Evolution, 50(1), 1996, pp. 360–373

RESOURCE-ASSOCIATED POPULATION SUBDIVISION IN A SYMBIOTIC CORAL-REEF SHRIMP

J. EMMETT DUFFY[1]

Center for Population Biology, and Section of Evolution and Ecology, University of California, Davis, California 95616

Abstract.—The importance of sympatric speciation remains controversial. An empirical observation frequently offered in its support is the occurrence of sister taxa living in sympatry but using different resources. To examine the possibility of sympatric differentiation in producing such cases, I measured genetic, behavioral, and demographic differentiation between populations of the tropical sponge-dwelling shrimp *Synalpheus brooksi* occupying two alternate host species on three reefs in Caribbean Panama. This species belongs to an apparently monophyletic group of ≥ 30 species of mostly obligate, host-specific sponge-dwellers, many of which occur in sympatry. Demographic data demonstrated the potential for disruptive selection imposed by the two host species: shrimp demes from the sponge *Agelas clathrodes* were consistently denser, poorer in mature females, more heavily parasitized by branchial bopyrid isopods, and less parasitized by thoracic isopods, than conspecific shrimp from the sponge *Spheciospongia vesparium*. Laboratory assays demonstrated divergence in host preference: shrimp on all three reefs tended to choose their native sponge species more often than did conspecific shrimp from the other host. Because *S. brooksi* mates within the host, this habitat selection should foster assortative mating by host species. A hierarchical survey of protein-electrophoretic variation also supported host-mediated divergence, revealing the following: (1) shrimp from the two hosts are conspecific, as evidenced by absence of fixed allelic differences at any of nine allozyme loci scored; (2) strong genetic subdivision among populations of this philopatric shrimp on reefs separated by 1–3 km; and (3) significant host-associated genetic differentiation within two of the three reefs. Finally, intersexual aggression (a proxy for mating incompatibility) between shrimp from different host species was significantly elevated on the one reef where host-associated genetic differences were strongest, demonstrating concordance between genetic and behavioral estimates of divergence. Adjacent reefs appear to be semi-independent sites of host-associated differentiation, as evidenced by differences in the degree of host-associated behavioral and genetic differentiation, and in the specific loci involved, on different reefs. In philopatric organisms with highly subdivided populations, such as *S. brooksi*, resource-associated differentiation can occur independently in different populations, thus providing multiple "experiments" in differentiation and resulting in a mosaic pattern of polymorphism as reflected by neutral genetic markers. Several freshwater fishes, an amphipod, and a snail similarly show independent but remarkably convergent patterns of resource-associated divergence in different conspecific populations, often in the absence of obvious spatial barriers. In each case, substantial differentiation has occurred in the face of continuing gene flow.

Key words.—Allozymes, habitat selection, host races, parasitism, population structure, speciation, symbiosis, sympatric differentiation, *Synalpheus*.

Received April 27, 1994. Accepted October 2, 1994.

Genetic subdivision among populations is a crucial component of the evolution of phenotypic and ecological diversity within species and ultimately in the splitting of lineages to form new species. Genetic and phenotypic discontinuities within species are commonly produced by geographic barriers to dispersal (Mayr 1942), and the ultimate genetic isolation of such divided taxa, i.e., allopatric speciation via vicariance, may be the most common way in which new species originate (Lynch 1989). Nevertheless, a lingering controversy centers on the question of how effective ecological factors, in the absence of strong geographic barriers, can be in promoting genetic subdivision of populations. More specifically, can differential resource use alone sufficiently reduce gene flow among segments of a population to result ultimately in their reproductive isolation?

Sympatric speciation involves the invasion of a new niche by part of a population and its subsequent divergence, via disruptive selection on resource use and assortative mating, despite the presence of significant gene flow with the ancestral population (Maynard Smith 1966; Bush 1975). For decades, the consensus has been that sympatric speciation is possible only under such stringent conditions that it is likely to occur very rarely, if at all, in nature (Mayr 1942, 1963; Futuyma and Mayer 1980; Felsenstein 1981). In recent years, however, both theoretical (Rice 1984, 1987; Diehl and Bush 1989) and experimental evidence (reviewed by Rice and Hostert 1993) has provided increasingly strong support for the efficacy of selection in producing strong divergence in the face of substantial gene flow. Most importantly, these studies have focused attention on the situation in which assortative mating, and thus reduced gene flow, results as an automatic consequence of habitat selection. This condition will be met whenever mating occurs in association with the habitat (or host), as is true of most animals proposed as candidates for sympatric speciation. In such cases, disruptive selection on habitat or resource use alone can produce divergence (the "single-variation model," Rice and Hostert 1993), obviating the strongest theoretical objection to sympatric speciation, i.e., the problem of establishing tight linkage between separate traits for fitness in a given habitat and for habitat selection (the "double-variation model"). Thus, the most important factors promoting resource-associated divergence in the face of gene flow are mating in association with the resource, which results in assortative mating as a pleiotropic consequence of habitat selection, and "strong, discontinuous,

[1] Present address: College of William and Mary, School of Marine Science and Virginia Institute of Marine Science, Gloucester Point, Virginia 23062-1346. E-mail: jeduffy@vims.edu

and multifarious divergent selection" (Rice and Hostert 1993, p. 1637). The process should also be fostered by strong resource specificity (Bush 1975; Price 1980) and independent regulation of population size on the two resources (Levene 1953; Rosenzweig 1978; Wilson and Turelli 1986).

Empirical studies of sympatric speciation have considered primarily phytophagous (plant-feeding) insects, in part because the ecology of several phytophagous taxa appears amenable to divergence in the absence of spatial barriers but also because distributions of many insect taxa are not easily explained by allopatric models of speciation. Specifically, many insect groups contain complexes of very closely related taxa that occur in broad sympatry and often differ obviously along only a single resource axis, commonly the habitat or host organism they occupy (Bush 1975; Price 1980; Diehl and Bush 1984; Tauber and Tauber 1989). Indeed, Bush (1975) suggested that sympatric speciation accounts for the majority of parasitic and phytophagous insect species and thus a large proportion of animal diversity. The central evidence offered in support of sympatric divergence in such cases involves differentiation of genetic markers and host affiliation among conspecific populations or recently diverged species that presently occur in sympatry (Guttman et al. 1981; Feder et al. 1990a,b). Although less thoroughly documented, superficially similar situations occur in other animal groups, including marine shrimp (Duffy 1992, 1993), an estuarine amphipod (Stanhope 1993), an intertidal snail (Johannesson et al. 1993), and several groups of freshwater fishes that comprise ecologically, morphologically, and sometimes genetically differentiated sister taxa living sympatrically (Echelle and Kornfield 1984; Hindar et al. 1986; Keenleyside 1990; Taylor and Bentzen 1993). In all of these cases, the sister taxa appear to differ primarily in resource use, i.e., diet or microhabitat preference, and sympatric origins have been suggested for many of them.

Coral reefs represent the archetypes of marine diversity and characteristically support a high frequency of closely related, sympatric species (Kohn 1968; Choat and Bellwood 1992; Knowlton and Jackson 1994). Snapping shrimp in the genus *Synalpheus* (Decapoda: Alpheidae) provide a striking example. This is among the most diverse genera of decapod crustaceans, with well over 100 species worldwide (Chace 1989). Of particular interest in the context of resource-mediated speciation is the gambarelloides species group of *Synalpheus*. This group comprises 19 described, and probably a similar number of undescribed, species (J. E. Duffy pers. obs.) that share several derived morphological characters (Dardeau 1984), suggesting the group is monophyletic. Most of the gambarelloides species are obligate sponge dwellers, and nearly all are restricted to the tropical West Atlantic, where 14 or more can be found in sympatry (Dardeau 1984; Duffy 1992). Sponge-dwelling *Synalpheus* species feed, at least in part, on tissues of their host sponges (Ruetzler 1976; Erdman and Blake 1987; J. E. Duffy pers. obs.), many of which contain bioactive secondary metabolites (Faulkner 1984; Pawlik 1993). Most important, these shrimp exhibit several characteristics likely to promote host-associated differentiation in the absence of spatial barriers (Dardeau 1984; Duffy 1992, 1993): (1) many species are highly host specific, using only one or a few sponge species in a given area; (2) conspecific populations can use different hosts in different geographic areas, confirming that host shifts in fact do occur; (3) adults are sedentary, probably spending their entire post-larval lives within the internal canals of a single host sponge; (4) mating occurs within the sponge, resulting in positive assortative mating among members of host-associated demes; and (5) dispersal appears restricted, as evidenced by direct or abbreviated larval development (Dobkin 1965, 1969). These shrimp thus represent promising candidates for sympatric divergence under the single-variation model of Rice and Hostert (1993).

Evaluating modes of speciation can be approached in one of two ways. From a macroevolutionary perspective, geographic distributions and character states (e.g., resource use patterns) can be mapped onto a species cladogram to assess their associations with speciation events (Lynch 1989; Brooks and McLennan 1991). The microevolutionary perspective, however, focuses on the genetic relationships among conspecific populations relative to the distribution of resources believed to be driving their divergence (e.g., Feder et al. 1990a,b). Progress toward speciation should be evidenced by reduced genetic exchange between populations and ultimately by decreased reproductive (pre-and/or postmating) compatibility between them. These criteria follow from the biological-species concept, which defines species as groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups (Mayr 1963). In fact, estimates of reduced behavioral (i.e., premating) compatibility between isolated populations or species correlate well with genetic distance in *Drosophila* (Coyne and Orr 1989), salamanders (Tilley et al. 1990), and alpheid shrimp (Knowlton et al. 1993), demonstrating that behavioral differentiation often accompanies genetic divergence during speciation.

In this study, I employ the latter, microevolutionary approach to assess the degree to which association with different host species has promoted population subdivision in the common Caribbean shrimp *Synalpheus brooksi*. Like most members of the gambarelloides species group, *S. brooksi* is an obligate sponge associate. On the Caribbean coast of Panama, *S. brooksi* inhabits the sponges *Spheciospongia vesparium* and *Agelas clathrodes*. Embryos of this shrimp hatch as benthic juveniles, passing the otherwise planktonic stage within the egg (Dobkin 1965) and therefore probably disperse only very short distances. A pilot genetic study (Duffy 1993) revealed preliminary evidence for differentiation among populations of *S. brooksi* occupying different host species on the scale of hundreds of meters to a few kilometers. Here, I present an analysis of population genetic structure in *S. brooksi* at several hierarchical spatial scales, together with complementary data on shrimp host-choice behavior and intersexual aggression. I ask how spatial isolation and host affiliation interact to produce genetic and phenotypic divergence within this species.

Materials and Methods

Natural History

The snapping shrimp *Synalpheus "brooksi"* actually comprises a complex of at least four cryptic species that are

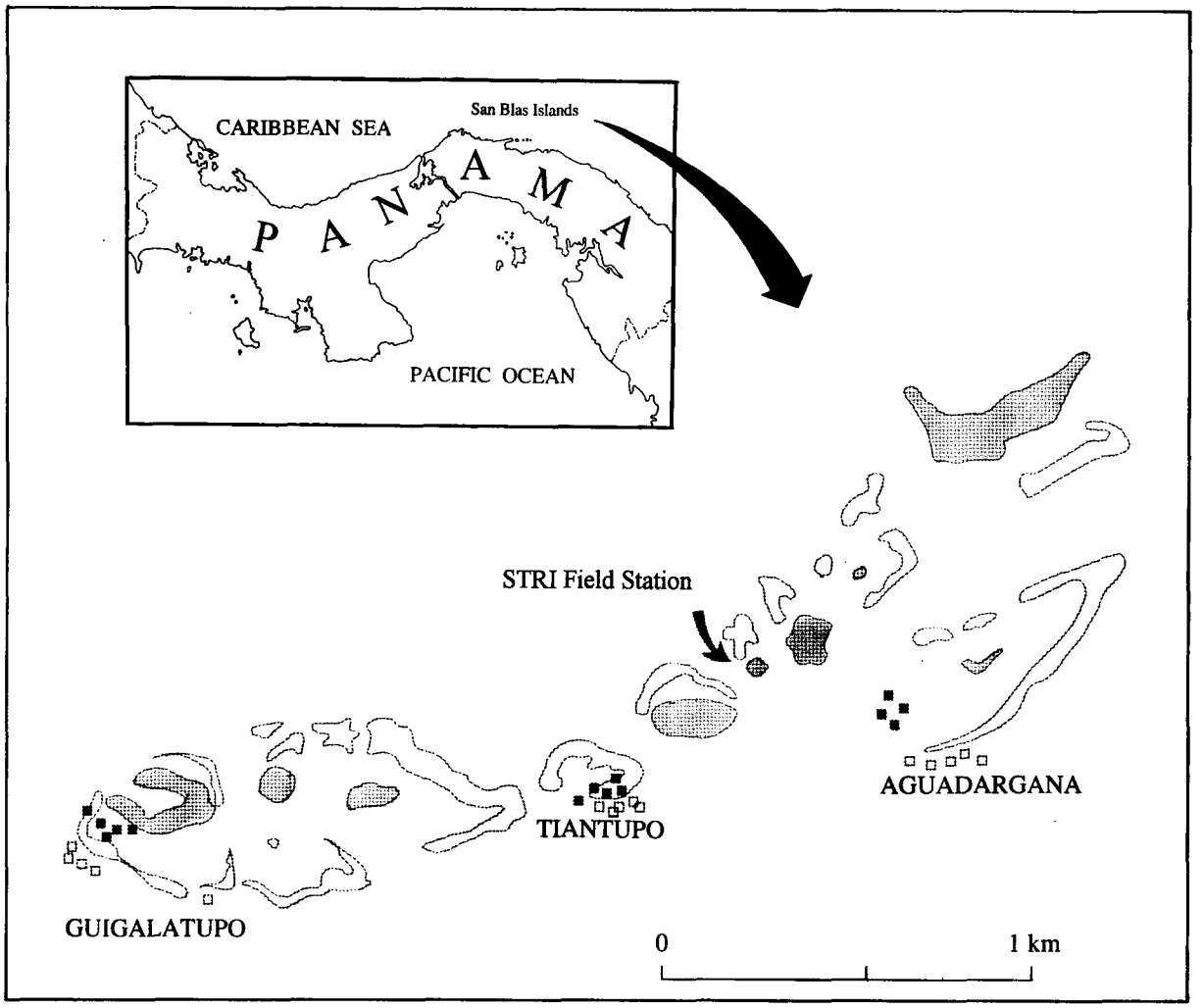

FIG. 1. The study area in the vicinity of the Smithsonian Tropical Research Institute's (STRI) field station in the San Blas islands on the Caribbean coast of Panama. Islands are shaded; submerged reefs are unshaded. Filled and unfilled symbols denote approximate positions of sampled *Spheciospongia vesparium* and *Agelas clathrodes*, respectively.

extremely similar morphologically but are distinguished by subtle differences in color, in the suite of hosts used, and by fixed allozyme differences (J. E. Duffy, unpubl. data). I studied one of these four species of the *S. brooksi* complex in the San Blas Islands of Panama, in an area of ~3 km^2 in the vicinity of the Smithsonian Tropical Research Institute's field station (9°34'N, 78°58'W, Fig. 1). The site comprises a large number of islands and shallow reefs that support diverse and fairly dense sponge assemblages, and are separated by deeper (~10–30 m) sand channels essentially devoid of sponges. Initial sampling of most of the common sponges in this area (Duffy 1992, unpubl. data) produced 13 sponge species that harbored *Synalpheus*; of these, only *Spheciospongia vesparium, Agelas clathrodes,* and an occasional *Agelas dispar* supported *S. brooksi. Spheciospongia vesparium* and *A. clathrodes* both occur on most of the reefs in the study area, though they differ somewhat in habitat distribution (J. E. Duffy, pers. obs.). *Spheciospongia vesparium* is most often found in shallow seagrass beds on banktops (< ~5 m depth) and is rarer in deeper water. *Agelas clathrodes,* in contrast, is most common on reef slopes below ~15 m, although occasional specimens grow as shallow as 8 m in San Blas. Sponge population densities in the field were not measured, but there were no obvious differences in the relative abundances of the two sponge species among the three reefs from which shrimp were sampled.

Population Structure in Different Hosts

I assessed the degree of subdivision between *S. brooksi* populations occupying different host-sponge species by measuring demographic parameters, allozyme frequencies, and behavioral differences in shrimp demes from the co-occurring sponges *Sp. vesparium* and *A. clathrodes.* As *S. brooksi* is an obligate sponge associate (Duffy 1992), I consider the sample of shrimp from a single sponge to represent a deme, and I use "deme" hereafter to refer to the shrimp in a single, individual sponge. Although both sponge species are fairly common in the study area, *Sp. vesparium* is patchily distributed everywhere, and scarce on some reefs. Moreover, *S.*

brooksi does not occupy all *Sp. vesparium* individuals at this site (Duffy 1992). Thus, in 1992, I was able to locate two reefs and, in 1993, one additional reef, where *S. brooksi* occupied both species of sponge (Fig. 1). I obtained shrimp by collecting portions of sponges from the substratum while scuba diving, dissecting sponges in the lab, and removing live shrimp. In 1992, I sampled shrimp for allozyme analysis from three individual sponges of each species on Tiantupo reef, and two sponges of each species on Guigalatupo reef. In 1993, I sampled shrimp from five individual sponges of each host species on each of Tiantupo, Guigalatupo, and Aguadargana reefs (except that on Aguadargana, I sampled shrimp from only four *Sp. vesparium* for demographic parameters and from three of these same sponges for allozymes). Because the two sponge species occupied different habitats, collections of *Sp. vesparium* were generally separated from those of *A. clathrodes* on the same reef by tens of meters, i.e., collections of the two species were usually not interspersed. Collections from different reefs were separated by 1–3 km. In the field, I removed a sample from each sponge, which was then divided in half in the lab. I measured the volume of the first half by displacement (these samples ranged from 200–1,400 ml tissue volume, mean = 528 ml), removed the shrimp and preserved them in 10% formalin for analysis of shrimp population structure and prevalence of parasites. I then removed the shrimp from the second half and froze them in liquid nitrogen for allozyme electrophoresis.

For each of the formalin-preserved samples, I counted the total number of mature female, male/juvenile, and postlarval (i.e., very young juvenile) *S. brooksi*. Mature females were identified by the presence of embryos in the brood chamber or ovarian tissue visible through the thoracic body wall. Individuals were classed as postlarvae if the carpus of the second pereopod consisted of four segments, as compared with five in the adult. All nonfemale individuals with five carpal segments were classed as males/juveniles, because mature males show no external morphological characters that distinguish them from juveniles. Each shrimp was also examined for parasitic epicaridean isopods.

Behavioral Responses to Different Hosts

I conducted laboratory assays to assess whether shrimp from *Sp. vesparium* and *A. clathrodes* differ in their behavioral responses to these two host sponges. The assay consisted of offering pieces of each of the two sponges to individual shrimp in the laboratory and recording which one was occupied after several hours. For each of the three reefs, Aguadargana, Tiantupo, and Guigalatupo, I conducted separate assays, identical in design, as follows. I first collected portions from three sponges each of *Sp. vesparium* and *A. clathrodes* on a single reef. In the lab, I sliced the sponge portions into pieces approximately 4 × 4 × 1 cm in size, removed and set aside all shrimp, then suspended the sponge pieces in a mesh bag submerged in the field for ≥ 30 min prior to assays. After this recovery period, one piece of each of the two sponge species was placed in a plastic bowl containing ~400 ml of seawater, and a single shrimp was then added to the bowl. The assays began in the evening, and shrimp were allowed to distribute themselves for 2–3 h in the darkness. After this time, I quickly removed each sponge piece from the bowl, dipped it in freshwater to dislodge the shrimp, and recorded which sponge piece was occupied by the shrimp in each replicate bowl.

To test for differences in response between shrimp from different host species, it was necessary to ensure that shrimp from all demes were offered identical choices of sponges. Because sponge pieces for the assay were derived from three individual sponges of each species, there were nine possible pairwise combinations (i.e., of one *Sp. vesparium* and one *A. clathrodes*); I set up two replicates of each of these combinations for each shrimp deme, resulting in 18 shrimp from each deme tested. All of the shrimp from a given reef (6 demes × 18 shrimp) were assayed simultaneously. Thus, any difference among demes in mean response to the two host species should be attributable to shrimp behavior, because variation in host quality and experimental conditions were controlled.

Results of host-choice assays were analyzed with heterogeneity *G*-tests (Sokal and Rohlf 1981), separately by reef. First, a *G*-value was calculated for overall heterogeneity in host choice among the six demes (three from *A. clathrodes* and three from *S. vesparium*) on a given reef (df = 5). Next, separate *G*-tests were computed for each host species, measuring heterogeneity among the three demes from one host species (df = 2 for each host species). Finally, the two *G*-values for among-deme heterogeneity were subtracted from the overall *G*, leaving a *G*-value with 1 df that tests for heterogeneity in host-choice between conspecific shrimp from the two different sponge species.

Genetic Structure of Shrimp Populations

Sampling and Electrophoresis.—I used horizontal starch-gel electrophoresis to characterize spatial and host-associated genetic subdivision among shrimp demes. In general, I analyzed 10 to 13 shrimp from each deme (individual sponge). Live shrimps were dissected from sponges and maintained alive for up to 8 d before being snap frozen in liquid nitrogen and stored at −80°C until electrophoresis. Immediately before electrophoresis, frozen shrimps were placed in ceramic spot plates with 40–50 μl of 0.25 M sucrose in 2% (v/v) 2-phenoxyethanol and ground manually with a glass pestle. Homogenates were absorbed onto filter-paper wicks and analyzed using standard electrophoretic methods (Selander et al. 1971) and staining procedures (Brewer 1970; Harris and Hopkinson 1976). Nine enzyme loci were resolved: *Pgi, Pgm, Mdh-1,* and *Mdh-2* on citrate pH 6.0 (Clayton and Tretiak 1972); *Tpi* on lithium hydroxide ph 8.1/8.4 (System 2 of Selander et al. 1971); *Idh-1, Idh-2, Mpi,* and *Aat* on tris-citrate pH 8.0 (System 5 of Selander et al. 1971). Samples from different locations and host species were interspersed on a given gel to facilitate comparison of alleles; all alleles were named according to their mobility relative to the most common allele in *S. brooksi* from Tiantupo, Panama, which was designated 100.

Analysis of Genetic Data.—Allele frequencies, genotype frequencies, and exact tests for conformance of genotype frequencies to Hardy-Weinberg expectations were calculated

by BIOSYS-1 (Swofford and Selander 1981). I measured genetic subdivision among shrimp demes by testing for allele frequency heterogeneity using *G*-tests and by estimating Wright's (1978) *F*-statistics at three levels: (1) among demes (conspecific sponges) within reefs, (2) between host sponge species within reefs, and (3) among reefs.

As traditionally employed (e.g., Feder et al. 1990a; Waring et al. 1990; Rank 1992), hierarchical analysis involves pooling all individuals in a sample, tallying the numbers of different alleles in the sample, and comparing those allelic frequencies with the frequencies in other samples. This procedure is appropriate for comparing approximately panmictic units (e.g., in this case demes within reefs). To test for allele-frequency heterogeneity at more inclusive levels (e.g., reefs within total), however, it has been common practice in population genetic studies to pool the demes (e.g., within a reef), and conduct a second *G*-test at the more inclusive level, effectively ignoring the variance among demes within that level. Consequently, all heterogeneity in allele frequencies detected in this second test is implicitly attributed to the level currently being tested, and the *P*-value of the test is artifically inflated. This problem, moreover, is not necessarily solved when there is no significant difference in an initial *G*-test of heterogeneity among demes.

To avoid this problem, I subdivided the total heterogeneity in allele frequencies on a given reef. First, I obtained an overall *G*-value by computing *G* for heterogeneity among all demes on a reef (e.g., all five demes from *A. clathrodes* plus all five demes from *Sp. vesparium*; df = 9). Second, I calculated a *G* for heterogeneity among demes within each of the two host species (df = 4 each). Then, making use of the additivity of *G* values (Sokal and Rohlf 1981), I subtracted these two among-deme *G*'s from the overall *G*; the remainder measures heterogeneity between the two host species (df = 1). Finally, I calculated a grand overall *G* for heterogeneity among all samples on all three reefs; when the overall *G*-values for each reef were subtracted from this, the difference reflected heterogeneity among reefs (df = 2). These hierarchical *G*-tests were calculated for all locus-reef combinations at which the overall frequency of the most common allele was $\leq$ 90%. Tablewise probability values were computed using the sequential Bonferroni procedure (Rice 1989).

Wright's (1978) *F*-statistics measure reduction in heterozygosity relative to that expected in a panmictic population, partitioning the total inbreeding (F_{IT}) into components due to inbreeding within populations (F_{IS}) and subdivision among populations (F_{ST}). I estimated these parameters using Weir and Cockerham's (1984) formulae, which account for finite sample size and among-locus variance in allele frequencies. Patterns revealed by *F*-statistics were qualitatively similar to those of the *G*-tests. However, the small number of polymorphic loci resulted in large standard errors for *F*-statistics (obtained by jackknifing over loci) and therefore low power of the tests for their significance. F_{ST} values are therefore reported for comparative purposes but significance of allele frequency heterogeneity is evaluated by *G*-tests as described above.

Intersexual Aggression Assays

As a proxy for mating incompatibility between shrimp from different populations, I measured aggression in heterosexual pairs of shrimp, exploiting the fact that alpheids are generally territorial and highly aggressive toward nonmates (Nolan and Salmon 1970) and especially so toward heterospecifics (Knowlton and Keller 1983). To estimate the degree of mating incompatibility between shrimp from different host species, I compared the level of aggression between male and female shrimp taken from different co-occuring host species on the same reef with that of control pairs of shrimp taken from the same host species (but different individual sponges).

The assay consisted of placing one female and one male shrimp together in a small plastic chamber filled with seawater, observing the pair for five (1992) or 10 (1993) min (the clock starting upon first contact between them) and recording the number of each of the following behaviors that occurred during each 30-s interval: antennal tapping, body contact, crawling over one another, face-off (shrimp facing one another just out of range of contact), escape response (rapid flip of the tail propelling the shrimp backwards), and snaps with the major chela. I considered snaps, face-offs, and escape responses as indicating incompatibility. For each pair, I calculated an index of incompatibility by summing the total number of 30-s intervals in which one or more of these incompatible behaviors occurred and dividing this by the total number of intervals in which any kind of behavior was observed (i.e., excluding intervals in which both shrimp were inactive and not in contact). The first 30-s interval was excluded from these calculations because shrimp often appeared agitated immediately after introduction to the chamber.

The assay included three treatments: (1) both shrimp from *Sp. vesparium,* (2) both shrimp from *A. clathrodes,* and (3) one shrimp from each of the two hosts. No shrimp was used more than once. In all treatments, the two shrimp in a pair came from different individual sponges; thus the treatments should test the effects of host species of origin rather than individual sponge of origin. Each treatment combination was replicated six times. In almost all cases, the six replicates of a given treatment combination included three females from one sponge and three females from the other. Pairs were excluded from the analysis if behavior was recorded in fewer than four intervals. As a consequence, some treatment combinations had fewer than six replicates.

I conducted these assays separately for Tiantupo and Guigalatupo reefs in 1992 and for both of these as well as Aguadargana reef in 1993. Because the two assays for a given reef were conducted a year apart, and differed in duration (5 vs. 10 min) and source of sponges, I analyzed each assay separately with a model I ANOVA; however, because the experiments in 1992 and 1993 tested the same biological (though not statistical) hypothesis, I combined the probability values from the two assays on a given reef, as suggested by Sokal and Rohlf (1981, p. 779).

The outcome of aggressive contests between alpheid shrimp depends strongly on the difference in size between contestants (Schein 1977), and in the field, heterosexual pairs of alpheids are more closely matched by chela size than by body length (Knowlton 1980). Therefore, to determine whether the results of the aggression assays might be influenced by variance in the shrimp size differential among treatments, I used ANOVAs to test whether the percent difference

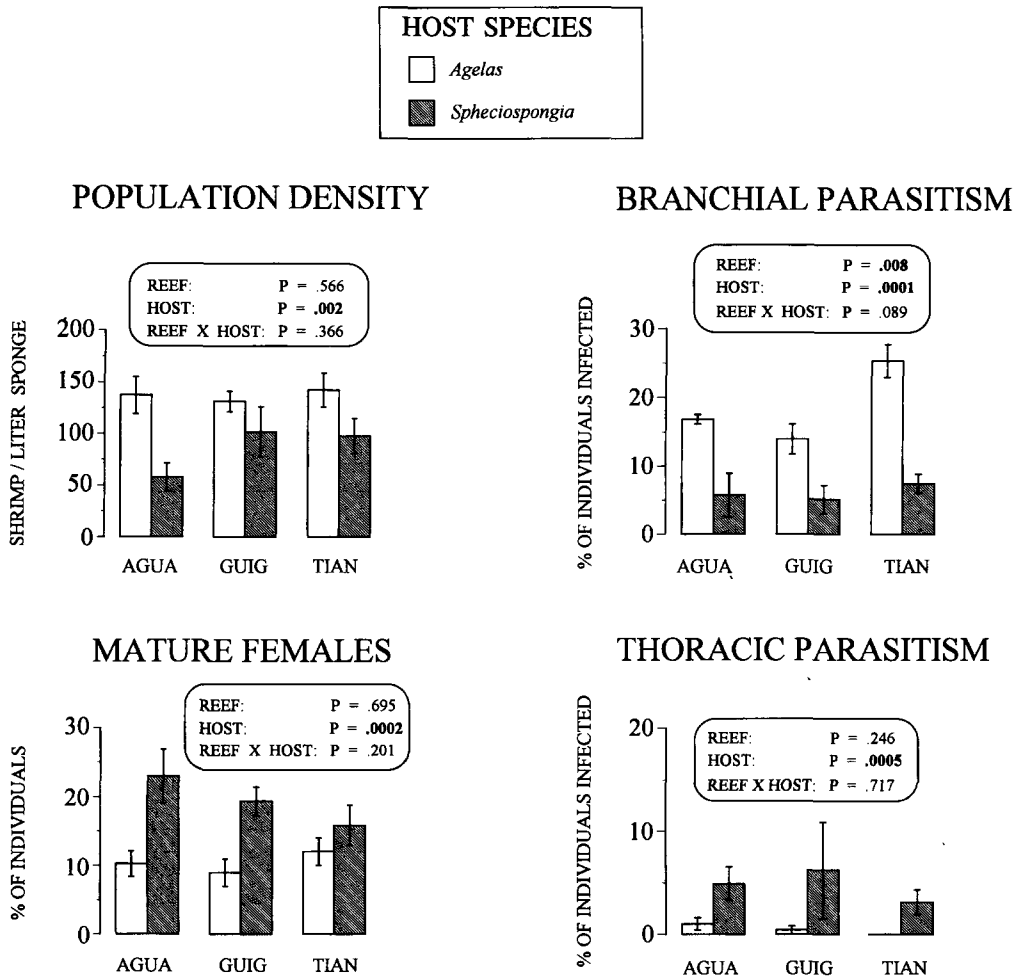

FIG. 2. Population characteristics of *Synalpheus brooksi* from the host sponges *Agelas clathrodes* and *Spheciospongia vesparium* on three reefs in Caribbean Panama. *P*-values are from two-factor, model-I ANOVAs testing the main effects REEF and HOST (data on thoracic parasites were log transformed). $N = 5$ demes of each host-sponge species sampled from each reef (except *Sp. vesparium* on Aguadargana, $N = 4$). AGUA, Aguadargana reef, GUIG, Guigalatupo, TIAN, Tiantupo.

in body size (carapace length), or in major chela size, between members of a pair varied significantly among the three treatments.

RESULTS

Population Structure in Different Hosts

Synalpheus brooksi populations occupying different sponge species differed strikingly in several population characteristics (Fig. 2). Shrimp demes from *A. clathrodes* were denser, and supported fewer mature females, than co-occuring demes from *Sp. vesparium.* Shrimp in alternate hosts also experienced different levels of exposure to enemies. *S. longicarpus,* a larger and more aggressive congener of *S. brooksi,* was present in all *Sp. vesparium* sampled, whereas the alternate host *A. clathrodes* was nearly devoid of potentially competing congeners. Prevalence of thoracic (probably entoniscid) isopod parasites was also several-fold higher in shrimp populations from *Sp. vesparium* than in those from *A. clathrodes.* Conversely, prevalence of bopyrid isopods infesting the branchial region was two to three times higher in populations from the latter sponge (Fig. 2). For each of these population parameters, two-way ANOVAs showed that host species had a highly significant effect ($P \leq 0.002$ in all cases), whereas variation among the three reefs was generally nonsignificant. Thus, the two host-sponge species present significantly different environments to *S. brooksi,* and these host-associated effects appear to override variation among sites, at least on a scale of a few kilometers, in influencing the parameters of shrimp populations studied here.

Behavioral Responses to Different Hosts

In the lab, *S. brooksi* tended to choose the sponge species from which they originated more often than did sympatric conspecifics from the foreign host (Fig. 3), although these host-associated differences in response were relatively subtle. On Aguadargana, shrimp from the two sponge species showed clear differences in host choice ($P = 0.013$), whereas the patterns on Tiantupo ($P = 0.049$) and Guigalatupo ($P = 0.055$) were near the border of statistical significance. Interestingly, on Tiantupo, there were also substantial differences

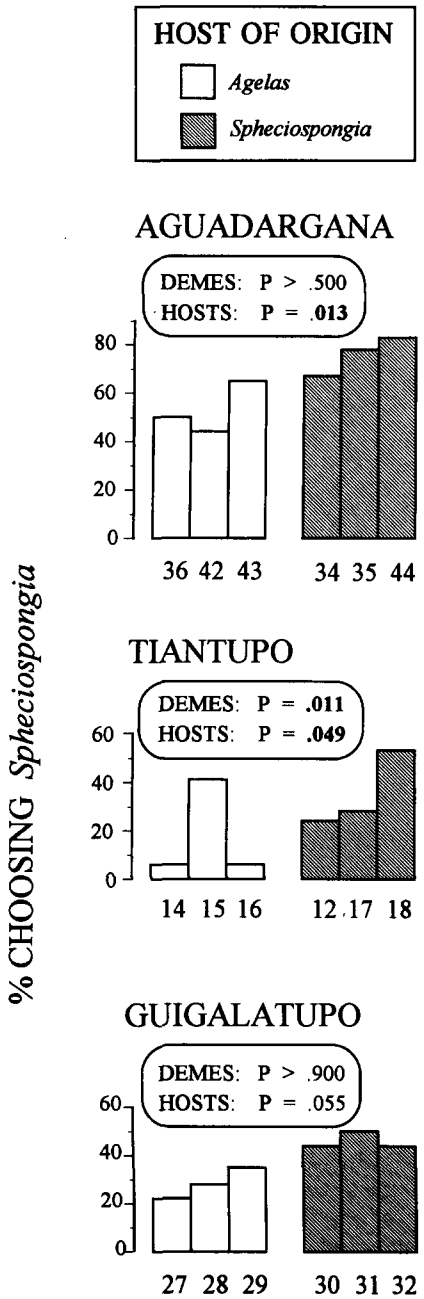

FIG. 3. Results of laboratory assays testing shrimp choice between two host-sponge species as a function of the host of origin. Each bar represents the proportion of 18 shrimp from a single individual sponge (i.e., deme) that occupied *Spheciospongia vesparium* when given a choice between that sponge and *Agelas clathrodes*. Numbers below each bar represent the populations (Appendix 1) from which shrimp originated. Assays were conducted separately for each of the three reefs. *P*-values are from hierarchically partitioned *G*-tests (see Materials and Methods: *Behavioral Responses to Alternate Hosts*). DEMES represents heterogeneity among demes from different conspecific sponges; HOSTS represents heterogeneity between populations from different host species.

in host preference among demes (i.e., groups of shrimp from individual conspecific sponges, $P = 0.011$). Because each of the six shrimp demes from a given reef was exposed to all nine possible combinations of paired host sponges from that reef, and because all six demes were assayed simultaneously, the variation among demes in response must be attributable to differences in shrimp behavior among them. The data also suggest that the strength of preference between the two sponge species may differ among the three reefs as well, but it is impossible to evaluate this possibility because assays on the different reefs used different individual sponges.

Genetic Structure of Shrimp Populations

Of the nine loci scored, *Mdh-1* was monomorphic, and *Pgi, Pgm, Mdh-2,* and *Idh-2* were nearly so; these loci are not considered further. Allele frequencies at the remaining loci are presented in Appendix 1. Of a total of 178 tests for goodness-of-fit to Hardy-Weinberg equilibrium, only four were significant at the nominal $\alpha = 0.05$.

Populations of *S. brooksi* were strongly subdivided within the restricted area sampled (~3 km long, Fig. 1). At the largest spatial scale, *G*-tests of allele frequency heterogeneity revealed strongly significant subdivision among reefs in both 1992 and 1993 (Table 1). Estimates of F_{ST} among reefs calculated separately for the two host species in each year, ranged from 0.104–0.147. Values of F_{ST} can be used to calculate a rough estimate of gene flow, i.e., the number of migrants (Nm) exchanged among subpopulations per generation, using the expression $Nm = (1/F_{ST} - 1)/4$ (Slatkin 1985). These values correspond to estimates (Nm) of about two migrants exchanged among reefs each generation. The restricted gene flow among reefs implied by these values necessitates treating the three reefs separately when considering genetic subdivision among populations using different hosts.

Genetic subdivision by host species was clearly evident, but both the degree of differentiation and the particular loci involved varied among the three reefs. In 1993, host-associated heterogeneity in allele frequencies was highly significant on both Aguadargana and Tiantupo, whereas no such differences were found on Guigalatupo (Table 1). The limited data from 1992 are consistent with these patterns insofar as significant host-associated differences occurred on Tiantupo but not on Guigalatupo in each year. Despite rather small sample sizes, the robustness of the host-associated differences in allele frequencies is supported by consistency among the replicate demes on a given reef (Appendix 1), consistency between years on Guigalatupo and Tiantupo, and by high *G*-values (Table 1). Values of F_{ST} for host-associated subdivision within reefs show a similar pattern. F_{ST} between host species (mean ± 1 SE) was 0.206 ± 0.111 for Aguadargana and 0.135 ± 0.053 for Tiantupo in 1993, whereas values of F_{ST} among demes within host species for the same reefs were much smaller (0.010–0.084). This indicates that genetic differentiation occurred primarily between hosts, even though there was also significant differentiation among reefs. Estimates of the number of migrants per generation exchanged between the two host species, calculated from these F_{ST} values, were 0.096 on Aguadargana, 1.60 on Tiantupo, and 10.62 on Guigalatupo. [These calculations are likely to underestimate gene flow to some extent in cases in which population structure conforms more closely to a stepping-stone than an island model, and where extinctions and recolonizations oc-

TABLE 1. Hierarchical components of allele frequency heterogeneity in *Synalpheus brooksi*. Table entries are *G*-values for heterogeneity, calculated as described in Materials and Methods: Genetic Structure of Shrimp Populations; only locus/reef combinations for which overall frequency of the most common allele at that level in the hierarchy was ≤90% were included (cells marked by a horizontal line did not meet this criterion). * $P < 0.05$ at the adjusted alpha, which controls for type-I error over all comparisons at that hierarchical level, as determined by the sequential Bonferroni procedure (Rice 1990). df, degrees of freedom.

Hierarchical level / Year / Reef/host	Locus			
	Tpi-2	*Idh-1*	*Mpi*	*Aat*
Among demes within host species				
1992				
Gugalatupo				
Agelas (df = 1)	1.43	—	—	—
Spheciospongia (df = 1)	1.68	—	—	—
Tiantupo				
Agelas (df = 2)	3.59	14.86	2.18	—
Spheciospongia (df = 2)	0.56	6.84	1.66	—
1993				
Aguadargana				
Agelas (df = 4)	8.95	9.40	15.28	—
Spheciospongia (df = 2)	3.12	0.36	6.86	—
Guigalatupo				
Agelas (df = 4)	6.75	—	3.83	9.21
Spheciospongia (df = 4)	5.32	—	1.32	1.16
Tiantupo				
Agelas (df = 4)	3.82	7.99	—	3.77
Spheciospongia (df = 4)	11.49	7.04	—	11.45
Between host species within reefs (df = 1 for all comparisons)				
1992				
Guigalatupo	0.06	—	—	—
Tiantupo	0.69	0.79	12.90*	—
1993				
Aguadargana	31.83*	1.64	9.00*	—
Guigalatupo	1.13	—	3.84	4.22
Tiantupo	13.03*	26.81*	—	4.86
Among reefs				
1992 (df = 1)	14.89*	22.37*	—	—
1993 (df = 2)	7.14*	84.03*	8.37*	—

cur (Slatkin 1985), as are likely for *S. brooksi*]. The degree of host-associated subdivision appeared to be lower in 1992, although there are too few data to assess this rigorously.

At the smallest spatial scale, there was no evidence for significant genetic subdivision among demes from conspecific sponges, which are separated by meters to tens of meters, within reefs. Estimates of F_{ST} among demes ranged from 0.010–0.110 (Nm = 2.02–24.75), and none of the values for allele frequency heterogeneity among demes (Table 1) differed significantly from zero.

In summary, the genetic data reveal strong subdivision among reefs separated by a few kilometers, and equally strong subdivision between populations occupying different host species (separated by tens to hundreds of meters) within some, but not all, reefs. Thus, patterns of host-associated differentiation, where present, differ among reefs. In contrast to the subdivision seen at larger scales, there is no evidence of significant differentiation among demes from conspecific sponges within reefs.

TABLE 2. Tests for variance in body size and major chela size among treatments in the intersexual aggression assays (Fig. 4). Variables analyzed are the percent difference in size (body or major chela) between the two members of a pair. Size variables were measured, and these tests computed, only for the assays conducted in 1993. The *F* reported is for the main effect, type of pairing, in one-way ANOVAs computed separately for each reef.

Variable / Reef	*F*	df	*P*
Percentage difference in body size (carapace length)			
Aguadargana	0.90	2, 33	0.415
Guigalatupo	0.12	2, 15	0.891
Tiantupo	0.50	2, 15	0.615
Percentage difference in major chela length			
Aguadargana	0.09	2, 33	0.911
Guigalatupo	0.52	2, 15	0.607
Tiantupo	0.89	2, 15	0.432

Intersexual-Aggression Assays

The level of aggression between shrimp from different hosts varied considerably among the three reefs (Fig. 4). On one reef, Tiantupo, shrimp from different host species behaved significantly more aggressively toward one another than shrimp in control pairs taken from the same host species (P = 0.021, combined probabilities from separate ANOVAs for the two years), showing nearly double the level of aggression, on average, of pairs taken from the same host species (Fig. 4). Interestingly, the two aggression assays using shrimp from Tiantupo, though conducted a year apart with different sponges and shrimp, showed a similar pattern of elevated aggression in pairs from different host species, relative to same-host controls. Likewise, on Aguadargana the pattern of differences among treatments was similar in the two experiments (which were conducted in the same year on this reef but with shrimp from different individual sponges). Thus the host-associated behavioral differences on Tiantupo, and lack thereof on the other reefs, are not readily explained by heterogeneity between the replicate experiments in the individual source sponges. Nor were these patterns attributable to differences in body size, or in size of the major chela (claw), between shrimp from different sources. Analyses of variance showed that neither the difference in body size between contestants (P = 0.415–0.891), nor the difference in major chela size between them (P = 0.432–0.911), differed significantly among the three treatments on any of the reefs (Table 2).

DISCUSSION

The prevalence of co-occurring, ecologically differentiated sister taxa in several groups of animals continues to fuel debate over the existence and importance of sympatric speciation. Given the host specificity of sponge-dwelling *Synalpheus*, the habit of mating within the sponge, and the great number of congeneric species living sympatrically, these shrimp would appear to be as likely candidates for sympatric speciation as many of the insects and fishes that have been proposed. Indeed, the most obvious ecological difference between sympatric species of *Synalpheus* generally is their association with different host species (Duffy 1992).

Assessing whether sympatric divergence is occurring in a given case involves two separate, although related, issues that have sometimes been confounded: the existence of resource-mediated differences between morphs or populations, and the spatial context (sympatric or not) in which those differences originated. With regard to the first, this study provides several lines of evidence for host-associated divergence. First, consistent and highly significant differences in population density, proportion of mature females, and prevalence of parasites among populations of *S. brooksi* occupying different hosts (Fig. 2) confirm that these sponges present substantially different environments for shrimp (see also Duffy 1992), although they do not establish definitively the more important point, that the hosts impose disruptive selection on them. Second, shrimp taken from the two sponges differed in host preference (Fig. 3). These differences could reflect genetic differences between the races or conditioning based on experience, either of which would enhance assortative mating and thus reduce gene flow between races. In fact, the distinction is complicated in practice by the direct development and sedentariness of *S. brooksi,* which result in juveniles being born into the mother's sponge and probably remaining there for most or all of their lives. It is unclear whether shrimp disperse as adults (adults were used in this assay) or as juveniles, but in either case, some period of experience with the natal sponge would already have occurred prior to dispersal. The third source of evidence for host-associated divergence is genetic (discussed below). Finally, the aggression assays provide preliminary evidence that, on one of the reefs, divergence between host races has proceeded to the point where reproductive compatibility between them is reduced (Fig. 4). Taken together, these data suggest that *S. brooksi* fulfills the two principal preconditions for sympatric speciation by habitat specialization (Rice 1987, p. 310–311): "that organisms mate locally within relatively discrete spatially and/or temporally separated habitats, and that the environmental demands . . . be sufficiently different between habitats."

As with all putative cases of sympatric speciation, the critical and much more difficult question is the degree to which populations were spatially isolated as these host-associated differences arose. At a minimum, claims of sympatric differentiation (a necessary, but not sufficient, condition for sympatric speciation) require demonstration of significant genetic differentiation between conspecific populations using different resources within "normal cruising range" of one another (Jaenike 1981, p. 830). Restriction in gene flow between the host races must be attributable to intrinsic differences in host-selection behavior between them, rather than simply to spatial isolation caused, for example, by extreme philopatry (Mayr 1942; Futuyma and Mayer 1980; Diehl and Bush 1989). The data presented here allow an assessment of this criterion for *S. brooksi.* The overall pattern in the genetic data is one of strong subdivision (Table 1), presumably a consequence of the absence of planktonic dispersal in *S. brooksi* (Dobkin 1965) and this shrimp's obligate association with sponges (Dardeau 1984; Duffy 1992), which result in extreme philopatry. One implication of this subdivision is that populations on different reefs can be considered semi-independent when examining host-associated patterns of pop-

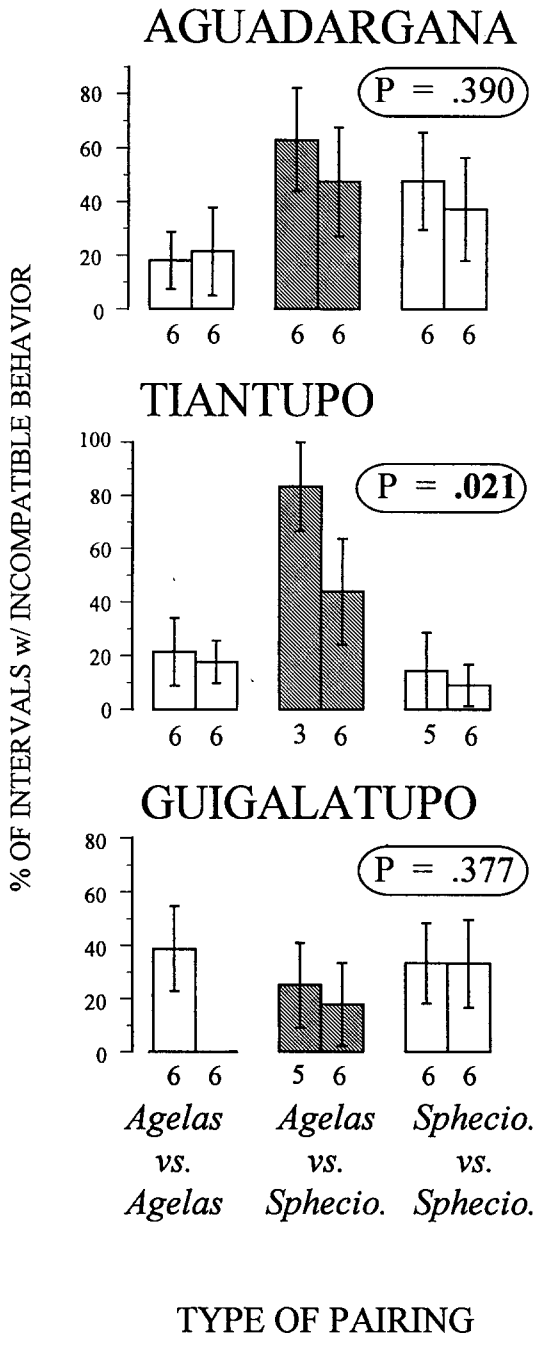

FIG. 4. Assays of aggressive behavior in heterosexual pairs of *Synalpheus brooksi* when pairs came from different conspecific sponges (*Agelas* vs. *Agelas,* and *Sphecio* vs. *Sphecio.*) or different species of sponges (*Agelas* vs. *Sphecio*). Incompatible behaviors are defined in Materials and Methods: *Intersexual Aggression Assays.* The two adjacent bars shown for each treatment represent the two "replicate" experiments conducted for each reef. Numbers below bars = number of replicate pairs of shrimp. *P*-values shown are combined *P*-values (Sokal and Rohlf 1981) from separate one-factor, model-I ANOVAs of the two separate experiments conducted for each reef.

ulation structure in this species. More important, the extreme philopatry of *S. brooksi* calls into question the assumption that shrimp in alternate host-sponge species on a given reef are within normal cruising range of one another. Given *S. brooksi*'s sedentary habits, and the different (although overlapping) habitat and depth distributions of its two host sponges, the host-associated divergence documented here may be caused as much by extrinsic factors (spatial isolation) as by intrinsic ones (host selection).

The scale of genetic subdivision in *S. brooksi* illustrates a general problem with the empirical demonstration of sympatric differentiation. The patchy distribution of suitable hosts, together with the sedentary habits of most life-history stages, create conditions favorable for local differentiation (fostered by strong spatial subdivision, i.e., an extrinsic isolating mechanism) in many of the same animals proposed as candidates for sympatric speciation. It is thus little wonder that assessment of sympatric divergence in nature has been so difficult. If resource-associated differences can arise among populations in as close proximity as those of *S. brooksi* in this study, such differences would seem all the more likely in the completely isolated (allopatric) populations that must commonly arise in such philopatric organisms (Futuyma 1983). Thus, the likelihood of allopatric (or "microallopatric") speciation seems just as high in *Synalpheus* as in most other animals. Although host shifts in phytophagous insects are not infrequent, even on an ecological time scale (Fox and Morrow 1981; Thomas et al. 1987), the currently sympatric occurrence of differentiated host races or species, which has been cited in support of sympatric speciation (Bush 1975; Wood and Guttman 1982; Tauber and Tauber 1989), does not offer especially strong support, given the dynamic nature of host communities on the scale of hundreds and even tens of parasite generations.

The strongly subdivided population structure of *S. brooksi* puts the issue of host-race divergence into a somewhat different light: how do host-associated differences arise and spread in such a system? In effect, the three reefs provide three semi-independent opportunities for host-associated differentiation to occur, as illustrated both by genetic and behavioral data. First, host-associated genetic differentiation occurred at different loci on Aguadargana and Tiantupo and was absent on Guigalatupo (Table 1). The difference among reefs in the degree of polymorphism at particular loci is somewhat puzzling; this might be explained if reefs are colonized by a small number of individuals (as seems likely given their poor dispersal ability), which randomly sample only a fraction of the existing genetic variation. Under such conditions, groups of colonists reaching different reefs may differ substantially in allele frequencies purely by chance. If host-associated divergence then occurs independently on different reefs, inconsistency among reefs in the loci involved would be expected of neutral genetic markers. Second, independence also is supported by differences among reefs in the degree of host-associated behavioral differentiation. Shrimp from different host species showed elevated levels of intersexual aggression only on Tiantupo (Fig. 4), which was also the reef with the strongest genetic differentiation among hosts (Table 1). There is thus a rough correspondence, in comparisons among reefs, between genetic and behavioral estimates of host-race divergence, and substantial variance among reefs in the degree of differentiation. Interestingly, such subdivided populations may provide precisely the kind of situation envisioned in Wright's (1931) shifting-balance model and may be particularly amenable to rapid, resource-mediated divergence because of the multiple opportunities they experience.

These results present an interesting contrast with better-known cases of putative sympatric speciation. The most celebrated, and by far the best documented, of these is that of the apple maggot fly *Rhagoletis pomonella*. Within the last 150 y, populations of this tephritid fly have colonized introduced apples from the native host, hawthorn, and established largely allochronic "host races" that are sympatric throughout the species' range (Bush 1969). Feder et al. (1990a,b) documented host-associated allozyme differences between these races on both local (hundreds of meters) and geographic (throughout eastern North America) scales that are consistent in both the particular loci involved and the direction of the allele-frequency differences. Their genetic data demonstrated that the host races are largely reproductively isolated from one another in nature as a consequence of association with (and mating on) different host species. Feder et al. (1990a, p. 595) then suggested that such consistency of genetic differences in time and space should provide a general criterion for demonstrating the existence of host races as it distinguishes "resource-related polymorphism . . . from variation caused by stochastic processes such as founder events and genetic drift." It is not clear, however, that this need generally be true. Instead, broad-scale spatial consistency of genetic markers (i.e., same loci and same directions) is expected only in weakly geographically structured species, such as *R. pomonella*. In such cases the consistency of genetic differences in time and space does indeed demonstrate that recent gene flow within races has been much greater than that between races and, thus, that the races are isolated, as Feder et al. (1990a) conclude.

In highly subdivided species, by contrast, differentiation is unlikely to occur at the same loci throughout the species' range if it is measured by neutral markers. This is because resource-associated differentiation in such species can occur independently in a number of semi-isolated populations, as in *S. brooksi*. Thus, if the genetic markers examined are in fact neutral, the particular loci involved, the directions, and the magnitudes of resource-associated differences should vary among populations, even if resource-associated selection acts on the same traits in the independent populations. Many of the groups that have been suggested as exemplars of sympatric speciation—including various phytophagous and parasitic insects, snails, and freshwater fishes—are characterized by philopatric species with strongly subdivided populations (Carson and Kaneshiro 1976; Futuyma and Peterson 1985; Guttman and Weigt 1989; Gittenberger 1991; Ribbink 1991). There is in fact evidence for multiple origins of resource-associated differentiation within several such species. For a number of freshwater fishes (salmonids, Hindar et al. 1986; sticklebacks, Schluter and McPhail 1992; smelt, Taylor and Bentzen 1993), an estuarine amphipod (Stanhope 1993), and an intertidal snail (Johannesson et al. 1993), genetic data reveal that remarkably convergent processes of morphological and ecological differentiation have occurred

independently in different conspecific populations, often in the absence of obvious spatial barriers to gene flow. Thus, the pattern of differentiation expected in the initial stages of resource-associated speciation may be quite different in philopatric versus widely dispersing organisms.

Finally the question remains whether the population-level patterns documented here can be extrapolated to the level of speciation. Are these populations of *S. brooksi* progressing toward full, reproductively isolated species? Given the subtlety and spatial variation of host-associated differentiation in this species, this seems unlikely. Again, comparison with *R. pomonella* is instructive. In contrast to this fly, whose host shift is known to have occurred within the last 150 y, *S. brooksi* has likely occupied the two sponges studied in San Blas as long as these reefs have existed, probably at least since sea level approached its present level after the last glaciation, 7,000–9,000 y ago (Jackson 1992). Any host races arising during this period thus may already have achieved reproductive isolation, perhaps contributing to the considerable number of sibling species of Caribbean *Synalpheus*. In contrast, extant conspecific populations, such as *S. brooksi* in the two sponges studied here, may represent populations that have achieved stable polymorphisms or phenotypic plasticity for host use. The question might also be approached from a macroevolutionary perspective. In this case, the answer requires data on host ranges, geographic ranges, and phylogenetic relationships, most of which are poorly known for *Synalpheus* species. Of the four known species within the nominal taxon *S. "brooksi,"* however, three include *Sp. vesparium* in their host ranges, indicating that host shifts alone are insufficient to explain most speciation events in the *brooksi* complex. However, no two of the species (in this or any other complex within *Synalpheus*) uses the same suite of hosts, i.e., changes in host use appear to be consistently associated with speciation events. Whether, and how often, these events are initiated by sympatric host shifts will remain a mystery pending better resolution of the distribution and phylogeny of these curious shrimp.

Acknowledgments

For able assistance and cameraderie in the field, I am grateful to P. Marko and C. Morrison. The paper benefited from discussions with D. Ayre, R. Grosberg, M. Hellberg, N. Knowlton, D. Levitan, P. Marko, and B. Shaffer. I am also grateful for thoughtful readings of the manuscript by C. Fox, R. Grosberg, J. Hilbish, N. Knowlton, P. Marko, and an especially thorough analysis by an anonymous reviewer. I am particularly indebted to R. Grosberg for advice, encouragement, and the use of his nice, clean lab. This research was supported by a Postdoctoral Fellowship in Environmental Biology from the National Science Foundation (NSF) and by NSF grant 90-00153 to R. K. Grosberg. I thank the Kuna Nation and the government of the Republic of Panama for permission to work in San Blas.

Literature Cited

Brewer, G. J. 1970. Introduction to Isozyme Techniques. Academic Press, New York.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behavior. University of Chicago Press, Chicago.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23:237–251.

———. 1975. Modes of animal speciation. Annual Review of Ecology and Systematics 6:339–364.

Carson, H. L., and K. Y. Kaneshiro. 1976. *Drosophila* of Hawaii: Ecological genetics and speciation. Annual Review of Ecology and Systematics 7:311–345.

Chace, F. A., Jr. 1989. The caridean shrimps (Crustacea: Decapoda) of the *Albatross* Phillipine expedition, 1907–1910, Part 5: Family Alpheidae. Smithsonian Contributions to Zoology 46:1–99.

Choat, J. H., and D. R. Bellwood. 1992. Reef fishes: Their history and evolution. Pp. 39–66 *in* P. F. Sale, ed. The ecology of fishes on coral reefs. Academic Press, New York.

Clayton, J. W., and D. N. Tretiak. 1972. Amine citrate buffers in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29:1169–1172.

Coyne, J. A., and H. A. Orr. 1989. Patterns of speciation in *Drosophila*. Evolution 43:362–381.

Dardeau, M. R. 1984. *Synalpheus* shrimps (Crustacea: Decapoda: Alpheidae). I. The Gambarelloides group, with a description of a new species. Memoirs of the Hourglass Cruises 7, Part 2:1–125.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Annual Review of Entomology 29:471–504.

———. 1989. The role of habitat preference in adapatation and speciation. Pp. 345–365 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Dobkin, S. R. 1965. The first post-embryonic stage of *Synalpheus brooksi* Coutiere. Bulletin of Marine Science 15:450–462.

———. 1969. Abbreviated larval development in caridean shrimps and its significance in the artifical culture of these animals. FAO Fisheries Reports 57:935–946

Duffy, J. E. 1992. Host use patterns and demography in a guild of tropical sponge-dwelling shrimps. Marine Ecology Progress Series 90:127–138.

———. 1993. Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. Marine Biology 116:459–470.

Echelle, A. A., and I. Kornfield. 1984. Evolution of fish species flocks. University of Maine Press, Orono, ME.

Erdman, R. B., and N. J. Blake. 1987. Population dynamics of the sponge-dwelling alpheid *Synalpheus longicarpus*, with observations on *S. brooksi* and *S. pectiniger*, in shallow-water assemblages of the eastern Gulf of Mexico. Journal of Crustacean Biology 7:328–337.

Faulkner, D. J. 1984. Marine natural products: Metabolites of marine invertebrates. Natural Products Reports 1:551–598.

Feder J. L., C. A. Chilcote, and G. L. Bush. 1990a. Regional, local, and microgeographic allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in western Michigan. Evolution 44:595–608.

———. 1990b. The geographic pattern of genetic differentiation between host-associated populations of *Rhagoletis pomonella* (Diptera: Tephritidae) in the eastern United States and Canada. Evolution 44:570–594.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124–138.

Fox, L. R., and P. A. Morrow. 1981. Specialization: Species property or local phenomenon? Science 211:887–893.

Futuyma, D. J. 1983. Evolutionary interactions among herbivorous insects and plants. Pp. 207–231 *in* D. J. Futuyma and M. Slatkin, eds. Coevolution, Sinauer, Sunderland, MA.

Futuyma, D. J., and G. C. Mayer. 1980. Non-allopatric speciation in animals. Systematic Zoology 29:254–271.

Futuyma, D. J., and S. C. Peterson. 1985. Genetic variation in the use of resources by insects. Annual Review of Entomology 30: 217–238.

Gittenberger, E. 1991. What about non-adaptive radiation? Biological Journal of the Linnean Society 43:263–272.

Guttman, S. I., and L. A. Weigt. 1989. Macrogeographic genetic variation in the *Enchenopa binotata* complex (Homoptera: Men-

bracidae). Annals of the Entomological Society of America 82: 156–165.
Guttman, S. I., T. K. Wood, and A. A. Karlin. 1981. Genetic differentiation along host plant lines in the sympatric *Enchenopa binotata* Say complex (Homoptera: Membracidae). Evolution 35: 205–217.
Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. American Elsevier, New York.
Hindar, K., N. Ryman, and G. Stahl. 1986. Genetic differentiation among local populations and morphotypes of Arctic Charr, *Salvelinus alpinus*. Biological Journal of the Linnean Society 27: 269–285.
Jaenike, J. 1981. Criteria for ascertaining the existence of host races. American Naturalist 117:830–834.
Jackson, J. B. C. 1992. Pleistocene perspectives on coral reef community structure. American Zoologist 32:719–731.
Johannesson, K., B. Johannesson, and E. Rolán-Alvarez. 1993. Morphological differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail *Littorina saxatilis*. Evolution 47:1770–1787.
Keenleyside, M. H. A., ed. 1990. Cichlid Fishes. Behaviour, ecology and evolution. Chapman and Hall, London.
Knowlton, N. 1980. Sexual selection and dimorphism in two demes of a symbiotic, pair-bonding snapping shrimp. Evolution 34: 161–173.
Knowlton, N., and J. B. C. Jackson. 1994. New taxonomy and niche partitioning on coral reefs: Jack of all trades or master of some? Trends in Ecology and Evolution 9:7–9.
Knowlton, N., and B. D. Keller. 1983. A new, sibling species of snapping shrimp associated with the Caribbean sea anemone *Bartholomea annulata*. Bulletin of Marine Science 33:353–362.
Knowlton, N., L. A. Weigt, L. A. Solorzano, D. K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the isthmus of Panama. Science 260:1629–1632.
Kohn, A. J. 1968. Microhabitats, abundance and food of *Conus* on atoll reefs in the Maldive and Chagos islands. Ecology 49:1046–1061.
Levene, H. 1953. Genetic equilibrium when more than one niche is available. American Naturalist 87:331–333.
Lynch, J. D. 1989. The Gauge of speciation. Pp. 527–553 *in* D. Otte, and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.
Maynard Smith, J. 1966. Sympatric speciation. American Naturalist 100:637–650.
Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.
———. 1963. Animal species and evolution. Harvard University Press, Cambridge, MA.
Nolan, B. A., and M. Salmon. 1970. The behavior and ecology of snapping shrimp (Crustacea: *Alpheus heterochelis* and *Alpheus normanni*). Forma et Functio 2:289–335.
Pawlik, J. R. 1993. Marine invertebrate chemical defenses. Chemical Reviews 93:1911–1922.
Price, P. W. 1980. Evolutionary biology of parasites. Princeton University Press, Princeton, NJ.
Rank, N. E. 1992. A hierarchical analysis of genetic differentiation in a montane leaf beetle *Chrysomela aenicollis* (Coleoptera: Chrysomelidae). Evolution 46:1097–1111.
Ribbink, A. J. 1991. Distribution and ecology of the cichlids of the African Great Lakes. Pp. 36–59 *in* M. H. A. Keenleyside, ed. Cichlid fishes. Behaviour, ecology and evolution. Chapman and Hall, London.
Rice, W. R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38:1251–1260.
———. 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evolutionary Ecology 1:301–314.
———. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.
Rice, W. R., and E. E. Hostert. 1993. Perspective: Laboratory experiments on speciation: What have we learned in forty years? Evolution 47:1637–1653.
Rosenzweig, M. L. 1978. Competitive speciation. Biological Journal of the Linnean Society 10:275–289.
Ruetzler, K. 1976. Ecology of Tunisian commercial sponges. Tethys 7:249–264.
Schein, H. 1977. The role of snapping in *Alpheus heterochaelis* Say, 1818, the big-clawed snapping shrimp. Crustaceana 33: 182–188.
Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.
Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Studies in Genetics IV. University of Texas Publication 7103:49–90.
Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 16:393–430.
Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Freeman, San Francisco, CA.
Stanhope, M. J. 1993. Molecular phylogeographic evidence for multiple shifts in habitat preference in the diversification of an amphipod species. Molecular Ecology 2:99–112.
Swofford, D. L., and R. K. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281–283.
Tauber, C. A., and M. J. Tauber. 1989. Sympatric speciation in insects: Perception and perspective. Pp. 307–344 *in*: D. Otte, and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.
Taylor, E. B., and P. Bentzen. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. Evolution 47:813–832.
Thomas, C. D., D. Ng, M. C. Singer, J. L. B. Mallet, C. Parmesan, and H. L. Billington. 1987. Incorporation of a European weed into the diet of a North American herbivore. Evolution 41:892–901.
Tilley, S. G., P. A. Verrell, and S. J. Arnold. 1990. Correspondence between sexual isolation and allozyme differentiation: A test in the salamander *Desmognathus ochrophaeus*. Proceedings of the National Academy of Sciences, USA 87:2715–2719.
Waring, G. L., W. G. Abrahamson, and D. J. Howard. 1990. Genetic differentiation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). Evolution 44: 1648–1655.
Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.
Wilson, D. S., and M. Turelli. 1986. Stable underdominance and the evolutionary invasion of empty niches. American Naturalist 127:835–850.
Wood, T. K., and S. I. Guttman. 1982. Ecological and behavioral basis for reproductive isolation in the sympatric *Enchenopa binotata* complex (Homoptera: Membracidae). Evolution 36:233–242.
Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 97–159.
———. 1978. Evolution and the genetics of populations, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

Corresponding Editor: T. Hilbish

APPENDIX 1

Allele frequencies in populations of *Synalpheus brooksi*, 1992. Populations are listed hierarchically by reef, host species, and population number.

	Guigalatupo				Tiantupo					
	Agelas		*Spheciospongia*		*Agelas*			*Spheciospongia*		
Locus / Allele	57	68	49	50	542	56	71	55	65	67
Tpi-2										
(*N*)	12	12	10	10	11	11	10	10	10	10
123	0.542	0.708	0.500	0.700	0.182	0.318	0.450	0.350	0.450	0.350
100	0.458	0.292	0.500	0.300	0.818	0.682	0.550	0.650	0.550	0.650
Idh-1										
(*N*)	12	10	10	10	11	11	10	8	10	10
100	0.958	0.900	0.900	1.000	0.909	0.818	0.400	0.375	0.750	0.750
85	0.042	0.100	0.100		0.091	0.182	0.600	0.625	0.250	0.250
Mpi										
(*N*)	11	10	10	10	10	10	10	8	10	10
104										0.050
100	1.000	0.900	0.850	0.950	0.750	0.650	0.850	1.000	0.950	0.950
96		0.100	0.150	0.050	0.250	0.350	0.150		0.050	
Aat										
(*N*)	4	10	4	10	6	10	10	4	10	10
121			0.125	0.100	0.167	0.100			0.050	0.200
100	1.000	1.000	0.875	0.900	0.833	0.900	1.000	1.000	0.950	0.800
96										
76										

Allele frequencies in populations of *Synalpheus brooksi*, 1993.

	Aguadargana							
	Agelas					*Spheciospongia*		
Locus / Allele	11	36	42	43	53	34	35	44
Tpi-2								
(*N*)	10	8	10	10	10	12	12	12
123	0.300	0.500	0.100	0.150	0.250	0.750	0.542	0.750
100	0.700	0.500	0.900	0.850	0.750	0.250	0.458	0.250
Idh-1								
(*N*)	10	8	10	10	10	12	12	11
100	0.900	0.688	1.000	0.800	0.950	0.833	0.833	0.773
85	0.100	0.313		0.200	0.050	0.167	0.167	0.227
Mpi								
(*N*)	10	8	10	10	10	12	12	12
104	0.050			0.100	0.050			
100	0.950	1.000	0.600	0.800	0.750	1.000	0.875	1.000
96			0.400	0.100	0.200		0.125	
Aat								
(*N*)	10	8	10	10	10	12	12	12
121						0.125	0.375	
100	1.000	1.000	1.000	0.900	1.000	0.875	0.625	1.000
96								
76				0.100				

	Guigalatupo									
	Agelas					*Spheciospongia*				
Locus / Allele	27	28	29	62	63	30	31	32	59	60
Tpi-2										
(*N*)	10	10	10	10	10	10	12	10	10	10
123	0.450	0.700	0.550	0.700	0.800	0.600	0.458	0.450	0.750	0.600
100	0.550	0.300	0.450	0.300	0.200	0.400	0.542	0.550	0.250	0.400
Idh-1										
(*N*)	7	10	7	5	10	7	7	7	10	10
100	1.000	1.000	1.000	1.000	1.000	0.714	0.857	1.000	1.000	0.850
85						0.286	0.143			0.150
Mpi										
(*N*)	7	5	7	5	10	7	7	7	10	5
104										
100	0.929	1.000	0.786	0.900	0.900	0.714	0.786	0.786	0.800	0.900
96	0.071		0.214	0.100	0.100	0.286	0.214	0.214	0.200	0.100

APPENDIX 1. Continued.

	Guigalatupo									
	Agelas					*Spheciospongia*				
Locus / Allele	27	28	29	62	63	30	31	32	59	60
Aat										
(*N*)	7	10	7	5	10	7	7	7	10	10
121	0.214	0.300		0.300	0.100	0.071	0.071	0.143	0.050	0.050
100	0.786	0.700	1.000	0.700	0.900	0.929	0.929	0.857	0.950	0.950
96										
76										

	Tiantupo									
	Agelas					*Spheciospongia*				
Locus / Allele	14	15	16	58	61	12	17	18	54	55
Tpi-2										
(*N*)	10	10	10	10	10	10	10	10	10	10
123	0.400	0.500	0.650	0.650	0.500	0.100	0.550	0.250	0.350	0.200
100	0.600	0.500	0.350	0.350	0.500	0.900	0.450	0.750	0.650	0.800
Idh-1										
(*N*)	10	10	10	10	10	10	10	10	9	10
100	0.700	0.650	0.950	0.850	0.850	0.450	0.250	0.650	0.500	0.400
85	0.300	0.350	0.050	0.150	0.150	0.550	0.750	0.350	0.500	0.600
Mpi										
(*N*)	9	10	10	10	7	10	10	10	10	10
104				0.050						
100	0.889	1.000	1.000	0.700	1.000	0.850	1.000	1.000	1.000	0.950
96	0.111			0.250		0.150				0.050
Aat										
(*N*)	10	10	10	10	10	10	10	10	10	10
121	0.100	0.100	0.050	0.050		0.200		0.350	0.150	0.150
100	0.900	0.900	0.900	0.950	1.000	0.800	1.000	0.650	0.850	0.850
96			0.050							
76										