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Gene flow at three spatial scales in a coral reef fish, the three-spot dascyllus, *Dascyllus trimaculatus*

Abstract Dispersal in coral reef fishes occurs predominantly during the larval planktonic stage of their life cycle. With relatively brief larval stages, damselfishes (Pomacentridae) are likely to exhibit limited dispersal. This study evaluates gene flow at three spatial scales in one species of coral reef damselfish, *Dascyllus trimaculatus*. Samples were collected at seven locations at Moorea, Society Islands, French Polynesia. Phylogenetic relationships and gene flow based on mitochondrial control region DNA sequences between these locations were evaluated (first spatial scale). Although spatial structure was not found, molecular markers showed clear temporal structure, which may be because pulses of settling larvae have distinct genetic composition. Moorea samples were then compared with individuals from a distant island (750 km), Rangiroa, Tuamotu Archipelago, French Polynesia (second spatial scale). Post-recruitment events (selection) and gene flow were probably responsible for the lack of structure observed between populations from Moorea and Rangiroa. Finally, samples from six Indo-West Pacific locations, Zanzibar, Indonesia, Japan, Christmas Island, Hawaii, and French Polynesia were compared (third spatial scale). Strong population structure was observed between Indo-West Pacific populations.

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Introduction

Most coral reef fishes have a bipartite life history, with pelagic early life stages and reef-associated older stages. Dispersal during the benthic adult stage is generally limited. In contrast, the pelagic larval stage is thought to provide an opportunity for dispersal over great distances (Leis 1991). While the evolutionary and ecological significance of dispersal is of pivotal importance (Warner 1997), estimating dispersal capabilities of coral reef fishes remains a formidable challenge. Techniques to investigate dispersal have included direct approaches, such as tag and recapture of individuals (Jones et al. 1999), as well as indirect techniques such as the use of plankton tows, larval traps, the study of artificial drifters, and the evaluation of meristic and morphological characters of fish populations. Molecular markers (Gyllensten 1985; Doherty et al. 1995; Shulman and Bermingham 1995; Schulman 1998), and more recently otolith microchemistry (Thorrold et al. 1998; Arai et al. 1999; Swearer et al. 1999) have been added to the list of indirect methods to infer dispersal.

When finite populations are separated for a sufficient time, a set of unique mutations accumulate in each population, due to genetic drift or natural selection. These differences disappear when individuals from a population migrate into another population and mix their genetic material with local residents (gene flow). Coupling molecular data with mathematical models allows estimation of gene flow (Slatkin and Barton 1989; Hudson et al. 1992; Whitlock and McCauley 1999) and dispersal (Neigel et al. 1991; Neigel 1997). As a result of the development of PCR amplification and DNA sequencing, significant data sets from even very small individuals can be obtained (Shulman 1998). These advances afford the potential for molecular techniques to provide powerful insight into cryptic ecological phenomena such as dispersal.

Reef fishes tend to live a sedentary life as adults and be more mobile as eggs, larvae, or juveniles. Long pel-

agic larval stages could result in high dispersal capabilities, which should, in turn, be associated with high levels of gene flow. In theory, high levels of gene flow should prevent the development of population structure and ultimately speciation (Hansen 1980, 1982; Shaklee et al. 1982; Palumbi 1992, 1994). A large number of fish species, however, are typically found on reefs (Shulman 1998), and this has prompted a number of studies relating gene flow levels and dispersal capabilities (e.g. Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Bernardi 2000). As underscored by Shulman (1998) and Cunningham and Collins (1998), no simple relationship has emerged between dispersal capability and gene flow. There are several possible explanations for these results. Local extinctions and subsequent recolonization by neighboring populations may obscure the relationships between populations (Planes 1993; Cunningham and Collins 1998). More importantly, dispersal capabilities depend not only on the degree of mobility of eggs and larvae, but reflect a combination of dispersal capabilities at different stages including egg, larva, juvenile, and adult.

The goal of this study was to explore phylogenetic relationships among individuals of a coral reef damselfish, *Dascyllus trimaculatus,* from Indo-Pacific localities using the mitochondrial control region (also called D-loop) to estimate gene flow at different geographic scales. The damselfish genus *Dascyllus* (Pomacentridae) comprises nine species restricted to the coral reefs of the Indo-West Pacific (Randall and Allen 1977; Allen 1991). These species have been the focus of a large number of ecological, behavioral, physiological, and genetic studies (e.g. Holzberg 1973; Planes et al. 1993; Schmitt and Holbrook 1996, 1999a, b; Bernardi and Crane 1999). The species *D. trimaculatus,* also called three-spot dascyllus (or domino damselfish), is part of a complex of three species (Godwin 1995; Bernardi and Crane 1999) that comprises *D. trimaculatus, D. albisella,* and *D. strasburgi.* Whereas *D. trimaculatus* is widespread across the Indo-West Pacific, *D. albisella* and *D. strasburgi* are endemic to the Hawaiian and Marquesas Islands, respectively. The taxonomic status of the two latter species is still unresolved. They have occasionally been considered subspecies of the more widespread *D. trimaculatus* (Randall and Allen 1977). In this study we conservatively consider *D. albisella* as a population of *D. trimaculatus* although our goal is not to infer any systematic ranking or engage in debate on classification issues.

The reproductive and life history traits of *D. trimaculatus* suggest moderate dispersal capability in the species. Eggs of *D. trimaculatus* are laid on the sea bottom and fertilized externally. Larvae hatch after about 3 days (Garnaud 1957; Fricke and Holzberg 1974; Thresher 1984). After approximately 22–26 days in the water column (Wellington and Victor 1989), larvae settle on anemones (Fautin and Allen 1992; Schmitt and Holbrook 1999b, c; Holbrook and Schmitt 2000) where they remain until the sub-adult stage. Adults do not occupy anemones but shelter in nearby reef crevices. There are two 3- to 5-day-long pulses of settlement around the quarter moons of each lunar month, with relatively little settlement in between (Schmitt and Holbrook 1999a; Holbrook and Schmitt 2000).

Molecular biogeography and population genetics studies have shown that the biogeography of the Indo-Pacific region is complex (e.g. Planes 1993; Benzie and Williams 1995; Lacson and Clark 1995; Briggs 1999; McMillan et al. 1999). Fish larval dispersal has been particularly difficult to study because of complicating factors such as geological history, unknown natural histories, hybridizations, and complex mating behaviors (Doherty et al. 1995; Lacson and Clark 1995; McMillan and Palumbi 1995, 1997; McMillan et al. 1999).

In this study, we used the mitochondrial control region to estimate gene flow in *D. trimaculatus* at three different geographic scales: (1) within the island of Moorea, French Polynesia; (2) within French Polynesia, between the islands of Moorea (Society Islands) and Rangiroa (Tuamotus); and (3) between six sites that are representative of the Indo-Pacific range of *D. trimaculatus* [East-Africa, Indonesia, Japan, Hawaii, Christmas Island (Kiritimati), and French Polynesia].

Materials and methods

Samples and DNA extraction

D. trimaculatus individuals were collected by divers using hand nets or spears. Newly recruited individuals were taken from the island of Moorea (French Polynesia) by hand nets and measured (total length, TL) to the nearest millimeter at the following locations: Gump Reef (GR), Octogonal Church (OC), Soccer Field (SF), Haapiti (HI), Maatea (MT), Temae (TE), and Maharepa (MR) (Fig. 1) on 14–15 December 1996. Body sizes of these fish indicated that they had settled on the reef within the past 6 weeks (i.e. the three most recent settlement pulses, Holbrook et al. 1997). Juvenile individuals were collected at Manado (north Sulawesi, Indonesia) by hand nets. All other individuals were adults collected by spear at Tiputa Pass in Rangiroa (French Polynesia), Akajima and Okinawa (Japan), Christmas Island (Kiritimati, Kiribati), Mnemba Atoll (Zanzibar, Tanzania), and Kona (Hawaii) (Fig. 1). Numbers per locality are listed in Table 1. The closely related species *D. reticulatus* and *D. carneus* were used as outgroups, following Bernardi and Crane (1999).

Juvenile individuals collected in Moorea were preserved whole in 95% ethanol. Liver tissue was extracted immediately upon capture from adult individuals and preserved in 95% ethanol at ambient temperature in the field and then stored at 4 °C in the laboratory. Tissues were digested overnight at 55 °C in 500 µl of extraction buffer (NaCl 400 mM, Tris 10 mM, EDTA 2 mM, SDS 1%). We purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook et al. 1989).

PCR and sequence analysis

Amplification of the mitochondrial control region was accomplished with universal primers CR-A and CR-E described in Woo-Jai et al. (1995). Each 100 µl reaction contained 10–100 ng of DNA, 10 mM Tris HCL (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 2.5 units of Taq DNA polymerase (Perkin-Elmer, Norwalk, Conn.), 150 mM of each dNTP, and 0.3 mM of each primer and was amplified with a cycling profile of 45 s at 94 °C, 45 s at 48 °C,

1 min at 72 °C, for 35 cycles. After purification following the manufacturer's protocol (Applied Biosystems, Foster City, Calif.) sequencing was performed in both directions with the primers used in the PCR amplification on an ABI 373 automated sequencer (Applied Biosystems, Foster City, Calif.).
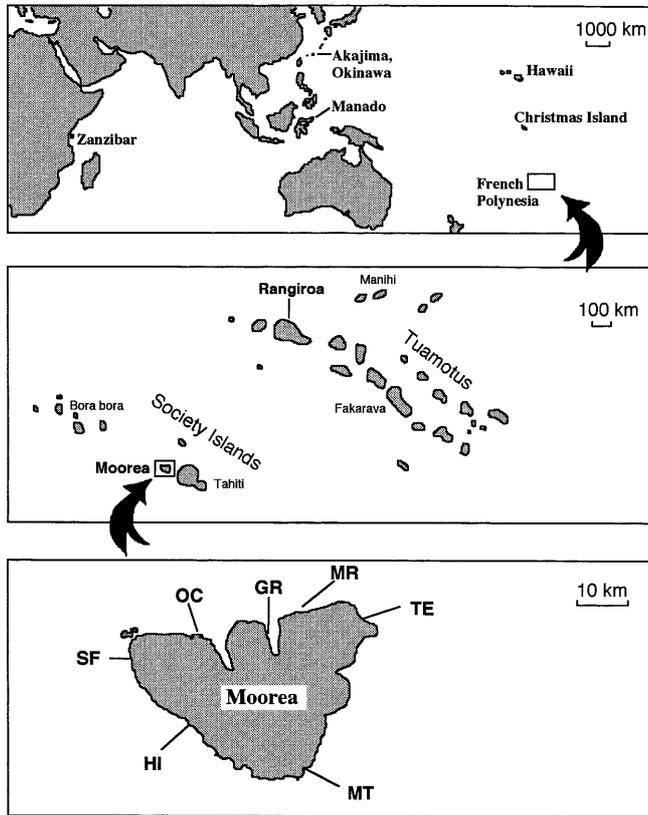

Fig. 1 *Dascyllus trimaculatus* sampling localities at three different geographic scales. *Bottom panel* Within the island of Moorea: Gump Reef (*GR*), Octogonal Church (*OC*), Soccer Field (*SF*), Haapiti (*HI*), Maatea (*MT*), Temae (*TE*), Maharepa (MR). *Central panel* Within French Polynesia – relative position of Moorea (Society Islands) and Rangiroa (Tuamotus). *Top panel* Indo-Pacific – six sampling sites were located at Zanzibar (Tanzania), Manado (Sulawesi, Indonesia), Akajima (Japan), Christmas Island (Kiritimati), Kona (Hawaii), and French Polynesia (Moorea and Rangiroa)

We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the mitochondrial control region sequences. Phylogenetic relationships were assessed using the neighbor-joining (uncorrected distances) method and the maximum parsimony method implemented by the Software package PAUP* (Phylogenetic Analyses Using Parsimony, version 4.0, Swofford 1998). Maximum parsimony using common algorithms could not be employed due to prohibitive computer time. Thus, topological confidence was evaluated with 500 bootstrap replicates (Felsenstein 1985) for neighbor-joining and also with 500 replicates using the fast-step method for maximum parsimony (only one tree kept at each replicate). In both neighbor-joining and maximum parsimony, bootstrapping analysis was performed with equal weighting of transitions and transversions, as well as with transversions weighted three times as much as transitions. Alternative tree topologies were tested using the Kishino and Hasegawa (1989) method implemented by PAUP. Gene flow (F_{st}), and haplotype diversity were calculated using the software package DNAsp (Rozas and Rozas 1997) following Hudson et al. (1992).

Results

A total of 98 *D. trimacultus* were collected and sequenced. Average within-population sequence divergences are given in Table 1. Haplotype diversity was found to be very high. Out of 98 sampled individuals, 87 had unique haplotypes (diversity = 0.99).

Aligned control regions comprised an 11-bp repeated palyndromic portion on the 5′ end (TAATTTAAATT) that was present in 1–8 copies. We did not find any heteroplasmic individuals (i.e. individuals containing several types of molecules differing in repeat copy numbers). Repeated regions on the 5′ end of the control region are not uncommon in fish species (reviewed in Woo-Jai et al. 1995; Faber and Stepien 1999). Because this repeated region was identical in sequence and variable in numbers of repeats between individuals, it was not included in our analysis. The remaining sequenced region was 378 base pairs (bp) long, which did not comprise any deletions or insertions. Of these 378 bp, 145 were variable and 101 were phylogenetically informative. As expected, transitions were more frequent

Table 1 Summary of *Dascyllus trimaculatus* sequence variation within and between populations. Number of haplotypes, number of variable sites, and mean sequence divergence were calculated using DNAsp (Rozas and Rozas 1997)

Region name	Locality name	n	Number of haplotypes	Number of variable sites	Mean percent divergence
All samples		98	87	145	5.1%
Hawaii		10	9	19	2.3%
Zanzibar		5	5	27	3.5%
Manado		10	10	11	0.7%
Japan		9	9	17	1.6%
Christmas Island		2	2	1	0.3%
French Polynesia		62	56	62	2.5%
	Rangiroa	6	6	21	2.1%
	Moorea	56	51	15	2.5%
	MR	9	9	26	1.7%
	MT	7	7	23	2.1%
	SF	7	6	24	2.5%
	GR	11	10	51	3.4%
	HI	8	8	18	1.5%
	OC	7	7	34	2.5%
	TE	7	7	25	2.2%

than transversions (ratio 2.8). Tree topologies using unweighted and 3:1 weighting were identical. Phylogenetic relationships between individuals based on mitochondrial control regions are shown in Fig. 2. Consensus maximum parsimony bootstrapped trees were identical to the neighbor-joining ones (Fig. 2).

Fig. 2 *Dascyllus trimaculatus* phylogenetic relationships using the neighbor-joining method (unrooted tree). The *right panel* indicates a list of abbreviations used in the tree and in Fig. 1. Numbers above the major nodes indicate bootstrap support using the neighbor-joining method with equal weights for transitions and transversions (first number), and when transversions are weighted three times as much as transitions (second number). Numbers below the major nodes correspond to bootstrap support using the maximum parsimony method with equal weights and 3:1 weights for transversions and transitions. In all cases, 500 bootstrap replicates were used. Other numbers indicate bootstrap support using the neighbor-joining method, with equal weights for transitions and transversions (only values above 85% are shown)
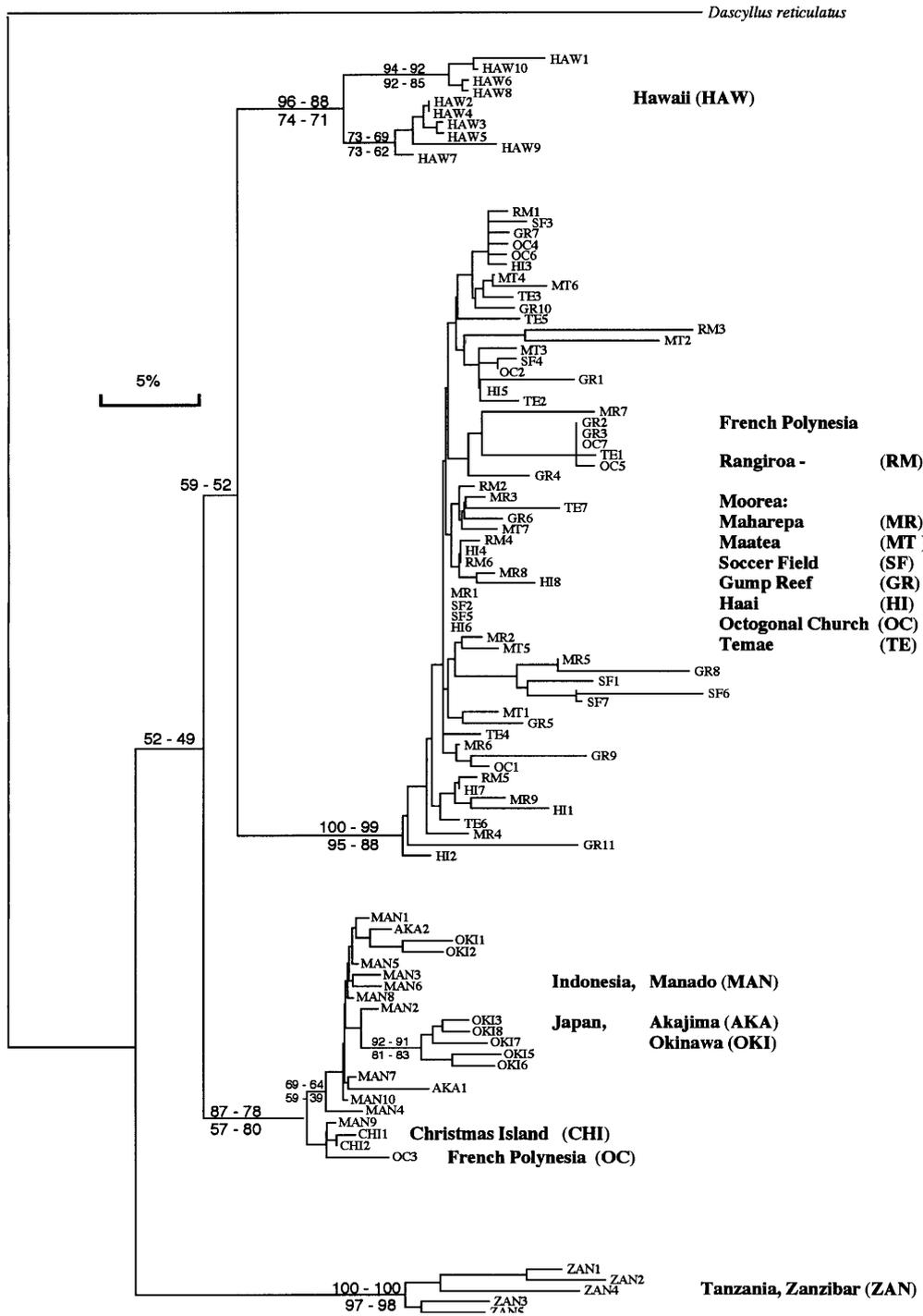
First geographic scale: within the island of Moorea

Whereas the average sequence divergence (2.5%) and haplotype diversity (0.98) was high within the island, control region sequences did not separate individuals according to their geographic origin. This is illustrated by the phylogenetic relationships shown in Fig. 2. To reconcile geographic origin and phylogenetic relationships, we constrained a tree topology that would group individuals by sampling origin. This new topology required an additional 61 steps, which is significantly worse than the most parsimonious topology by a Kishino and Hasegawa (1989) test ($P < 0.001$). Thus our result is not feasibly explained by a partitioning of haplotypes by geographic location within the island of Moorea. This result was not unexpected as it is due to the relatively high level of gene flow between close localities, as shown in Table 2. Indeed, average gene flow between locations within the island of Moorea was high ($F_{st} = 0.048$; $N_m = 25.0$).

The genetic constitution of populations may be influenced by local recruitment, which in turn may be driven by oceanographic patterns and local currents. Local recruitment is subject to spatial and temporal change. We first tested the possibility that recruitment in Moorea is partitioned geographically. *D. trimaculatus* settlement in Moorea is greater on the north shore than on the south shore (Schmitt and Holbrook 1999a). Therefore, we evaluated gene flow levels between the north and south shores, and also between the east and west shores of Moorea. No restriction of gene flow was observed between the north and south shores (i.e. SF,OC,GR,MR,TE vs. HI,MT; $F_{st} = 0.030$; $N_m = 16.33$) and between the east and west shores (TE-MT vs. SF-HI; $F_{st} = 0.042$, $N_m = 11.38$). In both cases, gene flow was not found to be significantly lower than the average level of gene flow between any two populations within the island (*t* test, $P > 0.1$), indicating that gene flow was not spatially restricted.

We also tested for differences that could have arisen due to temporal patterns of recruitment (Fig. 3). Individuals were similar in size within a locality, but different between localities. These patterns may correspond to different recent recruitment pulses (cohorts) predominating at each site at the time of sampling. When superimposing phylogenetic relationships of locales (using
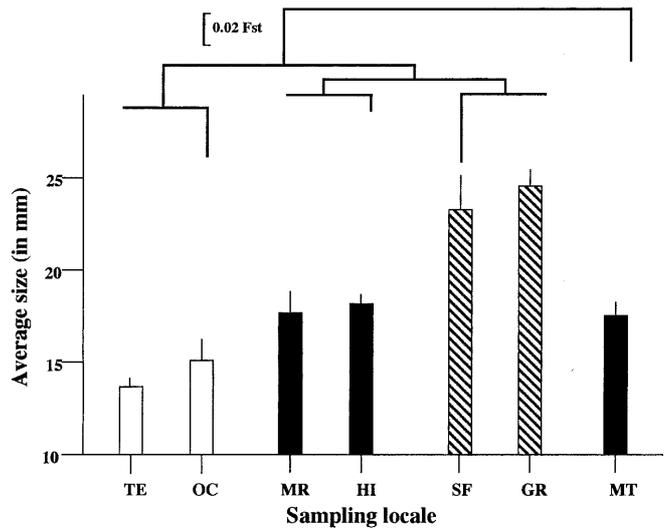

Fig. 3 *Dascyllus trimaculatus* relationships between average size per locality and F_{st} distances between Moorea populations . Histograms represent the average size (total length) of the fish collected (*white bars* small sizes; *black bars* medium sizes; *hatched bars* large sizes). Sizes are given in millimeters; *bars* represent standard error. Sampling locality abbreviations and geographic locations are given in Fig. 1. A tree based on F_{st} distances (neighbor-joining method) represents the relationships between locales

F_{st} distances) with average fish sizes from these locales, individuals with similar sizes grouped together (Fig. 3). Only one sampling site (MT) did not group with "similar size" groups (MR, HI).

Second geographic scale: between the islands of Moorea and Rangiroa

Phylogenetic reconstructions did not separate individuals from Moorea and Rangiroa (Fig. 2). When topologies were forced to separate these two geographic locations, an additional 19 steps were required, which were found to be statistically significant (Kishino and Hasegawa test, $P < 0.01$). Gene flow levels between these two islands were also very high ($F_{st} = 0.01$; $N_m = 41.1$). Given that individuals collected in Rangiroa were all adults, it is not possible to separate the effects of differential recruitment from the larger effects of inter-island gene flow.

Third geographic scale: Indo-Pacific Ocean

Gene flow between our six Indo-Pacific sites based on mitochondrial control region sequences was extremely low overall (average $F_{st} = 0.72$; $N_m = 0.21$; Table 3). In a phylogenetic analysis, our data could separate *D. trimaculatus* populations into two major sister clades. One strongly supported monophyletic clade (97–100% bootstrap) corresponded to individuals from the western Indian Ocean (Zanzibar). The other major clade com-

Table 2 *Dascyllus trimaculatus* F_{st} and N_m values between Moorea populations. F_{st} values are shown below the diagonal and N_m values above the diagonal

	MR	MT	SF	GR	HI	OC	TE
MR		7.96	9.50	94.9	125.8	8.39	45.5
MT	0.059		3.48	6.20	5.15	4.75	8.75
SF	0.050	0.126		12.20	5.26	5.59	6.27
GR	0.005	0.075	0.039		16.72	16.16	166.1
HI	0.004	0.088	0.086	0.029		10.10	23.70
OC	0.060	0.095	0.082	0.030	0.047		74.2
TE	0.010	0.054	0.074	0.003	0.020	0.007	

Table 3 *Dascyllus trimaculatus* F_{st} and N_m values between Indo-Pacific populations. F_{st} values are shown below the diagonal and N_m values above the diagonal

	Zanzibar	Hawaii	Japan	Christmas I.	Indonesia	French Polynesia
Zanzibar		0.18	0.23	0.15	0.19	0.18
Hawaii	0.73		0.18	0.12	0.14	0.20
Japan	0.69	0.74		0.43	2.35	0.18
Christmas Island	0.77	0.80	0.54		0.38	0.11
Indonesia	0.72	0.78	0.17	0.57		0.14
French Polynesia	0.74	0.66	0.72	0.82	0.78	

prised individuals from the Pacific. The Pacific clade in turn separated into three sub-clades. One basal sub-clade included the Indonesian, Japanese, and Christmas Island populations. Although individuals within this sub-clade did not form distinct groups (with the exception of Christmas Island individuals, but with a very small sample size), gene flow between Indonesia, Japan, and Christmas Island was found to be reduced. Values of F_{st} between these regions ranged from 0.57 to 0.17; N_m values ranged from 0.38 to 2.35.

From this basal clade, two derived clades branch off with one clade including fish from French Polynesia and the other clade including fish from Hawaii. Individuals from Hawaii grouped in a well-supported monophyletic clade (itself divided into two sub-clades). The French Polynesia clade was strongly supported by bootstrap analysis (97–100% of the replicates). Only one French Polynesian individual (out of 62) was not included in this clade (sample OC3). This sample grouped with fish from Indonesia, Japan, and Christmas Island.

To determine if the large sample size from French Polynesia could alter the topology of the phylogenetic tree, we randomly excluded French Polynesia samples to match the sample sizes from other localities. This alteration in sample sizes never changed the overall topology of the tree (not shown).

Discussion

Gene flow within Moorea and within French Polynesia

Gene flow at small spatial scales between Pacific marine populations can be very high (Benzie and Williams 1995; Lacson and Clark 1995; Lavery et al. 1995; Williams and Benzie 1998). For example, the zebra humbug *D. aruanus*, a species closely related to *D. trimaculatus*, did not exhibit population structure over the French Polynesian region (Planes 1993). Planes attributed this lack of population structure to the reduction of suitable habitat (shallow lagoons) during the last glaciation and the recent rapid recolonization of the entire range of the species (thus lowering the genetic diversity). By contrast, *D. trimaculatus* lives both inside and outside lagoons and should not have encountered similar bottlenecks. Indeed, the mitochondrial control region in *D. trimaculatus* was found to be highly variable (diversity indexes higher than 0.8, Table 3), indicating that the species has

not gone through a recent bottleneck. In addition, population structure was found to be absent at the level of the island of Moorea. Similarly, while Moorea and Rangiroa are approximately 750 km apart, little genetic divergence was found between these islands. These results are not entirely surprising since *D. trimaculatus* has been shown to be an effective disperser at small scale (Danilowicz 1997), as it is usually among the first species to recruit on artificial reefs (Golani and Diamant 1999).

Recruitment events in Moorea

Spatial and temporal patterns of recruitment in *D. trimaculatus* are highly variable (Schmitt and Holbrook 1999a, b), and little is known regarding the source and transport of the larval stages. Data suggest, however, that larval stages may travel great distances prior to settlement (Danilowicz 1997). For instance, self-recruitment was not observed in Kaneohe Bay, Hawaii (Danilowicz 1997). Considering spawning times and local current patterns, Danilowicz (1997) predicted that sources of recruits that settled in Kaneohe Bay were located in upcurrent islands. Uneven settling events have been shown to be responsible for creating genetic differences between cohorts of recruits in several species of invertebrates (Hedgecock 1986; Watts et al. 1990; Kordos and Burton 1993; Edmands et al. 1996; Moberg and Burton 2000). Similarly, Moorea settlers were shown to arrive on the island in cohorts that may have been produced elsewhere (Schmitt and Holbrook 1999a). Thus, genetic differences that were observed between individuals with different body sizes on Moorea may be a reflection of independent genetic sources. Nearby source islands include Tahiti (about 20 km east of Moorea), Tetiaroa (40 km northwest), and Maiao (30 km southwest). Variation in oceanographic conditions could result in transport of larvae from one or more of these islands to Moorea during different settlement pulses. Alternatively, genetic differences may be derived from the recruitment of genetically dissimilar larval pools. Long pelagic larval stages combined with high mortality rates could provide a means for pre-settlement selection. Regardless of the mechanism, our finding of genetic differences among recently settled groups is not contradicted by the overall lack of population structure at the spatial level on Moorea or when comparing Moorea and Rangiroa. Indeed, several subsequent factors, such as additional settlement from dif-

ferent sources, movements of adults, or post-settlement selection (Hedgecock 1986), may have obscured the original genetic signature of settlement.

Gene flow across the Indo-Pacific

D. trimaculatus populations from our six Indo-Pacific sampling sites were found to be genetically distinct from each other. Estimated gene flow between these localities was very low, in most cases less than 1 individual per generation (average $N_m = 0.21$). Furthermore, phylogenetic relationships helped us reconstruct the evolutionary history of the species, which may in turn reflect its main dispersal routes. Within the genus *Dascyllus*, *D. trimaculatus* has a derived position and joins with the central Pacific *D. reticulatus* and the Indian Ocean *D. carneus* (Bernardi and Crane 1999). Thus, the origin of *D. trimaculatus* is likely to be located in the central Pacific or in the Indian Ocean (Randall and Allen 1977). The separation of *D. trimaculatus* individuals into two major clades, corresponding to the Indian Ocean and the Pacific, with little gene flow between these two basins ($F_{st} = 0.72$, $N_m = 0.19$), also suggests that the origin of dispersal in *D. trimaculatus* is likely to be the central Pacific.

These results are in agreement with previous studies. Indeed, differential dispersal between the Indian Ocean and the Pacific was shown to have created two distinct groups. Currently, only limited dispersal is possible between these two regions (Gordon and Fine 1996; Gordon 1998). This is reflected in both the pattern of distribution of species (Briggs 1999) and the genetic structure of populations within species. Populations of crabs (Lavery et al. 1995), starfish (Williams and Briggs 1998), butterflyfishes (McMillan and Palumbi 1995, 1997; McMillan et al. 1999), prawns (Duda and Palumbi 1999), surgeonfishes (Planes, personal communication), and damselfishes (Lacson 1994; Lacson and Clark 1995) show clear genetic separation between the Indian Ocean (except western Australia in some cases) and Pacific Basins. These genetic separations are a reflection of dispersal barriers between these regions.

The biogeography of the Pacific region is complex, probably resulting from different dispersal modes (Palumbi 1997; Briggs 1999). Three major modes and routes of dispersal have been proposed to explain the distribution of species in this region (Benzie 1999; Briggs 1999; reviewed in Planes and Galzin 1999). Briefly, one pattern of dispersal results from the export of propagules from a center of high biodiversity, located in the Indonesia–Philippines region; one pattern results from exactly the opposite dispersal direction: neighboring regions contribute to propagules that aggregate in this center. Lastly, one pattern results from an overlap of distributions in that center, with no particular dispersal trend. These dispersal alternatives are still under debate.

Although the Johnston Atoll, Christmas Island, and the Tuamotus are geologically linked (Woodroffe and McLean 1998), *D. trimaculatus* populations from Hawaii, Christmas Island, and French Polynesia are well separated. In fact, Japanese, Indonesian, and Christmas Island samples are closely related (within the same clade). This is surprising, as both Japan and Christmas Island are geographically closer to Hawaii than they are to each other. Interestingly, this pattern was previously observed in other fish species, the milkfish *Chanos chanos* (Winans 1980) and the goatfish *Mulloidichthys vanicolensis* (Stepien et al. 1994). This pattern was attributed to the isolation of the Hawaii Archipelago, favoring rare colonization events resulting in founder effects and genetic isolation (Stepien et al. 1994). The species of *Dascyllus* from Hawaii (and Johnston Atoll; P. Lobel, personal communication) has been described as *D. albisella*, a close relative of *D. trimaculatus* (Randall and Allen 1977). *D. albisella* is very similar morphologically and ecologically to *D. trimaculatus*. The morphological differences observed in Hawaiian individuals that prompted its ranking as a separate species are consistent with our results that place this group as a separate population. However, while Hawaiian samples form a strongly supported clade, it is nested within the *D. trimaculatus* complex.

French Polynesia individuals are geographically the most distant from the Zanzibar individuals and are also the most genetically derived. One individual from French Polynesia (OC3) and one individual from Indonesia (MAN9) grouped with the Christmas Island clade. These individuals were sequenced several times independently to ensure that the results were not an artifact. The sequences differed from any other sequence, suggesting that PCR contamination was unlikely. These individuals may be an historical indicator of low levels of migration between these regions (1 out of 62 French Polynesia individuals) between the Christmas Island–Japan–Indonesia clade and French Polynesia.

Conclusion: control regions and the study of gene flow

Although mitochondrial DNA markers have been shown to present some important limitations (e.g. maternal inheritance), mitochondrial control regions have been regarded as a molecular marker of choice for studying rapidly evolving populations (Sturmbauer and Meyer 1992). In Indo-West Pacific butterflyfish populations, the control region was found to be extremely variable, up to 33–43 times faster than the cytochrome *b* region, and approximately 2–10 times faster than other typical fish control regions (McMillan and Palumbi 1997). In *D. trimaculatus*, another tropical Indo-Pacific species, the control region was found to be on average 5.8 times faster than the cytochrome *b* (data not shown). The rate of evolution for cytochrome *b* regions in vertebrates is approximately 1.0–2.5% per million years (Irwin et al. 1991; Martin et al. 1992). Thus, the rate of evolution in *D. trimaculatus* control regions is approximately 5.8–14.5% per million years. Control regions

were found to differ by 2.3–10.5% between Indo-Pacific populations of *D. trimaculatus*. These sequence divergences correspond to divergence times of between 160,000 and 1.8 million years ago. Dispersal in *D. trimaculatus* should therefore be viewed in the perspective of these large time scales.

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