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Mitochondrial gene diversity of *Skistodiaptomus mississippiensis* in impoundments of the Upper Coastal Plain near Aiken, South Carolina, USA

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With 4 figures and 5 tables

Abstract: Genetic diversity of the calanoid copepod *Skistodiaptomus mississippiensis* was assessed by sequencing a 348-base pair fragment of a mitochondrial gene, cytochrome *b* apoenzyme, from 165 copepods in ponds from the Savannah River and Edisto River watersheds near Aiken, South Carolina, USA. Eight ponds were sampled, including four on the Savannah River Site (SRS) that had been used to cool effluent from nuclear reactors. All of the ponds were man-made, and the potential times since establishment of the copepod populations ranged from 10–70 yr. *Skistodiaptomus mississippiensis* is nearly unknown from natural habitats of the area, but high genetic diversity within and among populations in the man-made ponds suggests that the species colonized from local sources. Haplotypes were not randomly distributed among ponds or between drainages. Results of a nested cladistic analysis revealed that primary routes of colonization were most likely due to contiguous range expansion of haplotypes predominant within the area. Among abandoned cooling ponds of the SRS, the larger ponds showed greater proportions of haplotypes confined to the Savannah River drainage. These haplotypes were poorly represented in the two smaller ponds,

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where more extreme temperatures or extensive flushing may have opened the habitats for more extensive colonization by haplotypes predominantly occurring in the Edisto River drainage.

Key words: mtDNA, population genetics, thermal impact, geographic variation, genetic diversity.

Introduction

Throughout much of the southeastern US, man-made impoundments are an aquatic habitat with few natural analogs. Although these impoundments support diverse assemblages of pelagic zooplankton, some of the predominant species that occur in them are absent from local natural habitats. One of these, *Skistodiaptomus mississippiensis* (MARSH), a species that occurs throughout southeastern North America (WILSON 1959), is often abundant in impoundments of the Savannah River Site (SRS) and adjacent areas of Upper Coastal Plain of South Carolina (TAYLOR & DEBIASE, unpubl.). However, it is nearly absent from Carolina bays and wetland ponds, the most common natural lentic habitats of the area (MAHONEY et al. 1990, DEBIASE & TAYLOR, unpubl.). Other species with similar distributions include the planktonic cladoceran *Daphnia ambigua*, several less common *Skistodiaptomus* species, and two recently invading temorid copepods. The temorids appear to have colonized the SRS in the late 1980s (DEBIASE & TAYLOR 1993). The source of *Epischura fluviatilis*, which was extremely rare, remains unknown; *Eurytemora affinis* probably invaded from brackish coastal waters (LEE 1999).

Species in the impoundments may be domestic invaders from distant drainages or long-distance dispersers, having spread into South Carolina after the impoundments were created. Alternatively, they may be derived from indigenous populations within the drainage, perhaps in habitats that are poorly studied or now rare, with traits allowing for rapid proliferation in the new environment [sensu "exaptation" of GOULD & VRBA (1982)].

Genetic diversity of a new population depends on the number of successfully reproducing immigrants and the genetic diversity of the source. If strong negative selection is not acting on the genetic composition, a large number of successful immigrants will establish a population with diversity similar to the source. Diversity may be lower in a population established by a small number of immigrants, which may not represent all of the haplotypes in the source.

The two models of invasion should have distinct consequences for genetic diversity. Under the long-distance dispersal model, small numbers of highly dispersive individuals from outside the region establish local populations. Genetic diversity should be low due to founder effects. If the species spreads by means of a series of colonizations (BOILEAU & HEBERT 1991), population di-

versity should attenuate with distance from the source area. Many invading exotics do have low genetic diversity (e.g., BERG & GARTON 1994, TSUTUSI et al. 2000). In contrast, if the species has been established in the region for a long time, as hypothesized in the indigenous source model, diversity within all populations is likely to be high.

Copepods may enter impoundments through tributaries from upstream sources, flooding from nearby drainages (HAVEL et al. 2000), co-transport with fish stocks, or transport by waterfowl (PROCTOR 1964, PROCTOR & MALONE 1965). Because populations of copepods can expand rapidly to enormous size ($>10^6$) in impoundments (TAYLOR & MAHONEY 1988, TAYLOR et al. 1993, LEEPER & TAYLOR 1995), differences among populations are more likely arise from founder events rather than genetic drift. Additional immigrants should have minimal impact if the population remains large.

In this study, we sampled *Skistodiaptomus mississippiensis* from 8 impoundments on or near the SRS in South Carolina to determine whether patterns of genetic diversity were more consistent with the long-distance dispersal model or the indigenous source model. We assessed diversity by sequencing a mitochondrial DNA (mtDNA) marker cytochrome *b* apoenzyme (cyt*b*). We estimated potential ages of the populations from scientific literature, SRS records, historical documents, and other sources. Four of the SRS impoundments had been used to cool effluents from nuclear reactors. In the hottest of these, reactor effluent imposed repeated episodes of local extinction due to thermal stress followed by recolonization (TAYLOR et al. 1988, LEEPER & TAYLOR 1995). We described patterns of diversity within and among populations with standard measures and tests of molecular population parameters, and we used a nested clade analysis to evaluate distribution of haplotypes across their habitats.

Study area

The study area is situated on the Upper Atlantic Coastal Plain of South Carolina in watersheds of the Savannah and Edisto Rivers (Fig. 1). The Savannah River arises in the Appalachian Mountains, and its drainage basin includes the Piedmont and Coastal Plain. The Edisto River is contained entirely within the Coastal Plain.

Except for oxbows, natural lakes are essentially absent from the landscape of South Carolina. Impoundments are numerous. Construction of small impoundments for farm and millponds began in the 18th Century; most large impoundments were built during the mid- to late 20th Century (KOVACIK & WINBERRY 1987). Small beaver ponds have recently become common, as beaver populations have recovered from near extinction in the region in the late 18th century (HACKNEY & ADAMS 1992).

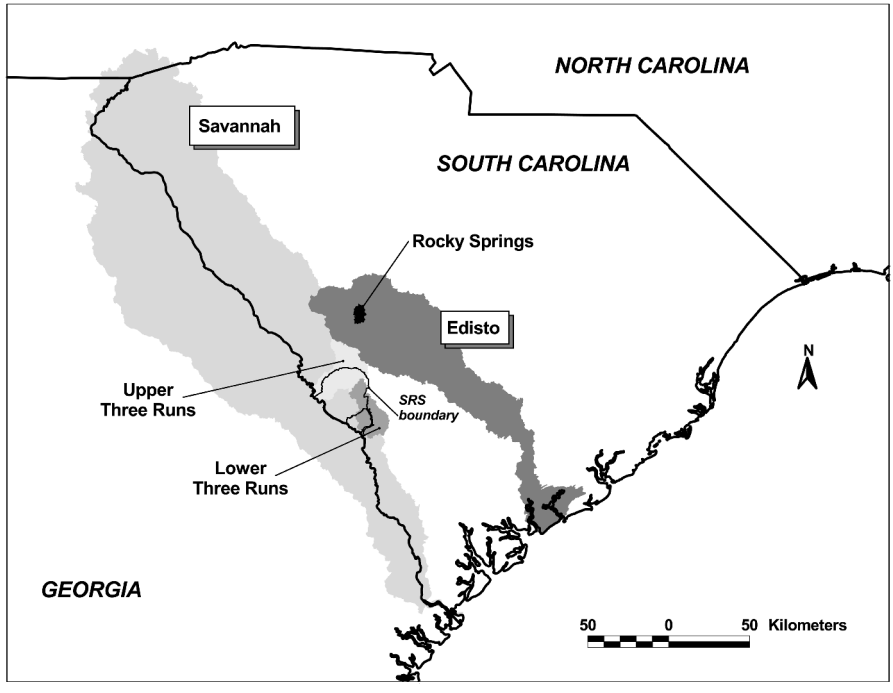

Fig. 1. Watersheds of Upper Three Runs Creek, Lower Three Runs Creek, and Rocky Springs Creek within the Savannah River and Edisto River drainages. These watershed boundaries are depicted by different shadings, according to United States Geologic Survey maps of hydrologic units for South Carolina and Georgia.

All of the study ponds were man-made (Table 1). Dicks Pond, in the Upper Three Runs watershed, predates the SRS. Federal land acquisition records note a fishpond in 1951 on the parcel including Dicks Pond. We surmise that the pond was constructed for recreational fishing and that it was probably stocked with fish. Since the SRS was created, recreation has officially been prohibited, but Dicks Pond has been used for experimental ecological research.

The ponds on the SRS in the Lower Three Runs watershed were built to provide and receive cooling water for two nuclear reactors, P Reactor and R Reactor (Fig. 2). Water from Par Pond was supplemented with water drawn from the Savannah River at the pump houses downstream of the mouth of Upper Three Runs Creek.

When the nuclear reactors were operating, thermal discharges had substantial impact on biota of the impoundments. Water temperatures throughout Pond C sometimes exceeded 40 °C in summer (Leeper & Taylor 1995), eliminating calanoid copepods and most other zooplankton. Thermal effects would have been more extreme in Ponds 2 and 4, which were smaller and closer to the source of the effluent.

Table 1. Sizes and histories of the impoundments. Sources of information include: Dicks Pond – US Department of Agriculture Barnwell County soil map of 1912, aerial photographs from 1943 and 1951, US Atomic Energy Commission Real Estate map of 1951, BENKE & BENKE (1975); Par Pond, Pond B, Pond 2 Pond 4 – WILDE (1985) and other SRS documents; Brooks Pond, Snaggy Pond, Ready Pond – US Geologic Survey Aiken Quadrangle (15′) map of 1921, plat of Ready tract in 1937, aerial photographs from 1938, 1943, 1951, 1955, 1959, 1966, 1971, and 1979, USGS Foxtown Quadrangle (7.5′) map of 1982.

Impoundment	Area (ha)	Construction Date	Years as Potential Copepod Habitat	Purpose
Dicks Pond	0.9	Between 1943 & 1951; breached & repaired in 1967	47–55	Recreational pond
Par Pond	1012	1958	40	Cooling reservoir with moderate temperatures, 1958–1988; partial drawdown 1991–1995 to facilitate repair of dam
Pond B	81	1961	34	Cooling reservoir with extremely warm temperatures, 1961–1964
Pond 2	6.7	1958	10	Cooling reservoir with extremely warm temperatures, 1958–1988
Pond 4	11	1958	10	Cooling reservoir with extremely warm temperatures, 1958–1988
Brooks Pond	9	Between 1955 & 1959	39–43	Recreational pond
Snaggy Pond	2.6	Between 1971 & 1979	19–27	Retention basin for sediment from kaolin mine
Ready Pond	6.2	Original dam built before 1918, dam relocated before 1937	60–80	Recreational pond

Par Pond was partially refilled with unheated water from the Savannah River after the dam was repaired in 1995. Most of this water was pumped directly into the west arm of the pond (T. HINTON, SREL, pers. comm.), but flow through P Canal may also have occurred. Pumping of water to Pond B ceased entirely in 1964 after the reactor was shut down.

The three ponds on Rocky Springs Creek (Fig. 3) are used mainly for recreational fishing. Fishermen often move fish among these and other local ponds (BET, personal observation). Holley Pond, just upstream of Snaggy Pond, was stocked with hatchery grass carp in the early 1990s (C. SUMMER, SREL, pers. comm.). Ready Pond and Brooks Pond may also have been stocked with hatchery fish. Water in these ponds is occasionally drawn down, either intentionally to manage fish stocks or aquatic vegetation or catastrophically due to breaches in the earthen dams.

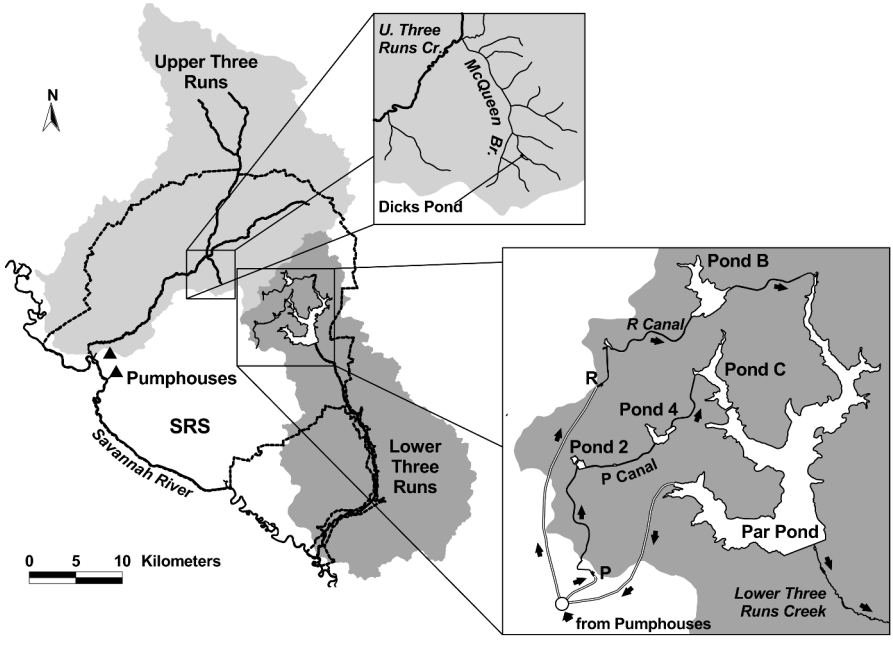

Fig. 2. Impoundments sampled on the Savannah River Site (SRS). Depiction of water pipes and pumps in the Par Pond system is schematic; arrows show directions of water flow during times of reactor operations. Streams and waterbodies are from SRS GIS coverages. Watershed boundaries, as in Fig. 1; other features depicted according to SRS GIS maps. "P" represents the P Reactor, which was the heat source affecting ponds along the P canal in the Lower Three Runs system.

Methods

Collection of material

165 adult *Skistodiaptomus mississippiensis* were collected from 8 ponds from 23 June 1998 to 23 September 1999 (Figs. 2 & 3, Table 2). Animals were collected with a hand-cast 125-µm-mesh plankton net. Contents of each tow were concentrated on a 63-µm sieve and specimens were fixed immediately in 95 % ethanol. Individual copepods were identified under a dissecting microscope and were either used immediately for nucleic acid extraction or stored in 95 % ethanol at 4 °C for later extraction.

DNA amplification and sequencing

DNA from individual copepods was extracted according to the procedure of SCHIZAS et al. (1997) except extraction volumes were doubled to 20 µl. Small aliquots of extracted nucleic acids (typically 2–3 µl) were used as template for polymerase chain reaction (PCR) amplification (SAIKI et al. 1988). Cyt*b* amplifications used the follow-

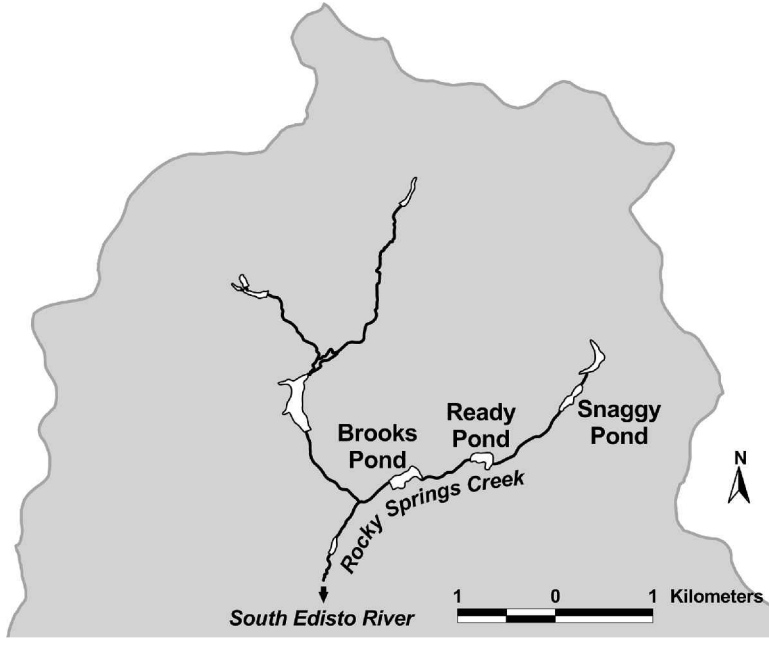

Fig. 3. Impoundments sampled in the Rocky Springs Creek watershed. Streams depicted according to 1982 7.5' Foxtown Quadrangle map (USGS 1982). Locations of ponds determined from the Foxtown Quadrangle map and orthorectified 1979 aerial photograph from the United States Department of Agriculture.

Table 2. Dates, locations and number on individuals assayed (N) for each collection at Savannah River Site.

Location	Lat./Long.	Date	N
Dicks Pond	33° 17′ 28″/81° 37′ 30″ W	23 June 1998	16
Par Pond	33° 15′ 50″/81° 32′ 29″ W	23 June 1998	22
Pond B	33° 17′ 45″/81° 32′ 45″ W	23 June 1998	17
Pond 2	33° 15′ 30″/81° 35′ 15″ W	23 June 1998	16
Pond 4	33° 15′ 30″/81° 33′ 30″ W	23 June 1998	18
Brooks Pond	33° 42′ 55″/81° 31′ 50″ W	24 June 1998	20
Snaggy Pond	33° 43′ 21″/81° 30′ 44″ W	23 September 1999	22
Ready Pond	33° 43′02″/81° 31′ 19″ W	23 September 1999	34

ing conditions: 50 mM KCl, 10 mM Tris-HCl, pH 8.3 (Perkin-Elmer Cetus), 3.0 mM $MgCl_2$, 200 µM dNTP (Pharmacia), 5 pmol forward and reverse primer, and 1 unit Taq DNA polymerase (Promega). Amplifications used primers 151F (5′-TGTGGRGCNA-CYGTWATYACTAA-3′) and 270R (5′-AANAGGAARTAYCAYTCNGGYTG-3′) (Merritt et al. 1998). A "hotstart" was facilitated using non-barrier wax beads containing the $MgCl_2$ (Lumitekk, Salt Lake City, UT, USA), at 3.0 mM final concentration. Template DNA and negative controls were initially denatured at 94 °C for 3 min

followed by 10 cycles of 94 °C for 30 sec, 47 °C for 30 sec and 72 °C for 45 sec. This protocol was followed immediately by 30 cycles of 94 °C for 30 sec, 53 °C for 30 sec, and 72 °C for 45 sec. Prior to sequencing, products were purified by polyethylene glycol precipitation (KUSUKAWA et al. 1990). An aliquot (200–500 ng) of the purified PCR product was used as template for fluorescent sequencing using PRISM™ (Perkin-Elmer) chemistry, labeled with Big Dye™ terminators (dideoxynucleotides) and products were sequenced in both directions. Standard procedures were followed except terminator chemistry was diluted by half using *half*BD™ Reagent (GENPAK Inc., Stony Brook, NY, USA) and reactions were run at 10-µl volume. Sequencing reactions were analyzed on an ABI 377XL sequencer (Perkin-Elmer) using 4.5 % acrylamide gels. Complementary sequence strands were assembled using Sequencher 3.1™ (Gene Codes, Ann Arbor, Michigan, USA) and edited by eye. Nucleotides were translated into amino acids using the invertebrate mtDNA code.

Data analyses

Statistics were computed using MEGA version 2 (KUMAR et al. 2001). Haplotype nucleotide data were aligned by inferred amino acids, and the aligned sequences are available from GenBank (Accession Nos. AY257554–AY257647). A network of haplotypes was constructed using TCS ver. 1.13 (CLEMENT et al. 2000), which implements the statistical parsimony procedure of TEMPLETON et al. (1993). Reticulations or circularities in the network were solved as prescribed in PFENNINGER & POSADA (2002).

No outgroup is used in attempts to root the network as the difficulties associated with rooting an intraspecific phylogeny are well known (CRANDALL & TEMPLETON 1993, POSADA & CRANDALL 2001), especially when a suitable outgroup is unknown (WHEELER 1990). We used the criterion of maximum root probability (CASTELLOE & TEMPLETON 1994) to assign the ancestral haplotype for our network. A nested cladistic analysis (NCA; TEMPLETON & SING 1992) was implemented using GeoDis 2.0 (POSADA et al. 2000). NCA has the advantage over traditional population-genetic analyses, like the *F* statistics of WRIGHT (1951), in that it can differentiate factors producing genetic patterns at multiple levels and assign statistical support for these patterns based on the dispersion of haplotypes across a geographical zone (TEMPLETON et al. 1995, TEMPLETON 1998).

Results

The rate of errors in the DNA sequences due to amplification appeared to be low. Seven singleton haplotypes contained substitutions at the second position of a three-nucleotide codon. The substitutions would change the amino acid specified by the codon. These non-silent substitutions, which accounted for all of the second-position substitutions in our data, probably represent errors. If sequencing errors occur at a similar rate at all three codon positions, the probability that any base pair is incorrectly determined is 0.035 %. The probability that any individual sequence of 348 base pairs contains no error is 88 %; the

Table 3. Distribution of shared haplotypes from sampled ponds. Single occurrence haplotypes (see Fig. 4) are listed by number for each pond.

	1	2	3	7	11	12	13	17	19	22	23	25	30	33	37	47	50	52	Single Haplotypes
Dicks Pond															3	3	1		38, 39, 40, 41, 44, 45, 46, 48, 49
Par Pond	2	1		1							1	1	2	2			7	2	34, 42, 51, 53
Pond B											2	1	3				7		14, 20, 21, 35
Pond 2	1						4	1			3	1	3				1		5, 27
Pond 4	2						1					1	4				5	1	24, 26, 34
Brooks Pond	3			2	1		2		1		2	4							8, 9, 10, 32, 36
Snaggy Pond	2	1	2	1	1	1	2		2	1	1	3							4, 15, 16, 28
Ready Pond	3		1			2	3	1	5	1	1	13							6, 18, 29, 31
Totals	13	2	3	4	2	3	12	2	8	2	10	25	12	2	3	3	21	3	35

Table 4. Gene diversity (h) and nucleotide diversity (π) are calculated after the formulae of Nei (1987, equations 8.4 and 10.5) for each sampling locale. Values are means ± the standard error (SE).

Locale	$h \pm$ SE	$\pi \pm$ SE
Upper Three Runs Creek – *Savannah River Watershed*		
Dicks Pond	0.95 ± 0.041	0.027 ± 0.014
Lower Three Runs Creek/Par Pond cooling system – *Savannah River Watershed*		
Par Pond	0.90 ± 0.050	0.040 ± 0.020
Pond B	0.82 ± 0.081	0.041 ± 0.022
Pond 2	0.90 ± 0.050	0.017 ± 0.0095
Pond 4	0.88 ± 0.051	0.036 ± 0.019
Rocky Springs Creek – *Edisto River Watershed*		
Brooks Pond	0.94 ± 0.033	0.0084 ± 0.0052
Snaggy Pond	0.97 ± 0.024	0.0088 ± 0.0053
Ready Pond	0.83 ± 0.054	0.0079 ± 0.0048

probability that it contains just one error is 11%; the probability of more than one error is <1%.

Our study found 53 DNA haplotypes among the 8 populations of *Skistodiaptomus mississippiensis* (Table 3). At Dicks Pond, only one haplotype was shared with any other pond. At all of the other ponds, most of the haplotypes were shared. Of the 15 shared haplotypes, about half were shared between two ponds; one was shared among seven ponds, and none was found in all eight ponds.

Diversities of haplotypes and nucleotides were computed for the unadjusted sequences (Table 4). Sequencing errors might inflate these estimates, but should have little effect on relative values. Haplotype diversity was high in all ponds. Similar values occurred in both the Savannah and Edisto watersheds. Nucleotide diversity differed more substantially among ponds, and it

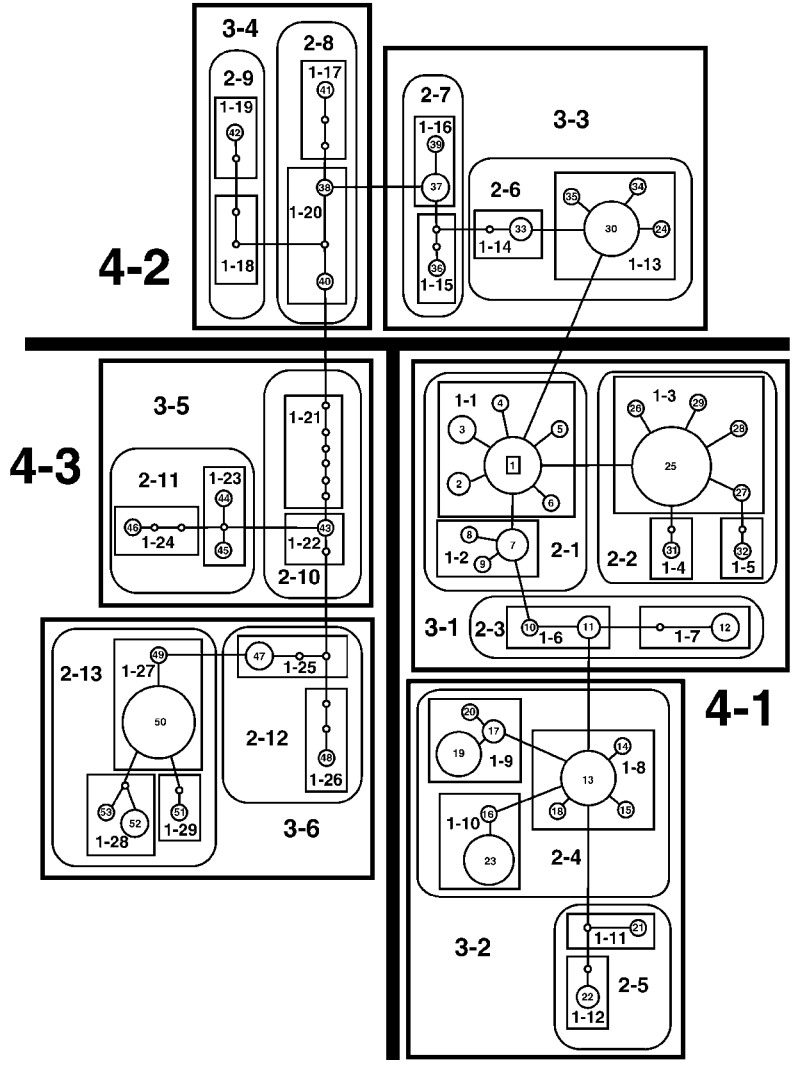

Fig. 4. Haplotype network using statistical parsimony of TEMPLETON et al. (1992). Area of each circle represents frequency of each haplotype with the smallest N = 1 (e.g., haplotype 4, 5, or 6) and the largest N = 25 (haplotype 25). Haplotype 1 (number boxed, N = 13) possessed the highest root probability (CASTELLOE & TEMPLETON 1994). Nesting procedure clusters clades of same relative mutational level up to the 4-*i* level.

was lower by nearly an order of magnitude between ponds on the SRS and those on Rocky Springs Creek.

A network shows separation of haplotypes into distinct lineages (Fig. 4). Statistical parsimony could reliably connect haplotypes separated by as many

Table 5. Results of the contingency test for geographical association of clades by the NCA analysis, and the biological interpretation of those significant nested clades (see Fig. 4). *P* is the probability of producing a larger χ^2 statistic based on 9999 randomizations of the contingency table from our data. Inference steps are derived from a dichotomous key from TEMPLETON (1998), and available from: http://inbio.byu.edu/Faculty/kac/crancdall_lab/geodis.htm

Clades nested within	Permutational χ^2 statistic	*P*	Inference steps	Biological inference
Clade 3–3	22.00	0.000	Tip/Interior status Cannot be Determined	Inconclusive outcome
Clade 3–6	19.13	0.001	2–11–12–13–14-NO	Contiguous range expansion or Long distance colonization
Clade 4–1	12.87	0.040	2–11–12-NO	Contiguous range expansion
Entire cladogram	97.61	0.000	2–11–12-NO	Contiguous range expansion

as seven steps ($P > 0.95$), which included all haplotypes in this study. Haplotype 1 had the highest relative root probability (P = 0.12). The network links 53 haplotypes across 165 sampled individuals.

The network is clustered initially into a series of one-step clades (1-*i*) starting at the tips of the network and progressing inward to more interior clades (Fig. 4). One-step clades are then nested into two-step clades (e.g., 2-*i*), two-step clades into three-step clades (3-*i*), and finally four-step clades (4-*i*, see Fig. 4). A permutational contingency analysis assesses significance of genetic pattern data, and significant nested clades are reported in Table 5. Significant associations between haplotype clades and geographic distributions only occurred at higher nesting levels.

All conclusive inferences point toward range expansions within the areas sampled. Clade 3–6 is most closely associated with the development of the SRS facility cooling ponds as habitat. Within Clade 2–13, haplotypes 50–53 are relatively isolated in the cooling ponds with only a single occurrence of haplotype 50 in Dicks Pond. Closely related types 47–49 are restricted to Dicks Pond, alone. Both Clade 4–1 and the entire cladogram are significant and consistent with a pattern of range expansion. Clearly, 4–1 has representatives of all sampled sites except Dicks Pond, which is consistent with the hypothesis of contiguous range expansion into newly formed habitats and possibly broader distribution by disruption via thermal impacts on the cooling ponds.

Discussion

Skistodiaptomus mississippiensis is rare in natural habitats of the study area in South Carolina, but high diversity of the mitochondrial gene cytochrome *b* within and among populations in the man-made ponds indicates that the spe-

cies colonized from sources with high diversity. Although this could also represent multiple colonizations from diverse sources, this seems unlikely since the majority of this diversity is closely related to individuals from the single, unimpacted pond in our study (Dicks), and is not found in the neighboring Edisto drainage. The genetic evidence does clearly not support the long-distance dispersal model, which predicts low diversity of haplotypes and low differentiation due to limited numbers of founders in a region. High diversity within populations (Table 4) and patterns of contiguous range expansion inferred from NCA analyses (Table 5) suggest regional colonization from local riverine populations is most likely, supporting the indigenous source model. Geographic distributions of haplotypes from divergent mitochondrial lineages further indicate at least two genetically distinct sources of these colonists.

The discovery of divergent mitochondrial lineages was surprising. Haplotypes 37–53 differ from the remaining haplotypes by inferred silent, first-position changes at either one or three different codons. Substitutions at the first position are relatively rare, as compared with substitutions at the third position (the majority of substitutions in a population-level study, including this one). According to the general estimate that mitochondrial sequences diverge at 1–1.5 % per million years for invertebrates (Palumbi & Wilson 1991), divergence of widespread haplotypes encompass evolutionary differentiation of 2.5–6 million years; haplotypes of Dicks Pond and the SRS alone, perhaps 1.3–2 million years. Unless change is unusually rapid in these mitochondrial genomes, we may reasonably infer that the local source populations have a long evolutionary history in the southeastern US, far exceeding the brief existences of their impoundment habitats.

Some rapid and recent invasions of other aquatic habitats have yielded genetic patterns inconsistent with genetic predictions of the long-distance dispersal model. Studies have demonstrated surprising levels of diversity in exotic invasive invertebrates and fishes of the Laurentian Great Lakes (Demelo & Hebert 1994, Dillon & Stepien 2001). However, shipping and other anthropogenic forces heavily influence the Great Lakes biota, and the case histories suggest repeated introductions of large numbers of founders. Except for pumping of water from the Savannah River into the Par cooling system, ponds in our study did not receive large volumes of material from sources outside their immediate watersheds.

Among the ponds on the SRS, only Dicks Pond did not receive water directly from the Savannah River. Dicks Pond contained haplotypes predominately from Clades 1–16 and higher (Table 3 & Fig. 4). These haplotypes might represent either a local source of copepods or perhaps a specific hatchery source. The presence of haplotypes from these clades in the Par cooling system, which was not stocked with hatchery fish, supports the idea that these haplotypes arise from a local source. One haplotype (50) is shared among all

five of the SRS ponds. It is also possible that copepods discharged from Dicks Pond into Upper Three Runs Creek and then into the Savannah River could have been pumped into the Par cooling system or that copepods were transported overland by birds, clandestine fishermen, ecologists, or other such vectors. In the latter case, the absence of haplotypes in Clades 2–6 or 4–1 from Dicks Pond would imply that transport was effectively unidirectional.

However, individuals from Clades 2–6 and 4–1 predominated in the Par cooling system, and water from the Savannah River may have been the source for haplotypes from this lineage. *Skistodiaptomus mississippiensis* does occur upstream in large impoundments of the Savannah River (WILDE 1998). We speculate that repeated episodes of severe reduction and reestablishment of these populations facilitated the expansion of individuals from Clades 2–6 and 4–1 into these populations. At Pond C, a larger pond downstream of Ponds 2 and 4 (Fig. 2), *S. mississippiensis* was absent during reactor operation, but reappeared during extended outages of the reactor (TAYLOR & MAHONEY 1988, LEEPER & TAYLOR 1995). Flushing rates were also high. Retention time at Pond C averaged a few days during reactor operation, when flow through P Canal was 12 $m^3 sec^{-1}$, to a week during reactor shutdown (LEEPER & TAYLOR 1995). Ponds 2 and 4 were smaller and closer to the reactor, so effects of flow and temperature were certainly more extreme. In contrast, the size and configuration of Par Pond allowed large areas to remain at ambient conditions during reactor operations, and copepods were not severely reduced by reactor operation (CHIMNEY et al. 1986; reported values combine cyclopoids and calanoids). Among ponds that were connected by P Canal (2, 4, and Par), the proportion of haplotypes in Clades 2–6 and 4–1 increased relative to the severity of thermal stress when the reactor was operating.

Only haplotypes from Clades 2–6 (plus 36) and 4–1 were present in the three ponds on Rocky Springs Creek. The genetic similarity among Rocky Springs Creek copepod populations is consistent with their adjacent positions and apparent similarity of habitats. Among the SRS impoundments, Dicks Pond is most similar in history and condition to ponds on Rocky Springs Creek, but there is no overlap of related haplotypes. Thus, the genetic similarity of the ponds on Rocky Springs Creek to ponds in the Par cooling system, particularly Pond 2, is unexpected. One haplotype is shared among all seven of these ponds, and several are shared among subsets, implying at least one similar source for the populations in the Savannah and Edisto drainages.

Although *Skistodiaptomus mississippiensis* seems now to be rare except in impoundments, we speculate that it occurred during prehistoric times in oxbow lakes in the broad floodplains of the Savannah and Edisto Rivers and their larger tributaries. TURNER's (1910) report of this species in a brickyard pond near the Savannah River at Augusta, Georgia, (30 km upstream of the SRS) may reflect its presence on the floodplain. This occurrence predates

great alterations during the 20th century to the river and floodplain by large impoundments upstream.

Distributions of many organisms in this region follow physiographic provinces, principally the Coastal Plain, Piedmont, and Blue Ridge Mountains, or river drainages (for examples, see RADFORD et al. 1968 – vascular plants; ROHDE et al. 1994 – freshwater fishes; MARTOF et al. 1980 – amphibians and reptiles). Haplotypes from Clades 3–4 and 4–3 may have been indigenous to the central Savannah watershed; the remainder, to the upper Savannah and the Edisto. One or both may have been redistributed by anthropogenic activity, including fisheries management. The two groups may be different ecotypes, although there is no association with obvious differences among the ponds. Extending the geographic scope of this survey would provide some basis for distinguishing among these alternatives. Further, we cannot reject the possibility that cryptic speciation may be occurring within Clade 4–3 of *Skistodiaptomus mississippiensis*. Certainly within other freshwater microcrustaceans, unrecognized speciation has been cited as the root of extreme ecological variation within apparently cohesive cosmopolitan species (FREY 1986).

This study illustrates that modern biodiversity may have a complicated history, even in newly created habitats. It also illustrates the importance of using multiple sources of reference data and placing local genetics within a larger biogeographic context (STATON et al. 2001). This study was undertaken originally to detect genetic markers for environmental contaminants. If samples only from the SRS had been analyzed, we might have concluded that Clade 4–1 haplotypes were biomarkers for persistent effects of thermal effluents or other contaminants due to activities of the Department of Energy. While the presence of individuals from Clade 4–1 does likely mark operation of the Par cooling system on the SRS, its presence in apparently unimpacted ponds on Rocky Springs Creek shows that it is not diagnostic for thermal effluents or associated contaminants but is, instead, the result of secondary invasion by a novel set of haplotypes from thermal disruption of the impoundments of the SRS.

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