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

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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.

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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

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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*-*Pagurus bernhardus*, *H. polyclina*-*P. acadianus*, *H. symbiolongicarpus*-*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; Mercado 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 from an EtBr-CsCl density gradient as described by Maniatis et al. (1982). DNA extracted 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-

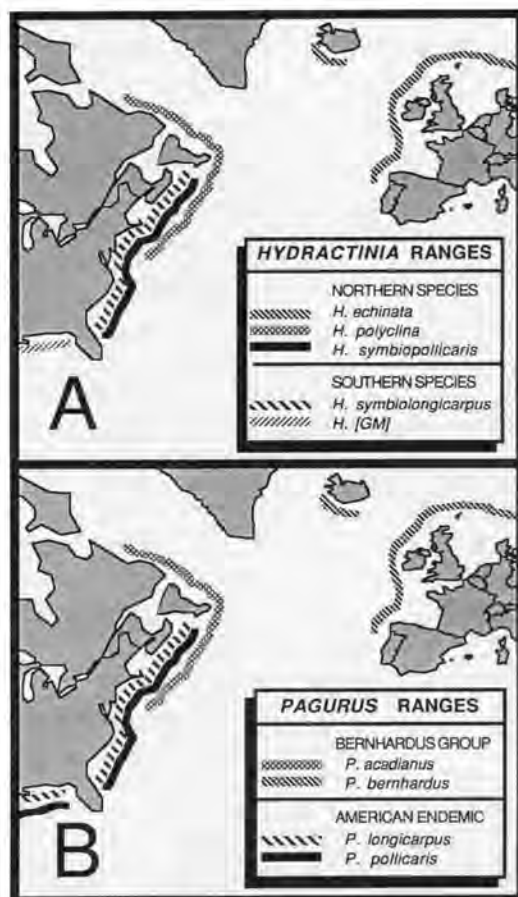


FIG. 1. North Atlantic distributions of (A) members of the hydroid genus *Hydractinia* and (B) its hermit crab host genus *Pagurus*. *Hydractinia* distributions in New England are drawn from Buss and Yund (1989) and, outside New England, are inferred on the basis of patterns of host-specificity detailed 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 C_0t 1,000 (EC_{0t} , moles \times sec \times liters) instead of EC_{0t} 100 (excess repetitive DNA reassociates by EC_{0t} 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 H^3 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 μ l 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.

Hydroid species	N	SH	Host species	Collection locality
<i>Hydractinia</i>	4	+	<i>Pagurus longicarpus</i>	Old Quarry Harbor, Guilford CT
<i>symbiolongicarpus</i>	1	+	<i>Pagurus longicarpus</i>	Wolf's Head Harbor, Freeport, ME
<i>Hydractinia symbiopollicaris</i>	1	+	<i>Pagurus pollicaris</i>	Woods Hole, MA
<i>Hydractinia polyclina</i>	1	+	<i>Pagurus acadianus</i>	Bothbay Harbor, ME
<i>Hydractinia echinata</i>	1	+	<i>Pagurus bernhardus</i>	North Sea, FRG
<i>Hydractinia [GM]</i>	2	+	<i>Pagurus longicarpus</i>	Dickerson Bay, Wakulla County, FL
	2	+	<i>Pagurus pollicaris</i>	Shell Point Reef, Wakulla County, FL
<i>Hydractinia milleri</i>	1	–	Rocks	Bodega Bay, CA
<i>Hydractinia serrata</i>	1	–	<i>Pagurus aleuticus</i>	Bering Sea
<i>Podocoryne carnea</i>	1	–	<i>Pagurus longicarpus</i>	Old Quarry Harbor, CT
<i>Stylactis hooperi</i>	1	–	<i>Nassarius</i> sp.	Monterey Bay, CA
<i>Stylactis inabai</i>	1	–	unknown snail	Japan

carried out as described by Sibley and Ahlquist (1981) except that $>40,000$ cpm of H^3 labeled tracer DNA was added to each hybrid and hybrids were incubated at $50^\circ C$ to EC_{50} 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 $55^\circ C$ and ending at $95^\circ C$, allowing 4 min temperature equilibration before each wash. Each elution was counted by scintillation with Optifluor 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_{median} or T_{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 (Δ), is obtained, expressed in $^\circ C$ (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 (*NPR*) of heteroduplexes, normalized against the homoduplex, and 100% yields a third dissimilarity measure (ΔNPR).

T_{median} , T_{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 $\geq 62.5^\circ C$. The dissimilarity measures of thermal stability (T_{median} and T_{mode}) were

calculated from melting curves drawn by expressing the number of counts eluted at each temperature as a percentage of all counts eluted $\geq 62.5^\circ C$. T_{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_{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 (Δ) 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 Amphiatlantic-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-

junction 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 spe-

cies 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-

land, 1986). 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 *Bundusoma 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 (ΔT_{median} , ΔT_{mode} , ΔNPR) 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 ΔT_{median} and ΔT_{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, ΔNPR 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

TABLE 2. Means and standard errors for three DNA-DNA hybridization dissimilarity measures. Sample size of heteroduplexes in parenthesis. Standard errors for Δ values are based on variances of homo- and heteroduplexes as described by Caccone et al. (1987). Tracer species are radioactively labeled, while driver species are not.

Tracer species	Driver species				
	<i>H. symbiolongicarpus</i>	<i>H. symbiopollicaris</i>	<i>H. polyclina</i>	<i>H. [GM]</i>	<i>H. echinata</i>
<i>H. symbiolongicarpus</i>					
ΔT_{median}		6.33 ± 0.23 (4)	6.20 ± 0.27 (4)	2.23 ± 0.23 (3)	6.41 ± 0.21 (4)
ΔT_{mode}		6.65 ± 0.27 (4)	6.62 ± 0.24 (4)	1.94 ± 0.22 (3)	6.73 ± 0.31 (4)
ΔNPR		4.66 ± 8.13 (7)	-1.34 ± 9.33 (8)	-12.70 ± 7.53 (7)	-2.39 ± 9.72 (7)
<i>H. symbiopollicaris</i>					
ΔT_{median}	NA		0.98 ± 0.12 (4)	NA	2.36 ± 0.11 (4)
ΔT_{mode}	NA		0.62 ± 0.12 (4)	NA	1.16 ± 0.13 (4)
ΔNPR	33.12 ± 1.12 (4)		7.08 ± 0.65 (4)	19.96 ± 1.84 (4)	18.70 ± 1.31 (4)
<i>H. polyclina</i>					
ΔT_{median}	7.78 ± 0.34 (7)	0.85 ± 0.32 (4)		7.81 ± 0.34 (4)	1.53 ± 0.37 (4)
ΔT_{mode}	7.89 ± 0.47 (7)	0.54 ± 0.18 (4)		7.79 ± 0.31 (4)	0.87 ± 0.23 (4)
ΔNPR	21.03 ± 6.30 (7)	-0.54 ± 0.72 (4)		15.06 ± 0.93 (4)	7.34 ± 1.00 (4)
<i>H. (GM)</i>					
ΔT_{median}	2.96 ± 0.22 (3)	8.19 ± 0.19 (4)	7.79 ± 0.33 (3)		8.60 ± 0.15 (2)
ΔT_{mode}	2.21 ± 0.27 (3)	7.02 ± 0.22 (4)	10.96 ± 0.35 (3)		12.02 ± 0.13 (2)
ΔNPR	6.59 ± 0.84 (3)	13.88 ± 1.64 (4)	30.18 ± 6.02 (3)		31.88 ± 2.72 (2)
<i>H. echinata</i>					
ΔT_{median}	7.09 ± 0.13 (4)	0.87 ± 0.15 (4)	0.92 ± 0.18 (4)	8.39 ± 0.16 (4)	
ΔT_{mode}	7.96 ± 0.21 (4)	1.06 ± 0.13 (4)	1.19 ± 0.23 (4)	10.55 ± 0.30 (4)	
ΔNPR	19.66 ± 1.18 (4)	-6.63 ± 1.34 (4)	0.60 ± 4.18 (4)	16.74 ± 1.26 (4)	

NA: measurement not available in this direction.

TABLE 3. Weighted means and standard errors of reciprocal measurements for the three DNA-DNA hybridization dissimilarity metrics in Table 2, with sample size of heteroduplex measurements in parentheses. Means have been weighted towards the reciprocal measurement with lowest standard error as described by Caccone et al. (1987).

	<i>H. symbiolongicarpus</i>	<i>H. symbiopollicaris</i>	<i>H. polyclina</i>	<i>H. (GM)</i>
<i>H. symbiopollicaris</i>				
ΔT_{median}	6.33 \pm 0.23 (4)*			
ΔT_{mode}	6.65 \pm 0.27 (4)*			
ΔNPR	32.59 \pm 1.11 (11)			
<i>H. polyclina</i>				
ΔT_{median}	6.81 \pm 0.21 (11)	0.96 \pm 0.11 (8)		
ΔT_{mode}	6.88 \pm 0.21 (11)	0.60 \pm 0.10 (8)		
ΔNPR	18.90 \pm 2.88 (15)	3.66 \pm 0.48 (8)		
<i>H. [GM]</i>				
ΔT_{median}	2.61 \pm 0.16 (6)	8.19 \pm 0.19 (4)*	7.80 \pm 0.24 (7)	
ΔT_{mode}	2.05 \pm 0.17 (6)	7.02 \pm 0.22 (4)*	9.18 \pm 0.23 (7)	
ΔNPR	6.35 \pm 0.83 (9)	16.57 \pm 1.22 (8)	19.22 \pm 1.28 (7)	
<i>H. echinata</i>				
ΔT_{median}	6.90 \pm 0.11 (8)	1.84 \pm 0.09 (8)	1.04 \pm 0.16 (11)	8.50 \pm 0.11 (6)
ΔT_{mode}	7.57 \pm 0.17 (8)	1.11 \pm 0.09 (8)	1.03 \pm 0.16 (11)	11.79 \pm 0.12 (6)
ΔNPR	19.21 \pm 1.17 (11)	6.32 \pm 0.94 (8)	6.98 \pm 0.97 (11)	19.42 \pm 1.14 (6)

* Measured in one direction only.

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 ΔT_{median} showed a lower *MPN* than ΔT_{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 ΔNPR is 175.00%, indicating that the mean difference between reciprocals is actually greater than the mean sum of the reciprocals. *MPN* for ΔNPR is significantly greater than either ΔT_{median} or ΔT_{mode} ($P < 0.002$, Mann Whitney *U*-test, two-tailed). In sum, while ΔT_{median} appears to obey the axiom of symmetry somewhat better than does ΔT_{mode} , both measures are far superior to ΔNPR in this respect. The values for ΔT_{median} also obeyed the triangle inequality (as per Bledsoe and Sheldon, 1989) for all 10 3-taxon combinations, as compared to only 7 for ΔNPR , and only 5 for ΔT_{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 ΔT_{median} was able to consistently distinguish between intraspecific and interspecific levels of divergence.

Of the three dissimilarity measures, ΔT_{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. ΔNPR 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 ΔNPR 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 ΔNPR measurements, this statistic appears to give phylogenetic information that is comparable to that ob-

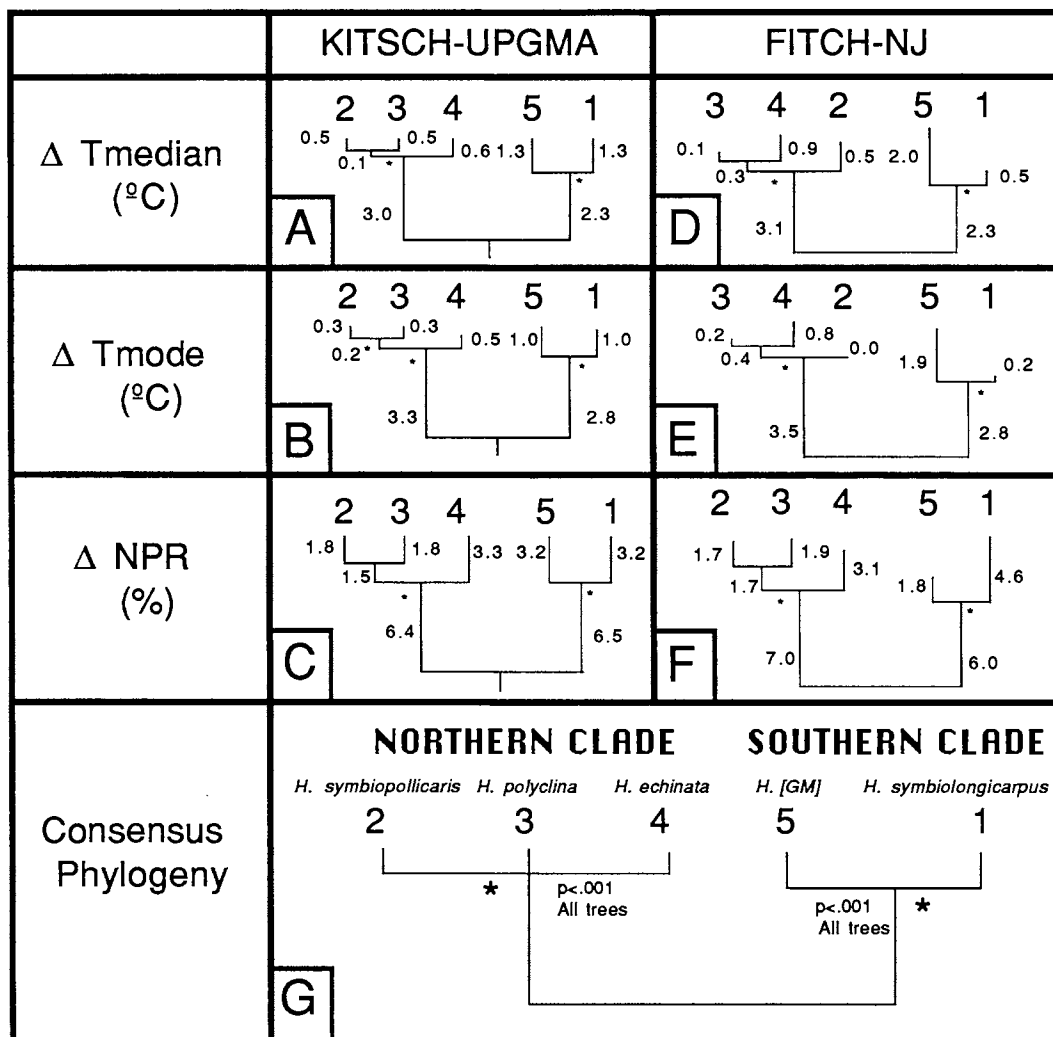


FIG. 2. Dendrograms based on three different DNA-DNA hybridization dissimilarity measures (ΔT_{median} , ΔT_{mode} , and ΔNPR) and four different tree building algorithms (KITSCH, UPGMA, FITCH, and NJ) as described in text. Since the trees presented here disagree on the relationship of *H. symbiopollicaris*, *H. polyclina*, 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).

tained from dissimilarity measures based on thermal stability of duplexes. This congruence greatly increases our confidence in the consensus phylogeny (Fig. 2G).

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. polyclina*, 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*.

Tracer (Individuals)	Driver	N (#)	ΔT_{median} (°C)	(MSR) (°C)	ΔT_{mode} (°C)	(MSR) (°C)	ΔNPR (%)	(MSR) (%)
Intraspecific heteroduplexes								
5A	5B	4	0.39	(0.73)	0.41	(0.81)	-0.63	(7.8)
5A	5C	4	-0.13	(0.73)	0.06	(0.81)	-1.70	(7.8)
5B	5A	2	0.46	(0.89)	0.64	(0.99)	-0.40	(9.5)
5B	5D	3	0.72	(0.73)	0.67	(0.81)	-3.67	(7.8)
Interspecific heteroduplexes								
2A	3A	4	0.98*	(0.63)	0.62	(0.70)	7.08	(7.8)
3A	2A	4	0.85*	(0.73)	0.54	(0.81)	-0.54	(7.8)

* $P < 0.05$.

spondence between phylogenetic information and vicariance patterns can be used to infer shared evolutionary history of host and symbiont lineages.

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 reproductively isolated 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-

tablished for host lineages on the basis of geologic evidence scale to measures of sequence divergence between symbiont sister taxa presently divided by the same biogeographic barriers, then the coincidence of vicariant events in host and symbiont lineages is supported.

The discussion above has suggested two ages for vicariance events leading to *Hydractinia* 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 *bernhardus* group hermit crab populations were divided by the onset of Northern Hemispheric glaciation 2.5–3.1 mya. The disjunction of the southern clade on both sides of the Florida peninsula is suggested to have occurred when *P. longicarpus* populations were divided by the final closure of the Suwannee 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 formation of Maryland, USA, first appearing in the PP-1 stratum of the mid-Miocene Plum Point Member (16.5–17.5 mya. Kidwell, 1982, 1984; recorrelated by Olsson et al., 1987) and persisting through the Pleistocene (Gernant, 1970; Gibson, 1971; Buss and Yund, 1988). Since the Florida vicariance occurred before the hypothesized invasion of the northern clade from the Pacific, 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 *minimum* 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 vicariance events. Since ΔT_{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 reciprocity and triangle inequality, it has been used to test this hypothesis. The ΔT_{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 ΔT_{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 ± 0.53 (95% confidence interval), which is indistinguishable from the value of 1.0 predicted for isochrony. A second, nonparametric test was unable to reject the hypothesis (required for isochrony) that rate of sequence divergence is independent of distance from the origin ($P > .20$, Puri and Sen Test: as in Gingerich, 1986). Thus, this analysis was unable to reject an assumption of a relatively constant rate of single-copy sequence divergence in Atlantic *Hydractinia*. The overall rate of change calculated for all three dates is $0.43 \pm 0.14^\circ\text{C}/\text{million}$ years (95% interval; Fig. 3).

To be confident in our overall estimate of sequence divergence, rates calculated independently for each of the three geologically determined estimates of absolute time should fall within a narrow range, which is the case for our data: 1) Onset of glaciation = $0.46\text{--}0.58^\circ\text{C}/\text{million}$ years (from upper and lower limits of geological estimate); 2) closure of the Suwannee straits = $0.63\text{--}0.69^\circ\text{C}/\text{million}$ years (from upper and lower limits of geological estimate); and 3) Maryland Miocene fossils $<0.45\text{--}0.47^\circ\text{C}/\text{million}$ years (this date is a minimum date of divergence). If only one of these three dates had been available, our estimate still would have fallen within the narrow range of $0.45\text{--}0.69^\circ\text{C}/\text{million}$ years. These rates of single-copy DNA change are comparable to rates for ΔT_{median} estimated for other invertebrates, including sea urchins ($0.50\text{--}1.0^\circ\text{C}/\text{million}$ years; Britten, 1986; Smith, 1988) and *Drosophila* ($0.11\text{--}1.11^\circ\text{C}/\text{million}$ years (Britten, 1986).

To the limits of our resolution the timing of cladogenic events, established on the basis of a measure of sequence divergence, and the timing of the vicariance events suspected of generating them, established on

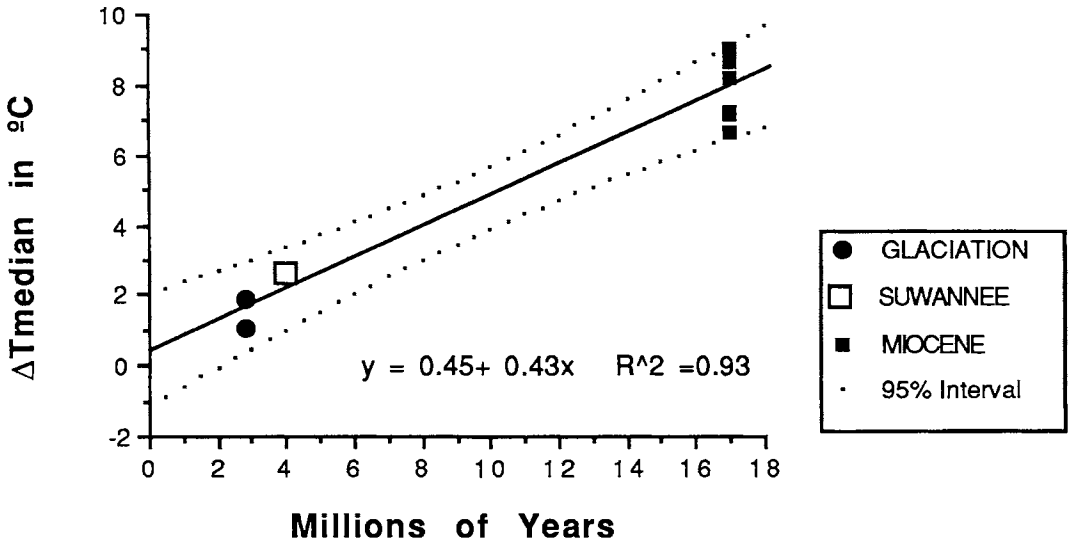


FIG. 3. Temporal scaling between ΔT_{median} and three geological estimates of cladogenic events in the *Hydractinia* lineage. Regression and 95% confidence intervals shown were calculated for X with >1 value of Y (Sokal and Rohlf, 1981 p. 483). This regression is highly significant in both parametric ($P < 0.001$, 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. symbiolongicarpus* (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.

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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_{median} and T_{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_{median} and T_{mode} .

Tracer	Driver	N	$T_{\text{median}} (s^2)$	$T_{\text{mode}} (s^2)$	$PR (s^2)$	$NPR (s^2)$
1A	1B	4	81.23 (0.01)	83.70 (0.01)	70.70 (1.40)	115.53 ((3.40)
1A	1C	3	80.51 (0.01)	83.28 (0.01)	53.10 (0.03)	83.77 (0.07)
1A	1D	3	80.52 (0.02)	83.35 (0.05)	63.93 (0.82)	100.86 (2.05)
1A	2A	4	74.48 (0.17)	76.76 (0.26)	66.58 (3.13)	105.02 (7.78)
1A	3A	4	74.61 (0.25)	76.79 (0.24)	74.61 (0.25)	117.70 (0.62)
1A	4A	4	74.40 (0.12)	76.68 (0.31)	74.40 (0.12)	117.38 (0.27)
1A	5A	3	78.57 (0.12)	81.47 (0.13)	78.57 (0.12)	123.95 (0.31)
1B	1B	4	NA	NA	55.98 (1.10)	95.47 (3.18)
1B	1E	2	NA	NA	63.95 (0.41)	109.07 (1.19)
1B	2A	3	NA	NA	48.33 (11.04)	82.44 (32.11)
1B	3A	4	NA	NA	49.83 (0.95)	84.98 (2.76)
1B	4A	3	NA	NA	48.33 (11.04)	82.44 (32.11)
1B	5A	4	NA	NA	61.13 (0.38)	104.26 (1.11)
2A	2A	4	79.07 (0.01)	82.48 (0.02)	71.62 (0.72)	100.00 (1.40)
2A	3A	4	78.09 (0.05)	81.86 (0.04)	66.55 (0.16)	92.92 (0.32)
2A	4A	4	76.71 (0.04)	81.32 (0.05)	58.22 (2.82)	8.30 (5.51)
2A	1B	4	NA	NA	47.90 (1.85)	66.88 (3.60)
2A	5A	4	NA	NA	57.33 (6.21)	80.05 (12.09)
3A	3A	3	81.62 (0.28)	83.80 (0.08)	83.15 (0.99)	100.00 (1.44)
3A	1B	4	74.14 (0.04)	76.73 (0.52)	60.10 (4.13)	72.28 (5.99)
3A	1E	4	73.45 (0.05)	74.81 (0.10)	73.08 (1.05)	87.88 (1.51)
3A	2A	4	80.77 (0.03)	83.26 (0.03)	83.60 (0.10)	100.54 (0.14)
3A	4A	3	80.09 (0.12)	82.92 (0.08)	77.05 (2.65)	92.66 (3.83)
3A	5A	4	73.81 (0.09)	76.01 (0.29)	70.63 (1.04)	84.94 (1.52)
4A	4A	4	79.01 (0.05)	83.45 (0.03)	66.00 (1.42)	100.00 (3.26)
4A	1B	4	72.81 (0.03)	75.49 (0.16)	53.03 (3.56)	80.34 (8.22)
4A	2A	4	79.04 (0.05)	82.39 (0.04)	70.38 (1.72)	106.63 (3.94)
4A	3A	4	79.24 (0.15)	82.63 (0.06)	67.03 (0.55)	101.57 (1.26)
4A	5A	4	71.52 (0.05)	72.90 (0.34)	54.95 (1.03)	83.26 (2.36)
4A	4A	1	83.55 (0.00)	86.21 (0.00)	89.10 (0.00)	100.00 (0.00)
4A	3A	3	82.28 (0.03)	84.53 (0.21)	86.00 (3.99)	96.52 (5.03)
5A	5A	3	83.73 (0.02)	85.61 (0.10)	89.90 (8.13)	99.15 (9.90)
5A	5B	4	83.35 (0.03)	85.21 (0.02)	80.48 (6.65)	99.78 (8.11)
5A	5C	4	83.86 (0.24)	85.55 (0.43)	91.45 (2.30)	100.86 (2.79)
5A	1B	3	80.68 (0.12)	83.23 (0.10)	84.70 (0.25)	93.41 (0.30)
5A	3A	4	75.45 (0.10)	78.42 (0.14)	78.09 (5.17)	86.12 (6.29)
5B	5B	3	80.56 (0.12)	83.58 (0.00)	68.77 (34.38)	98.52 (70.52)
5B	5A	2	80.10 (0.08)	82.95 (0.02)	69.05 (0.61)	98.93 (1.23)
5B	5D	3	79.84 (0.02)	82.91 (0.05)	71.33 (1.56)	102.20 (3.22)
5B	3A	3	72.28 (0.27)	72.21 (0.31)	48.73 (48.42)	69.82 (99.39)
5B	4A	2	71.57 (0.01)	71.16 (0.01)	47.55 (4.21)	68.12 (8.65)