184	130	142	179	187	160	160	185	217	128	140	102	124	137	141
Tca5-20	133	167	199	199	150	154	160	160	179	187	156	160	225	233	132	136	114	126	125	133
Tca5-21	141	147	196	199	0	0	152	168	167	187	160	160	221	225	116	124	114	122	141	157
Tca5-22	135	167	196	199	154	158	168	180	187	199	160	160	241	265	120	150	118	126	121	137
Tca5-23	133	161	190	199	0	0	160	172	167	191	160	160	233	249	132	136	122	122	133	137
Tca5-24	127	149	190	199	154	164	148	168	207	211	156	160	217	241	124	124	114	122	129	133
Tca5-25	149	149	196	199	150	184	138	160	179	187	148	156	213	225	120	128	122	122	137	167
Tca5-26	139	149	193	193	168	180	156	156	183	219	160	160	225	241	132	140	114	126	157	175
Tca5-27	133	133	196	199	0	0	152	172	203	207	160	160	217	245	116	128	114	122	129	141
Tca5-28	141	149	187	196	158	158	148	148	199	203	160	160	201	233	120	136	122	136	125	145
Tca5-29	127	133	199	202	168	184	160	164	187	231	148	156	185	257	132	136	114	126	129	153
Tca5-30	151	167	202	202	164	164	142	148	175	183	148	160	237	241	132	132	114	122	125	133
Tca5-31	163	163	193	199	134	176	118	180	187	207	160	160	213	225	124	136	114	136	129	137
Tca5-32	137	161	196	199	164	180	130	152	191	231	156	160	185	201	124	128	114	118	121	121
Tca5-33	133	161	199	199	154	158	142	152	183	187	164	168	201	225	120	128	114	122	137	145
Tca5-34	139	139	193	199	126	196	134	142	207	207	148	164	225	225	120	128	122	122	121	137
Tca5-35	133	163	199	199	180	192	0	0	167	187	160	160	221	233	124	132	122	124	125	163
Tca5-36	147	149	196	196	0	0	156	156	167	207	156	156	213	261	136	144	114	118	137	187
Tca5-37	147	171	187	199	158	164	148	148	179	199	156	164	217	233	120	136	122	136	121	137
Tca5-38	139	145	193	199	158	196	134	156	207	207	148	160	229	245	120	128	122	124	133	137
Tca5-39	139	141	199	199	168	188	152	156	191	199	160	160	217	249	120	124	122	136	121	129
Tca5-40	133	135	190	196	154	196	152	152	179	195	156	160	201	221	124	124	114	124	137	145

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca5-41	139	141	196	199	154	158	148	168	179	183	148	156	233	237	132	132	114	122	137	153
Tca5-42	135	145	193	196	164	164	148	180	167	187	148	160	213	233	128	128	122	124	133	141
Tca5-43	133	139	196	199	184	188	134	134	195	203	160	164	221	237	120	132	122	136	121	145
Tca5-45	149	161	196	199	0	0	118	168	187	211	160	160	185	201	112	132	114	136	121	137
Tca5-54	127	139	193	199	0	0	164	180	179	199	156	156	221	237	116	116	102	126	171	179
Tca5-56	139	147	199	199	0	0	134	164	195	215	160	160	233	253	120	136	118	162	137	137
Tca5-59	141	171	190	196	142	188	148	152	199	203	160	168	217	245	0	0	114	118	0	0
Tca5-61	133	139	193	193	142	164	152	160	187	215	160	160	201	201	124	136	114	124	133	157
Tca5-72	139	139	193	199	164	172	160	172	187	203	148	160	229	233	120	124	114	124	125	167
Tca5-74	133	141	196	199	164	164	130	148	187	187	148	160	217	237	124	132	114	118	137	157
Tca5-76	127	127	196	199	0	0	142	156	187	195	156	160	241	245	116	120	122	148	157	167
Tca5-78	163	163	199	199	164	196	130	192	183	203	156	156	217	225	120	128	114	148	129	133
Rock Creek Park																				
Tca8-01	139	167	193	199	0	0	148	160	203	203	148	160	201	253	124	124	114	122	153	167
Tca8-02	141	167	193	196	176	180	130	142	199	207	160	160	201	229	128	136	122	148	133	175
Tca8-03	133	149	196	199	150	150	152	152	187	199	156	160	205	229	124	124	114	122	133	145
Tca8-04	141	167	199	199	158	180	168	184	191	199	148	160	213	217	116	124	118	162	133	137
Tca8-05	141	145	193	199	158	188	152	156	183	195	160	160	213	261	116	124	114	122	0	0
Tca8-06	133	139	190	199	0	0	130	164	183	211	156	156	217	217	124	128	118	124	0	0
Tca8-07	131	133	199	199	142	184	168	184	167	199	160	160	201	217	124	124	114	114	125	129
Tca8-08	133	139	187	199	142	146	168	168	191	211	160	160	213	249	124	124	102	126	0	0
Tca8-09	141	141	193	199	154	168	134	148	187	203	160	164	201	213	124	124	114	124	137	145
Tca8-10	135	141	196	199	158	196	156	168	187	219	148	160	217	237	116	116	114	130	125	133
Tca8-11	131	133	196	199	158	176	138	168	187	195	156	160	213	249	120	120	114	124	133	163
Tca8-12	147	167	199	199	180	180	164	176	167	183	156	160	217	217	120	124	122	126	149	157
Tca8-13	149	159	193	199	146	168	130	156	183	187	156	160	217	241	120	128	122	130	157	163
Tca8-14	141	169	196	196	142	158	130	130	183	195	148	156	205	225	124	132	114	114	133	137
Tca8-15	139	161	199	199	146	164	152	156	187	207	156	160	201	225	120	124	102	114	121	167
Tca8-16	127	141	196	196	154	184	164	168	199	211	156	160	225	253	128	132	124	162	137	179
Tca8-17	135	143	199	199	158	180	134	168	187	187	160	164	225	245	120	124	124	126	167	175

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
	Casey																			
Tca9-01	0	0	190	199	0	0	0	0	207	239	160	160	225	237	120	132	0	0	129	137
Tca9-02	133	149	196	199	154	180	160	172	175	187	148	148	237	257	120	128	114	122	137	145
Tca9-03	141	167	196	199	168	200	152	176	211	211	156	160	205	225	128	140	114	114	167	171
Tca9-04	139	147	199	199	154	168	142	164	167	167	148	160	217	249	120	128	0	0	153	167
Tca9-05	141	141	193	199	164	180	134	148	167	215	160	160	221	257	124	166	114	122	141	145
Tca9-06	135	139	193	199	168	180	152	152	187	211	156	168	205	257	120	124	118	118	121	121
Tca9-07	127	167	199	199	142	172	156	156	187	203	156	156	225	241	120	124	114	122	133	171
Tca9-08	135	147	199	199	164	184	148	168	187	199	160	160	201	265	124	128	136	148	121	137
Tca9-09	147	167	193	196	150	200	148	156	183	207	148	160	221	233	128	136	122	122	153	163
Tca9-10	167	167	193	199	142	158	138	156	167	191	148	156	237	237	128	140	114	114	137	137
Tca9-11	133	145	196	196	130	158	160	168	167	187	148	160	213	237	116	150	122	124	133	171
Tca9-12	133	161	199	199	142	184	152	164	191	195	156	160	241	261	128	128	126	154	121	137
Tca9-13	141	147	193	199	158	180	118	142	187	215	156	160	221	233	128	128	122	124	121	137
Tca9-14	141	145	196	196	158	184	168	184	183	187	160	160	213	217	128	128	122	122	133	163
Tca9-15	139	159	199	199	154	172	130	164	187	207	160	160	213	221	124	132	114	136	133	171
Tca9-16	0	0	193	196	142	168	134	168	183	187	156	160	201	241	112	140	122	136	141	163
Tca9-17	147	157	199	199	126	192	164	164	199	203	156	160	201	237	112	120	114	114	149	153
Tca9-18	167	167	193	193	0	0	152	172	207	207	148	160	237	241	120	124	122	122	133	167
Tca9-19	133	141	196	202	164	164	130	148	199	211	156	160	185	201	120	124	114	114	125	171
Tca9-20	133	149	190	199	130	158	142	160	183	215	160	160	201	233	124	136	114	124	133	153
Tca9-21	141	149	196	199	180	200	152	160	183	187	156	164	221	237	128	128	122	130	125	125
Tca9-23	133	167	193	199	146	172	142	148	191	191	148	160	217	225	104	120	114	124	133	137
Tca9-24	147	167	199	199	164	180	130	152	191	207	148	156	217	229	136	150	114	122	121	137
Tca9-25	141	147	190	196	0	0	152	152	167	179	160	160	217	257	124	124	114	124	153	157
Tca9-26	133	141	196	199	154	168	148	172	183	207	148	156	229	241	128	140	114	154	137	153
Tca9-27	147	167	190	193	164	176	152	152	183	191	148	160	233	245	120	128	118	148	129	163
Tca9-28	133	167	187	199	168	168	148	148	183	191	148	156	185	233	124	140	114	124	137	171
Tca9-29	139	145	196	196	142	196	124	160	187	215	156	160	221	249	124	140	124	130	133	137
Tca9-30	133	167	187	199	168	168	160	160	167	207	160	160	201	217	124	132	114	118	121	137
Tca9-31	167	167	199	205	154	154	156	176	203	203	148	156	213	221	124	128	124	126	137	137

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca9-32	167	167	196	199	154	180	148	156	183	223	156	156	213	217	128	128	118	126	121	137
Tca9-33	139	149	196	196	142	142	124	148	191	203	156	160	213	233	120	140	122	124	133	137
Tca9-34	143	147	193	199	158	180	142	148	167	215	156	160	213	221	124	128	114	122	121	157
Tca9-35	147	167	193	199	154	158	148	156	191	215	148	160	213	245	132	132	114	136	0	0
Tca9-37	131	167	190	196	168	188	130	176	191	203	156	160	229	249	128	132	114	124	175	183
Tca9-38	133	133	193	196	142	188	124	156	191	195	156	160	221	253	132	144	102	136	133	137
Tca9-39	133	167	193	196	154	158	148	176	187	211	156	164	221	253	104	140	126	154	145	145
Tca9-40	141	171	187	196	164	168	130	168	187	187	156	164	233	237	120	136	122	136	129	157
Tca9-41	139	139	196	199	142	168	152	180	183	203	160	160	229	233	120	124	114	154	145	163
Egmont Key National Wildlife Refuge																				
Tca10-01	131	167	196	196	158	158	142	180	179	187	160	160	185	193	120	140	136	136	121	121
Tca10-02	133	141	193	199	184	192	138	148	183	183	148	148	229	229	120	140	126	136	121	121
Tca10-03	131	133	193	193	158	158	138	142	183	183	160	160	185	229	128	154	136	136	149	149
Tca10-04	131	167	193	193	158	188	152	164	183	203	148	148	201	225	120	144	110	126	121	121
Tca10-05	133	149	193	193	168	192	164	180	183	203	160	160	185	225	116	128	126	136	121	121
Tca10-06	131	159	193	193	158	192	138	156	179	203	156	156	229	229	120	144	110	136	133	149
Tca10-07	131	139	196	196	158	192	138	168	179	179	148	148	193	229	132	132	110	136	133	193
Tca10-08	131	131	193	199	160	204	142	156	183	187	160	160	185	225	128	154	136	136	121	149
Tca10-09	131	167	193	199	158	158	142	180	183	183	148	156	185	229	144	150	136	136	149	153
Tca10-11	139	167	193	193	158	204	138	180	183	183	148	156	193	229	120	144	126	136	121	121
Tca10-12	157	167	196	199	158	158	142	164	183	183	148	156	225	225	120	144	136	136	121	121
Tca10-13	131	157	193	196	158	184	142	168	183	183	160	160	225	225	132	150	136	150	121	153
Tca10-15	139	141	196	196	158	188	156	164	179	179	148	156	185	209	132	150	110	136	121	133
Tca10-16	131	131	193	193	158	158	138	142	179	203	148	156	185	229	120	128	136	136	121	121
Tca10-17	157	157	193	193	158	172	138	176	179	183	148	156	185	193	0	0	136	136	121	121
Tca10-18	131	157	193	193	158	188	138	156	183	183	156	156	225	229	132	150	136	136	121	121
Tca10-19	159	167	193	202	158	158	142	142	183	211	148	148	225	225	120	150	136	136	121	121
Tca10-20	157	167	0	0	0	0	138	138	0	0	160	160	225	225	0	0	0	0	121	121
Tca10-21	131	157	193	196	158	180	138	138	183	211	148	148	225	229	128	154	136	136	121	133
Tca10-22	131	157	193	199	172	192	148	176	183	203	148	148	225	225	120	140	136	136	121	121
Tca10-23	139	167	199	199	158	192	152	156	183	183	148	148	225	225	140	154	136	150	121	121

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca10-24	157	167	196	199	0	0	138	168	183	183	0	0	185	225	132	140	122	136	121	121
Tca10-25	131	167	193	199	0	0	138	148	179	183	148	148	225	229	116	116	0	0	0	0
Tca10-26	0	0	193	196	0	0	138	148	179	179	0	0	201	225	132	136	0	0	0	0
Tca10-27	131	157	193	199	176	184	138	172	183	187	148	148	225	225	140	154	110	136	121	121
Tca10-28	157	157	193	202	0	0	156	164	179	179	148	148	185	205	0	0	0	0	121	121
Tca10-29	133	141	190	199	158	196	142	156	179	183	160	160	225	225	0	0	136	136	121	121
Tca10-30	139	167	193	199	188	204	138	138	203	203	148	156	225	225	128	140	126	150	121	149
Tca10-31	157	157	193	199	158	180	138	142	179	203	0	0	225	225	120	140	136	150	121	149
Tca10-32	131	157	193	202	158	192	142	172	183	183	0	0	201	225	0	0	126	150	121	149
Tca10-33	0	0	193	193	0	0	138	156	179	183	148	156	225	225	0	0	122	150	121	133
Tca10-34	131	157	199	199	158	176	156	160	183	183	160	160	225	229	120	120	136	136	121	149
Tca10-35	131	167	193	199	158	172	138	142	179	207	148	148	225	225	132	144	136	136	121	121
Tca10-36	139	167	193	193	0	0	148	148	179	183	148	156	193	229	140	154	110	136	121	149
Tca10-37	139	167	193	199	158	176	152	152	179	183	0	0	205	225	0	0	0	0	121	153
Tca10-38	157	159	199	199	158	192	148	156	183	183	148	148	201	225	140	154	136	136	121	153
Tca10-39	131	131	193	199	176	188	138	164	203	203	156	156	201	225	116	128	136	136	121	121
Tca10-41	131	131	196	199	0	0	142	164	179	179	0	0	185	193	136	144	114	130	121	121
Tca10-42	131	167	199	199	0	0	148	148	183	207	148	148	201	229	132	136	136	136	121	121
Tca10-43	131	133	193	193	158	192	164	164	179	183	148	148	185	225	150	154	126	136	121	121
Tca10-44	159	167	193	199	0	0	138	168	183	203	148	148	193	225	120	132	136	150	121	121
Tca10-45	131	157	193	199	158	158	138	172	183	183	0	0	205	225	0	0	0	0	121	121
Tca10-46	155	163	193	193	176	196	142	142	183	183	160	160	225	229	0	0	136	136	121	121
Tca10-47	141	161	193	196	172	188	138	142	179	183	160	160	193	225	128	154	126	136	121	121
Tca10-48	131	157	196	199	0	0	0	0	183	211	160	160	225	225	144	154	136	136	121	121
Tca10-49	131	139	193	193	158	192	142	180	179	183	148	156	229	229	132	136	136	136	121	149
Tca10-50	167	167	193	202	188	192	138	180	179	187	148	148	185	185	128	150	110	136	121	121
Tca10-51	157	167	193	193	158	184	138	138	183	207	160	160	225	225	0	0	110	136	0	0
Tca10-53	131	167	193	199	158	158	148	148	183	183	148	160	185	205	150	154	126	136	121	121
Tca10-54	141	157	193	196	0	0	138	142	179	183	148	160	225	225	140	150	136	162	121	121
Tca10-56	133	167	0	0	0	0	142	148	179	183	148	156	201	229	116	120	136	136	121	121
Tca10-58	131	157	193	199	176	192	138	172	179	183	148	148	193	225	120	154	136	136	121	153

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca10-59	167	167	193	199	158	184	142	168	179	183	148	156	225	229	136	144	114	136	121	125
Tca10-61	131	131	193	193	0	0	148	172	179	183	148	160	193	229	140	144	136	136	121	121
Tca10-63	167	167	193	196	158	180	142	168	179	183	148	156	185	225	116	120	136	150	149	149
Tca10-64	133	167	193	193	158	196	138	164	179	203	160	160	193	225	128	150	136	136	121	133
Tca10-65	131	157	193	199	172	176	138	142	183	183	160	160	193	225	144	154	126	136	121	149
Tca10-66	131	131	199	199	0	0	164	164	179	207	148	160	185	229	144	154	136	136	121	121
Tca10-67	131	131	193	199	0	0	142	142	179	183	148	160	185	201	0	0	136	136	121	133
Tca10-68	141	157	193	193	158	168	138	152	179	183	160	160	205	225	150	154	110	150	121	121
Gettysburg National Military Park																				
Tca11-01	145	145	193	196	0	0	156	176	195	199	160	164	225	257	132	140	114	114	125	175
Tca11-02	137	141	187	196	184	188	142	160	203	215	160	160	213	229	132	132	102	122	125	145
Tca11-03	143	145	196	199	176	180	148	168	203	207	156	160	185	217	124	128	114	124	133	163
Tca11-04	133	167	193	199	164	164	124	148	187	199	156	160	221	229	124	128	118	154	153	167
Tca11-05	133	149	199	199	154	164	124	152	199	203	160	164	221	233	124	128	122	124	129	153
Tca11-06	147	175	190	193	176	188	152	164	167	199	148	160	217	237	128	136	114	122	133	137
Tca11-07	131	149	187	196	126	158	130	142	207	211	148	156	185	213	128	140	114	114	125	167
Tca11-09	131	167	187	202	168	168	138	160	187	211	156	164	201	233	124	128	114	134	133	163
Tca11-10	133	135	190	196	188	196	152	152	167	203	148	160	201	241	120	132	136	154	137	163
Tca11-11	133	147	190	196	168	184	130	130	199	203	160	164	205	237	132	132	118	124	129	137
Tca11-12	147	147	199	202	158	176	130	130	167	203	156	160	213	237	104	124	122	124	137	137
Tca11-13	139	167	193	199	184	204	142	156	167	199	148	156	237	245	124	124	102	122	0	0
Tca11-14	127	139	196	199	158	184	130	138	207	227	160	160	201	201	124	124	118	118	133	137
Tca11-15	141	147	199	199	164	188	152	152	187	219	148	156	237	245	128	132	114	124	133	133
Tca11-16	149	161	196	199	154	184	160	176	167	223	160	164	217	225	120	128	102	122	171	171
Tca11-17	143	143	190	190	168	176	148	172	199	211	156	160	185	225	124	128	114	126	133	175
Tca11-18	133	149	199	199	158	192	156	160	167	183	156	160	185	221	120	128	122	136	153	171
Tca11-19	133	133	196	199	158	168	168	168	187	195	156	160	233	241	128	128	124	124	133	145
Tca11-20	133	163	199	199	164	184	124	130	183	207	148	164	221	233	128	128	122	124	129	129
Tca11-22	141	145	199	199	164	168	148	152	183	211	156	160	237	241	116	128	118	134	137	145
Tca11-23	131	167	187	196	184	184	160	176	183	215	160	160	229	245	132	132	102	122	125	137
Tca11-24	141	145	196	199	180	188	126	168	195	211	148	164	225	245	116	120	114	140	141	145

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca11-25	131	137	196	196	150	184	142	176	195	215	160	184	185	229	132	132	102	114	137	145
Tca11-26	133	133	196	199	164	168	134	134	183	215	156	160	229	241	124	124	122	134	145	145
Tca11-27	149	149	196	199	158	184	176	176	167	195	148	160	221	237	104	120	122	124	129	133
Tca11-28	135	167	196	196	158	164	152	152	183	199	148	160	201	241	124	144	102	118	121	157
Tca11-30	139	163	199	199	0	0	160	164	183	187	156	156	225	241	124	132	118	122	145	163
Tca11-31	141	141	196	199	168	168	148	148	167	191	148	160	217	229	136	144	114	124	137	153
Tca11-32	127	133	193	199	142	150	156	168	179	203	156	160	201	217	124	150	122	154	163	167
Tca11-33	149	161	199	199	0	0	152	160	187	195	160	164	217	225	120	120	102	122	133	171
Tca11-34	145	147	193	199	184	188	152	152	191	211	148	156	201	225	124	128	118	124	121	125
Tca11-35	133	149	199	199	0	0	138	148	199	199	156	160	221	225	116	132	114	122	137	157
Tca11-36	143	147	187	199	142	180	118	168	199	203	160	164	213	253	128	136	114	118	137	137
	Isle of Wight Wildlife Mangement Area																			
Tca12-01	149	155	199	199	188	204	160	168	187	207	156	160	221	233	116	140	102	124	157	157
Tca12-02	143	167	193	196	176	180	118	168	191	207	156	160	201	233	124	128	114	118	145	157
Tca12-03	141	167	193	199	168	184	118	134	211	231	156	156	229	233	120	144	118	124	145	145
Tca12-04	131	131	196	196	0	0	138	160	183	203	156	160	185	229	120	128	114	148	137	145
Tca12-05	159	167	196	199	154	176	156	168	167	191	160	160	217	241	120	120	114	114	137	179
Tca12-06	137	161	193	196	158	192	134	152	187	199	160	160	185	233	124	136	114	118	125	137
Tca12-07	141	149	193	196	180	188	152	160	187	203	148	160	217	225	116	136	114	122	125	157
Tca12-08	141	147	190	199	0	0	160	160	211	211	148	148	213	217	124	140	114	124	137	167
Tca12-09	147	149	196	199	0	0	134	156	191	203	148	156	217	229	132	144	114	124	129	137
Tca12-10	127	141	196	199	0	0	130	160	179	203	156	164	201	233	120	132	114	126	125	191
Tca12-11	135	167	199	199	130	188	152	152	207	211	160	160	201	213	120	124	102	124	125	137
Tca12-12	131	139	190	196	168	180	148	148	191	195	156	160	233	233	132	132	118	122	125	133
Tca12-13	147	155	187	193	0	0	148	156	183	211	160	160	225	237	120	136	114	122	129	145
Tca12-14	131	149	193	196	0	0	152	156	203	227	156	160	185	241	120	128	114	122	129	137
Tca12-15	135	139	196	196	0	0	130	134	191	211	160	160	201	229	144	154	114	122	137	175
Tca12-16	127	147	193	196	138	150	160	160	191	207	160	160	217	237	120	120	114	124	137	153
Tca12-17	133	163	202	202	0	0	148	160	211	211	160	160	217	237	132	150	122	154	133	137
Tca12-18	141	147	187	196	172	180	118	118	207	207	160	160	217	237	120	124	102	102	133	133
Tca12-19	141	147	190	196	0	0	156	160	207	211	160	160	185	229	124	124	114	122	133	179

Appendix A. Continued

	Locus																			
Sample	*Gmu*A19		*Gmu*B08		*Gmu*D16		*Gmu*D40		*Gmu*D55		*Gmu*D79		*Gmu*D87		*Gmu*D88		*Gmu*D90		*Gmu*D121	
Tca12-20	141	167	193	199	0	0	118	134	167	211	156	156	217	229	120	144	118	124	145	145
Tca12-21	141	167	199	199	0	0	130	160	167	199	156	156	225	233	128	144	114	118	145	163
Tca12-22	133	149	196	202	0	0	152	180	191	191	148	148	225	229	120	132	124	148	145	163
Tca12-23	131	141	199	199	0	0	168	168	0	0	148	160	213	241	120	120	114	158	137	149
Tca12-24	141	147	202	202	130	176	118	152	191	207	156	160	217	217	120	140	114	148	163	167
Tca12-25	131	141	199	202	0	0	142	152	211	211	156	156	225	241	120	136	114	158	145	145
Tca12-26	135	149	196	199	0	0	118	118	203	211	156	156	217	221	124	124	114	148	163	163
Tca12-27	131	141	187	196	154	184	148	160	167	211	156	156	185	237	128	140	114	136	145	163
Tca12-28	133	163	196	199	164	192	134	180	187	199	156	160	185	217	128	136	114	122	125	133
Tca12-29	139	163	196	199	0	0	152	168	207	211	156	156	225	241	132	132	118	148	145	163
Tca12-30	133	143	193	199	0	0	130	152	195	211	156	160	225	241	120	132	124	124	125	179
Tca12-31	141	167	193	196	0	0	118	148	207	211	148	156	217	225	120	136	118	148	133	145
Tca12-32	133	167	187	202	0	0	118	164	187	207	148	156	225	241	124	140	114	148	163	163

Appendix B. Allele frequencies, sample size (*N*), observed heterozygosity by locus (*H*), mean heterozygosity, and mean number of alleles per locus at 10 microsatellite DNA loci in eastern box turtle populations in Maryland, Pennsylvania and Florida. Parenthetical following locus designation is the number of alleles identified at that locus. See Table 5.1 for population abbreviations.

	Study Site									
Allele	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
					***Gmu*A19 (21)**					
N	51	33	51	42	57	51	17	37	32	58
127	0.039	0.030	0.000	0.000	0.000	0.088	0.029	0.014	0.031	0.000
131	0.000	0.061	0.020	0.000	0.009	0.000	0.059	0.014	0.109	0.319
133	0.167	0.182	0.108	0.155	0.167	0.167	0.147	0.162	0.078	0.060
135	0.010	0.030	0.029	0.012	0.035	0.039	0.059	0.027	0.047	0.000
137	0.010	0.030	0.000	0.000	0.009	0.020	0.000	0.000	0.016	0.000
139	0.049	0.045	0.078	0.119	0.079	0.167	0.118	0.095	0.047	0.069
141	0.147	0.091	0.245	0.190	0.175	0.108	0.235	0.135	0.203	0.052
143	0.039	0.061	0.049	0.036	0.044	0.020	0.029	0.014	0.031	0.000
145	0.000	0.091	0.029	0.024	0.044	0.039	0.029	0.041	0.000	0.000
147	0.078	0.106	0.078	0.024	0.053	0.069	0.029	0.135	0.109	0.000
149	0.176	0.121	0.088	0.048	0.096	0.108	0.059	0.054	0.094	0.009
151	0.000	0.000	0.020	0.000	0.009	0.010	0.000	0.000	0.000	0.000
155	0.020	0.000	0.059	0.012	0.026	0.000	0.000	0.000	0.031	0.009
157	0.020	0.000	0.020	0.036	0.009	0.000	0.000	0.014	0.000	0.207
159	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.014	0.016	0.034
161	0.029	0.030	0.029	0.036	0.026	0.039	0.029	0.014	0.016	0.009
163	0.059	0.030	0.000	0.060	0.053	0.059	0.000	0.000	0.047	0.009
167	0.127	0.076	0.137	0.214	0.158	0.049	0.118	0.257	0.125	0.224
169	0.010	0.000	0.010	0.000	0.000	0.000	0.029	0.000	0.000	0.000
171	0.020	0.000	0.000	0.024	0.009	0.020	0.000	0.014	0.000	0.000
175	0.000	0.015	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
H	0.843	0.788	0.784	0.857	0.842	0.824	0.941	0.811	0.969	0.776
Allele					***Gmu*B08 (7)**					
N	49	33	51	43	58	51	17	39	32	58
187	0.051	0.076	0.010	0.035	0.017	0.029	0.029	0.038	0.063	0.000
190	0.061	0.076	0.118	0.035	0.043	0.049	0.029	0.064	0.047	0.009
193	0.153	0.091	0.127	0.256	0.241	0.157	0.147	0.179	0.156	0.534
196	0.255	0.273	0.235	0.233	0.241	0.294	0.235	0.282	0.328	0.138
199	0.459	0.455	0.490	0.407	0.388	0.431	0.559	0.410	0.297	0.284
202	0.010	0.030	0.020	0.023	0.060	0.029	0.000	0.013	0.109	0.034
205	0.010	0.000	0.000	0.012	0.009	0.010	0.000	0.013	0.000	0.000
H	0.673	0.667	0.647	0.698	0.741	0.686	0.588	0.692	0.750	0.569

Appendix B. Continued

	Study Site									
Allele	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
					***Gmu*D16 (23)**					
N	41	29	49	39	46	40	15	36	13	44
126	0.024	0.017	0.041	0.000	0.022	0.025	0.000	0.014	0.000	0.000
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.077	0.000
134	0.000	0.000	0.000	0.000	0.011	0.013	0.000	0.000	0.000	0.000
138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000
142	0.037	0.034	0.020	0.103	0.033	0.063	0.100	0.125	0.000	0.000
146	0.012	0.000	0.051	0.026	0.022	0.000	0.100	0.014	0.000	0.000
150	0.037	0.034	0.041	0.000	0.043	0.038	0.067	0.014	0.038	0.000
154	0.049	0.034	0.061	0.090	0.109	0.163	0.067	0.125	0.077	0.000
158	0.134	0.121	0.143	0.090	0.163	0.113	0.200	0.111	0.038	0.455
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
164	0.122	0.138	0.071	0.154	0.152	0.200	0.033	0.097	0.038	0.000
168	0.073	0.155	0.051	0.103	0.076	0.063	0.067	0.167	0.077	0.023
172	0.061	0.000	0.082	0.064	0.054	0.050	0.000	0.042	0.038	0.057
176	0.134	0.069	0.010	0.026	0.043	0.013	0.067	0.014	0.115	0.080
180	0.085	0.052	0.122	0.064	0.022	0.075	0.167	0.111	0.154	0.034
184	0.098	0.190	0.051	0.051	0.076	0.075	0.067	0.042	0.077	0.057
188	0.037	0.103	0.071	0.115	0.043	0.050	0.033	0.028	0.115	0.080
192	0.037	0.017	0.051	0.026	0.065	0.013	0.000	0.014	0.077	0.136
196	0.024	0.017	0.031	0.038	0.022	0.050	0.033	0.014	0.000	0.034
200	0.024	0.000	0.082	0.026	0.033	0.000	0.000	0.042	0.000	0.000
204	0.012	0.017	0.010	0.013	0.000	0.000	0.000	0.000	0.038	0.034
208	0.000	0.000	0.010	0.000	0.011	0.000	0.000	0.000	0.000	0.000
216	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
H	0.902	0.862	0.918	0.923	0.935	0.850	0.867	0.861	1.000	0.818
					***Gmu*D40 (19)**					
N	50	33	51	44	56	49	17	38	32	59
114	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000
118	0.040	0.015	0.020	0.023	0.018	0.041	0.000	0.013	0.156	0.000
124	0.010	0.045	0.010	0.034	0.000	0.010	0.000	0.039	0.000	0.000
126	0.020	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
130	0.070	0.106	0.157	0.205	0.063	0.041	0.147	0.066	0.063	0.000
134	0.050	0.030	0.039	0.091	0.054	0.071	0.059	0.026	0.094	0.000
138	0.010	0.045	0.000	0.011	0.027	0.010	0.029	0.013	0.016	0.280
142	0.040	0.061	0.059	0.057	0.054	0.061	0.029	0.066	0.016	0.212

Appendix B. Continued

	Study Site									
Allele	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
148	0.130	0.106	0.098	0.102	0.152	0.163	0.059	0.171	0.094	0.110
152	0.120	0.182	0.108	0.170	0.125	0.143	0.118	0.158	0.156	0.042
156	0.120	0.061	0.196	0.057	0.152	0.082	0.118	0.105	0.078	0.085
160	0.120	0.106	0.157	0.091	0.116	0.102	0.029	0.092	0.188	0.008
164	0.050	0.030	0.029	0.034	0.098	0.061	0.088	0.066	0.016	0.102
168	0.060	0.091	0.039	0.080	0.045	0.092	0.235	0.066	0.094	0.051
172	0.050	0.015	0.039	0.023	0.054	0.051	0.000	0.039	0.000	0.042
176	0.050	0.091	0.020	0.011	0.009	0.010	0.029	0.053	0.000	0.017
180	0.030	0.000	0.000	0.000	0.027	0.051	0.000	0.013	0.031	0.051
184	0.030	0.000	0.029	0.011	0.000	0.000	0.059	0.013	0.000	0.000
192	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
H	0.700	0.697	0.686	0.795	0.732	0.837	0.824	0.816	0.781	0.780
					***Gmu*D55 (19)**					
N	51	33	51	44	57	51	17	39	31	59
167	0.069	0.121	0.059	0.080	0.079	0.059	0.059	0.103	0.065	0.000
175	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.013	0.000	0.000
179	0.000	0.015	0.020	0.000	0.000	0.069	0.000	0.013	0.016	0.297
183	0.020	0.106	0.088	0.091	0.149	0.078	0.147	0.128	0.032	0.508
187	0.049	0.091	0.157	0.182	0.167	0.225	0.235	0.179	0.081	0.034
191	0.127	0.030	0.206	0.091	0.149	0.059	0.059	0.141	0.145	0.000
195	0.118	0.091	0.059	0.057	0.079	0.049	0.088	0.026	0.032	0.000
199	0.108	0.167	0.059	0.125	0.088	0.157	0.147	0.038	0.048	0.000
203	0.196	0.121	0.147	0.102	0.114	0.069	0.088	0.090	0.097	0.102
207	0.069	0.061	0.069	0.080	0.070	0.118	0.059	0.103	0.177	0.034
211	0.069	0.091	0.069	0.045	0.035	0.039	0.088	0.064	0.274	0.025
215	0.088	0.061	0.020	0.034	0.026	0.020	0.000	0.077	0.000	0.000
219	0.010	0.015	0.010	0.034	0.018	0.020	0.029	0.000	0.000	0.000
223	0.029	0.015	0.020	0.057	0.000	0.000	0.000	0.013	0.000	0.000
227	0.029	0.015	0.000	0.011	0.000	0.000	0.000	0.000	0.016	0.000
231	0.010	0.000	0.010	0.000	0.018	0.020	0.000	0.000	0.016	0.000
235	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
239	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.013	0.000	0.000
243	0.010	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000
H	0.902	0.970	0.902	0.909	0.947	0.922	0.882	0.846	0.839	0.610

Appendix B. Continued

	Study Site									
Allele	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
					***Gmu*D79 (8)**					
N	51	33	51	44	57	50	17	39	32	53
148	0.216	0.167	0.147	0.159	0.061	0.110	0.118	0.179	0.141	0.491
156	0.324	0.258	0.265	0.375	0.281	0.240	0.265	0.308	0.406	0.179
160	0.382	0.424	0.471	0.409	0.561	0.560	0.559	0.462	0.438	0.330
164	0.059	0.136	0.118	0.000	0.053	0.050	0.059	0.038	0.016	0.000
168	0.000	0.000	0.000	0.045	0.026	0.040	0.000	0.013	0.000	0.000
184	0.010	0.015	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
188	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
192	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
H	0.588	0.879	0.608	0.591	0.561	0.540	0.706	0.692	0.438	0.340
					***Gmu*D87 (21)**					
N	51	33	51	44	58	50	17	39	32	60
177	0.039	0.000	0.020	0.011	0.000	0.000	0.000	0.000	0.000	0.000
185	0.010	0.076	0.020	0.034	0.034	0.040	0.000	0.026	0.094	0.150
193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
201	0.127	0.106	0.157	0.216	0.112	0.080	0.147	0.077	0.063	0.067
205	0.020	0.015	0.020	0.000	0.017	0.000	0.059	0.026	0.000	0.042
209	0.010	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.008
213	0.078	0.061	0.118	0.057	0.103	0.040	0.147	0.103	0.047	0.000
217	0.108	0.091	0.078	0.125	0.181	0.120	0.235	0.090	0.203	0.000
221	0.059	0.091	0.059	0.045	0.060	0.090	0.000	0.128	0.031	0.000
225	0.069	0.121	0.108	0.091	0.095	0.140	0.118	0.051	0.141	0.450
229	0.137	0.091	0.108	0.045	0.052	0.070	0.059	0.051	0.109	0.183
233	0.039	0.061	0.069	0.068	0.103	0.100	0.000	0.103	0.125	0.000
237	0.039	0.106	0.059	0.091	0.078	0.080	0.029	0.115	0.078	0.000
241	0.108	0.091	0.069	0.080	0.052	0.060	0.029	0.064	0.109	0.000
245	0.000	0.061	0.020	0.057	0.034	0.060	0.029	0.026	0.000	0.000
249	0.059	0.000	0.029	0.045	0.026	0.030	0.059	0.038	0.000	0.000
253	0.039	0.015	0.039	0.000	0.017	0.050	0.059	0.026	0.000	0.000
257	0.049	0.015	0.010	0.011	0.017	0.020	0.000	0.051	0.000	0.000
261	0.010	0.000	0.010	0.011	0.009	0.010	0.029	0.013	0.000	0.000
265	0.000	0.000	0.010	0.000	0.000	0.010	0.000	0.013	0.000	0.000
273	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000
H	0.902	0.970	0.843	0.886	0.879	0.940	0.882	0.974	0.938	0.683

Appendix B. Continued

	Study Site									
Allele	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
					***Gmu*D88 (15)**					
N	51	33	51	42	57	49	17	39	32	49
104	0.010	0.030	0.020	0.012	0.009	0.000	0.000	0.026	0.000	0.000
112	0.000	0.000	0.010	0.024	0.009	0.031	0.000	0.026	0.000	0.000
116	0.010	0.045	0.039	0.012	0.079	0.051	0.118	0.013	0.031	0.061
120	0.108	0.106	0.147	0.155	0.088	0.153	0.176	0.167	0.297	0.163
124	0.294	0.242	0.333	0.250	0.228	0.204	0.500	0.205	0.156	0.000
128	0.127	0.258	0.137	0.190	0.184	0.204	0.118	0.269	0.094	0.102
132	0.196	0.197	0.186	0.119	0.123	0.184	0.059	0.090	0.141	0.112
136	0.088	0.045	0.069	0.143	0.096	0.122	0.029	0.051	0.094	0.051
140	0.039	0.030	0.010	0.012	0.105	0.020	0.000	0.103	0.078	0.122
144	0.078	0.030	0.020	0.012	0.026	0.010	0.000	0.013	0.078	0.122
150	0.020	0.015	0.020	0.060	0.026	0.020	0.000	0.026	0.016	0.112
154	0.020	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.016	0.153
158	0.010	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.010	0.000	0.009	0.000	0.000	0.000	0.000	0.000
166	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
H	0.765	0.697	0.863	0.714	0.754	0.755	0.588	0.821	0.781	0.939
					***Gmu*D90 (17)**					
N	51	33	51	44	56	50	17	37	32	54
102	0.029	0.106	0.069	0.057	0.036	0.040	0.059	0.014	0.063	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083
114	0.275	0.212	0.255	0.295	0.223	0.290	0.324	0.311	0.344	0.019
118	0.049	0.136	0.039	0.148	0.080	0.100	0.059	0.068	0.125	0.000
122	0.176	0.227	0.216	0.182	0.286	0.270	0.176	0.230	0.125	0.019
124	0.196	0.167	0.186	0.125	0.143	0.120	0.147	0.135	0.156	0.000
126	0.078	0.015	0.088	0.034	0.063	0.080	0.088	0.054	0.016	0.093
130	0.039	0.000	0.020	0.023	0.045	0.000	0.059	0.027	0.000	0.009
134	0.000	0.045	0.010	0.034	0.009	0.000	0.000	0.000	0.000	0.000
136	0.059	0.030	0.039	0.023	0.036	0.060	0.000	0.081	0.016	0.685
140	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
148	0.010	0.000	0.049	0.000	0.009	0.020	0.029	0.027	0.109	0.000
150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083
154	0.039	0.045	0.020	0.068	0.054	0.000	0.000	0.054	0.016	0.000
158	0.000	0.000	0.000	0.011	0.009	0.000	0.000	0.000	0.031	0.000

Appendix B. Continued

	Study Site									
Allele	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK
162	0.029	0.000	0.010	0.000	0.009	0.020	0.059	0.000	0.000	0.009
166	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.765	0.879	0.804	0.773	0.786	0.820	0.882	0.784	0.906	0.519
					***Gmu*D121 (19)**					
N	51	32	51	43	56	48	14	38	32	57
121	0.088	0.031	0.059	0.116	0.152	0.115	0.036	0.118	0.000	0.754
125	0.039	0.078	0.078	0.081	0.054	0.073	0.071	0.039	0.109	0.009
129	0.098	0.078	0.118	0.035	0.080	0.094	0.036	0.039	0.047	0.000
133	0.137	0.156	0.147	0.151	0.089	0.156	0.214	0.132	0.109	0.061
137	0.176	0.203	0.196	0.163	0.196	0.208	0.143	0.237	0.172	0.000
141	0.000	0.016	0.020	0.035	0.018	0.042	0.000	0.026	0.000	0.000
145	0.049	0.125	0.098	0.070	0.080	0.052	0.071	0.066	0.219	0.000
149	0.020	0.000	0.000	0.047	0.009	0.000	0.036	0.013	0.016	0.123
153	0.039	0.063	0.049	0.047	0.071	0.042	0.036	0.079	0.016	0.044
157	0.088	0.031	0.127	0.047	0.018	0.063	0.071	0.039	0.063	0.000
163	0.127	0.078	0.020	0.058	0.027	0.021	0.071	0.066	0.141	0.000
167	0.029	0.047	0.059	0.047	0.054	0.052	0.107	0.039	0.031	0.000
171	0.059	0.063	0.010	0.081	0.125	0.021	0.000	0.079	0.000	0.000
175	0.039	0.031	0.020	0.000	0.018	0.021	0.071	0.013	0.016	0.000
179	0.000	0.000	0.000	0.000	0.009	0.031	0.036	0.000	0.047	0.000
183	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.013	0.000	0.000
187	0.010	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000
193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009
H	0.902	0.813	0.863	0.907	0.786	0.896	1.000	0.868	0.781	0.368
					Means and SEs					
H	0.794	0.822	0.792	0.805	0.796	0.807	0.816	0.817	0.818	0.640
S.E.	0.036	0.035	0.034	0.035	0.036	0.038	0.045	0.026	0.051	0.062
Alleles	13.300	11.600	12.700	12.500	13.400	12.200	9.700	12.800	10.600	7.700
SE	1.283	1.166	1.453	1.204	1.284	1.289	1.126	1.332	1.087	0.883

Appendix C. Estimation of *F*-statistics following Wier and Cockerman (1984).

Locus	Allele	F_{IT}	F_{ST}	F_{IS}
*Gmu*A19	**127**	0.089	0.03	0.061
	131	0.266	0.198	0.085
	133	0.078	0.004	0.074
	135	-0.025	0	-0.025
	137	-0.007	0.001	-0.008
	139	0.085	0.007	0.078
	141	0.06	0.019	0.042
	143	0.274	-0.004	0.277
	145	0.145	0.013	0.134
	147	-0.029	0.017	-0.047
	149	0.086	0.019	0.068
	151	0.498	-0.007	0.502
	155	0.12	0.009	0.112
	157	0.162	0.125	0.041
	159	-0.006	0.011	-0.017
	161	0.067	-0.008	0.075
	163	0.11	0.009	0.102
	167	0.024	0.023	0.002
	169	-0.002	0.003	-0.005
	171	-0.008	-0.001	-0.007
	175	-0.001	0.001	-0.002
	All	0.085	0.029	0.057
*Gmu*B08	**187**	-0.03	0.007	-0.037
	190	0.083	0.007	0.077
	193	0.116	0.101	0.017
	196	0.063	0.003	0.06
	199	0.033	0.013	0.02
	202	0.181	0.011	0.172
	205	-0.005	-0.006	0.001
	All	0.068	0.031	0.038
*Gmu*D16	**126**	-0.016	-0.002	-0.014
	130	0	0.039	-0.041
	134	-0.002	-0.005	0.003
	138	0.003	0.028	-0.026
	142	0.012	0.024	-0.013

Appendix C. Continued

Locus	Allele	F_{IT}	F_{ST}	F_{IS}
	146	0.128	0.017	0.114
	150	0.075	-0.001	0.076
	154	-0.041	0.022	-0.064
	158	0.054	0.089	-0.038
	160	0	-0.003	0.003
	164	0.06	0.028	0.034
	168	0.138	0.012	0.128
	172	0.006	-0.003	0.009
	176	0.009	0.026	-0.017
	180	0.036	0.012	0.025
	184	0.084	0.008	0.077
	188	-0.068	0.003	-0.071
	192	-0.045	0.026	-0.073
	196	0.177	-0.012	0.186
	200	-0.023	0.019	-0.043
	204	-0.01	0	-0.01
	208	-0.002	-0.006	0.004
	216	0	-0.002	0.001
	All	0.036	0.025	0.011
*Gmu*D40	**114**	0	-0.003	0.003
	118	0.133	0.043	0.094
	124	-0.012	0.009	-0.022
	126	-0.002	0.005	-0.007
	130	0.27	0.038	0.241
	134	0.25	0.005	0.247
	138	0.15	0.176	-0.032
	142	0.131	0.041	0.094
	148	0.118	-0.004	0.122
	152	0.276	0.002	0.275
	156	0.218	0.007	0.212
	160	0.197	0.016	0.184
	164	0.188	0.004	0.186
	168	0.064	0.012	0.052
	172	0.03	-0.003	0.033

Appendix C. Continued

Locus	Allele	F_{IT}	F_{ST}	F_{IS}
	176	0.153	0.013	0.142
	180	0.08	0.007	0.074
	184	0.192	0.009	0.185
	192	0	-0.002	0.001
	All	0.174	0.021	0.155
*Gmu*D55	**167**	-0.031	0.007	-0.038
	175	-0.002	0.004	-0.006
	179	0.186	0.198	-0.015
	183	0.174	0.183	-0.011
	187	-0.05	0.034	-0.087
	191	0.066	0.037	0.03
	195	-0.059	0.014	-0.074
	199	0.023	0.028	-0.005
	203	0.052	0.002	0.05
	207	0.038	0.007	0.031
	211	0.075	0.052	0.025
	215	0.04	0.02	0.021
	219	-0.012	-0.001	-0.011
	223	0.157	0.013	0.146
	227	-0.005	0.005	-0.011
	231	-0.007	-0.003	-0.004
	235	0	-0.002	0.002
	239	-0.001	-0.001	-0.001
	243	-0.002	-0.004	0.002
	All	0.05	0.055	-0.005
*Gmu*D79	**148**	0.229	0.097	0.145
	156	0.107	0.009	0.099
	160	0.157	0.014	0.145
	164	0.093	0.029	0.066
	168	-0.012	0.014	-0.026
	184	-0.003	-0.002	0
	188	0	-0.002	0.002
	192	-0.001	0.005	-0.006
	All	0.148	0.032	0.12

Appendix C. Continued

Locus	Allele	F_{IT}	F_{ST}	F_{IS}
*Gmu*D87	**177**	-0.006	0.013	-0.019
	185	-0.003	0.037	-0.041
	193	-0.003	0.087	-0.1
	201	0.079	0.011	0.069
	205	-0.017	0.004	-0.021
	209	-0.003	-0.004	0.002
	213	0.033	0.016	0.018
	217	0.088	0.031	0.058
	221	-0.058	0.016	-0.076
	225	0.144	0.121	0.026
	229	0.084	0.015	0.07
	233	0.039	0.016	0.024
	237	0.045	0.01	0.035
	241	-0.028	0.009	-0.038
	245	-0.026	0.012	-0.038
	249	-0.027	0.006	-0.033
	253	-0.022	0.007	-0.029
	257	-0.017	0.008	-0.025
	261	-0.008	-0.006	-0.002
	265	-0.003	-0.003	0.001
	273	0	-0.003	0.003
	All	0.046	0.032	0.015
*Gmu*D88	**104**	-0.01	-0.001	-0.009
	112	-0.009	0.002	-0.012
	116	0.247	0.006	0.242
	120	0.034	0.011	0.024
	124	0.217	0.06	0.167
	128	0.155	0.012	0.145
	132	0.078	0.003	0.075
	136	-0.027	0.004	-0.031
	140	-0.01	0.029	-0.04
	144	0.02	0.03	-0.01
	150	-0.032	0.021	-0.055
	154	-0.013	0.099	-0.124

Appendix C. Continued

Locus	Allele	F_{IT}	F_{ST}	F_{IS}
	158	-0.001	-0.003	0.001
	162	-0.002	-0.004	0.003
	166	0	0.001	-0.001
	All	0.094	0.023	0.072
*Gmu*D90	**102**	0.07	0.009	0.061
	110	-0.002	0.072	-0.079
	114	0.044	0.041	0.003
	118	0.087	0.02	0.068
	122	0.041	0.034	0.007
	124	0.071	0.019	0.053
	126	0.092	0.001	0.091
	130	0.093	0.004	0.09
	134	-0.007	0.014	-0.021
	136	0.473	0.449	0.044
	140	0	0.004	-0.003
	148	-0.018	0.033	-0.053
	150	-0.002	0.072	-0.079
	154	-0.029	0.011	-0.041
	158	-0.003	0.006	-0.009
	162	-0.01	0.006	-0.016
	166	0	0.008	-0.008
	All	0.106	0.082	0.026
*Gmu*D121	**121**	0.452	0.383	0.111
	125	0.066	0.003	0.063
	129	0.127	0.012	0.116
	133	-0.037	0.002	-0.039
	137	0.079	0.026	0.054
	141	0.13	0.001	0.129
	145	0.159	0.03	0.132
	149	0.148	0.051	0.102
	153	-0.052	-0.005	-0.047
	157	-0.006	0.021	-0.027
	163	0.085	0.032	0.054
	167	-0.042	0.002	-0.044

Appendix C. Continued

Locus	Allele	F_{IT}	F_{ST}	F_{IS}
	171	0.164	0.032	0.136
	175	-0.018	0.003	-0.021
	179	-0.007	0.017	-0.024
	183	-0.002	0.007	-0.008
	187	-0.002	-0.003	0.002
	191	0	0.004	-0.004
	193	0	-0.004	0.003
	All	0.124	0.079	0.049
Over all loci		0.092	0.041	0.053

Appendix D. Conformance to Hardy-Weinberg equilibrium, by locus within population. The *P*-value of loci out of conformance after sequential Bonferroni correction appears in bold type. See Table 5.1 for population abbreviations.

Population	Locus	*P*-value	S.E.
PWRC			
	*Gmu*A19	0.126	0.013
	*Gmu*B08	0.445	0.0097
	*Gmu*D16	0.2681	0.0182
	*Gmu*D40	**0**	0
	*Gmu*D55	0.6297	0.0153
	*Gmu*D79	0.007	0.001
	*Gmu*D87	0.5062	0.0211
	*Gmu*D88	0.2129	0.0125
	*Gmu*D90	0.0197	0.003
	*Gmu*D121	0.3424	0.013
GNMP			
	*Gmu*A19	0.0387	0.0062
	*Gmu*B08	0.2325	0.005
	*Gmu*D16	0.3771	0.0175
	*Gmu*D40	**0.0002**	0.0002
	*Gmu*D55	0.9571	0.0048
	*Gmu*D79	0.993	0.0006
	*Gmu*D87	0.8796	0.0095
	*Gmu*D88	0.0845	0.0059
	*Gmu*D90	0.6175	0.0104
	*Gmu*D121	0.0651	0.0068
JBWS			
	*Gmu*A19	**0**	0
	*Gmu*B08	0.1887	0.0052
	*Gmu*D16	0.0596	0.0099
	*Gmu*D40	**0.0005**	0.0004
	*Gmu*D55	0.746	0.0148
	*Gmu*D79	0.0465	-
	*Gmu*D87	0.0327	0.0058
	*Gmu*D88	0.8534	0.013
	*Gmu*D90	0.3121	0.0157
	*Gmu*D121	0.0297	0.0043

Appendix D. Continued

Population	Locus	*P*-value	S.E.
BHP			
	*Gmu*A19	0.0413	0.0065
	*Gmu*B08	0.3588	0.0102
	*Gmu*D16	0.1528	0.0142
	*Gmu*D40	0.0132	0.0032
	*Gmu*D55	0.3517	0.0167
	*Gmu*D79	0.1388	0.0041
	*Gmu*D87	0.5913	0.0198
	*Gmu*D88	0.0553	0.0077
	*Gmu*D90	0.1548	0.0099
	*Gmu*D121	0.654	0.0141
MB			
	*Gmu*A19	0.0988	0.0117
	*Gmu*B08	0.5651	0.0103
	*Gmu*D16	0.8021	0.0178
	*Gmu*D40	0.0035	0.0013
	*Gmu*D55	0.9153	0.0074
	*Gmu*D79	0.4123	0.0084
	*Gmu*D87	0.2821	0.0165
	*Gmu*D88	0.0006	0.0003
	*Gmu*D90	0.1169	0.0096
	*Gmu*D121	0.0184	0.0036
WRP			
	*Gmu*A19	0.0513	0.0067
	*Gmu*B08	0.0286	0.0019
	*Gmu*D16	0.127	0.0097
	*Gmu*D40	0.044	0.0074
	*Gmu*D55	0.745	0.0144
	*Gmu*D79	0.3105	0.0057
	*Gmu*D87	0.5706	0.016
	*Gmu*D88	0.058	0.0044
	*Gmu*D90	0.3485	0.0106
	*Gmu*D121	0.5682	0.0173

Appendix D. Continued

Population	Locus	*P*-value	S.E.
RCP			
	*Gmu*A19	0.8215	0.0154
	*Gmu*B08	0.3387	0.0053
	*Gmu*D16	0.1281	0.0098
	*Gmu*D40	0.1968	0.0119
	*Gmu*D55	0.3258	0.0102
	*Gmu*D79	0.7946	-
	*Gmu*D87	0.5892	0.0145
	*Gmu*D88	0.1213	0.0036
	*Gmu*D90	0.8149	0.0076
	*Gmu*D121	1	0
Casey			
	*Gmu*A19	0.2063	0.0178
	*Gmu*B08	0.4177	0.0099
	*Gmu*D16	0.2391	0.0207
	*Gmu*D40	0.0509	0.0068
	*Gmu*D55	0.0833	0.0078
	*Gmu*D79	0.6999	0.0058
	*Gmu*D87	0.8909	0.0111
	*Gmu*D88	0.3823	0.0179
	*Gmu*D90	0.2628	0.0099
	*Gmu*D121	0.0865	0.0086
IW			
	*Gmu*A19	0.8816	0.0106
	*Gmu*B08	0.2187	0.0046
	*Gmu*D16	1	0
	*Gmu*D40	0.0538	0.0057
	*Gmu*D55	0.4814	0.0152
	*Gmu*D79	0.0085	-
	*Gmu*D87	0.8353	0.0062
	*Gmu*D88	0.2215	0.0097
	*Gmu*D90	0.5094	0.0128
	*Gmu*D121	0.0597	0.0072

Appendix D. Continued

Population	Locus	*P*-value	S.E.
EK			
	*Gmu*A19	0.5095	0.0169
	*Gmu*B08	0.108	0.0038
	*Gmu*D16	0.9573	0.0053
	*Gmu*D40	0.035	0.0042
	*Gmu*D55	0.1912	0.0057
	*Gmu*D79	**0**	-
	*Gmu*D87	0.4923	0.0083
	*Gmu*D88	0.73	0.0063
	*Gmu*D90	0.7826	0.0087
	*Gmu*D121	0.243	0.007

Appendix E. Genotypic disequilibrium, by population and locus pair. All loci are products of cross-species amplification of the developed *Glyptemys muhlenbergii* (*Gmu*) microsatellite markers. Asterisks indicate that scores were missing at one or both loci. *P*-values in bold type indicate that the locus pair was significantly linked. See Table 5.1 for population abbreviations.

Population	Locus#1	Locus#2	*P*-value	S.E.
PWRC	A19	B08	0.973	0.009
PWRC	A19	D16	0.110	0.027
PWRC	B08	D16	0.601	0.037
PWRC	A19	D40	0.203	0.038
PWRC	B08	D40	0.075	0.021
PWRC	D16	D40	0.152	0.034
PWRC	A19	D55	1.000	0.000
PWRC	B08	D55	0.934	0.017
PWRC	D16	D55	0.168	0.036
PWRC	D40	D55	0.120	0.032
PWRC	A19	D79	0.655	0.026
PWRC	B08	D79	0.971	0.005
PWRC	D16	D79	0.628	0.031
PWRC	D40	D79	0.764	0.030
PWRC	D55	D79	0.787	0.026
PWRC	A19	D87	0.028	0.015
PWRC	B08	D87	0.721	0.033
PWRC	D16	D87	**0.000**	0.000
PWRC	D40	D87	0.225	0.040
PWRC	D55	D87	0.132	0.032
PWRC	D79	D87	0.693	0.029
PWRC	A19	D88	0.384	0.042
PWRC	B08	D88	0.635	0.028
PWRC	D16	D88	1.000	0.000
PWRC	D40	D88	1.000	0.000
PWRC	D55	D88	0.586	0.043
PWRC	D79	D88	0.480	0.024
PWRC	D87	D88	0.597	0.044
PWRC	A19	D90	1.000	0.000
PWRC	B08	D90	0.864	0.018
PWRC	D16	D90	1.000	0.000
PWRC	D40	D90	1.000	0.000
PWRC	D55	D90	1.000	0.000
PWRC	D79	D90	0.500	0.028
PWRC	D87	D90	1.000	0.000
PWRC	D88	D90	0.848	0.028
PWRC	A19	D121	1.000	0.000
PWRC	B08	D121	0.306	0.030
PWRC	D16	D121	0.263	0.041
PWRC	D40	D121	0.193	0.038
PWRC	D55	D121	0.007	0.005
PWRC	D79	D121	0.059	0.012

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
PWRC	D87	D121	0.238	0.041
PWRC	D88	D121	1.000	0.000
PWRC	D90	D121	1.000	0.000
GNMP	A19	B08	0.001	0.001
GNMP	A19	D16	1.000	0.000
GNMP	B08	D16	0.402	0.030
GNMP	A19	D40	1.000	0.000
GNMP	B08	D40	1.000	0.000
GNMP	D16	D40	1.000	0.000
GNMP	A19	D55	1.000	0.000
GNMP	B08	D55	1.000	0.000
GNMP	D16	D55	1.000	0.000
GNMP	D40	D55	1.000	0.000
GNMP	A19	D79	0.062	0.010
GNMP	B08	D79	0.276	0.014
GNMP	D16	D79	0.521	0.023
GNMP	D40	D79	0.263	0.025
GNMP	D55	D79	0.077	0.016
GNMP	A19	D87	0.060	0.022
GNMP	B08	D87	0.390	0.029
GNMP	D16	D87	1.000	0.000
GNMP	D40	D87	0.044	0.020
GNMP	D55	D87	1.000	0.000
GNMP	D79	D87	0.007	0.004
GNMP	A19	D88	1.000	0.000
GNMP	B08	D88	0.633	0.026
GNMP	D16	D88	1.000	0.000
GNMP	D40	D88	1.000	0.000
GNMP	D55	D88	1.000	0.000
GNMP	D79	D88	0.597	0.022
GNMP	D87	D88	1.000	0.000
GNMP	A19	D90	0.237	0.037
GNMP	B08	D90	0.488	0.028
GNMP	D16	D90	1.000	0.000
GNMP	D40	D90	0.256	0.038
GNMP	D55	D90	1.000	0.000
GNMP	D79	D90	0.898	0.011
GNMP	D87	D90	0.006	0.005
GNMP	D88	D90	0.735	0.032
GNMP	A19	D121	1.000	0.000
GNMP	B08	D121	1.000	0.000
GNMP	D16	D121	1.000	0.000
GNMP	D40	D121	1.000	0.000
GNMP	D55	D121	1.000	0.000
GNMP	D79	D121	1.000	0.000

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
GNMP	D87	D121	1.000	0.000
GNMP	D88	D121	0.224	0.036
GNMP	D90	D121	1.000	0.000
JBWS	A19	B08	0.519	0.033
JBWS	A19	D16	1.000	0.000
JBWS	B08	D16	0.745	0.028
JBWS	A19	D40	1.000	0.000
JBWS	B08	D40	0.598	0.030
JBWS	D16	D40	1.000	0.000
JBWS	A19	D55	0.322	0.045
JBWS	B08	D55	0.825	0.023
JBWS	D16	D55	0.201	0.039
JBWS	D40	D55	1.000	0.000
JBWS	A19	D79	0.844	0.018
JBWS	B08	D79	0.403	0.014
JBWS	D16	D79	0.396	0.029
JBWS	D40	D79	0.852	0.016
JBWS	D55	D79	0.230	0.021
JBWS	A19	D87	1.000	0.000
JBWS	B08	D87	0.651	0.029
JBWS	D16	D87	1.000	0.000
JBWS	D40	D87	0.354	0.047
JBWS	D55	D87	0.344	0.044
JBWS	D79	D87	0.693	0.027
JBWS	A19	D88	0.618	0.043
JBWS	B08	D88	0.942	0.012
JBWS	D16	D88	1.000	0.000
JBWS	D40	D88	**0.000**	0.000
JBWS	D55	D88	0.111	0.026
JBWS	D79	D88	0.347	0.020
JBWS	D87	D88	0.347	0.040
JBWS	A19	D90	1.000	0.000
JBWS	B08	D90	0.522	0.028
JBWS	D16	D90	0.199	0.038
JBWS	D40	D90	0.638	0.043
JBWS	D55	D90	0.296	0.041
JBWS	D79	D90	0.984	0.004
JBWS	D87	D90	0.112	0.030
JBWS	D88	D90	0.198	0.031
JBWS	A19	D121	1.000	0.000
JBWS	B08	D121	0.849	0.020
JBWS	D16	D121	1.000	0.000
JBWS	D40	D121	1.000	0.000
JBWS	D55	D121	0.589	0.045
JBWS	D79	D121	0.082	0.013

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
JBWS	D87	D121	1.000	0.000
JBWS	D88	D121	0.784	0.035
JBWS	D90	D121	1.000	0.000
BHP	A19	B08	0.601	0.031
BHP	A19	D16	1.000	0.000
BHP	B08	D16	0.541	0.036
BHP	A19	D40	1.000	0.000
BHP	B08	D40	0.716	0.030
BHP	D16	D40	1.000	0.000
BHP	A19	D55	1.000	0.000
BHP	B08	D55	0.515	0.035
BHP	D16	D55	0.183	0.035
BHP	D40	D55	1.000	0.000
BHP	A19	D79	0.824	0.017
BHP	B08	D79	0.988	0.002
BHP	D16	D79	0.725	0.024
BHP	D40	D79	0.326	0.026
BHP	D55	D79	0.686	0.024
BHP	A19	D87	1.000	0.000
BHP	B08	D87	0.777	0.025
BHP	D16	D87	1.000	0.000
BHP	D40	D87	1.000	0.000
BHP	D55	D87	1.000	0.000
BHP	D79	D87	1.000	0.000
BHP	A19	D88	0.664	0.039
BHP	B08	D88	0.428	0.027
BHP	D16	D88	1.000	0.000
BHP	D40	D88	0.035	0.015
BHP	D55	D88	1.000	0.000
BHP	D79	D88	0.380	0.020
BHP	D87	D88	1.000	0.000
BHP	A19	D90	0.511	0.045
BHP	B08	D90	0.713	0.025
BHP	D16	D90	1.000	0.000
BHP	D40	D90	1.000	0.000
BHP	D55	D90	0.037	0.017
BHP	D79	D90	0.574	0.023
BHP	D87	D90	1.000	0.000
BHP	D88	D90	0.741	0.032
BHP	A19	D121	1.000	0.000
BHP	B08	D121	0.713	0.026
BHP	D16	D121	1.000	0.000
BHP	D40	D121	1.000	0.000
BHP	D55	D121	1.000	0.000
BHP	D79	D121	0.400	0.031

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
BHP	D87	D121	1.000	0.000
BHP	D88	D121	0.014	0.008
BHP	D90	D121	0.016	0.011
MB	A19	B08	0.460	0.032
MB	A19	D16	0.378	0.046
MB	B08	D16	0.753	0.027
MB	A19	D40	0.034	0.017
MB	B08	D40	0.424	0.027
MB	D16	D40	1.000	0.000
MB	A19	D55	0.038	0.019
MB	B08	D55	0.807	0.021
MB	D16	D55	1.000	0.000
MB	D40	D55	0.542	0.047
MB	A19	D79	0.765	0.027
MB	B08	D79	0.774	0.012
MB	D16	D79	0.936	0.012
MB	D40	D79	0.755	0.027
MB	D55	D79	0.910	0.013
MB	A19	D87	1.000	0.000
MB	B08	D87	0.910	0.017
MB	D16	D87	1.000	0.000
MB	D40	D87	1.000	0.000
MB	D55	D87	1.000	0.000
MB	D79	D87	0.119	0.018
MB	A19	D88	1.000	0.000
MB	B08	D88	0.234	0.021
MB	D16	D88	1.000	0.000
MB	D40	D88	0.531	0.044
MB	D55	D88	0.627	0.044
MB	D79	D88	0.131	0.015
MB	D87	D88	0.133	0.030
MB	A19	D90	0.558	0.046
MB	B08	D90	0.638	0.028
MB	D16	D90	0.289	0.040
MB	D40	D90	0.305	0.041
MB	D55	D90	0.684	0.041
MB	D79	D90	0.394	0.028
MB	D87	D90	0.295	0.044
MB	D88	D90	0.843	0.029
MB	A19	D121	0.525	0.047
MB	B08	D121	0.259	0.025
MB	D16	D121	0.280	0.042
MB	D40	D121	1.000	0.000
MB	D55	D121	0.468	0.046
MB	D79	D121	0.179	0.023

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
MB	D87	D121	0.146	0.034
MB	D88	D121	1.000	0.000
MB	D90	D121	1.000	0.000
WRP	A19	B08	0.164	0.025
WRP	A19	D16	1.000	0.000
WRP	B08	D16	0.832	0.024
WRP	A19	D40	1.000	0.000
WRP	B08	D40	0.869	0.020
WRP	D16	D40	0.274	0.040
WRP	A19	D55	1.000	0.000
WRP	B08	D55	0.486	0.033
WRP	D16	D55	1.000	0.000
WRP	D40	D55	0.415	0.047
WRP	A19	D79	0.966	0.007
WRP	B08	D79	0.173	0.012
WRP	D16	D79	0.060	0.014
WRP	D40	D79	1.000	0.000
WRP	D55	D79	0.483	0.027
WRP	A19	D87	1.000	0.000
WRP	B08	D87	0.752	0.030
WRP	D16	D87	1.000	0.000
WRP	D40	D87	1.000	0.000
WRP	D55	D87	1.000	0.000
WRP	D79	D87	0.639	0.027
WRP	A19	D88	1.000	0.000
WRP	B08	D88	0.908	0.013
WRP	D16	D88	1.000	0.000
WRP	D40	D88	0.016	0.010
WRP	D55	D88	0.606	0.042
WRP	D79	D88	0.921	0.009
WRP	D87	D88	1.000	0.000
WRP	A19	D90	1.000	0.000
WRP	B08	D90	0.641	0.027
WRP	D16	D90	0.662	0.038
WRP	D40	D90	0.049	0.018
WRP	D55	D90	0.736	0.036
WRP	D79	D90	0.920	0.010
WRP	D87	D90	1.000	0.000
WRP	D88	D90	0.014	0.006
WRP	A19	D121	1.000	0.000
WRP	B08	D121	0.933	0.015
WRP	D16	D121	1.000	0.000
WRP	D40	D121	1.000	0.000
WRP	D55	D121	1.000	0.000
WRP	D79	D121	0.885	0.016

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
WRP	D87	D121	1.000	0.000
WRP	D88	D121	1.000	0.000
WRP	D90	D121	1.000	0.000
RCP	A19	B08	1.000	0.000
RCP	A19	D16	1.000	0.000
RCP	B08	D16	0.171	0.011
RCP	A19	D40	1.000	0.000
RCP	B08	D40	0.265	0.013
RCP	D16	D40	1.000	0.000
RCP	A19	D55	1.000	0.000
RCP	B08	D55	1.000	0.000
RCP	D16	D55	1.000	0.000
RCP	D40	D55	1.000	0.000
RCP	A19	D79	1.000	0.000
RCP	B08	D79	0.945	0.003
RCP	D16	D79	1.000	0.000
RCP	D40	D79	1.000	0.000
RCP	D55	D79	1.000	0.000
RCP	A19	D87	1.000	0.000
RCP	B08	D87	1.000	0.000
RCP	D16	D87	1.000	0.000
RCP	D40	D87	1.000	0.000
RCP	D55	D87	1.000	0.000
RCP	D79	D87	1.000	0.000
RCP	A19	D88	1.000	0.000
RCP	B08	D88	0.122	0.007
RCP	D16	D88	1.000	0.000
RCP	D40	D88	1.000	0.000
RCP	D55	D88	1.000	0.000
RCP	D79	D88	0.778	0.007
RCP	D87	D88	1.000	0.000
RCP	A19	D90	1.000	0.000
RCP	B08	D90	0.561	0.013
RCP	D16	D90	1.000	0.000
RCP	D40	D90	1.000	0.000
RCP	D55	D90	1.000	0.000
RCP	D79	D90	1.000	0.000
RCP	D87	D90	1.000	0.000
RCP	D88	D90	0.423	0.021
RCP	A19	D121	1.000	0.000
RCP	B08	D121	1.000	0.000
RCP	D16	D121	1.000	0.000
RCP	D40	D121	1.000	0.000
RCP	D55	D121	*	*
RCP	D79	D121	1.000	0.000

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
RCP	D87	D121	*	*
RCP	D88	D121	1.000	0.000
RCP	D90	D121	1.000	0.000
Casey	A19	B08	0.933	0.012
Casey	A19	D16	0.248	0.039
Casey	B08	D16	0.069	0.015
Casey	A19	D40	0.134	0.031
Casey	B08	D40	0.460	0.033
Casey	D16	D40	1.000	0.000
Casey	A19	D55	1.000	0.000
Casey	B08	D55	0.654	0.032
Casey	D16	D55	1.000	0.000
Casey	D40	D55	1.000	0.000
Casey	A19	D79	0.439	0.021
Casey	B08	D79	0.972	0.003
Casey	D16	D79	0.767	0.018
Casey	D40	D79	0.773	0.021
Casey	D55	D79	0.233	0.019
Casey	A19	D87	1.000	0.000
Casey	B08	D87	0.521	0.036
Casey	D16	D87	1.000	0.000
Casey	D40	D87	1.000	0.000
Casey	D55	D87	1.000	0.000
Casey	D79	D87	1.000	0.000
Casey	A19	D88	1.000	0.000
Casey	B08	D88	0.864	0.017
Casey	D16	D88	1.000	0.000
Casey	D40	D88	1.000	0.000
Casey	D55	D88	0.332	0.040
Casey	D79	D88	0.756	0.015
Casey	D87	D88	0.020	0.012
Casey	A19	D90	0.730	0.032
Casey	B08	D90	0.207	0.017
Casey	D16	D90	0.341	0.038
Casey	D40	D90	1.000	0.000
Casey	D55	D90	0.417	0.040
Casey	D79	D90	0.550	0.017
Casey	D87	D90	1.000	0.000
Casey	D88	D90	0.817	0.025
Casey	A19	D121	0.504	0.043
Casey	B08	D121	0.067	0.015
Casey	D16	D121	1.000	0.000
Casey	D40	D121	1.000	0.000
Casey	D55	D121	1.000	0.000
Casey	D79	D121	0.194	0.018

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
Casey	D88	D121	0.028	0.010
Casey	D90	D121	1.000	0.000
IW	A19	B08	0.823	0.020
IW	A19	D16	*	*
IW	B08	D16	*	*
IW	A19	D40	0.117	0.026
IW	B08	D40	0.346	0.031
IW	D16	D40	*	*
IW	A19	D55	1.000	0.000
IW	B08	D55	0.723	0.024
IW	D16	D55	*	*
IW	D40	D55	1.000	0.000
IW	A19	D79	0.134	0.014
IW	B08	D79	0.981	0.002
IW	D16	D79	*	*
IW	D40	D79	0.633	0.018
IW	D55	D79	0.541	0.018
IW	A19	D87	1.000	0.000
IW	B08	D87	0.728	0.025
IW	D16	D87	*	*
IW	D40	D87	1.000	0.000
IW	D55	D87	1.000	0.000
IW	D79	D87	0.180	0.014
IW	A19	D88	0.353	0.038
IW	B08	D88	0.755	0.021
IW	D16	D88	*	*
IW	D40	D88	0.156	0.030
IW	D55	D88	1.000	0.000
IW	D79	D88	0.251	0.014
IW	D87	D88	1.000	0.000
IW	A19	D90	0.254	0.029
IW	B08	D90	0.190	0.019
IW	D16	D90	*	*
IW	D40	D90	0.016	0.006
IW	D55	D90	0.670	0.033
IW	D79	D90	0.459	0.012
IW	D87	D90	1.000	0.000
IW	D88	D90	0.626	0.029
IW	A19	D121	0.304	0.038
IW	B08	D121	0.676	0.026
IW	D16	D121	*	*
IW	D40	D121	0.133	0.029
IW	D55	D121	1.000	0.000
IW	D79	D121	0.002	0.001
IW	D87	D121	1.000	0.000

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
IW	D88	D121	0.298	0.034
IW	D90	D121	0.141	0.023
EK	A19	B08	0.539	0.018
EK	A19	D16	0.662	0.031
EK	B08	D16	0.873	0.011
EK	A19	D40	0.065	0.015
EK	B08	D40	0.163	0.014
EK	D16	D40	0.228	0.030
EK	A19	D55	0.508	0.021
EK	B08	D55	0.019	0.003
EK	D16	D55	0.397	0.028
EK	D40	D55	0.968	0.006
EK	A19	D79	0.158	0.008
EK	B08	D79	0.672	0.006
EK	D16	D79	0.589	0.012
EK	D40	D79	0.381	0.012
EK	D55	D79	0.612	0.007
EK	A19	D87	0.165	0.019
EK	B08	D87	0.903	0.007
EK	D16	D87	0.308	0.027
EK	D40	D87	0.152	0.024
EK	D55	D87	0.970	0.005
EK	D79	D87	0.640	0.009
EK	A19	D88	0.563	0.035
EK	B08	D88	0.377	0.017
EK	D16	D88	0.578	0.035
EK	D40	D88	0.821	0.030
EK	D55	D88	0.625	0.024
EK	D79	D88	0.293	0.010
EK	D87	D88	0.914	0.015
EK	A19	D90	0.258	0.022
EK	B08	D90	0.428	0.017
EK	D16	D90	0.185	0.014
EK	D40	D90	0.331	0.028
EK	D55	D90	0.723	0.015
EK	D79	D90	0.949	0.003
EK	D87	D90	0.210	0.019
EK	D88	D90	0.753	0.020
EK	A19	D121	0.279	0.019
EK	B08	D121	0.849	0.008
EK	D16	D121	0.737	0.022
EK	D40	D121	0.947	0.009
EK	D55	D121	0.944	0.007
EK	D79	D121	0.364	0.007
EK	D87	D121	0.842	0.013

Appendix E. Continued

Population	Locus#1	Locus#2	*P*-value	S.E.
EK	D88	D121	0.838	0.017
EK	D90	D121	0.427	0.023

Appendix F. Results of maximum likelihood assignment tests for 10 eastern box turtle populations from Maryland, Pennsylvania and Florida derived from 10 microsatellite DNA markers. Those below the defined threshold of α = 0.01 are shaded. See Table 5.1 for population abbreviations.

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
PWRC	0.065	0.041	0.006	0.005	0.018	0.001	0.013	0.018	0.072	0.000	10
PWRC	0.082	0.088	0.005	0.040	0.031	0.001	0.001	0.036	0.002	0.000	10
PWRC	0.676	0.091	0.248	0.147	0.215	0.204	0.025	0.070	0.117	0.000	9
PWRC	0.169	0.013	0.059	0.049	0.089	0.181	0.032	0.080	0.001	0.000	10
PWRC	0.526	0.213	0.046	0.261	0.242	0.002	0.030	0.203	0.005	0.000	10
PWRC	0.080	0.137	0.021	0.025	0.050	0.013	0.233	0.006	0.043	0.000	10
PWRC	0.729	0.679	0.863	0.634	0.665	0.575	0.212	0.780	0.037	0.000	10
PWRC	0.236	0.030	0.052	0.055	0.020	0.028	0.034	0.012	0.002	0.000	10
PWRC	0.132	0.008	0.011	0.063	0.024	0.005	0.003	0.018	0.010	0.000	10
PWRC	0.724	0.409	0.370	0.603	0.394	0.523	0.106	0.178	0.090	0.000	10
PWRC	0.423	0.157	0.276	0.351	0.071	0.150	0.373	0.360	0.259	0.000	10
PWRC	0.526	0.121	0.435	0.083	0.267	0.082	0.061	0.317	0.077	0.000	10
PWRC	0.453	0.140	0.065	0.073	0.138	0.075	0.086	0.077	0.030	0.000	10
PWRC	0.731	0.454	0.564	0.684	0.671	0.255	0.178	0.428	0.316	0.000	10
PWRC	0.134	0.086	0.045	0.019	0.242	0.048	0.056	0.036	0.008	0.000	10
PWRC	0.200	0.025	0.042	0.036	0.001	0.005	0.014	0.034	0.017	0.000	10
PWRC	0.587	0.095	0.141	0.084	0.011	0.056	0.024	0.105	0.092	0.000	10
PWRC	0.889	0.691	0.817	0.413	0.607	0.391	0.400	0.375	0.129	0.000	10
PWRC	0.557	0.314	0.329	0.479	0.663	0.242	0.056	0.700	0.023	0.000	10
PWRC	0.166	0.205	0.040	0.001	0.016	0.055	0.024	0.012	0.000	0.000	10
PWRC	0.656	0.478	0.131	0.183	0.269	0.165	0.041	0.354	0.012	0.000	10
PWRC	0.196	0.113	0.019	0.025	0.225	0.078	0.041	0.045	0.017	0.000	10
PWRC	0.875	0.735	0.475	0.261	0.591	0.144	0.334	0.511	0.041	0.000	9
PWRC	0.330	0.025	0.006	0.022	0.015	0.011	0.015	0.009	0.008	0.000	10
PWRC	0.746	0.320	0.867	0.354	0.224	0.123	0.306	0.232	0.252	0.000	10
PWRC	0.700	0.259	0.132	0.025	0.193	0.148	0.042	0.211	0.012	0.000	10
PWRC	0.163	0.058	0.051	0.189	0.010	0.005	0.052	0.073	0.037	0.000	10
PWRC	0.059	0.005	0.015	0.005	0.014	0.001	0.012	0.005	0.000	0.000	10
PWRC	0.685	0.751	0.488	0.377	0.742	0.438	0.098	0.369	0.058	0.000	10
PWRC	0.203	0.009	0.141	0.272	0.125	0.210	0.034	0.055	0.007	0.000	10
PWRC	0.177	0.101	0.100	0.030	0.197	0.107	0.035	0.088	0.002	0.000	9
PWRC	0.560	0.076	0.176	0.054	0.121	0.007	0.060	0.058	0.038	0.000	10
PWRC	0.638	0.601	0.244	0.440	0.468	0.575	0.122	0.482	0.083	0.000	10
PWRC	0.528	0.394	0.403	0.467	0.207	0.167	0.158	0.194	0.044	0.000	10
PWRC	0.227	0.226	0.125	0.042	0.073	0.132	0.047	0.044	0.002	0.000	10
PWRC	0.420	0.056	0.004	0.005	0.004	0.000	0.006	0.007	0.007	0.000	10
PWRC	0.968	0.818	0.577	0.755	0.642	0.855	0.242	0.583	0.350	0.000	9
PWRC	0.792	0.733	0.559	0.239	0.455	0.682	0.122	0.603	0.110	0.000	10
PWRC	0.825	0.471	0.531	0.182	0.468	0.677	0.065	0.208	0.150	0.000	9
PWRC	0.144	0.028	0.009	0.091	0.001	0.021	0.080	0.013	0.003	0.000	9
PWRC	0.839	0.826	0.842	0.715	0.687	0.646	0.206	0.910	0.702	0.000	9
PWRC	0.561	0.022	0.014	0.015	0.010	0.003	0.015	0.005	0.010	0.000	10
PWRC	0.528	0.062	0.225	0.057	0.056	0.028	0.047	0.042	0.038	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
PWRC	0.150	0.031	0.021	0.046	0.018	0.053	0.017	0.169	0.003	0.000	10
PWRC	0.835	0.401	0.102	0.553	0.414	0.416	0.209	0.378	0.123	0.000	9
PWRC	0.726	0.339	0.034	0.144	0.267	0.135	0.055	0.375	0.039	0.000	9
PWRC	0.702	0.138	0.342	0.117	0.553	0.369	0.206	0.399	0.079	0.000	9
PWRC	0.901	0.761	0.822	0.734	0.669	0.583	0.405	0.490	0.525	0.000	9
PWRC	0.155	0.084	0.007	0.043	0.019	0.017	0.020	0.011	0.002	0.000	10
PWRC	0.600	0.182	0.346	0.165	0.204	0.416	0.177	0.234	0.151	0.000	9
PWRC	0.572	0.275	0.110	0.018	0.102	0.158	0.083	0.105	0.004	0.000	9
GNMP	0.029	0.222	0.010	0.001	0.025	0.033	0.021	0.017	0.001	0.000	9
GNMP	0.173	0.550	0.131	0.062	0.056	0.060	0.015	0.020	0.062	0.000	10
GNMP	0.647	0.869	0.251	0.536	0.410	0.358	0.506	0.468	0.481	0.000	10
GNMP	0.324	0.681	0.136	0.747	0.619	0.406	0.052	0.642	0.005	0.000	10
GNMP	0.581	0.905	0.523	0.350	0.689	0.699	0.059	0.603	0.012	0.000	10
GNMP	0.334	0.542	0.083	0.329	0.292	0.249	0.075	0.253	0.086	0.000	10
GNMP	0.040	0.181	0.019	0.015	0.011	0.011	0.032	0.059	0.061	0.000	10
GNMP	0.013	0.308	0.006	0.027	0.008	0.001	0.020	0.046	0.042	0.000	10
GNMP	0.255	0.364	0.094	0.206	0.051	0.065	0.020	0.156	0.038	0.000	10
GNMP	0.290	0.764	0.272	0.051	0.123	0.098	0.042	0.099	0.015	0.000	10
GNMP	0.308	0.613	0.322	0.129	0.288	0.038	0.094	0.300	0.076	0.000	10
GNMP	0.178	0.500	0.211	0.393	0.238	0.153	0.130	0.156	0.013	0.000	9
GNMP	0.261	0.509	0.123	0.373	0.174	0.275	0.354	0.088	0.037	0.000	10
GNMP	0.327	0.829	0.456	0.783	0.332	0.655	0.186	0.549	0.093	0.000	10
GNMP	0.160	0.454	0.020	0.066	0.156	0.050	0.020	0.059	0.004	0.000	10
GNMP	0.117	0.179	0.028	0.010	0.035	0.021	0.034	0.009	0.008	0.000	10
GNMP	0.330	0.614	0.297	0.266	0.769	0.302	0.029	0.632	0.023	0.000	10
GNMP	0.555	0.910	0.429	0.687	0.621	0.647	0.438	0.687	0.143	0.000	10
GNMP	0.140	0.617	0.108	0.097	0.195	0.232	0.009	0.158	0.003	0.000	10
GNMP	0.085	0.905	0.190	0.490	0.506	0.335	0.114	0.464	0.090	0.000	10
GNMP	0.077	0.680	0.023	0.024	0.018	0.027	0.020	0.033	0.017	0.000	10
GNMP	0.004	0.101	0.015	0.004	0.002	0.011	0.047	0.004	0.004	0.000	10
GNMP	0.016	0.288	0.006	0.001	0.001	0.001	0.004	0.002	0.004	0.000	10
GNMP	0.312	0.640	0.136	0.487	0.283	0.181	0.088	0.217	0.052	0.000	10
GNMP	0.433	0.775	0.115	0.017	0.040	0.075	0.039	0.179	0.004	0.000	10
GNMP	0.257	0.419	0.179	0.400	0.231	0.192	0.116	0.123	0.028	0.000	10
GNMP	0.411	0.676	0.237	0.635	0.614	0.597	0.211	0.281	0.274	0.000	9
GNMP	0.741	0.456	0.512	0.487	0.614	0.275	0.122	0.673	0.306	0.000	10
GNMP	0.240	0.111	0.035	0.178	0.138	0.127	0.208	0.099	0.010	0.000	10
GNMP	0.399	0.748	0.352	0.271	0.501	0.522	0.244	0.124	0.102	0.000	9
GNMP	0.357	0.737	0.418	0.620	0.376	0.307	0.131	0.380	0.148	0.000	10
GNMP	0.649	0.903	0.472	0.294	0.649	0.850	0.329	0.223	0.118	0.000	9
GNMP	0.234	0.253	0.052	0.043	0.029	0.169	0.091	0.103	0.019	0.000	10
JBWS	0.116	0.024	0.104	0.019	0.036	0.045	0.041	0.012	0.055	0.000	10
JBWS	0.239	0.252	0.550	0.003	0.099	0.065	0.012	0.059	0.005	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
JBWS	0.576	0.065	0.900	0.427	0.605	0.043	0.083	0.663	0.082	0.000	10
JBWS	0.570	0.290	0.792	0.414	0.330	0.181	0.265	0.237	0.052	0.000	10
JBWS	0.161	0.105	0.587	0.001	0.012	0.029	0.010	0.057	0.007	0.000	10
JBWS	0.545	0.417	0.690	0.047	0.483	0.401	0.142	0.310	0.015	0.000	10
JBWS	0.512	0.286	0.672	0.312	0.468	0.345	0.444	0.186	0.079	0.000	10
JBWS	0.354	0.198	0.759	0.150	0.271	0.158	0.155	0.307	0.291	0.000	10
JBWS	0.479	0.117	0.847	0.449	0.636	0.190	0.259	0.434	0.064	0.000	10
JBWS	0.619	0.123	0.898	0.405	0.556	0.234	0.047	0.245	0.154	0.000	10
JBWS	0.482	0.274	0.213	0.059	0.120	0.135	0.019	0.123	0.028	0.000	10
JBWS	0.498	0.274	0.885	0.400	0.347	0.344	0.295	0.148	0.324	0.000	10
JBWS	0.381	0.105	0.576	0.178	0.418	0.087	0.047	0.278	0.025	0.000	10
JBWS	0.407	0.828	0.751	0.629	0.794	0.832	0.705	0.819	0.289	0.000	9
JBWS	0.250	0.180	0.463	0.042	0.147	0.116	0.092	0.072	0.076	0.000	10
JBWS	0.103	0.010	0.231	0.180	0.130	0.233	0.081	0.051	0.004	0.000	10
JBWS	0.227	0.180	0.297	0.139	0.496	0.135	0.250	0.142	0.034	0.000	10
JBWS	0.442	0.287	0.823	0.125	0.101	0.309	0.262	0.338	0.117	0.000	10
JBWS	0.173	0.339	0.484	0.099	0.140	0.166	0.182	0.375	0.038	0.000	10
JBWS	0.436	0.196	0.702	0.272	0.433	0.220	0.060	0.534	0.083	0.000	10
JBWS	0.172	0.034	0.153	0.079	0.174	0.062	0.032	0.140	0.002	0.000	10
JBWS	0.169	0.184	0.449	0.310	0.205	0.225	0.094	0.428	0.093	0.000	10
JBWS	0.269	0.067	0.372	0.242	0.126	0.368	0.090	0.079	0.004	0.001	9
JBWS	0.954	0.867	0.983	0.863	0.807	0.826	0.791	0.879	0.276	0.000	10
JBWS	0.466	0.123	0.448	0.250	0.157	0.056	0.173	0.283	0.154	0.000	10
JBWS	0.528	0.739	0.861	0.542	0.663	0.566	0.358	0.474	0.154	0.000	10
JBWS	0.048	0.062	0.190	0.046	0.131	0.012	0.039	0.017	0.034	0.000	10
JBWS	0.638	0.237	0.647	0.352	0.259	0.649	0.450	0.571	0.154	0.000	10
JBWS	0.376	0.167	0.576	0.513	0.214	0.140	0.041	0.158	0.091	0.000	10
JBWS	0.052	0.313	0.110	0.540	0.299	0.231	0.104	0.191	0.005	0.000	10
JBWS	0.608	0.182	0.821	0.248	0.316	0.285	0.401	0.447	0.174	0.000	10
JBWS	0.916	0.764	0.954	0.808	0.791	0.590	0.331	0.746	0.250	0.000	10
JBWS	0.723	0.478	0.753	0.377	0.478	0.515	0.459	0.593	0.063	0.000	10
JBWS	0.104	0.007	0.188	0.015	0.063	0.032	0.042	0.102	0.010	0.000	10
JBWS	0.241	0.138	0.345	0.043	0.271	0.166	0.084	0.057	0.028	0.000	10
JBWS	0.098	0.280	0.322	0.225	0.324	0.325	0.041	0.099	0.038	0.000	10
JBWS	0.325	0.270	0.294	0.259	0.109	0.119	0.088	0.105	0.005	0.000	10
JBWS	0.495	0.635	0.798	0.685	0.620	0.482	0.151	0.468	0.051	0.000	10
JBWS	0.319	0.209	0.526	0.336	0.211	0.105	0.125	0.299	0.079	0.000	10
JBWS	0.010	0.004	0.046	0.007	0.018	0.001	0.010	0.012	0.002	0.000	10
JBWS	0.233	0.406	0.759	0.489	0.605	0.558	0.438	0.415	0.106	0.000	10
JBWS	0.092	0.117	0.211	0.179	0.192	0.119	0.020	0.071	0.038	0.000	10
JBWS	0.612	0.585	0.638	0.302	0.157	0.245	0.123	0.233	0.375	0.000	10
JBWS	0.048	0.034	0.142	0.166	0.076	0.087	0.063	0.105	0.014	0.000	10
JBWS	0.110	0.031	0.106	0.043	0.010	0.001	0.223	0.017	0.028	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
JBWS	0.460	0.494	0.359	0.307	0.418	0.456	0.164	0.300	0.012	0.000	10
JBWS	0.019	0.014	0.130	0.033	0.021	0.005	0.024	0.036	0.000	0.000	10
JBWS	0.155	0.548	0.478	0.547	0.290	0.245	0.201	0.213	0.052	0.000	10
JBWS	0.415	0.202	0.518	0.241	0.316	0.133	0.129	0.300	0.028	0.000	10
JBWS	0.350	0.138	0.408	0.307	0.193	0.105	0.117	0.224	0.010	0.000	10
JBWS	0.319	0.563	0.411	0.217	0.273	0.172	0.131	0.183	0.007	0.000	10
BHP	0.318	0.284	0.173	0.547	0.258	0.617	0.156	0.399	0.020	0.000	9
BHP	0.257	0.220	0.518	0.743	0.343	0.078	0.100	0.455	0.017	0.000	10
BHP	0.423	0.051	0.085	0.214	0.205	0.062	0.033	0.124	0.016	0.000	10
BHP	0.095	0.132	0.023	0.553	0.082	0.160	0.059	0.073	0.037	0.000	8
BHP	0.119	0.153	0.115	0.419	0.210	0.114	0.060	0.176	0.021	0.000	10
BHP	0.374	0.145	0.164	0.751	0.377	0.753	0.110	0.221	0.113	0.000	9
BHP	0.155	0.071	0.015	0.421	0.315	0.268	0.024	0.431	0.038	0.000	10
BHP	0.271	0.290	0.096	0.517	0.250	0.125	0.135	0.054	0.300	0.000	9
BHP	0.229	0.054	0.079	0.714	0.044	0.028	0.118	0.221	0.003	0.000	10
BHP	0.422	0.802	0.496	0.610	0.246	0.234	0.227	0.437	0.058	0.000	10
BHP	0.025	0.196	0.046	0.446	0.090	0.053	0.069	0.166	0.004	0.000	10
BHP	0.175	0.580	0.272	0.851	0.644	0.582	0.330	0.641	0.047	0.000	10
BHP	0.592	0.888	0.522	0.691	0.541	0.541	0.133	0.583	0.269	0.000	10
BHP	0.418	0.209	0.501	0.613	0.260	0.292	0.088	0.300	0.063	0.000	10
BHP	0.065	0.153	0.042	0.184	0.205	0.030	0.013	0.145	0.004	0.000	10
BHP	0.330	0.326	0.579	0.513	0.734	0.296	0.085	0.422	0.144	0.000	9
BHP	0.255	0.273	0.201	0.643	0.224	0.091	0.091	0.299	0.035	0.000	10
BHP	0.235	0.179	0.249	0.707	0.636	0.591	0.074	0.549	0.028	0.000	10
BHP	0.085	0.051	0.011	0.163	0.010	0.027	0.047	0.006	0.004	0.000	10
BHP	0.374	0.364	0.410	0.648	0.245	0.223	0.216	0.448	0.038	0.000	10
BHP	0.518	0.248	0.634	0.689	0.666	0.327	0.167	0.327	0.036	0.000	10
BHP	0.429	0.122	0.463	0.611	0.330	0.127	0.131	0.223	0.116	0.000	10
BHP	0.278	0.076	0.189	0.549	0.253	0.087	0.024	0.085	0.034	0.000	10
BHP	0.061	0.346	0.022	0.234	0.054	0.062	0.063	0.012	0.007	0.000	10
BHP	0.408	0.320	0.140	0.656	0.365	0.302	0.254	0.535	0.068	0.000	10
BHP	0.168	0.374	0.036	0.341	0.280	0.206	0.041	0.329	0.028	0.000	10
BHP	0.255	0.733	0.162	0.819	0.488	0.433	0.155	0.732	0.287	0.000	10
BHP	0.237	0.248	0.081	0.401	0.150	0.151	0.210	0.107	0.003	0.000	6
BHP	0.173	0.164	0.097	0.391	0.178	0.100	0.027	0.223	0.035	0.000	10
BHP	0.245	0.121	0.042	0.569	0.193	0.249	0.044	0.118	0.079	0.000	10
BHP	0.118	0.392	0.128	0.478	0.284	0.396	0.130	0.329	0.012	0.000	10
BHP	0.220	0.115	0.167	0.287	0.267	0.163	0.117	0.645	0.005	0.000	10
BHP	0.245	0.242	0.130	0.524	0.353	0.135	0.034	0.158	0.081	0.000	10
BHP	0.261	0.337	0.231	0.417	0.271	0.231	0.069	0.102	0.028	0.000	10
BHP	0.616	0.545	0.726	0.848	0.544	0.442	0.626	0.688	0.058	0.000	10
BHP	0.233	0.070	0.142	0.567	0.347	0.241	0.254	0.112	0.005	0.000	10
BHP	0.599	0.511	0.771	0.872	0.673	0.812	0.552	0.727	0.383	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
BHP	0.356	0.131	0.083	0.129	0.123	0.050	0.082	0.105	0.017	0.000	10
BHP	0.028	0.102	0.042	0.400	0.130	0.112	0.020	0.100	0.024	0.000	10
BHP	0.042	0.027	0.016	0.097	0.010	0.001	0.020	0.012	0.003	0.000	10
BHP	0.142	0.056	0.223	0.709	0.274	0.225	0.171	0.139	0.012	0.000	10
BHP	0.085	0.109	0.052	0.362	0.270	0.141	0.029	0.094	0.005	0.000	10
BHP	0.422	0.088	0.215	0.597	0.280	0.076	0.015	0.143	0.058	0.000	10
BHP	0.168	0.099	0.055	0.517	0.043	0.079	0.039	0.049	0.037	0.000	9
MB	0.341	0.273	0.525	0.133	0.531	0.227	0.122	0.149	0.024	0.000	10
MB	0.157	0.161	0.270	0.099	0.336	0.146	0.320	0.311	0.008	0.000	10
MB	0.104	0.101	0.018	0.051	0.401	0.080	0.006	0.093	0.004	0.000	10
MB	0.038	0.067	0.202	0.010	0.466	0.123	0.173	0.059	0.004	0.000	10
MB	0.183	0.209	0.207	0.322	0.689	0.188	0.188	0.193	0.113	0.000	10
MB	0.124	0.287	0.306	0.143	0.230	0.050	0.038	0.114	0.023	0.000	10
MB	0.495	0.385	0.727	0.760	0.515	0.345	0.250	0.491	0.218	0.000	10
MB	0.010	0.034	0.028	0.047	0.500	0.099	0.020	0.102	0.034	0.000	10
MB	0.058	0.078	0.021	0.129	0.287	0.069	0.042	0.186	0.000	0.000	10
MB	0.166	0.095	0.182	0.649	0.629	0.553	0.042	0.462	0.018	0.000	10
MB	0.887	0.795	0.810	0.940	0.893	0.845	0.598	0.652	0.161	0.000	6
MB	0.479	0.683	0.206	0.707	0.802	0.498	0.163	0.688	0.068	0.000	10
MB	0.414	0.202	0.070	0.053	0.443	0.218	0.061	0.025	0.010	0.000	10
MB	0.255	0.593	0.281	0.657	0.529	0.399	0.266	0.428	0.063	0.000	10
MB	0.124	0.084	0.127	0.055	0.527	0.070	0.030	0.102	0.038	0.000	10
MB	0.015	0.031	0.009	0.042	0.134	0.066	0.015	0.031	0.016	0.000	10
MB	0.216	0.197	0.035	0.109	0.658	0.345	0.167	0.424	0.004	0.000	10
MB	0.362	0.192	0.599	0.383	0.807	0.201	0.041	0.667	0.037	0.000	10
MB	0.402	0.265	0.284	0.663	0.866	0.454	0.068	0.651	0.014	0.000	10
MB	0.227	0.067	0.270	0.339	0.792	0.543	0.063	0.615	0.043	0.000	10
MB	0.016	0.060	0.010	0.003	0.055	0.020	0.052	0.017	0.000	0.000	10
MB	0.304	0.161	0.199	0.157	0.332	0.543	0.042	0.593	0.055	0.000	10
MB	0.255	0.153	0.115	0.372	0.365	0.244	0.027	0.025	0.079	0.000	10
MB	0.239	0.117	0.156	0.164	0.125	0.123	0.049	0.282	0.122	0.000	8
MB	0.415	0.672	0.558	0.641	0.662	0.235	0.223	0.581	0.057	0.000	10
MB	0.335	0.467	0.398	0.384	0.490	0.258	0.104	0.195	0.035	0.000	9
MB	0.648	0.283	0.569	0.663	0.753	0.567	0.179	0.328	0.028	0.000	10
MB	0.653	0.914	0.853	0.417	0.722	0.830	0.323	0.883	0.570	0.017	4
MB	0.459	0.413	0.431	0.489	0.748	0.518	0.092	0.329	0.063	0.000	10
MB	0.267	0.391	0.656	0.494	0.389	0.275	0.161	0.153	0.067	0.000	9
MB	0.138	0.086	0.201	0.187	0.290	0.152	0.314	0.251	0.060	0.000	10
MB	0.118	0.049	0.490	0.359	0.217	0.382	0.281	0.132	0.154	0.000	10
MB	0.330	0.153	0.064	0.250	0.209	0.099	0.061	0.030	0.100	0.000	10
MB	0.203	0.085	0.097	0.191	0.506	0.103	0.015	0.111	0.002	0.000	10
MB	0.557	0.703	0.813	0.689	0.739	0.523	0.226	0.861	0.185	0.000	10
MB	0.873	0.800	0.438	0.554	0.835	0.548	0.237	0.490	0.314	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
MB	0.099	0.031	0.025	0.065	0.491	0.050	0.071	0.235	0.038	0.000	10
MB	0.648	0.329	0.112	0.377	0.744	0.361	0.034	0.300	0.041	0.000	10
MB	0.489	0.210	0.217	0.227	0.605	0.095	0.106	0.428	0.004	0.000	10
MB	0.041	0.149	0.007	0.099	0.201	0.018	0.002	0.084	0.002	0.000	10
MB	0.173	0.243	0.114	0.329	0.200	0.153	0.014	0.267	0.016	0.000	10
MB	0.212	0.531	0.456	0.373	0.578	0.427	0.564	0.511	0.106	0.000	10
MB	0.209	0.079	0.208	0.206	0.428	0.050	0.047	0.186	0.004	0.000	10
MB	0.058	0.175	0.025	0.158	0.242	0.192	0.039	0.223	0.028	0.000	10
MB	0.085	0.054	0.023	0.002	0.116	0.090	0.039	0.046	0.015	0.000	10
MB	0.065	0.173	0.062	0.078	0.159	0.128	0.010	0.165	0.010	0.000	10
MB	0.179	0.051	0.126	0.143	0.532	0.213	0.026	0.215	0.003	0.003	9
MB	0.614	0.209	0.178	0.453	0.571	0.076	0.078	0.454	0.028	0.000	10
MB	0.644	0.456	0.144	0.584	0.632	0.404	0.171	0.094	0.032	0.000	10
MB	0.224	0.459	0.040	0.156	0.179	0.072	0.059	0.158	0.127	0.000	9
MB	0.820	0.722	0.500	0.722	0.894	0.778	0.584	0.814	0.821	0.000	9
MB	0.019	0.007	0.010	0.049	0.318	0.047	0.035	0.091	0.000	0.000	9
MB	0.195	0.182	0.036	0.072	0.116	0.105	0.047	0.051	0.008	0.000	10
MB	0.157	0.016	0.099	0.008	0.217	0.055	0.004	0.109	0.004	0.000	9
MB	0.254	0.139	0.275	0.250	0.220	0.214	0.088	0.300	0.016	0.000	10
MB	0.203	0.297	0.297	0.320	0.433	0.509	0.013	0.142	0.091	0.000	9
MB	0.375	0.286	0.278	0.481	0.851	0.639	0.304	0.498	0.053	0.000	9
MB	0.029	0.018	0.010	0.033	0.293	0.005	0.002	0.017	0.000	0.000	9
WRP	0.042	0.056	0.006	0.063	0.040	0.285	0.055	0.043	0.005	0.000	10
WRP	0.541	0.187	0.108	0.432	0.500	0.527	0.108	0.250	0.015	0.000	10
WRP	0.293	0.698	0.275	0.209	0.210	0.242	0.292	0.348	0.008	0.000	10
WRP	0.049	0.036	0.115	0.029	0.033	0.285	0.050	0.059	0.004	0.000	10
WRP	0.174	0.085	0.149	0.216	0.213	0.288	0.134	0.097	0.158	0.000	3
WRP	0.205	0.567	0.581	0.250	0.503	0.324	0.113	0.191	0.028	0.000	10
WRP	0.471	0.480	0.365	0.588	0.463	0.911	0.140	0.373	0.128	0.000	10
WRP	0.324	0.499	0.094	0.303	0.115	0.318	0.207	0.090	0.053	0.000	9
WRP	0.118	0.076	0.016	0.031	0.024	0.433	0.015	0.105	0.028	0.000	10
WRP	0.311	0.478	0.284	0.563	0.411	0.796	0.257	0.393	0.076	0.000	10
WRP	0.389	0.511	0.275	0.363	0.580	0.715	0.142	0.595	0.076	0.000	10
WRP	0.306	0.269	0.179	0.614	0.563	0.608	0.224	0.157	0.138	0.000	9
WRP	0.362	0.288	0.144	0.552	0.536	0.754	0.374	0.565	0.072	0.000	10
WRP	0.341	0.255	0.186	0.445	0.443	0.537	0.104	0.519	0.049	0.000	9
WRP	0.118	0.150	0.212	0.351	0.447	0.762	0.068	0.536	0.010	0.000	10
WRP	0.439	0.100	0.199	0.158	0.284	0.466	0.049	0.176	0.010	0.000	10
WRP	0.763	0.326	0.491	0.413	0.267	0.472	0.293	0.601	0.186	0.000	10
WRP	0.025	0.342	0.046	0.061	0.131	0.169	0.039	0.057	0.019	0.000	10
WRP	0.357	0.250	0.691	0.437	0.571	0.806	0.096	0.342	0.189	0.000	10
WRP	0.356	0.787	0.726	0.412	0.610	0.893	0.526	0.651	0.306	0.000	9
WRP	0.089	0.038	0.046	0.191	0.242	0.389	0.110	0.181	0.010	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
WRP	0.585	0.247	0.544	0.297	0.632	0.632	0.026	0.366	0.041	0.000	9
WRP	0.799	0.703	0.546	0.437	0.524	0.860	0.379	0.530	0.341	0.000	10
WRP	0.219	0.515	0.272	0.052	0.294	0.425	0.144	0.127	0.068	0.000	10
WRP	0.118	0.025	0.086	0.032	0.116	0.107	0.088	0.091	0.014	0.000	10
WRP	0.281	0.589	0.322	0.335	0.776	0.815	0.212	0.377	0.046	0.000	9
WRP	0.478	0.433	0.301	0.341	0.515	0.493	0.066	0.280	0.087	0.000	10
WRP	0.060	0.046	0.007	0.006	0.050	0.119	0.006	0.025	0.004	0.000	10
WRP	0.010	0.084	0.019	0.047	0.056	0.075	0.002	0.025	0.004	0.000	10
WRP	0.234	0.024	0.009	0.047	0.173	0.232	0.015	0.009	0.063	0.000	10
WRP	0.199	0.249	0.173	0.603	0.284	0.357	0.060	0.242	0.018	0.000	10
WRP	0.140	0.571	0.530	0.542	0.625	0.694	0.326	0.458	0.015	0.000	10
WRP	0.057	0.041	0.104	0.084	0.089	0.410	0.039	0.077	0.003	0.000	10
WRP	0.586	0.625	0.540	0.526	0.559	0.545	0.116	0.453	0.280	0.000	9
WRP	0.136	0.060	0.025	0.024	0.052	0.033	0.023	0.061	0.096	0.000	9
WRP	0.158	0.104	0.022	0.039	0.123	0.419	0.006	0.139	0.004	0.000	10
WRP	0.237	0.363	0.411	0.458	0.347	0.680	0.255	0.393	0.042	0.000	10
WRP	0.720	0.354	0.799	0.721	0.786	0.742	0.431	0.660	0.072	0.000	10
WRP	0.304	0.452	0.461	0.297	0.327	0.530	0.181	0.300	0.070	0.000	10
WRP	0.319	0.543	0.568	0.581	0.620	0.846	0.137	0.736	0.093	0.000	10
WRP	0.063	0.283	0.080	0.181	0.382	0.517	0.025	0.413	0.005	0.000	10
WRP	0.304	0.422	0.212	0.211	0.302	0.425	0.035	0.128	0.023	0.000	10
WRP	0.201	0.206	0.095	0.197	0.142	0.577	0.050	0.150	0.084	0.000	9
WRP	0.000	0.001	0.000	0.001	0.003	0.055	0.002	0.001	0.000	0.000	9
WRP	0.231	0.085	0.033	0.067	0.130	0.351	0.053	0.139	0.015	0.000	9
WRP	0.081	0.201	0.026	0.470	0.107	0.261	0.055	0.072	0.010	0.000	8
WRP	0.648	0.374	0.547	0.889	0.575	0.700	0.299	0.651	0.037	0.000	10
WRP	0.464	0.179	0.743	0.659	0.540	0.717	0.092	0.481	0.093	0.000	10
WRP	0.758	0.893	0.901	0.932	0.822	0.929	0.520	0.852	0.264	0.000	10
WRP	0.021	0.072	0.056	0.006	0.013	0.306	0.111	0.024	0.010	0.000	9
WRP	0.118	0.110	0.079	0.271	0.125	0.200	0.054	0.028	0.038	0.000	10
RCP	0.846	0.510	0.834	0.686	0.722	0.535	0.492	0.732	0.063	0.000	9
RCP	0.436	0.263	0.353	0.413	0.193	0.190	0.341	0.191	0.133	0.000	10
RCP	0.695	0.820	0.699	0.351	0.638	0.666	0.761	0.357	0.177	0.000	10
RCP	0.482	0.251	0.502	0.316	0.271	0.229	0.873	0.263	0.076	0.000	10
RCP	0.196	0.564	0.668	0.326	0.782	0.321	0.733	0.310	0.026	0.000	9
RCP	0.315	0.599	0.333	0.574	0.565	0.365	0.536	0.456	0.164	0.000	8
RCP	0.502	0.696	0.348	0.476	0.283	0.416	0.820	0.213	0.079	0.000	10
RCP	0.129	0.027	0.083	0.126	0.034	0.056	0.601	0.086	0.007	0.000	9
RCP	0.737	0.652	0.961	0.786	0.855	0.636	0.756	0.770	0.258	0.000	10
RCP	0.016	0.063	0.096	0.047	0.210	0.166	0.539	0.033	0.012	0.000	10
RCP	0.479	0.484	0.109	0.253	0.184	0.099	0.757	0.223	0.158	0.000	10
RCP	0.333	0.122	0.282	0.178	0.077	0.119	0.554	0.518	0.043	0.000	10
RCP	0.215	0.137	0.243	0.341	0.253	0.075	0.452	0.302	0.045	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
RCP	0.325	0.388	0.449	0.537	0.193	0.151	0.618	0.300	0.028	0.000	10
RCP	0.340	0.355	0.795	0.790	0.629	0.704	0.619	0.336	0.054	0.000	10
RCP	0.227	0.153	0.025	0.032	0.050	0.461	0.286	0.050	0.063	0.000	10
RCP	0.045	0.124	0.212	0.038	0.077	0.232	0.579	0.036	0.004	0.000	10
Casey	0.222	0.456	0.445	0.155	0.268	0.574	0.037	0.314	0.237	0.000	6
Casey	0.371	0.274	0.257	0.233	0.173	0.512	0.053	0.665	0.081	0.000	10
Casey	0.257	0.320	0.090	0.150	0.227	0.016	0.112	0.636	0.079	0.000	10
Casey	0.180	0.219	0.110	0.230	0.287	0.291	0.168	0.654	0.006	0.000	9
Casey	0.297	0.143	0.134	0.277	0.205	0.233	0.042	0.458	0.025	0.000	10
Casey	0.019	0.017	0.006	0.121	0.032	0.078	0.048	0.152	0.004	0.000	10
Casey	0.689	0.297	0.529	0.694	0.571	0.486	0.307	0.663	0.081	0.000	10
Casey	0.208	0.358	0.212	0.163	0.316	0.580	0.146	0.405	0.015	0.000	10
Casey	0.205	0.187	0.228	0.134	0.469	0.176	0.018	0.661	0.037	0.000	10
Casey	0.300	0.261	0.148	0.425	0.580	0.092	0.088	0.877	0.064	0.000	10
Casey	0.058	0.317	0.055	0.119	0.270	0.111	0.063	0.286	0.010	0.000	10
Casey	0.347	0.143	0.070	0.295	0.333	0.163	0.086	0.308	0.002	0.000	10
Casey	0.541	0.482	0.558	0.523	0.636	0.682	0.052	0.889	0.058	0.000	10
Casey	0.216	0.654	0.296	0.343	0.582	0.399	0.680	0.571	0.036	0.000	10
Casey	0.297	0.113	0.301	0.417	0.494	0.465	0.122	0.615	0.010	0.000	10
Casey	0.034	0.053	0.002	0.221	0.078	0.084	0.024	0.367	0.007	0.000	9
Casey	0.084	0.022	0.022	0.060	0.036	0.025	0.031	0.051	0.002	0.000	10
Casey	0.326	0.187	0.265	0.541	0.378	0.315	0.093	0.615	0.173	0.000	9
Casey	0.395	0.717	0.244	0.802	0.645	0.472	0.128	0.326	0.214	0.000	10
Casey	0.442	0.514	0.527	0.372	0.531	0.346	0.064	0.573	0.033	0.000	10
Casey	0.108	0.322	0.478	0.172	0.294	0.181	0.059	0.417	0.019	0.000	10
Casey	0.482	0.120	0.771	0.603	0.502	0.241	0.208	0.645	0.113	0.000	10
Casey	0.764	0.365	0.726	0.809	0.574	0.524	0.155	0.811	0.225	0.000	10
Casey	0.456	0.343	0.325	0.116	0.186	0.366	0.131	0.414	0.108	0.000	9
Casey	0.475	0.442	0.190	0.421	0.644	0.313	0.047	0.861	0.079	0.000	10
Casey	0.084	0.187	0.043	0.106	0.090	0.050	0.015	0.242	0.084	0.000	10
Casey	0.304	0.494	0.046	0.383	0.582	0.110	0.025	0.845	0.093	0.000	10
Casey	0.082	0.063	0.016	0.097	0.079	0.068	0.033	0.366	0.001	0.000	10
Casey	0.722	0.822	0.421	0.822	0.748	0.574	0.192	0.814	0.262	0.000	10
Casey	0.405	0.090	0.289	0.119	0.179	0.046	0.063	0.606	0.006	0.000	10
Casey	0.339	0.123	0.386	0.663	0.596	0.252	0.214	0.798	0.029	0.000	10
Casey	0.344	0.239	0.142	0.363	0.428	0.295	0.084	0.816	0.060	0.000	10
Casey	0.728	0.567	0.675	0.452	0.535	0.465	0.136	0.836	0.017	0.000	10
Casey	0.587	0.234	0.520	0.234	0.510	0.311	0.034	0.750	0.014	0.000	9
Casey	0.250	0.231	0.151	0.083	0.015	0.005	0.056	0.111	0.035	0.000	10
Casey	0.245	0.157	0.085	0.083	0.079	0.084	0.020	0.112	0.005	0.000	10
Casey	0.034	0.036	0.013	0.002	0.033	0.003	0.010	0.171	0.002	0.000	10
Casey	0.067	0.097	0.071	0.114	0.036	0.181	0.016	0.123	0.010	0.000	10
Casey	0.291	0.384	0.142	0.540	0.354	0.346	0.189	0.632	0.107	0.000	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
IW	0.038	0.061	0.096	0.009	0.033	0.055	0.012	0.012	0.083	0.000	10
IW	0.517	0.271	0.325	0.588	0.271	0.200	0.191	0.300	0.611	0.000	10
IW	0.124	0.105	0.039	0.097	0.068	0.020	0.018	0.041	0.530	0.000	10
IW	0.012	0.472	0.098	0.025	0.030	0.039	0.067	0.083	0.615	0.000	9
IW	0.423	0.149	0.188	0.320	0.332	0.262	0.477	0.469	0.525	0.000	10
IW	0.100	0.307	0.126	0.503	0.421	0.486	0.053	0.077	0.136	0.000	10
IW	0.481	0.480	0.813	0.659	0.509	0.694	0.403	0.264	0.539	0.000	10
IW	0.460	0.356	0.361	0.048	0.150	0.067	0.099	0.416	0.503	0.000	9
IW	0.987	0.606	0.796	0.202	0.602	0.439	0.105	0.333	0.768	0.000	9
IW	0.185	0.136	0.297	0.053	0.047	0.288	0.032	0.050	0.064	0.000	9
IW	0.218	0.527	0.568	0.462	0.275	0.201	0.401	0.345	0.479	0.000	10
IW	0.145	0.325	0.231	0.174	0.158	0.285	0.024	0.240	0.163	0.000	10
IW	0.154	0.317	0.289	0.109	0.403	0.297	0.052	0.246	0.406	0.000	9
IW	0.561	0.773	0.385	0.276	0.439	0.429	0.109	0.348	0.634	0.000	9
IW	0.233	0.087	0.122	0.038	0.122	0.088	0.145	0.048	0.182	0.000	9
IW	0.234	0.134	0.190	0.059	0.224	0.308	0.047	0.287	0.395	0.000	10
IW	0.034	0.157	0.008	0.076	0.180	0.052	0.001	0.026	0.147	0.000	9
IW	0.079	0.041	0.046	0.047	0.005	0.099	0.034	0.019	0.324	0.000	10
IW	0.478	0.534	0.669	0.155	0.330	0.504	0.233	0.375	0.758	0.000	9
IW	0.317	0.191	0.098	0.250	0.175	0.041	0.065	0.078	0.930	0.000	9
IW	0.617	0.822	0.349	0.723	0.522	0.212	0.148	0.402	0.751	0.000	9
IW	0.173	0.038	0.059	0.013	0.016	0.042	0.006	0.028	0.464	0.000	9
IW	0.140	0.166	0.061	0.157	0.014	0.052	0.369	0.110	0.381	0.000	8
IW	0.011	0.004	0.004	0.001	0.015	0.001	0.009	0.011	0.579	0.000	10
IW	0.014	0.147	0.029	0.053	0.009	0.014	0.015	0.011	0.755	0.000	9
IW	0.307	0.069	0.029	0.019	0.015	0.037	0.046	0.044	0.685	0.000	9
IW	0.082	0.340	0.006	0.035	0.054	0.026	0.011	0.223	0.352	0.000	10
IW	0.408	0.442	0.140	0.723	0.748	0.826	0.091	0.135	0.255	0.000	10
IW	0.215	0.337	0.039	0.207	0.050	0.246	0.037	0.060	0.795	0.000	9
IW	0.504	0.621	0.415	0.436	0.229	0.281	0.249	0.095	0.489	0.000	9
IW	0.246	0.120	0.190	0.389	0.125	0.251	0.106	0.229	0.963	0.000	9
IW	0.050	0.022	0.000	0.012	0.007	0.006	0.010	0.063	0.275	0.000	9
EK	0.002	0.003	0.002	0.001	0.010	0.014	0.001	0.028	0.000	0.346	10
EK	0.058	0.013	0.013	0.005	0.099	0.011	0.008	0.080	0.001	0.364	10
EK	0.000	0.004	0.001	0.001	0.002	0.000	0.004	0.003	0.000	0.551	10
EK	0.038	0.006	0.011	0.040	0.021	0.002	0.023	0.018	0.001	0.382	10
EK	0.027	0.013	0.009	0.015	0.370	0.111	0.012	0.046	0.000	0.072	10
EK	0.004	0.001	0.001	0.000	0.001	0.000	0.002	0.000	0.002	0.121	10
EK	0.000	0.002	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.035	10
EK	0.000	0.004	0.002	0.001	0.001	0.001	0.002	0.004	0.000	0.221	10
EK	0.001	0.004	0.002	0.001	0.002	0.000	0.002	0.006	0.000	0.528	10
EK	0.004	0.001	0.002	0.001	0.008	0.001	0.002	0.006	0.000	0.476	10
EK	0.038	0.012	0.019	0.027	0.062	0.021	0.012	0.093	0.000	0.843	10

Appendix F. Continued

	Probability										
Population	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	No. Alleles
EK	0.004	0.027	0.006	0.011	0.016	0.012	0.013	0.011	0.000	0.441	10
EK	0.004	0.003	0.006	0.003	0.002	0.021	0.001	0.004	0.000	0.027	10
EK	0.001	0.004	0.002	0.001	0.001	0.001	0.001	0.005	0.000	0.815	10
EK	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.564	9
EK	0.001	0.004	0.006	0.004	0.009	0.001	0.002	0.005	0.000	0.750	10
EK	0.001	0.002	0.004	0.003	0.002	0.001	0.001	0.008	0.000	0.525	10
EK	0.016	0.026	0.008	0.058	0.086	0.022	0.049	0.028	0.000	0.862	5
EK	0.004	0.007	0.004	0.001	0.001	0.000	0.005	0.005	0.000	0.527	10
EK	0.013	0.003	0.006	0.001	0.002	0.001	0.000	0.013	0.000	0.575	10
EK	0.010	0.004	0.009	0.005	0.062	0.005	0.006	0.049	0.000	0.528	10
EK	0.007	0.077	0.010	0.038	0.217	0.055	0.006	0.143	0.000	0.490	8
EK	0.000	0.062	0.008	0.000	0.007	0.013	0.021	0.002	0.001	0.738	7
EK	0.019	0.045	0.033	0.037	0.052	0.281	0.008	0.010	0.016	0.338	5
EK	0.001	0.002	0.000	0.000	0.001	0.000	0.000	0.002	0.000	0.473	10
EK	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.168	7
EK	0.045	0.023	0.148	0.030	0.156	0.301	0.019	0.089	0.000	0.250	9
EK	0.005	0.004	0.002	0.005	0.005	0.000	0.006	0.004	0.001	0.107	10
EK	0.000	0.001	0.000	0.003	0.001	0.001	0.003	0.001	0.000	0.570	9
EK	0.000	0.001	0.003	0.000	0.007	0.001	0.002	0.000	0.000	0.269	8
EK	0.011	0.019	0.009	0.026	0.047	0.045	0.020	0.028	0.003	0.241	7
EK	0.041	0.030	0.027	0.017	0.023	0.013	0.039	0.039	0.004	0.374	10
EK	0.011	0.003	0.006	0.001	0.002	0.003	0.000	0.005	0.000	0.780	10
EK	0.000	0.000	0.000	0.003	0.001	0.000	0.000	0.004	0.000	0.365	9
EK	0.116	0.152	0.082	0.226	0.376	0.175	0.239	0.275	0.005	0.175	7
EK	0.005	0.003	0.006	0.001	0.010	0.001	0.002	0.013	0.000	0.369	10
EK	0.010	0.022	0.004	0.001	0.010	0.001	0.011	0.003	0.001	0.227	10
EK	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.030	8
EK	0.130	0.030	0.040	0.055	0.036	0.077	0.001	0.177	0.000	0.305	9
EK	0.001	0.000	0.001	0.001	0.002	0.001	0.000	0.002	0.000	0.653	10
EK	0.003	0.002	0.003	0.016	0.005	0.001	0.002	0.016	0.000	0.521	9
EK	0.008	0.011	0.011	0.011	0.061	0.012	0.048	0.029	0.000	0.824	7
EK	0.007	0.003	0.000	0.003	0.034	0.006	0.000	0.006	0.000	0.158	9
EK	0.004	0.002	0.006	0.005	0.018	0.021	0.002	0.005	0.000	0.205	10
EK	0.005	0.006	0.003	0.000	0.004	0.008	0.001	0.005	0.001	0.598	8
EK	0.005	0.001	0.004	0.001	0.001	0.003	0.000	0.003	0.000	0.517	10
EK	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.000	0.123	10
EK	0.001	0.024	0.001	0.002	0.025	0.002	0.007	0.005	0.000	0.578	8
EK	0.004	0.004	0.006	0.005	0.042	0.003	0.009	0.057	0.000	0.641	10
EK	0.001	0.001	0.000	0.004	0.002	0.006	0.002	0.004	0.000	0.604	9
EK	0.039	0.018	0.037	0.037	0.094	0.148	0.006	0.164	0.000	0.585	8
EK	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.620	10
EK	0.173	0.090	0.085	0.158	0.138	0.117	0.039	0.128	0.010	0.181	10
EK	0.000	0.000	0.000	0.000	0.001	0.002	0.000	0.002	0.000	0.746	9

Appendix F. Continued

Population	Probability										No. Alleles
	PWRC	GNMP	JBWS	BHP	MB	WRP	RCP	Casey	IW	EK	
EK	0.001	0.004	0.005	0.006	0.001	0.001	0.018	0.008	0.002	0.222	10
EK	0.017	0.004	0.006	0.013	0.040	0.029	0.006	0.030	0.000	0.339	10
EK	0.001	0.000	0.001	0.001	0.001	0.000	0.002	0.001	0.000	0.556	10
EK	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.460	9
EK	0.001	0.038	0.006	0.000	0.001	0.011	0.002	0.016	0.000	0.712	8
EK	0.000	0.000	0.001	0.001	0.002	0.000	0.002	0.002	0.000	0.209	10

Appendix G. Identification number, age, gender and encounter history for turtles found in study plots in BHP, MB and WRP. A "1" indicates that a turtle was seen during a survey period, and a "0" that it was not. See Table 5.1 for population abbreviations in identification numbers.

Identification No.	Age	Gender	Encounter History
076/BHP04	Adult	I	10
078/BHP06	Adult	M	10
079/BHP07	Adult	F	10
082/BHP10	Adult	M	10
083/BHP11	Adult	M	11
092/BHP14	Subadult	M	10
093/BHP15	Adult	M	10
094/BHP16	Adult	M	10
095/BHP17	Adult	M	10
097/BHP19	Juv	I	10
098/BHP20	Adult	M	10
203/BHP22	Adult	M	10
204/BHP23	Adult	M	10
205/BHP24	Adult	M	10
207/BHP26	Adult	M	10
210/BHP29	Adult	M	11
218/BHP35	Adult	M	10
222/BHP36	Juv	I	10
223/BHP37	Juv	I	10
224/BHP38	Adult	M	10
338/BHP42	Subadult	I	01
341/BHP45	Adult	M	01
349/BHP50	Adult	M	01
350/BHP51	Adult	M	01
352/BHP52	Adult	M	01
353/BHP53	Adult	M	01
354/BHP54	Adult	M	01
411/BHP58	Subadult	I	01
412/BHP59	Adult	M	01
420/BHP60	Adult	F	01
499/BHP61	Subadult	M	01
500/BHP62	Adult	M	01
501/BHP63	Adult	M	01
012/MB002	Adult	M	100
013/MB003	Adult	M	100
014/MB004	Juv	I	100
015/MB005	Adult	M	111
016/MB006	Adult	M	100

Appendix G. Continued

Identification No.	Age	Gender	Encounter History
017/MB007	Adult	M	111
018/MB008	Juv	I	100
019/MB009	Adult	M	100
020/MB010	Subadult	I	101
021/MB011	Adult	M	101
022/MB012	Juv	I	101
023/MB013	Adult	F	101
024/MB014	Adult	M	100
025/MB015	Adult	M	100
026/MB016	Adult	M	101
027/MB017	Adult	M	100
028/MB018	Adult	M	100
029/MB019	Adult	F	101
030/MB020	Adult	M	100
031/MB021	Adult	M	111
032/MB022	Adult	M	101
033/MB023	Adult	M	100
036/MB025	Adult	F	100
037/MB026	Adult	M	100
039/MB028	Adult	F	101
041/MB030	Adult	F	110
042/MB031	Adult	M	111
043/MB032	Adult	M	101
044/MB033	Adult	M	101
049/MB035	Adult	F	100
085/MB037	Adult	M	011
086/MB038	Adult	F	010
087/MB039	Adult	M	010
088/MB040	Adult	F	010
089/MB041	Juv	I	010
090/MB042	Adult	M	011
099/MB043	Adult	I	010
100/MB044	Juv	I	010
106/MB045	Adult	M	010
107/MB046	Adult	M	010
108/MB047	Adult	I	010
109/MB048	Adult	F	010
110/MB049	Adult	M	010
111/MB050	Adult	I	010

Appendix G. Continued

Identification No.	Age	Gender	Encounter History
112/MB051	Adult	M	011
113/MB052	Subadult	I	010
123/MB053	Adult	M	010
124/MB054	Adult	M	011
125/MB055	Subadult	M	010
126/MB056	Adult	M	010
129/MB059	Adult	F	011
153/MB062	Adult	M	010
154/MB063	Adult	F	010
167/MB065	Adult	M	010
182/MB070	Adult	M	010
183/MB071	Adult	F	010
184/MB072	Adult	M	010
185/MB073	Adult	M	010
186/MB074	Adult	M	010
187/MB075	Adult	M	011
188/MB076	Adult	F	010
438/MB077	Adult	M	001
460/MB078	Adult	M	001
461/MB079	Adult	F	001
462/MB080	Adult	M	001
464/MB081	Adult	M	001
465/MB082	Subadult	I	001
466/MB083	Adult	M	001
467/MB084	Adult	F	001
468/MB085	Subadult	I	001
470/MB087	Adult	M	001
471/MB088	Adult	M	001
472/MB089	Adult	M	001
474/MB090	Adult	F	001
475/MB091	Adult	M	001
476/MB092	Juv	I	001
477/MB093	Juv	I	001
478/MB094	Adult	M	001
479/MB095	Adult	F	001
480/MB096	Adult	I	001
483/MB099	Adult	M	001
484/MB100	Adult	M	001
486/MB102	Adult	F	001

Appendix G. Continued

Identification No.	Age	Gender	Encounter History
487/MB103	Juv	I	001
489/MB105	Adult	M	001
490/MB106	Adult	F	001
491/MB107	Adult	M	001
492/MB108	Adult	M	001
051/WP001	Adult	F	1010
052/WP002	Adult	M	1000
053/WP003	Juv	I	1000
054/WP004	Adult	F	1001
055/WP005	Adult	F	1001
056/WP006	Adult	M	1100
057/WP007	Adult	M	1000
058/WP008	Adult	M	1100
059/WP009	Adult	M	1110
060/WP010	Adult	M	1101
063/WP011	Adult	M	1100
064/WP012	Juv	I	1010
066/WP014	Adult	M	1000
067/WP015	Adult	M	1010
068/WP016	Adult	M	1010
069/WP017	Adult	F	1010
070/WP018	Adult	M	0101
071/WP019	Adult	M	0101
072/WP020	Adult	M	0100
117/WP024	Juv	I	0100
118/WP025	Adult	M	0101
140/WP026	Juv	I	0100
141/WP027	Adult	M	0111
142/WP028	Juv	I	0100
143/WP029	Juv	I	0100
156/WP029	Adult	F	0110
157/WP030	Adult	M	0111
158/WP031	Adult	F	0100
159/WP032	Subadult	F	0110
162/WP033	Adult	F	0100
163/WP034	Adult	M	0110
164/WP035	Subadult	F	0100
165/WP036	Adult	F	0100

Appendix G. Continued

Identification No.	Age	Gender	Encounter History
166/WP037	Subadult	F	0100
175/WP038	Adult	F	0100
176/WP039	Adult	M	0101
177/WP040	Juv	I	0100
178/WP041	Adult	M	0100
198/WP042	Subadult	F	0100
199/WP043	Adult	M	0101
200/WP044	Adult	M	0101
201/WP045	Adult	M	0100
213/WP046	Adult	M	0101
214/WP047	Adult	M	0101
219/WP048	Juv	I	0100
220/WP049	Adult	M	0101
221/WP050	Adult	M	0101
225/WP051	Adult	M	0100
226/WP052	Adult	I	0100
227/WP053	Adult	M	0101
228/WP054	Adult	M	0101
229/WP055	Adult	F	0100
359/WP058	Adult	M	0010
361/WP060	Adult	I	0010
362/WP061	Adult	M	0010
363/WP062	Adult	M	0010
371/WP064	Subadult	M	0010
378/WP065	Juv	I	0011
379/WP066	Juv	I	0010
380/WP067	Adult	M	0010
381/WP068	Adult	M	0010
382/WP069	Adult	M	0011
415/WP070	Adult	F	0010
416/WP071	Adult	M	0010
417/WP072	Adult	F	0010
418/WP073	Adult	F	0011
419/WP074	Juv	I	0011
437/WP075	Juv	I	0010
445/WP076	Adult	F	0011
454/WP077	Juv	I	0010
493/WP078	Adult	F	0010

Appendix G. Continued

Identification No.	Age	Gender	Encounter History
520/WP079	Adult	M	0001
521/WP080	Adult	M	0001
522/WP081	Adult	M	0001
527/WP086	Adult	M	0001
528/WP087	Adult	M	0001
529/WP088	Adult	F	0001
530/WP089	Subadult	I	0001
531/WP090	Adult	M	0001
532/WP091	Adult	F	0001
533/WP092	Adult	I	0001
534/WP093	Adult	F	0001
535/WP094	Juv	I	0001
536/WP095	Juv	I	0001
537/WP096	Juv	I	0001
538/WP097	Juv	I	0001
539/WP098	Adult	F	0001
540/WP099	Adult	M	0001
541/WP100	Adult	F	0001
542/WP101	Juv	I	0001
543/WP102	Juv	I	0001
544/WP103	Adult	F	0001
545/WP104	Juv	I	0001
546/WP105	Adult	M	0001
547/WP106	Juv	I	0001
548/WP107	Adult	M	0001

Appendix H. Box turtle road survey dates and results, by park. Time searched recorded to the nearest quarter hour. “L” indicates turtle was alive when found. “D” indicates that the turtle was dead. “A” indicates an adult turtle (M for male, F for female), and “J” a juvenile. “I” indicates age or gender was indeterminate. Times in italics indicate surveys conducted only by vehicle. Numbers in parentheses in total rows are live turtles included in road mortality probability estimates. See Table 5.1 for population abbreviations.

	BHP						MB						WRP					
Year	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex
2005	22-May	3.75	0	0			22-May	2.50	0	0			12-May	2.75	0	1[a]	A	I
	1-Jun	4.75	0	0			1-Jun	1.50	0	0			26-May	4.25	0	1	A	F
	4-Jun	3.00	0	0			4-Jun	1.75	0	0			2-Jun	*0.75*	0	0		
	8-Jun	*0.75*	0	0			8-Jun	*0.50*	0	0			3-Jun	3.75	0	0		
	14-Jun	6.75	0	3	J	I	14-Jun	1.00	0	2	A	I	8-Jun	0.75	0	0		
					A	I					A	I	9-Jun	3.00	0	0		
					A	I	18-Jun	*0.50*	0	0			15-Jun	4.75	0	1	A	I
	18-Jun	*0.50*	0	0			23-Jun	*0.75*	0	0			18-Jun	*0.75*	0	0		
	23-Jun	*0.75*	0	0			25-Jun	1.50	0	0			22-Jun	4.50	0	0		
	25-Jun	4.25	0	0			30-Jun	*0.50*	0	0			23-Jun	*0.75*	0	0		
	30-Jun	*0.75*	0	0			2-Jul	*0.50*	0	0			30-Jun	*0.75*	0	0		
	2-Jul	*0.50*	0	0			7-Jul	1.50	0	0			2-Jul	*0.75*	0	0		
	7-Jul	4.50	0	1	I	I	9-Jul	*0.75*	0	0			6-Jul	4.50	2	0	A	F[b]
	9-Jul	*0.50*	0	0			13-Jul	*0.50*	0	0							A	F[c(1)]
	13-Jul	*0.75*	0	0			15-Jul	0.75	1[b]	0	A	F	9-Jul	4.50	0	0		
	15-Jul	3.50	0	1	A	F	19-Jul	*0.75*	0	0			13-Jul	*1.00*	0	0		
	19-Jul	*1.25*	0	1	A	I	20-Jul	*0.75*	0	0			17-Jul	3.75	0	1	A	F
	20-Jul	1.00	0	0			25-Jul	*0.75*	0	0			19-Jul	1.25	0	0		
	25-Jul	1.50	0	0			5-Aug	*0.50*	0	0			20-Jul	*1.00*	0	0		
	5-Aug	*0.75*	0	0			6-Aug	*0.75*	0	0			25-Jul	*2.00*	0	1	A	M

Appendix H. Continued

	BHP						MB						WRP					
Year	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex
2005	6-Aug	*0.75*	0	0			10-Aug	*0.75*	0	0			5-Aug	*1.00*	0	0		
	10-Aug	*1.25*	0	0			12-Aug	*0.75*	0	0			6-Aug	*1.25*	0	0		
	12-Aug	*1.00*	0	0									10-Aug	*0.75*	0	0		
													12-Aug	*1.00*	0	0		
	Total	42.50	0	6				19.50	1 (0)	2				49.50	2 (1)	5		
		7 hrs/turtle						6.5 hrs/turtle						6.2 hrs/turtle				
2006	16-May	*0.75*	0	0			16-May	*0.50*	0	0			16-May	*0.75*	0	0		
	18-May	*0.50*	0	0			18-May	*0.50*	0	0			18-May	*0.75*	0	0		
	25-May	3.25	0	0			25-May	*0.75*	0	0			24-May	5.00	1	0	A	M^d
	26-May	*0.25*	1	1	A	M^d	26-May	1.25	0	0			26-May^e	9.50	2	0	A	$F^{c(1)}$
					A	M	27-May	0.75	0	0							J	$I^{c(1)}$
	27-May	4.00	1	0	A	$F^{c(2)}$	28-May	*1.00*	0	0			27-May	3.50	1	0	A	F^d
	28-May	2.25	0	0			29-May	*0.75*	0	1	A	I	28-May	2.25	0	0		
	29-May	*0.50*	0	0			30-May	*0.50*	0	0			29-May	4.25	0	0		
	30-May	1.25	0	0			31-May	0.75	0	0			30-May	3.00	1	0	A	$M^{f(1)}$
	31-May	3.75	0	0			1-Jun	*0.75*	0	0			31-May	2.50	0	0		
	1-Jun	*1.25*	0	1	A	F	2-Jun	*0.75*	0	0			1-Jun	*1.25*	0	0		
	2-Jun	7.00	1	0	A	$F^{c(3)}$	3-Jun	1.25	0	0			3-Jun	3.25	0	0		
	3-Jun	4.00	0	0			4-Jun	1.25	0	0			4-Jun	2.25	0	0		
	4-Jun	3.75	0	0			5-Jun	*0.75*	0	0			5-Jun	2.25	0	0		
	5-Jun	2.00	0	0			6-Jun	*0.50*	0	0			6-Jun	2.50	0	1	A	I
							7-Jun	*0.75*	0	0			7-Jun	2.00	0	0		

Appendix H. Continued

	BHP						MB						WRP					
Year	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex
2006	6-Jun	2.00	0	0			8-Jun	*2.50*	0	0			8-Jun	*0.75*	0	0		
	7-Jun	2.50	1 (F)	1 (M)	A	F[g]	9-Jun	*0.75*	0	0			9-Jun	3.75	0	0		
					A	M	10-Jun	*0.50*	0	0			10-Jun	*1.00*	0	0		
	8-Jun	*1.00*	0	0			11-Jun	*0.50*	0	0			11-Jun	*0.75*	0	0		
	9-Jun	*0.75*	0	0			12-Jun	*1.25*	0	0			12-Jun	2.25	0	0		
	10-Jun	2.75	0	0			13-Jun	*0.50*	0	0			13-Jun	*1.00*	0	0		
	11-Jun	*0.75*	0	0			14-Jun	*1.25*	0	2	A	F	14-Jun	3.75	0	0		
	12-Jun	2.25	0	0							A	I	15-Jun	2.25	0	0		
	13-Jun	4.75	0	0			15-Jun	1.50	0	0								
	14-Jun	*0.75*	0	0			16-Jun	1.00	0	0			16-Jun	1.75	0	0		
	15-Jun	1.75	0	0			17-Jun	*0.75*	0	0			17-Jun	2.25	0	1	A	I
	16-Jun	3.50	0	0			18-Jun	*1.25*	0	0			18-Jun	2.25	0	0		
	17-Jun	3.25	1	0	A	M[c(3)]	19-Jun	*0.50*	0	0			19-Jun	*1.00*	0	0		
	18-Jun	1.75	0	0			20-Jun	*1.00*	0	0			20-Jun	2.25	1	0	A	F[c(5)]
	19-Jun	*1.00*	0	0			21-Jun	2.25	1	0	A	F[c(4)]	21-Jun	2.50	0	1	A	I
	20-Jun	2.25	0	0			22-Jun	2.25	0	0			22-Jun	*0.75*	0	0		
	21-Jun	*0.75*	0	0			23-Jun	*1.00*	0	1	A	F	23-Jun	2.75	0	0		
	22-Jun	*1.50*	1	1	A	F[c(2)]	24-Jun[h]		0	1	A	F	26-Jun	*0.75*	0	0		
					A	F	26-Jun	*0.50*	0	0			28-Jun[h]		2	0	A	F[c(6)]
	23-Jun	*1.00*	0	1	A	F	29-Jun	*0.50*	0	0							A	M[d]
	25-Jun[h]		1	0	A	M[c(3)]	3-Jul	*0.75*	0	0			29-Jun	*1.00*	0	0		
	26-Jun	*0.75*	0	0			4-Jul	*1.25*	0	0			3-Jul	*1.00*	0	0		

Appendix H. Continued

	BHP						MB						WRP					
Year	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex
2006	29-Jun	*1.00*	0	0			6-Jul	*1.00*	0	1	I	I	4-Jul	1.75	0	1	A	M[i]
	30-Jun		0	1	A	M	8-Jul	*1.00*	0	0			6-Jul	*1.00*	0	0		
	3-Jul	3.00	0	0			9-Jul	*0.50*	0	0			8-Jul	1.75	0	0		
	4-Jul	2.00	0	0			11-Jul	*1.00*	0	0			9-Jul	2.00	0	0		
	6-Jul	*1.00*	0	1	I	I	12-Jul	*1.00*	0	0			11-Jul	1.75	0	0		
	8-Jul	2.00	0	2	A	I	13-Jul	*0.75*	0	0			12-Jul	1.75	0	0		
					A	I	14-Jul	1.00	0	0			13-Jul	3.00	1	0	A	F[b]
	9-Jul	1.75	0	0			16-Jul	*0.50*	0	0			14-Jul	*1.00*	0	0		
	11-Jul	*1.25*	0	1	J	I	17-Jul	*0.50*	0	0			16-Jul	*0.75*	0	0		
	12-Jul	1.75	0	0			18-Jul	*0.75*	0	0			17-Jul	*0.75*	0	0		
	13-Jul	*1.25*	0	1	A	M	19-Jul	1.75	0	0			18-Jul	1.75	0	0		
	14-Jul	3.00	1	0	A	M[c(2)]	20-Jul	1.75	0	0			19-Jul	2.00	0	0		
	16-Jul	*0.75*	0	0			22-Jul	*1.75*	0	0			20-Jul	*0.75*	0	0		
	17 Jul [e]	5.50	0	1	A	I	25-Jul	*1.00*	0	1	A	M	22-Jul	1.75	0	0		
	18-Jul	1.25	0	0			27-Jul	*0.75*	0	0			25-Jul	1.75	0	0		
	19-Jul	2.25	0	0			28-Jul	*0.50*	0	0			27-Jul	2.25	0	0		
	20-Jul	*1.00*	0	0			30-Jul	2.25	0	0			28-Jul	*1.00*	0	0		
	22-Jul	*1.50*	0	0			31-Jul	*0.50*	0	0			30-Jul	2.25	0	0		
	25-Jul	1.25	0	1	A	I	2-Aug	*1.00*	0	0			31-Jul	*0.75*	0	0		
	27-Jul	*0.75*	0	0			3-Aug	*0.75*	0	0			2-Aug	*1.00*	0	0		
	28-Jul	*0.75*	0	0			5-Aug	*1.00*	0	0			3-Aug	*1.00*	0	0		
	30-Jul	*1.00*	0	0			7-Aug	*1.00*	0	0			5-Aug	1.50	0	0		

Appendix H. Continued

	BHP						MB						WRP					
Year	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex
2006	31-Jul	*0.75*	0	0			8-Aug	*0.75*	0	0			7-Aug	1.75	0	0		
	2-Aug	2.75	0	0			10-Aug	*0.75*	0	0			8-Aug	*1.00*	0	0		
	3-Aug	*1.00*	0	0			12-Aug	*0.50*	0	0			10-Aug	*1.75*	0	0		
	5-Aug	*1.25*	0	0			14-Aug	*0.50*	0	0			12-Aug	3.25	0	0		
	7-Aug	1.50	0	0			16-Aug	*1.00*	0	0			14-Aug	*0.75*	0	0		
	8-Aug	2.50	0	1	I	I	18-Aug	*0.75*	0	0			16-Aug	1.50	0	0		
	10-Aug	2.25	0	0			20-Aug	*1.25*	0	0			18-Aug	*1.00*	0	0		
	12-Aug	*0.75*	0	0			23-Aug	1.75	0	0			20-Aug	*0.75*	0	0		
	14-Aug	*0.75*	0	0			24-Aug	*1.00*	0	0			23-Aug	*1.00*	0	0		
	16-Aug	1.50	0	0			26-Aug	*1.25*	0	0			24-Aug	1.75	0	0		
	18-Aug	1.50	0	1	I	I	27-Aug	*1.75*	0	1	A	M	26-Aug	2.25	0	0		
	20-Aug	2.75	0	0			29-Aug	*1.25*	0	1	A	I	27-Aug	1.50	0	0		
	23-Aug	*0.75*	0	0			30-Aug	*0.75*	0	0			29-Aug	*1.00*	0	0		
	24-Aug	*1.25*	0	0			31-Aug	*0.50*	0	0			30-Aug	1.75	0	0		
	26-Aug	1.75	0	0			Sept 1[h]		0	0			31-Aug	*0.75*	0	0		
	27-Aug	1.50	0	0			3-Sep	*1.25*	0	1	J	I	1-Sep[h]		0	0		
	29-Aug	1.75	0	0			6-Sep	1.25	0	1	A	M	3-Sep	2.00	0	0		
	30-Aug	*0.75*	0	0			7-Sep	*0.50*	0	0			6-Sep	*0.75*	0	0		
	31-Aug	*1.00*	0	0			8-Sep	1.25	0	0			7-Sep	*1.00*	0	0		
	1-Sep[h]		0	0			9-Sep	1.25	0	0			8-Sep	2.25	0	0		
	3-Sep	1.75	0	0			10-Sep	*1.00*	0	0			9-Sep	*1.25*	0	0		
	6-Sep	1.25	0	0			12-Sep	*0.75*	0	0			10-Sep	2.25	1[d]	1	A	M[j]

Appendix H. Continued

	BHP						MB						WRP					
Year	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex	Date	Time	L	D	Age	Sex
2006	7-Sep	*0.75*	0	0			14-Sep	1.00	0	0							A	M
	8-Sep	1.75	0	0			15-Sep[h]		0	1	A	I	12-Sep	*1.00*	0	0		
	9-Sep	2.75	0	0			16-Sep	*1.25*	0	0			14-Sep	2.00	1	0	A	M[j]
	10-Sep	1.75	0	0			17-Sep	0.75	0	0			16-Sep	2.00	0	0		
	12-Sep	2.25	0	0			19-Sep	1.50	0	0			17-Sep	*1.25*	0	0		
	14-Sep	1.50	0	0			20-Sep	*1.00*	0	0			19-Sep	*0.75*	0	0		
	16-Sep	1.50	0	0			21-Sep	*1.00*	0	0			20-Sep	1.00	0	0		
	17-Sep	2.75	0	0			22-Sep	1.25	0	0			21-Sep	1.75	0	0		
	19-Sep	*1.00*	0	0			25-Sep	*1.00*	0	0			22-Sep	2.25	0	0		
	20-Sep	3.25	0	0			26-Sep	*0.50*	0	0			25-Sep	2.75	0	1	A	I
	21-Sep	1.25	0	0									26-Sep	*0.75*	0	0		
	22-Sep	1.50	0	0														
	25-Sep	*0.75*	0	0														
	26-Sep	*0.75*	0	0														
	Total	158.00	8 (3)	15				86.75	1(0)	12				158.75	11(5)	6		
	6.9 hrs/turtle						7.2 hrs/turtle						9.3 hrs/turtle					

[a] May have been killed by a mower.

[b] Successfully crossed road on his/her own; not included in probability estimate.

[c] Moved out of road by field assistant.

[d] No indication that road crossing attempt was imminent. Turtle not included in probability estimate.

[e] Combined time of two assistants.

[f] Recapture of male found on May 24, 2006 at WRP. Turtle was in the road on May 30, and is included in probability estimate.

[g] Recapture of female found June 2, 2006. Turtle had just successfully crossed road when found.

[h] Search time unknown.

Appendix H. Continued

[i] Male turtle initially found in roadside survey of June 28.

[j] Same male found on both Sept 10 and 14th, and in both cases was attempting to access road, though confined by fence. Included as one crossing attempt in probability estimate.

Roads where found:
1) Glenallen Avenue
2) River Road
3) Pennyfield Lock Road
4) Quince Orchard Road
5) Orebaugh Avenue
6) Kempmill Road

LITERATURE CITED

Adams NA, Claussen DL, Skillings J (1989) Effects of temperature on voluntary locomotion of the eastern box turtle (*Terrapene carolina carolina)*. Copeia 1989:905-915

Adams LW, Geis AD (1981) Effects of highways on wildlife. Rep No FHWA/RD-81/067, U.S. Dep Trans, Fed Highway Admin, Washington, DC

Allard HA (1948) The eastern box turtle and its behavior. J Tenn Acad Sci 23:307-321

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell, Malden, Massachusetts

Aresco MJ (2005) The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biol Conserv 123:37-44

Ashley, EP, Robinson JT (1996) Road mortality of amphibians, reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. Can Field-Nat 110:403-412

Avise JC, Bowen BW, Lamb T, Meylan AB, Bermingham E (1992) Mitochondrial DNA evolution at a turtle's pace: Evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Mol Biol Evol 9:457-473

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155-165

Bickham JW (1981) Two-hundred-million-year-old chromosomes: deceleration of the rate of karyotypic evolution in turtles. Science 212:1291-1293

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. Trends Ecol Evol 13:202-206

Boucher TP (1999) Population, growth and thermal ecology of the eastern box turtle, *Terrapene carolina carolina,* (L.), in Fairfax County, Virginia. Dissertation, George Mason University

Brisbin IL Jr., Kennamer RA, Peters EL, Karapatakis DJ (2008) A long-term study of eastern box turtles (*Terrapene c. carolina*) in a suburban neighborhood: survival characteristics and interactions with humans and conspecifics. In: Mitchell JC, Jung Brown RE, Bartholomew B (eds) Urban Herpetology, Herpetological Conservation No 3, Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, pp 373-385

Brooks RJ, Brown GP, Galbraith DA (1991) Effects of a sudden increase in natural mortality of adults on a population of the common snapping turtle (*Chelydra serpentina*). Can J Zool 69:1314-1320

Budischak, SA, Hester, JM, Price SJ, Dorcas ME (2006) Natural history of *Terrapene carolina* (box turtles) in an urbanized landscape. Southeast Nat 5:191-204

Butler JM (2008) Morphology, molecules and delineation of the Gulf Coast box turtle. Thesis, University of Florida

Cagle, FR (1939) A system of marking turtles for future identification. Copeia 1939**:**170-173

Castellano CM, Behler JL, Amato G (2008) Genetic diversity and population genetic structure of the wood turtle (*Glyptemys insculpta*) at Delaware Water Gap National Recreation Area, USA. Conserv Genet DOI 10.1007/s10592-008-9743-6

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Am J Hum Genet 19:233-257

Ciofi C, Milinkovitch MC, Gibbs JP, Caccone A, Powell JR (2002) Microsatellite analysis of genetic divergence among populations of giant Galápagos tortoises. Mol Ecol 11:2265-2283

Congdon JD, Dunham AE, van Loben Sels RC (1993) Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): Implications for conservation and management of long-lived organisms. Conserv Biol 7:826-833

Congdon JD, Dunham AE, van Loben Sels RC (1994) Demographics of common snapping turtles (*Chelydra serpentina*): Implications for conservation and management of long-lived organisms. Amer Zool 34:397-408

Converse SJ, Iverson JB, Savidge JA (2005) Demographics of an ornate box turtle population experiencing minimal human-induced disturbances. Ecol Appl 15:2171-2179

Dodd CK Jr. (1997) Population structure and the evolution of sexual size dimorphism and sex ratios in an insular population of Florida box turtles (*Terrapene carolina bauri*). Can J Zool 75:1495-1507

Dodd CK Jr. (2001) North American box turtles: A natural history. University of Oklahoma Press, Norman

Dodd CK Jr., Enge KM, Stuart JN (1989) Reptiles on highways in north-central Alabama, USA. J Herpetol 23:197-200

Dodd CK Jr., Franz R, Smith LL (1994) Activity patterns and habitat use of box turtles (*Terrapene carolina bauri*) on a Florida island, with recommendations for management. Chelonian Conserv Biol 1:97-106

Dolbeer, RA (1969) A study of population density, seasonal movements and weight changes, and winter behavior of the Eastern box turtle, *Terrapene c. carolina*, in eastern Tennessee. Thesis, University of Tennessee

Doroff AM, Keith LB (1990) Demography and ecology of an ornate box turtle (*Terrapene ornata*) population in south-central Wisconsin. Copeia 1990:387-399

Ernst CH, Lovich JE (2009) Turtles of the United States and Canada. Johns Hopkins University Press, Baltimore

Ernst CH, Lovich JE, Barbour RW (1994) Turtles of the United States and Canada. Smithsonian Institution Press, Washington, DC

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611-2620

Ewing HE (1943) Continued fertility in female box turtles following mating. Copeia 1943:112-114

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. Genetics 131:479-491

Fahrig L (1997) Relative effects of habitat loss and fragmentation on population extinction. J Wildl Manage 61:603-610

Feldman CR, Parham JF (2002) Molecular phylogenetics of Emydine turtles: Taxonomic revision and the evolution of shell kinesis. Mol Phylogen Evol 22:388-398

Ferebee KB, Henry PFP (2008) Movements and distribution of *Terrapene carolina* in a large urban area, Rock Creek National Park, Washington, D.C. In: Mitchell JC, Jung Brown RE, Bartholomew B (eds) Urban Herpetology, Herpetological Conservation No 3, Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, pp 365-372

Festin SM (1996) Summary of national and regional travel trends: 1970-1995. Office of Highway Information Management, Federal Highway Administration. Available via http://www.fhwa.dot.gov/ohim/bluebook.pdf. Accessed 14 June 2009

Fisher RA (1935) The logic of inductive inference. J Royal Stat Soc 98:39-82

FitzSimmons NN, Moritz C, Moore SS (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol Biol Evol 12:432-440

Forman RTT, Alexander LE (1998) Roads and their major ecological effects. Ann Rev Ecol Syst 29:207-231

Forman RTT, Sperling D, Bissonette JA, Clevenger AP, Cutshall CD, Dale VH, Fahrig L, France R, Goldman CR, Heanue K, Jones JA, Swanson FJ, Turrentine T, Winter TC (2003) Road ecology: Science and solutions. Island Press, Washington, DC

Forsythe P, Flitz B, Mullin SJ (2004) Radio telemetry and post-emergent habitat selection of neonate box turtles (Emydidae: *Terrapene carolina)* in central Illinois. Herpetol Rev 35:333-335

Fraser DF, Gilliam JF, Daley MJ, Le AN, Skalski GT (2001) Explaining leptokurtic movement distributions: Intrapopulation variation in boldness and exploration. Am Nat 158:124-135

Gibbons, JW (1987) Why do turtles live so long? BioScience 37:262-269

Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT (2000) The global decline of reptiles, déjà vu amphibians. Bioscience 50:653-666

Gibbs JP, Amato GD (2000) Genetics and demography in turtle conservation. In: Klemens MW (ed) Turtle conservation. Smithsonian Institution Press, Washington, DC, pp 207-217

Gibbs, JP, Shriver WG (2002) Estimating the effects of road mortality on turtle populations. Conserv Biol 16:1647-1652

Gibbs JP, Steen DA (2005) Trends in sex ratios of turtles in the United States: Implications of road morality. Conserv Biol 19:552-556

Glista DJ, DeVault TL, DeWoody JA (2007) Vertebrate road mortality predominantly impacts amphibians. Herpetol Conserv Biol 3:77-87

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html

Gray EM (1995) DNA fingerprinting reveals a lack of genetic variation in northern populations of the western pond turtle (*Clemmys marmorata*). Conserv Biol 9:1244-1255

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372

Hagood S, Bartles MJ (2008) Use of existing culverts by eastern box turtles (*Terrapene c. carolina*) to safely navigate roads. In: Mitchell JC, Jung Brown RE, Bartholomew B (eds) Urban Herpetology, Herpetological Conservation No 3, Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, pp 169-170

Hall RJ, Henry PFP, Bunck CM (1999) Fifty-year trends in a box turtle population in Maryland. Biol Conserv 88:165-172

Hallgren-Scaffidi L (1986) Habitat, home range and population study of the Eastern box turtle (*Terrapene carolina*). Thesis, University of Maryland

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:1-9

Hartl DL, Clark AG (1997) Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts

Hauswaldt JS and Glenn TC (2005) Populations genetics of the diamondback terrapin (*Malaclemys terrapin*). Mol Ecol 14:723-732

Haxton T (2000) Road mortality of snapping turtles, *Chelydra serpentina*, in Central Ontario during their nesting season. Can Field-Nat 114:106-110

Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. Ann Rev Ecol Syst 31:139-162

Hels T, Buchwald E (2001) The effect of road kills on amphibian populations. Biol Conserv 99:331-340

Holman JA (1987) Herpetofauna of the Egelhoff Site (Miocene: Barstovian) of North-Central Nebraska. J Vert Paleon 7:109-120

Howeth JG, McGaugh SE, Hendrickson DA (2008) Contrasting demographic and genetic estimates of dispersal in the endangered Coahuilan box turtle: a contemporary approach to conservation. Mol Ecol DOI 10.1111/j.1365-294X.2008.03904.x

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Res 9:1322-1332

Iuell B, Bekker GJ, Cuperus R, Dufek J, Fry G, Hicks C, Hlaváč V, Keller VB, Rosell C, Sangwine T, Torslov N, Wandall B le Maire B (2003) Wildlife and traffic: a European handbook for identifying conflicts and designing solutions. KNNV Publishers, The Netherlands

Jaeger JAG, Bowman J, Brennan J, Fahrig L, Bert D, Bouchard J, Charbonneau N, Frank K, Gruber B, Tluk von Toschanowitz K (2005) Predicting when animal populations are at risk from roads: an interactive model of road avoidance behavior. Ecol Model 185:329-348

Karl SA, Bowen BW, Avise JC (1992) Global population genetic structure and male-mediated gene flow in the green turtle *(Chelonia mydas):* RFLP analyses of anonymous nuclear loci. Genetics 131:163-173

Kiester AR, Schwartz CW, Schwartz ER (1982) Promotion of gene flow by transient individuals in an otherwise sedentary population of box turtles (*Terrapene carolina triunguis*). Evolution 36:617-619

Killebrew FC (1977) Mitotic chromosomes of turtles, Part 4: The Emydidae. Texas J Sci 29:245-253

King TL, Julian SE (2004) Conservation of microsatellite DNA flanking sequence across 13 Emydid genera assayed with novel bog turtle (*Glyptemys muhlenbergii*) loci. Conserv Genet 5:719-725

Kuo C-H, Janzen FJ (2004) Genetic effects of a persistent bottleneck on a natural population of ornate box turtles (*Terrapene ornata*). Conserv Genet 5:425-437

Langen TA, Machniak A, Crowe EK, Mangan C, Marker DF, Liddle N, Roden B (2007) Methodologies for surveying herpetofauna mortality on rural highways. J Wildl Manage 71:1361-1368

Litzgus JD (2006) Sex differences in longevity in the spotted turtle (*Clemmys guttata*). Copeia 2006:281-288

Lowe A, Harris S, Ashton P (2004) Ecological Genetics: Design, Analysis and Application. Blackwell, Malden, Massachusetts

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189-197

Marchand MN, Litvaitis JA (2004) Effects of habitat features and landscape composition on the population structure of a common aquatic turtle in a region undergoing rapid development. Conserv Biol 18:758-767

Marsack K, Swanson BJ (2009) A genetic analysis of the impact of generation time and road-based habitat fragmentation on eastern box turtles (*Terrapene c. carolina*). Copeia 4:647-652

Maryland Dept of Natural Resources (2009) No Net Loss of Forest Task Force: final report and recommendations. Available via: http://www.dnr.state.md.us/dnrnews/pdfs/NNLTFFINALREPORT1.pdf . Accessed 1 Aug 2009

Maryland State Highway Admin (2009) Maryland's traffic volume maps by county. Available via http://www.marylandroads.com/shaservices/mapsbrochures/maps/oppe/tvmaps.asp. Accessed 7 Sept 2009

McCallum ML, McCallum JL, Trauth SE (2009) Predicted climate change may spark box turtle declines. Amphibia-Reptilia 30:259-264

McDougal J (2000) Conservation of tortoises and terrestrial turtles. In: Klemens MW (ed) Turtle conservation. Smithsonian Institution Press, Washington, DC, pp 180-206

Miller MP (2005) Alleles In Space (AIS): Computer software for the joint analysis of interindividual spatial and genetic information. J Hered 96:722-724

Mitchell JC, Klemens MW (2000) Primary and secondary effects of habitat alteration. In Klemens MW (eds) editor. Turtle conservation. Smithsonian Institution Press, Washington, pp 5-32

Mockford SW, Snyder M, Heman TB (1999) A preliminary examination of genetic variation in a peripheral population of Blanding's turtles, *Emydoidea blandingii.* Mol Ecol 8:323-327

Muegel LA, Claussen DL (1994) Effects of slope on voluntary locomotor performance in the turtle, *Terrapene carolina carolina*. J. Herpetol 28:6-11

Mühlmann-Diaz MC, Ulsh BA, Whicker FW, Hinton TG, Congdon JD, Robinson JF, Bedford JS (2001) Conservation of chromosome 1 in turtles over 66 million years. Cytogenet Cell Genet 92:139-143

Paetkau, D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol Notes 4:347-354

Parks SDE (2001) Trypanotolerance in West African cattle and the population genetic effects of selection. Dissertation, University of Dublin Avail from: http://animalgenomics.ucd.ie/sdepark/ms-toolkit Accessed 18 Aug 2009

Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295

Piry S, Alapetite A, Cornuet, JM, Paetkau D, Baudouin, L, Estoup, A (2004) GeneClass2: A software for genetic assignment and first-generation migrant detection. J Hered 95:536-539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Raymond M, Rousset F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248-249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223-225

Richtsmeier RJ, Bernstein NP, Demastes JW, Black RW (2008) Migration, gene flow, and genetic diversity within and among Iowa populations of ornate box turtles (*Terrapene ornata ornata*). Chelonian Conserv Biol 7:3-11

Rosen PC, Lowe CH (1994) Highway mortality of snakes in the Sonoran Desert of Southern Arizona. Biol Conserv 68:143-148

Rosenbaum PA, Robertson JM, Zamudio KR (2007) Unexpectedly low genetic divergences among populations of the threatened bog turtle (*Glyptemys muhlenbergii*). Conserv Genet 8:331-342

Row JR, Blouin-Demers G, Weatherhead PJ (2007) Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). Biol Conserv 137:117-124

Rubin CS, Warner RE, Bouzat JL, Paige KN (2001) Population genetic structure of Blanding's turtles (*Emydoidea blandingii*) in an urban landscape. Biol Conserv 99:323-330

Schwartz TS, Karl SA (2005) Population and conservation genetics of the gopher tortoise (*Gopherus polyphemus*). Conserv Genet 6:917-928

Schwartz TS, Osentoski M, Lamb T, Karl SA (2003) Microsatellite loci for the North American tortoises (genus *Gopherus*) and their applicability to other turtle species. Mol Ecol Notes 3:283-286

Schwartz CW, Schwartz ER (1974) The three-toed box turtle in central Missouri: its population, home range, and movements. Terrestrial Ser No 5, Missouri Dept Conserv, Jefferson City, Missouri

Schwartz ER, Schwartz CW (1991) A quarter-century study of survivorship in a population of three-toed box turtles in Missouri. Copeia 1991:1120-1123

Schwartz ER, Schwartz CW, Kiester AR (1984) The three-toed box turtle in central Missouri, Part II: a nineteen-year study of home range, movements and population. Missouri Dep of Conserv Terrestrial Series 12:1-28

Scribner KT, Evans JE, Morreale SJ, Smith MH, Gibbons JW (1986) Genetic divergence among populations of the yellow-bellied slider turtle (*Pseudemys scripta*) separated by aquatic and terrestrial habitats. Copeia 3:691-700

Seigel RA (2004) The importance of population demography in the conservation of box turtles: What do we know and what do we need to learn? In: Swarth C, Hagood S (eds) Summary of the Eastern Box Turtle Regional Conservation Workshop, Laurel, MD

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Letters 9:615-629

Shepard DB, Kuhns AR, Dreslik MJ, Phillips CA (2008a) Roads as barriers to animal movement in fragmented landscapes. Anim Conserv 11:288-296

Shepard DB, Dreslik MJ, Jellen BC, Phillips CA (2008b) Reptile road mortality around an oasis in the Illinois Corn Desert with emphasis on the endangered Eastern Massasauga. Copeia 2:350-359

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462

Smith LL, Dodd CK Jr. (2003) Wildlife mortality on U.S. Highway 441 across Paynes Prairie, Alachua County, Florida. Fla Sci 66:128-140

Soulé ME, Bolger DT, Alberts AC, Wright J, Sorice M, Hill S (1988) Reconstructed dynamics of rapid extinctions of chaparral-requiring birds in urban habitat islands. Conserv Biol 2:75-92

Steen DA, Aresco MJ, Beilke SG, Compton BW, Condon EP, Dodd CK Jr., Forrester H, Gibbons JW, Greene JL, Johnson G, Langen TA, Oldham MJ, Oxier DN, Saumure RA, Schueler FW, Sleeman JM, Smith LL, Tucker JK, Gibbs JP (2006) Relative vulnerability of female turtles to road mortality. Anim Conserv 9:269-273

Steen DA, Gibbs JP (2004) Effects of roads on the structure of freshwater turtle populations. Conserv Biol 18:1143-1148

Steen DA, Smith LL (2006) Road surveys for turtles: Consideration of possible sampling biases. Herpetol Conserv Biol 1: 9-15

Stickel LF (1950) Populations and home range relationships of the box turtle, *Terrapene c. carolina* (Linnaeus). Ecol Monogr 20:353-378

Stickel LF (1978) Changes in a box turtle population during three decades. Copeia 1978:221-225

Stickel LF (1989) Home range behavior among box turtles (*Terrapene c. carolina*) of a bottomland forest in Maryland. J Herpetol 23:40-44

Stickel LF, Bunck CM (1989) Growth and morphometrics of the box turtle, *Terrapene c. carolina*. J Herpetol 23:216-223

Swofford DL, Selander RB (1981) BIOSYS-1: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72:281-283

Szerlag S, McRobert SP (2006) Road occurrence and mortality of the northern diamondback terrapin. Applied Herpetol 3:27-37

Tamura K, Dudley J, Nei M Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596-1599

Tessier N, Paquette SR, Lapointe F-J (2005) Conservation genetics of the wood turtle (*Glyptemys insculpta*) in Quebec, Canada. Can J Zool 83:765-772

Thorbjarnarson J, Lagueux CJ, Bolze D, Klemens MW, Meylen AB (2000) Human use of turtles: a worldwide perspective. In: Klemens MW (ed) Turtle conservation. Smithsonian Institution Press, Washington, DC, pp 33-84

Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. Conserv Biol 14:18-30

U.S. Fish and Wildlife Service (1996) Export of box turtles from the United States in 1996. Federal Register 61:3894-3898

von Seckendorff Hoff K, Marlow RW (2002) Impacts of vehicle road traffic on desert tortoise populations with consideration of conservation of tortoise habitat in southern Nevada. Chelonian Conserv Biol 4:449-456

Weir BS, Cockerham, CC (1984) Estimating F-Statistics for the analysis of population structure. Evolution 38:1358–1370

White, GC, Burnham KP (1999) Program MARK: Survival estimation from populations of marked animals. Bird Study 46 (Supplement):120-138

Wilcox BA, Murphy DD (1985) Conservation strategy: The effects of fragmentation on extinction. Am Nat 125:879-887

Williams EC, Parker WS (1987) A long-term study of a box turtle (*Terrapene carolina*) population at Allee Memorial Woods, Indiana, with emphasis on survivorship. Herpetologica 43:328-335

Wilson GL, Ernst CH (2005) Reproductive ecology of the *Terrapene carolina carolina* (eastern box turtle) in central Virginia. Southeast Nat 4:689-702

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97-159

Wright S (1951) The genetical structure of populations. Annals Eugenics 15:323-354

Yahner, RH (1974) Weight change, survival and home range change in the box turtle, *Terrapene carolina*. Copeia 1974:546-548