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THE QUANTITATIVE GENETICS OF INCIPIENT SPECIATION: HERITABILITY AND GENETIC CORRELATIONS OF SKELETAL TRAITS IN POPULATIONS OF DIVERGING *FAVIA FRAGUM* ECOMORPHS

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Recent speciation events provide potential opportunities to understand the microevolution of reproductive isolation. We used a marker-based approach and a common garden to estimate the additive genetic variation in skeletal traits in a system of two ecomorphs within the coral species *Favia fragum*: a Tall ecomorph that is a seagrass specialist, and a Short ecomorph that is most abundant on coral reefs. Considering both ecomorphs, we found significant narrow-sense heritability (h^2) in a suite of measurements that define corallite architecture, and could partition additive and nonadditive variation for some traits. We found positive genetic correlations for homologous height and length measurements among different types of vertical plates (costosepta) within corallites, but negative correlations between height and length within, as well as between costosepta. Within ecomorphs, h^2 estimates were generally lower, compared to the combined ecomorph analysis. Marker-based estimates of h^2 were comparable to broad-sense heritability (H) obtained from parent–offspring regressions in a common garden for most traits, and similar genetic co-variance matrices for common garden and wild populations may indicate relatively small G × E interactions. The patterns of additive genetic variation in this system invite hypotheses of divergent selection or genetic drift as potential evolutionary drivers of reproductive isolation.

KEY WORDS: **Corallite, G matrix, genetic correlation, heritability, speciation**

Incipient species provide opportunity to understand the key evolutionary processes that drive reproductive isolation and phenotypic diversification. Systems of closely related but phenotypically differentiated populations with some form of partial reproductive isolation have been used to illuminate the role of natural and sexual selection in speciation (Schluter 2000; Rundle and Nosil 2005), the genetics of reproductive isolation (Coyne and Orr 2004), and to test models of sympatric speciation (Via 2001). Indeed, many of these systems are now familiar (e.g., three-spine sticklebacks *Gasterosteus*, monkeyflowers *Mimulus*, apple maggot flies *Rhagoletis*), because these models hold the potential to answer many different kinds of questions united by the complex process of speciation.

In a previous paper, Carlon and Budd (2002) described a pair of incipient species in the tropical coral *Favia fragum* associated

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with strong ecological gradients that appears to have evolved by an ecological model of speciation (sensu Rundle and Nosil 2005). Briefly, two distinct phenotypes (hereafter the "Tall" and "Short" ecomorphs) are partially isolated by adjacent seagrass and reef habitats; and had diverged in allele frequencies at five allozyme loci, to the point of near fixation at the *Pgm* locus. Moreover, the two ecomorphs remain phenotypically distinct within a narrow zone of ecological overlap, supporting the hypotheses: (1) some mechanism limits gene flow between ecomorphs within the putative hybrid zone, and (2) that morphological differences are under genetic control. Carlon and Lippé (2011) have recently addressed the dynamics of gene flow by showing that a highly selfing mating system ($s > 0.90$) strongly limits gene flow between ecomorphs, but have also shown evidence for hybridization between ecomorphs. In this article, we focus on the latter hypothesis by estimating the amount of quantitative genetic variation within natural populations for key skeletal features that distinguish the two ecomorphs.

An understanding of the quantitative genetics of incipient species is essential to answer a number of questions related to the microevolutionary process that leads to reproductive isolation. First, although genetic variation is parsed into two key quantities: additive and nonadditive components (the latter due to dominance effects and epistatic interactions), it is the additive variation that is visible to the processes of natural selection or genetic drift during diversification. Thus, if divergence in a putatively important ecological trait is the result of natural selection, the sum of the phenotypic variation must have an additive genetic basis. For the same reason, comparisons of the amount of additive genetic variation within and between populations can provide insight into the nature of microevolutionary processes in the past (Lande 1976). Lastly, genetic correlations among traits provide insight into genetic constraint and the possible directions of evolutionary change (Schluter 1996). Thus a full quantitative description of set of ecologically relevant traits would include the narrow-sense heritability values (formally h^2, the additive genetic variation divided by the total phenotypic variation), as well as the genetic correlations among traits. The **G** matrix provides a convenient summary of this information with h^2 estimates as diagonal elements and the genetic correlations as nondiagonal elements.

With regard to scleractinian corals, there have only been a few attempts to estimate these key quantitative parameters. Meyer et al. (2009) employed a half-sibling design to find significant additive and nonadditive genetic components of larval behavior and physiology in the Indo-Pacific coral *Acropora millepora*. A second recent study by Császár et al. (2010) used a clonal design in the same species to estimate broad-sense heritability (H, the sum of additive and nonadditive genetic variation divided by the total phenotypic variation) in a suite of thermal tolerance traits expressed by either the coral host, or their symbiotic dinoflagellates, or traits that result from host–symbiont interaction. These approaches have limited applications to other traits or for other coral species. Experimental crosses are possible for many ecologically important broadcasting species (eggs and sperm released into the water column during synchronized spawning events), however cultivating the resulting F1 long enough to measure juvenile and adult traits is technically challenging in species that typically take several years, or much more, to mature (Hughes et al. 1992). For other species that brood larvae after internal fertilization, experimental crosses are complicated by less-synchronized spawning patterns and in some cases limited outcrossing (Carlon 1999). On the other hand, clonal designs can be employed for many different physiological, morphological, and behavioral traits, but present difficulties in partitioning genetic variation into additive and nonadditive components (Lynch and Walsh 1998). A clearer understanding of quantitative genetic parameters in scleractinian corals demands alternative approaches.

A marker-based approach offers several advantages over more traditional breeding designs (Ritland 2000). First, it avoids once source of discrepancy between experimental estimates and the true parameters: the influence of genotype × environment interactions (G × E) resulting from the difference in environment between wild and experimental conditions. Second, the marker-based approach is particularly appropriate for nonmodel systems such as scleractinian corals, where life-history characteristics make either controlled crosses or long-term laboratory culture difficult, if not impossible. Third, with the full model it is possible to estimate both additive and nonadditive sources of genetic variation under natural conditions. This feature is particularly advantageous for species that regularly inbreed, or alternate between asexual and sexual generations, as nonadditive variation becomes an important evolutionary component of quantitative variation in these cases (Walsh 2005). Drawbacks of the method include the fact that it cannot be applied to population structures or sampling schemes that contain too little variation in relatedness (e.g., outbred vertebrates: Csillery et al. 2006). A second drawback is that statistical and genetic sampling variance in estimates of relatedness tends to bias h^2 downward compared to methods that employ high-quality pedigree information, even when suitable panels of markers are employed (Coltman 2005). Finally, covariance between relatedness and environments can potentially inflate h^2 estimates when phenotypic variation is caused by within-generation phenotypic plasticity or several mechanisms of nongenetic inheritance (sensu Bonduriansky and Day 2009). Nonetheless, the method has proven successful at contrasting h^2 among different kinds of quantitative traits, and in identifying significant genetic correlations between traits in terrestrial plants. These include a variety of morphological and phenological traits in the original application in *Mimulus guttatus* (Ritland and Ritland 1996), growth rate in conifers of the genus *Abies* (Ritland

and Travis 2004), chemical defenses in the genus *Eucalyptus* (Andrew et al. 2005), wood density in *Pinus radiata* (Kumar and Richardson 2005), and leaf morphology in *Prosopis alba* (Bessega et al. 2009).

Here, we apply the marker-based method to natural populations of the Tall and Short ecomorph of *F. fragum* in the Bocas del Toro of Panama. We have three primary objectives: (1) to test the hypothesis that there is significant additive genetic variation and co-variation among key skeletal traits that contribute to phenotypic differences between ecomorphs, (2) to compare h^2 in traits estimated from both ecomorphs to estimates from within ecomorphs, and (3) given that fine-scale genetic structure is correlated with environmental variation in this system, we partially control for environmental contributions by exploiting brooding larval development and an inbred mating system to culture full siblings in a common-garden experiment for one year. Both marker-based and common-garden methods reveal substantial and significant additive genetic variation and genetic correlations in suite of skeletal traits that define the two phenotypes, but less variation and fewer significant values within ecomorphs.

Methods

SAMPLE COLLECTION

The Tall and Short ecomorphs of *F. fragum* are ecologically partitioned between adjacent shallow seagrass and deep reef habitats in the Bahiá Almirante of the Bocas del Toro, Panama (Carlon and Budd 2002). At broader biogeographic scales within the Bocas del Toro region, populations of the Tall ecomorph are limited in distribution to within the Bahiá Almirante. In contrast, the Short ecomorph is distributed more broadly on exposed and protected shores around the outer islands (D. Carlon, unpubl. data). We sampled Tall and Short ecomorphs at a site we aptly named "STRI Point" because of its proximity to the Smithsonian Tropical Research Institute's (STRI) Bocas del Toro field station (Carlon and Lippé 2011). For the purposes of this article, we refer to STRI Point as the "lagoon site" because of its protected location inside the Bahiá Almirante. At this site, habitat at shallow depths (< 1 m) is dominated by turtle grass *Thalassia testudinum* (see Carruthers et al. 2005). Corals and other sessile invertebrates colonize fragments of coral rubble that lie on the substrata of this soft-bottom community. As one dives deeper, to depths of 3–4 m, the habitat transitions to patch reefs of *Montastraea* spp. and eventually a continuous reef system below ~ 6 m. In seagrass habitat, the Tall and Short ecomorphs can co-occur in sympatry, but the Tall ecomorph is more common here. In contrast, in reef habitats $>$ 3 m depth only the Short ecomorph is found. For comparative purposes, we also sampled a population of the Short ecomorph at an exposed reef site on the NE shore of Isla Colon (9° 22′417″N 82°14′244″W), which we refer to as the "exposed reef site." Only the Short ecomorph occurred at this site. Within each of these sites, we collected adult corals ($>$2.5 cm diameter) by swimming along haphazardly positioned line transects and collecting a single colony every 2–3 m. A small plug of tissue from each live colony was removed and preserved in 90% isopropyl alcohol. Entire colonies were soaked in a 50:50 Clorox bleach/fresh water solution overnight, rinsed several times with fresh water, dried, and shipped to the University of Iowa for morphometric analyses.

We made estimates of marker-based heritability from a total of five populations: (1) the combined samples of Tall and Short ecomorphs from the lagoon site, (2) Short ecomorph samples only from the lagoon site, (3) Tall ecomorphs only from the lagoon site, (4) Short ecomorphs from the exposed reef site, and (5) the juveniles from the common-garden experiment, described in the next section.

COMMON-GARDEN EXPERIMENT

To compare the heritability estimates between the marker-based method and parent–offspring regressions, we set up a common-garden experiment inside the lagoon. In *F. fragum*, gametogenesis is synchronized to lunar cycles, and brooded larvae are released over an approximately one-week period before full moons (Szmant-Froelich et al. 1985). In the laboratory, we noninvasively harvested larvae from known wild-caught parents and then settled them onto artificial substrata, which we then back-transplanted to seagrass habitat. Using the transect method described previously, we collected a total of 30 dams from the lagoon site, with 10 dams sampled from each of three ecomorph $\times$ habitat combinations: (1) Short ecomorph, reef habitat, (2) Short ecomorph, seagrass habitat, and (3) Tall ecomorph, seagrass habitat. All dams were collected 15 days prior to the September 7, 2006 full moon. Corals were maintained in a running seawater system designed to capture larvae from individual dams immediately after release (Jokiel and Richmond 1984). Larvae were released over three to five successive nights, and sibs were settled on numbered 2.5 $\times$ 2.5 cm $\times$ 4 mm acrylic plates that had been pre-conditioned in the field for several months. A total of 2093 larvae were settled on plates, at an average density of 20 individuals/plate (SD $=$ 8). Plates were then bolted to flat cinder blocks in 3 $\times$ 5 spatial arrays (15 plates/block). Each plate was elevated 5 cm off the cinder block, and randomly assigned to one of eight blocks. The majority of larvae settled on the cryptic surface of the plate (surface facing down) and this orientation was maintained on cinder blocks. Blocks were transported to a seagrass habitat north of the STRI Bocas del Toro station (9°22′368″N; 82°17′653″W), and positioned single-file along a 2-m depth contour $\sim$25 cm apart. Water flow was moderate at this site, and transplanted corals thrived there. The experiment was ended after 11 months, at which point blocks were retrieved. Plates with 11-month-old juveniles were bleached, rinsed, and shipped to the University of Iowa for

downstream morphometric analyses. Survivorship in the common garden was slightly higher in the Short Ecomorph (49%) compared to the Tall ecomorph (41%). Surviving corals provided sufficient replication to estimate within- and between-sibship variance, as well as ecomorph × habitat effects. Because we knew that the mating system of both ecomorphs approaches complete selfing (Carlon and Lippé 2011), we could infer the genotypes of larvae from the genotypes of adults (see Models and estimation) and therefore compare marker-based and parent–offspring estimates of heritability from the same phenotypes.

MOLECULAR MARKERS

We used 15 microsatellite (SSR) markers cloned from *F. fragum* in the Bocas del Toro (Carlon and Lippé 2008), and follow the DNA extraction and genotyping protocols as described in this paper. We used Microsatellite Analyzer (version 4.05 Dieringer and Schlotterer 2003) to calculate summary statistics for these loci, listed in Table 1. Genotyping patterns revealed clear evidence for null alleles at three loci. Specifically, single-locus PCR failure at *Ffr12*, *Ffr127*, and *Ffr25* at many samples in the exposed reef site suggests null alleles at high frequency. In contrast, although these same loci failed to amplify in some lagoon samples, the frequency of hypothesized nulls was much lower (Table 1). We can explain the differences in the frequencies of null alleles among populations by the fact that microsatellite libraries were developed from genomic DNA extracted from the lagoon population, and considerable small-scale population structure in this species (Carlon and Budd 2002). Given the low heterozygosity in the dataset, these null loci should minimally bias relatedness estimators via scoring errors (such as scoring null/+ as +/+). We retained all 15 loci to estimate relatedness in this article.

QUANTITATIVE TRAITS

We used three-dimensional (3D) landmark morphometrics to measure morphometric characters that define corallite architecture. Corallites are the system of skeletal elements and plates that support and protect the tissue of individual polyps, budded from a single founder to build a colonial colony. In *F. fragum*, corallite structure is defined by a series of radial vertical plates, termed costosepta, that repeat in cycles of four plate types (Cuif and Perrin 1999). Each of the four costoseptal plate types may have a unique size and shape (Appendix S1). We were specifically interested in phenotypic and genetic correlations among cycles, because it is the coordination of development and growth among plate types within a cycle that determine corallite shape, as well as other aspects of colony morphology. We used landmark methods and traditional morphometric measurements, but the former eliminate variation due to size differences alone (Bookstein 1991). This is a useful property for this application as we are interested in phenotypes of juvenile corals grown out in a common garden (<1 year old) and those of larger corals sampled from natural populations. Our landmark scheme is identical to that used in Carlon and Budd (2002), which was chosen to specifically characterize the shape of the septal margin (the uppermost growing edge) and the development of costal extensions of primary (1^o), secondary (2^o), and tertiary (3^o) costosepta. For photographs, figures, and descriptions of landmarks see Appendix S1.

The Cartesian coordinates (*x*-*y*-*z*) of landmarks were digitized in 3D using a standard motor-driven Reflex Microscope at the University of Iowa (serial # 050). Size and shape coordinates (Bookstein 1991; Zelditch et al. 2004) were calculated for the landmark data using the computer program Simple3D in the IMP software series (Integrated Morphometrics Package, 2004, written by H. David Sheets, available at http://www2.canisius.edu/~sheets/morphsoft.html). Centroid size was calculated by summing the squared distances from each of the 20 landmarks to a common centroid. Shape coordinates were calculated for triplets of points in which points 5 (0,0,0), 18 (1,0,0), and 11 (z = 0) served as the baseline plane, and we use these shape coordinates in our analyses of quantitative traits. In general, *z*-values are related to spacing between septa, *x*-values are related to costal, septal, and calice elevation, whereas *y*-values are related to costal and septal lengths. In addition to size and shape coordinates, we also used the landmark data to calculate six traditional skeletal traits (see Appendix S1 for landmark pairs and verbal descriptions). For these linear distances, distances between landmarks were calculated using the computer program WireMan6.exe in the IMP software series. For analyses of natural populations ($n = 130$ individual colonies) we measured three corallites from each colony, and colony averages were used in all statistical analyses. The common garden dataset consisted of smaller colonies (two to six corallites, $n = 264$ colonies), and only one or two corallites were measured. If two corallites could be measured, the average was used as the phenotypic measurement.

To extract complex phenotypic correlations among shape coordinates, we conducted multivariate analyses on the landmark data. Preliminary statistical analyses indicated relatively low variance in *z*-values (spacing of costosepta), so we therefore performed canonical variates analyses on each dataset using a total of 31 variables consisting of *x*- and *y*-values for all landmarks (except 6 and 17) and centroid size. In the canonical variates analyses of natural populations, a priori groups were defined by: (A) three ecomorph × habitat combinations [(1) Short ecomorph, reef habitat; (2) Short ecomorph, seagrass habitat; (3) Tall ecomorph, seagrass habitat] and (B) four ecomorph × habitat × site combinations [(1) Short ecomorph, reef habitat, exposed site; (2) Short ecomorph, reef habitat, lagoon site; (3) Short ecomorph, seagrass habitat, lagoon site; (4) Tall ecomorph, seagrass habitat, lagoon site]. In the canonical variates analyses involving the common garden dataset, a priori groups were defined by three groups as in (A) above and the 22 individual parent colonies.

Table 1. **Summary statistics for 15 microsatellite loci in five populations.**

Locus		*D3*	*Ffr9*	*A12*	*B11*	*B12*	*D10*	*D7*	*Ffr12*	*Ffr31*	*Ffr127*	*Ffr25*	*Ffr53*	*Ffr83*	*Ffr111*	*Ffr68*	Mean	min	max
Exposed reef	*N*	35	31	35	34	35	35	33	4	35	19	7	35	35	35	33	29.4	4	35
	A	4	3	4	3	3	2	7	3	3	2	4	5	3	4	3	3.5	2	7
	H_o	0.029	0.065	0.057	0.000	0.029	0.000	0.091	0.000	0.029	0.053	0.000	0.029	0.029	0.000	0.000	0.027	0.000	0.091
	H_e	0.586	0.283	0.646	0.304	0.312	0.056	0.664	0.714	0.084	0.371	0.747	0.518	0.233	0.389	0.386	0.420	0.056	0.747
	F	0.951	0.772	0.911	1.000	0.908	1.000	0.863	1.000	0.659	0.858	1.000	0.945	0.877	1.000	1.000	0.916	0.659	1.000
Lagoon	*N*	115	114	115	115	114	114	114	104	114	92	115	115	107	115	69	108.8	69	115
	A	9	7	9	6	4	6	10	11	23	3	3	7	10	6	6	8.0	3	23
	H_o	0.061	0.035	0.035	0.026	0.026	0.026	0.035	0.010	0.079	0.022	0.009	0.035	0.019	0.017	0.000	0.029	0.000	0.079
	H_e	0.746	0.688	0.773	0.671	0.611	0.719	0.775	0.860	0.855	0.178	0.026	0.804	0.734	0.633	0.564	0.642	0.026	0.860
	F	0.918	0.949	0.955	0.961	0.957	0.963	0.955	0.989	0.908	0.878	0.664	0.957	0.975	0.973	1.000	0.933	0.664	1.000
Lagoon (Short ecomorph)	*N*	67	66	67	67	66	66	66	60	66	51	67	67	67	67	52	64.1	51	67
	A	5	5	7	5	4	6	8	7	17	2	2	6	10	6	5	6.3	2	17
	H_o	0.104	0.061	0.060	0.045	0.045	0.045	0.045	0.017	0.106	0.020	0.015	0.060	0.030	0.030	0.000	0.046	0.000	0.106
	H_e	0.613	0.272	0.678	0.547	0.623	0.560	0.749	0.774	0.886	0.281	0.015	0.760	0.750	0.531	0.395	0.562	0.015	0.886
	F	0.829	0.777	0.912	0.918	0.927	0.919	0.939	0.978	0.880	0.930	−0.004	0.921	0.960	0.944	1.000	0.855	−0.004	1.000
Lagoon (Tall ecomorph)	*N*	48	48	48	48	48	48	48	44	48	41	48	48	40	48	17	44.6	17	48
	A	7	6	4	3	2	4	5	5	11	2	2	5	3	3	4	4.4	2	11
	H_o	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.042	0.024	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.042
	H_e	0.702	0.764	0.627	0.341	0.449	0.554	0.737	0.652	0.652	0.024	0.041	0.727	0.647	0.476	0.699	0.540	0.024	0.737
	F	1.000	1.000	1.000	1.000	1.000	1.000	0.972	1.000	0.936	−0.006	1.000	1.000	1.000	1.000	1.000	0.927	−0.006	1.000
Lagoon (common garden)	*N*	27	27	27	27	27	27	27	24	27	25	27	27	27	27	12	25.6	12	27
	A	5	6	5	3	3	5	7	7	9	2	3	6	6	5	2	4.9	2	9
	H_o	0.111	0.037	0.037	0.000	0.037	0.037	0.074	0.000	0.074	0.000	0.037	0.074	0.037	0.037	0.000	0.040	0.000	0.074
	H_e	0.670	0.718	0.734	0.668	0.532	0.683	0.772	0.762	0.730	0.078	0.174	0.766	0.533	0.385	0.159	0.558	0.078	0.772
	F	0.834	0.948	0.950	1.000	0.930	0.946	0.904	1.000	0.898	1.000	0.787	0.903	0.930	0.904	1.000	0.929	0.787	1.000

N = No. of genotyped individuals, A = No. of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, F = inbreeding coefficient.

MODELS AND ESTIMATION

Marker-based heritability

The marker-based approach developed by Ritland (1996) measures heritability and genetic correlations in natural populations by estimating relatedness among un-manipulated individuals with molecular markers and exploiting the linear relationship between relatedness and phenotypic similarity. Because standard least squares regression requires that the predictor variable (in this case relatedness) be known without error, a key innovation of Ritland's method was to develop an estimator for the actual variance of relatedness within populations. We used a derivation of Ritland's full multiregression model, which permits the decomposition of phenotypic variance into additive, nonadditive, and shared inbreeding components

$$Z_{ij} = h^2 r_{ij} + \left(H - h^2\right) \Delta_{ij} + b_f^2 f_{2ij} + a_e + e_{ij}.$$

In this case, the dependent variable for individuals i and j is Z_{ij}, the phenotypic covariance. The explanatory variables for individuals i and j are: r_{ij}, two-gene relatedness; Δ_{ij} four-gene relatedness (both defined in Lynch and Ritland 1999); and f_{2ij}, a measure of shared inbreeding co-efficients (defined by Ritland and Ritland 1996). This model estimates the parameters: h^2, narrow-sense heritability; H, broad-sense heritability; b_f^2, inbreeding depression; and a_e the intercept. Although Ritland included an additional parameter (b_e) to measure the effect of phenotypic similarity due to shared environments, we did not map positions of corals in field populations, and thus cannot estimate this parameter here. In the case of the common garden data, we have largely eliminated this source of phenotypic similarity, as the experiment was designed to minimize environmental variation. We used the matrix formulation of the model above to estimate heritability, inbreeding, and genetic correlations (Ritland 1996). Genetic covariance and correlation among traits were estimated from this matrix approach, and the significance of the sign of covariance was tested using a bootstrap percentile test. Since Ritland's initial paper, more precise estimators of relatedness and actual variance have coevolved with applications of SSR markers (Lynch and Ritland 1999; Wang 2002; 2007). For our microsatellite dataset, we used Lynch and Ritland's (1999) estimates of r_{ij} and Δ_{ij}, specifically designed for the high allelic diversity of these markers. These were originally formulated as asymmetrical relationship estimators that calculate a probability for one individual in the pair using the other individual as a reference (proband). Symmetric estimators are inherently preferable, as they use more of the available information. The ideal approach of averaging the two multilocus estimates (Lynch and Ritland 1999) was not available to us because of the need for locus-specific weights and estimates. Instead, we obtained symmetric locus-specific estimates and weights by averaging the asymmetric versions for each pair (Andrew et al. 2005), but these estimates were less stable than the asymmetric estimators for the *F. fragum* data. Consequently, we used the asymmetric estimators for all analyses and randomized the order of the samples to avoid introducing bias. We used a modified bootstrap across individuals to estimate sampling error, and test the hypotheses that model parameters were >0, and note these are conservative tests, because bootstrapping individuals tends to overestimate sampling error by 10–100% (Thomas et al. 2002). We excluded any comparisons between identical individuals in this procedure. All parameter estimates, genetic correlations, and bootstrapping were done with a modified version of K. Ritland's program MARK (version 2.1, available at: http://genetics.forestry.ubc.ca/ritland/programs.html). Unlike MARK, the modified code can estimate the full model of Ritland (1996) and can carry out additional bootstrapping and permutation tests. A Fortran95 program is available from R. Andrew by request. To protect against excessive false positives in large tables of tests, we used the Q-statistic, a measure of the false discovery rate (FDR)(Storey et al. 2003). Given α, a Q-value measures the probability that a significant value is a false positive. We used the software QVALUE (http://www.genomics.princeton.edu/storeylab) to calculate these statistics.

In addition to natural populations, we also estimated heritability in the common-garden experiment based on wild-caught parents from the lagoon site. In this case, we did not genotype offspring, but inferred offspring genotypes from parents under the assumption that $s = 1.0$. Because 14% of sampled parents were heterozygous at one or more loci, our approach introduces error in predicting offspring genotypes from adult genotypes in cases where adults were heterozygous. Assuming linkage equilibrium among loci, the probability that the offspring genotype will differ from the parent genotype is equal to the product of the number of heterozygous loci and 0.5, because the fraction of offspring homozygous for either allele will be 0.5. Thus, the genotypes of half of the offspring of a parent that is heterozygous at a single locus will be incorrect. To determine the effects of incorrectly predicting offspring genotypes from parental genotypes on parameter estimates obtained from the marker-based approach, we ran an additional model in which we excluded all sibs with heterozygous parents. This reduced the dataset from 264 to 204 juveniles.

The Lynch and Ritland estimator of relatedness has been shown to underestimate relatedness in inbred mating systems compared to a maximum-likelihood estimator (MLE) based on triadic relationships (Wang 2007). However, the actual variance in relatedness required for the marker-based approach has not been derived for these MLE estimators. To measure potential bias in the Lynch and Ritland estimator, we simulated datasets of microsatellite genotypes under a selfing rate (s) of 0.90 using the program CoAncestry (written by Jinliang Wang). We created 100 genotype pairs for nine classes of relatedness and compared the

performance of estimated relatedness to actual relatedness. We used allele frequencies from the combined Tall and Short ecomorph samples from the Lagoon site, and calculated the nine IBD coefficients for different relatives by considering selfing and outcrossing jointly. For example, considering the case for full-siblings, the IBD coefficients $[\Delta_1 - \Delta_9]$ for two selfed offspring from the same parent are: [1/8, 1/8, 1/4, 0, 1/4, 0, 1/4, 0, 0]. For two outbred full-siblings, the IBD coefficients are: [0, 0, 0, 0, 0, 0, 1/4, 1/2, 1/4]. Therefore for a selfing rate of s and no biparental inbreeding, two full-siblings on average have IBD coefficients

$$s[1/8, 1/8, 1/4, 0, 1/4, 0, 1/4, 0, 0] + (1 - s)[0, 0, 0, 0, 0, 0, 1/4, 1/2, 1/4].$$

Plots and regressions of actual relatedness versus marker-estimated relatedness were used to assess patterns and magnitude of bias.

Parent–offspring regressions

We used two methods to estimate heritability from the common-garden data, independent of the marker approach. The first was a linear regression of the offspring phenotypes grown in the common garden on their wild-caught parent phenotype. Because all offspring were assumed to result from selfing, we used the regression coefficient (b_{op}) as an estimate of broad-sense heritability (H). Second, we used the Riska estimator (Riska et al. 1989) equivalent to $b^2_{OP}(\frac{V_{PN}}{V_{GL}})$, where b_{op} is the regression coefficient as above, V_{PN} is the phenotypic variance in the field, and V_{GL} is genetic variance in the common garden. In this case, we estimated V_{GL} as the between-family variance in a single factor ANOVA, assuming that families represent inbred lines. This analysis was first performed on three datasets: each ecomorph treated separately, the ecomorphs combined without regard for morph identity. A further analysis based on the combined dataset, but estimating the slopes and variance components within ecomorphs only, provided an estimate of average within-ecomorph heritability. We tested for a difference in regression slopes between ecomorphs using a likelihood-ratio test comparing the model containing the morph × parental phenotype term with the model omitting this interaction.

Variance component based estimation of H

The *R* package nlme was used for estimation of variance components. We again assumed that families in the common-garden represent inbred lines and estimated V_G, the variance due to genetic effects, as the variance among families in a linear model with random-effects only. We used the linear model: $Y_{ij} = \mu + F_i + e_{ij,}$ where Y is the trait value, μ is the average trait value, F_i is the family-specific random effect, and e_{ij} is the individual-specific error. Heritability was estimated as $H = (\frac{V_G}{V_P})$, where V_P is the total phenotypic variance in the common garden, which is the sum of the residual variance and the family variance. This analysis was performed on all data, the Short ecomorphs samples only, and the Tall ecomorph samples only. Individuals were bootstrapped (with replacements) 5000 times to obtain standard errors and confidence limits for H.

Results

PHENOTYPIC PATTERNS

The amount of phenotypic variance differed among populations (Table 2). Levels of phenotypic variance, ranked by coefficients of variation (CVs), were as follows: Tall ecomorph, lagoon > Short ecomorph, lagoon ∼ Short ecomorph, exposed reef. Not surprisingly, the phenotypic variance of the combined samples of Tall and Short ecomorphs from the lagoon was considerably larger than any population of one ecomorph type. The amount of phenotypic variation expressed in the common-garden experiment was similar to that sampled for wild-caught adults. There was considerable phenotypic covariance among individual measurements. Multivariate analyses of phenotypic variation in the adult datasets revealed differentiation among three multivariate axes (Fig. 1). Canonical variate 1 explained 69.4% of the variation and is strongly correlated with costa height (shape coordinates x01, x02, x03, x09, x10, x13, x14, x15). This axis clearly separates Tall and Short ecomorphs. Canonical variate 2 explained 26.3% of the variation and is strongly correlated with septal length (shape coordinates y07, y19). This axis separates Short ecomorph populations collected from reef environments from the same ecomorph collected from seagrass. Canonical variate 3 explained 4.3% of the variation and is strongly correlated with overall size (csize).

MARKER-BASED HERITABILITY AND GENETIC CORRELATIONS IN NATURAL POPULATIONS

Average two-gene relatedness was negative or near 0.0, but four-gene relatedness was >0.0 in all natural study populations and the common garden (Table 3). There was significant actual variance in two-gene relatedness, four-gene relatedness, and shared inbreeding in all populations except the exposed reef population, where the variance in shared inbreeding was not significant. Although we measured a total of 38 quantitative characters, we present marker-based results for a subset of 13 linear measurements and shape coordinates. Characters included in this subset had high canonical variate loadings on the three major multivariate axes and therefore explain much of the phenotypic variation in this system (see Fig. 1). Further, we included homologous measurements across the three major plate types of the septal cycle to estimate genetic correlations across septal plate types. The complete analysis is available from the lead author by request.

Table 2. The mean, standard error (SE), and coefficient of variation (CV) for 13 corallite characters across five datasets analyzed in this article. 1°, 2°, and 3° refer to the primary, secondary, and tertiary costoseptal plates. See Appendix S1 for additional character descriptions. The first seven characters are linear measurements, all listed in millimeters except the volume centroid size (mm^3). The last six characters are Bookstein shape coordinates and are dimensionless.

		Lagoon-combined			Lagoon-Tall			Lagoon-Short			Exposed reef			Common garden		
Character	Description	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV
csize	centroid size	3.698	0.074	0.193	4.134	0.122	0.189	3.340	0.052	0.110	2.454	0.037	0.090	2.928	0.032	0.175
d3_4	1° costa length	1.242	0.063	0.486	1.755	0.078	0.284	0.821	0.034	0.296	0.455	0.025	0.329	0.946	0.023	0.397
d8_7	1° calice depth	0.963	0.026	0.259	1.101	0.038	0.223	0.851	0.027	0.229	0.767	0.021	0.162	0.877	0.017	0.309
d10_11	3° costa length	0.959	0.049	0.486	1.311	0.072	0.352	0.670	0.025	0.264	0.344	0.013	0.232	0.582	0.017	0.469
d5_7	1° septum length	1.064	0.019	0.170	0.998	0.027	0.171	1.119	0.025	0.157	0.817	0.016	0.118	0.703	0.010	0.233
d5_4	1° costoseptum height	0.672	0.018	0.256	0.793	0.021	0.172	0.574	0.018	0.224	0.570	0.016	0.169	0.702	0.014	0.325
d4_11	septal spacing	0.498	0.010	0.191	0.536	0.016	0.187	0.467	0.011	0.167	0.439	0.012	0.165	0.490	0.009	0.299
x04	1° costoseptum height	0.560	0.019	0.329	0.714	0.020	0.183	0.434	0.015	0.249	0.462	0.015	0.193	0.627	0.014	0.360
x16	2° costoseptum height	0.539	0.019	0.340	0.699	0.020	0.182	0.408	0.013	0.231	0.439	0.017	0.236	0.596	0.013	0.366
x11	3° costoseptum height	0.386	0.009	0.209	0.417	0.012	0.188	0.361	0.011	0.211	0.345	0.015	0.263	0.472	0.010	0.329
y02	1° costa length	0.792	0.034	0.408	1.042	0.041	0.251	0.588	0.028	0.340	0.293	0.019	0.391	0.619	0.015	0.386
y14	2° costa length	0.745	0.032	0.410	0.986	0.039	0.256	0.548	0.025	0.316	0.296	0.019	0.382	0.587	0.014	0.389
y10	3° costa length	0.697	0.031	0.426	0.905	0.045	0.319	0.526	0.023	0.308	0.271	0.014	0.302	0.484	0.013	0.424

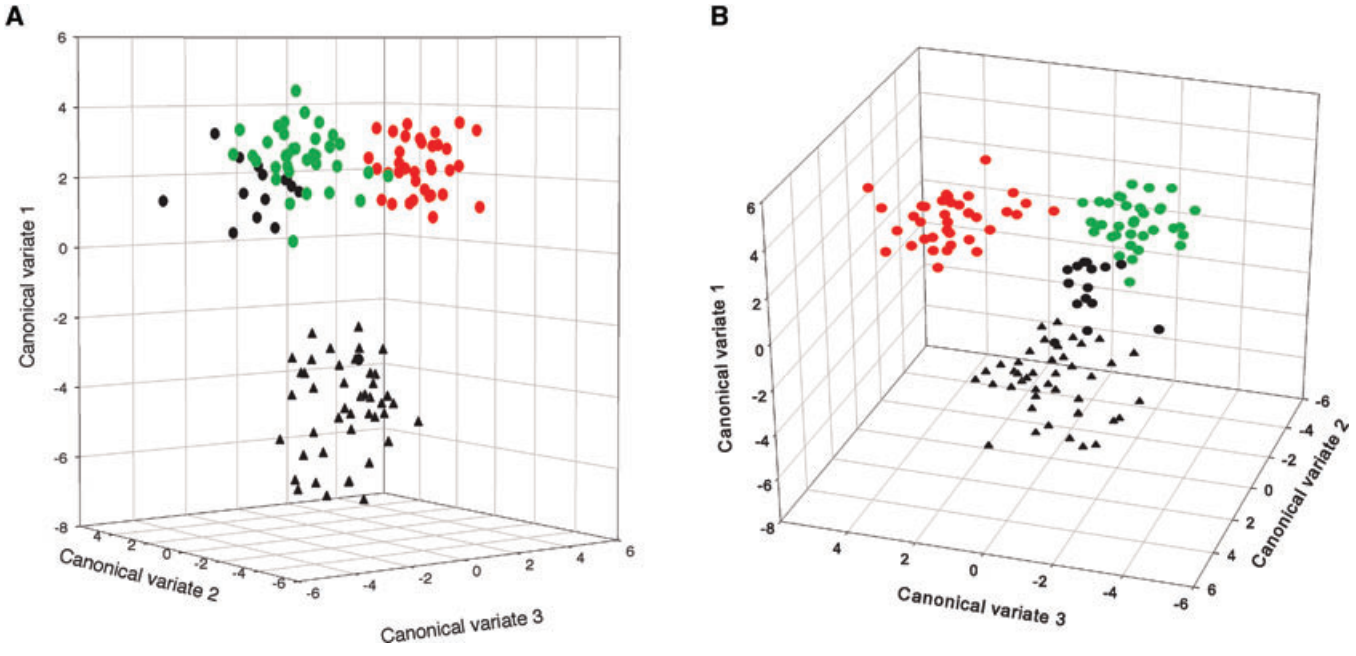

Figure 1. Phenotypic variation in natural populations plotted as scores along three major canonical variates (CVs). (A) Perspective emphasizing variation in CV 1. (B) Perspective emphasizing variation in CVs 1 and 3. Red circles = Short ecomorph, reef habitat, lagoon; black circles = Short ecomorph, seagrass habitat, lagoon; green circles = Short ecomorph, exposed reef; black triangles = Tall ecomorph, seagrass, lagoon. Canonical variate 1 explained 69.4% of the variation and is strongly correlated with costa height (shape coordinates x01, x02, x03, x09, x10, x13, x14, x15). Canonical variate 2 explained 26.3% of the variation and is strongly correlated with septal length (shape coordinates y07, y19). Canonical variate 3 explained 4.3% of the variation and is strongly correlated with overall size (csize).

In general, we found significant h^2 for at least one of the traits in all natural populations, except for the exposed reef population (Table 4). Although nonadditive variance ($H - h^2$) was positive for a number of traits, standard errors tended to be large so that significant nonadditive variation was detected in only a single trait (1° costoseptum height) in the lagoon-combined sample and in the lagoon-Tall ecomorph sample. We did not find significant variation attributable to inbreeding (b^2) in any of the populations. The proportion of additive variance was significantly >0 for all traits except septal spacing in the analysis of both ecomorphs in the lagoon. Estimates of h^2 for linear dimensions ranged from –0.13 for septal spacing to 1.10 for 1° calice depth. Also in both ecomorphs, h^2 for traits based on shape coordinates related to costa length were all >0.50 and in fact the three shape coordinates related to costa length were all ≥1.0. Within single ecomorphs, there were fewer significant h^2 values, and their magnitudes were

Table 3. Average two-gene relatedness (*r*), four-gene relatedness (Δ), shared level of inbreeding (f_2), and estimated inbreeding coefficient of two individuals (*f*). Actual variances within populations are listed in the last three rows. Standard error is given in parenthesis, and asterisks denote significance of *P* values determined by bootstrapping.

	Lagoon-combined		Lagoon-Tall		Lagoon-Short		Exposed reef		Common garden	
Coefficient										
r	−0.015**	(0.005)	−0.033**	(0.016)	0.007	(0.017)	−0.031**	(0.017)	0.027*	(0.004)
Δ	0.131**	(0.022)	0.302**	(0.083)	0.145**	(0.085)	0.213**	(0.064)	0.212**	(0.021)
f_2	−0.002*	(0.004)	−0.026**	(0.010)	−0.001*	(0.011)	0.019	(0.033)	−0.002**	(0.001)
f	0.826**	(0.039)	0.923**	(0.025)	0.747**	(0.077)	1.061**	(0.151)	0.696**	(0.009)
Actual variance										
V(r)	0.076**	(0.016)	0.198**	(0.070)	0.112**	(0.069)	0.047**	(0.023)	0.143**	(0.021)
V(Δ)	0.054**	(0.009)	0.100**	(0.048)	0.071**	(0.051)	0.139**	(0.076)	0.097**	(0.007)
V(f_2)	0.304**	(0.200)	1.006**	(0.541)	0.250**	(0.275)	0.586	(0.876)	0.168**	(0.172)

*$P < 0.05$, **$P < 0.01$.

Table 4. Marker-based estimates of narrow-sense heritability h^2, Nonadditive variation $H - h^2$, and inbreeding b^2 for 13 corallite characters in four different populations. Shaded characters are linear measurements, nonshaded are Bookstein shape coordinates. Standard errors SE determined by bootstrapping, and asterisks indicate significance of *P* values,. See Appendix S4 for *Q*-values.

	Lagoon-combined						Lagoon-Short						Lagoon-Tall					
Character	h^2	SE	$H - h^2$	SE	b^2	SE	h^2	SE	$H - h^2$	SE	b^2	SE	h^2	SE	$H - h^2$	SE	b^2	SE
centroid size	0.80***	0.25	−0.06	0.17	−0.02	0.07	0.05	0.17	0.19	0.16	−0.03	0.09	0.30	0.22	−0.09	0.16	−0.01	0.17
1^0 costa length	0.22**	0.26	0.24	0.19	0.19	0.13	0.00*	0.23	0.49	0.16	0.15	0.25	0.21	0.21	−0.08	0.17	0.09	0.16
1^0 calice depth	1.10***	0.30	−0.24	0.19	−0.07	0.07	−0.04	0.14	0.13	0.15	−0.01	0.09	0.43*	0.25	−0.22	0.16	−0.05	0.14
3^0 costa length	0.07*	0.15	0.12	0.12	−0.02	0.05	−0.02**	0.23	0.58	0.16	−0.06	0.11	0.06	0.14	−0.09	0.16	0.11	0.17
1^0 septum length	0.09**	0.34	0.64	0.40	0.17	0.14	0.83***	0.25	0.45	0.24	0.15	0.24	0.16	0.17	−0.13	0.54	−0.06	0.19
1^0 costoseptum height	0.07***	0.01	0.04***	0.01	−0.01	0.01	0.07***	0.04	0.06**	0.02	−0.01	0.03	0.04	0.04	0.02	0.02	−0.05	0.04
septal spacing	−0.13	0.23	0.51	0.19	0.14	0.11	0.54***	0.29	0.54	0.26	0.17	0.20	0.00	0.14	−0.04	0.12	−0.02	0.11
1^0 costoseptum height	0.57***	0.34	0.13	0.23	0.04	0.09	0.32**	0.26	0.54	0.22	0.17	0.19	0.02	0.16	0.00	0.12	−0.04	0.07
2^0 costoseptum height	0.75***	0.32	0.03	0.23	0.00	0.10	−0.01**	0.26	0.85	0.22	0.31	0.30	0.12	0.18	−0.02	0.13	−0.06	0.14
3^0 costoseptum height	0.46*	0.25	−0.14	0.13	−0.03	0.04	0.13	0.24	0.12	0.18	−0.05	0.11	−0.05	0.14	0.04	0.13	0.00	0.10
1^0 costa length	1.24***	0.23	−0.59	0.19	−0.08	0.07	0.22	0.15	−0.02	0.15	−0.07	0.11	0.02	0.13	−0.07	0.12	−0.01	0.11
2^0 costa length	1.17***	0.25	−0.50	0.19	−0.07	0.09	0.06	0.14	−0.03	0.14	−0.05	0.09	0.08	0.15	−0.11	0.12	−0.05	0.18
3^0 costa length	1.00***	0.22	−0.42	0.17	−0.08	0.08	0.17	0.17	0.03	0.17	−0.04	0.09	0.13	0.17	−0.14	0.13	−0.05	0.20

	Exposed reef- Short ecomorph					
Character	h^2	SE	$H - h^2$	SE	b^2	SE
centroid size	0.35	9.33	0.07	0.78	−0.08	31.40
1^0 costa length	0.21	3.07	0.04	0.29	−0.13	6.65
1^0 calice depth	0.18	5.22	0.02	0.45	−0.06	31.15
3^0 costa length	−0.23	9.52	−0.03	0.77	−0.06	8.93
1^0 septum length	0.37	4.67	−0.04	0.76	0.05	2.30
1^0 costoseptum height	0.02	0.02	0.00	0.02	−0.02	0.02
septal spacing	−0.01	2.80	−0.04	0.24	−0.07	1.46
1^0 costoseptum height	−0.16	7.97	−0.06	0.64	0.04	3.55
2^0 costoseptum height	0.13	5.28	−0.07	0.45	−0.04	20.49
3^0 costoseptum height	0.20	5.10	−0.08	0.43	−0.04	18.95
1^0 costa length	−0.04	5.31	0.00	0.44	0.01	1.71
2^0 costa length	0.27	2.99	0.00	0.28	−0.07	5.80
3^0 costa length	0.06	5.59	0.07	0.40	−0.03	26.21

*$P < 0.10$, **$P < 0.05$, ***$P < 0.01$.

lower, compared to the combined ecomorph estimates. This pattern was more extreme in the Tall ecomorph compared to the Short ecomorph, in the former significant h^2 was found for only a single trait (1° calice depth, $h^2 = 0.43$).

For the combined ecomorph analysis, we found significant positive and negative genetic correlations among traits (Table 5). The structure of positive and negative correlations indicated coordinated development and growth among the three septal cycles (Fig. 2). For example, homologous *x* or *y* values (Bookstein coordinates) were strongly positively correlated across the three types of costoseptal plates, whereas *x* and *y* values were strongly negatively correlated both within plate types, as well as among plate types. This pattern indicates a trade-off between the height and width of plates. Linear distances do not always show this trade-off, for example 1° costa height is positively correlated with 1° costoseptum length, but because these characters are derived from landmarks, they include the effects of changes in the *x* and *y* dimensions, and should therefore reflect interactions between these two spatial dimensions. The structure of the genetic correlation matrix described above remained constant if only the Tall ecomorphs were analyzed, but some correlations reversed sign in the sample of Short ecomorphs (**G** matrices in Appendix S2).

COMMON-GARDEN EXPERIMENT

The ranking of mean trait values estimated for wild Tall and Short ecomorphs were generally similar to those of the common garden (ANOVAs and means in Appendix S3). For linear measurements, the differences among means were generally smaller in the common garden compared to the wild. In the common garden, a mixed-model ANOVA with population (ecomorph, habitat) as a fixed factor and family as a random factor, generally found differences between the Tall and Short ecomorphs. On the other hand, in three unique traits (S1 septum length [d5_7], S1 costoseptum height [d5_4 and x04], septal spacing [d4_11]) there were significant differences between the Short ecomorph living on the reef and a group that included both Short and Tall ecomorphs living in seagrass. Thus, the Short ecomorph, seagrass population is expressing some differences from the Short ecomorph, reef population in the common-garden experiment.

Although absolute values were generally lower, marker-based h^2 in the common-garden experiment were significant for nearly all characters (Table 6). As in the natural populations, we detected nonadditive variance for 1° costoseptum height. Removing offspring from heterozygous parents had a small effect on marker-based h^2 estimates in the common garden, by reducing estimates slightly, however it had no effect on patterns of significance (results not shown). Estimates based on variance components, parent–offspring regressions, and the Riska method were comparable to the marker-derived estimates in the common garden (Table 6) and the pattern of more quantitative variation in the total dataset compared to within individual ecomorphs was evident in these estimates as well. With the exception of one trait (1° septum length), there were positive associations between estimates derived from parent–offspring regression and both variance components and molecular markers in the common garden (Appendix S5). The h^2 estimate from wild populations showed no real pattern with the variance-based common-garden estimates, however the nonadditive estimate ($H - h^2$) showed a stronger association with other estimates.

We could detect a strong phenotypic effect of microsatellite genotype in the common-garden experiment. Among the six Short ecomorph dams collected from seagrass habitat, we sampled only two unique 15 locus microsatellite genotypes, which differed at six of 15 loci (*Ffr9*, *B12*, *D10*, *Ffr12*, *Ffr31*, and *Ffr83*). We refer to these genotypes as A and B. The offspring of the single dam with genotype A were clearly separated in multivariate phenotype space from all the offspring of five dams with genotype B (Fig. 3). Phenotypic differences between these two genotypes were mainly expressed along CV 2, which in this case was most strongly associated with costal tooth elevation (shape coordinates x03 and x15) and length of costosepta (y2, y10, and y13). Scanning electron micrographs of offspring cultured in the common garden illustrate the major differences in skeletal architecture (Fig. 4). All the phenotypes have a paddle-shaped costoseptal dentition that is diagnostic of Atlantic faviids, but the height of the costosepta varies considerably across ecomorphs. Costosepta are low in offspring from Short ecomorph, reef environment dams (Sr); intermediate in offspring from Short ecomorph, seagrass environment dams (Ss); and high in offspring from Tall ecomorph dams (T). Additionally, calices extend deeper into the corallum in T offspring, than in either Sr or Ss offspring. Septal cycles are equal in height in Sr and Ss offspring, but tertiary septa are low relative to primary and secondary septa in T offspring. Finally, at even finer scales, the costoseptal teeth are low in Sr offspring, intermediate in Ss offspring, and high in T offspring.

BIAS IN MARKER-BASED RELATEDNESS

Assuming a mixed mating system of s = 0.90, and the panel of SSR markers employed here, our simulation shows that bias in the Lynch and Ritland estimator increases with actual relatedness (Fig. 5A). On the other hand, Wang's maximum likelihood estimators, that were developed specifically to accommodate inbreeding, show an opposite trend in bias: increasing positive bias as relatedness decreases (Fig. 5B).

Discussion

With the marker-based method, we have found considerable amounts of quantitative genetic variation in morphological traits segregating between ecomorphs of *F. fragum*. Indeed, narrow-sense heritability (h^2) estimates consistently exceeded 0.50 for

Table 5. Marker-based genetic correlations for the lagoon- combined dataset among 11 corallite traits. Shaded characters are linear measurements, nonshaded are Bookstein shape coordinates. Significance of the sign of correlations determined by bootstrapping, asterisks indicate *P* values,. The corresponding *Q*-values = 0.08, 0.03, and 0.01. Empty cell results from correlation that could not be estimated from regression model. See Figure 2 for a graphical depiction of positive and negative correlations among Bookstein height and length measurements, and Appendix S2 for correlation matrices for other populations.

	centroid size	1^0 costa length	1^0 calice depth	3^0 costa length	1^0 septum length	1^0 costoseptum height	1^0 costoseptum height	2^0 costoseptum height	3^0 costoseptum height	1^0costa length	2^0 costa length
centroid size											
1^0 costa length	1.01***										
1^0 calice depth	1.20***	1.10***									
3^0 costa length	1.00***	1.00***	1.15***								
1^0 septum length	−0.97	−0.96*	−1.11**	−0.92							
1^0 costoseptum height	1.60***	1.31***	1.21***	1.47**	−1.10**						
1^0 costoseptum height	1.12***	1.05***	1.03***	1.09***	−1.01**	1.08***					
2^0 costoseptum height	1.08***	1.04***	1.03***	1.06***	−0.96**	1.09***	1.01***				
3^0 costoseptum height	1.08***	1.15***	1.29**	1.10***	−1.30	1.69*	1.23***	1.22***			
1^0 costa length	−0.97***	−1.00***	−1.09***	−0.98***	1.02	−1.33**	−1.07***	−1.06***	−1.14***		
2^0 costa length	−0.97***	−1.00***		−0.99***	1.09	−1.33**	−1.07***	−1.05***	−1.13***	1.00***	
3^0 costa length	−0.98***	−1.01***	−1.11***	−1.00***	0.99	−1.40**	−1.09***	−1.07***	−1.13***	1.00***	1.00***

*$P < 0.10$, **$P < 0.05$, ***$P < 0.01$.

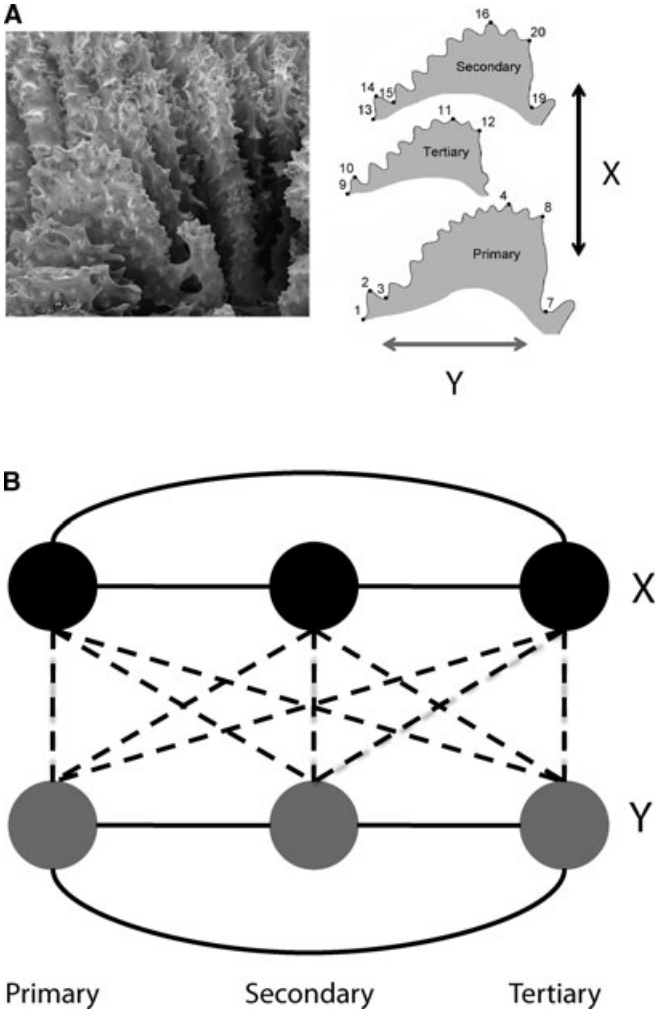

Figure 2. Genetic correlations within and among costosepta. (A) Left panel—Photograph of the interior of a corallite, showing outward radiating cycles of costoseptae. Right panel—Three types of costoseptum in X (height) and Y (length) dimensions, with positions of landmarks. Numbers are positions of landmarks. (B) A digraph illustrating genetic correlations among six homologous height and length measurements among three types of costoseptal plates as illustrated in (A). Each node represents a measurement, nodes in the same row are all homologous measurements across the three kinds of costoseptal plates, whereas nodes in the same column represent X and Y measurements on a single costoseptal plate, of type given in the column headings. Vertices indicate significant genetic correlations among measurements (*P*-values < 0.01, *Q*-values < 0.01). Solid lines are positive genetic correlations, whereas dotted lines are negative correlations. Note strong positive genetic correlations among homologous X or Y measurements among different kinds of costoseptal plates, while there were strong negative correlations between height and width within an individual costoseptum, and among the three types of costosepta. Data are from the combined Tall and Short ecomorphs from the lagoon. See Table 5 for the complete correlation matrix.

traits that define the shape of costoseptal plates in two dimensions. Tall and narrow plates are characteristic of the Tall ecomorph, whereas short and wide plates are characteristic of the Short ecomorph. Positive and negative correlations among plate measurements indicate genetic constraint on the direction of morphological evolution if corallite morphology results from the pleiotropic effects of a small number of loci. Alternatively, genetic correlations may be caused by linkage disequilibrium among a larger number of quantitative trait loci. Compared to the combined population of both ecomorphs, the amount of quantitative genetic variation within ecomorphs was considerably less, as indicated by smaller h^2 estimates of individual corallite traits, and fewer significant tests. Although the error in h^2 estimates from the marker-based method can be considerable, and is affected by both the panel of markers and the number of individuals assayed (Ritland 2000; Wang 2007), differences in the magnitude and significance of h^2 based on samples from both ecomorphs versus samples within ecomorphs are unlikely to be explained by sample sizes alone. We note that standard errors for h^2 estimates are similar for both combined and single ecomorphs, ranging between 0.10 and 0.30. Sample size was more of an issue for the exposed reef sample, which had the least phenotypic variation, the smallest sample size ($n = 35$), and standard errors that all exceeded 1.0. Given suitable polymorphic markers and adequate sample sizes, the marker-based technique shows considerable promise for systems containing the requisite variance in relatedness.

An intriguing pattern of this incipient speciation demands an evolutionary explanation: the large quantitative divergence between ecomorphs that is associated with low quantitative variation within ecomorphs. Perhaps the most parsimonious explanation for this pattern is that environmental differences between habitats result in divergent selection for different trait optima. There are a number of quantitative and qualitative environmental differences between habitats that could shift the direction of selection on skeletal traits. For example, shallow seagrass habitats experience greater salinity fluctuations, greater disturbance of substrata, and warmer temperatures compared to deeper, reef habitats (Guzman and Guevara 1998, D. Carlon, unpubl. data). These factors would favor skeletal traits that increase the growth rate in more disturbed seagrass habitat. A second environmental difference is that reef habitats harbor populations of coralivores (parrotfish), whereas these predators do not make forays into adjacent seagrass habitats (D. Carlon, unpubl. data). Thus we expect skeletal traits that increase defense and decrease lethal damage by scraping, biting, and crushing parrotfish to be favored in reef habitats. Although these associations between phenotypic traits and environmental variables are inviting, an alternative hypothesis can also explain the data based solely on genetic drift. In this case phenotypic divergence between isolated populations accrues at a rate proportional to the time since isolation, while the quantitative variation

Table 6. Comparisons of four different estimators of heritability in the common garden experiment for 13 corallite characters: (1). marker based, (2) variance components, (3) Parent–offspring regressions, and (4) the Riska estimator (Riska et al. 1989). Marker based estimates are decomposed into additive, h^2; no-additive, $H - h^2$; and inbreeding, b^2; components. Estimates based on variance components and parent–offspring regressions are broad-sense heritability (H) and were calculated from all data (H_A), the Short ecomorph samples only (H_S), and the Tall ecomorph samples only (H_T). Shaded characters are linear measurements, nonshaded are Bookstein shape coordinates. Standard errors (SE) were determined by bootstrapping, and significant values ($P < 0.05$) are indicated by bold text. See Appendix S4 for Q-values.

	Marker based						Variance components						Parent–offspring regression						Riska
Character	h^2	SE	$H - h^2$	SE	b^2	SE	H_A	SE	H_S	SE	H_T	SE	H_A	SE	H_S	SE	H_T	SE	H_A
centroid size	**0.32**	0.09	−0.04	0.05	−0.01	0.03	**0.28**	0.05	0.27	0.07	0.03	0.07	**0.15**	0.07	0.04	0.20	−0.07	0.09	**0.21**
1^0 costa length	**0.32**	0.10	0.01	0.05	0.08	0.06	**0.44**	0.05	0.20	0.08	0.02	0.08	**0.26**	0.06	−0.17	0.16	−0.10	0.10	**0.53**
1^0 calice depth	**0.33**	0.09	−0.06	0.05	−0.02	0.03	**0.32**	0.05	0.25	0.07	0.00	0.07	**0.36**	0.12	−0.04	0.35	0.06	0.08	**0.34**
3^0 costa length	0.04	0.04	0.02	0.03	−0.02	0.02	0.17	0.06	0.05	0.06	0.00	0.07	**0.16**	0.04	−0.01	0.11	−0.02	0.06	**0.70**
1^0 septum length	**1.14**	0.11	−0.55	0.98	−0.13	0.06	**0.09**	0.05	0.17	0.07	0.00	0.06	−0.06	0.10	−0.07	0.23	0.03	0.08	0.00
1^0 costoseptum height	**0.78**	0.12	−0.37	0.06	−0.09	0.04	**0.53**	0.04	0.42	0.07	0.09	0.09	**0.75**	0.08	**0.67**	0.10	0.36	0.25	**0.92**
septal spacing	**0.07**	0.02	**0.04**	0.01	0.00	0.00	**0.38**	0.05	0.42	0.09	0.00	0.06	**0.58**	0.16	0.40	0.25	−0.04	0.14	**0.41**
1^0 costoseptum height	**0.79**	0.11	−0.35	0.05	−0.09	0.05	**0.36**	0.05	0.25	0.08	0.04	0.08	**0.58**	0.07	**0.70**	0.14	0.25	0.17	**0.91**
2^0 costoseptum height	**0.67**	0.12	−0.25	0.05	−0.08	0.05	**0.35**	0.05	0.15	0.07	0.05	0.09	**0.53**	0.10	0.44	0.24	−0.11	0.23	**0.69**
3^0 costoseptum height	0.03	0.04	0.03	0.03	−0.02	0.02	**0.12**	0.05	0.15	0.08	0.04	0.08	0.17	0.19	0.23	0.32	−0.09	0.28	0.06
1^0 costa length	0.04	0.06	0.09	0.04	−0.01	0.02	**0.13**	0.05	0.13	0.05	0.06	0.08	0.04	0.06	−0.07	0.14	−0.08	0.10	0.02
2^0 costa length	**0.10**	0.07	0.04	0.04	−0.02	0.02	**0.11**	0.05	0.12	0.07	0.03	0.07	0.07	0.06	−0.10	0.15	−0.06	0.09	0.10
3^0 costa length	**0.11**	0.07	0.03	0.04	−0.01	0.02	0.09	0.05	0.07	0.06	0.00	0.08	0.09	0.05	−0.06	0.13	−0.03	0.06	0.24

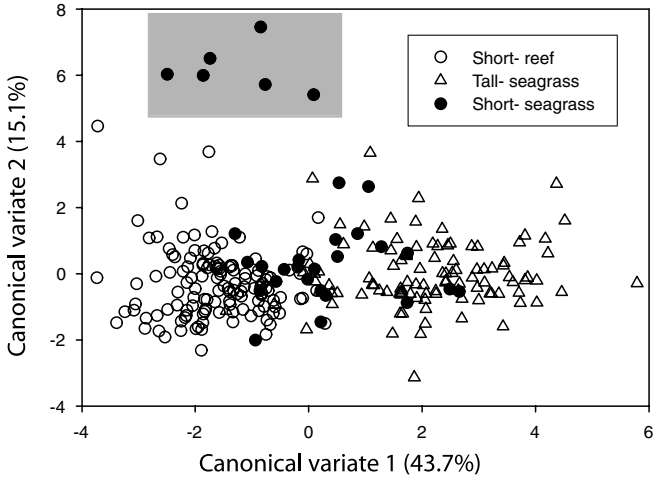

Figure 3. Phenotypic variation within and between ecomorphs in the common-garden experiment plotted as the scores of two major canonical variates. Open circles = offspring from Short ecomorph dams, reef habitat; Filled circles = offspring from Short ecomorph dams, seagrass habitat; Open triangles = offspring from Tall ecomorph dams, Seagrass habitat. Filled circles in the shaded box are six sibs from a dam with multilocus microsatellite genotype A. The remainder of filled circles are from dams with genotype B. Canonical variate 1 is correlated with variables related to the inverse of costoseptum height and the development of the third septal cycle (shape coordinates x04, x16, -x02, -x10, -x14, -x01, -x09, -x13). Canonical variate 2 is correlated with costal tooth elevation (x03 and x15) and length of costosepta (y2, y10, and y13).

within populations will depend on effective population size (N_e) (Lynch and Hill 1986). In the case of small effective population size ($N_e < 1000$), as is likely in subdivided populations of *F. fragum* (Carlon and Lippe 2011), genetic drift will rapidly eliminate new variation generated by mutation resulting in low h^2. A strong test of the role of divergent natural selection versus genetic drift in this system awaits an assessment of fitness "trade-offs" between habitats (sensu Schluter 2000). A forthcoming paper will provide such a test by reporting on ecomorph × habitat measures of ecological and evolutionary performance in the Bocas del Toro.

A second important finding of this article is that we have demonstrated the potential to use the marker-based approach to estimate nonadditive variation in natural populations. This goal has eluded previous applications to terrestrial plant populations (Ritland and Ritland 1996, Ritland and Travis 2004, Andrew et al. 2005, Kumar and Richardson 2005, Bessega et al. 2009). Part of our success may be explained by the fact we employed more loci with higher polymorphism than previous studies allowing greater precision in estimates of relatedness. Lynch and Ritland (1999) simulated different kinds of marker sets by permuting number of loci with polymorphism and found that with a minimum of 10 loci the greatest gains in minimizing sampling variance in relatedness was achieved by increasing the number of alleles rather than the number of loci. Thus earlier work that used allozyme datasets and their inherently low polymorphism suffered from relatively low statistical power to differentiate different classes of relatives (Ritland and Ritland 1996, Ritland and Travis 2004). Power may be less of a factor in more recent studies that used six to eight microsatellite loci with greater polymorphism (Andrew et al. 2005, Kumar and Richardson 2005, Bessega et al. 2009). Our relative success at finding nonadditive variation can be explained by a combination of a larger set of markers (15 microsatellite loci) with a study system that couples high selfing with limited larval dispersal. Still, most of our nonadditive estimates ($H - h^2$) were characterized by large standard errors and we found significant $H - h^2$ in only one character. Given the important role that nonadditive variation plays in the response to selection in clonal and inbred populations (Kelly 1999; Kelly and Williamson 2000; Walsh 2005; Holeski and Kelly 2006), there is a clear need to determine the magnitude and dynamics of this component of quantitative variation in many natural systems. Complex breeding designs are one solution to this problem (e.g., Kelly and Arathi 2003) but our application demonstrates that marker-based approaches may also be useful. Indeed, the conversion of nonadditive variation that is "hidden" by epistasis to additive variation (Lynch 1984, reviewed by Neiman and Linksvayer 2006) could be the key to understanding episodes of adaptive divergence in such nonrandom mating systems.

Beyond predicting the response to selection, mixed mating systems also present new technical challenges to employing marker-based techniques. Wang (2007) has shown that a moderate level of inbreeding negatively biases the Ritland and Lynch estimator of relatedness. In our simulations based on the panel of markers employed here, and s = 0.90, we show this effect increases as r approaches 1.0 (Fig. 5). It is unclear exactly how such bias in r affects marker-derived h^2, which depends partly on the estimation of actual variance in relatedness, which may also be influenced by bias due to inbreeding. Second, the method assumes that markers are in linkage equilibrium with quantitative traits. In the common garden, we have shown large effects of multilocus microsatellite genotypes on corallite phenotypes (Figs. 4 and 5). In inbreeding systems, a lack of effective recombination leads to linkage disequilibrium (LD) extending further distances along chromosomes. It remains unclear how such patterns of LD bias quantitative estimates (see Chapter 12; Lynch and Walsh 1998), but the few discrepancies between the marker-based and regression-based estimates of h^2 from the same sample of phenotypes in the common garden may be symptomatic of such LD effects.

Although both the marker-based approach and the common garden experiment found significant heritability in a number of skeletal traits, it is important to consider how environmental effects may positively bias estimates of h^2 and H derived from

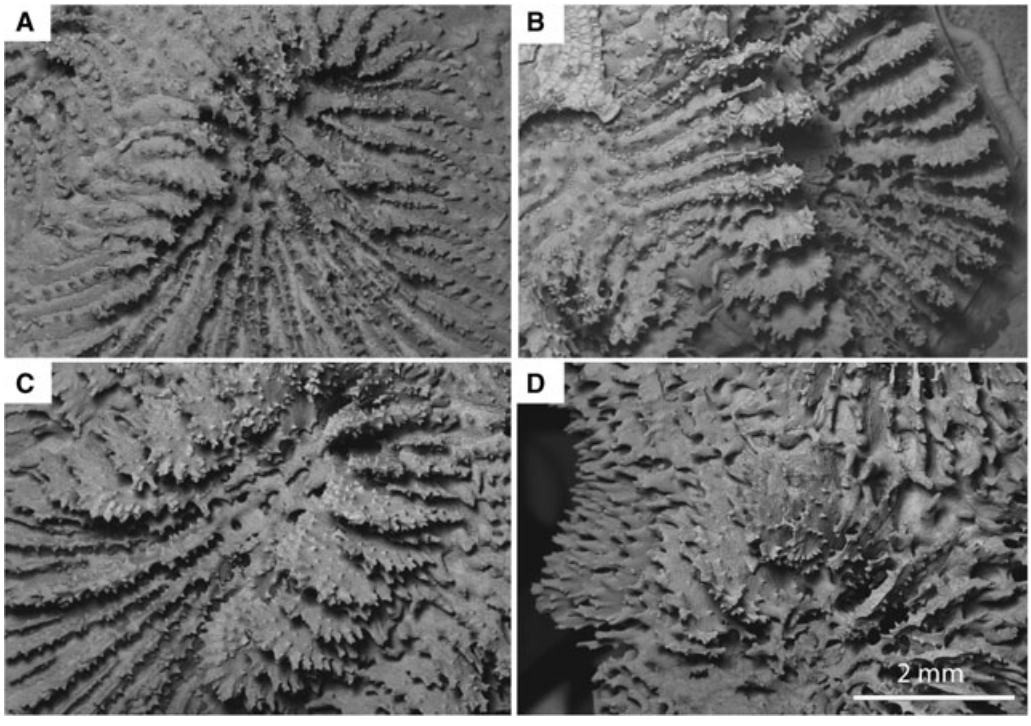

Figure 4. Scanning electron micrographs of corallites from offspring grown in the common garden. The four dams where: (A) Short ecomorph, reef environment; (B) Tall ecomorph, seagrass environment; (C) Short ecomorph, seagrass environment, genotpe A; (D) Short ecomorph, seagrass environment, genotype B. Scale bar is 2 mm for all panels.

both methods. If we assume a component of phenotypic plasticity to the phenotypic variance, then correlations between environment and relatedness would inflate h^2 from our application of the marker-based approach. Ritland's original model (1996) includes an environmental covariate in the form of distance between individuals, however we did not map individual corals in our sampling design and therefore could not include this term in our model. Such environmental effects should be reduced in the common garden, and indeed this can explain why narrow-sense heritability values (h^2) generally exceeded broad-sense heritability values (H) from the common garden. Further, because fertilization occurred naturally in the field, the offspring we cultured in the common garden originated from wild-caught dams and spent several weeks developing within the parental environment (Szmant-Froelich et al. 1985). If the trajectory of ontogeny is determined early in development by cues associated with the early environment, then an effect of phenotypic plasticity could persist in the common garden. Although neither approach completely eliminates the environmental component of phenotypic variation, phenotypic patterns in natural populations are not consistent with the idea that phenotypic plasticity (determined early or late in ontogeny) plays a major role in this morphological divergence. Specifically, the Short ecomorph co-occurs with the Tall ecomorph in seagrass habitats, and both field observations of dispersing larvae (Carlon and Olson 1993) and fine-scale genetic structure (Carlon and Lippe 2011) indicate that seagrass population of the Short ecomorph is self-recruiting. A second potential source of positive bias in both our field and common-garden estimates of heritability are mechanisms of nongenetic inheritance (Bonduriansky and Day 2009). These include the transmission of the epigenetic state (genomic imprinting) across generations, or the inheritance of cytoplasmic factors or nutrients from the parent. Nongenetic inheritance will be particularly challenging to parse from nuclear inheritance when genomic imprinting is correlated with the nuclear genotype. Given these caveats, an interesting pattern that emerged between the common garden and wild populations was the similarity in positive and negative genetic correlations among Bookstein shape coordinates (compare Table 5 with Appendix S2). Stability in genetic correlations across changing environmental conditions suggests that G $\times$ E interactions play relatively minor roles in the expression of the major features of corallite morphology (Steppan et al. 2002). For other coral species, reciprocal transplant experiments using replicate clones have found strong G $\times$ E components in both corallite and colony level skeletal features (Bruno and Edmunds 1997; Todd et al. 2004; Todd 2008). Assuming environmental components are not overwhelming the genetic signal, our results may indicate something fundamentally different about the genetic architecture of skeletal morphology in this particular coral system.

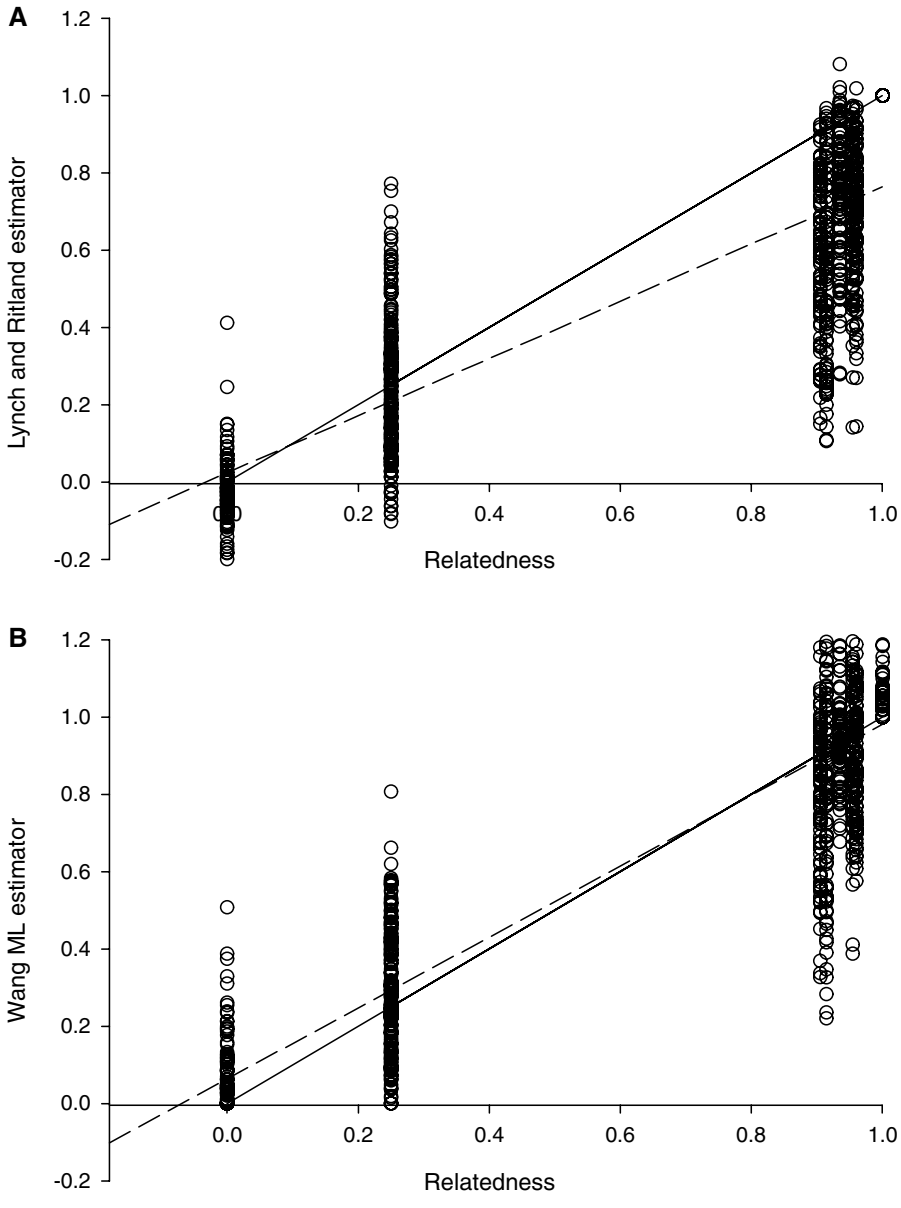

Figure 5. **Bias in estimators of relatedness assuming a mating system of s = 0.90 and the panel of markers used in this study. Actual relatedness is skewed toward 0 and 1.0 because of the opposing forces of outbreeding via outcrossing and inbreeding via selfing on the probability of identity by decent. (A) Lynch and Ritland's estimator (1999). (B) Wang's triadic maximum likelihood estimator (Wang 2007). Solid line is isometric for actual relatedness, whereas dotted line is the linear regression of estimated relatedness on actual relatedness.**

Lastly, our result of finding an additive genetic basis for quantitative variation in traits that determine corallite morphology has implications for morphological species boundaries in other groups. Although nominal species based on traditional qualitative assessment of morphology may not represent genetically meaningful entities (e.g., Eytan et al. 2009), quantitative measures of corallite morphology are increasingly being found to agree with genetic data in many different coral genera (e.g., Flot et al. 2008; Forsman et al. 2010). A prime example is the *Montastraea annularis* complex, which consists of three genetically distinct species that were not recognized until recently due to reliance in species identification on traditional morphological features, such as number of septa per corallite and gross corallite diameter (Knowlton et al. 1992; Weil and Knowlton 1994). Concordance between morphological and genetic data was discovered in the complex using a three-dimensional geometric morphometrics approach (Fukami et al. 2004; Budd and Pandolfi 2010), similar to the approach used herein. This approach emphasizes morphological features such as calical elevation, costa length, and the structure of the corallite wall (Budd and Klaus 2001), and is effective at detecting not only genetically distinct species but also distances among species (Fukami et al. 2004; Budd and Pandolfi 2010). In both

a previous (Carlon and Budd 2002) and the present study, these features clearly distinguish the two ecomorphs of *F. fragum*, and in the present study, they are found to be heritable. Nevertheless, the morphologic features that concur with genetic groupings vary among scleractinian clades. For example, Forsman et al. (2010) found the size and shape of verrucae (wart-like bumps on the colony surface) to agree best with genetic groupings of *Montipora*.

CONCLUSIONS

The phenotypic divergence between ecomorphs has substantial genetic components. In comparison to relatively little additive genetic variation within ecomorphs, this divergence may be explained by divergent natural selection between habitats or alternatively by the neutral forces of genetic drift. Slightly lower values of heritability in the common-garden compared to values of h^2 in the wild may be explained by environmental effects on the latter, which may positively bias the true estimates. Regardless of such bias, strong positive and negative genetic correlations among the dimensions of costoseptal plates can explain the observed shifts in skeletal architecture between Tall and Short ecomorphs. Further, the signs of these correlations remain constant between wild-caught individuals and those cultured in the common garden. We have successfully demonstrated a quantitative genetic basis for traits that are hypothesized to have played a major functional role in this incipient speciation across environmental gradients. This tropical coral system now provides a rare opportunity to explore the relative roles of natural selection and genetic drift in the evolution of reproductive isolation.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Diagrams and descriptions of morphological measurements.
Appendix S2. Marker-based genetic correlations for natural populations of Short (A) and Tall (B) ecomorphs sampled from the lagoon, and the common garden experiment (C).
Appendix S3. ANOVA tables for the wild populations (1. Wild) and the common garden experiment (2. Common).
Appendix S4. Parameter estimates, *P*-values, and *Q*-values (false discovery rates) for Table 4 (A–C) and Table 6 (D, E).
Appendix S5. Four estimates of heritability plotted against the parent offspring estimate.

Supporting Information may be found in the online version of this article.

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