

Project Report

for

“EVALUATION OF HYBRIDIZATION AND INTROGRESSION OF *TRACHEMYS*
GAIGEA AND *TRACHEMYS SCRIPTA* IN BIG BEND NATIONAL PARK”

Submitted to Raymond Skiles, BBNP

by

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Abstract: We know from past efforts that Red-eared Sliders (*Trachemys scripta elegans*) from the pet trade have been released into the wild in the Big Bend Region of Texas. It has also been argued in past literature that they can hybridize with the Big Bend Slider (*Trachemys gaigeae*), potentially leading to introgressive events and loss of genetic diversity and specialized adaptation in Big Bend Sliders. In the summers of 2005 and 2006, turtles were trapped in Big Bend National Park and adjacent areas. All slider turtles were individually marked, and exotic or potential hybrid individuals were removed. Capture frequency of exotic *Trachemys scripta elegans* was compared to similar data from 1997-8 to evaluate the efficiency of removal as a remediation strategy for introduced exotics. Genetic data were applied to evaluate our ability to detect hybrid individuals by eye in the field. The frequency of exotics has not changed significantly since 1998. Removal of exotic individuals does reduce their capture frequency the next year. Discrimination between *Trachemys gaigeae* and *Trachemys scripta elegans* by eye is not problematic, however identification of hybrids is difficult. Genetic data shows that there are hybrids that we are unable to detect phenotypically. It also shows that the Big Bend Slider population in the Big Bend region of Texas is significantly different from the New Mexico population, and should be managed as a distinct population. Mark recapture results demonstrated that Big Bend Sliders have significant dispersal ability such that areas adjacent to BBNP must be included in management of the park's population.

Introduction: The Big Bend slider (*Trachemys gaigeae*) is an Emydid turtle that was once common throughout the Rio Grande/Rio Bravo River from west Texas into New Mexico. Ernst (1992) shows that there are presently several large gaps in their distribution along this section of the river. Despite significant anthropogenic changes to the river including construction of reservoirs, channelization, and the reduction of in-stream flows and pulse flooding event that have altered or destroyed much of their habitat, the species remains common in some areas such as Big Bend National park (Forstner et al. 1999, *in press*). Virtually no data exists for the evaluation of the species in the Rio Conchos, which is assumed to have held a similarly abundant population, but is also likely to have suffered the same consequences resulting from similar anthropogenic pressures. The long-term effects of these changes to the river on watershed as an ecosystem are not clear, nor have the impacts to this species been quantified. If enough of the habitat is lost or made unsuitable, once contiguous populations become fragmented and isolated resulting in reduced or interrupted gene flow with attendant losses to genetic diversity.

It is likely that this species' division into subpopulations is already manifest in the Rio Grande/Rio Bravo basin by loss of in-stream flows and existing impoundments. A population genetic evaluation provides a logical approach to evaluating that speculation. Molecular markers are an ideal tool for the evaluation of population structure and, with a variety of suitable markers, can simultaneously evaluate historical and more recent population connectivity features. Turtles are known to accumulate changes in their mitochondrial DNA (mtDNA) much more slowly than most organisms (Avisé et al. ,

1992). This means that a significantly greater amount of time may need to pass before the changes in haplotype diversity can be observed. Faster evolving genetic markers are available (i.e. microsatellites) and can provide higher resolution of population genetic parameters than most other marker systems.

Trachemys gaigeae populations now face another threat in both the Rio Grande of New Mexico and in the Rio Grande/Rio Bravo of Texas, the introduction of the red-eared slider, *Trachemys scripta elegans*. The red-eared slider is a common pond turtle that is often sold in the pet trade. The introduction of red-eared sliders has adversely affected populations of other species around the globe, as they are strong competitors and have shown an ability to reduce survivorship of other turtle species in France (Cadi and Joly, 2004). They are not historically native to the Big Bend area, but have been introduced, most likely by people releasing their pet turtles when they no longer wish to care for them (Dixon, 2000). Some evidence for range expansion of native Texas *T. s. elegans* has also been shown (Forstner et al., *in press*). So while there are native Texas *T. s. elegans* in the Pecos River, those native individuals did not historically disperse upstream on the Rio Grande/Rio Bravo beyond the Sanderson County line (Forstner et al., 1999). This may have been, in part, a consequence of the historical significant rapids of the Lower Canyons of the Rio Grande/Rio Bravo. The concern to the Big Bend Slider (*T. gaigeae*) populations are less likely to come from expansion of these native populations as reproductive isolating mechanisms are more likely to be observed at contact zones between closely related native taxa than we would expect in a contact zone with non-native introduced *T. s. elegans*. Thus, the concern would be that Red-eared sliders

introduced from the eastern USA would be more likely to generate a hybrid population, to say nothing of the potential for novel diseases or other direct impacts on the native fauna.

Indeed, Degenhardt et al. (1996) suggest that hybridization between *T. gaigeae* and *T. scripta* in New Mexico only occurs where *T. s. elegans* is introduced, which is the case we see in Big Bend (Forstner et al., *in press*). This is a serious threat, as the exotics may not only compete for food and other resources with the Big Bend Slider, but can also reproduce with them. Hybridization may lead to loss of genetic diversity in *T. gaigeae*, and possibly to the replacement of parts of their genome by portions of the *T. s. elegans* genome (introgression). This is a particularly unique danger for turtles, which have already been shown to already possess lower levels of genetic diversity than other organisms (Avice et al., 1992). In addition, these processes can contribute to the loss of a species, especially a rare taxon (Rhymer and Simberloff, 1996). Other consequences of hybridization include outbreeding depression, which may result in the loss of locally adapted gene complexes otherwise contributing to the success of the native species. In these cases the hybridization event disrupts linked groups of genes that are particularly suited to the local environment. This has the immediate result of decreased suitability of the hybrids in the local environment for the conditions those genes convey but depending on the conditions at any one time, may not show an immediate consequence, allowing the hybrids to persist. In this situation, we could speculate that genes providing *T. gaigeae*

with the ability to prosper in the extreme variation of conditions in west Texas are unlikely to be found in *T. s. elegans* adapted for the stability of the eastern USA. By hybridization, the gene complexes conveying the advantage in the variable environments are broken apart in the recombination event of hybridization, which, if it were to spread through the population, would then lower the overall survivorship of any involved individuals.

Molecular markers can be used to evaluate the extent of such hybridization (Scribner et al., 2001) as well as evaluate the extent of any introgression (Gerber et al., 2001), allowing an appropriate management strategy to be devised in order to preserve the genetic integrity of *T. gaigeae*. The key to our evaluation was to determine the extent of hybridization in the population within BBNP and to compare our results to both the study by Forstner et al. (1999) and between years of our current study. We sought to determine the distribution of exotic turtles in BBNP and then to determine whether direct control by removal was likely to have a positive effect as a management strategy. With most exotics by the time the invasion is noted, there is little hope of removal. However, turtles are a unique case, long-lived, but with low juvenile survivorship. The removal of adult exotic turtles would mean a reduction in reproduction and every adult removed increases the probability that future hybridization would decrease in its rate of recurrence. The work of Forstner et al. (1999) indicated a low overall number of hybrids within BBNP and a relatively concentrated occurrence of exotic turtles. Thus, we would argue that turtles provide a life history and persistence that would lend itself to success in the

removal of introduced individuals and if the restricted distribution within BBNP remained true now that removal was a potentially successful option. To accomplish this the exotic must be safely and efficiently distinguished from native turtles and this would be particularly facilitated if hybrids could be phenotypically distinguished as well.

Materials and Methods: Turtles were collected using baited hoop net traps placed in the Rio Grande/Rio Bravo River and adjacent water bodies, by wading or by canoe, from 15 May through 29 July 2005, and 5 May through 20 June in 2006, in and adjacent to Big Bend National Park (BBNP). The sampling period in 2006 was adjusted to occur during the period we experienced highest capture success in 2005. Trapping was focused on eight popular access regions: Santa Elena Canyon, Cottonwood campground/Castolon, Black Dike, Loop Camp, Talley, Solis, La Clocha/Gravel Pit, and the area from Hot Springs Canyon through Rio Grande Village (including Beaver Pond) to Boquillas crossing (Figure 1) within BBNP, as well as Paso Lajitas and Big Bend Ranch State Park.

All captured turtles were measured, photographed, and a blood sample was taken for DNA analyses. Carapace length and width, plastron length and width, body depth, and weight were recorded. *T. gaigeae* were individually marked along their marginal scutes according to a standardized individual numbering system with a cordless Dremel tool, and released. Dorsal, ventral, as well as cranial dorsal and ventral photographs were taken of each *Trachemys* captured, and all photos were annotated with specimen information. *T. s. elegans* were removed from the park to a captive facility. All suspected hybrids (those possessing phenotypic characteristics of both species) were also

moved to a captive facility. The locality of each capture was recorded with a handheld GPS unit, as were all trap locations. GPS coordinates for trap locations were mapped using National Geographic TOPO! for Mac OSX. The localities of all recaptures of marked individuals were also recorded. Data collected from the recapture of marked *T. gaigeae* were analyzed using MARK, under both CJS and POPAN models.

Blood samples were immediately stored in blood storage buffer (0.1M EDTA, 0.1M Tris, 1% SDS). All samples were accessioned into the Fortner tissue catalog on return from the field, along with all associated data. DNA extractions were completed using a Qiagen DNeasy kit, following the Qiagen nucleated blood extraction protocol, or using a Beckman Coulter Biomek 3000 fluidics robot. Amplification of mitochondrial DNA was carried out on 72 samples, selected from the total to span all capture sites and included all *T. s. elegans* and putative hybrids using a standard Promega *Taq* polymerase protocol, for the ND4-tRNA^{LEU} mitochondrial gene region. Polymerase chain reactions (hereafter PCR) were attempted at annealing temperatures of 48, 50, and 55 degrees C. PCR was also attempted using tDNA full strength, 1:100 dilutions, and varying amounts of tDNA. One PCR was conducted using 1% DMSO. All PCR reactions were assayed using agarose gel electrophoresis. Successful PCR reactions were cleaned of remaining unincorporated primers and other PCR reaction components using Agencourt Ampure Bead Solution and accompanying protocol. Successfully cleaned PCR products were sequenced using a Beckman Coulter DCTS cycle sequencing kit and accompanying protocols. Cycle sequence reactions were purified using ethanol precipitation as described in the aforementioned DCTS kit protocol. Sequence reaction analysis was

conducted on a Beckman coulter CEQ 8800 automated DNA sequencer. Sequences were aligned using Sequencher v4.7. Mitochondrial sequences were analyzed using PAUP v4.0.

Microsatellite analyses were conducted using the same set of individuals examined for mtDNA haplotypes, but included additional *T. gaigeae* individuals from New Mexico, and *T. s. elegans* individuals from lower Rio Grande/Rio Bravo river from Langtry to Brownsville and individuals from the Pecos and Nueces rivers. A total of 127 individuals were eventually included in the microsatellite evaluation using 7 polymorphic microsatellite loci. PCR was conducted for each primer pair using labeled forward primers and Promega *Taq* following standard protocols, with annealing temperatures of 55 and 65 degrees C. Microsatellite reactions were analyzed using a Beckman Coulter CEQ 8800 automated DNA sequencer and accompanying fragment analysis software.

The genotypic data were then subjected to Bayesian assignment probability analysis as implemented in Structure 2.0 (Pritchard et al., 2000) (Figure 3) to determine the appropriate number of populations, as well as, to which each individual belonged according to their genotype. In the structure analysis, an admixture model was used, and allele frequencies among populations were assumed to be correlated (Falush et al., 2003). The Markov chain process was burned in for 100,000 repetitions, and then run for 10,000. Hybrid identity and determination of potential higher order crosses (backcrosses, F2s, etc...) was also analyzed by NewHybrids 1.1 (Anderson and Thompson, 2002), using a Jeffrey's-like prior for both mixing proportions and allele frequency (π and Θ).

T. s. elegans individuals from outside potential hybrid zones (areas where *T. gaigeae* do not occur) were used as priors for that species. *T. gaigeae* from Indio Mt. Ranch, Hudspeth County, TX, were used as priors for *T. gaigeae*, as this remains the most isolated remaining population of *T. gaigeae* in the USA, and therefore the least likely to have allele frequencies influenced by hybridization with *T. s. elegans*. The MCMC analysis was burned in for 100,000 generations, and then run for 100,000 more. Individuals whose multilocus genotype contained >10% missing data and resulted in an assignment to conflicting genotypic classes were removed from the analyses.

All loci were tested for Hardy Weinberg equilibrium with Fisher's exact test for each population designated by the Structure 2.0 analysis. Sequential Bonferroni correction was applied to the Big Bend *T. gaigeae* population. Pair wise F_{st} values were calculated among any significant populations using Arlequin 3.1. Allele frequencies for each population were estimated by Structure 2.0.

Results: Over 600 turtles of 5 species were captured in 1266 trap days (number of traps x number of days in place) inside and adjacent to Big Bend National Park. Twenty (9%) of the 212 Big Bend sliders individually marked in 2005 were recaptured during the 2006 survey period. One female and 4 male *Pseudemys gorzugi* were captured in Beaver Pond, a species that had never previously been recorded in Big Bend National Park. Six *Kinosternon flavescens* were also captured, 4 in Beaver Pond, 1 at Talley, and 1 DOR (road kill) near the Santa Elena Canyon overlook. For the more abundantly collected taxa, 335 *T. gaigeae* were captured, 8 *T. s. elegans*, 4 potential hybrids of the two species (based on intermediate phenotype), and 360 *Apalone spinifera*. Measurement data from

all *Trachemys*, including those captured by Forstner et al. (1999), were summarized by population and by time period (Table 1).

Analysis of genetic data further substantiates that there are, in fact, hybrids of *T. gaigeae* and *T. s. elegans* in the Big Bend region. The mitochondrial DNA results reveal that the majority of hybridization events appear to be the result of male *T. s. elegans* mating with female *T. gaigeae*, although a single instance of the reciprocal cross was detected. There is no apparent geographic structure within *T. gaigeae* in the mtDNA results. These results echo those of earlier work (Forstner et al., 1999) and also met our expectations that mtDNA analyses were not adequate to explicitly evaluate the hybridization events within BBNP. However, also expectedly these results allow an initial view of the direction of hybridization in the system.

Analysis of multi-locus genotypes using Structure 2.0 yields 3 significant population groups (Figure 2): New Mexico *T. gaigeae*, Big Bend region *T. gaigeae*, and *T. s. elegans*. Calculation of pair wise F_{st} values for these three populations substantiates their significant divergence from each other (Table 2). Significant deviation from Hardy Weinberg Equilibrium expectations was found in all populations tested. In all cases the deviation was a deficit of heterozygotes. NewHybrids analysis reveals 8 individuals likely to be F2, and 2 potential backcross individuals. Genetic diversity for each population identified by the Bayesian assignments in Structure is summarized for each of the three statistically significant populations (Table 3).

Discussion:

In 1997 and 1998, Forstner et al. (1999, *in press*) conducted surveys for this species in BBNP. Our own work in the present study both confirms and expands on the results from a decade prior. The trapping efforts for the current study were significantly more intense and, in replication, have stronger inferential capability on the location of exotic turtles within BBNP and adjacent areas. Significantly more turtles were captured during the past two years of surveys than were captured in the study of a decade ago. With more than six hundred individual turtles captured the field effort itself was very successful.

Genetic results are likewise unambiguous, both exotic turtles and hybrids exist within BBNP. The relative frequency has not changed significantly from a decade ago to today, which is likely good news for the species and for the likely success of a resolution to the introduced turtles. We assume that the introduction of exotic turtles is less likely to be currently ongoing within the park and, with a more informed public, it is less likely to begin again in the future. Thus, we speculate that the “core” of the introduction event was decades ago and that the situation we find ourselves in today is one of a historical introduction of exotics without consistent subsequent reintroductions recently.

Field data From the results, it is apparent that while we rarely encountered *T. s. elegans*, nearly all the ones captured inside the National Park were in a single isolated body of water, the Beaver Pond at Rio Grande Village, one additional hybrid was captured in the river by Rio Grande Village, at a location where the river flows within 40

yards of the Beaver Pond. If we examine this site alone, exotic turtles or hybrids made up 14 % of the Emydid turtle community of this pond. Two of the three hybrids here were taken in the pond itself, and the third was caught in the river proximal to the pond. This is consistent with the observations of Seidel et al. (1999), and suggests that while the greatest density of invaders is in the pond rather than the river, progeny from hybridization that occurs in the pond can easily disperse into the Rio Grande/Rio Bravo. Individuals originally marked in the river were recaptured in the beaver pond, and vice versa, illustrating free movement (and potential gene flow) between the two habitats. This would allow exotics to easily contaminate the *T. gaigeae* populations in the river by back crossing, even if the *T. s. elegans* were confining themselves to the pond. It is likely that there are red-eared sliders in the river as well, and that we have simply failed to capture them in the river up to this point.

The removal of captured *T. s. elegans* and potential hybrids in 2005 resulted in a 3% reduction of their frequency in our sampling during 2006. While the low total numbers of exotic capture does not allow us to make the claim that this is a significant percentage, empirically we are convinced that the removal of these exotics over time is both feasible and will be effective with subsequent follow-ups on our efforts. Additional individuals collected near Santa Elena and Black Dike were phenotypically atypical for *T. gaigeae*, and several additional *T. s. elegans* and a hybrid were trapped near Lajitas. This was as expected given the previous results (Forstner et al., 1999), and, we feel that the maintenance of the locations, without significant expansion of locations for exotics and hybrids across the intervening years, is further good news for *T. gaigeae*.

One particularly valuable result from our work during the past two years that was virtually unknown prior to this study is the remarkable dispersal capability for the species in relatively short period of time. The mark recapture data reveal that *T. gaigeae* individuals are capable of moving great distances along the river. Individuals were recaptured in 2006 from 8 to a maximum of 35 river miles **upstream** from their point of original capture and release in 2005. This demonstrates that they have a significant ability to disperse.

One other unexpected result was a reduction in overall capture success despite identical efforts in both years. We captured roughly half as many *T. gaigeae* in 2006 compared with the 2005 field season. This is even after having specifically optimized the 2006 fieldwork to coincide with the period of greatest trapping success (calendar period) in the 2005 season. Given that the flow during the survey period in 2006 was higher than during the 2005 survey period (Figure 3) it is likely that these results demonstrate that the turtles are forced to more densely occupy smaller areas of habitat when the river is very low, and that they disperse widely into suitable habitat created by higher water. This was also evident in the Beaver Pond, where trapping success was negligible in 2006 when there was more flow in the river. While one could argue that this was due to trap aversion or some other factor, sight surveys for basking turtles also clearly showed that the pond was virtually uninhabited in 2006 relative to 2005. Unfortunately, our ability to generate useful abundance estimates using mark recapture models is impeded by the low frequency of recaptures, as well as the small number of sampling occasions. At least three more replications of the trapping effort would be necessary to have the potential to generate estimates of abundance or population size that are not exceeded by the estimates

of error (Lettink and Armstrong, 2003). Thus, we will rely on genetic data to provide a deeper perspective on the underlying pressures and fragmentation effects on the remaining *T. gaigeae* populations.

DNA analysis From the results of the mtDNA analysis, it is apparent that *T. gaigeae* is a monophyletic group with very little resolution within either taxon (native or exotic) across the entire sampling areas using mtDNA sequence. It is also apparent that we are, in fact, easily able to distinguish between *T. s. elegans* and *T. gaigeae* based on external morphology alone. We had a one to one correspondence of phenotypic identification and genetic identification, thus the opportunity exists to rely on phenotypic characteristics without the necessity of genetic verification in the removal efforts. The situation for hybrids at the F1 level and beyond is less encouraging.

The analysis of the genotypic data using Structure (Figure 2) shows that individuals that we designated *T. gaigeae* or *T. s. elegans* in the field have a high probability of being assigned to that group by their genotype. It also illustrates that there are individuals we called *T. gaigeae* that have similar assignment probabilities to the individuals that were determined to be hybrids by external morphology, revealing that there are hybrids that we were not able to detect using external morphology alone. This is corroborated by the results of the NewHybrids analysis, which was able to detect hybrid and backcross identities of individuals that appear phenotypically to be *T. gaigeae* (Figure 4). At the same time, some of the individuals assumed to be hybrids by visual examination are not strongly assigned to hybrid genotypic classes by NewHybrids. On

such individual was collected in Terlingua creek and despite morphological and phenotypic characters believed to be hybrid in origin, was not strongly assigned to this category.

In contrast to the results of Forstner et al. (1998), we have found alleles at some loci that appear unique to *T. gaigeae* (Table 3), however the frequency of these alleles is 3% or less. In other words, they are extremely rare. Noting that our sample size for *T. gaigeae* is slightly more than twice that of *T. s. elegans*, and the rarity of these alleles, it is possible that we merely failed to detect them in *T. s. elegans* consequent of reduced sample size in the exotics. The difficulty in detection of hybrids, even with genetic data, largely stems from our inability to be completely sure of the purity of any *T. gaigeae* we capture, since *T. s. elegans* appear in virtually all known USA populations of *T. gaigeae*, however infrequently. The identity of at least some pure individuals must be known in order to set priors for the Bayesian analysis. Without the ability to set priors, one must face a reduction in the power of the analysis.

It has also been demonstrated through simulation experiments that one of the most critical factors effecting both the efficiency and accuracy of this type of analysis method in detecting hybrids is the frequency of hybrid individuals in the data set (population)(Juha-Pekka and Primmer 2006). These simulations demonstrate that populations where hybrids occur at frequencies similar to what we would expect in BBNP (~1%) present the greatest hurdle to identification by microsatellite data unless unrealistically large numbers of markers are available.

It is hoped that future additions to sample size, especially of the more rare species (*T. s. elegans*) will increase the power of our analyses. While we are clearly able to detect some hybrids and backcrosses using multi locus genotypic data, it is interesting to note that they still appear rather infrequently. Given that *T. s. elegans* have been found in the Rio Grande/Rio Bravo in sympatry with *T. gaigeae*, especially in New Mexico, for some time, this relatively low frequency of occurrence suggests to the authors that there is likely some form of reproductive isolating mechanism in place between the two species that is maintaining the genetic integrity of the two species to some degree. This should be accepted with caution, however; we should not rely upon this speculative mechanism alone until we have enough data to determine what and how effective it is. Likewise, the exotics pose more than simply a problem of hybridization and ultimately are consumptive of the resources shared by the native turtles.

The Structure analysis also assigned the *T. gaigeae* captured in Hudspeth County, Texas, to the New Mexico *T. gaigeae* group. This is further evidence that this species historically inhabited the region of the Rio Grande between this area and the New Mexico populations, which are currently centered in the Elephant Butte Reservoir area North to Bosque del Apache WMA. Sampling of this region by Forstner et al. (1999) demonstrated that the species is most likely extirpated from the area between the current New Mexico and Texas populations. This inference is reinforced when the previously mentioned conservation of turtle DNA and its slower evolutionary rate is considered.

Analysis of the microsatellite data also resulted in the assignment of *T. s. elegans* from both Texas and New Mexico to one population. This is an interesting contrast to the assignment of *T. gaigeae* from the two states to significantly different populations.

There are several possible interpretations of these results each with similar overall conclusions. The differences found between the *T. gaigeae* populations were weakly present in 1997-1998 (Forstner et al. 1999) and may be a result of the two populations being isolated from each other. If so then we would predict that the *T. s. elegans* populations introduced to each location would likewise be differentiated. However, as the exotics have been released this is analogous to a novel migration via introduction of captive *T. s. elegans* into the environment. Combined with the likelihood of pet trade sources for the individuals we have a solution to the close genetic similarity of the exotics found in both New Mexico and Texas. Thus, while the exotics in both Texas and New Mexico are separated by the same factors differentiating the native turtles, their hypothesized pet trade origin and relatively recent introduction are actually supported by their genetic similarity.

Conclusion From the results obtained, it appears that the invasion of *T. s. elegans* in Big Bend National Park itself is concentrated around the Beaver Pond. This suggests that efforts to remove the invaders in order to thwart introgression as well as monitoring efforts should be concentrated here. This should not be done at the cost of monitoring other sites, however, since it is obvious given our dispersal documentation that the captured *T. s. elegans* or hybrids at other sites (Lajitas, Santa Elena, etc...) are able to move easily across the park boundaries. It appears that *T. s. elegans* has not been able to explosively invade the area, as their frequency is about the same as it was in 1998 (Forstner et al., 1999). This may mean that a barrier to their colonization and extensive

hybridization exists, or it may be that they just have not had enough time to become fully established. It is also important to note that we have only explicitly considered the threat to *T. gaigeae* from introgression, and there remains the issue of competition with *T. s. elegans*, which is equally insidious, especially in an environment as harsh and overtaxed as the Rio Grande/Rio Bravo.

Management Implications

The infrequency of both hybrids and *Trachemys scripta elegans* in our survey data appears to be good news for *T. gaigeae*. As is always possible with any animal trapping effort, that individuals were present that we were unable to detect. It is also likely that as new methods are developed in the field in the coming years we will have increased statistical power to detect hybrids within our genetic data set. The removal of exotic turtles remains a feasible and rationale approach to directly mitigating the introduction events. However, as with any potentially invasive species, future releases must also be prevented through education and awareness within the communities along the Rio Grande/Rio Bravo.

The two biggest advances that would help us in the coming years are; 1) collection of data from “pure” stock *T. gaigeae*, with the Rio Conchos providing an obvious potential source and 2) the inclusion of additional exotics as the removal efforts proceed. Given that removal of individuals of undesirable or questionable ancestry reduced the frequency of their appearance in the data set the following year, continued removal efforts could not hurt the present situation. It is also important to remember that

the introduction of *Trachemys scripta elegans* also introduced competitors for *T. gaigeae*, who are already experiencing a reduction of habitat and resources, adding to the jeopardy they are currently facing.

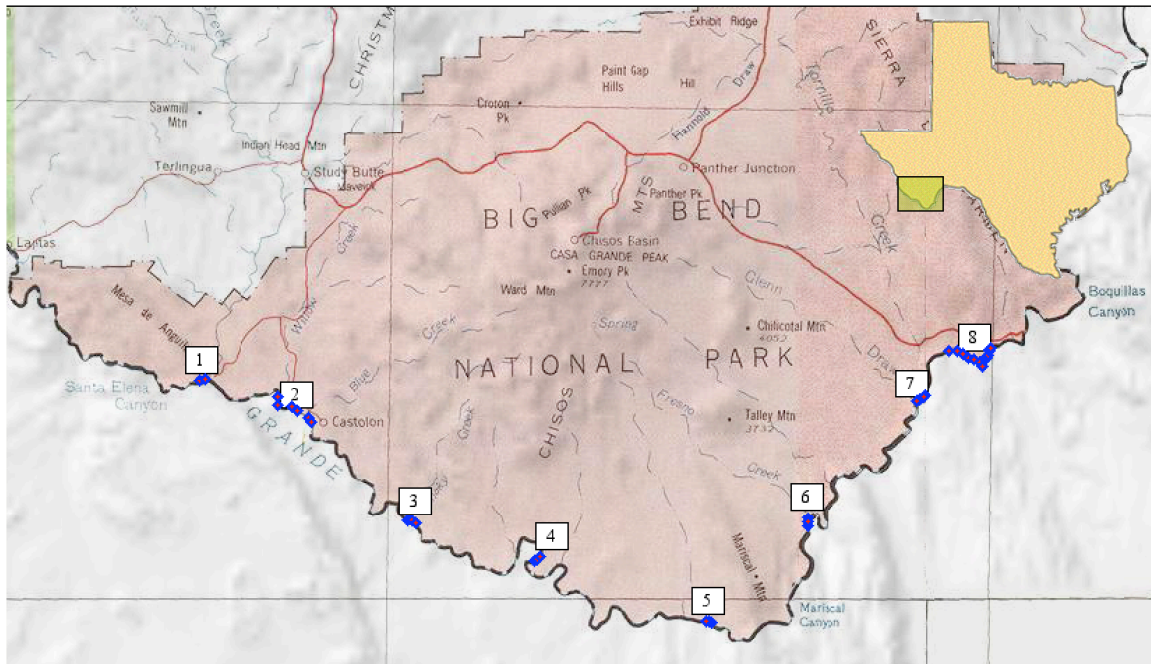
Evidence from the analysis of genetic data illustrates that the *T. gaigeae* population in the Big Bend Region is unique to other known and characterized populations of *T. gaigeae*. We speculate that this difference has, in part, been established by immigrants from the Rio Conchos drainage, but we will be unable to ascertain this until sufficient samples have been collected there. Given that these populations are nearly as divergent from one another as they are from *T. s. elegans*, the solution to their subdivision is complicated. It is possible that we may be able to mitigate the loss of genetic diversity by artificially transferring individuals between populations. If not, the two populations need to be managed as distinct and independent groups.

We detected evidence from the analysis of multilocus genetic data of deviations from Hardy Weinberg equilibrium (in each case having excess homozygosity relative to theoretical expectations). This indicates a departure from the expectation of random mating, it also could be interpreted to show a clear signal of a severe contraction of the size of populations (bottleneck). Estimates of population size will help to discern the functional aspects of why we have determined these inbreeding results, for example is this a mating system issue or is it truly the result of a steep decline in the total number of individuals in the system. If so, then the resulting loss of genetic diversity is cause for continued and increased concern for the species and its future.

We have demonstrated that the dispersal ability of these organisms is great enough that areas adjacent to Big Bend National Park (Lajitas, Big Bend Ranch State Park, Black Gap WMA) cannot be ignored in the management of this species. Given that we have established that this species has experienced a significant population reduction and loss of genetic diversity, we must strive to include as much of the population as possible in future management plans to retain what diversity remains.

Continuation of the mark recapture portion of this project would be very useful. We have many individuals already marked, but insufficient recapture data to yield useful estimates of population parameters. The long-term continuation (minimally three years of additional trapping at similar effort as the preceding two) of this project could serve as a useful monitoring tool for *T. gaigeae*, and eventually yield enough data to estimate survivorship, etc. It would also facilitate continued removal of any introduced turtles, and allow possible detection of any change in their frequency. We will continue our efforts this summer and that will leave just two additional seasons that would be necessary for a mark recapture evaluation to yield substantial results.

Compliance: This project was conducted under the following permits: BBNP permit number BIBE-2005-SCI-0027, and IACUC NIHAUH_03. All traps were placed on the Texas side of the river.



Map created with TOPO!® ©2003 National Geographic (www.nationalgeographic.com/topo)

Figure 1. Numbers represent approximate areas where traps were located in the eight main areas of Big Bend National Park surveyed in 2005 and 2006: Santa Elena Canyon (1), Cottonwood/Castolon (2), Black Dike (3), Loop Camp (4), Talley (5), Solis (6), La Clocha/Gravel Pit (7), and Rio Grande Village (including Beaver Pond) (8).

Table 1. Summary of trapping results for *Trachemys gaigeae* in the Rio Grande river drainage system during 1997-1998 and 2005-2006. Means of measurements taken are included accompanied by their standard deviation.

State	Sex	#	Plastron length (sd) mm	Plastron width (sd) mm	Carapace length (sd) mm	Weight (sd) g
New Mexico						
1997/98						
	F	19	193.22 (+/- 41.23)	120.42 (+/- 24.91)	210.44 (+/- 44.30)	1324.00 (+/- 650.70)
	M	27	145.15 (+/- 24.72)	94.65 (+/-15.36)	164.11 (+/- 28.13)	564.59 (+/- 254.42)
	?	4	34.5 (+/- 5.97)	27.75 (+/- 4.11)	38 (+/- 6.22)	10.90 (+/- 5.20)
		n = 50				
Texas						
1997/98						
	F	69	142.92 (+/- 32.69)	95.12 (+/- 19.97)	156.82 (+/- 36.06)	580.98 (+/- 360.98)
	M	83	113.51 (+/- 25.03)	75.31 (+/- 14.17)	128.18 (+/- 26.52)	299.36 (+/- 213.75)
	?	1	85	60	97	120
		n =153				
Texas						
2005/06						
	F	135	163.75 (+/- 39.99)	106.58 (+/- 16.49)	177 (+/- 29.14)	813.99 (+/- 350.67)
	M	182	118.83 (+/- 19.34)	79.73 (+/- 15.29)	134 (+/- 16.56)	311.84 (+/- 128.71)
	JV	4	88 (+/- 11)	59.5 (+/- 7.5)	93 (+/- 10)	120 (+/- 40)
		n =321				

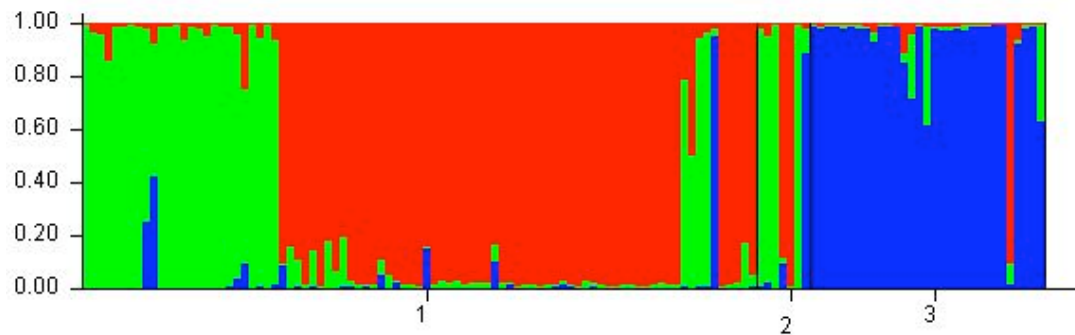


Figure 2. Graphical output from analysis of multilocus genotypic data using Structure 2.0. Group 1 were individuals identified in the field as *Trachemys gaigeae*, group 2 those identified as hybrids, and group 3 those identified as *T. scripta elegans*. Blue represents the probability of assignment of that individual to *T. s. elegans* based on that individual's genotype, green represents probability of assignment to the New Mexico population, and red represents probability of assignment to the Big Bend population. It is evident from this graph that there are a number of individuals that were identified as *T. gaigeae* based on their external morphology that are potentially hybrids according to their nuclear genotype.

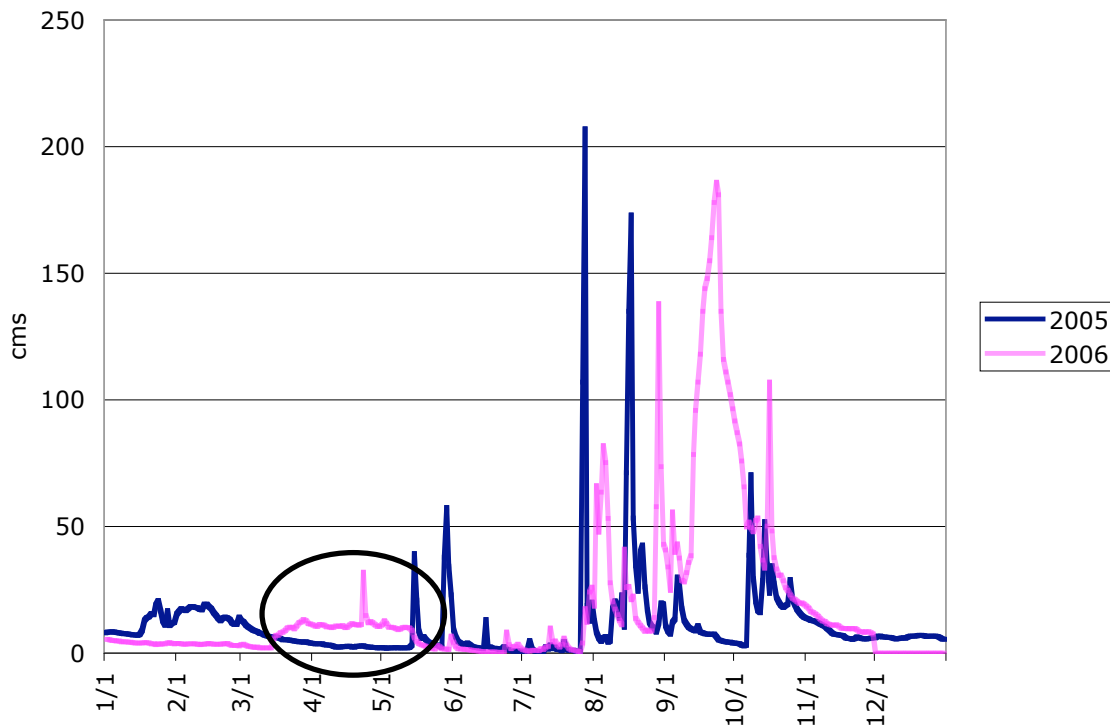


Figure 3. Flow (in cubic meters/sec) at Johnson's ranch for 2005 and 2006. Circled is the period of the year immediately preceding sampling events, and including the beginning of sampling in 2005, and most of the period of sampling in 2006. This illustrates that during the months preceding sampling in 2006 the flow was greater, and that for the same period in 2005 it was extremely low. This is likely have resulted in greater dispersal of turtles during the 2006 sampling period, contributing to lower capture success than in 2005. This could be interpreted as the minimum flow necessary for *Trachemys gaigeae* to disperse throughout the park.

Table 2. Pair wise Fst values for the three significant populations of turtles resolved from the multilocus genetic data. While the Big Bend and New Mexico populations are strongly divergent from one another, note that they are more strongly, and nearly equally, divergent from *Trachemys scripta elegans*.

	Big Bend	New Mexico	<i>T. s. elegans</i>
Big Bend	0	*	*
New Mexico	0.29505	0	*
T. s. elegans	0.33041	0.33465	0

Table 3. Number of alleles per locus for each of the three populations of turtles resolved from the multilocus dataset. Note the overall higher allelic diversity of *Trachemys scripta elegans*. While this table does illustrate the presence of potentially unique alleles in *T. gaigeae*, they occur at estimated frequencies of less than 5%. Their appearance here as "unique" may merely be an artifact of disparate sample size between the two species, or stochasticity of the sampling process.

Locus	Big Bend	New Mexico	<i>T. s. elegans</i>	mean	total
mt3	12	8	15	11.66	26
tufu2	13	9	11	11	21
pseud4-128	5	1	14	6.66	17
pseud225-2	6	3	7	5.33	12
gmuA19	7	5	13	8.33	16
gmuB08	11	8	9	9.33	15
gmuD70	11	11	21	14.33	28
mean	9.28	6.42	12.85	9.52	19.28
n	66	29	32		

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