

THE IMPORTANCE OF ODOR RECEPTORS TO THE CHEMOSENSORY BEHAVIOR OF SEA TURTLES

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AIM AND SUMMARY

The project's goal was to assess the importance of olfaction in the life history of sea turtles and to use this information to identify deterrent chemicals that might be applied to fish bait. The approach was to identify and characterize odor receptor (OR) genes, determining their relative importance to the life history of the turtles based on evolutionary selection. This approach was entirely noninvasive as appropriate for endangered species, requiring only aliquots of pre-existing blood samples for genetic analysis. Consequently, we successfully identified a subpopulation of OR genes in several sea turtle species. These genes were initially compared among themselves and with OR genes from other species. Subsequently, sequence variation of select sea turtle OR genes was characterized from discrete turtle populations to determine the degree of evolutionary selection acting on these genes. Low sequence variation would be interpreted as indicating positive selection and the importance of these genes to the life history of the species. We identified two OR genes that are highly conserved among two and three sea turtle species, respectively, and observed that a pattern of allelic variation among these and the less conserved OR genes were consistent with our hypothesis. Sea turtles appear to have a reduced complement of OR genes relative to their non-marine relatives, but the OR genes appear to be under positive selection suggesting that the olfactory pathways are indeed important to sea turtle behavior. We are currently performing tests to estimate the strength of the evolutionary selection. These results provide genetic evidence that validates efforts aimed at developing olfactory-based strategies for controlling the behavior of sea turtles, such as repelling them from fishing baits.

BACKGROUND

Sea turtles possess three major chemosensory systems or modalities: ciliary olfactory neurons, microvillus olfactory neurons, and taste neurons. Little is known about what molecules each modality perceives, as there have been only a handful of studies examining chemosensory responses of sea turtles. Since these animals are aquatic, it is possible that all three modalities respond to waterborne stimulants; although there is some conjecture that the ciliary neurons may respond to airborne odorants and that the microvillus neurons may respond to waterborne odorants. Where two modalities might respond to waterborne stimulants, these stimulants might be divided into more or less nonoverlapping chemical classes of molecules. Alternatively, or additionally, the possession of seemingly parallel chemosensory pathways (ciliary, microvillus, and taste) may have allowed these pathways to

evolve to convey information towards different behavioral contexts. In this case, ciliary and microvillar olfactory systems might be used for the detection of airborne or waterborne odors coming from a distant source, while the taste system might be used for the immediate assessment of the suitability of candidate food sources.

A considerable amount is known about the receptor genes of ciliary olfactory neurons; these are referred to specifically as OR genes and they encode the predominant chemosensory receptors involved in attractant type behaviors in vertebrate animals ranging from fish to mammals. The receptor genes associated with ciliary olfactory neurons, the OR genes, were the main focus of our study. The receptor genes of microvillus olfactory neurons were poorly understood at the outset of our project. Microvillus olfactory neurons associate with the vomeronasal organ in mammals and may have roles in pheromone detection and the modulation of social behavior. During the course of our study, others identified classes of these receptors in mammals and one subclass in fish. We used this information to identify such receptors in sea turtles, but have been unsuccessful to date. Microvillus olfactory neurons are abundant in the turtle nasal cavity, along with ciliary olfactory neurons, and thus characterizations of the associating receptors seem desirable. The receptor genes of taste neurons are not sufficiently characterized across vertebrate animals (fish to mammal) to make them suitable for broad scale characterization at this time. However, considering their likely role of assessing the quality of candidate food sources during biting, information of these receptors would as well seem desirable.

I. Identification of Ciliary Olfactory Odor Receptor (OR) Genes in Sea Turtles (manuscript near completion).

We identified OR genes from loggerhead (*Caretta caretta*), leatherback (*Dermochelys coriacea*), and green (*Chelonia mydas*) sea turtles. For comparison, we also identified OR genes from several species of terrestrial turtles ranging in degree of aquatic habitat (musk turtle, *Sternotherus odoratus*; box turtle, *Terrapene carolina*; painted turtle, *Chrysemys picta bellii*; and gopher tortoise, *Gopherus polyphemus*). And, for more distant comparison, we also identified OR genes from the American alligator (*Alligator mississippiensis*). All sequences were characterized for the presence of internal stop codons; such presence identifies them as pseudogenes or nonfunctional ORs. All sequences were also characterized against known OR sequences to determine their phylogenetic relationships among the larger OR gene family.

Our approach was to design Polymerase Chain Reaction (PCR) primers that should universally recognize OR genes in vertebrate animals ranging from fish to mammal and use these to amplify candidate DNA fragments from turtle genomic DNA obtained from provided blood samples. PCR products were cloned (i.e., inserted into bacterial plasmids), resulting in the construction of small DNA libraries enriched with OR genes. Approximately 200 clones were sequenced from each species' DNA library; OR sequences were identified based on significant sequence similarity to previously known olfactory receptor genes. All OR gene sequences have been submitted to and published in the GenBank database.

Sea turtles may have fewer OR genes than freshwater or terrestrial turtles. Table 1 summarizes the number of clones sequenced for each species and the number of unique OR genes encountered among those clones; many of these OR genes were encountered multiple times within the species-specific libraries. Sea turtles yielded fewer OR genes compared to freshwater and terrestrial turtles. This reduction is consistent with at least one report that sea turtles have fewer chemosensory neurons within their nasal epithelium than terrestrial turtles.

Table 1: Summary of olfactory receptor genes identified.

Species	# clones sequenced	# unique OR genes identified	Percent (number) of pseudogenes	Source of tissue
Loggerhead (sw) <i>Caretta caretta</i>	260	20	25 (5)	Dr. David Whitaker (South Carolina DNR)
Green (sw) <i>Chelonia mydas</i>	160	14	40 (6)	Dr. Richard Brill (NMFS—while at Honolulu Lab)
Leatherback (sw) <i>Dermochelys coriacea</i>	285	17	44 (7)	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Musk (fw) <i>Sternotherus odoratus</i>	200	22	9 (2)	Judy Greene (Savannah River Ecology Lab—Aiken SC)
Painted (fw) <i>Chrysemys picta bellii</i>	235	63	27 (17)	Judy Greene (Savannah River Ecology Lab—Aiken SC)
Box (terr) <i>Terrapene carolina</i>	225	42	2 (1)	Judy Greene (Savannah River Ecology Lab—Aiken SC)
Gopher (terr) <i>Gopherus polyphemus</i>	225	45	9 (4)	Judy Greene—(Savannah River Ecology Lab—Aiken SC)
Alligator <i>Alligator mississippiensis</i>	200	22	18 (4)	Dr. Travis Glenn—(Biological Sciences, University of South Carolina)

Sea turtles may have a higher percentage of OR pseudogenes than freshwater or terrestrial turtles.

One aspect of the evolutionary dynamics of a gene family is the expansion or contraction of the size of that gene family among species. One expression of this dynamics is the recognizable presence of pseudogenes. In the case of OR genes, these are recognized by the presence of internal stop codons, which would yield truncated and presumably nonfunctional gene products. Humans, for example, have a much higher proportion of OR pseudogenes than mice and consequently a much lower number of functional OR genes

(perhaps 300 compared to perhaps 1000 in mice). The meaning of such a difference is unclear, as olfaction is clearly important to humans. Nevertheless, the greater or lesser presence of pseudogenes suggests something regarding the olfactory capability of a species, perhaps relating to the diversity or complexity of olfactory-related behaviors of that species. In our study, sea turtles appeared to have a considerably higher proportion of OR pseudogenes than freshwater or terrestrial turtles; together with their reduced total number of OR genes, this suggests that odor detection, at least by ciliated olfactory neurons, may be less important to the life history of sea turtles than it is to the life history of freshwater or terrestrial turtles. However, "less important" may be misleading. It may be that sea turtles use ciliated olfactory neurons for airborne odor detection, and the detection of airborne odors may have a more focused or specific role in sea turtle behavior than it does in freshwater or terrestrial turtles. Alternatively, the complexity of airborne odors may be much less over marine environments than it is in terrestrial environments. Again, reduction of capacity does not necessarily mean reduction in importance.

Sea turtle OR genes represent subclasses of mammalian OR genes and perhaps a total number similar to humans (200–300).

Two broad classes of OR genes have been identified in vertebrate animals: one class that ranges from fish to amphibians, and a second class that ranges throughout tetrapod vertebrates, including amphibians, birds, and mammals, but that is not seen in fish. This suggests that an expansion of the OR gene family accompanied the establishment of the tetrapod vertebrate lineage. Within amphibians, the fish-like ORs are thought to detect waterborne odorants while the mammalian-like ORs are thought to detect airborne odorants. The sea turtle OR genes we identified clearly belong to non-fish or mammalian-like class of OR genes, based on sequence comparisons. A survey of the presence or absence of our PCR primers within the mouse OR genome indicates that the sea turtle genes we identified represent a broad range, though not all, of mouse OR gene subfamilies. These numbers and a limited number of studies on the structure of terrestrial turtle olfactory bulbs suggest that turtles in general may have a similar number of OR genes as mammals (perhaps 1000), but that the number of functional OR genes in sea turtles may be similarly reduced as in humans, perhaps to a range of functional 200–300 OR genes.

Two identified sea turtle OR genes are highly conserved between sea turtle species.

Our study identified two genes that are highly conserved between sea turtle species. One gene encodes the identical amino acid sequence in loggerhead, green, and leatherback sea turtles, and a second gene encodes the identical amino acid sequence in loggerhead and green sea turtles. Such conservation is difficult to resolve unless these genes play both a significantly important and significantly similar role in the life history of these species. Given the relatively small sample size in our data set, it is highly likely that more such conserved OR genes are present in these species. It would be of great interest to know what odors stimulate these receptors and how odor detection is used in the behaviors of these species.

II. Population Genetics of Sea Turtle OR Genes: Evidence for Evolutionary Selection (manuscript in preparation).

We tested the importance of OR genes by comparing the sequences of specific genes among individuals among and between discrete populations. The relative prominence of one haplotype over others within a population of animals would suggest that the gene is important to the life history of the animal, its prominence a result of evolutionary selection that favors individuals possessing that haplotype (fitness is dependent on the gene). A more equal representation of multiple haplotypes within a population suggests that the gene is less important to the life history of the animal, the tolerated diversity is the result of a more relaxed evolutionary selection (fitness is less dependent on the gene).

"Haplotypes" are different versions of the same gene, where each nucleotide difference represents an individual "allele." In the evolutionary history of the species, each nucleotide change is presumed to have occurred independently. The generation of two haplotypes differing by, say, four nucleotides means that at least five versions (haplotypes) of that gene must be in existence, the original gene plus a version for each accumulated nucleotide change.

Whether or not a change in gene nucleotide (creating a new allele) results in a gene product (protein) with new properties depends on several things. Each amino acid is encoded by a triplet codon (three nucleotides). Changes in the third nucleotide position of a codon often do not alter the amino acid, while changes in the first or second nucleotide position do often change the amino acid. Nucleotide changes that do not alter the amino acid are referred to as "synonymous," while changes that alter the amino acid are referred to as "non-synonymous." Even if an amino acid is changed, the change may or may not alter the properties of the resulting protein, depending on the exact position of that amino acid within the protein (near or far from a sensitive region), and whether the change is to an amino acid with profoundly different chemical properties (charged to uncharged, hydrophilic to hydrophobic).

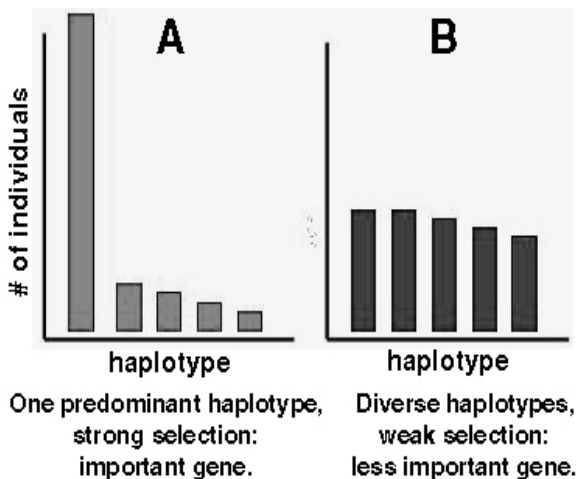


Figure 1. Hypothetical distribution of haplotypes in a population.

In Figure 1, two genes are represented within a population of animals. In both graphs, five haplotypes are shown where the height of the column indicates the proportion of individual animals possessing that allele. In Figure 1A, one haplotype is observed to predominate. In Figure 2B, all five haplotypes are more or less equally represented within the population. The predominance of one haplotype over all others in Figure 1A suggests that that haplotype provides some selective advantage; that individuals possessing that allele tend to "do better" than individuals possessing the other alleles, and the haplotype has emerged as a predominant version of that gene within the population. Such a pattern suggests that the gene is important to the life history of the animal.

Our approach was to examine the sequence variation among several sea turtle OR genes among individuals of defined populations and among different populations. Our hypothesis was that the importance of an OR gene would be reflected in the predominance of an individual haplotype. To test this hypothesis, we compared three sets of genes: one set that we had reason to believe was important, a second set of undetermined importance, and a third set that we had reason to believe was not important. Set 1 included the two OR genes already shown to be highly conserved between sea turtle species. Such conservation is difficult to resolve unless the genes are important to both species, and therefore the Set 1 genes represented OR genes with a high probability of importance. Set 2 included two OR genes for which no obvious orthologues were observed among species, but which also contained no internal stop codons. Set 3 included two to three OR genes which did contain internal stop codons, and which therefore were interpreted as not important based on their presumed nonfunctional status.

Blood samples were obtained for multiple individuals of loggerhead, green, and leatherback turtles from various sources. Four distinct loggerhead populations were characterized (Pacific, Atlantic, Mediterranean, Arabic Sea) while single populations were characterized for green (Pacific) and leatherback (Atlantic). The species and gene studies are summarized in the tables below.

Table 2: Summary of species, populations and numbers of individuals surveyed in the population genetics study.

Species, population, and gene summary	# of individuals	Source of tissue (blood samples)
Loggerhead—Pacific (LH-P)	10	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Loggerhead—Atlantic (LH-A)	20	Dr. David Whitaker (South Carolina DNR) Dr. Joe Quattro (University of South Carolina)
Loggerhead—Mediterranean (LH-M)	6	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Loggerhead—Arabian Sea / Oman (LH-O)	12	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Green—Pacific (G-P)	18	Dr. Richard Brill (NMFS—while at Honolulu Laboratory)
Leatherback—Atlantic (LB-A)	18	Dr. Peter Dutton (NMFS—La Jolla Laboratory)

Table 3: Summary of OR genes examined for each species.

	Taxon name	Accession number	# nucleotides analyzed	Set #
LH1 (conserved 1)	LHOR11	AY686461	339 (552)*	1
LH2 (conserved 2)	LHOR6	AY686456	329 (549)	1
LH3	LHOR12	AY686462	373 (507)	2
LH4	LHOR15	AY686464	272 (552)	2
LH5 (pseudogene)	LHOR3	AY686453	332 (537)	3
LH6 (pseudogene)	LHOR4	AY686454	299 (522)	3
G1 (conserved 1)	GTOR11	AY686407	339 (552)	1
G2 (conserved 2)	GTOR9	AY686405	329 (546)	1
G3	GTOR1	AY686397	326 (552)	2
G4 (pseudogene)	GTOR6	AY686402	348 (543)	3
G5 (pseudogene)	GTOR14	AY686410	326 (555)	3
LB1 (conserved 1)	LBOR12	AY686445	339 (549)	1
LB2	LBOR15	AY686448	372 (552)	2
LB3	LBOR10	AY686443	385 (552)	2
LB4 (pseudogene)	LBOR5	AY686438	379 (552)	3
LB5 (pseudogene)	LBOR14	AY686447	404 (552)	3

The number of nucleotides analyzed is noted, along with the total nucleotide length under the indicated accession number. Only a portion of the available sequence was used for these analyses because of restrictions in the availability of suitable unique PCR primer sites.

Analysis of Set 1 (conserved) genes—all species/populations.

Highly conserved Set 1 genes show remarkably little variation among populations and species, suggesting they are under strong positive selection and may play an important role in the chemosensory behavior of these animals. Highly conserved genes (Set 1) were represented by only a single haplotype for each population sample set; small but consistent differences were observed among the Pacific, Atlantic, and combined Mediterranean/Arabic Sea loggerhead populations. These OR haplotypes may be useful in large-scale characterizations of loggerhead populations; if so, the presence of common haplotypes in the Mediterranean and Arabic Sea populations suggests these two populations are sharing genetic material, perhaps by emigrational movement through the Suez Canal and Persian Gulf. Sequence variation was less between loggerhead and green than it was between either loggerhead or green and leatherback. This pattern is consistent with the close polygenetic relationship between loggerhead and green turtles and the more distant relationship of leatherbacks.

Table 4: Haplotype distribution and variation for two conserved OR genes found in loggerhead, green, and leatherback sea turtles (Set 1).

A. Conserved 1 (Haplotype Distribution)

	LH-A	LH-M/O	LH-P	G-P
Ha 1	20			
Ha 2		18		
Ha 3			10	
Ha 4				18

C. % difference in sequence (pair-wise comparisons)

Conserved 1	Ha 1 (LH-A)	Ha 2 (LH-M/O)	Ha 3 (LH-P)	Ha 4 (G-P)
Ha 1(L-HA)		<i>1.8</i>	<i>3.7</i>	<i>7.3</i>
Ha 2(LH-M/O)	0.6		<i>1.8</i>	<i>5.5</i>
Ha 3(LH-P)	1.2	0.6		<i>5.5</i>
Ha 4(G-P)	2.7	2.1	2.1	

B. Conserved 2 (Haplotype Distribution)

	LH-A	LH-M/O	LH-P	LB-A	G-P
Ha 1	20				
Ha 2		18			
Ha 3			10		
Ha 4				18	
Ha 5					18

D. % difference in sequence (pair-wise comparisons)

Conserved 2	Ha 1 (LH-A)	Ha 2 (LH-M/O)	Ha 3 (LH-P)	Ha 4 (LB-A)	Ha 5 (G-P)
Ha 1(L-HA)		<i>1.8</i>	<i>0.9</i>	<i>0.9</i>	<i>8.2</i>
Ha 2 (LH-M/O)	0.6		<i>0.9</i>	<i>2.8</i>	<i>9.1</i>
Ha 3 (LH-P)	0.3	0.3		<i>1.9</i>	<i>8.1</i>
Ha 4 (LB-A)	0.9	1.5	1.2		<i>7.1</i>
Ha 5 (G-P)	4.7	50.0	4.7	4.3	

Tables 4A and 4B indicate the haplotype distribution among the respective species and populations. Numbers indicate the number of individuals sampled; only single haplotypes were observed in any population for both conserved OR genes. Tables 4C and 4D indicate the percent differences in nucleotide (normal font) and amino acid (italics) sequences, comparing indicated pairs of haplotypes. Since only single haplotypes were observed for each population, these numbers also indicate differences among populations.

OR haplotype variation among four populations of loggerhead sea turtles.

Figure 2 and Table summarize the data for the three populations of loggerhead turtles. In general, the two conserved genes (LH1, LH2), as stated above, do not show haplotype variation within populations; however, they show small variation among populations, the degree of which is also noted above. The two pseudogenes (LH5, LH6) are represented by several alleles, while the two genes that do not contain internal stop codons (LH3, LH4) show an intermediate number of alleles. Assuming these three sets represent a range of importance to the animals' life history, these results appear to support our hypothesis that importance of a gene is reflected in the restriction of allelic variation.

It is noteworthy that the range in variation between haplotype sequences is somewhat similar, regardless of the gene examined (see tables following page). Rather, the prominent difference observed is in the prevalence of minor haplotypes: the conserved OR genes show only single haplotypes, while the presumably nonselected pseudogenes show the greatest diversity of haplotypes.

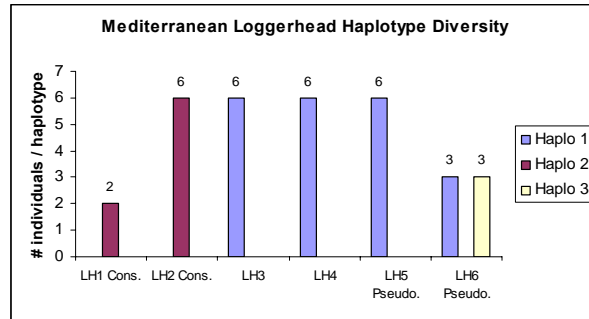
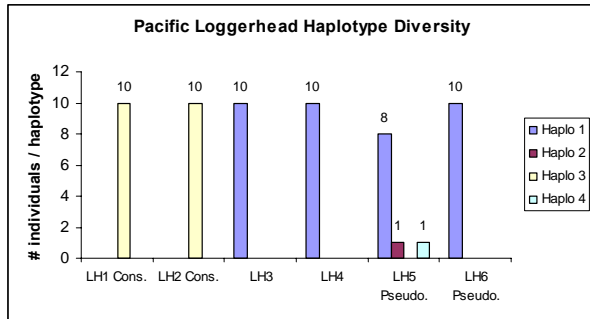
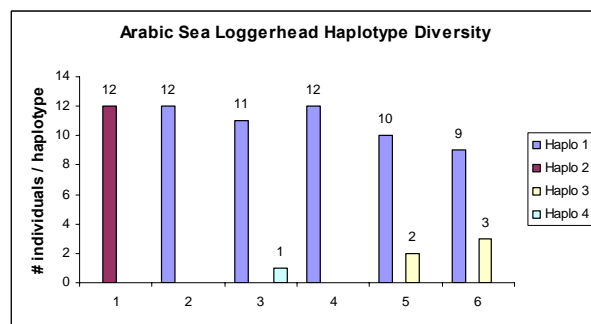
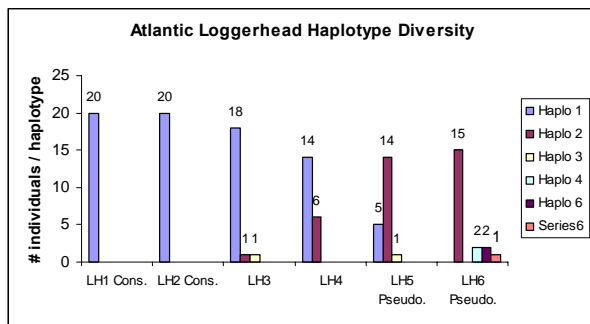


Figure 2. Haplotype diversity of loggerhead OR genes.

Table 5: Table 5 summarizes the data regarding the four non-conserved loggerhead OR genes (Sets 2 and 3).

A. LH3 (Haplotype Distribution)

	LH-A	LH-O	LH-M	LH-P	Total
Ha 1	18	11	6	10	45
Ha 2	1				1
Ha 3	1				1
Ha 4		1			1

E. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2	Ha 3	Ha 4
Ha 1		<i>0.8</i>	<i>0.0</i>	<i>0.8</i>
Ha 2	0.3		<i>0.8</i>	<i>1.6</i>
Ha 3	0.3	0.5		<i>0.8</i>
Ha 4	0.3	0.5	0.5	

B. LH4 (Haplotype Distribution)

	LH-A	LH-O	LH-M	LH-P	Total
Ha 1	14	12	6	10	42
Ha 2	6				6

F. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2
Ha 1		<i>0.0</i>
Ha 2	0.4	

C. LH5-pseudo (Haplotype Distribution)

	LH-A	LH-O	LH-M	LH-P	Total
Ha 1	5	10	6	8	29
Ha 2	14			1	15
Ha 3	1	2			3
Ha 4				1	1

G. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2	Ha 3	Ha 4
Ha 1		<i>1.9</i>	<i>0.9</i>	<i>0.9</i>
Ha 2	0.6		<i>0.9</i>	<i>0.9</i>
Ha 3	0.3	0.3		<i>1.9</i>
Ha 4	0.3	0.3	0.6	

D. LH6-pseudo (Haplotype Distribution)

	LH-A	LH-O	LH-M	LH-P	Total
Ha 1		9	3	10	22
Ha 2	15				15
Ha 3		3	3		6
Ha 4	2				2
Ha 5	2				2
Ha 6	1				1

H. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2	Ha 3	Ha 4	Ha 5	Ha 6
Ha 1		<i>1.0</i>	<i>0.0</i>	<i>2.0</i>	<i>1.0</i>	<i>3.0</i>
Ha 2	0.7		<i>1.0</i>	<i>1.0</i>	<i>0.0</i>	<i>2.0</i>
Ha 3	0.3	1.0		<i>2.0</i>	<i>1.0</i>	<i>3.0</i>
Ha 4	1.3	0.7	1.0		<i>1.0</i>	<i>1.0</i>
Ha 5	1.0	0.3	0.7	0.3		<i>2.0</i>
Ha 6	1.7	1.0	1.3	0.3	0.7	

Tables 5A – 5D indicate the haplotype distribution among the respective populations. Numbers indicate the number of individuals sampled. Tables 5E – 5H indicate the percent differences in nucleotide (normal font) and amino acid (*italics*) sequences, comparing indicated pairs of haplotypes.

OR haplotype variation among a Pacific population of green sea turtles.

Figure 3 summarizes data for the green turtles. The same general trend is observed as in loggerheads. The conserved OR genes (G1, G2) show only a single haplotype, respectively; the pseudogenes (G4, G5) show the most allelic diversity. The presumably expressed OR G3 (no internal stop codon) shows an intermediate level of diversity.

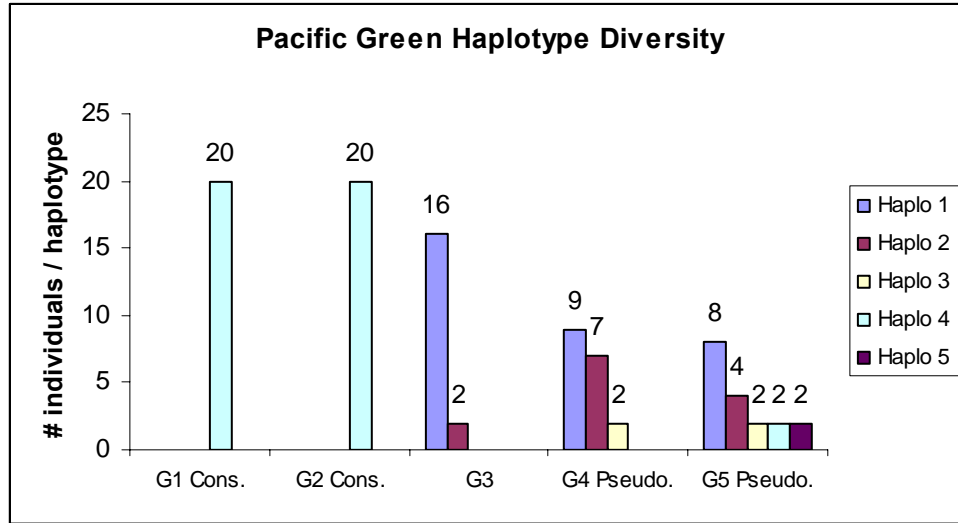


Figure 3. OR haplotype variation among a Pacific population of green sea turtles.

OR haplotype diversity among an Atlantic population of leatherback sea turtles.

Figure 4 summarizes the data for leatherbacks. The presumed expressed sequences (no internal stop codons) show the same trend as described above: conserved LB2 is represented by only one haplotype, while the less conserved LB3 and LB4 show considerably greater allelic diversity. A striking difference among the leatherback data and that of loggerhead and green turtles is the apparent conservation among the pseudogenes; all individuals possessed identical DNA sequences for genes containing internal stop codons. This unquestionably seems quite unexpected if these pseudogenes are indeed without function.

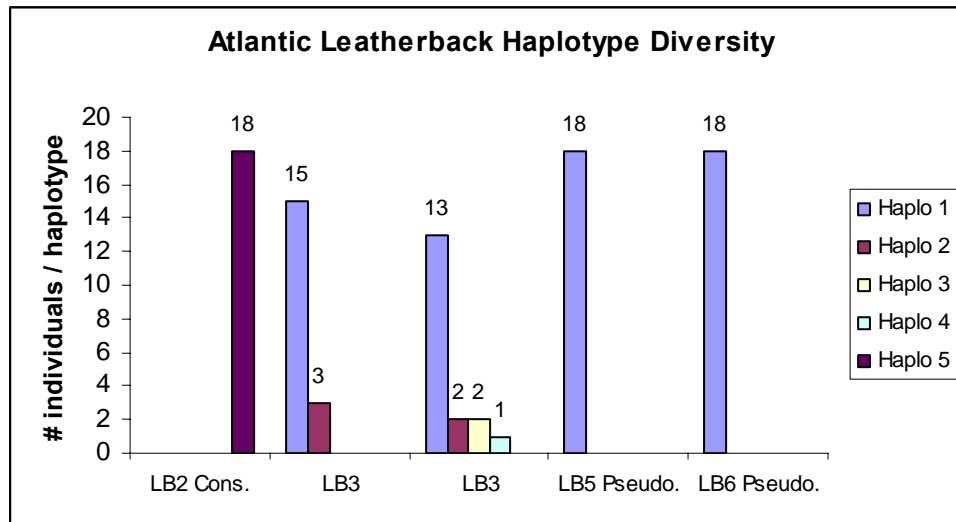


Figure 4. OR haplotype diversity among an Atlantic population of leatherback sea turtles.

SUMMARY

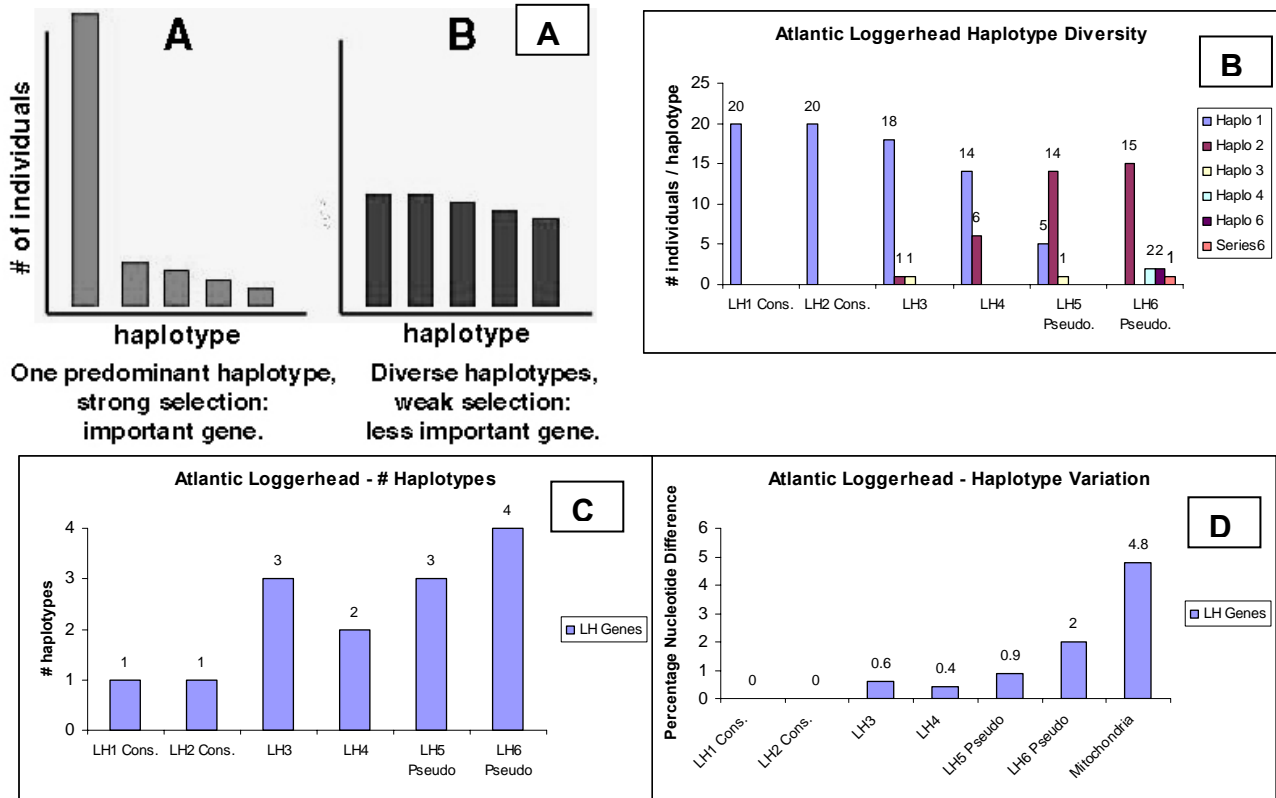


Figure 5. Summary of hypothesis and Atlantic loggerhead results.

Figure 5A illustrates our hypothesis that the importance of a gene may be reflected in the diversity of gene haplotypes. Figure 5B represents our results (previously shown) for the Atlantic population of loggerhead turtles (samples collected off the Carolina and Georgia coasts by the South Carolina Department of Natural Resources). These data appear to support our hypothesis. The two genes we interpret as being important, based on their high degree of conservation among different species, have a single dominant haplotype. The pseudogenes, arguably nonfunctional, show diverse haplotypes, while the two arbitrarily chosen genes (LH3 and LH4) show a somewhat intermediate pattern. Figure 5C indicates the number of haplotypes observed for each gene, and this, too, seems consistent with the hypothesis, with the conserved genes showing less diversity than the less conserved genes. Figure 5D compares the nucleotide differences among haplotypes of the respective OR genes with a mitochondrial gene, all for the Atlantic population of loggerheads. This mitochondrial sequence represents a neutral region of DNA and has been used to characterize the loggerhead populations (Anderson, Quattro, unpublished). Two haplotypes are predominant within the Atlantic population (accession numbers AJ001074 and AJ001075) and they differ in nucleic acid sequence by 4.8%. Because this region is under neutral selection, this variation can be viewed as an upper level variation one might expect. Again, the conserved genes show variation, the pseudogenes the greatest, though considerably less than the mitochondrial genes, and LH3 and LH4 show an intermediate level of variation.

We interpret these data to support our hypothesis that it is feasible to assess the importance of a gene based on its haplotype diversity and variation within a population. And based on the pattern of conservation of the two conserved genes, this study suggests that these genes and the odors their products convey are likely of considerable importance to these animals.

Relative conservation among pseudogenes.

When we began this study, we expected pseudogenes to be highly degenerate, on the verge of being unrecognizable. If they are nonfunctional, then evolutionary selection will no longer be acting on the genes, and they should be accumulating nucleotide replacements. Eventually, they should accumulate so many nucleotide replacements that they will no longer be recognizable. The data seem inconsistent with this point of view; especially with respect to the leatherback pseudogenes (Leatherback tissue samples were taken across a wide region of the Caribbean and therefore not likely from closely related individuals).

One possible explanation is that the pseudogenes have not entirely lost all function. OR genes are known to play a broader role than just detecting odors; they are also known to play a role in axon guidance, directing sensory neurons to their appropriate brain targets during development, and they are known to contribute to the regulation of which OR genes are expressed in a given sensory neuron. It may be that these pseudogenes are still playing a role and that selection is somehow still acting.

Perhaps a more interesting possibility is that these genes have only become pseudogenes relatively recently. In this view, the OR genome would be under constant expansion (gene duplication) and contraction (gene loss). Comparisons of the OR genomes of mouse and human suggest expansion and contraction of OR gene subfamilies. Certainly, we recognize the pseudogenes based on their retained similarity to the presumed functional genes. Perhaps their conservation indicates something of the genetic dynamics the olfactory system is capable of on a relatively short, but nevertheless evolutionary, time scale. Still, the conserved loggerhead pseudogenes remain perplexing.

III. Evidence of positive selection among sea turtle OR genes (under study).

The work described above presents a largely theoretical perspective on the importance of the OR genome in sea turtle behavior. We are currently applying mathematical analyses to these data to confirm (or reject) the proposed hypotheses.

CONCLUSIONS

We set out to determine if one could assess the importance of OR genes in the life history of an endangered species using largely non-invasive methods. We identified and compared the olfactory genomes of three species of sea turtles and, in so doing, identified two highly conserved OR genes suggesting an important and common use for olfactory processing for all three species. We suggested the possibility that airborne odorants play an important role in sea turtle behavior. We established an indirect genetic test of the importance olfaction has in the life history of these animals.

A goal of using these genetic data was to identify odors that might actually be employed to discourage turtles from fishing baits. Perhaps the most valid way to do this would be to take odor receptors which are presumed to be important, and identify the odors that activate them. This is feasible, but techniques are still under development. Perhaps this will be doable in the near future, but right now it remains a technical difficulty. Far easier, cheaper, and faster would be a physiological study recording electrodes on the olfactory nerves of a turtle and screening the types of ecologically relevant odor molecules that the turtle can perceive. Such a study should be coupled with careful behavioral analyses, measuring responses to these odors so that desired answers to these problems may be found.