

Genetic relatedness in groups of the humbug damselfish *Dascyllus aruanus*: small, similar-sized individuals may be close kin

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Abstract

Kin selection plays an important role in the evolution of social behaviour in terrestrial systems. The extent to which kin selection influences the evolution of social behaviour in marine systems is largely unexplored. Generally, it is considered that kin selection is irrelevant in marine systems, because it is assumed that the dispersing larval phase of marine organisms will break up kin associations. Here, we challenge this assumption and investigate the opportunity for kin selection in a coral reef fish: the humbug damselfish *Dascyllus aruanus*. This fish lives in groups composed of a large male and a number of smaller females and nonbreeders. We use 10 polymorphic microsatellite loci to assess the relatedness of 265 individuals from 35 groups. The mean coefficient of relatedness among group members is 0.01 ± 0.04 , suggesting that individuals are not associated with close relatives. However, the distribution of pairwise relatedness of individuals within groups has an overabundance of positive values, and indicates that there might be 35 pairs of close relatives within groups. Further analyses reveal that close relatives likely are similar in size and small in size, suggesting that they might have recruited together. We conclude that it is possible for kin selection to operate in *D. aruanus*, but kin recognition will be a prerequisite for such selection. This study reveals that individuals can be associated with close relatives, and there is a hidden potential for kin selection, during certain parts of the life cycle of coral reef fishes.

Keywords: *Dascyllus aruanus*, kinship, larval dispersal, microsatellites, recruitment, social evolution, sweepstakes hypothesis

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Introduction

Kin selection theory provides the foundation for our understanding of the evolution of social behaviour (Hamilton 1963, 1964a,b; West-Eberhard 1975). Hamilton's key insight was that the evolution of social behaviour would be influenced by relatedness (Hamilton 1963). The likelihood of kin groups forming and the

efficacy of kin selection are dependent on the pattern of dispersal (Hamilton 1964a,b; Taylor 1992a,b; Queller 1994; West *et al.* 2002; Johnstone 2008). Many terrestrial organisms with limited dispersal form groups composed of kin, and the degree of relatedness influences individual behaviour in these groups (Bourke & Franks 1995; Emlen 1997; Keller & Reeve 2002; Griffin & West 2003). Indeed, the vast majority of empirical work on kin selection and social evolution has been conducted on terrestrial organisms and theoretical work has developed in parallel.

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Marine organisms tend to be absent from the thinking of theoreticians and because of this they provide an opportunity to test the robustness of current theories of social evolution (e.g. Buston 2004a,b; Wong 2009) and generate new insights (e.g. Buston 2003; Wong *et al.* 2007, 2008). It tends to be assumed that kin selection will play a minor role in the evolution of social behaviour of marine organisms, because it is hard to imagine how relatives would stay together during, or find each other following, the dispersing larval phase that is part of the life cycle of many marine organisms (Leis 1991; Kinlan & Gaines 2003). However, observations of limited dispersal in teleost fishes (Jones *et al.* 2005; Miller-Sims *et al.* 2008) and kin associations in taxa as diverse as ascidians (Grosberg & Quinn 1986), shrimp (Duffy 1996) and teleost fish (Selkoe *et al.* 2006) underscore the importance of testing this assumption (Buston *et al.* 2007).

The humbug damselfish *Dascyllus aruanus* exhibits high levels of plasticity in individual behaviour, making it a good system for testing models of social evolution. *D. aruanus* are found throughout the Indopacific (Allen 1991) in close association with acroporan or pocilloporan corals (Sale 1972; Holbrook *et al.* 2000). Settling larvae locate suitable corals and conspecifics using chemical cues (Sweatman 1985, 1988). The coral provides the fish with protection from predators and a place to lay their eggs (Coates 1980a; Mizushima *et al.* 2000). Within each coral head, there is a single group of *D. aruanus* (Sale 1971; Forrester 1990). Groups have an average size of <10 individuals, although they may be as large as 80 individuals (Sale 1972; Forrester 1991; Holbrook *et al.* 2000). Within each group, there is a size-based dominance hierarchy (Coates 1980b; Forrester 1991); the largest individual is male and smaller individuals are females or nonbreeders (Cole 2002; Asoh 2003). The fish are protogynous hermaphrodites (Cole 2002; Asoh 2003); if the male of the group disappears, a large female from the group or another nearby group changes sex and takes his place (Fricke & Holzberg 1974; Coates 1982). *D. aruanus* breed on a lunar or semi-lunar cycle; females deposit eggs in a nest and the male cares for them for 2–5 days before they hatch (Sale 1970; Mizushima *et al.* 2000). Once hatched, the larvae disperse for 16–24 days before settling to the reef (Wellington & Victor 1989), resulting in genetic homogeneity at small, within-archipelago, spatial scales (Planes *et al.* 1993).

The extent to which the behaviour of individuals within groups of *D. aruanus* might be influenced by kin selection is unknown. Yet, these fish live in a context where kin selection may be important. Here, we test four hypotheses concerning the kin structure of *D. aruanus* groups: (i) the mean coefficient of relatedness of individuals within groups will be greater than

zero – many individuals within groups will be closely related; (ii) the distribution of pairwise relatedness of individuals within groups will have an overabundance of positive values, relative to the distribution of pairwise relatedness between groups – a few individuals within groups will be closely related; (iii) pairwise relatedness will vary as a function of size similarity, providing insight into whether close relatives are sibling pairs or parent–offspring pairs; and (iv) pairwise relatedness will vary as a function of absolute size, providing insight into the size and age at which close relatives are associated. The results provide us with a good picture of the genetic context of *D. aruanus* social behaviour, and lay the foundation for future tests of models of social evolution.

Materials and methods

Study population

This study was conducted using a population of humbug damselfish, *Dascyllus aruanus* (Fam: Pomacentridae), studied in July and August of 2007, in the lagoon of Moorea, French Polynesia (17°29'18" S, 149°54'53" E). All fieldwork was conducted using SCUBA and/or snorkel. Three hundred and seventeen groups of *D. aruanus*, 2537 individuals, were located between two small islands at the northwestern tip of Moorea (an area ~400 m × 400 m). Each group was associated with a single coral head, or cluster of coral heads, ranging in diameter from 24 to 240 cm (mean diameter of coral or coral cluster ± SD = 73 ± 47 cm, $n = 317$ groups). The fish were associated with four genera of corals (*Acropora*, *Montipora*, *Pocillopora* and *Porites*) and with three species of coral within the most common genus (*Pocillopora eydouxi*, *Pocillopora meandrina* and *Pocillopora verrucosa*). Group size ranged from 1 to 44 individuals (mean number of individuals per group ± SD = 8 ± 8, $n = 317$ groups).

We randomly selected 35 groups out of 228 groups found on *Pocillopora* corals. From these groups, all individuals ($n = 270$) were captured using a combination of hand nets, fence net and clove oil, and taken to the surface in Ziploc plastic bags. There, the standard length (SL) of each individual was measured to 0.1 mm using calipers. A fish's SL is the straight-line distance from the tip of the snout to the base of the caudal fin. SL ranged from 7.7 to 59.5 mm (mean SL ± SD = 30.2 ± 9.9 mm, $n = 270$ individuals). We used SL as our metric of body size, rather than mass, because SL is less likely to change from day to day. Individuals were ranked based on their size relative to other individuals within the same group, with the largest being ranked 1. We determined the ratio of SL of individuals adjacent in rank within each group

as SL_n/SL_{n+1} , where n is an individual's rank (see also Buston & Cant 2006; Wong *et al.* 2007; Kohda *et al.* 2008). We used the ratio as our measure of similarity in size, rather than absolute size difference, because the ratio controls for absolute size. A fin-clip was taken from each of these individuals ($n = 270$) and preserved in 95% EtOH for genetic analyses.

Polymerase chain reaction and genotyping

Genomic DNA was isolated from each fin-clip using a DNA Purification Kit (Puregene). For each individual, polymerase chain reactions (PCRs) were conducted to amplify 16 nuclear microsatellite loci (Fauvelot *et al.* 2009). Three independent multiplex PCR mixes were conducted (with six, five and five loci respectively) using the Qiagen Multiplex PCR kit (QIAGEN). For each locus, the forward primer was labelled with Beckman-Coulter dyes D2, D3 or D4, as described in Fauvelot *et al.* (2009). Amplified fragments were separated on a Beckman-Coulter CEQTM 8000 Genetic Analysis System, with a 400-bp internal size standard (Beckman-Coulter). Genotypes and allele sizes were scored ($n = 265$ individuals) using Beckman-Coulter CEQTM 8000 Genetic Analysis System-associated software.

Summary statistics, tests of Hardy–Weinberg equilibrium and linkage disequilibrium

Number of alleles, observed heterozygosity and expected heterozygosity based on Hardy–Weinberg proportions were estimated using GENETIX (Belkhir *et al.* 2002). Heterozygosity estimates were made for each locus and over all loci, and for each colony and over all colonies. Tests for gametic disequilibrium and departure from Hardy–Weinberg proportions were performed using exact tests implemented in Genepop version 3.4 (Raymond & Rousset 1995). Markov chain method was used to estimate without bias exact P -values using 1000 dememorizations, 100 batches and 1000 iterations per batch. The presence of null alleles was detected using MICRO-CHECKER (van Oosterhout *et al.* 2004).

Genetic relatedness

We analysed relatedness of 265 individuals from 35 groups using 10 polymorphic microsatellite loci. We did not use 5 of the original 270 individuals because no genotype could be obtained. We did not use 6 of the original 16 loci because they were not in Hardy–Weinberg equilibrium and had null alleles present (see below). We determined the mean coefficient of relatedness of individuals within groups and the pairwise relatedness of all individuals in the population using the program

RELATEDNESS 5.0.8 (Queller & Goodnight 1989). This program calculates genetic relatedness using a regression measure of relatedness, accounting for population allele frequencies (Queller & Goodnight 1989). The mean coefficient of relatedness for unrelated individuals from the same population is zero ($r = 0$), for half-siblings $r = 0.25$ and for full siblings $r = 0.5$. Relatedness was estimated with respect to the allele frequencies in the entire study population rather than each group and individuals were weighted equally. The justification for this being that previous studies indicate that larvae may travel long distances and that there is genetic homogeneity at small spatial scales (Wellington & Victor 1989; Planes *et al.* 1993); we confirmed genetic homogeneity among groups of individuals using a Bayesian approach implemented in the program STRUCTURE version 2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003, 2007).

Statistical analyses

We investigated the four hypotheses using a variety of statistics. We investigated whether the mean coefficient of relatedness of individuals within groups is greater than zero using a one-sample t -test. We investigated whether the distribution of pairwise relatedness of individuals within groups has an overabundance of positive values, relative to the distribution within groups, using a chi-squared test for multiple classes with subsequent subdivision of the chi-squared analysis (Zar 1984). The test statistic and significance associated with this analysis should be treated with caution because there is more than one measure of pairwise relatedness per individual and multiple measures of pairwise relatedness involving the same individual are not independent. We investigated whether pairwise relatedness varies as a function of size similarity or as a function of absolute size using mixed model analyses (SAS PROC MIXED). In these analyses, we dealt with the fact that there is more than one measure of pairwise relatedness per individual, by entering individual identification numbers (for both individuals involved in each pairwise relatedness value) as random effects. This approach enabled us to test for the effects of interest, while controlling for the lack of independence among pairwise relatedness values involving the same individual. Statistical analyses were carried out using SAS 9.1.3, employing an alpha level of 0.05.

Results

Summary statistics, tests of Hardy–Weinberg equilibrium and linkage disequilibrium

In this population of *Dascyllus aruanus* we observed from 7 to 50 alleles per locus (Appendix S1). Over all

colonies, observed heterozygosity ranged from 0.307 to 0.955 per locus and expected heterozygosity from 0.697 to 0.953. Within colonies, observed heterozygosity ranged from 0.625 to 0.891 and expected heterozygosity from 0.650 to 0.813. No significant gametic disequilibrium was found for any pair of loci ($P > 0.01$). Significant heterozygote deficiencies were found in six loci (Da314, Da371, Da408, Da432, Da479 and Da603; all $P < 0.003$, the threshold following Bonferroni standard correction; Appendix S1). Null alleles were detected for these loci using MICRO-CHECKER software, which identified a homozygote excess for most allele size classes (combined probability for all classes $P < 0.001$). The presence of null alleles was confirmed by the consistent nonamplification of these loci in several individuals despite repeated PCR attempts, but successful amplification of unaffected loci in the same individuals. We removed these six loci from subsequent analyses to avoid introducing bias due to null allele occurrence.

The mean coefficient of relatedness of individuals within groups

First, as is standard in this type of study (Emlen 1997; Griffin & West 2003), we hypothesized that many individuals within groups will be closely related. If many individuals within groups are closely related, then the mean coefficient of relatedness among individuals within groups should be greater than zero. The mean coefficient of relatedness among group members (mean \pm SD = 0.01 ± 0.04 , $n = 35$ groups) is not significantly different from zero (one-sample t -test: d.f. = 34, $t = 0.912$, $P = 0.3683$; Fig. 1). This indicates that the probability that two individuals from the same group share an allele is no different from the probability that two individuals from the study population share an

allele. On average, individuals within groups are not closely related.

Pairwise relatedness of individuals within and between groups

If only a few, rather than many, individuals within groups are closely related, then their presence might not be detected using the mean coefficient of relatedness (Fig. 1). Therefore, we hypothesized that a few individuals within groups will be closely related. Faced with similar situations, previous studies have attempted to determine the likelihood that a focal pair of individuals has a specific relationship, based on their pairwise relatedness and the simulated distributions of pairwise relatedness of relatives and nonrelatives (Blouin *et al.* 1996; Goodnight & Queller 1999; Selkoe *et al.* 2006). However, this approach has problems when the relative frequency of relatives and nonrelatives in the population is unknown (Appendix S1; Reeve 1989; Csilléry *et al.* 2006). Problems arise because simulated distributions of relatedness for relatives and nonrelatives overlap greatly, e.g. simulated relatedness of half-sibs can range from <0 to more than 0.5 (Appendix S1). In the absence of information on population relatedness composition, the safest prediction for our hypothesis is that, if some individuals within groups are closely related, then the distribution of pairwise relatedness of individuals within groups should have more positive values than expected by chance.

We assigned pairwise relatedness (r) values to classes of width $r = 0.05$. This class width was chosen *a priori* to strike a balance between classes that were too small, which would increase the effect of stochastic processes, and classes that were too large, which would decrease the amount of information available. We compared the observed distribution of pairwise relatedness of individuals within groups of *D. aruanus* to the expected distribution. The expected was based on the distribution of pairwise relatedness of individuals between groups of *D. aruanus*. Only classes with an expected count >1 were included in the analysis, and no more than 20% of classes had an expected count of <5 . The observed and expected distributions of pairwise relatedness are different (chi-squared test: d.f. = 15, $\chi^2 = 27.23$, $P < 0.05$; Fig. 2). Subsequent subdivision of the chi-square revealed that 40% was attributable to the observed number of positive relatedness values being greater than the expected number; comparison of observed and expected counts indicates that there are 35 more pairs of close relatives within groups than expected by chance. In sum, a few individuals within groups are closely related. The results of this test should be treated with caution, because of nonindependence of pairwise

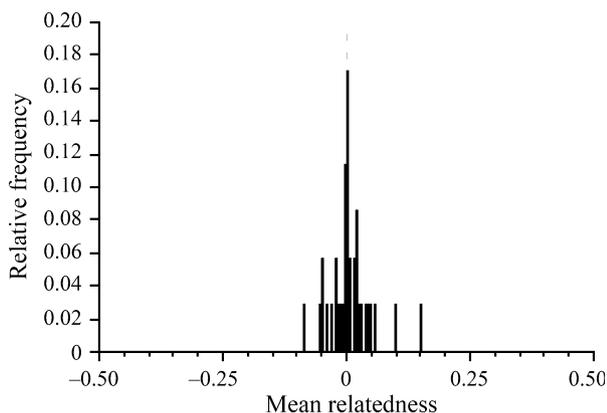


Fig. 1 Distribution of the average relatedness among *Dascyllus aruanus* group members (35 groups), in Moorea, French Polynesia.

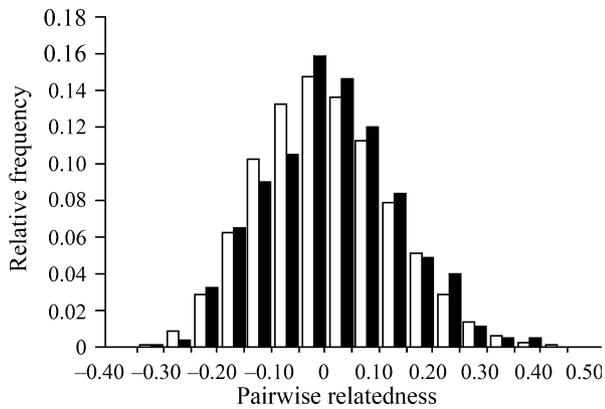


Fig. 2 Distributions of the pairwise relatedness of individuals from the same group (black; 1147 pairwise comparisons) and different groups (white; 33 833 pairwise comparisons) of *Dascyllus aruanus* in Moorea. Pairwise relatedness (r) values are assigned to classes of width $r = 0.05$.

relatedness values involving the same individual. However, the results are consistent with the results of subsequent analyses in which we controlled for the lack of independence by the use of random effects.

Pairwise relatedness of individuals as a function of size similarity

Following our observation that some pairs of individuals within groups might be close relatives (Fig. 2), we hypothesized that pairwise relatedness would vary as a function of size similarity. Specifically, we predicted that the smallest individuals in a group of *D. aruanus* might be the offspring of the largest individuals in the group, as is seen in the cooperative breeding cichlid fish *Neolamprologus pulcher* (Dierkes *et al.* 2005), in which case high relatedness would be associated with size dissimilarity. Alternatively, we predicted that siblings might recruit together, as is seen in the kelp bass *Paralabrax clathratus* (Selkoe *et al.* 2006), in which case high relatedness would be associated with size similarity.

We compared the pairwise relatedness of individuals that were similar in body size (SL ratio ≥ 0.75) and dissimilar in body size (SL ratio < 0.75). These categories of body size ratio were chosen to create the most balanced design for statistical analysis. Within groups of *D. aruanus*, the pairwise relatedness of individuals that are similar in size is greater than the pairwise relatedness of individuals that are dissimilar in size (PROC MIXED: d.f. = 1, $F = 4.84$, $P = 0.0281$; Fig. 3). This effect occurs within but not between groups of *D. aruanus* (PROC MIXED: d.f. = 1, $F = 0.02$, $P = 0.8931$; Fig. 3). We do not suggest that the mean pairwise relatedness of 0.0128 found among similar size individuals within groups of

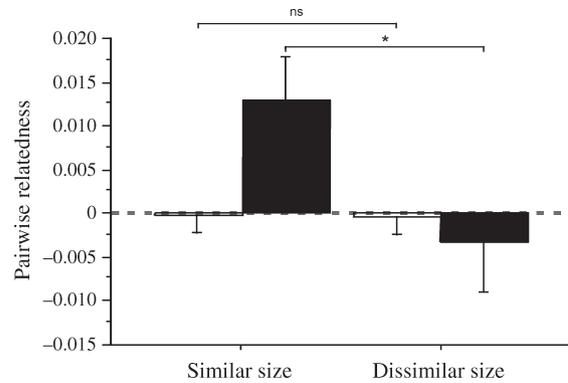


Fig. 3 The relationship between pairwise relatedness and size similarity of individuals [similar size, standard length (SL) ratios ≥ 0.75 ; dissimilar size, SL ratios < 0.75] from the same group (black bars) and different groups (white bars) of *Dascyllus aruanus*. Bars represent the least squares mean estimate and lines are standard errors of this estimate; ns, nonsignificant comparison; *, significant comparison.

D. aruanus is indicative of close relatedness (Fig. 3). Rather we suggest that 32 pairs of close relatives, with an average relatedness of 0.25, would be sufficient to shift the mean pairwise relatedness of individuals similar in size from -0.0002 (the between-group mean) to $+0.0128$ (the within-group mean, $n = 622$). This can be demonstrated using the formula for a mean, $+0.0128 = [-0.0002(n - x) + 0.25(x)]/n$, where n is the sample size and x is the number of close relatives. This analysis (i) provides further evidence that pairs of close relatives are occasionally found within groups and (ii) indicates that close relatives are likely to be similar in size.

Pairwise relatedness of individuals as a function of absolute size

Following our observation that relatives tend to be similar in size (Fig. 3), we hypothesized that pairwise relatedness would vary as a function of absolute size. Specifically, we predicted that relatives would be more common at large body sizes, perhaps because of a survival advantage of individuals that recruit with relatives. Alternatively, we predicted that relatives would be less common at large body sizes, perhaps because random mortality of individuals following recruitment would tend to break up kin associations over time.

We compared the pairwise relatedness of large individuals (SL ≥ 30.0 mm) and small individuals (SL < 30.0 mm), where large and small individuals were defined in reference to median body size (30.0 mm), thereby creating the most balanced design for statistical analysis. Within groups of *D. aruanus*, the pairwise relatedness of small individuals is greater than the

pairwise relatedness of large individuals (PROC MIXED: d.f. = 1, $F = 5.60$, $P = 0.0186$; Fig. 4). This effect occurs within but not between groups of *D. aruanus* (PROC MIXED: d.f. = 1, $F = 1.50$, $P = 0.2211$; Fig. 4). Once again, we do not suggest that the mean pairwise relatedness of 0.024 found among small individuals within groups of *D. aruanus* is indicative of close relatedness, rather we suggest that the presence of close relatives within groups could shift the mean pairwise relatedness of small individuals from the between group mean to the within group mean (Fig. 4). This analysis (i) provides further evidence that pairs of close relatives are occasionally found within groups and (ii) indicates that close relatives are likely to be small in size.

Discussion

Kin selection (Hamilton 1964a,b; West-Eberhard 1975) plays an important role in the evolution of social behaviour in terrestrial systems (Bourke & Franks 1995; Emlen 1997; Keller & Reeve 2002; Griffin & West 2003) but is considered to play a trivial role in marine systems because the dispersing larval phase of marine organisms is assumed to break up kin associations (Leis 1991; Kinlan & Gaines 2003; Buston *et al.* 2007; Selkoe *et al.* 2008). Here, we show that kin associations do form in one of the most widespread and heavily studied of coral reef fishes – the humbug damselfish *Dascyllus aruanus*. Although individuals within groups are not closely related on average, i.e. the mean coefficient of relatedness is zero (Fig. 1), some individuals within groups are closely related (Fig. 2). The closely related individuals likely are similar in size (Fig. 3) and small

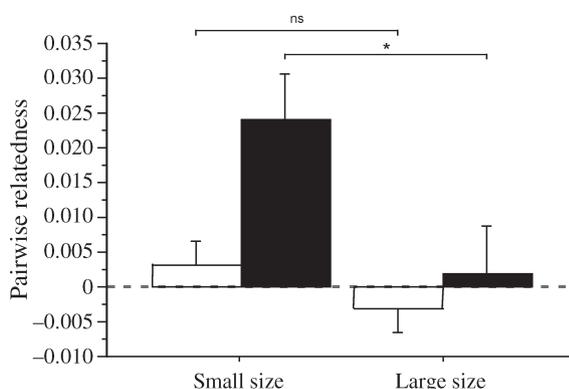


Fig. 4 Relationship between pairwise relatedness and absolute size of individuals [small size, standard length (SL) <30 mm; large size, SL ≥30 mm] from the same group (black bars) and different groups (white bars) of *Dascyllus aruanus*. Bars represent the least squares mean estimate and lines are standard errors of this estimate; ns, nonsignificant comparison; *, significant comparison.

in size (Fig. 4). A plausible explanation for these patterns is that close relatives are siblings that recruit together, but kin associations break-up with time post-recruitment. The fact that individuals have been found to be associated with close relatives in four out of eight studies that have explicitly investigated relatedness in marine fish (relatives: this study; Planes *et al.* 2002; Selkoe *et al.* 2006; Miller-Sims *et al.* 2008; non-relatives: Avise & Shapiro 1986; Kolm *et al.* 2005; Buston *et al.* 2007; Palm *et al.* 2008) suggests that there may be a greater potential for kin selection to operate in the marine environment than previously acknowledged.

The potential for kin selection

The potential for kin selection to influence the evolution of social behaviour in *D.* can be viewed from a glass half-empty or glass half-full perspective. On the one hand, if we consider that there are only 35 pairs of close relatives in groups, then this indicates that only 35 out of a possible 1147 within group interactions (3% of interactions; Fig. 2) occur between close relatives. On the other hand, if we consider that 35 pairs of close relatives involve 70 individuals, then this indicates that 70 out of 265 individuals (26% of the population; 52% of small individuals) have the opportunity to discriminate between close relatives and nonrelatives. Clearly, indiscriminate altruism will not be expected to evolve by kin selection in *D. aruanus*, because the mean coefficient of relatedness is zero (Fig. 1; Hamilton 1963, 1964a). However, discriminate, targeted, cooperative behaviour may be predicted to evolve by kin selection, because some *D. aruanus* likely are associated with close relatives (Figs 2–4; Hamilton 1964b). Paradoxically, targeted cooperative behaviour may be more likely to evolve as a product of kin selection during certain parts of the life cycle in *D. aruanus* than in many terrestrial vertebrates, because the relatedness between the actor and other, nonrecipient, individuals within the group is almost certainly zero, i.e. because there is no need to account for local kin competition (Hamilton 1975; West-Eberhard 1975; Taylor 1992a,b; Queller 1994; West *et al.* 2002; Johnstone 2008).

The possible cues for kin recognition

If cooperative acts are to be targeted at close relatives then *D. aruanus* must have some form of kin recognition at their disposal (Pfennig & Sherman 1995; Sherman *et al.* 1997). This does not mean that the donor of a cooperative act has to know the genotype of the recipient, but rather the donor must have some simple rule that enables it to discriminate kin from nonkin with a reasonable degree of certainty and behave

accordingly (Reeve 1989; Sherman *et al.* 1997). This study identifies three cues, or rules of thumb, that individual *D. aruanus* might use to recognize kin. First, close relatives tend to be similar in size (Fig. 3), thus individuals might use phenotype matching, assessing their own size and the size of others, to recognize kin (Holmes & Sherman 1982; Hauber & Sherman 2001). Second, close relatives tend to be small in size (Fig. 4), thus individuals might use this contextual cue to help identify kin (Hoogland & Sherman 1976). Third, the observation that close relatives tend to be small and similar in size suggests that they likely have recruited together (see also Planes *et al.* 2002; Selkoe *et al.* 2006), in which case individuals might recognize individuals that they were reared with, dispersed and recruited with, as kin (Holmes & Sherman 1982). These cues, combined with the potential for olfactory cues in fishes (Brown & Brown 1996; Mann *et al.* 2003; Neff & Sherman 2003, 2005; Gerlach & Lysiak 2006) may enable *D. aruanus* to discriminate kin from nonkin and dispense cooperative behaviour accordingly.

Patterns of dispersal and recruitment

In addition to providing insight into the potential for kin selection and possible cues for kin recognition, our results enable us to make some inferences about the patterns of dispersal and recruitment in *D. aruanus*. The observation that close relatives tend to be small and similar in size (Figs 3 and 4) suggests that they are siblings that have dispersed together and recruited together. At first this seems remarkable, given that *D. aruanus* spend 16–24 days as larvae, but the same pattern is also seen in the bluespine unicorn fish *Naso unicornis* (Planes *et al.* 2002) and kelp bass *Palabrax clathratus* (Selkoe *et al.* 2006). This pattern may be a simple effect of larvae being caught up in the same packet of water (Siegel *et al.* 2003) or, alternatively, it may be caused by larvae actively trying to stay together.

The same observation (Figs 3 and 4) implies that multiple new recruits to a single group can be close relatives and this provides support for the sweepstakes reproductive success hypothesis (Hedgercock 1986, 1994; Hedrick 2005; Selkoe *et al.* 2006; Hedgercock *et al.* 2007). As with many problems in ecology and behaviour, evidence for the sweepstakes hypothesis will be contingent on spatial scale and the measure of reproductive success. Here, although there is a sweepstakes effect when 'production of offspring' (small individuals) is used as the metric of reproductive success, the effect apparently disappears when 'production of offspring that survive to breed' (large individuals) is used as the metric of reproductive success (Fig. 4). That is to say that, the

sweepstakes effect documented in *D. aruanus* is an ecological phenomenon that seems to have no evolutionary consequences.

Conclusion

This study reveals that individuals can be associated with close relatives, and there is the potential for kin selection to operate, in the humbug damselfish *Dascyllus aruanus*. This adds to the growing number of studies that have found kin associations and hence the opportunity for kin selection in the marine environment. More generally, it highlights the possibility of kin selection in marine and terrestrial systems even when the mean coefficient of relatedness among individuals within groups is zero. This is because groups in which the mean relatedness is zero may still contain some pairs of relatives.

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1. Genetic relatedness in groups of the humbug damselfish *Dascyllus aruanus*: small, similar-sized individuals may be close kin.

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1 **Genetic relatedness in groups of the humbug damselfish *Dascyllus aruanus*:**
2 **small, similarly-sized individuals may be close kin**

3

4 Peter M. Buston, Cécile Fauvelot, Marian Y. L. Wong & Serge Planes

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7 **SUPPLEMENTARY INFORMATION**

8

9

9 **RESULTS**

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11 ***Summary statistics, tests of Hardy-Weinberg equilibrium and linkage disequilibrium***

12 In this population of *Dascyllus aruanus* we observed from 7 to 50 alleles per locus (Table 1). Over all
13 colonies, observed heterozygosity ranged from 0.307 to 0.955 per locus and expected heterozygosity from
14 0.697 to 0.953. Within colonies, observed heterozygosity ranged from 0.625 to 0.891 and expected
15 heterozygosity from 0.650 to 0.813. No significant gametic disequilibrium was found for any pair of loci
16 ($P > 0.01$). Significant heterozygote deficiencies were found in six loci (Da314, Da371, Da408, Da432,
17 Da479, and Da603; all $P < 0.003$, the threshold following Bonferroni standard correction; Table 1).

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INSERT TABLE 1 HERE

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20 **Table 1.** Heterozygosity of sixteen *Dascyllus aruanus* microsatellite loci, estimated based on a sample of
 21 265 individuals from 35 groups, in Moorea. N_A , number of alleles; H_O , observed heterozygosity; H_E ,
 22 expected heterozygosity; F_{IS} , Wright's fixation index; * significant heterozygote deficiency.

23

Locus	GenBank Accession no.	N_A	H_O	H_E	F_{IS}
Da 304	FJ225427	19	0.809	0.805	- 0.003
Da 314	FJ225428	13	0.307	0.727	0.579 *
Da 331	FJ225429	7	0.719	0.697	- 0.030
Da 360	FJ225430	17	0.782	0.788	0.009
Da 371	FJ225431	11	0.368	0.707	0.481 *
Da 408	FJ225432	26	0.527	0.811	0.353 *
Da 432	FJ225433	18	0.785	0.865	0.095 *
Da 479	FJ225434	17	0.565	0.839	0.329 *
Da 494	FJ225435	40	0.861	0.887	0.030
Da 523	FJ225436	23	0.838	0.855	0.021
Da 542	FJ225437	32	0.948	0.937	- 0.009
Da 565	FJ225438	14	0.772	0.782	0.015
Da 589	FJ225439	33	0.955	0.938	- 0.017
Da 590	FJ225440	36	0.910	0.918	0.010
Da 593	FJ225441	27	0.834	0.876	0.050
Da 603	FJ225442	50	0.518	0.953	0.458 *

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25 *Pairwise relatedness of individuals within and between groups*

26 If only a few individuals within groups are closely related, then their presence might not be detected using
27 the mean coefficient of relatedness. Therefore, we hypothesized that a few individuals within groups will
28 be closely related. Faced with similar situations, previous studies have attempted to determine the
29 likelihood that a focal pair of individuals has a specific relationship, based on their pairwise relatedness
30 and the simulated distributions of pairwise relatedness of close relatives and non-relatives (Goodnight &
31 Queller 1999; Blouin et al. 1996; Selkoe et al. 2006). However, this approach has problems when the
32 relative frequency of relatives and non-relatives in the population is unknown. Problems arise because
33 simulated distributions of relatedness for relatives and non-relatives overlap greatly (Figure 5).

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INSERT FIGURE 5 HERE

36

37 In these situations, molecular ecologists are faced with a classic discrimination problem (Reeve
38 1989). Discrimination problems occur when there are two types of objects (e.g., good rivets or bad rivets,
39 or close relatives and non-relatives) that have overlapping distributions of attributes. If it were possible,
40 one would like to take any given object and determine its type without error. However, this will not be
41 possible when distributions of attributes overlap: some type A objects will be classified as type B objects
42 and *vice versa* (Figure 5). In these cases one must select a threshold above which objects are classified as
43 one type and below which they are classified as the other type. The major question is, where should the
44 threshold be placed to achieve an optimal balance between the two kinds of discrimination errors?

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The optimal location of the threshold depends on the relative costs of the two sorts of error, the
shape of the objects' distributions of attributes, and the relative frequency of the two objects (see Reeve
1989 for the mathematics underlying this result). In the current situation, we can assume that the costs of
the two types of errors are equal. However, in the absence of pedigree data we do not know the shape of
the distribution of pairwise relatedness for close relatives (Csilléry et al. 2006). Furthermore, in the
absence of dispersal data we do not know the relative frequency of non-relatives and close relatives in the

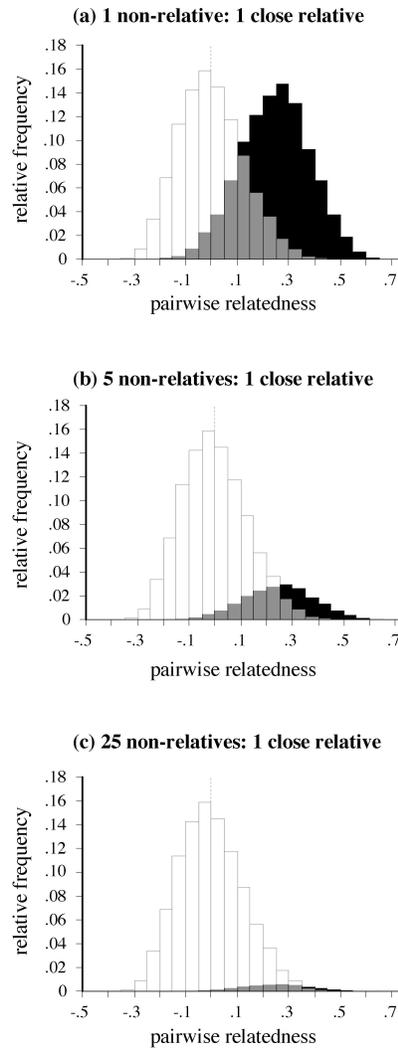
51 study population (Figure 5). This means that it is not possible to determine the likelihood that a particular
52 pairwise relatedness value represents a close relative or non-relative. That is, it is not possible to *a priori*
53 determine optimal placement of the threshold.

54 Programs such as KINSHIP (Goodnight & Queller 1999) and ML-relate (Kalinowski et al. 2006)
55 will estimate the relative likelihood that a focal pairwise relatedness represents a non-relative or close
56 relative, but the estimate is based on a key, simplifying, assumption. These programs have no difficulty
57 in estimating the likelihood of obtaining a particular pairwise relatedness if the true relationship is known,
58 e.g., both programs can estimate the likelihood that half sibs (true $r = 0.25$) have an observed r of 0.20,
59 and both programs can estimate the likelihood that non-relatives (true $r = 0$) have an observed r of 0.20
60 (Figure 5). However, difficulties arise when these programs try to estimate the relative likelihood that a
61 particular pairwise r (e.g., 0.20) represents one relationship or another (e.g., non-relative or half-sib). To
62 do this the programs make the simplifying assumption that the two relationships occur in equal frequency
63 (Figure 5a). This assumption will often be violated, causing these programs to overestimate the likelihood
64 that a focal pairwise relatedness represents a close relative in many situations (e.g., Figure 5b & c).

65 In the absence of information on population relatedness composition, the safest prediction for our
66 hypothesis is that, if some individuals within groups are closely related, then the distribution of pairwise
67 relatedness of individuals within groups should have an overabundance of positive values. We test this
68 prediction using a simple chi-square test with multiple classes, followed by subdivision of the chi-square.
69 The beauty of this approach is that it not only avoids but also helps to solve the problems outlined above.
70 We are able to estimate that there are 35 more pairs of close relatives than expected within groups (Figure
71 2 and associated analyses). More precisely, we are able to estimate that there are 35 pairs of close
72 relatives with a mean relatedness of 0.25 (Figure 3 and associated analyses). Given that there are 1147
73 pairs within groups, this indicates that pairs of non-relatives will outnumber pairs of close relatives by
74 32:1 within groups. Information derived in this way could be entered as a prior in the next generation of
75 programs like KINSHIP and ML-RELATE, helping to refine estimates regarding whether a focal pairwise
76 relatedness represents one relationship or another.

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81 **Figure 5** Distributions of the pairwise relatedness of non-relatives (white; mean relatedness \pm SD = 0.00

82 \pm 0.13, n = 10,000) and half-sibs (black; mean relatedness \pm SD = 0.25 \pm 0.13, n = 10000), generated by

83 simulation based on the allele frequencies in the population using the program KINSHIP 1.3.1 (overlap of

84 distributions is shaded grey; Queller & Goodnight 1989): **a**) pairs of non-relatives and pairs of half-sibs

85 occur in a 1: 1 ratio; **b**) pairs of non-relatives and pairs of half-sibs occur in a 5:1 ratio; **c**) pairs of non-

86 relatives and pairs of half-sibs occur in a 25:1 ratio.