accumulation, B (positive for melting), and the difference in ice flux between the two gates per unit area, $\partial H / \partial t = A - B + (\phi_{GL} - \phi_{DG})/ISA$, where ϕ_{GL} and ϕ_{DG} are the ice flux for the GL and the DG, respectively. Under steady-state conditions, $\partial H / \partial t = 0$, and neglecting surface melt and accumulation (<0.5 m/year) compared with basal melting, B is equal to the decrease in ice flux per unit area. If the ice shelf is thickening (thinning) and ice flow is steady, then B would be lower (higher) than calculated under steady-state conditions.

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Hybridization and the Evolution of Reef Coral Diversity

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Hundreds of coral species coexist sympatrically on reefs, reproducing in mass-spawning events where hybridization appears common. In the Caribbean, DNA sequence data from all three sympatric *Acropora* corals show that mass spawning does not erode species barriers. Species *A. cervicornis* and *A. palmata* are distinct at two nuclear loci or share ancestral alleles. Morphotypes historically given the name *Acropora prolifera* are entirely F_1 hybrids of these two species, showing morphologies that depend on which species provides the egg for hybridization. Although selection limits the evolutionary potential of hybrids, F_1 individuals can reproduce asexually and form long-lived, potentially immortal hybrids with unique morphologies.

Diverse reef-building coral assemblages have served as the foundation for complex reef ecosystems with exceptional biodiversity and productivity. Yet, the evolutionary genesis of coral diversity remains mired in a paradox. As many as 105 coral species from 36 genera and 11 families reproduce in yearly, synchronous mass-spawning events (*1*), thereby providing overwhelming opportunities for hybridization among congenerics (*2*). Laboratory crosses from a number of mass-spawning genera demonstrate that viable hybrids occur among congenerics (*2*, *3*). Interspecific hybridization should blur coral species boundaries and stifle species diversification, yet many mass-spawning coral groups have rapidly diversified. The juxtaposition of high hybridization potential and high species diversity in mass-spawning corals has confused the picture of coral evolution and cast such doubt on the cohesiveness of coral species boundaries (*4*) that some species-rich genera have been considered hybrid swarms (*3*). *Acropora*, the world's most speciose coral group (*5*), exemplify this view (*2*–*4*). Most of the 115 species of *Acropora* arose over the past 5 million years (My) (*6*, *7*), and many are capable of hybridizing with sympatric congenerics in laboratory crosses (*2*, *8*). One prominent hypothesis proposes that interspecific hybridization promotes reticulate evolution and morphological diversification in the absence of genetically distinct species (*3*), even though a genetic mechanism for this

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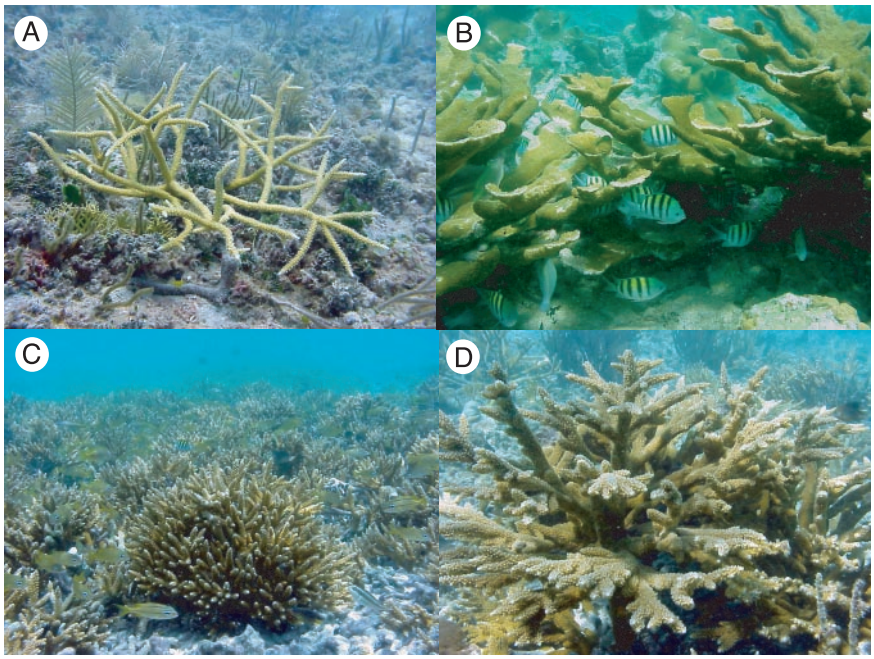

Fig. 1. The Caribbean *Acropora* species: (**A**) *A. cervicornis* and (**B**) *A. palmata*, and (**C**) the bushy and (**D**) palmate F_1 hybrid *A. prolifera* morphs from Puerto Rico.

hypothesis is lacking. Polyphyletic sequence data for corals continue to be taken as direct evidence of reticulate evolution (*8*–*11*) without due consideration to alternatives such as incomplete lineage sorting.

To examine the potential role of hybridization in coral speciation, we analyzed DNA sequence variation at three loci in the three sympatric species of Caribbean *Acropora* (Fig. 1). *Acropora cervicornis* and *A. palmata* are sister species with fossil records dating back at least 3 to 3.6 My (*12*, *13*). Both have distinct morphologies and habitat preferences. The arborescent "staghorn" coral *A. cervicornis* occurs throughout forereef and backreef habitats, whereas the robust "elkhorn" coral *A. palmata* occurs primarily in high–wave energy reef-crest habitats (*14*, *15*). Both species spawn synchronously over a few nights each summer (*16*) and can potentially hybridize. The third species, *Acropora prolifera*, occurs Caribbean-wide, where it varies from being locally rare to occurring in large patches (*7*, *14*, *15*). It is morphologically intermediate between *A. cervicornis* and *A. palmata*, causing many to consider it a species of hybrid origin (*7*, *15*). *Pax*-C intron data showing high heterozygosity support this possibility (*10*). Morphological variation in *A. prolifera* is high and yet surprisingly discrete. In Puerto Rico, for example, there are two discrete *A. prolifera* morphs—a thin, highly branched form we term the "bushy" morph (Fig. 1C), and a thicker form with palmate, flattened branches we call the "palmate" morph (Fig. 1D).

We obtained sequence data for the Caribbean *Acropora* species at introns of the nuclear minicollagen and calmodulin genes, and at the mitochondrial putative control region (*17*). The nuclear data indicate that the species *A. cervicornis* and *A. palmata* are genetically distinct and that the morphologically intermediate species *A. prolifera* is actually a first-generation (F_1) hybrid. *Acropora cervicornis* and *A. palmata* were reciprocally monophyletic at minicollagen (Fig. 2A). All of the *A. prolifera* (n = 22) were heterozygous at minicollagen, containing one allele from each of the two species' clades. The calmodulin data for *A. cervicornis* and *A. palmata* formed three distinct alleles: A, B, and B′ (Fig. 2B). Allele A was exclusive to *A. cervicornis*. B alleles were exclusive to *A. palmata*, but the variant B′ was shared between species, making it either a shared ancestral allele or an introgressed allele from recent or historical hybridization. As with minicollagen, all of the *A. prolifera* (n = 28) were heterozygous at calmodulin (A/B = 26; B/B′ = 2). The complete heterozygosity of *A. prolifera* at these two nuclear loci strongly suggests that every individual sampled was a F_1 hybrid.

Mitochondrial data show that the 45 unique haplotypes form a polytomy with three clades (Fig. 2C), labeled as haplotypes A, B, and C. The A and C haplotypes contained only *A. cervicornis* and hybrid *A. prolifera*. The B haplotypes contained all three taxa: *A. palmata*, *A. cervicornis*, and hybrid *A. prolifera*. All three haplotypes were found in *A. prolifera*, indicating that hybrid crosses occur in both directions. Hybrids receive maternally inherited mitochondrial DNAs from either *A. palmata* (B haplotype) or *A. cervicornis* (A haplotype) "mothers."

Although hybrid crosses occur in either direction, mitochondrial DNA (mtDNA) introgression appears unidirectional because *A. cervicornis* colonies possess all three haplotype clades, but *A. palmata* colonies do not. The data indicate that "*palmata*" (B) haplotypes are passed to *A. cervicornis* through backcrossing of *A. cervicornis* with hybrid *A. prolifera*. Introgressed B haplotypes in *A. cervicornis* were common (~20%) and sampled at every site. The presence of multiple B variants in *A. cervicornis* indicates the mtDNA introgression has occurred more than once. Because nuclear loci should sort more slowly than maternally inherited mtDNAs (*18*, *19*), polyphyletic patterns in the mitochondrial data but not the minicollagen data are consistent with recent introgression rather than incomplete lineage sorting.

In Puerto Rico, we sampled two distinct morphs of *A. prolifera*, i.e., the bushy and palmate morphs (Fig. 1, C and D). Although all individuals, irrespective of morphology, are F_1 hybrids, they differ in which species donated its egg and mitochondrion to the hybridization event. All bushy hybrids had a *palmata* maternal and mitochondrial background, whereas all of the palmate hybrids had a *cervicornis* background. This suggests

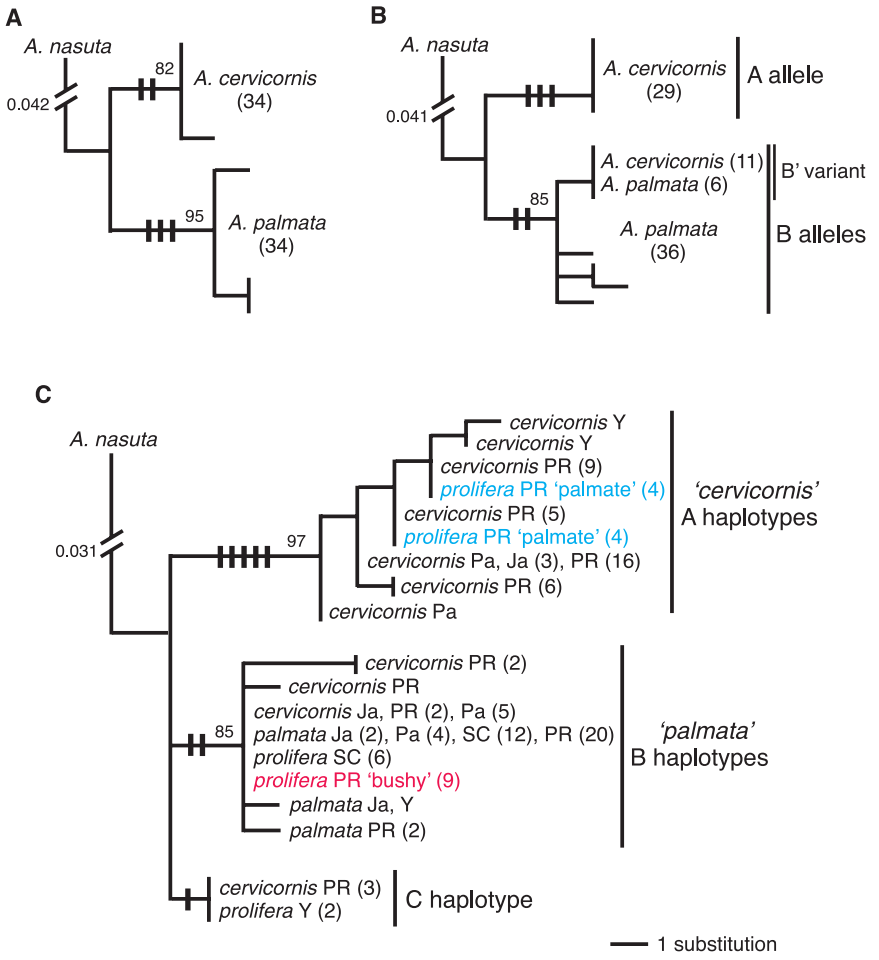

Fig. 2. Maximum likelihood (ML) trees for (**A**) minicollagen, (**B**) calmodulin, and (**C**) mitochondrial putative control region. Likelihood searches were conducted in PAUP* 4.0b8 (*31*) with estimated model parameters and 25 random-addition heuristic searches with tree-bisection-reconnection branch swapping. Models of sequence evolution were evaluated on distance-based topologies with hierarchical likelihood ratio tests (*32*) in MODELTEST 3.06 (*33*). Major allele/haplotype clades are labeled. Tick marks along major branches indicate substitutions. Sample sizes (alleles or haplotypes) are labeled in parentheses (n). Site abbreviations: Yucatan (Y); Panama (Pa); Jamaica (Ja); Puerto Rico (PR); St. Croix (SC). Bootstrap values (>50%) from 300 replicates are labeled on relevant nodes. The Pacific congener *Acropora nasuta* was used as the outgroup. Sequences are available in GenBank (accession numbers AF507116 to AF507373). (A) Minicollagen ML tree constructed with a K80 model (ln score = 654.81). (B) Calmodulin ML tree constructed with a HKY model (1 of 4 trees; ln score = 592.86). (C) Mitochondrial putative control region ML tree constructed with a F81 + Γ model (ln score = 2014.96). Palmate *A. prolifera* hybrids are shown in blue; bushy hybrids are in red.

that maternal and/or cytoplasmic effects account for the marked differences in these two hybrid morphotypes. Thus, coral morphology appears sensitive to not only nuclear genetic effects, but also to nuclear-cytoplasmic interactions within a hybrid nuclear genome.

Differential introgression of loci characterizes many terrestrial hybridization systems (*20*); however, a rarely explored alternative is that the pattern is due to ancestral polymorphism. We applied a two-population Bayesian coalescent model (*21*) to our data and the published *Pax*-C data (*10*) to estimate the rate of introgression [as migration (M) in units $2 \times$ the product of effective population size (N_e) and migration (m)] and test null hypotheses of no introgression ($M = 0$) using likelihood ratio tests (LRTs) (*22*). Results [Table 1 and supplemental material (*23*)] indicate that the mitochondrial data are consistent with low levels of introgression ($M = 0.20$), roughly equivalent to one haplotype crossing the species boundary every $5N_f$ (i.e., mtDNA effective population size) generations. For the nuclear loci, the *Pax*-C data were also consistent with low levels of introgression ($M = 0.30$), whereas the minicollagen and calmodulin data were both consistent with no introgression, suggesting that the shared B′ allele at calmodulin is a retained ancestral allele. Such differential cytoplasmic and nuclear introgression is consistent with selection against hybrid genotypes that is thought to result from selection against nuclear genes in foreign genetic backgrounds (*24*), and/or the breakup of coadapted gene complexes in backcrossed individuals (*25*).

The existence of hybrid *A. prolifera* shows that complete barriers to hybridization have not evolved between *A. palmata* and *A. cervicornis*. However, the observation that *A. prolifera* hybrid populations are composed almost entirely of F_1 individuals suggests that the reproductive potential of hybrid *A. prolifera* is severely limited or that hybrid breakdown occurs in later generations. Some hybrid *A. prolifera* are reproductive, produce viable gametes, and are interfertile with *A. cervicornis*. Yet, the limited introgression suggests that they are essentially sterile "mules," which have little genetic impact on either parent species. Strict F_1 hybrids are often ecologically rare in natural hybridization systems (*26*). Where F_1 hybrids dominate, selection manifest as hybrid infertility or hybrid breakdown has been inferred, as here (*27*). Such F_1 hybrids should be common only when hybridization is frequent or F_1 offspring are long-lived. Like many corals (*28*), hybrid *A. prolifera* can propagate clonally by fragmentation (*29*), allowing for long-lived, potentially immortal hybrid genotypes. These "immortal mules" may accumulate over time, providing the opportunity for rare backcrosses, and for the ecological persistence of a diverse suite of *Acropora* morphotypes that is greater than the number of species on reefs.

Table 1. Estimated genetic introgression. Results of the Bayesian coalescent modeling for each gene showing the estimated rates of introgression (M in $2N_em$ units) and the results of the likelihood ratio tests (LRTs). NS, not significant; $*P = 0.05$; $**P = 0.01$.

Gene	$2N_em$	LRT	P
Minicollagen	0.00	0.00	1.000 (NS)
Calmodulin	0.08	2.17	0.071 (NS)
Pax-C	0.30	6.02	0.007**
MtDNA control region	0.20	4.31	0.019*

The Caribbean *Acropora* show that reef-building corals diversify not only through conventional species formation, but also through the unprecedented formation of long-lived coral hybrid morphotypes. In effect, hybridization, through the formation of asexual coral hybrid lines, generates new morphologies and potentially new ecotypes without speciation. Similar clonal niche partitioning is known for rare parthenogenetic taxa (*30*), but has never been postulated for an ecosystem-defining group like reef-building corals. Although it remains to be seen how pervasive coral hybrid "mules" are, the variety of intermediate morphologies in corals, especially in regional endemics and putative subspecies (*5*), suggests that morphologically unique hybrids may be common. Because of the potential for natural hybridization in mass-spawning corals, the coral reticulate evolution hypothesis suggested that genetic exchange between "species" generates discrete coral morphologies (*3*) without genetic isolation. Instead, we suggest that reef-building coral diversity is enhanced by hybridization through the production of long-lived asexual hybrid morphotypes, which have little evolutionary potential.

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