Defining Turtle Diversity: Proceedings of a Workshop on Genetics, Ethics, and Taxonomy of Freshwater Turtles and Tortoises
H. Bradley Shaffer, Nancy N. FitzSimmons, Arthur Georges, and Anders G.J. Rhodin, Eds.
Chelonian Research Monographs 4:107–123 • © 2007 by Chelonian Research Foundation

Genetic Issues in Freshwater Turtle and Tortoise Conservation

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ABSTRACT. – Freshwater and terrestrial turtles are among the most imperiled biota on the planet, with nearly half of all extant taxa threatened with extinction. Active science-based management is required for the persistence of many species. Evolutionary genetic principles are often overlooked in the development of conservation and management plans, yet genetic data and theory can be critical to program success. Conservation biologists are encouraged to consider using genetic data and concepts when developing conservation strategies for turtles. We identify general areas where genetic principles and empirical data can be profitably used in conservation planning and provide examples from the turtle literature. Finally, we suggest important areas for future research in chelonian conservation genetics.

KEY WORDS. – Reptilia; Testudines; adaptive potential; conservation; forensics; genetic diversity; genetic drift; gene flow; inbreeding; management units; mating systems; outbreeding; taxonomy; trade; turtle

Turtles and tortoises are threatened globally. Approximately 40% (129 taxa) of over 300 extant taxa are regarded as vulnerable or endangered, and many face extinction if effective conservation measures are not implemented. Widespread declines in abundance and distribution documented in recent decades have been caused by habitat destruction, pollution, and overexploitation for trade in meat, pets, and traditional medicines (Gibbons et al., 2000; van Dijk et al., 2000; Turtle Conservation Fund, 2002; Moll and Moll, 2004). The number and intensity of pressures continue to mount, with climate change looming as a new threat, particularly for species with temperature-dependent sex determination (Janzen, 1994; Davenport, 1997; Nelson et al., 2002; Miller et al., 2004; Booth, 2006). Removal or amelioration of immediate threats does not necessarily ensure the persistence of endangered taxa or populations. Remnant populations are more often than not, small and highly fragmented, attributes that exacerbate their vulnerability to extinction from stochastic events and loss of genetic diversity (Lande, 1998; Hager, 1998).

Genetic diversity represents the raw material to facilitate adaptation to changing environmental conditions through natural selection. Hence, loss of genetic diversity can result in the loss of adaptive potential. Global environmental change is occurring at a rate unseen in the history of our planet (Hare and Meinshausen, 2006; Lenton, 2006; Li et al., 2006). If chelonian species are to adapt and persist in the face of future changes, they will likely require active human intervention. Maintaining required levels of genetic diversity is only possible through conservation planning.

Knowledge of genetics is increasingly recognized as a critical element of conservation biology (Moritz, 1994; Soltis and Gitzendanner, 1999). Molecular techniques and methods of statistical analysis derived from evolutionary theory can be used to estimate how genetic diversity is apportioned spatially, how rapidly diversity will be lost over time, to identify crucial forces (anthropogenic or otherwise) contributing to present and future loss of diversity, to gain insight into fundamental aspects of an organism's biology, and to provide informed guidance for conservation and management (Moritz, 1999; Reed and Frankham, 2003; DeYoung and Honeycutt, 2005; Whiteley et al., 2006). Despite the clear importance of genetics as a foundation for understanding turtle biology and directing turtle conservation actions, there is a paucity of turtle genetic studies relative to many other taxa.

We describe how population genetic theory and data can contribute to greater understanding of turtle biology and how this knowledge can be applied to achieve conservation objectives. We address eight major genetic issues that we believe are most relevant to turtle conservation: 1) genetic diversity and potential for future adaptation; 2) genetic drift; 3) inbreeding and outbreeding; 4) selection; 5) gene flow and identification of management units; 6) clarifying taxonomy;

7) elucidating aspects of species' behavior and ecology; and 8) forensics. We provide a glossary of terms (highlighted in bold in the text) that are widely used in population genetics but may not be well known to biologists interested in turtles. Boxes are also included to emphasize several important concepts discussed in the text.

We have written the text to be accessible to the non-specialist and have minimized the use of technical terms. Background theory and concepts are developed and empirical examples are presented to show relevance in areas of turtle conservation. We conclude by suggesting future priorities and directions. We advocate the use of genetics as only one component of a comprehensive conservation toolkit. Genetic principles and data should be complemented with biological, ecological, zoogeographic, socio-economic and other relevant data in order to better direct decisions regarding chelonian conservation and management.

Genetic Diversity and Adaptive Potential

Genetic diversity is a fundamental component of life on earth. Without it, there can be no evolution, no diversification, and thus, little or no biodiversity at any level of biological organization. In a contemporary sense, without genetic diversity, populations cannot respond to biological or environmental changes through natural selection, be those changes natural or anthropogenic in origin (Frankham, 1995a, 2005; Amos and Balmford, 2001).

The **phenotype** of an organism (its observable properties) is determined by an individual's **genotype**, the expression of which is modified by the environment. Adaptation occurs when the phenotypic composition of a population shifts in response to environmental change. The new generation will preferentially represent the genetic composition of parents best able to cope with changes through their ability to survive and leave offspring. The resulting shift in genetic composition of the population reflects adaptation by **natural selection** (Orr, 2005). In the lifetime of an individual, responses to environmental change occur via **phenotypic plasticity** (non-heritable changes in phenotype such as faster growth when conditions are favorable). However, the capacity of an individual to be plastic also has a genetic basis. Variation is required at the level of **genes** coding for traits (Via, 1993; Bradshaw, 2006). Thus, phenotypic plasticity is itself an evolved trait.

The rate of adaptive **microevolution** is roughly proportional to the **additive genetic variance**. Loss of genetic diversity is a fundamental concern in conservation biology because a populations' ability to evolutionarily adapt to changing conditions is reduced when additive genetic variation is depleted (Amos and Balmford, 2001; Frankham, 2005). Given current rates of environmental change, the adaptive potential of populations will be critically linked to their probability of long-term persistence.

Levels of genetic diversity can be assayed by measuring variances and covariances in phenotypic traits among individuals. The field of **quantitative genetics** apportions variation in phenotypic traits resulting from complex interactions between heritable genetic and environmental sources of variation. **Quantitative trait loci** (QTL) are the most relevant targets of genetic studies of phenotypic adaptation (Falconer and MacKay, 1996; Lynch and Walsh, 1998; Barton and Keightley, 2002). However, quantitative genetic studies are difficult to conduct. Established pedigrees and/or large sample sizes are required to disentangle the effects of environment and genotype on quantitative traits (Falconer and MacKay, 1996; Lynch and Walsh, 1998; Barton and Keightley, 2002; Kirkpatrick and Meyer, 2004). It is often impossible to obtain large sample sizes from small wild populations, and establishing pedigrees is difficult and time-consuming. Small population sizes, long generation times, secretive mating habits, and the potential for long term sperm storage by females render turtles difficult subjects for quantitative genetic studies.

Genetic studies that employ **neutral genetic markers** are easier to conduct than quantitative genetic analyses. These two approaches differ because variation at neutral loci is presumably not subject to natural selection, but governed primarily by drift, **mutation**, and migration (Merila and Crnokrak, 2001; Holderegger et al., 2006). The adaptive potential of populations has frequently been inferred from population characteristics identified using neutral genetic markers, under the assumption that neutral and adaptive variations are positively correlated. Some empirical studies suggest that neutral markers can be predictive of variation at quantitative trait loci (Merila and Crnokrak, 2001), whereas other studies found no significant correlation (Reed and Frankham, 2001). The degree of correlation between the two measures of genetic variation will depend on the force of selection pressures on quantitative traits. Traits under the strongest local selection are expected to exhibit the greatest divergences from neutral variation. Traits that are not under selection will be largely shaped by the same microevolutionary forces as neutral regions (McKay and Latta, 2002). Neutral markers therefore must be evaluated carefully to infer adaptive variation. New emerging molecular technologies such as **genome**-wide scans will aid in development of measures of adaptive variation because these techniques can detect loci under selection in the absence of *a priori* knowledge of gene function (Schlotterer, 2003; Luikart et al., 2003; Nielsen, 2005; Storz, 2005; Kohn et al., 2006; see also McGaugh et al., 2007).

An on-going debate in conservation biology concerns the relative importance of adaptive versus neutral genetic variation when weighing conservation options (Merila and Crnokrak, 2001; McKay and Latta, 2002; Holderegger et al., 2006). **Heritability** measured for QTLs and **heterozygosity** (a measure of variation assayed using neutral molecular or biochemical markers) may both be related to current population **fitness** (Reed and Frankham, 2003). Thus, neutral genetic variation and trait heritability may both be useful as surrogates of population fitness and may be used to prioritize populations for conservation. The value of each approach for

conservation and management of chelonians will be highlighted by brief discussion of two published examples.

Janzen (1992) estimated the **heritability** of pivotal temperature (T_{piv}) determining sex (i.e., the incubation temperature that produces a 1:1 sex ratio) for common snapping turtles (*Chelydra serpentina*). A standard quantitative genetic breeding design was not possible because *C. serpentina* takes around 10 yrs or more to reach reproductive maturity (Iverson et al., 1997). Instead, eggs from 15 clutches were incubated near the T_{piv} for the population, such that the among-clutch variation in sex ratio could be interpreted statistically as quantitative genetic variation. Under controlled conditions, heritability of T_{piv} was estimated as 0.76 (possible range of 0 to 1) at 28ºC, suggesting substantial quantitative genetic variation for sex ratio. In nature, the temperatures of turtle nests are influenced by the environmental conditions in the area of the nest (e.g., soil moisture, canopy cover, aspect, etc.). When accounting for variations in the temperature of nests in a natural population of *C. serpentina* the *effective* heritability of T_{piv} reduced to 0.05, implying that genetic factors have a minimal effect on sex ratios compared to environmental factors. Anthropogenic habitat alterations to nest thermal environments can greatly influence offspring ratios in turtles with temperature-dependent sex determination. Active management may be required to maintain equitable sex ratios for populations nesting in thermally-altered habitats.

Molecular and/or biochemical genetic markers can also provide estimates of levels of genetic diversity. Beheregaray et al. (2003) used two different neutral genetic markers (nuclear **microsatellites** and mitochondrial DNA [**mtDNA**]) to estimate levels of genetic variability within and among four island populations of Galápagos tortoises (*Geochelone nigra*). Use of markers with different rates of mutation to new alleles facilitates estimation of the relative importance of contemporary vs. historical factors on population levels of genetic diversity. Microsatellites, with their faster rates of mutation, will illuminate the more contemporary situation compared to mtDNA (Avise et al., 1992). Analyses of sequence variation in the mtDNA control region revealed long-term evolutionary divergence *among* populations on the four islands that was concordant with the geographic history of the region. Interestingly, for the island of Pinzón, there was evidence of historical population growth and retention of high levels of diversity (estimated from 10 microsatellite loci) *within* the population despite the populations' near extinction in the 1920s from predation by the introduced black rat. Survivors of the island population had maintained higher levels of genetic diversity than expected from population genetic theory. Hence, conservation efforts for Galápagos tortoises may be best directed at retaining the relatively high existing genetic variability in two populations (Pinzón and La Caseta), and intensively managing to reduce further loss in two genetically depauperate populations (San Cristóbal and Cerro Fatal). Genetic studies as described above can be used to assess the merits of alternative management actions.

Genetic Drift

Genetic drift arises from chance fluctuations in **allele** frequencies from one generation to the next. Even if individuals mate randomly within populations, changes in **allele frequency** will occur each generation. Due to chance alone, not all alleles will be present in the next generation, because not all individuals will successfully reproduce. Genetic drift is often described as a 'sampling effect' in which individuals produced in each generation represents a sample of the alleles in the ancestral **gene pool** of previous generations. Genetic drift is greater in smaller relative to larger populations (Nei et al., 1975). For example, assume on average 70% of a turtle population is at a reproductive age. Not all sexually mature individuals will produce progeny for a given year for a variety of reasons, such as not finding a mate, poor nest site choice, predation of eggs, etc. Hence, effectively, only a fraction of the population will contribute genetically to the next generation and represents the **effective population size** (see Box 1). If the effective population size is small, then there is a greater chance that the "sample" will diverge in allelic composition from that of the overall gene pool. Thus the allele frequencies in the gene pool will drift.

If population numbers decline dramatically (i.e., the population experiences a **bottleneck**) or sex ratios become heavily skewed, or variance in male or female reproductive success is high, the effective population size (Ne) will be small and the probability that offspring represent a random sample from the original gene pool will be low. As a consequence of low Ne, alleles will be lost, particularly those present at low frequencies. When few alleles are present in the gene pool, opportunities for **heterozygous** combinations of alleles at a **locus** are reduced, and overall diversity will decline with each successive generation (see Box 2 for more detail). The rate of loss of diversity in a bottlenecked population depends on several related factors, including population size, severity and duration of the bottleneck, generation time, and gene flow (Allendorf, 1986; Hedrick and Miller, 1992; Richards and Leberg, 1995; Newman and Pilson, 1997; Garza and Williamson, 2001).

Kuo and Janzen (2004) used **neutral genetic markers** to compare the genetic diversity of a small, isolated population of imperiled ornate box turtles (*Terrapene ornata*) to that of a large population located within the main range of the species. Theory predicts that the small population size of the isolated population should over time lead to reduced genetic diversity due to the effects of genetic drift, relative to the large population. Genetic diversity was assessed using 11 polymorphic, nuclear microsatellite DNA loci for ca. 75 turtles from each population. Contrary to expectations, measures of genetic diversity did not differ between the two populations. However, the small population had a genetic signature that indicated a bottleneck in population size (that

Box 1: Calculating Effective Population Size

The effective population size is the number of individuals in an "ideal" population having the same magnitude of random genetic drift, or loss of genetic diversity, or increase in inbreeding as observed. Effective population size is often less than the total population size due to the fact that not all individuals contribute equal numbers of progeny to the next generation. Effective population size can be estimated either using population genetic data or demographic parameters.

N_e estimated using demographic data-- If the number adult males and females is known, effective population size can be estimated as:

$$N_e = 4N_mN_f/(N_m+N_f)$$

where N_m and N_f are the number of breeding age males and females respectively (Nunney and Elam, 1994). This equation defines the probability that 2 randomly selected genes in the current generation are copies of the same parental gene.

N_e estimated using empirical genetic data.-- Population allele frequencies change over time as a function of N_e and elapsed time in generations (t). Over small time intervals ($t<<2N_e$), and assuming that changes in allele frequency are due to drift, the expected variance in allele frequency [E(Fc)] is approximately $t/(2N_e)$. Using adults for a species which exhibits discrete non-overlapping generations, Waples (1989) defined the variance in allele frequency (Fc) between the 2 samples, which can be estimated for each locus as:

$$Fc = \left(\frac{1}{k}\right)\sum_{i=1}^{k}\frac{(x_i - y_i)}{(x_i + y_i)/2 - x_iy_i}$$

where x_i and y_i are the allele frequencies of the i^{th} of k alleles for adults in time periods t and t+1, respectively. Thus, Fc can be used to estimate N_e. Fc (variance in population allele frequency) must be estimated by Fc' (variance in sample allele frequency), which is also affected by random sampling errors in computing sample allele frequencies. Effective population size can be estimated by incorporating the variance in allele frequency due to the finite population size (genetic drift) and due to variation as a function of the finite number of samples used to estimate allele frequencies.

$$N_e = \frac{t}{2F_c - 1/(2S_o) - 1/(2S_t) + 1/N]}$$

where S_0 and S_t are the number of individuals samples in generations 0 and t. We can also estimate the effective number of breeders (not effective population size) using parent-offspring data (i.e., where t=1). This number can be adjusted to estimate effective population size. For example, for anadromous salmonids, Waples (1990) has shown that $N_e \sim gN_b$ where N_b is the number of breeders and g is the generation length (or average age of breeders) in the adult breeding population. With overlapping generations (i.e., breeding adults of several age classes contributing progeny to the next generation), estimating expected genetic drift becomes more difficult. N_e as defined above based on the temporal method must be corrected based on estimates of age-specific fecundity and survival (see Jorde and Ryman, 1995 for a review and for calculations).

had occurred based on theoretical expectations). Why was there no detectable difference in levels of genetic diversity between populations differing in current numerical abundance despite a bottleneck persisting for 100–200 yrs?

Ornate box turtles have a relatively long lifespan, living on average 22 yrs in the wild (Metcalf and Metcalf, 1985). This longevity, long generation times, and overlapping generations are life-history traits characteristic of turtles that might retard the negative effects of drift on population levels of genetic diversity. The long duration of the bottleneck spanning hundreds of years (and several generations) may have also influenced the retention of genetic diversity. Short, but severe bottlenecks were found by England et al. (2003) to have a greater impact on loss of alleles than bottlenecks of lower severity occurring over several generations.

Not all turtles have retained high levels of genetic diversity after experiencing population bottlenecks. Similar to the ornate box turtle, the gopher tortoise, *Gopherus polyphemus*, in the southeastern United States has suffered a bottleneck persisting for more than a century due to habitat destruction of favored longleaf pine forests, *Pinus palustris*, and harvesting of turtles for food. Populations were reduced numerically by up to 80% (Auffenberg and Franz, 1982). Schwartz and Karl (2005) estimated levels of genetic differ-

Box 2: Predicting the loss of genetic diversity in populations from drift.

Expected loss of genetic diversity from the effects of drift, as measured by heterozygosity, can be predicted based on the population size. Population measures of heterozygosity can be measured as the proportion of individuals heterozygous at a locus. The expected proportion of original heterozygosity remaining after a generation of drift is [1-1/2N]. If population size remains constant over many generations the heterozygosity after t generations (H_t) can be estimated as:

$$H_t = (1\text{-}1/2N)^t H_o$$

where H_o is the population heterozygosity in the present population, and N is the adult breeding population size.

Population size and stochastic changes in allele frequency due to drift also have demonstrable effects on other population measures of genetic diversity such as the number of alleles per locus. Consider a diploid locus with n alleles present in frequencies p_1, p_2, p_3.....p_n. The expected number of alleles remaining after a single generation (n') of random mating by N adults is:

$$E(n') = n - \sum_{i=1}^{n} (1 - pi)^{2N}$$

The probability that an allele will be lost is a function of the frequency of the allele in the population. Thus, alleles at greatest risk of loss are those that are rare (Allendorf, 1986).

entiation among and diversity within gopher tortoise populations in Florida and Georgia using nine microsatellite loci. Genetic divergence among populations in both regions were high (average pairwise F_{ST} of 0.37 ± 0.17 and 0.14 ± 0.05 among Florida and Georgia populations, respectively). Values of F_{ST} greater than 0.10 are considered to be high (Wright, 1969) indicating restricted migration or **gene flow** (see below and glossary). Populations which are reproductively isolated, for example within highly fragmented landscapes, are more susceptible to loss of genetic variation due to drift.

Founder effects have been well documented, where newly established populations have substantially reduced levels of genetic variance compared to sources (Leberg, 1992; Hedrick et al., 2001). For example, only a small proportion of animals in the captive breeding program of Galápagos tortoises (evaluated for 15 microsatellite markers) contributed to the repatriated population on the island of Española (Milinkovitch et al., 2004). Variance in adult contributions can be attributed to several factors, most likely acting in concert, such as unequal access to mates, variance in fertility, unequal sex ratios, and differential survivorship of offspring. Re-evaluation of the breeding adults to equalize contributions of breeders will ensure that diversity is not compromised in the supplemented island population by the 'sampling effects' (Ramirez et al., 2006; Sigg, 2006).

Inbreeding and Outbreeding

Matings can occur between relatives, even if mating occurs at random and the population size is large. Inbreeding can have severe genetic consequences. The probability of matings between relatives will increase when populations are small in size, particularly if population size remains small over several generations, and in the absence of behavioral mechanisms to preclude inbreeding such as kin avoidance during mate selection. The primary effect of inbreeding is to change genotypic frequencies in favor of homozygous **genotypes** (see Box 3). Inbreeding can also lead to decreased fitness (**inbreeding depression**) due to the expression of **deleterious recessive alleles** through matings with close relatives. Inbreeding depression and the loss of heterozygosity probably contribute to many components of phenotype and fitness, including metabolic efficiency, growth rate, reproductive physiology, and disease resistance (Gilpin and Soule, 1986). The detrimental effects of inbreeding in captive (Ralls and Ballou, 1983) and natural populations (Keller and Waller, 2002) are widely accepted.

Population risk of extinction is related to population intrinsic rate of increase (Lande, 1988). Declines in reproductive output and survival (the basic components affecting population growth) increase proportionally with levels of inbreeding (Falconer and MacKay, 1996). There is a considerable literature from case studies on captive populations (Lacy, 1997), laboratory populations (Frankham, 1995b; Reed et al., 2002), natural populations (e.g., Frankham, 1997; Crnokrak and Roff, 1999; Keller and Waller, 2002), and from meta-analyses (review in Frankham, 2005) and population viability simulations (Brook et al., 2002) that document the negative impact of inbreeding depression and loss of genetic diversity on probabilities of population persistence.

Inbreeding can be a major concern in natural and captive populations of turtles, particularly if populations are small and there is little or no exchange among populations. For many populations, exchange of individuals and genes among populations is becoming infrequent or impossible

Box 3: Estimating Inbreeding in populations.

There are numerous definitions and ways to estimate inbreeding (reviewed in Templeton and Read, 1996). At the population level, inbreeding (F) is a measure of deviation from random mating (Hardy-Weinberg). Population levels of inbreeding can be quantified empirically using molecular or biochemical markers by estimating the excess or deficiency of observed heterozygosity (H_o) relative to heterozygosity expected if populations were mating at random (i.e., under Hardy-Weinberg). For example, expected heterozygosity (H_e) for a locus with 2 alleles with frequencies p and q = (1-p) would be 2pq. F can be estimated as:

$$(H_e - H_o) / H_e \Longrightarrow 1 - (H_o/2pq)$$

Thus, if F is a measure of the proportional deviation of observed from expected heterozygosity observed heterozygosity can be expected to diminish as

$$H_o = 2pq\ (1 - F)$$

and the frequency of homozygous and heterozygous genotypes in the next generation can be estimated as:

Genotypes	AA	Aa	aa
Hardy Weinberg frequencies	p^2	2pq	q^2
Frequencies with inbreeding	$p^2 + pqF$	$2pq(1 - F)$	$q^2 + pqF$

due to habitat fragmentation and human development creating impenetrable barriers to gene flow (see below). Isolated populations of turtles are at high risk of loss of genetic diversity through drift and inbreeding. Since adults of many species are long-lived and have reproductive life spans extending over long periods of time, there is the potential that they could mate with their sons and daughters, even grandsons and granddaughters, as adults. If there are no mechanisms to prevent mating with close relatives (i.e., kin recognition), inbreeding would accelerate loss of genetic variability and could result in expression of lethal recessive alleles leading to lower probabilities of population persistence. Levels of inbreeding will accrue in captive populations with high probability, so considerable attention has been devoted to design of captive breeding programs (Miller and Hedrick, 1993; Ebenhard, 1995; Philippart, 1995; see also Syed et al., 2007).

One way to avoid inbreeding is to **outbreed**. The opposite of inbreeding depression is outbreeding enhancement, which is often referred to as **heterosis** or hybrid vigor (Lerner, 1954). Individuals from different populations are not likely to be homozygous for the same recessive alleles. Thus, outbreeding among individuals from different populations (wild or captive) can lead to masking of different **deleterious recessive alleles** present in different populations. If offspring from outbred matings subsequently contribute reproductively in future generations, and if the deleterious recessive alleles are present in low frequency, then these alleles are likely to be randomly lost from the population after several generations due to simple **Mendelian segregation** and genetic drift. The fitness of individuals and the long-term viability of an outbred population can be higher than that of either parental population due to the reduced frequency of these deleterious recessive alleles.

Outbreeding up to some threshold level (i.e., perhaps between individuals from lineages of divergent populations) would be expected to result in increased population mean fitness. If such a simplistic perspective were indeed true, one universal conservation prescription for turtle populations of conservation concern would be to advocate mating individuals from different populations. However, while inbreeding is essentially a concept formulated on a single locus basis, we

Box 4: Outbreeding depression causes a breakdown in co-adapted gene complexes.

Consider an outbreeding situation demonstrated using two loci. One locus has two alleles (A and a) and the second locus also has two alleles (B and b). There are two populations living in two different environments.

	Pop1	x	Pop2	F1 progeny	Progeny in later generations
Locus 1	AA	x	aa	Aa	AA or Aa or aa
Locus 2	bb	x	BB	Bb	BB or Bb or bb

Individuals in population 1 have 2 locus genotypes AA/bb whereas individuals in population 2 have genotypes aa/BB. If individuals from both populations inter-breed, offspring (F1 progeny) would all be Aa/Bb. The mixing of new alleles within the genetic background that has evolved within the environments inhabited by population 1 and population 2 can lead to problems. In the first generation, we may indeed see an increase in population fitness. If alleles A and B are primarily dominant to alleles a and b, then either AA or Aa genotypes or BB or Bb genotypes will still express the same phenotype. The initial reductions in the frequencies of homozygous recessive genotypes through outbreeding may actually be beneficial. However, expectations are that reductions in population fitness would be seen in later generations, where through Mendelian segregation, potentially maladaptive multi-locus genotypes (e.g., AA/BB, aa/bb) are present in the population.

need to consider outbreeding in the context of the entire **genome**. Declines in fitness can be realized over a much broader spectrum of outbred mating scenarios.

The phenomenon of **outbreeding depression** can be expressed in several ways. Under one scenario, declines in fitness for hybrids or outcrossed genotypes can occur due to "genetic swamping" of locally adaptive genes through gene flow or directed matings from another population that evolved under different ecological settings. We can consider two genotypes AA and BB that evolved in environments 1 and 2, respectively. AA has higher fitness in environment 1 than the BB genotype. Conversely, genotype BB has the higher fitness in environment 2. Hybrid genotype AB is not well adapted to either environment. The presence of inferior hybrid genotypes as a consequence of gene flow and subsequent reproduction will result in decreased population fitness.

The second way in which outbreeding depression can occur is by the breakdown of physiological or biochemical compatibilities between genes that have evolved in different populations. Interactions among alleles at several loci (**epistasis**) collectively affect fitness. Organisms have evolved in the context of specific environments and have evolved suites of genotypes across many genetic loci that are co-adapted to each environment. If new alleles are introduced via gene flow into the genetic background of the resident population, a loss in fitness may result from physiological or biochemical incompatibilities introduced through disruption of these co-adapted gene complexes (see Box 4). The fitness of the entire population could be compromised because outbred progeny are maladapted to either parental environment.

Outbreeding depression and inbreeding depression can occur simultaneously in a population. Fluctuations in population size and gene flow (either natural or directed) of maladaptive alleles can result in inbreeding or outbreeding depression, respectively, in natural populations, potentially reducing population fitness. Ultimately, in the design of breeding strategies, one must weigh the effects of potential past inbreeding in the population (which may have purged some deleterious alleles) relative to the effects of outbreeding on locally adaptive genotypic combinations. For many species of turtles, populations are numerically depressed, and in some cases, the species is only represented in captive populations, potentially represented by few individuals originating from geographically different locales, or even from different taxonomically recognized subspecies or **evolutionarily significant units**. Decisions to breed across genetically and ecologically differentiated groups must weigh the potential detrimental consequences of both inbreeding and outbreeding to probabilities of species persistence.

Selection

Natural selection acts on the phenotypic composition of a population, altering it via the differential survival and reproduction of individuals (Lande and Arnold, 1983). Phenotypes that are better adapted to their environment (i.e., individuals with greater 'fitness') will be preferentially transmitted to the next generation. When the characters under selection have a genetic basis and are inherited, natural selection may result in the differential success of genotypes passing gametes to future generations (Nielsen, 2005). Selection can be decomposed into components, by taking a cohort born at the same time and following changes in the phenotypic and/or genetic characteristics of this cohort through each stage of the life cycle. Selection components include *viability selection* (differential survivorship), *sexual selection* (differential mating success), and *fertility selection* (differential production of offspring).

Selection may be introduced by humans through environmental changes to biotic and abiotic features. In captive populations, selection may be intentional such as a deliberate selection program designed to change some characteristic of the population. Selection can also be an inadvertent side effect of sampling or husbandry procedures, for instance, by selecting a small segment of a population as breeders to produce the next generation. Selecting individuals with specific characteristics or phenotypes may increase the intensity of selection, and lead to loss of genetic variance. For example, in captive colonies of the Mallorcan midwife toad *Alytes muletensis* maintained as breeding stock for reintroductions, allelic richness and heterozygosity both declined in long-term captive bred stocks compared to short-term stocks and wild populations (Kraaijeveld-Smit et al., 2006). The consequences of selection may be a depression in fitness-related traits (e.g., fertility, disease resistance, growth rate) such as those that are related to survival and reproductive success. Consequences of selection in captive breeding programs are most important in situations where captive-reared individuals are released back into their native environment or when there is the possibility of breeding with wild individuals. Genetic monitoring of captive breeding and reintroduction programs is important to ensure that artificial selection does not impede continued success. For turtles and tortoises, there is currently little or no genetic monitoring of successful captive breeding and reintroduction programs (Ballou and Lacy, 1995; see also Syed et al., 2007).

Humans exert an ever-increasing influence on the direction and force of selection acting on species. Average global atmospheric temperatures have increased by approximately 0.6ºC from pre-industrial times to the year 2000, a rate of change much larger than that seen in the past 10,000 yrs (Houghton, 2005). By the year 2100, average global atmospheric temperatures are projected to rise by 2 to 6ºC (Mann and Jones, 2003). To put this predicted shift into perspective, this degree of climate change is one third of that seen in the last ice age that lasted a period of approximately 100,000 yrs (Houghton, 2005). Such dramatic climatic changes will exert strong selective pressure on species to evolve. For instance, even moderate temperature shifts (i.e., as little as 2ºC for the painted turtle, *Chrysemys picta*) can drastically skew sex ratios in reptiles with temperature-dependent sex determination (Janzen, 1994). Skewed sex ratios can result in smaller **effective population sizes**, elevating risks of inbreeding and loss of diversity via drift. Behavioral modi-

fications, such as nest-site choice and altered timing of the initiation of nesting, may compensate for the effects of these local climatic shifts on sex determination (Doody et al., 2006), although selection would also act on other aspects. For example, juvenile mortality may increase as turtles experience prolonged higher temperatures; reduced hatchling recruitment was found in *Chrysemys picta* after a particularly long hot summer in 1988 (Janzen, 1994). Given these startling projections, can turtles and tortoises evolve at a pace that is rapid enough to compensate for the negative fitness consequences of global warming?

Theory predicts that the maximum rate of sustainable evolution for a population, or conversely, the maximum rate of environmental change that can be tolerated, can be inferred on the basis of the interactions of evolutionary forces on quantitative genetic variation (Lynch and Walsh, 1998). In the absence of immigration, the rate of phenotypic evolution can become limited by the availability of **additive genetic variance**. If the rate of environmental change is too high, selective pressures (e.g., impacting survival and/or fecundity) could exceed a population's capacity to assimilate new genetic variation via mutation and maintain a positive growth rate, especially for organisms with long generation times such as turtles. If so, the inevitable outcome would be extinction. If the rate of environmental change is sufficiently slow, and if the amount of genetic variation relative to environmental variation is sufficiently high, the population may be able to evolve very rapidly in response to this change. Overall, the capabilities of turtles to respond to and survive the impacts of environmental change such as global warming will depend on the rate of climatic change (i.e., the intensity of selection) and the degree of genetic variance within each population for the key traits. In the face of global warming, maximizing the adaptive genetic diversity at the population, landscape, regional, and species scales is paramount to the survival of turtles and tortoises in the 21st century and beyond.

Gene Flow and Management Units

Gene flow is defined as the movement of alleles from one population to another. Such migration is an evolutionary force that counters the effects of genetic drift and inbreeding within each population. Gene flow among populations is often summarized as the average fraction of individuals in each population in each generation that has contributed genes derived from another. Gene flow can be measured directly from field techniques of mark-recapture and tracking individuals, and indirectly by applying various mathematical models of population structure to genetic data (i.e., the island model vs. stepping stone model vs. isolation-by-distance model).

There are several reasons to expect that direct measures of movements may differ from indirect measures of gene flow (Slatkin, 1985). First, gene flow in the strict sense refers to the transfer of genes from one population to another. Migration, as quantified by direct observations, documents the physical presence of an individual in more than one population at two or more time periods. Direct observations provide no information about the likelihood of breeding, and thus actual gene flow per se. Further, inferences from direct observations are only germane to those populations where observations were made. Gene flow can occur over much broader areas and the indirect genetic-based estimates can provide accurate measures from population to landscape scales.

Further, direct observations chronicle the extent of movements only over the period of observation but provide no information regarding historical levels of dispersal. Genetic measures of gene flow report the cumulative effects of past and contemporary gene flow. However, for many populations of conservation or management concern, present levels of gene flow are of special interest. If rates of gene flow and/or effective population size had historically been high, then estimates of gene flow may not reflect present conditions. For example, high levels of gene flow and little population genetic structuring (panmixis) were documented for the geometric tortoise (*Psammobates geometricus*). Populations of *P. geometricus* are now severely fragmented, and the indirect measures of gene flow reflect the historical high levels of connectivity rather than the current fragmented condition. In contrast, direct and indirect methods for estimating gene flow yielded similar results in the freshwater turtle *Hydromedusa maximiliani*, with very restricted movements suggesting a **metapopulation** structure within drainages (Souza et al., 2002).

Understanding the use of terrestrial and aquatic habitats by local breeding populations of amphibians and reptiles is critical for conservation and management (Semlitsch and Bodie, 2003). Freshwater turtles often require different habitats to carry out all life-history functions. Turtles often live and forage in temporary wetlands that are some distance from permanent wetlands. They use upland habitats to disperse seasonally between wintering, breeding, and foraging sites, for purposes of aestivation, feeding, and hibernation, and females use upland habitats to nest (Burke and Gibbons, 1995). For example, high levels of gene flow in the estuarine diamondback terrapin (*Malaclemys terrapin*) within estuaries are most likely promoted by mating aggregations during the breeding season and high juvenile dispersal (Hauswaldt and Glenn, 2005). These movements were not detected in long-term mark recapture studies (Gibbons et al., 2001) and may be important for inbreeding avoidance and maximizing genetic diversity in estuaries.

Landscape connectivity, the degree to which landscape features facilitate or impede movements and gene flow between populations (Taylor et al., 1993), is an essential feature of landscape structure because of effects on movements among populations, population persistence, and probabilities of recolonization. Landscape connectivity can be quantified in a relative sense based on indices that characterize the spatial dispersion of landscape habitat types and account for the proportional contributions of each landscape type to landscape matrices between populations. The degree

of genetic differentiation among populations has been widely used in wildlife studies as a surrogate measure of dispersal (Scribner et al., 2005). For example, Scribner et al. (1986) used protein allozymes to estimate genetic relationships among populations of slider turtles (*Trachemys scripta*) that were separated by different types of intervening habitats. Based on estimates of inter-population variance in **allele frequency**, these authors presented compelling evidence for higher rates of gene flow among populations from different embayments along contiguous lake shoreline relative to interspersed (but aquatically connected) riverine habitat. Populations in small ponds separated by upland terrestrial habitat had the lowest rates of gene flow compared to those in the other intervening habitat types.

Management strategies for populations need to account for the dispersal capabilities and natural history of the species. Where panmixis occurs, the populations may be managed as a single entity with a focus on maintenance of size and habitat quality. In contrast, where there is a high degree of structuring, each population contributes to overall species diversity. Managing these populations as separate units is important to ensure diversity is retained within each, and that overall species diversity is not compromised from increased gene flow and resultant genetic homogenization (DeYoung and Honeycutt, 2005; Moritz, 1994; Moritz, 1999). Mixing genetically differentiated populations can also cause outbreeding depression (see above). Management can be guided by the extent to which populations have diverged, with issues of outbreeding depression and isolation being of greatest concern among the most divergent units, referred to as **evolutionarily significant units** (ESUs; Moritz 1994), in comparison to less divergent populations referred to as **management units** (MUs).

Spinks and Shaffer (2005) defined management units for the vulnerable western pond turtle (*Emys [= Actinemys] marmorata)* with analyses of 1372bp of *ND4* and *tRNA* mitochondrial genes. Populations in northern California and farther north were genetically similar and formed a single management unit, whereas drainages farther south exhibited more structuring. In central and southern California, a large proportion of intraspecific diversity could be attributed to two populations. To retain diversity, these two populations should be a priority for conservation and management of the species.

Defining management units was a greater challenge for the giant Amazon river turtle, *Podocnemis expansa*. This species has an impressive dispersal capability, with females known to traverse up to 400 km between nesting beaches and feeding areas (Hildebrand et al., 1988). As predicted from theory, because of its dispersal capabilities and lack of barriers to dispersal, high levels of gene flow were found within basins (Pearse et al., 2006a). Based on this mtDNA analysis, an entire basin represents a management unit. Lack of structuring in basins was confirmed for nine microsatellite loci but these markers also revealed recent reductions in population size. Extensive harvesting has decimated populations of *P. expansa* and its continuation will result in loss of genetic diversity. Given the harvesting pressures, the units of management would be more appropriate at the population level to ensure local nesting beaches are not overexploited for eggs and mature females of *P. expansa.* Conservation biologists thus need to consider all threatening aspects from local to landscape scales when defining units for management in chelonians.

Clarifying Taxonomy

Inadequately informed management plans and a limited knowledge of biological richness are often the result of misunderstanding taxonomic status and relationships among taxa. If the units of evolutionary significance or taxonomic importance have not been identified and prioritized for conservation, biological diversity may not be protected adequately. Molecular methods are particularly amenable to resolving taxonomic relationships and identifying units for conservation, because they can uncover diversity in taxa not apparent from morphological analyses. Phylogenetics is a discipline that often uses genetic information to delimit species boundaries and divergent lineages within species, and then to estimate the evolutionary relationships amongst those units (Davis and Nixon, 1992; Avise and Wollenberg, 1997; Nei and Kumar, 2000; Iverson et al., 2007; Turtle Taxonomy Working Group, 2007a). We will illustrate how phylogenetics has contributed to resolving taxonomic issues in chelonians.

Taxonomy has traditionally used morphological characters to delimit species where a **holotype** is used as a reference specimen. However, the propensity of some turtles to hybridize with other species can result in difficulties. For example, at least two "species" of rare Chinese turtles were described from specimens purchased from the Hong Kong animal trade. Scientists were unable to find these animals in the wild and began to question their taxonomic validity. Allozyme and **mitochondrial** DNA analyses revealed that these "taxa" were not representative of species but rather they were distinct morphological forms resulting from **hybridization** events (Parham et al., 2001). Hybridization and **introgression** are fairly common in freshwater turtles (e.g., Georges et al., 2002; Stuart and Parham, 2004; Spinks and Shaffer, 2005). Neutral genetic markers may effectively resolve these taxonomic issues and have advantages over morphological traits as they are less subject to plasticity and presumably selection.

Phylogenetic studies can redefine taxonomies. Taxonomies have been refuted or supported by genetic evidence where phylogenetic criteria are used to delimit species and genera (reviewed in Turtle Taxonomy Working Group, 2007b). Delimiting species on the basis of combined molecular and morphological criteria is considered the best approach for resolution of taxonomies (Seberg et al., 2003; Blaxter, 2004; Dayrat, 2005). For turtles and tortoises, delimiting species boundaries can be even more difficult because interspecific hybridization frequently occurs even amongst distantly related taxa (e.g., Georges et al., 2002).

Phylogenetic methods can identify such instances of hybridization and resolve taxonomies to define groups constituting genera or species (Templeton, 2001; Sites and Marshall, 2004). For example, in a phylogenetic study of the Geoemydidae, not all recognized species appeared to be of the same evolutionary lineage. This suggested misclassification of several species (by some criteria), and instances of interspecific hybridization were documented. Based on this genetic evidence, taxonomic revision of this group was required (Spinks et al., 2004).

Phylogenetic or phylogeographic studies can identify cryptic species. Cryptic species are named because they comprise distinct genealogical lineages but in the absence of molecular or behavioral evidence, lack distinguishing morphologic characteristics or other diagnostic features to warrant recognition as species. For purposes of conservation, cryptic species are important units of diversity and may represent threatened taxa, previously unknown to conservation biologists (Georges and Adams, 1996; Georges et al., 1998; Walker et al., 1998; Fritz et al., 2005). In Asian softshell turtles, two species were formally recognized in the *Chitra* genus: *C. indica* and *C. chitra*. MtDNA sequence data revealed three deeply divergent **monophyletic groups** in *Chitra* (Engstrom et al., 2002). The third and previously unidentified form was subsequently named as a distinct species (*C. vandijki*) based on additional morphological data (McCord and Pritchard, 2002), and is a critically endangered species that warrants greater protection (Engstrom et al., 2002). As protection is usually only conferred to recognized species or subspecies in wildlife legislation, it is imperative that taxonomies are clearly defined for effective conservation (Soltis and Gitzendanner, 1999; George and Mayden, 2005; Turtle Taxonomy Working Group, 2007a).

Insights into Species Biology

Biologists have traditionally explored various aspects of the natural history of a species through observation. Turtles are notoriously difficult subjects for some observational studies, yet knowledge of many aspects of a species' biology is critical for successful conservation efforts. **Molecular markers** are providing new insights into turtle mating systems, dispersal (sex-specific or otherwise), population connectivity, and fluctuations of population sizes that can be difficult to ascertain from field and observational studies alone.

Female turtles have sperm storage structures in the oviducts (Gist and Jones, 1989), and captive females held in the absence of adult males have been known to produce viable eggs for as long as 7 yrs (Ewing, 1943; Magnusson, 1979). Molecular marker studies have revealed that freshwater turtles and tortoises in natural populations frequently use stored sperm to fertilize eggs (e.g., Gist and Congdon, 1998; Pearse and Avise, 2001; Roques et al., 2004). Indeed, microsatellite DNA analyses have revealed that some *Chrysemys picta* will produce fully-fertile clutches of eggs in nature without re-mating for 3 yrs (Pearse et al., 2002). However, lower hatching success and hatchling mass were found in clutches fertilized from stored sperm in the European pond turtle (*Emys orbicularis*), suggesting deterioration of stored sperm for some species (Roques et al., 2006).

The vast body of literature documents a substantial frequency of multiple paternity in non-marine turtles and tortoises (examples include Galbraith, 1993; Palmer et al., 1998; Moon et al., 2006), but there are exceptions. Low incidences of multiple paternity (less than 10% of clutches) have been documented for *Emys orbicularis*, resulting perhaps from competition of viable stored sperm to fertilize eggs (Roques et al., 2006). This finding contradicted observations of multiple *E. orbicularis* males mounting a single female during the breeding season (Rovero et al., 1999). Mating systems may also differ between populations of the same species. *Podocnemis expansa* exhibited 100% multiple paternity in smaller samples (Valenzuela, 2000) and 10 to 20% in larger samples (Pearse et al., 2006b). Molecular markers thus can shed light on mating systems in turtles and tortoises that may not be apparent from observational data.

Reproductive success is critical to population persistence. Only recently, based on applications of biochemical markers, have turtle biologists been able to extend estimates of annual recruitment to quantify reproductive contributions of individual adult males and females. Variance in reproductive success will greatly affect Ne and generational rates of loss of genetic diversity. Importantly, knowledge of phenotypic, demographic, and geographic (e.g., habitat) variables that can be linked to reproductive success and to inter-annual variation in recruitment will greatly aid in the development of conservation plans. Scribner et al. (1993) used allozymes to examine relationships between inter-annual variation in reproductive success and juvenile cohort measures of genetic diversity in *Chrysemys picta* that inhabits the E.S. George Reserve, a large protected wetland complex in southeastern Michigan. During years where few females successfully reproduced, offspring from these cohorts were characterized by higher **inbreeding coefficients** (F), lower heterozygosity (H), and higher genetic correlations among individuals (θ) compared to cohorts recruited in years when greater proportions of females contributed progeny. For conservation biologists, these findings emphasize that factors affecting inter-annual variation in recruitment also can impact cohort levels of genetic diversity.

Ecological characteristics are not alone predictive of how genetic variation is apportioned within and among populations. Closely related turtle species may display substantial variation in connectivity and structure that reflect important differences in natural history among species. For example, Roman et al. (1999) found strong phylogenetic structuring for the highly aquatic alligator snapping turtle (*Macrochelys temminckii*) across basins in a mtDNA control region analysis, suggesting limited dispersal of turtles. In contrast, *Chelydra serpentina* lacked structure for allozyme and mtDNA, reflecting its greater tendency to disperse over land and long distances in water (Phillips et al., 1996). Each species is different. The most informed conservation deci-

sions are formulated based on knowledge of fundamental aspects of a species' biology derived from joint studies of genetic structure and natural history.

Estimating the size of a population from mark-recapture analyses can be difficult and time-consuming, particularly for species that are difficult to capture or at low population densities. Obtaining genetic samples can be easier because individuals do not need to be subsequently re-caught to obtain data for estimating population size.

Molecular data can be used to estimate the effective population size, which is the size of the population that is actually reproducing, a parameter that may be more meaningful for conservation than the census size. The effective population size (Ne) can be monitored by assessing temporal changes of allele frequencies in the population (Richards and Leberg, 1995; Luikart et al., 1999). Genetic techniques can also provide point estimates of the number of breeding individuals in a population (Nb) from paternity (or maternity) microsatellite data. Pearse et al. (2001) developed a technique for estimating current reproductive size of a population of *Chrysemys picta* and provided additional information, such as the movement of breeding individuals, which was not possible based on capture-mark-recapture studies alone.

Forensics

Trade in turtles has increased dramatically and is considered to be the greatest threat to their survival (Asian Turtle Working Group, 1999; van Dijk et al., 2000). Turtle and tortoise trade can be classified into three main categories: trade for human consumption, pet shop trade, and traditional medicines (van Dijk et al., 2000; Turtle Conservation Fund, 2002). Consumption of turtles is by far the largest scale trade, and larger, more mature individuals tend to be targeted. Due to their life-history characteristics (great longevity, high juvenile mortality, and late onset of maturity), this type of trade probably has the greatest negative impact on chelonian populations (Smith, 1993; van Dijk et al., 2000). Exploitation of chelonians for the pet shop trade favors juveniles of unusual species and, as commodity values are often driven by rarity, this can rapidly contribute to the extinction of rare and endangered species (Ceballos and Fitzgerald, 2004; Gamble and Simons, 2004; Cheung and Dudgeon, 2006; Gong et al., 2006; Stuart et al., 2006). Finally, large numbers of turtles are frequently harvested primarily for their shells, which are ground to a powder or jelly, and sold for its alleged positive effects on longevity and virility in humans (van Dijk et al., 2000; Hsieh et al., 2006; Lo et al., 2006).

DNA-based forensic methods can be used to monitor illegal trade by verifying taxonomy and providing information on geographic origin of seizures. Traditionally, morphological characteristics were used for species identification. However, often seizures include small fragments of eggshells, carapace, cooked meat, or powdered turtle shell, where standard diagnostic features are no longer discernible. Molecular methods are ideal for forensics because they can be used on degraded or processed specimens, and can elucidate species, and even regional or population origins (Randi, 2003). Where commercial industries are established, genetic techniques may be the only means by which products derived from legal trade can be reliably distinguished from poaching activities. Further, genetic methods have the resolution to 'tag' individuals and establish paternities or maternities, technologies that are particularly useful for monitoring activities of licensed reptile breeders. The application of molecular techniques for wildlife forensics is still in its infancy. Approaches tend to be handled on a case-by-case basis and standard protocols have not been adopted. Currently only a few studies have applied molecular techniques for forensic issues in freshwater turtles and tortoises.

Legitimacy of turtle meat trade in Florida and Louisiana were investigated by Roman and Bowen (2000). Species composition was determined from 36 turtle meat products purported only to contain *Macrochelys*. The majority did not contain *Macrochelys*, but were predominantly *Chelydra serpentina*, as revealed by analyses of the control region and cytochrome *b* genes of mtDNA (394bp and 256bp respectively). This shift in trade to a species that is 50 kg lighter in weight and less favored for its flavor is speculated to reflect depletions of *Macrochelys* populations. With more catch effort required by harvesters to meet demand from these depleted populations, the market shifted to the more readily available *Chelydra*. In addition, softshell turtles (*Apalone* spp.) were present in a small proportion of the products. Impacts of this trade have not been investigated for any of these species, although current harvest rates may not be sustainable. Further research on the effects of harvesting and continued genetic monitoring of processed trade goods is recommended to prevent overexploitation or to minimize its impact in these species.

Molecular methodologies have analyzed species composition in cooked meat, eggs (Moore et al., 2003), and powdered turtle shell (Lo et al., 2006). Preparations of turtle shell in the Taiwanese market were analyzed with mitochondrial 12s ribosomal RNA and cytochrome *b* sequences (Lo et al., 2006). Reassuringly, CITES (Convention on International Trade of Endangered Species of Wild Fauna and Flora) listed species were not present in these turtle shell and jelly preparations. Also in Taiwan, methods have been developed for determining the presence of a CITES-listed endangered turtle (*Kachuga tecta*) in shell preparations (Hsieh et al., 2006).

Identifying geographic origins or provenance of seizures is required to repatriate animals to their wild populations without disrupting existing genetic structure or elevating risks of outbreeding depression. Molecular techniques can also be used for assessing origins of individuals. In the case of the Indian star tortoise (*Geochelone elegans*), the origins of 92 individuals seized from the Singapore airport were determined using mtDNA (control region, cytochrome *b*) and six microsatellites (Gaur et al., 2006). The rescued group of tortoises was found to be a mix of individuals from

different populations in southern India and possibly Sri Lanka. Exact localities for many of the individuals could not be identified because sampling was limited and not all diversity has been characterized across the range of *G. elegans*. With more extensive sampling, these methodologies will be able to identify source populations of seized chelonians, enabling them to be returned to their original geographic location(s). Overall, these studies highlight the power of molecular methods to monitor trade directly from a range of trade products for species identification and provenance delineation.

The utility of genetics in forensics is hindered by the limited markers available for chelonians. With more markers becoming available from genome sequencing projects, such as that proposed for *Chrysemys picta* (see http://www.reptilegenome.com for more information), genetics will play an ever-increasing role. New technologies, such as single nucleotide polymorphisms (SNP) markers will enable analyses of samples from more highly degraded samples, more rapidly and with greater resolution for addressing forensic issues. Advances in genetic technologies and marker development will pave the way for development of DNA registers for routine monitoring of trade activities. Such inventories are urgently required if we are to assess the threats of overexploitation to turtles and tortoises worldwide.

Concluding Remarks

We have discussed important genetic issues that conservation biologists should consider when planning and executing projects involving turtles. We have highlighted the importance of genetic diversity for future adaptive evolution and we outlined processes by which diversity is lost. Anthropogenic effects can exacerbate loss of genetic diversity owing to increased habitat fragmentation and diminished population size. Genetic approaches can be used to detect and monitor these effects at various temporal and spatial scales.

Understanding historical and contemporary evolutionary processes, at scales ranging from an individual to an entire landscape, provides valuable knowledge for development of short-term and long-term conservation plans. Conservation priorities can be identified and program success can be monitored using molecular methodologies. Aspects of turtle biology and mating systems that are exceedingly difficult or impossible to ascertain from field studies can be illuminated using genetic markers. Further, molecular methods are an emerging crime investigation tool for monitoring the turtle trade. Despite these applications and the inherent importance of genetic diversity to long-term viability of turtle populations, there is a general paucity of such genetic studies on freshwater turtles and tortoises (reviewed in FitzSimmons and Hart, 2007).

Due to the lack of studies, there is a limited repertoire of molecular markers currently available for turtle geneticists (Engstrom et al., 2007). With the ongoing genomic revolution, the number of available markers, their information content, and range of applications for chelonian conservation will greatly increase. For example, new genomic approaches offer exciting possibilities to investigate whether variation within specific gene regions can be tied to phenotypic or other traits that are tied to probabilities of survival or reproductive success. Emerging technologies hold great promise to link increasingly assessable modern technology to fundamental problems in turtle biology and conservation. Other technological advancements will enhance efficiency of DNA fingerprinting technologies and enable high throughput analyses, such as SNPs (**single nucleotide polymorphisms**) and **microarrays** (reviewed in McGaugh et al., 2007).

We conclude by listing what we perceive to be three crucial future directions in turtle conservation genetics:

1. Reconciling taxonomic uncertainties and identification of genetic discontinuities at landscape and species levels to delineate management units.

2. Predicting effects of landscape-level changes and concomitant changes in population demography and movement patterns on apportionment of genetic diversity within and among populations.

3. Monitoring trade and directing enforcement to protect overexploited turtle populations.

Each issue is a global concern that potentially influences every turtle species. While substantial progress has been made, the geographic and taxonomic coverage has been uneven and not necessarily focused on species of greatest concern (reviewed in FitzSimmons and Hart, 2007). Turtle geneticists should work closely with biologists, managers, local communities, and conservation organizations to bring state-of-the-art technology and methods of statistical inference to bear on pressing issues in turtle conservation.

Acknowledgments. — This material is based upon work supported by the NSF under grant # DEB-0507916 for the Turtle Genetics workshop held from 7–12 August 2005 at Harvard University. Additional financial support for the workshop came from the Museum of Comparative Zoology (Harvard University), Chelonian Research Foundation, and Conservation International.

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Glossary

Additive Genetic Variance. – Genetic variance that arises from the additive effects of genes on the phenotype.

Allele. – Alternative forms of a gene at a given locus on a chromosome.

Allele Frequency. – Also termed gene frequency. The proportion of an allele (or gene) in a population relative to other alleles (or genes) at its locus.

Allelic Richness. – The number of alleles in a population corrected for sample size. Used as a measure of genetic diversity.

Allozymes . – Forms of an enzyme that differ in amino acids and have different electrophoretic mobilities.

Chromosome. – A strand of DNA with associated proteins that is visible as a rod-shaped structure in cells that have been stained during cell division. Chromosomes contain the heritable genetic information within the DNA.

Deleterious Recessive Alleles. – The phenotypic effects of recessive alleles are masked in the phenotype of heterozygotes, and expressed in homozygotes. Deleterious alleles have negative fitness effects on individuals.

Effective Population Size. – The average number of breeding individuals in a population which are assumed to contribute equally to the next generation.

Epistasis. – The interaction between two nonallelic genes, such that one gene alters the expression of the other at a different locus.

Evolutionarily Significant Unit. – A population (or group of populations) reproductively isolated from other conspecific population units for long enough duration to display genetic isolation, and is an important component in the evolutionary legacy of the species.

Fitness. – The ability of an individual to produce offspring in a given environment. In a genetic sense, the relative reproductive success of a genotype.

Founder Effects. – The loss of genetic diversity when a new colony is formed by a very small number of individuals from a larger population; a form of genetic drift.

Gene. – A basic unit of inheritance transmitted through the gametes from generation to generation, occupying a specific locus on a chromosome and with a specific function.

Gene Pool. – All the genes available among reproductive members of a population at a given point in time.

Genetic Drift. – Changes in allele frequencies of populations due to random sampling effects because not all individuals (and their genes) will reproductively contribute to the next generation.

Gene Flow. – Movement of genes from one population to another by interbreeding or migration.

Genotype/genotypic. – The genetic constitution or expression of an individual.

Genome. – The entire complement of genetic material in a cell. In eukaryotes this refers to the genetic material in a single set of chromosomes.

Genotypic Frequency. – The proportion of a genotype in the population relative to all other genotypes.

Heritability. – The proportion of phenotypic variability for a given trait that is quantitatively genetically based; expressed as the ratio of phenotypic variance to genetic variance.

Heterosis. – Superiority or vigor of hybrid individuals compared to either parental stock.

Heterozygote. – A diploid individual with different alleles at a particular locus.

Holotype. – The single specimen designated or indicated as the name-bearing type of a nominal species or subspecies by the original author.

Homozygote. – A diploid individual with identical alleles at a particular locus.

Hybridization. – Crossbreeding of individuals of different genetic composition, typically belonging to different species or varieties to produce hybrid offspring.

Inbreeding. – Mating of related individuals.

Inbreeding Coefficient. – The probability that an individual contains copies of the same ancestral gene from both its parents because they are related.

Inbreeding Depression. – Reduction of fitness by increased homozygosity as a result of inbred matings.

Introgression. – The spread of genes from one species to another via hybridization and backcrossing.

Locus/loci. – The specific region on a chromosome where a gene is located.

Management Units. – Demographically independent sets of populations identified to aid short-term conservation management. Genetically divergent but not to the extent as observed in evolutionarily significant units.

Meiotic Drive. – Preferential production of certain gametes during meiosis (germ cell production). This alters the expected Mendelian segregation ratios in heterozygotes.

Mendelian Segregation. – Mendel's first law. The principle that the two different alleles of a gene pair segregate from each other during meiosis; each resultant gamete has an equal probability of obtaining either allele.

Metapopulation. – A group of spatially separated populations from the same species connected by immigration and emigration.

Microevolution. – Evolutionary events occurring over a shorter period of time, such as the changes in the gene pool of a population.

Microsatellites. – Tandem repeat motifs of DNA sequence interspersed throughout the eukaryotic genome in which the repeat unit is typically five or fewer bases in length.

Molecular marker. – A genetic polymorphism with multiple alleles and a simple mode of inheritance. Useful in pedigree studies, disease studies, studies of the distribution of genes in populations and linkage mapping.

Mutation. – A change in a gene or chromosome.

Microarrays. – A technique used to monitor gene expression in which genes or gene fragments are deposited typically on a glass, filter, or silicon wafer in a predetermined spatial order allowing them to be made available as probes.

Migration. – Movement of an individual or group from one location to another.

mtDNA. – Mitochondrial DNA: The circular, double-stranded DNA of the mitochondria. It typically has matrilineal inheritance, although paternal leakage has been documented for some taxa.

Monophyletic Group. – A group comprised of a single ancestral species and all its descendants. Also called a clade.

Natural Selection. – A primary mechanism for evolution in which individuals best suited to their environment have greater survival and reproductive success, thereby transmitting their genetic characteristics to succeeding generations.

Neutral Genetic Markers. – Genetic markers presumably not under the forces of natural selection and often residing in non-coding genomic regions.

Outbreeding. – The breeding of genetically unrelated or distantly related individuals.

Outbreeding Depression. – A reduction in the fitness of progeny from matings of individuals from different populations, possibly from the breakdown of co-adapted gene complexes or 'swamping' of locally adaptive genes.

Panmictic. – Pertaining to a genetically unstructured randomly mating population.

Phenotype/phenotypic. – The observed properties of an organism, resulting from the interaction of its genotype with the environment.

Phenotypic Plasticity. – The ability of an organism's phenotype to change in response to changes in the environment.

Population Bottleneck. – An evolutionary event resulting in a decrease in the size of a population and subsequent loss of genetic diversity via the effects of genetic drift.

Quantitative Genetics. – The study of the genetic basis of traits showing continuous variation.

Single Nucleotide Polymorphism. – Variations in DNA sequence that occur when a single nucleotide base (adenine, guanine, cytosine, or thymine) is altered via a mutation event.

Vicariance. – The splitting of closely related groups of taxa or biota by the formation of a natural barrier.