MOLECULAR AND GEOLOGIC EVIDENCE OF SHARED HISTORY BETWEEN HERMIT CRABS AND THE SYMBIOTIC GENUS HYDRACTERAIA

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Abstract. — The paleobiogeographic histories of three North Atlantic hermit crab lineages were compared with a single-copy DNA-DNA hybridization phylogeny of their symbiotic hydroid genus Hydractinia to test hypotheses of shared history between these host and symbiont lineages. A survey of the geologic literature suggests that two vicariance events in the Quaternary are responsible for existing range disjunctions of the host hermit crab lineages. The Hydractinia phylogeny revealed two distinct clades, one with a primarily northern and the other with a primarily southern distribution. In two of three cases, hydroids associated with closely related hermits on both sides of the range disjunction appear as sister taxa in the phylogeny. A linear scaling between a measure of hydroid sequence divergence and independent geologic estimates of the timing of the vicariant events believed to have established the hermit crab range disjunctions is consistent with the claim of temporal coincidence of cladogenic and vicariance events. These findings provide evidence for shared history of symbiotic associations in two of the three cases.

Key words. — DNA hybridization, hermit crabs, hydroids, symbiosis, temporal scaling, vicariance biogeography.

Received April 18, 1990. Accepted February 22, 1991.

Host-symbiont relationships have often been assumed to reflect a shared evolutionary history between host and symbiont lineages (see reviews by Mitter and Brooks, 1983; Brooks, 1985, 1988). Several authors have noted that these claims of shared history must be tested with historical information about the lineages concerned (Mitter and Brooks, 1983; Brooks, 1985, 1988; Humphries et al., 1986; Lyal, 1986). These workers have compared independently derived cladistic phylogenies of hosts and symbionts for elements of congruence, with congruence being interpreted as support for a hypothesis of shared history between host and symbiont lineages. To date, most of these comparisons have relied on cladistic phylogenies of morphological characters. But a morphological, exclusively neontological approach lacks the ability to detect cases of pseudocongruence; that is, cases where apparent cladistic congruence is the result of speciation events that actually took place at different geologic times in host and symbiont lineages.

Pseudocongruence can be detected only if an element of time can be introduced into studies of shared history. Hafner and Nadler (1988, 1990) and Page (1990) have convincingly argued that, used cautiously, genetic distances can provide an important source of historical information about the relative timing of speciation events in host and symbiont lineages. But again, without
geological and paleontological information, these workers are unable to assign actual dates to speciation events in host and symbiont lineages. In the same vein, Vermeij (1989b) has argued that without geologic information, the historical conclusions of exclusively neontological biogeographic studies can be misleading.

In this study we have compared a paleobiogeographic analysis of vicariance in three hermit crab host lineages with a molecular analysis of cladogenesis in the encrusting symbiont hydroid genus *Hydractinia*. In the temperate North Atlantic, the athecate hydroid *Hydractinia* is typically found in association with pagurid hermit crabs. This association first appears in the fossil record of the Western Atlantic in the Miocene (Gernant, 1970; Gibson, 1971; Kidwell, 1982). All three hermit crab lineages associated with *Hydractinia* in the temperate North Atlantic belong to marine biogeographic provinces characterized by extensive range disjunctions. The ages of these range disjunctions were estimated from a review of the geological literature. The phylogenetic relationships and genetic distances between the hermit-crab dwelling species of the genus *Hydractinia* in the North Atlantic were determined by single-copy DNA-DNA hybridization. Each approach yielded an independent estimate of the relative timing of vicariance events for hosts and symbionts. We find that the phylogenetic relationships and genetic distances of these hydroids are consistent with known geologic patterns of vicariance for two of the three hermit crab lineages.

We suggest that the interplay of phylogenetic information, genetic distance, and geologically inferred patterns of vicariance can, in some instances, constitute compelling evidence of shared history. Methods of inferring shared history have relevance far beyond host-symbiont systems and can be applied to species involved in almost any ecological interaction (Brooks, 1985).

**Materials and Methods**

**Biogeography of the Hydractinia/Hermit Crab Association**

The ranges of temperate North Atlantic *Hydractinia* and their host hermit crabs are presented in Figures 1A and 1B, respectively. Four of the five *Hydractinia* species are found predominantly on four species of pagurid hermit crabs in the following host-specific associations: *H. echinata*- *P. bernhardus*, *H. polyclina*- *P. acadianus*, *H. symbiopollicaris*- *P. longicarpus*, and *H. symbiopollicaris*- *P. pollicaris* (Table 1; Buss and Yund, 1989). These hydroid species occur only rarely on other available substrata (Karlson and Shenk, 1983; Mercando and Lytle, 1980; Yund and Parker, 1989). In addition to these four Atlantic species, *Hydractinia* is also found in the Gulf of Mexico where it encrusts the shells of both *P. longicarpus* and *P. pollicaris* (Table 1), and only rarely encrusts other available hosts (Wells, 1969; Fotheringham, 1976). Mating experiments between Gulf specimens and North Atlantic *Hydractinia* have shown this to be a new, undescribed species *Hydractinia [GM]* (Buss and Cunningham, unpubl. data).

**Hermit Crab Paleobiogeography**

The extant ranges of each of the three hermit crab lineages encrusted by *Hydractinia* in the temperate North Atlantic were used to place each lineage in a recognized marine biogeographic province. The probable causes and ages of the range disjunctions that characterize these marine provinces were evaluated from a review of the geologic literature, including studies of sea level fluctuations, paleoclimatology, and paleobiogeography.

**Hydractinia Single-Copy DNA-DNA Hybridization**

**Extraction and Labeling of Hydroid DNA.**—Hydroid tissue was homogenized in buffer (4M EDTA, 10mM Tris-HCl, 2% Sodium Sarkosyl, pH 9.4) and DNA isolated form an EtBr-CsCl density gradient as described by Maniatis et al. (1982). DNA isolated in this way was pure (1.7–1.8 OD, 260/280 ratio), with insignificant protein contamination, in contrast to our experience with phenol-extracted *Hydractinia* DNA. DNA concentration was determined by UV spectrophotometry and DNA was sheared by sonication to an average of 500 bp using a cell disruptor (Cole Palmer). Sin-
EVIDENCE OF SHARED HISTORY

Northern Species

- H. echinata
- H. polyclina
- H. symbiopollicaris

Southern Species

- H. symbiolongicarpus
- H. [GM]

BERNHARDUS GROUP

- P. acadianus
- P. bernhardus

AMERICAN ENDEMIC

- P. longicarpus
- P. pollicaris

Fig. 1. North Atlantic distributions of (A) members of the hydroid genus Hydractinia and (B) its hermit crab host genus Pagurus. Hydractinia distributions in New England are drawn from Buss and Yund (1989) and, outside New England, are inferred on the basis of patterns of host-specificity detailed by Buss and Yund (1989). Hermit crab distribution drawn from Williams (1984).

gle-copy sequences were isolated from whole genomic DNA as described by Sibley and Ahlquist (1981) with two modifications. DNA was incubated to equivalent Ceq 1,000 (ECot, moles × sec × liters) instead of ECot 100 (excess repetitive DNA reassociates by ECot 500 in Hydractinia; Cunningham, unpubl. data) and GENE CLEAN (BIO 101) was used to concentrate DNA and remove phosphates rather than dialysis. Single-copy DNA was then labeled with H3 dTTP (Amersham) using a random priming kit (Boehringer), and sized as described by Hunt et al. (1981). DNA labeled in this way produced tracers of an average size of 250–300 bp in length. The tracers were then brought to 0.48 M in mono-dibasic phosphate buffer (PB), denatured by boiling for 10 min, and allowed to reassociate for one hour at 50°C to allow self-similar “hairpin” DNA to reassociate. Self-similar DNA, which may be produced as an artifact of polymerase labeling (Maniatis et al., 1982), was then removed by diluting the sample to 0.12 M in PB and passing the tracer over 200 μl of hydroxylapatite (HAP). Additional size fractionation was achieved by passing over a 1 ml syringe filled with Sephacryl S-400 beads (Pharmacia) and washing with 1 ml of TE. By eliminating smaller DNA fragments, the average size of the first tracer fraction with significant radioactivity was generally increased to about 400–500 bp in length and was used in subsequent hybridization experiments.

DNA-DNA Hybridization Using the Hydroxylapatite (HAP) Method. — Preparation of hybrids for DNA-DNA hybridization was

Table 1. Host specificity and collection localities for individuals which did (+) and did not (−) form stable hybrids (SH) in DNA-DNA hybridizations with Atlantic Hydractinia. N = sample size.

<table>
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<th>Host species</th>
<th>Collection locality</th>
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<tbody>
<tr>
<td>Hydractinia</td>
<td></td>
<td>+</td>
<td>Pagurus longicarpus</td>
<td>Old Quarry Harbor, Guilford CT</td>
</tr>
<tr>
<td>symbiolongicarpus</td>
<td>4</td>
<td></td>
<td>Pagurus longicarpus</td>
<td>Wolf's Head Harbor, Freeport, ME</td>
</tr>
<tr>
<td>Hydractinia sylmipollicaris</td>
<td>1</td>
<td>+</td>
<td>Pagurus pollicaris</td>
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</tr>
<tr>
<td>Hydractinia echinata</td>
<td>1</td>
<td>+</td>
<td>Pagurus bernhardus</td>
<td>North Sea, FRG</td>
</tr>
<tr>
<td>Hydractinia [GM]</td>
<td>2</td>
<td>+</td>
<td>Pagurus longicarpus</td>
<td>Dickerson Bay, Wakulla County, FL</td>
</tr>
<tr>
<td>Hydractinia milleri</td>
<td>1</td>
<td>−</td>
<td>Pagurus pollicaris</td>
<td>Shell Point Reef, Wakulla County, FL</td>
</tr>
<tr>
<td>Hydractinia serrata</td>
<td>1</td>
<td>−</td>
<td>Pagurus aleuticus</td>
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<tr>
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carried out as described by Sibley and Ahlquist (1981) except that >40,000 cpm of H\textsuperscript{3} labeled tracer DNA was added to each hybrid and hybrids were incubated at 50°C to EC, t 8,000. Hybrids were then placed on ice, divided into 4 aliquots and added to 1.5 mls of HAP on a filter disk in 10 ml syringes and melted as described by Sibley and Ahlquist (1981) with two modifications. Delivery of buffer by the peristaltic pump and temperature control were controlled manually, and each syringe was washed with approximately 4 ml PB at 2.5 degree increments beginning at 55°C and ending at 95°C, allowing 4 min temperature equilibration before each wash. Each elution was counted by scintillation with Optiflour scintillation cocktail, and plotted against temperature to produce a melting curve for each hybrid. 

Data Analysis. — DNA-DNA hybridization results are of two types. The first is a measurement of thermal stability of reassociated hybrids. As species diverge, mutations accumulate in their DNA. By subtracting an index of thermal stability ($T_{\text{median}}$ or $T_{\text{mode}}$) for homoduplex DNA hybrids (within the same species) against the same index for heteroduplex DNA hybrids (between two species), a dissimilarity measure, or delta ($\Delta$), is obtained, expressed in °C (Britten et al., 1974; Bledsoe and Sheldon, 1989). A dissimilarity measure based on thermal stability, however, can measure only sequence divergence in portions of the genome that retain sufficient similarity to form a hybrid under the reaction conditions (approximately 80%, Britten et al., 1974). As species diverge, regions of their genome will exist that have diverged so greatly that they do not form stable hybrids when they are allowed to reassociate. The second result of hybridization studies is a measurement of the proportion of DNA molecules that form stable hybrids. The difference between percent reassociation ($\text{NPR}$) of heteroduplexes, normalized against the homoduplex, and 100% yields a third dissimilarity measure ($\text{ANPR}$).

$T_{\text{median}}$, $T_{\text{mode}}$ and NPR were calculated for each melting curve as described by Sheldon and Bledsoe (1989). Specifically, reassociation was defined as the percentage of counts eluted $\geq$62.5°C. The dissimilarity measures of thermal stability ($T_{\text{median}}$ and $T_{\text{mode}}$) were calculated from melting curves drawn by expressing the number of counts eluted at each temperature as a percentage of all counts eluted $\geq$62.5°C. $T_{\text{median}}$ was calculated by linear interpolation between the points above and below the median of a curve drawn between cumulative percentage and temperature (ideally a sigmoid curve), while $T_{\text{mode}}$ was calculated by locating the mode of a curve fitted by a five order polynomial equation between actual percentage of counts against temperature (ideally a unimodal curve, Sheldon and Bledsoe, 1989). Variances were calculated separately for homo- and heteroduplexes, and their variances were combined to give a combined standard error for the delta ($\Delta$) values (Caccone et al., 1987).

The PHYLIP computer program package (available from J. Felsenstein, Department of Genetics, University of Washington, Seattle) was used to produce FITCH and KITSCH phylogenies for each of the three dissimilarity measures calculated. KITSCH finds the least sum of squares using the Fitch and Margoliash (1967) algorithm assuming a constant rate of evolution to root the tree. The FITCH program uses the same algorithm, without this assumption, thereby producing unrooted trees. In addition, topologies produced by the Unweighted Pair Group Matrix Averaging (UPGMA, Sokal and Michener, 1958) and Neighbor-Joining (NJ, Saitou and Nei, 1987) algorithms were compared with KITSCH and FITCH for congruence. For each data set being analyzed by a particular algorithm, the jackknifing method of Lanyon (1985) was carried out to detect internal inconsistencies in the distance matrix. Finally, all three dissimilarity measures were evaluated for reciprocity, accordance with the triangle inequality, and level of taxonomic resolution (as per Bledsoe and Sheldon, 1989).

Results

Hermit Crab Paleobiogeography

Geologic History of the Disjunct Antarctic-Boreal Marine Province. — Two of the five Hydractinia species we studied encrust either P. acadianus or P. bernhardus. These two pagurids are sibling species found on opposite sides of the Atlantic with a dis-
juncture between Iceland and Canada (Williams, 1984; Heegard, 1941; Samuelson, 1970; Allen, 1967; Jensen and Bender, 1973) (Fig. 1b). These species are morphologically almost indistinguishable, and were once considered the same species (Benedict, 1901). Hermit crab taxonomists have assigned both species to the primarily Pacific bernhardus group of hermit crabs on the basis of both adult (McLaughlin, 1974; Ingle, 1985) and larval characteristics (Roberts, 1973). The distribution of these two crabs is typical of members of the Amphiatlantic-Boreal marine biota, characterized by a disjunction between Iceland and Canada, with strong affinities to the North Pacific fauna (Fig. 1, Ekman, 1953; Briggs, 1970, 1974; Pielou, 1979; Franz and Merrill, 1980).

The history of the Amphiatlantic-Boreal marine biota in the North Atlantic has been strongly influenced by two major events: the opening of the Bering Strait in the mid-Pliocene, about 3.5 mya (Hopkins, 1967; Durham and MacNeil, 1967; Herman and Hopkins, 1980; Vermeij, 1989a, 1989b) and the climatic deterioration in the Northern Hemisphere, with cooling beginning at about 3.1 mya and culminating in the first major glaciation 2.5 mya (Shackleton et al., 1984; Stanley, 1986; Loubere, 1988; Vermeij, 1989a, 1989b). The initial opening of the Bering Strait in the mid-Pliocene took place when Arctic temperatures were considerably warmer than at any time during the Pleistocene (Herman and Hopkins, 1980; Carter et al., 1986; Andrews, 1988) and coincided with the appearance in mid-Pliocene strata in Iceland of at least 125 molluscan taxa previously known only in the Pacific (Durham and MacNeil, 1967). The direction of this interchange between Pacific and Atlantic faunas was primarily from Pacific to Atlantic, with relatively little migration in the opposite direction (Durham and MacNeil, 1967; Franz and Merrill, 1980; Grant et al., 1984; Vermeij 1989a, but see Grant and Ståhl, 1988). The first pulses of glaciation have been interpreted as being responsible for the observed replacement of a temperate fauna by an Arctic fauna in the Icelandic fossil record (Durham and MacNeil, 1967; Stanley, 1986). While warm interglacial periods have allowed boreal species to return to Iceland (Einarsson and Albertsson, 1988), the ice sheets covering Greenland have remained stable for the last 2 million years (Andrews, 1988), maintaining the observed disjunction of the Amphiatlantic-Boreal marine biota across Greenland (Ekman, 1953; Briggs, 1970, 1974; Pielou, 1979; Vermeij, 1989b).

These considerations are consistent with the suggestion that the ancestor of P. acadianus and P. bernhardus migrated to the North Atlantic through the Bering Strait about 3.5 mya. Since neither hermit species is currently found north of Newfoundland in Canada (Williams, 1984) or in Greenland (Heegard, 1941), it is likely that the newly arrived ancestral population was subsequently divided into two amphiatlantic populations either by the onset of Northern Hemisphere cooling at 3.1 mya, or at the latest by the time of the onset of major glaciation at 2.5 mya, to yield the sibling species pair of P. acadianus and P. bernhardus.

Geologic History of the Disjunct Carolinian Marine Province.—The remaining three hermit crab-dwelling Hydractinia species in the temperate North Atlantic encrust two hermit crab lineages that consist of a single species each: P. longicarpus and P. pollicaris. Unlike the bernhardus group, both P. longicarpus and P. pollicaris represent lineages endemic to North America. These hermit crabs have similar distributions (Fig. 1B), displaying a disjunction around the Florida peninsula (Provenzano, 1959; Williams, 1984). This disjunction is typical for members of the Carolinian marine biota (Frey, 1965; Pielou, 1979; Bert, 1986).

The boundaries of this disjunction coincide with the location of the last direct waterway across northern Florida between the Atlantic Ocean and the Gulf of Mexico, known as the Suwannee Straits (McCommas, 1982; Riggs, 1984; Bert, 1986; Bert and Harrison, 1988). The Suwannee Straits were open during high sea level stands through the Miocene and were closed by a major regression in the late Miocene (Riggs, 1984; Haq et al., 1987). During the Pliocene there were several pulses of transgression along the American Atlantic Coast. Of these only the highest pulse was of sufficient magnitude to flood an arch such as the location of the Suwannee Straits (Ward and Strick-
The highest sea stand of the Pliocene began at about 5 mya, and ended between 3.8–4.2 mya when sea levels fell to near present levels (Haq et al., 1987). Sea stands since the Pliocene have not been sufficient to reinstate the Suwannee straits (Ward and Strickland, 1986; Haq et al., 1987), a conclusion supported by the discovery of a gap in a Pliocene relict shoreline sequence corresponding to the eastern mouth of the Suwannee straits, with no such gap appearing in Pleistocene relict shorelines (Winker and Howard, 1977).

The former location of the Suwannee straits not only coincides with the boundaries of the Carolinian marine biota (Bert, 1986; Bert and Harrison, 1988), but with a terrestrial suture zone between continental and peninsular biotas (Remington, 1968). Electrophoretic evidence from Atlantic and Gulf populations of the crab *Menippe adina* (Bert, 1986) and the anemone *Bundosoma cavernata* (McCommas, 1982) suggests that these populations have not resumed contact since the closure of the Suwannee straits. This contention is supported by a paleobiogeographic study of the American eastern seaboard that showed that the southern ranges of Carolinian ostracods were not significantly displaced south of northeastern Florida during glacial maxima (Cronin, 1988). These considerations are consistent with the suggestion that Gulf and Atlantic populations of Carolinian species such as *P. pollicaris* and *P. longicarpus* were divided by the closure of the Suwannee straits 3.8–4.2 mya and have had little or no direct contact during the Pleistocene glaciations.

It should be noted in passing that genetic discontinuities have been noted in northeastern Florida for species that are continuously distributed around the Florida peninsula. Unlike the temperate Carolinian fauna, which does show a disjunction around the Florida peninsula, genetic discontinuity in continuously distributed species appears to have been caused by one or more of the major regressions that followed the onset of glaciation 2.5–3.1 mya, considerably later than the smaller regression responsible for the closure of the Suwannee Straits (Saunders et al., 1986; Reeb and Avise, 1990; Ward and Strickland, 1986; Haq et al., 1987).

**Hydractinia Single-Copy DNA-DNA Hybridization**

The collection sites for the five North Atlantic *Hydractinia* species whose relationships were analyzed by single-copy DNA-DNA hybridization are presented in Table 1. Note that we tested several hydroids in addition to the five described above, including three *Hydractinia* species from the Pacific and two other genera of the family Hydractiniidae. In all of these cases, however, DNA-DNA hybridization attempts between these species and the five North Atlantic *Hydractinia* species failed to produce stable duplexes due to excessive sequence divergence and are not discussed further. DNA-DNA hybridization attempts among the three hermit crab lineages similarly failed to produce stable duplexes (Cunningham, unpubl. data).

Reciprocal means and standard errors for three dissimilarity measures ($\Delta T_{\text{median}}$, $\Delta T_{\text{mode}}$, $\Delta NPR$) between the five North Atlantic *Hydractinia* species are presented in Table 2. Means of reciprocals were weighted towards the reciprocal with the lowest standard error for all three dissimilarity measures (Caccone, et al., 1987) and are presented in Table 3. Mean values and variances of all three dissimilarity measures of replicates for all hybridizations performed are presented in Appendix 1.

The KITSCH-UPGMA trees for all three dissimilarity measures were congruent (Fig. 2A–C). FITCH-NJ trees for $\Delta T_{\text{median}}$ and $\Delta T_{\text{mode}}$ were congruent with one another (Fig. 2D and E), but were incongruent for one node with the KITSCH-UPGMA trees for the same dissimilarity measures (Fig. 2A and B). Of the three dissimilarity measures, $\Delta NPR$ was the only FITCH-NJ tree (Fig. 2F) congruent with its KITSCH-UPGMA tree. Jackknifing did not affect the topology of any tree. All methods of phylogenetic reconstruction showed broad congruence, agreeing that there are two distinct and widely separated clades of Atlantic *Hydractinia*. The nodes that were inconsistently resolved were separated by very small branch lengths and approached the limits of the resolution for the technique. For all dissimilarity measures within-clade measurements were significantly smaller than
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<tr>
<td><strong>H. symbiolongicarpus</strong></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>(\Delta T_{\text{median}})</td>
<td>6.33 ± 0.23 (4)</td>
<td>6.20 ± 0.27 (4)</td>
<td>2.23 ± 0.23 (3)</td>
<td>6.41 ± 0.21 (4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta T_{\text{mode}})</td>
<td>6.65 ± 0.27 (4)</td>
<td>6.62 ± 0.24 (4)</td>
<td>1.94 ± 0.22 (3)</td>
<td>6.73 ± 0.31 (4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta \text{NPR})</td>
<td>4.66 ± 8.13 (7)</td>
<td>-1.34 ± 9.33 (8)</td>
<td>-12.70 ± 7.53 (7)</td>
<td>-2.39 ± 9.72 (7)</td>
<td></td>
</tr>
<tr>
<td><strong>H. symbiopollicaris</strong></td>
<td></td>
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</tr>
<tr>
<td>(\Delta T_{\text{median}})</td>
<td>NA</td>
<td>0.98 ± 0.12 (4)</td>
<td>NA</td>
<td>2.36 ± 0.11 (4)</td>
<td></td>
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<tr>
<td>(\Delta T_{\text{mode}})</td>
<td>NA</td>
<td>0.62 ± 0.12 (4)</td>
<td>NA</td>
<td>1.16 ± 0.13 (4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta \text{NPR})</td>
<td>33.12 ± 1.12 (4)</td>
<td>7.08 ± 0.65 (4)</td>
<td>19.96 ± 1.84 (4)</td>
<td>18.70 ± 1.31 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>H. polyclina</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta T_{\text{median}})</td>
<td>7.78 ± 0.34 (7)</td>
<td>0.85 ± 0.32 (4)</td>
<td>7.81 ± 0.34 (4)</td>
<td>1.53 ± 0.37 (4)</td>
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<tr>
<td>(\Delta T_{\text{mode}})</td>
<td>7.89 ± 0.47 (7)</td>
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<td>7.79 ± 0.31 (4)</td>
<td>0.87 ± 0.23 (4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta \text{NPR})</td>
<td>21.03 ± 6.30 (7)</td>
<td>-0.54 ± 0.72 (4)</td>
<td>15.06 ± 0.93 (4)</td>
<td>7.34 ± 1.00 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>H. [GM]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta T_{\text{median}})</td>
<td>2.96 ± 0.22 (3)</td>
<td>8.19 ± 0.19 (4)</td>
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<td>8.60 ± 0.15 (2)</td>
<td></td>
</tr>
<tr>
<td>(\Delta T_{\text{mode}})</td>
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<td>12.02 ± 0.13 (2)</td>
<td></td>
</tr>
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<td>(\Delta \text{NPR})</td>
<td>6.59 ± 0.84 (3)</td>
<td>13.88 ± 1.64 (4)</td>
<td>30.18 ± 6.02 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H. echinata</strong></td>
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<td></td>
</tr>
<tr>
<td>(\Delta T_{\text{median}})</td>
<td>7.09 ± 0.13 (4)</td>
<td>0.87 ± 0.15 (4)</td>
<td>0.92 ± 0.18 (4)</td>
<td>8.39 ± 0.16 (4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta T_{\text{mode}})</td>
<td>7.96 ± 0.21 (4)</td>
<td>1.06 ± 0.13 (4)</td>
<td>1.19 ± 0.23 (4)</td>
<td>10.55 ± 0.30 (4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta \text{NPR})</td>
<td>19.66 ± 1.18 (4)</td>
<td>-6.63 ± 1.34 (4)</td>
<td>0.60 ± 4.18 (4)</td>
<td>16.74 ± 1.26 (4)</td>
<td></td>
</tr>
</tbody>
</table>

NA: measurement not available in this direction.
between-clade measurements (Fig. 2). A consensus phylogeny is presented in Figure 2G.

The three dissimilarity measures were evaluated for reciprocity by calculating mean percent nonreciprocity (MPN, Sarich and Cronin, 1976). While $\Delta T_{\text{median}}$ showed a lower MPN than $\Delta T_{\text{mode}}$ (7.08% versus 9.81%, data from Table 2), this difference is not significant ($P > 0.20$, Mann Whitney $U$-test, two-tailed). The value of MPN for $\Delta NPR$ is 175.00%, indicating that the mean difference between reciprocals is actually greater than the mean sum of the reciprocals. MPN for $\Delta NPR$ is significantly greater than either $\Delta T_{\text{median}}$ or $\Delta T_{\text{mode}}$ ($P < 0.002$, Mann Whitney $U$-test, two-tailed). In sum, while $\Delta T_{\text{median}}$ appears to obey the axiom of symmetry somewhat better than does $\Delta T_{\text{mode}}$, both measures are far superior to $\Delta NPR$ in this respect. The values for $\Delta T_{\text{median}}$ also obeyed the triangle inequality (as per Bledsoe and Sheldon, 1989) for all 10 3-taxon combinations, as compared to only 7 for $\Delta NPR$, and only 5 for $\Delta T_{\text{mode}}$.

For a dissimilarity measure to be useful for phylogenetic inference, intraspecific measurements should be significantly lower than interspecific measurements. Since Hydractinia [GM] has the broadest host range, four individuals of this species, taken from both hermit crab hosts and two different localities (Table 1), were chosen for an analysis of intraspecific variability. DNA from two of these individuals was labeled and homoduplexes were compared to conspecific heteroduplexes (Table 4). Values for interspecific heteroduplexes were included to determine the level of taxonomic resolution. Of the three dissimilarity measures, only $\Delta T_{\text{median}}$ was able to consistently distinguish between intraspecific and interspecific levels of divergence.

Of the three dissimilarity measures, $\Delta T_{\text{median}}$ behaved the best in terms of reciprocity, adherence to the triangle inequality, and ability to distinguish between intra and interspecific levels of genetic divergence. $\Delta NPR$ generally behaved the worst, which is not surprising because it is well known to be the dissimilarity measure with the highest variance (Caccone and Powell, 1989; Sheldon and Bledsoe, 1989). What is remarkable is that $\Delta NPR$ phylogenies showed broad congruence with other dissimilarity measures and was the only measure whose FITCH-UPGMA tree was congruent with its KITSCH-NJ tree (Fig. 2). So long as weighted means are used to reduce the influence of unreliable $\Delta NPR$ measurements, this statistic appears to give phylogenetic information that is comparable to that ob-
Fig. 2. Dendrograms based on three different DNA-DNA hybridization dissimilarity measures (\(\Delta T_{\text{median}}\), \(\Delta T_{\text{mode}}\), and \(\Delta \text{NPR}\)) and four different tree building algorithms (KITSCH, UPGMA, FITCH, and NJ) as described in text. Since the trees presented here disagree on the relationship of \(H.\) symbiopollicaris, \(H.\) polyyclina, and \(H.\) echinata, their relationship is presented as a trichotomy in the consensus phylogeny. The consensus phylogeny was midrooted, which requires a much weaker assumption of relatively constant rates of sequence divergence than required for the KITSCH and UPGMA algorithms (Farris, 1972). Significance of nodes determined by Mann Whitney \(U\)-Test, two-tailed (Fitch 1986).

**DISCUSSION**

All methods of phylogenetic reconstruction applied to the DNA-DNA hybridization data agree that there are two distinct and widely separated clades of Atlantic Hydractinia (Fig. 2). The first Hydractinia clade, composed of \(H.\) echinata, \(H.\) symbiopollicaris, and \(H.\) polyyclina, is primarily northern in distribution (Fig. 1A). The second clade, composed of \(H.\) symbiolongicarpus and Hydractinia [GM], is primarily southern in its distribution (Fig. 1A). We first consider the history of northern and southern Hydractinia clades in the context of the paleobiogeographic history of their hermit crab hosts presented above. Second, we address the conditions under which corre-
TABLE 4. Analysis of intraspecific variability for *H. (GM)*. Interspecific Δ values for the two closest species [between *H. symbiopollicaris* (2A) and *H. polyclina* (3A)] are included for comparison. Mean Δ values greater than the minimum significant range (MSR) are significantly different than the homoduplex (*P* < 0.05; T-Method for unplanned multiple comparisons between means adjusted for sample size, Sokal and Rohlf, 1981 p. 248). The Tukey-Kramer test gives identical result (Sokal and Rohlf 1981 p. 250). Individuals 5A, 5C from *P. longicarpus,* 5B, 5D from *P. pollicaris.*

<table>
<thead>
<tr>
<th>Intraspecific heteroduplexes</th>
<th>Tracer (Individuals)</th>
<th>Driver (Individuals)</th>
<th>N (#)</th>
<th>ΔT&lt;sub&gt;median&lt;/sub&gt; (°C)</th>
<th>ΔT&lt;sub&gt;mode&lt;/sub&gt; (°C)</th>
<th>ΔNPR (%)</th>
<th>MSR (%)</th>
</tr>
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<tr>
<td>5A 5B 4</td>
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<td>0.39 (0.73)</td>
<td>0.41 (0.81)</td>
<td>-0.63</td>
<td>7.8</td>
</tr>
<tr>
<td>5A 5C 4</td>
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<td></td>
<td></td>
<td>-0.13 (0.73)</td>
<td>0.06 (0.81)</td>
<td>-1.70</td>
<td>7.8</td>
</tr>
<tr>
<td>5B 5A 2</td>
<td></td>
<td></td>
<td></td>
<td>0.46 (0.89)</td>
<td>0.64 (0.99)</td>
<td>-0.40</td>
<td>9.5</td>
</tr>
<tr>
<td>5B 5D 3</td>
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<td></td>
<td></td>
<td>0.72 (0.73)</td>
<td>0.67 (0.81)</td>
<td>-3.67</td>
<td>7.8</td>
</tr>
<tr>
<td>Interspecific heteroduplexes</td>
<td>2A 3A 4</td>
<td></td>
<td></td>
<td>0.98* (0.63)</td>
<td>0.62 (0.70)</td>
<td>7.08</td>
<td>7.8</td>
</tr>
<tr>
<td>3A 2A 4</td>
<td></td>
<td></td>
<td></td>
<td>0.85* (0.73)</td>
<td>0.54 (0.81)</td>
<td>-0.54</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*P* < 0.05.

Phylogenetic Evidence for a Shared History of Hosts and Symbionts

A vicariant event that divides populations of a symbiotic association can conceivably give rise to speciation in either the host or symbiont lineage, or in both (Brooks, 1985). If the symbiont lineage undergoes speciation after such a division, then reconstruction of the phylogenetic relationships of symbionts should reveal that reproductive isolation of symbionts sharing the same or related hosts on opposite sides of the barrier are sister groups. Of the three host lineages we have considered, this pattern is consistent with our findings in two cases.

The range of a Pacific ancestor of the *bernhardus* group is hypothesized to have been divided into disjunct amphiatlantic populations. As predicted by a hypothesis of shared history, the symbionts encrusting the crabs of the *bernhardus* group on opposite sides of the Atlantic are sister taxa belonging to the northern clade (*H. echinata* and *H. polyclina*; Fig. 2). Similarly, the range of *Pagurus longicarpus* is hypothesized to have been divided into disjunct Atlantic and Gulf of Mexico populations by the closure of the Suwannee Straits. As predicted by a hypothesis of shared history, symbionts encrusting *P. longicarpus* on opposite sides of the barrier are sister taxa belonging to the southern clade (*H. symbiolongicarpus* and *H. [GM]*; Fig. 2).

The remaining member of the northern clade, *H. symbiopollicaris,* is associated with a hermit crab lineage distinct from the *bernhardus* group. In fact, *P. pollicaris* is associated with different hydroid lineages on opposite ends of its range; in the north it has been colonized by a member of the northern clade (*H. symbiopollicaris*) and in the south it has been colonized by a member of the southern clade (*Hydractinia [GM]*; Table 1, Fig. 2G). Of these two colonizations, the phylogenetic data show the northern colonization to be the source of *H. symbiopollicaris* (Fig. 2G) with no comparable speciation occurring in the south. There has been no clear allopatric division of marine populations along the eastern seaboard of the United States during the Pleistocene (Cronin, 1988) and the cause of the speciation event leading to *H. symbiopollicaris* remains obscure.

Temporal Scaling Evidence for a Shared History of Hosts and Symbionts

The mere concordance of phylogenetic data with known geologic patterns of vicariance cannot, however, be taken as compelling evidence for shared history between hosts and symbionts. Independent confirmation must exist that the cladogenic event in question is temporally coincident with the vicariance event presumed to generate it. In principle, molecular data permit such a test. If the ages of vicariance events es-
established for host lineages on the basis of
total evidence scale to measures of se-
sequence divergence between symbiont sister
taxa presently divided by the same bioge-
ographic barriers, then the coincidence of vi-
cariant events in host and symbiont lineages
is supported.

The discussion above has suggested two
ages for vicariance events leading to Hy-
dractinia speciation. The disjunction of
members of the northern Hydractinia clade
on both sides of the Atlantic is suggested to
have occurred at the earliest when bernhar-
dus group hermit crab populations were di-
vided by the onset of Northern Hemispheric
glaciation 2.5-3.1 mya. The disjunction of
the southern clade on both sides of the Flor-
da peninsula is suggested to have occurred
when P. longicarpus populations were di-
vided by the final closure of the Suwanee
Straits 3.8-4.2 mya. A third geologic date for
Hydractinia is available from the fossil
record. Fossil Hydractinia is common on
gastropod shells in the Calvert Cliffs for-
mation of Maryland, USA, first appearing
in the PP-1 stratum of the mid-Miocene
Plum Point Member (16.5-17.5 mya. Kid-
dwell, 1982, 1984; recorrelated by Olsson et
al., 1987) and persisting through the Pleis-
tocene (Gernant, 1970; Gibson, 1971; Buss
and Yund, 1988). Since the Florida vicar-
iance occurred before the hypothesized in-
vasion of the northern clade from the Pa-
cific, the southern clade is assumed to have
been in the Atlantic when the northern clade
arrived. On this basis, we treat the Calvert
Cliffs fossils as members of the southern
clade and use this fossil evidence as a min-
imum date for the divergence between the
northern and southern clades.

If these were the actual vicariance events
in the Hydractinia lineage and if sequence
divergence of the single-copy genome has
taken place at a relatively constant rate, then
estimates of sequence divergence should be
correlated with the relative ages of the vi-
cariance events. Since \( \Delta T_{\text{median}} \) is the only
one of the three dissimilarity measures
known to have a linear relationship with
sequence divergence (Caccone et al., 1988)
and to have best obeyed the tests of reci-
procity and triangle inequality, it has been
used to test this hypothesis. The \( \Delta T_{\text{median}} \)
values were corrected for multiple hits by
the Jukes and Cantor (1969) Additivity
Transformation as recommended for DNA-
DNA hybridization data by Springer and
Krajewski (1989). Using averages of each of
the three geologically determined dates as
measures of absolute time, we find a highly
significant linear relationship between
\( \Delta T_{\text{median}} \) and time (Fig. 3). The data were
further tested for linearity (isochrony) in two
ways suggested by Gingerich (1986). First,
a logarithmic regression of the data shown
in Figure 3 yielded a power function of 0.91
\( \pm 0.53 \) (95% confidence interval), which is
indistinguishable from the value of 1.0 pre-
dicted for isochrony. A second, nonpara-
metric test was unable to reject the hypoth-
thesis (required for isochrony) that rate of
sequence divergence is independent of dis-
cance from the origin (\( P > .20 \), Puri and Sen
Test: as in Gingerich, 1986). Thus, this anal-
ysis was unable to reject an assumption of
a relatively constant rate of single-copy se-
quence divergence in Atlantic Hydractinia.

The overall rate of change calculated for all
three dates is 0.43 \( \pm 0.14 \)°C/million years
(95% interval; Fig. 3).

To be confident in our overall estimate
of sequence divergence, rates calculated in-
dependently for each of the three geologi-
cally determined estimates of absolute time
should fall within a narrow range, which is
the case for our data: 1) Onset of glaciation
= 0.46-0.58°C/million years (from upper
and lower limits of geological estimate); 2)
closure of the Suwanee straits = 0.63-
0.69°C/million years (from upper and lower
limits of geological estimate); and 3) Mary-
land Miocene fossils \(<0.45-47°C/million
years (this date is a minimum date of di-
vergence). If only one of these three dates
had been available, our estimate still would
have fallen within the narrow range of 0.45-
0.69°C/million years. These rates of single-
copy DNA change are comparable to rates
for \( \Delta T_{\text{median}} \) estimated for other inverte-
brates, including sea urchins (0.50-1.0°C/
million years; Britten, 1986; Smith, 1988)
and Drosophila (0.11-1.11°C/million years
(Britten, 1986).

To the limits of our resolution the timing of
cladogenic events, established on the ba-
sis of a measure of sequence divergence, and
the timing of the vicariance events sus-
pected of generating them, established on

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Fig. 3. Temporal scaling between ΔT_{median} and three geological estimates of cladogenic events in the *Hydractinia* lineage. Regression and 95% confidence intervals shown were calculated for X with >1 value of Y (Sokal and Rohlf, 1981 p. 483). This regression is highly significant in both parametric (P < 0.001, F-test, Sokal and Rohlf, 1981 p. 485) and nonparametric tests (P < 0.005, Spearman’s Rank Correlation Test, corrected for ties, Gibbons, 1971 p. 234). (A) Onset of Northern Hemispheric Glaciation: Age shown is average of 3.1 and 2.5 mya estimates for this event. ΔT_{median} values are between *H. echinata* (European) and *H. polyclina* and *H. symbiopollicaris* (New England), respectively (Table 3). (B) Closure of Suwannee Straits: Age shown is average of 4.2 and 3.8 mya estimates for this event. ΔT_{median} values are between *H. symbiologicarpus* (New England) and *H. [GM]* (Gulf of Mexico) (Table 3). (C) Miocene *Hydractinia* fossils: Age shown represents a minimum estimate of divergence between Northern and Southern clades of *Hydractinia* between 16.5 and 17.5 mya. ΔT_{median} Values represent all between-clade measurements (Table 3).

the basis of geological evidence, are congruent and hence support a hypothesis of shared history in two of the three host lineages examined. While our analysis was limited by the number of species forming stable duplexes (Table 1), which in turn limited the number of potential vicariance events by which to assess temporal congruence (i.e., three points, Fig. 3), we suggest that claims of shared history in symbiotic associations may be profitably investigated whenever data are available (1) on the principal cladogenic events in a host or symbiont phylogeny, (2) on the principal vicariant events affecting the groups, and (3) on the concordance between the timing of cladogenic and vicariant events.

**CONCLUSIONS**

(1) A single-copy DNA-DNA hybridization phylogeny for North Atlantic, hermit crab-dwelling *Hydractinia* reveals distinct northern and southern clades.

(2) Two of three members of the northern clade appear on closely related host hermit crabs whose distribution is consistent with speciation of both host and symbiont following the vicariance events establishing the disjunction of the Amphiatlantic-Boreal marine biota.

(3) The two sister taxa of the southern clade occur on host hermit crabs whose distribution is consistent with speciation of the symbiont alone following the closing of the Suwannee Straits in northern Florida.

(4) Geological estimates of the timing of the two vicariant events, in addition to a date from fossil material, scale in a linear fashion to estimates of sequence divergence, supporting a claim for temporal coincidence of cladogenic and vicariant events.

**ACKNOWLEDGMENTS**

This work could not have been attempted without the support, encouragement, and technical expertise of A. Caccone, J. Powell, F. Sheldon, and D. Oppenheimer. We thank
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J. Taschner for technical assistance. The manuscript has benefited from comments by N. Blackstone, A. Caccone, R. DeSalle, M. Dick, F. Sheldon, J. Powell, B. Schierwater, A. Shenk, G. Vermeij, and K. Wei. The insightful comments of two anonymous reviewers significantly improved the quality of the final version. Our work was supported by the NSF (OCE-8712792, BSR-8805961, BSR-8806890), ONR (N00014-89-J-3046), and by Sigma Xi Grants in Aid of Research. We would like to thank two anonymous reviewers for their helpful comments, which contributed substantially to the presentation and organization of the manuscript.

LITERATURE CITED


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Corresponding Editor: C. Hickman
**APPENDIX 1.** Mean values and variances of three dissimilarity measures for all hybrids included in this study. Numerical designations for species as in Figure 2 and letter designation for specific individuals. Lines enclose single tracer runs. Data for all melting curves were included in calculations unless there was a technical failure in elution, or a failure in temperature control. In particular, one run of 35 replicates experienced a failure in temperature control causing the elutions for two temperature increments to be combined in a single fraction, although all radioactivity was eventually eluted. Since combining two temperature increments strongly affects the shape of the resulting melting curve, these replicates were excluded from calculation of $T_{\text{median}}$ and $T_{\text{mode}}$ (marked NA below). Since calculation of NPR is unaffected by such an error (see above), NPR values for these hybrids are included below. For this reason sample sizes for NPR in Tables 2 and 3 are greater than for $T_{\text{median}}$ and $T_{\text{mode}}$.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Driver</th>
<th>$N$</th>
<th>$T_{\text{median}}$ ($t^2$)</th>
<th>$T_{\text{mode}}$ ($t^2$)</th>
<th>$PR$ ($t^2$)</th>
<th>NPR ($t^2$)</th>
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<td>83.77 (0.07)</td>
<td></td>
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<tr>
<td>1A 1D</td>
<td>3</td>
<td>80.52 (0.02)</td>
<td>83.35 (0.05)</td>
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<tr>
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<td>NA</td>
<td>48.33 (11.04)</td>
<td>82.44 (32.11)</td>
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<tr>
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<td>100.00 (1.40)</td>
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