Hobbs J-P.A., van Herwerden L., Pratchett M.S., and Allen G.R. (2013) Hybridisation among butterflyfishes. Pages 48-69. In: Pratchett MS, Berumen ML, Kapoor B (Eds) Biology of Butterflyfishes. CRC Press, Boca Raton, FL

CHAPTER 3

Hybridisation among Butterflyfishes

Jean-Paul A. Hobbs,[1,]* Lynne van Herwerden,[2] Morgan S. Pratchett[3] and Gerald R. Allen[4]

INTRODUCTION

Hybridisation is defined as the interbreeding of individuals from two distinct populations (*sensu stricto* species), which are distinguishable on the basis of one or more heritable characters, following Harrison (1993). Hybrids have been recognised, based on intergrading of characteristic features from parent species, since at least the 1700's and have been documented among plants, corals, gastropods, crustaceans, insects, amphibians, reptiles, birds, mammals and fish (Barton and Hewitt, 1985; Schwenk, 1993; Bierne et al., 2003; van Oppen and Gates, 2006). At least 10% of animals and 25% of plant species are known to hybridise, although the true proportion is probably higher because hybridisation often goes unnoticed (Mallet, 2005, 2007). The fact that hybridisation is not a rare phenomenon but occurs in a considerable

[1]The Oceans Institute and School of Plant Biology, The University of Western Australia, Crawley 6009, Australia.
E-mail: jean-paul.hobbs@uwa.edu.au
[2]School of Marine and Tropical Biology, James Cook University, Townsville QLD 4811, Australia.
E-mail: lynne.vanherwerden@jcu.edu.au
[3]ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville QLD 4811, Australia.
E-mail: morgan.pratchett@jcu.edu.au
[4]Western Australian Museum Locked Bag 49, Welshpool DC, Perth, Western Australia 6986, Australia.
E-mail: gerald.allen@wa.gov.au
*Corresponding author

proportion of species, presents a significant challenge to the fundamental biological definition of a species (Barton and Hewitt, 1985; Mallet, 2005).

In addition to the taxonomic implications, the study of hybridisation is crucial to evolutionary biology for three reasons. Firstly, it provides unique insights into reproductive isolation and the speciation process. For example, a fundamental tenet of sympatric speciation is that there are behavioural or semi-permeable physical barriers to reproduction among distinct components of a population, leading through time to the eventual divergence of these populations into recognisably distinct species (e.g., Bush, 1969; Munday et al., 2004). The question then becomes what is it that limits interbreeding, and what level of interbreeding can occur while species continue to diverge? Secondly, hybridisation can increase genetic diversity and produce novel genotypes that enables hybrids to exploit new, unoccupied or altered habitats and these hybrids can evolve into new species (Seehausen, 2004; van Oppen and Gates, 2006; Mallet, 2007). This process has been linked to adaptive radiations of several groups of plants and animals (Seehausen, 2004). Thirdly, hybridisation can also decrease species richness through extinction (Rhymer and Simberloff, 1996) or reverse-speciation (Seehausen, 2006). Therefore, hybridisation can play a significant role in the generation and loss of species.

Hybridisation usually occurs in narrow and specific geographic areas where genetically distinct populations or species interbreed and produce offspring (Hewitt, 1988; Harrison, 1993; Arnold, 1997). These areas are known as hybrid zones and are the "natural laboratories" for studying hybridisation (Barton and Hewitt, 1989). Hybrid zones can arise within the overlapping geographic distributions of sympatric species (or populations) (e.g., van Herwerden et al., 2006; Yaakub et al., 2006), or through secondary contact of allopatric species (or populations) at or beyond the edge of a species, geographic range (Mayr, 1963; Hewitt, 1988, 2000). The latter (i.e., secondary hybrid zones) are believed to be the most common (Barton and Hewitt, 1985; Hewitt, 1988). Hybrid zones are not evenly distributed around the world and frequently occur in clusters (Hewitt, 2000). Clusters of hybrid zones often form where regional biotas, which have been separated by vicariant events, re-expand and come into secondary contact at biogeographic borders (termed a "suture zone": Remington, 1968; Hewitt, 2000; but see Swenson and Howard, 2004). In the marine environment hybrid zones often group on biogeographic borders (Gardner, 1997), for example, the Florida peninsula (America; Avise, 2000), the entrance of the Baltic Sea (Johannesson and Andre, 2006), and the Indo-Pacific border in the tropical eastern Indian Ocean (Hobbs et al., 2009).

In addition to secondary contact of allopatric species, hybridisation is also promoted by abundance disparities (Arnold, 1997), environmental gradients (Hewitt, 1988; Gardner, 1997), disruption to habitat (Rhymer and

Simberloff, 1996; Arnold, 1997; Seehausen et al.,1997; Coyne and Orr, 2004) and introduced species, including exotic predators (Rhymer and Simberloff, 1996; Roush, 1997; Taylor et al., 2006). Although these factors have been examined in detail in the terrestrial and freshwater environments we are only beginning to learn how these factors affect hybridisation in the marine environment (McMillan et al., 1999; Bierne et al., 2003; van Oppen and Gates, 2006; van Herwerden et al., 2006; Frisch and van Herwerden, 2006; Yaakub et al., 2006). The applicability of terrestrial based models to the marine environment may be limited given the distinct differences in the life history of terrestrial and marine organisms. For instance, dispersal ability plays a central role in the formation of hybrid zones and the dispersive larval phase coupled with high fecundities of most marine organisms means that their dispersal ability is many times greater than that of terrestrial organisms, thereby creating much wider hybrid zones (e.g., McMillan et al., 1999). Therefore, we cannot assume that the conditions conducive to hybridisation in the terrestrial environment are the same for the marine environment.

Hybridisation is most common among recently diverged taxa and there is a taxonomic bias with fishes being one of the most common groups of animals to hybridise (Mayr, 1979; Avise, 2004; Mallet, 2005). Traditionally, it was thought that freshwater fishes hybridise more than marine fishes, and temperate species more than tropical species, thus hybridisation was considered to be lacking in tropical marine fishes (Hubbs, 1955). However, this generalisation appears to be premature and reflected the lack of research on tropical marine fishes at the time. By 1956, there appeared to be only six instances of hybridisation in marine fishes, compared to 129 cases in freshwater fishes (Randall, 1956). Following the advent of SCUBA diving and an increase in marine research there has been a surge of new reports of hybrid marine fishes. By 1997 at least 172 marine species had been reported to hybridise, and bony fishes were the most common group accounting for 56% of the reported hybrids (Gardner, 1997; Fig. 3.1). Of the bony fishes, coral reef fishes have the most reported hybrids with at least 75 species implicated in hybridisation (Yaakub et al., 2006).

Among coral reef fishes, butterflyfishes (family Chaetodontidae) have the highest number of reported hybrids of any family, with approximately 30 species reported to form hybrids with congenerics (Yaakub et al., 2006). Butterflyfishes are also among the best-studied group of coral reef fishes, with considerable data available on species distributions (McMillan et al., 1999), phylogenetic relationships (Fessler and Westneat, 2007; Bellwood et al., 2009), ecology (e.g., Pratchett, 2005; Cole et al., 2008; Cole and Pratchett, Chapter 5), and behavioural interactions (Robert and Ormond, 1992; Wrathall et al., 1992; Berumen and Pratchett, 2006), thereby providing a unique opportunity to test terrestrially-derived theories of hybridisation in the world's most diverse vertebrate community. The primary aim of this
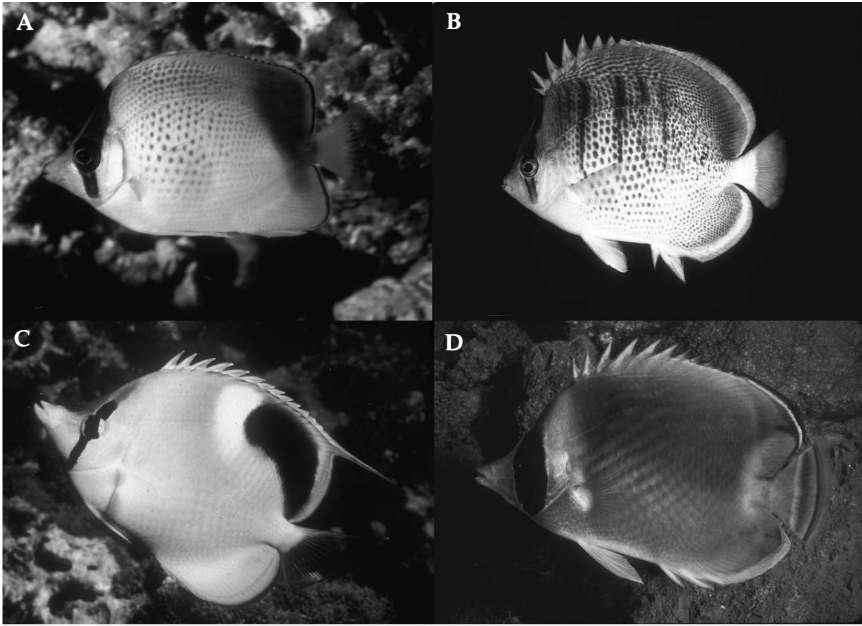
Fig. 3.1 Hybrid butterflyfishes. **(A)** *C. milliaris* x *C. tinkeri*, **(B)** *C. multicinctus* x *C. quadrimaculatus*, **(C)** *C. ephippium* x *C. semion*, **(D)** *C. auriga* x *C. lunula* (All photos: G. Allen).

Colour image of this figure appears in the colour plate section at the end of the book.

chapter is to document the range of hybrid butterflyfishes that have been recorded and consider whether there is a biogeographical, phylogenetic, ecological or behavioural basis to explain the occurrence of hybrids within this family.

In this review we use the term "hybrid" to include individuals that have been identified as hybrids based on intermediate colour (Fig. 3.1). Although genetic analyses are usually required to confirm hybridisation, these techniques have only been applied relatively recently for reef fish hybrids (McMillan et al., 1999; van Herwerden and Doherty, 2006; van Herwerden et al., 2006; Yaakub et al., 2006, 2007; Kuriiwa et al., 2007; Marie et al., 2007). Of the few molecular studies conducted so far on butterflyfishes, all confirm the status of hybrids identified based on intermediate colouration (McMillan et al., 1999; Montanari et al., 2012; Montanari et al., unpublished data). Therefore, intermediate colouration appears to be a good proxy for hybridisation in butterflyfish, although further genetic studies are required to confirm this assumption. This review also regards formation of heterospecific pairs (Fig. 3.2) as tentative evidence of hybridisation, because butterflyfishes mostly breed monogamously (Yabuta, 1997) and
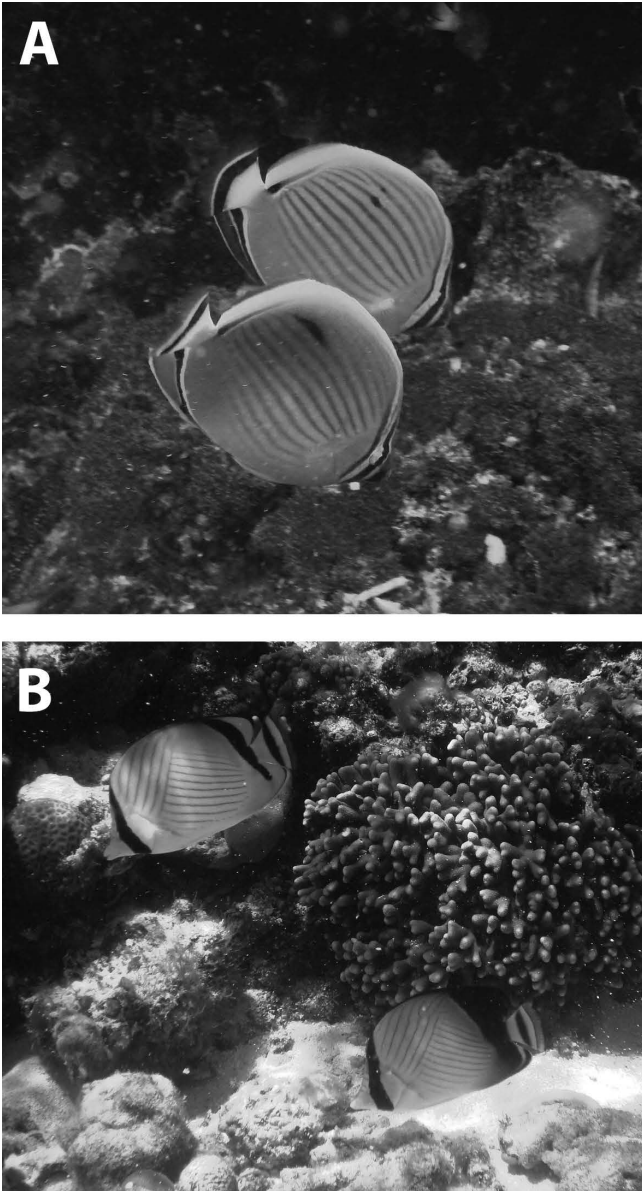
Fig. 3.2 Heterospecific pairs of butterflyfishes; **(A)** *C. trifasciatus* and *C. lunulatus* at Christmas Island (Indian Ocean), **(B)** *C. decussatus* and *C. vagabundus* at Ashmore Reef, Western Australia. More than 10 heterospecific pairs of these species were observed at each of the respective locations. Hybrid *C. trifasciatus* x *C. lunulatus* have also been observed at Christmas Island (Photos: JP Hobbs).

Colour image of this figure appears in the colour plate section at the end of the book.

pair-formation tends to correspond with the onset of sexual maturation and is likely to be for the purpose of reproduction (Pratchett et al., 2006a). As for pairings within species, heterospecific pairs of butterflyfishes examined so far are heterosexual with mature gonads (Hobbs, unpublished data) and are therefore probably interbreeding. Moreover, heterospecific pairs are often seen in locations where hybrids of these putative parental species are recorded (Hobbs et al., 2009). The formation of heterospecific pairs is a critical step in hybridisation because it shows a breakdown in assortative mating and identifies the conditions that enable this reproductive barrier to be overcome. For some heterospecific pairs, resulting hybrids have not been seen. This could be because the hybrids are rare, or they have the colour pattern of one of the parent species (McMillan et al., 1999), or interbreeding does not produce viable hybrid offspring. Failure to produce hybrids from interbreeding is important because it provides unique insights into the mechanism that establishes reproductive isolation (and therefore speciation) in butterflyfishes.

INCIDENCE OF HYBRIDISATION

Pyle and Randall (1994) reported that butterflyfishes are the family of marine fishes with the greatest number of hybrids, based on 15 reported crosses and at least 12 other known crosses at that time. As a proportion of species, Yakuub et al. (2006) suggested that the angelfishes (family Pomacanthidae) were the predominant family of marine fishes to hybridise, but they conceded that in absolute terms, there are more species of butterflyfishes than angelfishes that form hybrids. We know of 34 naturally occurring crosses, mostly based on observations of hybrid individuals with intermediate colouration (Table 3.1). There are also 6 further combinations of species that have been seen to form heterospecific pairs, which are likely to lead to interspecific reproduction even though hybrid offspring have not yet been reported. Excluding heterospecific pairs, there are 44 species of butterflyfishes that represent putative parental species of reported hybrids. Since there are nominally 114 species of butterflyfishes (Fessler and Westneat, 2007), the proportion of species that form hybrids within this family is 38.6%, which is much higher than has been reported within other families of marine fishes (Yakuub et al., 2006), and higher than the average proportion of hybridising plant (25%) and animal (10%) species (Mallet, 2005). The true proportion of hybridising butterflyfish species is likely to be even higher because hybrids often go undetected (Mallet, 2005, 2007) or observed crosses are yet to be reported.

For most coral reef fishes, hybridisation is restricted to a few genera within each family (Yakuub et al., 2006). Within the Labridae for example, most of the hybridising species belong to the genus *Thalassoma* (Walsh

Table 3.1 Parental species (and sub-genera) for reported hybrids. The feeding mode for each species is also given: O, obligate corallivores; F, facultative corallivores; and N, non-corallivore. Support for each hybrid in increasing order of significance is given: P, heterospecific pairing; C, intermediate colouration; M, morphological evidence (e.g., mersitics), and G, genetic confirmation. Citations given were selected to provide greatest support for each reported hybrid.

Species 1	Species 2	Diet [1,2]	Support	Key reference
C. adiergastos (Rhombochaetodon)	*C. collare* (Rhombochaetodon)	F, F	P	Hobbs (Pers. Obs.)
C. argentatus (Rhombochaetodon)	*C. mertensii* (Rhombochaetodon)	F, F	C	Allen et al. (1998)
C. argentatus (Rhombochaetodon)	*C. xanthurus* (Rhombochaetodon)	F, F	C	Michael (2004)
C. aureofasciatus (Discochaetodon)	*C. rainfordi* (Discochaetodon)	O, O	M	Randall et al. (1977)
C. auriga (Radophorus)	*C. ephippium* (Radophorus)	F, F	M	Randall et al. (1977)
C. auriga (Radophorus)	*C. vagabundus* (Radophorus)	F, F	P	Pratchett (Pers. Obs.)
C. auriga (Radophorus)	*C. ulientensis* (Radophorus)	F, F	P	Pratchett (Pers. Obs.)
C. auriga (Radophorus)	*C. fasciatus* (Chaetodontops)	F, F	C	Randall and Firdman (1981)
C. auriga (Radophorus)	*C. lunula* (Chaetodontops)	F, F	C	Kuiter (2002)
C. auriga (Radophorus)	*C. trifasciatus* (Corallochaetodon)	F, O	C	Schroeder (2006)
C. auriga (Radophorus)	*C. rafflesi* (Radophorus)	F, F	C	Hobbs (Pers. Obs.)
C. auripes (Chaetodontops)	*C. lunula* (Chaetodontops)	F, F	C	Michael (2004)
C. austriacus (Corallochaetodon)	*C. melapterus* (Corallochaetodon)	O, O	C	Kuiter (2002)
C. baronessa (Gonochaetodon)	*C. triangulum* (Gonochaetodon)	O, O	P	Kuiter (2002)
C. burgessi (Roaops)	*C. tinkeri* (Roaops)	N, N	C	Allen et al. (1998)
C. burgessi (Roaops)	*C. flavocoronatus* (Roaops)	N, N	C	Allen et al. (1998)
C. daedalma (Lepidochaetodon)	*C. nippon* (Lepidochaetodon)	F, F	C	Allen et al. (1998)
C. decussatus	*C. vagabundus* (Radophorus)	F, F	P	Hobbs (Pers. Obs.)
C. ephippium (Radophorus)	*C. semeion* (Radophorus)	F, N	M	Randall et al. (1977)
C. ephippium (Radophorus)	*C. xanthocephalus* (Radophorus)	F, F	C	Allen et al. (1998)

Table 3.1 contd....

C. falcula	*C. ulientensis* (Radophorus)	N, F	P	Hobbs (Pers. Obs.)
C. guentheri (Heterochaetodon)	*C. oxycephalus* (Rhaphorus)	N, F	C	Kuiter (2002)
C. guentheri (Heterochaetodon)	*C. daedallma* (Lepidochaetodon)	N, F	M	Sano et al. (1984)
C. guttatissimus (Exornator)	*C. punctatofasciatus* (Exornator)	F, F	G	S. Montanari (unpub. data)
C. kleinii (Lepidochaetodon)	*C. unimaculatus* (Lepidochaetodon)	F, F	M	Randall et al. (1977)
C. larvatus (Gonochaetodon)	*C. triangulum* (Gonochaetodon)	O, O	C	Michael (2004)
C. lineolatus (Radophorus)	*C. oxycephalus* (Radophorus)	N, F	C	Anon.
C. lunulatus (Corallochaetodon)	*C. trifasciatus* (Corallochaetodon)	O, O	G	Montanari et al., 2012
C. mertensii (Rhombochaetodon)	*C. xanthurus* (Rhombochaetodon)	F, F	C	Froese and Pauly (2009)
C. meyeri (Citharoedus)	*C. ornatissimus* (Citharoedus)	O, O	C	Allen et al. (1998)
C. miliaris (Heterochaetodon)	*C. multicinctus* (Exornator)	N, O	C	Michael (2004)
C. miliaris (Heterochaetodon)	*C. tinkeri* (Rhaphorus)	N, N	M	Randall et al. (1977)
C. multicinctus (Exornator)	*C. quadrimaculatus* (Exornator)	O, O	P	Fig 3.1B
C. multicinctus (Exornator)	*C. pelewensis* (Exornator)	O, F	P*	McMillan et al. (1999)
C. multicinctus (Exornator)	*C. punctatofasciatus* (Exornator)	O, F	P*	McMillan et al. (1999)
C. ocellatus (Chaetodon)	*C. striatus* (Chaetodon)	F, N	M	Clavijo (1985)
C. pelewensis (Exornator)	*C. punctatofasciatus* (Exornator)	F, F	G	McMillan et al. (1999)
C. rafflesi (Radophorus)	*C. vagabundus* (Radophorus)	F, F	C	Kuiter (2002)
C. reticulatus (Chaetodontops)	*C. meyeri* (Cithaoedus)	O, O	C	Senou et al. (2006)
C. reticulatus (Chaetodontops)	*C. ornatissimus* (Cithaoedus)	O, O	C	Senou et al. (2007)

P*—Allopatric species observed to form pairs in captivity.

and Randall, 2004). Similarly, all reported hybrids within the family Chaetodontidae occur among species from the genus *Chaetodon*. *Chaetodon* butterflyfishes account for the majority (87/114) of species within the family Chatodontidae, and more than half (44/87) of *Chaetodon* species appear to form hybrids. Some species, most notably *Chaetodon auriga,* appear to hybridise with a range of different species, whereas other species (e.g., *C. baronessa*) only hybridise with closely related sister species. There is little data on the occurrence of distinct hybrids, though most natural crosses have been described based on a single individual bearing distinctive colouration or markings. However, in areas where *C. pelewensis* and *C. punctatofasciatus* co-occur, up to 70% of individuals bear markings that appear to be intermediary between the two species, indicating very high levels of hybridisation (McMillan et al., 1999).

By any measure, *Chaetodon* butterflyfishes appear particularly predisposed to hybridisation. This may however, be a sampling artefact, as suggested by Randall and Fridman (1981) and Yakuub et al. (2006). The high number of reported hybrids within the family Chaetodontidae, and also Pomacanthidae, may be because they are very conspicuous reef fishes, occurring in relatively low densities, and are also very popular with underwater photographers and aquarists (Randall and Firdman, 1981; Pyle and Randall, 1994). Nominal species within these families are also fairly distinct and mostly have very consistent colouration (McMillan et al., 1999), meaning that hybrids may be more easily recognised than in most other families of coral reef fishes. If butterflyfishes really are predisposed to hybridisation, there are a number of evolutionary, ecological, and behavioural processes that may explain the high incidence of hybrids.

GEOGRAPHIC OCCURRENCE OF BUTTERFLYFISH HYBRIDS

The global distribution of marine hybrids is not uniform (Gardner, 1997), rather there are concentrations occurring in certain locations (Avise, 2000; Johannesson and Andre, 2006; Hobbs et al., 2009). Based on available reports, nearly 90% (34 of 38) of butterflyfish hybrids and heterospecific pairs are clustered in four geographic areas: southern Japan, Hawaii, Papua New Guinea-Micronesia and the eastern Indian Ocean (including Cocos (Keeling) Islands, Christmas Island, southern Indonesia and the offshore reefs of north-west Australia; Fig. 3.3). It is important to acknowledge that sampling intensity and species richness can affect geographic patterns of reported hybrids. For example, the lack of reported hybrid locations in the West Indian Ocean may be partly due to lower sampling intensity, while the dearth of hybrids in the Eastern Pacific-Atlantic area may reflect the low diversity of butterflyfishes in this region. However, there are many
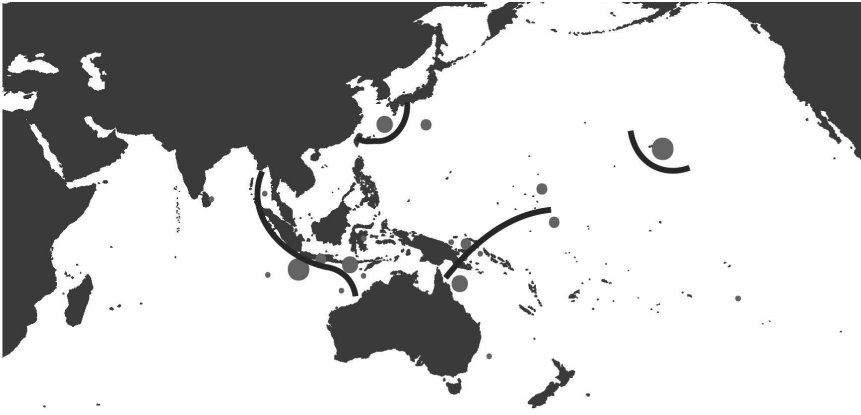
Fig. 3.3 The geographic distribution of reported locations of butterflyfish hybrids and heterospecific pairs. Some hybrids or heterospecific pairs have been observed at multiple locations and for these cases each reported location is represented by a dot (with the caveat that it is 300 km from another reported location). The size of the circle is representative of the number of species pairs that have formed heterospecific pairs or produced hybrids at a reported location, with the largest circles symbolising four cases. Not all instances from Table 3.1 are presented because locations were not always reported. The positions of four recognised biogeographic borders are illustrated with black lines (Blum, 1989; Bellwood and Wainwright, 2002; Hsu et al., 2007).

Colour image of this figure appears in the colour plate section at the end of the book.

well-studied and high diversity locations that lack reports of hybrids. Despite differences in sampling intensity and species richness, the four areas identified above as butterflyfish hybrid "hotspots" are also locations where hybrids of many other reef fish taxa have been found (Gardner, 1997; Pyle and Randall, 1994; Kuriiwa et al., 2007; Hobbs et al., 2009).

Importantly, these four areas of butterflyfish hybridisation align with recognised biogeographic borders (Blum, 1989; Bellwood and Wainwright, 2002; Hsu et al., 2007). Even the minority of cases that fall outside these four areas lie on other proposed biogeographic borders (Blum, 1989; Bellwood and Wainwright, 2002; Hsu et al., 2007). Why are butterflyfish forming heterospecific pairs and producing hybrids at biogeographic borders? This phenomenon occurs regularly in the terrestrial and freshwater environments because biogeographic borders represent areas of confluence between two regional biotas resulting in secondary contact between recently diverged, allopatric species (suture zones: Remington 1968; Avise, 2000; Hewitt, 2000). Examination of the geographic distribution of the species pairs in Table 3.1 (excluding cases involving endemics) reveals that only 13 of 35 cases involve pair formation or hybridisation between typically allopatric species. Thus, the possibility that hybridisation in butterflyfishes is due to secondary contact of allopatric species is only supported in about a third of cases. The remaining 22 cases represent hybridisation between sympatric species.

Given sympatric species co-occur throughout their geographic range, why do they only hybridise at biogeographic borders? Biogeographic borders by their very nature represent the edge of a range for many species and hybridisation is common at range edges (Mayr, 1963; Hewitt, 1988). Examination of locations where sympatric butterflyfish hybrids or heterospecific pairs have been reported revealed that in the majority of cases (14 of 17) at least one of the species is at its range edge (endemic species excluded from analysis). This indicates that butterflyfishes capable of hybridising or forming heterospecific pairs only do so under certain conditions that are present at peripheral locations. Species tend to have low abundances at the edge of their range (Brown, 1984), and therefore a lack of conspecific partners would increase the likelihood of hybridisation at the range edge. Many of these peripheral locations are also isolated islands and therefore likely to a have a relatively high proportion of both vagrants and resident species with small populations, and the lack of conspecifics would increase the chances of hybridisation (Rhymer and Simberloff, 1996; Rose and Polis, 2000; Hobbs et al., 2007, 2009). It is also possible that because species richness and abundance are generally low at peripheral and isolated locations, hybrids may be more prevalent as a result of utilising unoccupied niches (Lexer et al., 2003; Rieseberg et al., 2003; Willis et al., 2006). Either way, it is clear that hybridisation in butterflyfishes (both allopatric and sympatric species) is largely constrained to locations at the edge a species range.

PHYLOGENY OF HYBRID BUTTERFLYFISHES

Hybridisation involves the recombination of genetic material from two distinct species, requiring that parental species have compatible genotypes. As genotypes diverge, the probability of successful recombination of genetic material declines (Jiggins and Mallet, 2000), thereby maintaining stable species boundaries between species with incompatible genomes. It is expected therefore, that occurrence of hybrids is not random with respect to phylogenetic relationships within any given taxon. Rather, there would be a tendency to have the greatest occurrence of hybrids among most closely and recently diverged species pairs (Mallet, 2005). Butterflyfish are an excellent group to test this idea given the prevalence of hybridisation.

The most comprehensive phylogenetic study of butterflyfish published to date (Fessler and Westneat, 2007), includes at least 25 species pairs that are known to hybridise and another 10 species for which the hybrid partner was not sampled in the study. Four distinct phylogenetic clades were identified and hybridisation is reported within three of the four clades (Fig. 3.4). Only Clade 1 species, represented by two species, do not appear to undergo hybridisation. Clade 2 contains six closely related species pairs that are
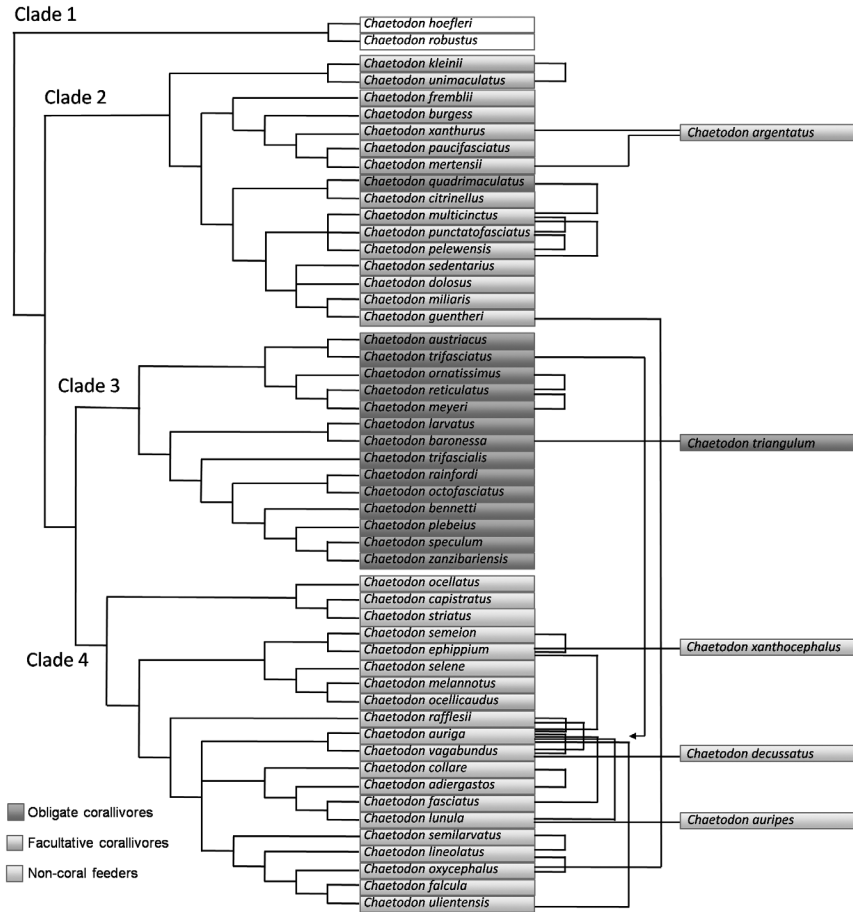
Fig. 3.4 Phylogenetic relationships among pairs of butterflyfishes known to hybridise. The phylogeny presented is based on Fessler and Westneat (2007), indicating 4 major clades that broadly correspond with major trophic guilds (indicated by colours). Hybridisations involving species within the current phylogeny are shown by linkages between species on the right side of the figure.

Colour image of this figure appears in the colour plate section at the end of the book.

hybridising and another 4 species for which their hybridising partner was not represented in the Fessler and Westneat (2007) study. Clade 3 species are less prone to hybridisation, with two species pairs crossing and another 5 species for which the other hybridising parent species was not sampled by Fessler and Westneat (2007). Hybridisation is most common in Clade 4, with 15 species pairs hybridising, not including the one species for which its hybrid partner was not sampled by Fessler and Westneat (Table 3.1;

Fig. 3.4). Only two species pairs hybridised between different clades and both of these were Clade 3 x Clade 4 crosses. One species, *C. auriga* hybridises with at least 9 other species. Overall Clade 4 contains nearly 50% of the hybridising butterflyfish species pairs (including the recently reported hybrid offspring of *C. semilarvatus* and *C. lineolatus* in the Red Sea). These observations suggest that hybridisation is most often observed between closely related butterflyfishes (as identified by molecular genetic data) and rarely between divergent species not sharing recent common ancestors, which is consistent with findings from the terrestrial environment (Mallet, 2005). Even so, it appears that certain monophyletic groups are more prone to hybridsation (e.g., Clade 4) compared to others (e.g., Clade 2).

ABUNDANCE DISPARITIES AND HABITAT OVERLAP

Hybridisation is also more likely where there are abundance disparities (Arnold, 1997) or if both parent species are rare. Based on the authors' unpublished data and observations, at least one of the parent species is rare (average density less than 2 per 1 hr dive or approximately 3000 m^2) in 11 of the 19 (58%) surveyed locations where butterflyfish hybrids or heterospecific pairs have been recorded. Thus, a lack of conspecific mates would increase the chance of hybridisation. However, the data also reveal that hybrids sometimes occur at locations where both parent species are moderately common or abundant (assuming the hybrids were produced at that location). Although a species may be common at a location, an individual of a common species may be restricted to a particular habitat (e.g., an isolated patch reef) where an absence of conspecific partners forces it to pair or mate with a congeneric. Alternatively, hybridisation between common species may occur due to sneak spawning, opportunistic interbreeding or accidental hybridisation, as has been suggested for some other common reef fishes (Frisch and van Herwerden, 2006; Yaakub et al., 2006, 2007; Walker and Ryen, 2007).

The formation of heterospecific pairs and subsequent hybridisation is more likely where two species overlap in their habitat use (Arnold, 1997). To test this, we used published estimates of the depth distribution of butterflyfishes (Allen et al., 1998) as a broad measure of habitat overlap. For species that hybridise or form heterospecific pairs, we found that in 36 of the 40 cases (90%) the species pairs overlap by 50% or more in their depth distribution. Although, some species pairs may differ in habitat use at the reef zone or microhabitat level, the overlap in depth distribution indicates that individuals are likely to have plenty of heterospecific encounters and this increases the possibility of hybridisation. Heterospecific encounters, and therefore the potential for hybridisation, would be much less likely

in reef fishes where congeners occupy discrete and separate habitats (e.g., coral gobies; Munday et al., 1997).

BEHAVIOURAL TRAITS OF HYBRID BUTTERFLYFISHES

While phylogenetic affinities, disparities in abundance and overlap in the depth distribution can all increase the likelihood of hybridisation, inter-specific variation in reproductive modes and behaviour can further affect the possibility of hybridisation. Differences in the timing or mode of reproduction among sympatric species represent impermeable barriers to heterospecific breeding (e.g., Palumbi, 1994), but different modes of reproduction are also more or less likely to result in hybridisation. *Thalassoma* wrasses (family Labridae) exhibit both mass-spawning, where males group-spawn with multiple females simultaneously (e.g., Warner, 1995), as well as pair-spawning, depending on the density of individuals and the sex and phase of the individuals (Sara et al., 2005). However, hybridisation between *Thalassoma* species (*T. quinquevittatum* and *T. jansenii*) at Holmes Reef in the Coral Seas is solely attributed to group-spawning, which enables initial phase males of *T. jansenii* to "sneak spawn" with harems of *T. quinquevittatum* (Yakuub et al., 2006). Sneak spawning has also been implicated in hybridisations among mass-spawning salmonid species (Garcia-Vazquez et al., 2002). However, Allen (1979) noted that it is the pair-spawning species of butterflyfishes and angelfishes that typically form hybrids. While some butterflyfishes (e.g., *C. melannotus*) are known to aggregate in certain locations and at specific times in the year, possibly for the purpose of mass-spawning (Pratchett et al., 2006a), the typical model of reproduction for butterflyfishes involves strong long-term pair formation and monogamous mating (Yabuta, 1997; Pratchett et al., 2006a; Yabuta and Berumen, Chapter 8). In this case, successful reproduction results from mutual consent by both partners. Monogamous mating combined with strong and consistent partner recognition (*sensu* Yabuta, 2000) should therefore provide significant barriers to hybridisation.

Assortative mating within and among species, whereby individuals choose mates of similar size and appearance, is considered to be an important process resulting in speciation and divergence of sympatric species that lack sexual dimorphism (e.g., Lande, 1981). Strong assortative mating and interspecific aggression will tend to reduce opportunities for hybridisation. However, many butterflyfishes form heterospecific pairs (Table 3.1), suggesting that assortative mating does not occur, or is relaxed, in some situations. McMillan et al. (1999) found that *C. pelewensis* and *C. punctatofasciatus*, which hybridise in a large contact zone, do not display assortative mating. When given a choice of mates, *C. pelewensis* and *C. punctatofasciatus* did not distinguish between conspecifics, congenerics

or hybrids. For these species, it appears that a different selective force is responsible for maintaining colour differences between the species, despite extensive levels of introgression beyond the hybrid zone (McMillan and Palumbi, 1995). Assortative mating has not been specifically tested for any other species of butterflyfishes, but the low incidence of hybrids in most locations suggest that strong assortative mating is important in maintaining species differences. It is probably only when conspecific mates are particularly scarce, that the desire to form pairs and reproduce overcomes strict adherence to conspecific mating. Even in these situations, innate aggression among some species may prevent pairing and interbreeding.

While partly confounded by phylogeny, butterflyfishes that feed exclusively on live corals (obligate corallivores) appear less likely to hybridise compared to facultative corallivores and non-coral feeders (Fig. 3.4). Obligate corallivores are highly territorial and very aggressive towards congenerics, whereas facultative corallivores and non-coral feeders tend to have large home-ranges, and rarely or only weakly defend these ranges against congenerics (Berumen and Pratchett, 2006). Strong interspecific aggression among obligate corallivores also begins almost immediately after settlement, which would largely prohibit formation of heterospecific pairs, which is fundamental to successful reproduction by monogamous pair-spawning species. Consequently, the number of hybrid butterflyfishes with one or more of their putative parents being an obligate corallivores is much less than expected base on the relative abundance of obligate corallivorous species versus facultative corallivores and non-coral feeders (Fig. 3.5). When obligate corallivores do hybridise, it is almost
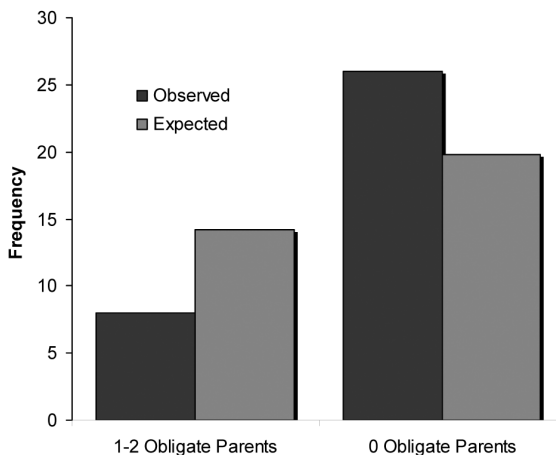

Fig. 3.5 Number of hybrid butterflyfishes with 1 or 2 versus 0 parental species that are obligate corallivores. There are significantly fewer than expected hybrids involving obligate corallivores ($\chi^2 = 28.7$, df = 1, p<0.05), possibly because strong aggression among obligate corallivores prevents inter-specific breeding.

always with their most recently diverged sibling species, which have similar appearance and habits, such as *C. trifasciatus* and *C. lunulatus*, *C. baronessa* and *C. trangulum*, or *C. austriacus* and *C. melapterus*. This species almost certainly evolved in allopatry and hybridisation resulted from secondary contact in small areas of overlap. Limited hybridisation among obligate corallivores may be due to genetic incompatibilities among more distantly related species, but this seems unlikely given that obligate coral-feeding species are no older than more generalist counterparts (Bellwood et al., 2009; Bellwood and Pratchett, Chapter 1). Rather, strong interspecific aggression may account for limited hybridisation within obligate corallivores and may even contribute to sympatric speciation within this group.

CONCLUSIONS

By combining data on biogeography, phylogeny, ecology and behaviour we can examine the conditions that facilitate the high level of hybridisation in butterflyfishes. Hybridisation occurs most often between recently diverged species at locations on the edge of their geographic range, where one or both parent species are rare and both species occupy the same habitat and do not aggressively defend territories from congenerics (e.g., Montanari et al., 2012). Any of these factors can promote hybridisation, however if these factors are associated then their combination can greatly increase the chance of hybridisation. For example, hybridisation amongst closely related species also means they probably have similar ecology, feeding and behavioural traits. The first three factors are also interrelated in that the edge of a species range often represents a biogeographic border and this may put a species in contact with a closely related sister species (Hobbs and Salmond, 2008). Species at the edge of their range also tend to have low abundances (Brown, 1984) and isolated locations also have a high proportion of vagrant species with low abundances (Rhymer and Simberloff, 1996; Rose and Polis, 2000; Hobbs et al., 2007, 2009). Thus it is not the locations *per se* that promote hybridisation, but rather the conditions that are associated with the biogeographic position of these locations.

Given the above conditions, the most likely scenario for hybridisation to occur is that at the edge of its geographic range a species has low abundance and therefore an individual may have difficulty finding a conspecific partner. However, because range edges usually coincide with biogeographic borders a species may come into contact with an allopatric sister species. For sympatric species at the edge of their range, searching for conspecifics may put them in contact with another closely related species with similar ecological and behavioural traits. The chances of heterospecific contact are increased if two species overlap in habitat use and the formation of a heterospecific pair is more likely if the two species are not territorial or

aggressive to congenerics (i.e., facultative corallivores and non-coral feeders). Once the assortative mating barrier has been overcome, heterospecific pairs are likely to interbreed and the production of hybrids occurs if there are no post-zygotic barriers and if the hybrids have sufficient fitness to survive and reproduce in a particular niche. This scenario would fit with most cases of butterflyfish hybridisation, however accidental hybridisation, opportunistic interbreeding or sneak spawning may be occurring at some locations where both parent species are common.

Hybridisation in butterflyfishes (and reef fishes in general) may become more common in the future as climate change and other anthropogenic impacts modify the marine the environment. Increasing habitat destruction due to multiple disturbances (particularly coral bleaching) can reduce the abundance of butterflyfishes in affected locations (Wilson et al., 2006; Pratchett et al., 2006b, 2008), and a lack of conspecifics partners would promote interbreeding and hybridisation. Anthropogenic modification of habitat structure may also increases hybridisation (Rhymer and Simberloff, 1996; Arnold, 1997; Seehausen et al., 1997; Coyne and Orr, 2004). In coastal marine habitats, the construction of break walls has created new contact zones leading to hybridisation between fish species that normally occupy different habitats within distinct depth ranges (Kimura and Munehara, 2009). Increased turbidity has also impaired mate recognition and led to hybridisation in freshwater fishes (Seehausen, 2006), and a similar scenario may occur on coral reefs as a consequence of sustained and ongoing sedimentation and eutrophication adjacent to major urban centres. The forecasted acidification of the ocean can also affect the sensory system of some reef fish and inhibit the ability to discriminate between suitable mates (Munday et al., 2009). Therefore, the conditions known to promote hybridisation (e.g., abundance disparities, overlap in habitat use, breakdown of assortative mating) are predicted to become more common in the future because of the increasing alteration of the physical and chemical properties of the marine environment brought about by a multitude of human impacts.

FUTURE DIRECTIONS

The combination of distinct colour patterns of adults and hybrids, a pair-forming social system, well known geographic distribution, established phylogeny and evidence of unprecedented levels of hybridisation make butterflyfish a fitting group in which to explore hybridisation in coral reef fishes. Because we know so little about hybridisation in reef fishes, areas of future interest are numerous. As a starting point we need to test if terrestrial theories apply to the marine environment. One area that sparks immediate interest is the overcoming of pre-zygotic reproductive isolation

mechanisms through the formation of heterospecific pairs. Assuming that these heterospecifc pairs interbreed and produce viable offspring (which needs to be confirmed) the breakdown of assortative mating is crucial in the formation of hybrids. A combination of mate choice experiments, the collection and sexing of heterospecific pairs and hybrids from natural populations, in combination with molecular evidence of unidirectional or bidirectional matings will elucidate how these pre-zygotic barriers are overcome.

Another area of importance relates to post-zygotic isolating mechanisms and determining if the hybrid offspring are viable, fertile and what their fitness is relative to parent species within and outside of the hybrid zone. This will determine whether hybrids are an evolutionary "dead-end" or whether they play a potentially significant role in the evolution of species. To ascertain fitness will require a combination of in-depth genetic analyses and some estimates of fitness (e.g., growth and fecundity) for hybrids and parent species. Tank experiments and *in vitro* fertilisation can be used to test if hybrids produce viable and compatible gametes (Frisch and van Herwerden, 2006). Genetic analysis will be able to determine whether the hybrids are successfully backcrossing with the parent species and the level and direction of introgression.

It has been argued that rapid radiations of species have occurred in the terrestrial and freshwater environments (and possibly in marine corals) because hybrids have occupied unexploited niches, often at the edge of their ranges where the abundances of the parent species are low (Seehausen, 2004; van Oppen and Gates, 2006; Willis et al., 2006). Given that butterflyfishes fit this pattern (i.e., a speciose group with hybridisation often occurring at range edges where abundances of parent species is often low) it is worth examining if hybrids exploit unoccupied niches. This could be achieved by comparing diet and habitat use of the hybrids and parent species, following Montanari et al. (2012).

Ultimately, genetic studies using both nuclear and mitochondrial (maternal) markers will allow us to confirm the hybrid status of putative hybrids and the parental contributors, the presence of uni- and/or bi-directional hybridisation and the extent of introgression. Previous studies have shown that producing viable first generation (F1) hybrids is most difficult, but once produced, the door is opened and may allow for reinforcement of reproductive barriers (Marshall et al., 2002), hybrid speciation (Hegarty and Hiscock, 2004), extinction (Rhymer and Simberloff, 1996) or reverse-speciation (Seehausen, 2006; Taylor et al., 2006). Molecular studies of butterflyfish hybrids provide the ideal opportunity to determine how hybridisation impacts on the generation, maintenance and loss of coral reef fish biodiversity.

REFERENCES

Allen, G.R. 1979. *Butterfly and angelfishes of the world.* Vol. 2, John Wiley and Sons, New York, USA.

Allen, G.R., R. Steene and M. Allen. 1998. *A guide to angelfishes and butterflyfishes*, Odyssey Publishing, Perth, Australia.

Arnold, M.L. 1997. *Natural hybridisation and evolution*, Oxford University Press, New York, USA.

Avise, J.C. 2000. *Phylogeography*, Harvard University Press, Cambridge, USA.

Avise, J.C. 2004. *Molecular markers, natural history, and evolution*, Sinauer Associates, Sunderland, USA.

Barton, N.H. and G.M. Hewitt. 1985. Analysis of hybrid zones. *Annual Review of Ecology Evolution and Systematics* 16: 113–148.

Barton, N.H. and G.M. Hewitt. 1989. Adaptation, speciation and hybrid zones. *Nature* 341: 497–503.

Bellwood, D.R. and P.C. Wainwright. 2002. The history and biogeography of fishes on coral reefs. In *Coral reef fishes: dynamics and diversity in a complex ecosystem*, P.F. Sale (ed.). Academic Press, San Diego, USA pp. 5–32.

Bellwood, D.R., S. Klanten, P.F. Cowman, M.S. Pratchett, N. Konow and L. van Herwerden. 2009. Evolutionary history of the butterflyfishes (f: Chaetodontidae) and the rise of coral feeding fishes. *Journal of Evolutionary Biology* 23: 335–349.

Berumen, M.L. and M.S. Pratchett. 2006. Effects of resource availability on the competitive behaviour of butterflyfishes (Chaetodontidae). *Proceedings of the 10th International Coral Reef Symposium, Okinawa* pp. 644–650.

Bierne, N., P. Borsa, C. Daguin, D. Jollivet, F. Viard, F. Bonhomme and P. David. 2003. Introgression patterns in the mosaic hybrid zone between *Mytilusedulis* and *M. galloprovincialis*. *Molecular Ecology* 12: 447–461.

Blum, S.D. 1989. Biogeography of the Chaetodontidae: an analysis of allopatry among closely related species. *Environmental Biology of Fishes* 25: 9–31.

Brown, J.H. 1984. On the relationship between abundance and distribution of species. *American Naturalist* 124: 255–279.

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

Cole, A.J., M.S. Pratchett and G.P. Jones. 2008. Diversity and functional importance of coral-feeding fishes on tropical coral reefs. *Fish and Fisheries* 9: 286–307.

Coyne, J.A. and H.A. Orr. 2004. *Speciation*, Sinauer Associates, Sunderland, USA.

Fessler, J.L. and M.W. Westneat. 2007. Molecular phylogenetics of the butterflyfishes (Chaetodontidae): Taxonomy and biogeography of a global coral reef fish family. *Molecular Phylogenetics and Evolution* 45: 50–68.

Frisch, A.J. and L. van Herwerden. 2006. Field and experimental studies of hybridisation between coral trouts, *Plectropomus leopardus* and *Plectropomus maculatus* (Serranidae), on the Great Barrier Reef, Australia. *Journal of Fish Biology* 68: 1013–1025.

Froese, R. and D. Pauly, eds. 2009. *FishBase*. World Wide Web electronic publication: www.fishbase.org, version (07/2009).

Garcia-Vazquez, E., P. Moran, J. Perez, J. Martinez, J. Izquierdo, B. De Gaudemar and E. Beall. 2002. Interspecific barriers between salmonids when hybridisation is due to sneak mating. *Heredity* 89: 288–292.

Gardner, J. 1997. Hybridisation in the sea. *Advances in Marine Biology* 31: 1–78.

Harrison, R.G. 1993. Hybrids and hybrid zones: historical perspective. In *Hybrid zones and the evolutionary process*, R.G. Harrison (ed.). Oxford University Press, Oxford, UK pp. 3–12.

Hegarty, M.J. and S.J. Hiscock. 2004. Hybrid speciation in plants: new insights from molecular studies. *New Phytologist* 165: 411–423.

Hewitt, G.M. 1988. Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution* 3: 158–167.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907–913.

Hobbs, J.P.A. and J. Salmond. 2008. Cohabitation of Indian and Pacific ocean species at Christmas and Cocos (Keeling) islands. *Coral Reefs* 27: 933–933.

Hobbs, J.P.A., A.J. Frisch, J. Hender and J.J. Gilligan. 2007. New records of Angelfishes (Pomacanthidae) and Butterflyfishes (Chaetodontidae) from Christmas and Cocos (Keeling) Islands, Indian Ocean. *Journal of the Royal Society of Western Australia* 90: 107–109.

Hobbs, J.P.A., A.J. Frisch, G.R. Allen and L. van Herwerden. 2009. Marine hybrid hotspot at Indo-Pacific biogeographic border. *Biology Letters* 5: 258–261.

Hsu, K.C., J.P. Chen and K.T. Shao. 2007. Molecular phylogeny of *Chaetodon* (Teleostei: Chaetodontidae) in the Indo-West Pacific: evolution in geminate species pairs and species groups. *The Raffles Bulletin of Zoology* s14: 77–86.

Hubbs, C.L. 1955. Hybridisation between fish species in nature. *Systematic Zoology* 4: 1–20.

Jiggins, C.D. and J. Mallet. 2000. Bimodal hybrid zones and speciation. *Trends in Ecology & Evolution* 15: 250–255.

Johannesson, K. and C. André. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Molecular Ecology* 15: 2013–2029.

Kimura, M.R. and H. Munehara. 2010. The disruption of habitat isolation among three Hexagrammos species by artificial habitat alterations that create mosaic-habitat. *Ecological Research* 25: 41–50.

Kuiter, R.H. 2002. *Butterflyfishes, Bannerfishes and their Relatives. A comprehensive Guide to Chaetodontidae and Microcanthidae*. The Marine Fish Families Series, TMC Publishing, Chorleywood, UK.

Kuriiwa, K., N. Hanzawa, T. Yoshino, S. Kimura and M. Nishida. 2007. Phylogenetic relationships and natural hybridisation in rabbitfishes (Teleostei: Siganidae) inferred from mitochondrial and nuclear DNA analyses. *Molecular Phylogenetics and Evolution* 45: 69–80.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences* 78: 3721–3725.

Lexer, C., M.E. Welch, O. Raymond and L.H. Rieseberg. 2003. The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habitat. *Evolution* 57: 1989–2000.

Mallet, J. 2005. Hybridisation as an invasion of the genome. *Trends in Ecology & Evolution* 20: 229–237.

Mallet, J. 2007. Hybrid speciation. *Nature* 446: 279–283.

Marie, A.D., L. van Herwerden, J.H. Choat and J.P.A. Hobbs. 2007. Hybridization of reef fishes at the Indo-Pacific biogeographic barrier: a case study. *Coral Reefs* 26: 841–850.

Marshall, J.L., M.L. Arnold and D.J. Howard. 2002. Reinforcement: the road not taken. *Trends in Ecology & Evolution* 17: 558–563.

Mayr, E. 1963. *Animal Species and Evolution*, Belknap Press of Harvard University Press, Cambridge, USA.

Mayr, E. 1979. *Animal Species and Evolution*. 6 ed, Belknap Press, Cambridge, USA.

McMillan, W.O. and S.R. Palumbi. 1995. Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 260: 229–236.

McMillan, W.O., L.A. Weigt and S.R. Palumbi. 1999. Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution* 247–260.

Michael, S.W. 2004. *Angelfishes & Butterflyfishes*, TFH Publications, Neptune City, USA.

Montanari, S.R., L. van Herwerden, M.S. Pratchett, J.A. Hobbs and A. Fugedi. 2012. Reef fish hybridisation: lessons learnt from butterflyfishes (genus *Chaetodon*). *Ecology and Evolution* 2: 310–328

Munday, P.L., G.P. Jones and M.J. Caley. 1997. Habitat specialisation and the distribution and abundance of coral-dwelling gobies. *Marine Ecology Progress Series* 152: 227–239.

Munday, P.L., L. van Herwerden and C.L. Dudgeon. 2004. Evidence for sympatric speciation by host shift in the sea. *Current Biology* 14: 1498–1504.

Munday, P.L., D.L. Dixson, J.M. Donelson, G.P. Jones, M.S. Pratchett, G.V. Devitsina and K.B. Doving. 2009. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences* 106: 1848–1852.

Palumbi, S.R. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology Evolution and Systematics* 25: 547–572.

Pratchett, M.S. 2005. Dietry overlap among coral-feeding butterflyfishes (Chaetodontidae) at Lizard Island, northern Great Barrier Reef. *Marine Biology* 148: 373–382.

Pratchett, M.S., O.A. Pradjakusuma and G.P. Jones. 2006a. Is there a reproductive basis to solitary living versus pair-formation in coral reef fishes? *Coral Reefs* 25: 85–92.

Pratchett, M.S., S.K. Wilson and A.H. Baird. 2006b. Declines in the abundance of *Chaetodon* butterflyfishes following extensive coral depletion. *Journal of Fish Biology* 69: 1269–1280.

Pratchett, M.S., P.L. Munday, S.K. Wilson, N.A.J. Graham, J.E. Cinner, D.R. Bellwood, G.P. Jones, N.V.C. Polunin and T.R. Mcclanahan. 2008. Effects of climate-induced coral bleaching on coral-reef fishes—Ecological and economic consequences. *Oceanography and Marine Biology: An Annual Review* 46: 251–296.

Pyle, R.L. and J.E. Randall. 1994. A review of hybridization in marine angelfishes (Perciformes: Pomacanthidae). *Environmental Biology of Fishes* 41: 127–145.

Randall, J.E. 1956. *Acanthurus rackliffei*, a possible hybrid surgeon fish (*A. achilles* x *A. glaucopareius*) from the Phoenix Islands. *Copeia* 1: 21–25.

Randall, J.E. and D. Fridman. 1981. *Chaetodon auriga* x *Chaetodon fasciatus*, a hybrid butterflyfish from the Red Sea. *Revue. fr. Aquariol* 7: 113–116.

Randall, J.E., G.R. Allen and R.C. Steene. 1977. Five probable hybrid butterfly fishes of the genus *Chaetodon* from the central and western Pacific. *Records of the Australian Museum* 6: 3–26.

Remington, C.L. 1968. Suture-zones of hybrid interaction between recently joined biotas. *Evolutionary Biology* 2: 321–428.

Rhymer, J.M. and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology Evolution and Systematics* 27: 83–109.

Rieseberg, L.H., O. Raymond, D.M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J.L. Durphy, A.E. Schwarzbach, L.A. Donovan and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211–1216.

Roberts, C.M. and R.F.G. Ormond. 1992. Butterflyfish social behaviour, with special reference to the incidence of territoriality: a review. *Environmental Biology of Fishes* 34: 79–93.

Rose, M.D. and G.A. Polis. 2000. On the insularity of islands. *Ecography* 23: 693–701.

Roush, W. 1997. Hybrids consummate species invasion. *Science* 277: 316–317.

Sano, M., M. Shimizu and Y. Nose. 1984. Food Habits of teleostean reef fishes in Okinawa Island, Southern Japan. *University Museum University of Tokyo Bulletin* 25.

Sara, G., C.N. Bianchi and C. Morri. 2005. Mating behaviour of the newly-established ornate wrasse *Thalassomapavo* (Osteichthyes: Labridae) in the Ligurian Sea (north-western Mediterranean). *Journal of the Marine Biological Association of the UK* 85: 191–196.

Schroeder, T.A. 2006. Annual Report for Fiscal Year 2006. Joint Institute for Marine and Atmospheric Research, University of Hawaii, Honolulu, USA, pp. 110.

Schwenk, K. 1993. Interspecific hybridization in *Daphnia*: distinction and origin of hybrid matrilines. *Molecular Biology and Evolution* 10: 1289–1302.

Seehausen, O. 2004. Hybridisation and adaptive radiation. *Trends in Ecology & Evolution* 19: 198–207.

Seehausen, O. 2006. Conservation: losing biodiversity by reverse speciation. *Current Biology* 16: R334–R337.

Seehausen, O., J.J.M. van Alphen and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277: 1808–1811.

Senou, H., H. Kodato, T. Nomura and K. Yunokawa. 2006. Coastal fishes of Ie-jima Island, the Ryukyu Islands, Okinawa, Japan. *Bulletin of the Kanagawa Prefectural Museum* 35: 67–92.

Senou, H., Y. Kobayashi and N. Kobayashi. 2007. Coastal fishes of the Miyako group, the Ryukyu islands, Japan. *Bulletin of the Kanagawa Prefectural Museum* 36: 47–74.

Swenson, N.G. and D.J. Howard. 2004. Do suture zones exist? *Evolution* 58: 2391–2397.

Taylor, E., J. Boughman, M. Groenenboom, M. Sniatynski, D. Schluter and J. Gow. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteusaculeatus*) species pair. *Molecular Ecology* 15: 343–355.

van Herwerden, L. and P.J. Doherty. 2006. Contrasting genetic structures across two hybrid zones of a tropical reef fish, *Acanthochromis polyacanthus* (Bleeker 1855). *Journal of Evolutionary Biology* 19: 239–252.

van Herwerden, L., J.H. Choat, C.L. Dudgeon, G. Carlos, S.J. Newman, A.J. Frisch and M.J.H. van Oppen. 2006. Contrasting patterns of genetic structure in two species of the coral trout *Plectropomus* (Serranidae) from east and west Australia: Introgressivehybridisation or ancestral polymorphisms. *Molecular Phylogenetics and Evolution* 41: 420–435.

van Oppen, M.J.H. and R.D. Gates. 2006. Conservation genetics and the resilience of reef-building corals. *Molecular Ecology* 15: 3863–3883.

Walker, S.P.W. and C. Ryen. 2007. Opportunistic hybridization between two congeneric tropical reef fish. *Coral Reefs* 26: 539–539.

Walsh, F. and J.E. Randall. 2004. *Thalassoma jansenii* x *T. quinquevittatum* and *T. nigrofasciatum* x *T. quinquevittatum*, hybrid labrid fishes from Indonesia and the Coral Sea. *Journal of Ichthyology and Aquatic Biology* 9: 69–74.

Warner, R.R. 1995. Large mating aggregations and daily long-distance spawning migrations in the bluehead wrasse, *Thalassoma bifasciatum*. *Environmental Biology of Fishes* 44: 337–345.

Willis, B.L., M.J.H. van Oppen, D.J. Miller, S.V. Vollmer and D.J. Ayre. 2006. The role of hybridization in the evolution of reef corals. *Annual Review of Ecology Evolution and Systematics* 37: 489–517.

Wilson, S.K., N.A.J. Graham, M.S. Pratchett, G.P. Jones and N.V.C. Polunin. 2006. Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? *Global Change Biology* 12: 2220–2234.

Wrathall, T., C. Roberts and R. Ormond. 1992. Territoriality in the butterflyfish *Chaetodon austriacus*. *Environmental Biology of Fishes* 34: 305–308.

Yaakub, S.M., D.R. Bellwood, L. van Herwerden and F. Walsh. 2006. Hybridization in coral reef fishes: introgression and bi-directional gene exchange in *Thalassoma* (family Labridae). *Molecular Phylogenetics and Evolution* 40: 84–100.

Yaakub, S.M., D.R. Bellwood and L. van Herwerden. 2007. A rare hybridization event in two common Caribbean wrasses (genus *Halichoeres*; family Labridae). *Coral Reefs* 26: 597–602.

Yabuta, S. 1997. Spawning migrations in the monogamous butterflyfish, *Chaetodon trifasciatus*. *Ichthyological Research* 44: 177–182.

Yabuta, S. 2000. Behaviors in agonistic interaction of the butterflyfish (*Chaetodon lunulatus*). *Journal of Ethology* 18: 11–15.