hybridization. Although selection limits the evolutionary potential of hybrids, F$_r$ 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 genetics 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 (I), thereby providing overwhelming opportunities for hybridization among congeners (2). Laboratory crosses from a number of mass-spawning genera demonstrate that viable hybrids occur among congeners (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 congeners 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

Hybridization and the Evolution of Reef Coral Diversity

Steven V. Vollmer* and Stephen R. Palumbi

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$_r$ 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$_r$ 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 genetics 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 (I), thereby providing overwhelming opportunities for hybridization among congeners (2). Laboratory crosses from a number of mass-spawning genera demonstrate that viable hybrids occur among congeners (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 congeners 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
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 (F1) 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 F1 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 F1 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
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) in units \( M = 0 \) 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 \( 3N_M \) 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 breakdown 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.

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 relictuate evolution hypothesis suggested that genetic exchange between “species” generates discrete coral morphologies (5) 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.

References and Notes

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>( 2N_M )</th>
<th>LRT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minicollagen</td>
<td>0.00</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>0.08</td>
<td>2.17</td>
<td>0.071</td>
</tr>
<tr>
<td>Pax-C</td>
<td>0.30</td>
<td>6.02</td>
<td>0.007*</td>
</tr>
<tr>
<td>MtDNA control region</td>
<td>0.20</td>
<td>4.31</td>
<td>0.019*</td>
</tr>
</tbody>
</table>

16. J. Paleontol.
17. C. C. Wallace, B. L. Willis, Proc. 8th Int. Coral Reef Symp.
20. J. C. Avise, Molecular Markers, Natural History and Evolution (Chapman & Hall, New York, 1994).
22. Acropora cervicornis and \( A. palmata \) were treated as separate populations. Models were run independently for each gene. The mode of the integrated posterior probability distribution for the migration parameter \( M \) was used to estimate the rate of introgression. LRTs compared probabilities of \( M \) versus \( M = 0 \) to test the null hypotheses of ancestral polymorphism. P values were divided by 2 after (21) multiple simulations confirm model convergence [supplemental material (23)]. The model searched for the best values of \( M \) and \( T \) within the bounds of 0 to 10 for both parameters.
23. Supplementary material is available on Science Online at www.sciencemag.org/cgi/content/full/296/5575/2023/DC1.