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Reproductive Isolation and Hybridization Dynamics in Threatened Caribbean Acroporid Corals

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REPRODUCTIVE ISOLATION AND HYBRIDIZATION DYNAMICS IN THREATENED CARIBBEAN ACROPORID CORALS

By

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I dedicate this dissertation to my fiancé Kevin, to my family, and to anyone who has followed through with a childhood dream.

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ABSTRACT

The Caribbean corals, *Acropora palmata* and *A. cervicornis*, are abundant in fossil records but have recently undergone drastic declines primarily as a result of disease. *Acropora prolifera*, a hybrid of these species, has no fossil record and was previously considered rare and to occupy nonparental habitats. Now, hybrids have equivalent or greater abundance than the parental species and have expanded into the parental habitat at some sites. Previous molecular studies have demonstrated regional variability in unidirectional introgression of *A. palmata* genes into *A. cervicornis*. The goals of this dissertation are **(1) to determine the strength of prezygotic mechanisms and to establish the likelihood of density dependent reproductive isolation, (2) to determine the strength of intrinsic and extrinsic postzygotic barriers, and (3) to ascertain if hybrid populations are composed of rare hybridization events that have asexually fragmented, or if colonies are genotypically distinct suggesting separate hybrid events.** Overall barriers to hybridization in this genus are weak, and the efficacy of these semipermeable isolating mechanisms may depend on density. In addition, hybrids are as viable as the parental species at a variety of life history stages and are less or equally susceptible to the typical afflictions that have lead to their decline. Most hybrid populations do not seem to be composed of a single hybridization event that has asexually propagated, but rather the genotypic diversity varies across sites with up to 17 different distinct genets in one population. Taken together, it appears that hybridization in a threatened Caribbean genus is evolutionarily significant with a range of possible outcomes from the benefit of novel alleles to the swamping of *A. cervicornis'* genome. These outcomes may hinge on the ability of the Caribbean acroporids to withstand the onslaught of threats that currently faces this genus (i.e.

Allee Effect, disease, predation, increased sea temperature, ocean acidification, and increased disturbances).

CHAPTER 1

INTRODUCTION

Botanists have long realized the importance of introgressive hybridization (i.e. gene flow between genetically different species via hybridization) as a key evolutionary process (Anderson 1949; Stebbins 1959; Grant 1981); however, only recently has the importance of introgressive hybridization been considered in animals (reviewed in Arnold 1997, 2007, Willis 2006). Outcomes of introgressive hybridization can vary from the exchange of novel alleles to the genetic swamping of one or both of the parental species. The likelihood of this outcome is dependent upon the strength of selection and direction of introgression. If there is sufficient selection against introgressed alleles, then ecological and morphological identity of the species will be maintained. Weak selection coupled with extensive hybridization, may result in genetic swamping driving one species, via unidirectional introgression, or both species to extinction through the fusion of both parental species genomes (Levin et al. 2002; Rhymer and Simberloff 1996). If introgressed alleles are favored by selection, it may lead to adaptive shortcuts for the recipient species (Stebbins 1959; Martinsen et al. 2001; Arnold 2006). Lastly, if hybrid populations stabilize and become reproductively isolated from the parental species, a new species may form (Riesberg 1997; Salzburger et al. 2002; Arnold 2006; Willis et al. 2006).

Another way of viewing introgression is that hybrids act as an evolutionary filter where selection will allow the introgression of beneficial alleles but prevent many deleterious alleles from introgressing (Martinsen et al. 2001). This balance of selection can be disrupted by ecological changes (i.e. species introductions, habitat destruction, abiotic fluctuations, and disease, predation, or parasitism outbreaks) that lead to high mortality in the parental species and a reduction in the efficacy of reproductive isolating barriers (Levin et al. 2002; Rhymer and Simberloff 1996; Dowling and Secor 1997). When this occurs, it is often the rarer of the two hybridizing species that suffers increased introgression and is threatened by genetic swamping (Hubbs 1955; Levin et al. 2002; Rhymer and Simberloff 1996; Wirtz 1999; Lepais et al. 2009). Endangered taxa are particularly vulnerable to genetic swamping; yet, these taxa are at risk of inbreeding depression and may actually benefit from the acquisition of genetic variation through introgression. In such a system, a sister species is sometimes introduced to enhance the genetic variation of the rare species as a conservation strategy (reviewed in Rhymer and Simberloff and Arnold 2006).

Reticulate evolution can be the result of extensive introgressive hybridization in a genus, where species undergo repeated fusion and separation over time. Reticulate evolution is well established as an evolutionary process in many plant species, but occurs to a lesser extent or at least understudied in animal species (Arnold 1992; Arnold 2006). Corals are one of the best known animal examples of introgressive hybridization. Corals share many life history characteristics with plants (e.g. sessile adults, broadcasting of gametes, lack of mating behavior, common hermaphroditism, and effective means of asexual propagation –Willis et al. 2006) and therefore appear to undergo similar evolutionary processes such as introgressive hybridization and reticulate evolution (Veron 1995; Hatta et al. 1999; Willis et al. 2006). The main evidence of reticulation of corals comes from their longevity in the fossil record, their ability to hybridize, their extensive diversity, their biogeographic patterns, and coral's mode of reproduction and long distant dispersal (Veron 1995). The genus *Acropora* is the most well studied coral genus likely because of its evolutionary success with more than 100 species, its dominance in shallow water reefs (Wallace 1999; Veron 2000), and its remarkable ability of effective clonal reproduction (Tunnicliffe 1981, 1983). Evidence from several molecular studies on Indo-Pacific acroporids species found high levels of gene pool sharing and suggest theses species should belong to a syngameon (i.e. a complex of interbreeding species). These findings are consistent with expectations of reticulate evolution (Hatta et al. 1999; van Oppen et al. 2001, 2002).

The Caribbean acroporid system is only composed of two species, *A. palmata* and *A. cervicornis* that form a hybrid, previously called *A. prolifera* (van Oppen et al. 2000; Vollmer and Palumbi 2002). Although *A. cervicornis* and *A. palmata* have been found in the fossil record for 6.6 (Budd and Johnson 1999) and 3.6-2.6 million years, respectively, the hybrid has no fossil record (Budd et al. 1994). Unidirectional introgression of genes from *A. palmata* to *A. cervicornis* occurs at varying frequencies across loci and across geographic sites (van Oppen 2000; Vollmer and Palumbi 2002, 2007; Hemond and Vollmer 2010). The role hybridization and reticulation play in this system has been controversial. Some scientist believe that these hybrids have little evolutionary significant but will persist through asexual fragmentation and the occupation of nonparental niches (Cairns 1982; Vollmer and Palumbi 2002), while others believe that even small amounts of introgression may increase genetic diversity needed for the resilience of these declining coral species (Miller and van Oppen 2003; Willis et al.2006). It has also been suggested that although reticulation is an important evolutionary force in the Indo-Pacific, the Caribbean is likely too uniform for reticulate pathways be created (Veron 1995).

Because a paucity of information exists on the ecology of the hybrid, the reproductive ecology of the parental species, and the strength of the reproductive isolating barriers, it is difficult to assess the evolutionary significance of hybridization in the Caribbean acroporids. Understanding the current ecology of the Caribbean acroporid system is particularly important because drastic ecological changes have occurred recently. In the past 30 years, *A. palmata* and *A. cervicornis* have undergone drastic declines of over 97% in abundance and coral cover (*Acropora* biological review team). This decline is primarily the result of white-band disease (Aronson and Prect 2001; Patterson et al. 2002; Williams and Miller 2006; Pandolfi and Jackson 2006), but predation, coral bleaching, disturbances, and other diseases (i.e. white pox) are factors that have also contributed to the dramatic loss of acroporids (Bruckner 2002). The loss of the parental species may have changed their reproductive success in two ways: (1) an overall decrease in the fertilization rates because gamete concentrations are too dilute (i.e. the Allee Effect Levitan and McGovern 2002), or (2) if eggs are not immediately swamped by conspecific sperm, they may drift unfertilized for longer periods of time increasing the probability of encountering heterospecific sperm. Understanding the level of compatibility between *A. palmata* and *A. cervicornis* will elucidate the probability of increased hybridization as a function of decreased parental species abundance.

If *A. palmata* and *A. cervicornis* are compatible, there may be postzygotic mechanisms that prevent the proliferation of the hybrid. Postzygotic mechanisms that may act on hybrids are intrinsic (i.e. hybrid sterility and hybrid inviability) or extrinsic (i.e. ecological inviability; Coyne and Orr 2004). For unidirectional introgression to occur between these species (*A. palmata* genes flowing into *A. cervicornis*), *A. cervicornis* must mate with hybrids demonstrating that hybrids are not sterile and therefore hybrid sterility can be ruled out as a possible postzygotic mechanism. Hybrid inviability could take place at a variety of different life cycle stages (i.e. larval, settlement, post-settlement, and adult). To summarize these stages, Caribbean acroporids are hermaphroditic corals that broadcast spawn gamete bundles full of eggs and sperm on a few nights in late summer (Szmant 1986). Fertilization takes place at the water's surface and the resulting larvae are competent (i.e. having the ability to settle) after four days (Fogarty pers.obs) but may not settle for a week or two (Szmant 1986). Settlement involves finding appropriate substrate through which chemical cues are used (Ritson-Williams et al. 2010). Because of their small size and slow growth rates, corals are particularly vulnerable during the post-settlement period (Ritson-Williams 2010) and take at least four years to reach sexual maturity (Wallace 1985). As adults, acroporids face numerous threats such as predation, disease, overgrowth, parasitism, coral bleaching, and disturbances. In order to adequately assess the hybrid inviability as a source of postzygotic selection, all of these stages should be assessed. Finally, ecological viability occurs when hybrids suffer lower fitness because they fail to find an appropriate ecological niche. Acroporid hybrids were thought to live in only nonparental niches (Carins 1982; Vollmer and Palumbi 2002; Willis et al. 2006); however, recently hybrids have also been observed to co-occur with both parental species (N.D. Fogarty pers. obs) which may suggest the expansion of their habitat range.

Not only might hybrids be expanding their range, but they may also be expanding their numbers. Although documented as rarer than the parental species, currently hybrid abundance is equivalent or greater than one or both parental species at some sites (N.D. Fogarty pers. obs.). A recent increase in hybrid abundance may be a result of an increase in the number of hybrid embryos formed, an increase in asexual fragmentation, and/or higher resistance of the hybrids to the factors that have led to the decline of the parental species.

Through use of field experiments and observations, laboratory experiments, and molecular techniques, this dissertation focused on the importance of hybridization in broadcast spawning clonal organisms. The goals of this dissertation are **(1) to determine the strength of prezygotic mechanisms and to establish the likelihood of density dependent reproductive isolation, (2) to determine the strength of intrinsic and extrinsic postzygotic barriers, and (3) to ascertain if hybrid populations are composed of rare hybridization events that have asexually fragmented, or if colonies are genotypically distinct suggesting separate hybrid events.**

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CHAPTER 2

WEAK PREZYGOTIC ISOLATING MECHANISMS IN THREATENED CARIBBEAN *ACROPORA* CORALS

2.1 ABSTRACT

The Caribbean corals, *Acropora palmata* and *A. cervicornis*, recently have undergone drastic declines primarily as a result of disease. Previous molecular studies have demonstrated that these species form a hybrid, formerly called *A. prolifera*, and that variability in unidirectional introgression of *A. palmata* genes into *A. cervicornis* exist across loci and across sites. Hybrid abundance varies from rare to locally abundant with no obvious geographic pattern. Here we examine the effectiveness of prezygotic reproductive isolating mechanisms within the Caribbean acroporid system including choice and no-choice fertilization crosses. We show that these species have subtle difference in mean spawning times, but overlapping ranges in spawning time and species-specific differences in gametic incompatibilities. *Acropora palmata* eggs were relatively resistant to hybridization, especially when conspecific sperm are available to outcompete heterospecific sperm. *Acropora cervicornis* eggs demonstrated no evidence for gametic incompatibility. This asymmetry in compatibility matches the genetic data on unidirectional introgression. Our data also suggest that these incomplete prezygotic isolating mechanisms may be density dependent. Under low abundances, eggs may remain unfertilized for longer periods, reducing the effectiveness of conspecific sperm precedence and subtle differences in spawning time in isolating these species.

2.2 INTRODUCTION

Although well established in plants, the evolutionary significance of introgressive hybridization is becoming more widely accepted in animals (Dowling and Secor 1997; Mallet 2005; Arnold 1997, 2006; Arnold and Fogarty 2009). Depending upon the strength of selection and the rate of introgression across loci, the outcome of introgressive hybridization may vary (Hunt and Selander 1973; Harrison 1986; Arnold et al. 1990; and Martinsen et al. 2001). If selection is weak and hybridization rates are high, then genetic swamping may eliminate one or both species, depending upon the directionality of introgression (Rhymer and Simberloff 1996). If there is sufficient selection against deleterious introgressed alleles, however, then the ecological and morphologic identity of the species will be maintained despite some low levels of gene exchange (Barton and Hewitt 1985; Martinsen et al. 2001). Increased genetic diversity from low levels of introgressed alleles may also lead to adaptive shortcuts for the recipient or may lead to reproductive isolation from the parental species resulting in a new species (Grant 1981; Dowling 1997; Riesberg 1997; Arnold 2006).

When ecological conditions change, the balance of selection and introgression may shift, become unstable, and lead to genetic swamping (Rhymer and Simberloff 1996). Anthropogenic activities (i.e. introduced species, excessive killing, and habitat alterations) are often the primary culprits that lead to destabilization of hybrid systems and increased introgression (Rhymer and Simberloff 1996; Dowling and Secor 1997). Natural declines in adult density from disease and predator outbreaks can also affect the degree of hybridization, as can environmental fluctuations that create an inhospitable environment for the parental species or a hospitable zone in which hybrids can thrive (Barton and Hewitt 1985). In many cases, reproductive isolation itself can be density-dependent such that the rarer species of a hybridizing pair is overwhelmed by abundant heterospecifics (Hubbs 1955; Rhymer and Simberloff 1996; Wirtz 1999). Thus