

Using molecular and ecological data to diagnose endangered populations of the puritan tiger beetle *Cicindela puritana*

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Abstract

Populations of the puritan tiger beetle *Cicindela puritana* in the eastern United States were found to be highly threatened at the Connecticut River, whereas several large populations on the western shore and newly discovered populations on the eastern shore of the Chesapeake Bay appeared to be less endangered. We assessed if the disjunct *C. puritana* subgroups are genetically distinct and therefore should be treated as separate units for conservation purposes. A total of 13 individuals from the Connecticut River and 27 individuals from the Chesapeake Bay were each analysed by sequencing of up to 837 base pairs of mitochondrial DNA per individual. Five different haplotypes could be distinguished. In a phylogenetic analysis of these DNA sequences that included four related *Cicindela* species as out-groups, haplotypes from the Chesapeake Bay represent a distinct clade. The conservation status of these populations was evaluated using a phylogenetic approach based on cladistic analysis and the framework of the phylogenetic species concept. According to this analysis, beetles from the Connecticut River and the Chesapeake Bay have to be considered as independent units. Populations from the eastern and western shore of Chesapeake Bay are not split in more than one unit using the same criteria, although they exhibited some degree of genetic subdivision. The results from the mtDNA analysis were corroborated by ecological parameters in that the Chesapeake Bay populations can be distinguished from all congeners by their different habitat association.

Keywords: conservation genetics, mitochondrial DNA, phylogenetic species concept, polymerase chain reaction

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Introduction

Tiger beetles comprise an insect family of over 2000 described species with a world-wide distribution (Pearson & Cassola 1992). They are ground surface predators that require open habitat free of vegetation, such as mudflats, sand dunes, water edges, saline flats, or sandy river banks

and ocean beaches. Habitat requirements are very specific (Shelford 1908; Willis 1967), and most species are sensitive to alterations of habitat quality and to human disturbance (Knisley & Hill 1992). In places where they occur these beetles are common and easily detected. Tiger beetles, therefore, have been proposed as indicator organisms for conservation studies and for monitoring of global biodiversity (Pearson & Cassola 1992). We intend to use tiger beetles as model organisms to resolve controversial conservation questions relating to population biology and genetics.

Currently two species, *Cicindela dorsalis* Say and *C. puritana* LeConte, are listed on the United States Endangered Species List which mandates action for their protec-

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tion. *Cicindela puritana* has a disjunct geographic range in the eastern United States, known historically from locations on the Connecticut River in Vermont, Massachusetts and Connecticut, and from a second area on the western shore of Chesapeake Bay in Maryland several hundred kilometres away. The Connecticut River populations have been affected by urbanization and by the construction of dams during the early parts of this century and the resulting flooding of river banks. *C. puritana* has not been recorded from the Connecticut River since the 1930s. The threat to Chesapeake Bay populations is more recent and results from waterfront development for housing, shoreline stabilization, and recreational purposes. No historical records exist for any location in between the Chesapeake Bay and Connecticut River sites indicating that the disjunct distribution is not the result of recent extinction of connecting populations due to human disturbance.

Management and recovery programs for endangered species require surveys of remaining populations and determination of potential conservation units. To preserve the full range of biotic diversity, efforts have to be made to identify and to maintain differentiated populations. The assessment of conservation units in *C. puritana* therefore has to include a survey of suitable habitat at both the Connecticut River and the Chesapeake Bay sites to evaluate the number and size of existing populations and their threats. Individuals can be subjected to genetic analysis in order to determine the degree of differentiation between and within populations from both major locations. For such analysis, mitochondrial DNA (mtDNA) is well-established as a tool for studying genetic subdivision within closely related groups of organisms (Lansman *et al.* 1981; Wilson *et al.* 1985; Avise *et al.* 1987; Harrison 1989). MtDNA has been used extensively to study geographic subdivision (e.g. Avise 1992) and taxonomy of closely related groups of endangered organisms (e.g. Avise & Nelson 1989; Wayne & Jenks 1991; Bowen *et al.* 1991).

Most conservationists agree that distinct groups within a 'species' deserve separate conservation status. The evolutionarily significant unit (ESU) has become an operational term for populations that should be managed as such units of conservation management (Ryder 1986; Woodruff 1989; Amato 1991). However, no consensus currently exists for the criteria that should be used to determine these groups. It has been proposed to base the evaluation of the conservation status on the assumptions of the biological species concept (O'Brien & Mayr 1991) or on phylogeographic parameters (Avise 1992; Dizon *et al.* 1992). As an alternative, the phylogenetic species concept could be used to identify evolutionarily distinct populations that should be the target of conservation measures.

The objectives of this study are to: 1 determine current distribution and abundance of *C. puritana* and describe its habitat requirements; 2 assess mtDNA differentiation

within and between populations from both of these sites; 3 analyse the phylogeny of the *C. puritana* group for inferences on its evolutionary history; 4 evaluate the detected genotypic and ecological differences between potential conservation units within this phylogenetic framework.

Materials and methods

Population surveys and sampling

Censuses of adult *C. puritana* were made at times of highest beetle abundance (early in July) by slowly walking through the site and counting all individuals observed. Breeding sites were determined by locating larval burrows in the ground surface. Description of habitats were made by observations during the field census. Soil and geological information was obtained from published geological surveys at these sites. A limited number of larvae was removed from their burrows for species determination. For genetic analyses, individuals from the Connecticut River population were sampled at Cromwell, Middlesex Co., Connecticut, and were taken at the end of the reproductive period in August 1990, and beetles from the eastern and western shore of Chesapeake Bay were collected in July 1991 and July 1992, respectively. Other specimens used in this study were from Shawnee Co., Kansas (*C. macra*), Gallia Co., Ohio (*C. cuprascens*), Calvert Co., Maryland (*C. marginata*) and Taylor Co., Florida (*C. hamata*).

DNA techniques

Total genomic DNA was isolated from the thorax of individual beetles as described previously (Vogler & DeSalle 1993). Three regions of the mitochondrial genome were PCR amplified, including the coding region for the cytochrome oxidase subunit III (COIII) (region I), the 5' end of NAD subunit I (NDI), the tRNA^{leu}, and the 3' end of the large subunit of rRNA (16SrRNA) (region II) and the central part of the 16SrRNA gene (region III). Primers for the amplification of regions I and II were specifically designed to match the DNA sequence of the related species *C. dorsalis* (Vogler & DeSalle 1993), and the 'universal' primers 16sar/16sbr (Simon *et al.* 1991) were used to amplify region III. PCR products were purified (GeneClean, Bio 101, LaJolla, CA) and sequenced directly using primers that bind to regions within the amplified fragment. An average of 200 base pairs could be reliably determined from a single sequencing reaction. A diagnostic restriction fragment length polymorphism (RFLP) for the endonuclease *Hae*III was assayed by adding restriction enzyme and the appropriate buffer directly to the PCR product and separating the restricted DNA on an ethidium-bromide-stained agarose gel.

Detection of polymorphisms

A total of 657 base pairs of *C. puritana* mtDNA sequenced from three regions of mtDNA (regions I–III) is known from an earlier study in which the species has been used as an out-group (Vogler *et al.* 1993). The search for polymorphic nucleotide positions was concentrated on these known mtDNA regions and an additional 180 bp in region I, hereafter designated region Ib. The total amount of mtDNA analysed in individual specimens is thus 837 bp, including 370 bp corresponding to position 5021–5390 of the *Drosophila yakuba* sequence (Clary & Wolstenholme 1985) (region Ia and Ib); 227 bp corresponding to position 12550–12776 (region II) encoding the 5'-end of NAD subunit I (NDI), the tRNA^{leu}, and the 3'-end of the large subunit of rRNA (16SrRNA); and 239 bp corresponding to position 12970–12308 (region III) encoding 16SrRNA. The sequences were deposited under Genbank accession nos. L20979, L20980 and L20981. After several polymorphic sites had been detected, additional individuals were analysed only for relevant regions as listed in Table 1.

Results

Current populations and habitats

The historic range of *C. puritana* along the Connecticut River largely coincides with the area formerly covered by ancient Lake Hitchcock, a Pleistocene lake which extended from Middletown, Connecticut, to north of Lyme, New Hampshire (Clark & Stearn 1960). Currently, the species exists at only two of eleven historic sites at Hadley, Massachusetts, and at Cromwell, Connecticut. The combined to-

tal adults at these sites is fewer than 500 individuals (Table 1). Both sites are sandy beaches which developed from deposition of sands near large bends of the river. Adults are active along the sandy beaches during summer while larvae occur in the medium grain sands of the back beach. The beach habitat is bordered at both sides by low (5–8-m-high) sandy loam cliffs of middle–late Triassic sedimentary material of the Newark formation. *C. puritana* is not found on these cliffs.

The Chesapeake Bay historic distribution is along the western shore in Calvert Co., Maryland. The 45-km Calvert County shoreline includes nine sites, four with current populations of over 500 adults. The habitat here includes very narrow sandy beaches backed by 20–30-m-high fossil-rich Miocene cliffs (Gernant *et al.* 1971). Larvae are restricted to the fine–medium-grained red–yellow sands of the St Mary Formation high on the cliff faces and in eroded deposits of this soil which frequently accumulates at the cliff base. Larvae do not occur in the dark gray, clay fossiliferous marls which make up most of the lower and middle strata of the cliffs. *C. puritana* is not present on sandy beaches that border most of the cliffs at both sides.

We found seven new *C. puritana* sites, including two with over 500 adults, in a disjunct area, about 70 km north-east across the Chesapeake Bay near the mouth of the Sassafras River in Kent Co., Maryland (Fig. 1). The habitat here is physically comparable to the Calvert County sites with narrow sandy beaches backed by 10–20-m-high cliffs. The cliff deposits here are yellow brown to yellow quartz sands of the Laurel Formation (Owens *et al.* 1970). Larvae of *C. puritana* are in the middle to upper strata but not present on the back beach.

Table 1 Populations of *C. puritana*. Listed are the populations surveyed; the approximate number of live adults (size); the number of individuals studied for mtDNA by sequencing region Ia, Ib, II and III, and by assaying a polymorphic *HaeIII* site; and the haplotype designation (Haplo) and the number of individuals exhibiting these haplotypes in each population

Population	County, State	Size	No. of individuals					Haplotype (n)
			Ia	Ib	II	III	<i>HaeIII</i>	
Connecticut River								
Hadley	Hampden, MA	150						
Cromwell	Middlesex, CT	300	6	3	13	2	13	<i>pur1-8</i> (1), <i>pur1-9</i> (7), <i>pur1-10</i> (5)
Chesapeake Bay, west								
Calvert Beach	Calvert, MD	2000	10	6	3	2	10	<i>pur2</i> (10)
Little Cove Point	Calvert, MD	1000	4	5	4		7	<i>pur2</i> (7)
Chesapeake Bay, east								
Turner Creek	Kent, MD	300				2	2	<i>pur2</i> (2)
Grove Point	Kent, MD	1000	8	1	5	1	8	<i>pur2</i> (6), <i>pur3</i> (2)

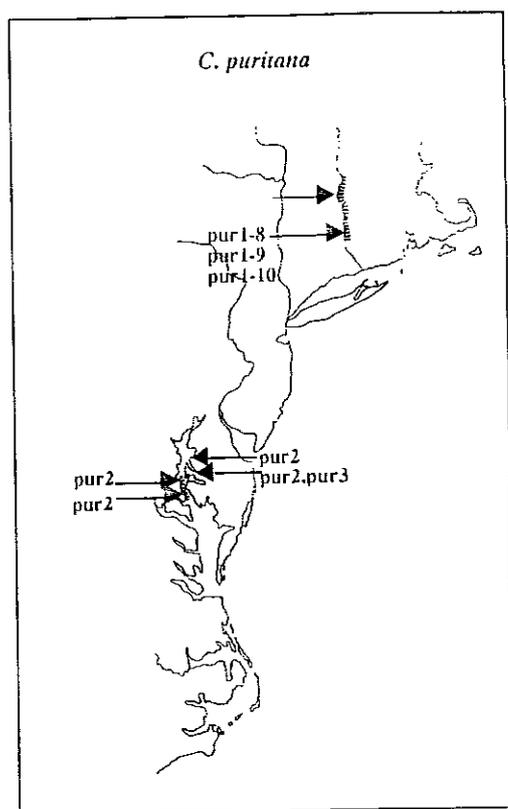


Fig. 1 Location of existing *C. puritana* populations and the mtDNA haplotypes encountered. The population at Hadley, Massachusetts, was not analyzed for mtDNA. The dotted lines indicate the areas of historical occurrences.

Genetic analysis

MtDNA studies were carried out to analyse genetic differentiation between and within the three principal *C. puritana* locations (Connecticut River, Chesapeake Bay east and west). At the Connecticut River, only the population in Cromwell, CT, was considered large enough for sampling of adult beetles, thus limiting the analysis of genetic differentiation of subpopulations. An initial search for polymorphic nucleotide positions was done using five individuals (two from the Connecticut River; two from the west side and one from the east side of Chesapeake Bay) and included sequencing of 837 bp from three regions of mtDNA. Five (0.6%) sites were polymorphic, distinguishing two different haplotypes that were confined to individuals from either the Connecticut River (haplotype designation pur1) or the Chesapeake Bay (haplotype pur2) location. A base-pair substitution in one of these polymorphic sites (bp 28 in region II; Table 2) disrupted a recognition site for the restriction endonuclease *Hae*III and thus provided an experimentally simple assay for the analysis of large numbers of individuals.

Table 2 Polymorphic nucleotide positions that distinguish haplotypes found in *C. puritana*. Also included is the character state at these positions in the out-group species. At position 170 the length of a stretch of dT (seven to ten dT) is given. In the out-groups, at one or two of these nucleotide position dT are changed to dA.

Haplotype	Region I		Region II				
	98	110	28*	52	130	140	170
Connecticut River							
<i>pur1-8</i>	T	T	G	C	T	T	T8
<i>pur1-9</i>	T	T	G	C	T	T	T9
<i>pur1-10</i>	T	T	G	C	T	T	T10
Chesapeake Bay							
<i>pur2</i>	C	T	A	T	C	C	T7
<i>pur3</i>	C	C	A	T	C	C	T7
Out-groups							
<i>C. cuprascens</i>	T	T	G	C	T	T	T7
<i>C. macra</i>	T	T	G	C	T	T	T7
<i>C. marginata</i>	C	T	A	T	C	T	T6,A1
<i>C. hamata</i>	C	T	A	T	C	T	T6,A1
<i>C. dorsalis</i>	T	T	G	T	A	C	T5,A2

**Hae*III.

In an analysis of additional specimens, only those mtDNA regions that appeared to be most variable (regions I and II) were sequenced as indicated in Table 1. An average of approximately 450 bp of sequence information was collected for 13 individuals from the Connecticut River and 22 individuals from the Chesapeake Bay was collected. A total of 40 individuals were analysed for the *Hae*III polymorphism. All five nucleotide polymorphisms were found to be fixed in either the Connecticut River or the Chesapeake Bay assemblage. An additional nucleotide polymorphism distinguished two individuals from the east of Chesapeake Bay (haplotype pur3). Interestingly, mtDNAs differed in length at the 3'-end of the presumed tRNA^{ku} gene. While individuals from the Chesapeake Bay exhibited a string of seven deoxythymidine (dT) residues at this position (T7), the number of dT in the Connecticut River specimens was found to be either eight, nine or ten bases (T8, T9, T10). Haplotypes were designated pur1-8, pur1-9 and pur1-10 (Fig. 2). Other species of the genus *Cicindela* exhibited seven base pairs in this region with one or two dTs changed to dA (Table 2).

Genetic subdivision was calculated using the method outlined by DeSalle *et al.* (1987) that corrects for small sample sizes. There was no overlap in the haplotypes detected in the Chesapeake Bay ($n = 27$) and Connecticut River ($n = 13$) populations, resulting in a trivial $F_{st} = 1$. Within the Chesapeake Bay, two of ten individuals at the eastern



Fig. 2 Length polymorphism in the coding sequence of the tRNA^{leu} gene. *C. puritana* DNA was amplified in region II. The PCR product was sequenced with primer CD10 (5'TTTTAGTACGAAAGGACCAA) using ddThymidine in the termination reaction. Sequencing reactions are shown for one individual from the Chesapeake Bay (lane 1) and three individuals from the Connecticut River (lanes 2-4).

shore exhibited the pur3 haplotype that was not encountered at the eastern shore. We calculated an $F_{st} = 0.07$ ($\chi^2_1 = 110.4, P < 0.005$) for both subpopulations. This F_{st} value and its associated chi square argue for the existence of two genetically differentiated groups within the Chesapeake Bay.

Phylogenetic analysis

Two closely related species ('sibling species'), *C. macra* and *C. cuprascens*, have been separated from *C. puritana* based on morphological characters (Willis 1967). Both species have a wide distribution in the midwestern United States, where they occur on sandy river banks in a habitat type similar to that of the Connecticut River populations of *C. puritana*. One individual from both of these taxa was used in a phylogenetic analysis of the *C. puritana* group. The analysis also included two out-group species, *C. marginata* and *C. hamata*, which like *C. puritana* are considered part of the subgenus *Ellipsoptera*, and the more distantly related *C. dorsalis* (subgenus *Habroscelimorpha*). A single most parsimonious tree of 172 steps with a consistency index excluding uninformative characters of 0.844 was found using the 'exact' branch-and-bound option in PAUP (Swofford 1990) as shown in Fig. 3. The length differences in the tRNA^{leu} gene were coded as a single multistate character. In this cladogram the *C. puritana* complex forms a well defined assemblage of closely related haplotypes in which the *C. cuprascens* haplotype appears to be in a position basal to the *C. macra* and *C. puritana* haplotypes. All haplotypes found in *C. puritana* form a monophyletic group that is diagnosed by three synapomorphies. The positions of *C.*



Fig. 3 Phylogenetic hypothesis for the relationship of taxa in the subgenus *Ellipsoptera*. The cladogram represents the single most parsimonious solution of 172 steps. *C. dorsalis* (subgenus *Habroscelimorpha*) was used as out-group taxon. The analysis is based on 837 base pairs of mtDNA for each of the taxa included in the analysis. Haplotypes at the Connecticut River location (haplotype designation pur1-8, pur1-9, and pur1-10) differed in length (T8, T9, T10) from the haplotypes in the Chesapeake Bay (T7) (designation pur2 and pur3). Rectangles represent mtDNA character changes that define the nodes in the cladogram. The decay index (D) is given for the node that defines the relative position of *C. macra* and *C. cuprascens* in the cladogram, and for the node that defines all *C. puritana* haplotypes as monophyletic. Also included in the figure is ecological and biogeographical information on the habitat type and the geographic location (Chesapeake Bay, ChBay; Connecticut River, ConnRi; Midwestern USA, Midwest) of populations.

macra and *C. cuprascens* relative to each other in the cladogram is only weakly supported due to the fact that few characters define branches between nodes whereas several changes define terminal branches. The node relevant to their relationships is not resolved in cladograms which are only one step longer than the most parsimonious solution (decay index, $D = 1$; Donoghue *et al.* 1991). The node that defines the monophyly of all *C. puritana* haplotypes remains resolved until the cladograms are two steps from parsimony ($D = 2$).

Discussion

The initial step in the determination of potential conservation units is a survey of existing populations. We found populations of *C. puritana* still existing in two of 11 historical locations at the Connecticut River and at least nine locations on the western shore of Chesapeake Bay. In addition, we discovered populations on the eastern shore of the Bay in an area for which no previous records were known. The occurrence of *C. puritana* on the eastern shore could be of very recent origin, but it is more likely that populations had not been detected before because the area was never adequately surveyed. The conservation status was very different at the two major *C. puritana* locations. Populations in the Chesapeake Bay appeared to be large and not immediately threatened. In contrast, the two remaining Connecticut River populations were small and widely separated, and the amount of habitat was relatively small. Also, at one of these sites (Hadley) population size has declined substantially since our first visit in 1987.

From these findings it appears that it will be more difficult to maintain the populations of *C. puritana* along the Connecticut River than in the Chesapeake Bay. There are several options as to how these findings can be translated into a conservation strategy. This includes the possibility of abandoning the Connecticut River populations in favor of enhanced conservation measures in the Chesapeake Bay, or, alternatively, to maintain the Connecticut River assemblage by restocking with Chesapeake Bay animals. If, however, both assemblages are determined to be significantly distinct they cannot be considered a single unit and conservation has to focus on both locations independently. We found both *C. puritana* assemblages to differ in their mtDNA sequences and in their habitat preferences. In the following, we address both of these parameters for their importance in determining conservation units.

A phylogenetic approach to the assessment of conservation units

Haplotypes in the Connecticut River assemblage (pur1-8, pur1-9, pur1-10) differed consistently by five single base mutations in 837 nucleotide positions of mtDNA sequence

and an additional length polymorphism in the gene encoding tRNA^{ku} from the main haplotype present in the Chesapeake Bay (pur2). An additional haplotype (pur3) was detected in two individuals from the eastern shore of the Chesapeake Bay that was not present in the western shore beetles. This finding may be an indication of the genetic subdivision of eastern and western Bay populations. Thus, there is a maximum of three potential conservation units that can be distinguished with the amount of sequence information generated in this study. Below, we discuss whether the observed differences are indicative of significant differentiation of these groups that require separate conservation management.

DNA sequence information can easily be used to establish a phylogenetic hypothesis if data for appropriate outgroups are available. Individual organisms represent the terminal entities in this analysis. The resulting cladogram can be used to investigate the question if phylogenetic structure exists *within* the traditionally recognized taxa (Vrana & Wheeler 1992). Any such phylogenetic pattern reflecting evolutionary history of the gene under study can be used as a basis for the determination of conservation units. Ideally, information from more than one gene or evidence from sources other than DNA should be used to infer the history of organisms.

In the cladistic analysis of the mtDNA data (Fig. 3) the *C. puritana* haplotypes appear as a monophyletic group within a closely related cluster that includes haplotypes found in *C. macra* and *C. cuprascens*. The related species *C. hamata* and *C. marginata* were included to polarize character states. Five synapomorphies group the Chesapeake Bay haplotypes and distinguish them from the Connecticut River haplotypes. The haplotype pur3 appears to be derived from pur2. The Chesapeake Bay haplotypes can therefore be inferred to have a common evolutionary history that was not shared by the haplotypes found in the Connecticut River. If the haplotype phylogeny is taken as an indicator for the phylogeny of the organisms, both *C. puritana* assemblages have to be considered as evolutionarily distinct and hence as separate conservation units.

The mtDNA data provide a less clear answer regarding the conservation status of both subgroups from the eastern and western part of Chesapeake Bay. The presence of the uncommon haplotype pur3 in the eastern populations can be taken as evidence that both subgroups exhibit a moderate degree of genetic subdivision. While this observation indicates the existence of two genetically differentiated groups within the Chesapeake Bay, these groups clearly are not phylogenetically separated. Therefore they do not attain the same level of evolutionary distinctiveness as the Connecticut River population and it is not obvious if they need to be managed as separate conservation groups.

We suggest that a decision on the conservation status can be derived from cladistic theory. A possible definition

for a distinct conservation units could be based on the definition for phylogenetic species (Barrowclough & Flesness, in press; Cracraft 1991; Dowling *et al.* 1992). Phylogenetically distinct populations are required to possess at least one unique character or a unique set of characters that consistently distinguishes them from all other populations. They are simply the smallest detectable samples of organisms which are distinguishable from other such samples. Hence, they are distinct populations diagnosable by characters (Nelson & Platnick 1981; Cracraft 1983, 1989; Nixon & Wheeler 1990). We will use the criterion of diagnosability to define evolutionarily significant units in *C. puritana*.

Individuals from the Connecticut River and from Chesapeake Bay are diagnosable because they have characters (the mtDNA haplotypes, or the nucleotide differences that distinguish these haplotypes) that are diagnostic for either one of the two assemblages. Consequently, they have to be considered as different phylogenetic lineages and they represent separate conservation units. Under the same criterion, the mtDNA data result in a different conclusion regarding the conservation status of both subgroups from the eastern and western shore of Chesapeake Bay. Although the uncommon haplotype pur3 is indicative of genetic subdivision of both groups, this haplotype (or the nucleotide change that differentiates it from the more common haplotype pur2) is not a diagnostic character because it does not consistently distinguish all individuals of this population from all of the individuals of the western shore populations. The eastern and western populations, therefore, do not represent diagnosable units and consequently the Chesapeake Bay assemblage is not split into more than one conservation unit.

Ecology and evolutionary history

Most species of tiger beetles occur in a narrow range of microhabitats which is limited by various physical factors, such as soil composition, moisture and temperature (Pearson 1988). The habitat choice is usually consistent throughout the geographic range of the species even at distant localities (Schultz 1989), presumably because of physiological requirements of the eggs and the larvae (Willis 1967; Knisley 1984, 1987). The precise choice of habitat and microhabitat for oviposition sites by adult females is apparently the key determinant of habitat selection for tiger beetle species (Shelford 1908; Knisley 1987). The strict dependence on specific microenvironmental parameters appears to be a consequence of genetically controlled factors specific to each species. Thus, habitat preference can be considered a heritable attribute. It can be used as an indicator of relationship and consequently as a phylogenetic character.

The habitat of larval *C. puritana* in the Chesapeake Bay (yellow-red sands of cliffs) is different from the habitat of *C. puritana* on the Connecticut River and of all other related species (sandy beaches). We assume that the altered habitat preference in the Chesapeake Bay is due to changes in the genetic composition of these beetles and, thus, represents an evolutionarily significant attribute. This assumption is supported by the fact that beach habitat is present immediately adjacent at the sides of cliffs in the Chesapeake Bay but is not utilized. Conversely, cliff habitat is present next to the beaches at the Connecticut River but is not utilized as larval habitat. Thus, the altered habitat association is not a result of the unavailability of the preferred habitat type.

In addition to results from genetic studies, information on ecology, behaviour, biogeography, or morphology can be used to delimit conservation units. This information can provide evidence for either 'splitting' or 'lumping' of conservation units (Dizon *et al.* 1992). The ecological or physiological differences between Chesapeake Bay and Connecticut River individuals corroborate the results of the genetic analysis and provide further reason to split both assemblages in two independent conservation units.

As a presumed heritable trait the ecological attributes can also be used as a character in a phylogenetic analysis. When mapped on the cladogram of the *C. puritana* complex (Fig. 3) the use of cliff habitat appears to represent a phylogenetically derived condition. In a phylogenetic sense, cliff habitat is a synapomorphy for all Chesapeake Bay individuals. This character unites populations from the east and west side of the Bay, diagnosing them as a single phylogenetic unit. Thus, the habitat preference can be used to distinguish between the two alternative interpretations of the mtDNA analysis regarding the subdivision of east and west coast populations. Ecological attributes group all Chesapeake Bay individuals together, to the exclusion of the Connecticut River populations.

Conclusions

Based on a combination of genetic and ecological information the status of *C. puritana* populations was evaluated. Phylogenetic patterns of variation were analysed using cladistic methodology, and evolutionary history of populations was inferred from these patterns. Under the criterion of diagnosability the Connecticut River and Chesapeake Bay assemblages represent separate conservation units. Populations from the eastern and western shore of Chesapeake Bay are grouped in a single unit although evidence for their genetic subdivision was found. As an operational procedure for the assessment of conservation status the phylogenetic approach seems objective and universally applicable.

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This work is a collaborative effort to use field and laboratory studies for the design of conservation strategies. A. Vogler and R. DeSalle work in the Molecular Systematics Laboratory of the American Museum of Natural History and use DNA techniques for conservation biology. S. Glueck assisted in this work during an internship. B. Knisley and J. Hill study the biogeography and ecology of tiger beetles. Their efforts resulted in the listing of *C. puritana* under the Federal Endangered Species Act.
