

# First genealogy for a wild marine fish population reveals multigenerational philopatry

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**Natal philopatry, the return of individuals to their natal area for reproduction, has advantages and disadvantages for animal populations. Natal philopatry may generate local genetic adaptation, but it may also increase the probability of inbreeding that can compromise persistence. Although natal philopatry is well documented in anadromous fishes, marine fish may also return to their birth site to spawn. How philopatry shapes wild fish populations is, however, unclear because it requires constructing multigenerational pedigrees that are currently lacking for marine fishes. Here we present the first multigenerational pedigree for a marine fish population by repeatedly genotyping all individuals in a population of the orange clownfish (*Amphiprion percula*) at Kimbe Island (Papua New Guinea) during a 10-y period. Based on 2927 individuals, our pedigree analysis revealed that longitudinal philopatry was recurrent over five generations. Progeny tended to settle close to their parents, with related individuals often sharing the same colony. However, successful inbreeding was rare, and genetic diversity remained high, suggesting occasional inbreeding does not impair local population persistence. Local reproductive success was dependent on the habitat larvae settled into, rather than the habitat they came from. Our study suggests that longitudinal philopatry can influence both population replenishment and local adaptation of marine fishes. Resolving multigenerational pedigrees during a relatively short period, as we present here, provides a framework for assessing the ability of marine populations to persist and adapt to accelerating climate change.**

*Amphiprion percula* | self-recruitment | multigenerational pedigree | inbreeding | parental effects

The dispersal of immature individuals away from their natal area in search of breeding sites can negatively affect population replenishment and, when not offset by immigration, put small populations at risk for extinction (1). Long-distance dispersal may also reduce the potential for local adaptation to environmental change, as individuals may be poorly adapted to distant habitats and suffer higher rates of mortality before reproduction (2). Natal philopatry, the return of individuals to their natal area (3), provides a mechanism to avoid at least some of the negative consequences of dispersal because genetically adapted individuals contribute to the renewal of their population of origin. However, philopatry is a double-edged sword, because although it can contribute to population replenishment, it may also increase inbreeding (3), which often has a deleterious effect on fertility and poses an extinction risk for small populations (4). Inbreeding may also reduce genetic diversity (5), which can reduce the potential of populations to adapt to changing conditions through natural selection (6). Therefore, understanding the balance between dispersal and philopatry is critical in animal

populations because of the implications for genetic diversity (7), adaptation (8), and conservation (9).

The vast majority of benthic marine species, including most coral reef fishes, have a dispersive larval stage in their early life history before settlement into adult habitat (10). Historically, larval offspring were considered as essentially passive particles carried by ocean currents to locations far from their natal population (11). More recently, it has been demonstrated that, similar to many anadromous species, marine fish may also return to their birth area (11–15). It has proved more difficult to verify that those individuals returning to their natal location later successfully reproduce as part of the adult population. Philopatric behavior has been widely documented in terrestrial vertebrate species, including birds and large mammals (16), using multigenerational pedigrees of local populations. Multigenerational pedigrees have yet to be constructed for a wild marine fish population, perhaps because of the presumed dissociation among parents, offspring, and their relatives (17). However, natal philopatry may be more prevalent in marine species than previously thought. Evidence for self-recruitment in coral reef fishes is accumulating (11–14, 18), and these findings raise questions about the role of philopatry in determining the structure and persistence of local populations. For example, do long-term family lineages

## Significance

**Evidence for natal philopatry, the return of individuals to their natal location for reproduction, is scarce in marine fish populations despite being common in anadromous fishes. The proportion of individuals returning to natal sites is an important metric for estimating the effects of inbreeding and the potential for local adaptation to generate resilience to climate change. Here, we present the first multigenerational pedigree for a wild marine fish. We resolved the genealogical tree of families of orange clownfish *Amphiprion percula* spanning up to five generations, using data from a 10-year genetic survey of a population at Kimbe Island, Papua New Guinea. We found that longitudinal philopatry plays a significant role in driving population renewal of the orange clownfish.**

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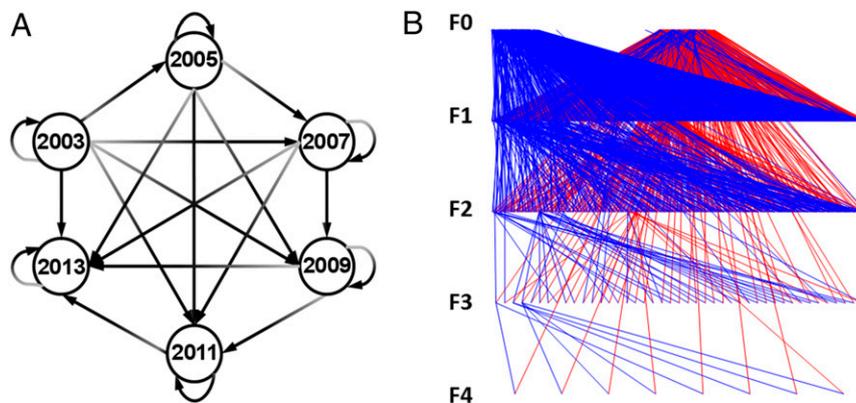
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**Fig. 1.** Philopatric events found in the wild marine orange clownfish population of Kimbe Island, spanning five generations. (A) Generational links among the six sampling periods (i.e., 2003, 2005, 2007, 2009, 2011, 2013) revealed by DNA parentage analysis. Orientation of the arrows gives the generation direction (i.e., from parent to offspring). (B) Pedigree representation: each line connects a parent with one of its offspring (blue and red lines represent, respectively, paternal and maternal links; an individual can be both mother and father). The generation of individuals is indicated on the left from first generation (F0) to fifth generation (F4).

contribute to generational turnover in local populations? Are the benefits of natal philopatry offset by the negative effects of inbreeding? Here, we resolve the first multifamily genealogical trees for a wild marine fish population, using a 10-y genetic survey, and evaluate the extent and effects of philopatry on inbreeding.

## Results

**Pedigree Reconstruction.** We found that self-recruitment [i.e., the ratio of locally produced settlers to settlers from all origins arriving at a given settlement site (19)] occurs over multiple generations in a wild population of the orange clownfish, *Amphiprion percula*, on coral reefs around Kimbe Island, Papua New Guinea. Self-recruitment averaged 56% during a 10-y period (20). We followed the fate of every fish (2927 individuals) and their philopatric progeny over the course of 10 y, using genotypic profiling and parentage analysis (Methods; Fig. 1A). The multigenerational pedigree we reconstructed contained 502 informative founders, 987 parent–progeny links, 1809 full-siblings, 412 maternal half-siblings and 248 paternal half-siblings, 278 paternal grandmothers and grandfathers, and 135 maternal grandmothers and grandfathers (Table 1). The pedigree included up to five generations of individuals within the same family during the 10-y period (Fig. 1B, and see examples of families in Fig. S1). We found examples of families that spread throughout the Kimbe Island population, and others that grouped in small geographic areas around the island (Fig. 2). On the smallest spatial scale, we found 57 instances of family members recruiting to the same anemone (2% of individuals from the local population), including cohabitation of parents and offspring, full-siblings and half-siblings (Table 1). The pedigree reconstruction revealed that 37% of adults (649 individuals) contributed nearly 56% to the renewal of the local orange clownfish population every 2 y (20). Clearly, some individuals contributed more than others to future generations, as is shown in the variety of family tree sizes reconstructed (Fig. S1). Our findings imply that intergenerational continuity in this insular population is dependent on a small proportion of the breeders contributing most of the local replenishment.

**Inbreeding and Extrapair Mating.** The potential downside of philopatry over multiple generations in marine fish populations is inbreeding (i.e., that mating partners are genetically related). Our pedigree reconstruction generally showed that most juveniles from the same family settled in different anemones from parents and siblings. Only two cases of successful inbreeding were observed: one between a brother and sister and one between a grandson and grandmother (Table 1). Whatever the mechanism, inbreeding in

the orange clownfish population was rare ( $F_{IS} = 0.018$ ), and average genetic diversity remained high ( $H_S = 0.714$ ; see Table S1 for detailed results).

Family trees were almost entirely based on adult pairs that cohabit the same anemone. We previously documented the presence of two cases of adults moving from their anemones to another anemone to reproduce [extrapair mating (20)]. The distance between the anemones of the two parents for these two cases were 13 and 133 m, respectively. Our data indicate extrapair mating was uncommon, presumably because individual *A. percula* are strongly site-attached and rarely stray more than a few meters beyond the periphery of their anemone (21, 22).

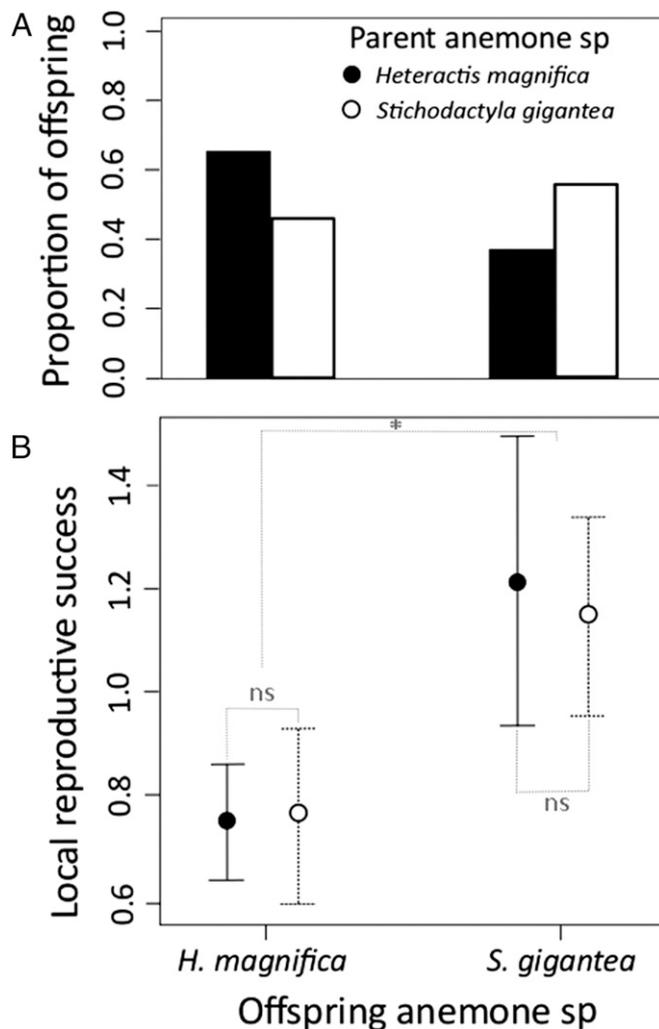
**Parental and Environmental Effects.** We tested the role that parental habitat and environment played in offspring survival and reproduction on Kimbe Island reefs. At the study location,

**Table 1. Pedigree statistics and inbreeding in the Kimbe Island *Amphiprion percula* wild population**

Pedigree statistic	Quantity	Pair of related individuals in anemone (number of inbred mating events)
<i>N</i> individuals	2927	57
<i>N</i> families	121	—
<i>N</i> founders	502	—
<i>N</i> maternal links	987	—
<i>N</i> paternal links	987	9
<i>N</i> full sibs	1809	24 (1)
<i>N</i> maternal half sibs	412	2
<i>N</i> paternal half sibs	248	5
<i>N</i> maternal grandmothers	135	—
<i>N</i> maternal grandfathers	135	—
<i>N</i> paternal grandmothers	278	—
<i>N</i> paternal grandfathers	278	3 (1)
<i>N</i> cousin	218	1
<i>N</i> uncle/aunt	308	10
<i>N</i> half uncle/aunt	165	2
<i>N</i> grand cousin	17	1
Pedigree maximum depth	4	—

The number of individuals, families, founders, and family links are listed in the central column. Total number of pairs of related individuals found within the same anemone are listed in the last column. Among these links, the number of cases of inbreeding are presented in brackets.





**Fig. 3.** Effect of parental anemone species on the local reproductive success of their offspring in orange clownfish from Kimbe Island. (A) Settlement choice distribution of offspring ( $n = 987$  individuals) on the two species of anemones, *H. magnifica* or *S. gigantea*, according to the anemone species of their parents. Offspring native from *H. magnifica* and those native from *S. gigantea* recruited in similar proportion in the two species of anemone ( $\chi^2 = 1.32$ ;  $P = 0.250$ ). (B) Effect of anemone species transmission from parent to offspring on the local reproductive success of offspring. Whatever the anemone species from which the clownfish progeny originated, the local reproductive success was always higher for individuals living in *S. gigantea* than for those living in *H. magnifica* ( $t = -2.41$ ;  $P = 0.016$ ). NS and \* mean Student's statistical test was not significant and significant, respectively.

of self-recruitment (~56%), the number of juveniles arriving from other reefs appears to be sufficient to maintain high genetic diversity in the study clownfish population. The two incidences of inbreeding we observed may be a result of the limited number of anemones available for settlement at any given time (28), which suggests it will be advantageous for settlers to remain at the first anemone they encounter (29). A dispersive larval phase and a hierarchical breeding system likely compensate for the potential inability of individuals to accurately identify and actively avoid mating with relatives. The hierarchical breeding system is a size-based queue for the two breeding positions in an anemone, which means it can take up to 10 y to become a breeder (30) (i.e., newly settled relatives that could engage in inbreeding may never get the chance).

High levels of self-recruitment do not preclude long-distance dispersal events in *A. percula*. Our observation of 56% self-recruitment means that 44% of the population arrived as larvae from reefs beyond Kimbe Island. Larvae that dispersed to the study population from other locations likely represented a significant influx of genetic diversity. Therefore, although the larval dispersal kernel may be weighted toward self-recruitment, immigration nonetheless has a significant effect on the genetic structure of the study clownfish population. It remains unknown whether local adaptation can occur in this *A. percula* population. Locally adapted genes may be swamped by gene flow from immigrants. However, evolutionary adaptive divergence, and therefore local adaptation, may occur despite gene flow if selection is strong (31). The high levels of self-recruitment suggest local adaptation is at least possible in *A. percula*. Identifying the possible extent, nature, and implications of local adaptations represents an important area for future research.

Our findings have consequences for the management of spatially structured marine fish populations and the ability of future generations to adapt to environmental change. Although it is well known that exploited populations can be protected in marine reserves such as Kimbe Island, our study has demonstrated that this benefit can ensure the protection of multiple generations. It is conceivable that such long-term protection may prove futile if coral reef fish species are unable to cope with rapid global climate change. Climate change may act to reduce the potential dispersal distance and functional connectivity between populations of many species in oceans (25, 32). Philopatric behavior may become the major source of local replenishment in the future. Although inbreeding is currently very low, the population may be at risk in the long term if immigration, and hence genetic diversity, decrease. However, our discovery of five-generation family trees in the space of 10 y suggests that the potential for local adaptation may be higher than previously thought in marine fish populations. The presence of local adaptation would, in turn, provide a mechanism for population resilience to climate-induced changes in environmental conditions.

## Materials and Methods

**Study Species and Data Collection.** Exhaustive sampling was carried out every 2 y from 2003 to 2013 on the entire orange clownfish (*A. percula*) population at Kimbe Island (5°12'22.56" S, 150°22'35.58" E), West New Britain Province, Papua New Guinea. Here, *A. percula* live in a mutualistic association with one of two host sea anemone species, *S. gigantea* and *H. magnifica*. Within each clownfish group (i.e., clownfish within one anemone) of typically three to five individuals, there is a size-based dominance hierarchy: the female is largest, the male is second largest, and the nonbreeders rank progressively lower in the hierarchy as they decrease in size. The bigger the group, the longer a settler has to wait to achieve reproductive status, and thus the lower the likelihood of surviving to maturity (33). If the single female adult of a group dies, then the male changes sex to female, and the largest juvenile from the anemone becomes sexually mature as male.

At Kimbe Island, reproduction occurs year round, with females laying several hundred eggs in a clutch near the pedal disk of the host anemone each lunar month. The eggs hatch after ~7 d of paternal care into larvae that spend ~10 d (34) in the pelagic environment before settling on an anemone that may or may not be on the natal reef (35).

The Kimbe Island population of *A. percula* consists of 310 different anemones (176 *H. magnifica* and 134 *S. gigantea*). Anemone positions were recorded with a GPS, and depth was measured using a dive computer. Anemones were surveyed during each biannual sampling period, and all fish within the anemones were counted, captured using hand nets, measured using calipers (total length, TL), and fin-clipped underwater for genetic analysis. Each fish was processed on SCUBA in situ, with each individual caught, processed next to the anemone, and then released back on the same anemone. The biggest fish in each anemone was identified as the female, the second largest individual was assumed to be the male, and all other individuals were classified as subadult (TL, >35 mm) or new recruits (TL, <35 mm). The small pieces of fin tissue were preserved in 95% ethanol in 2-mL vials. The new recruits were collected whole and preserved in vials with 95% ethanol.

We extracted DNA from a total of 4829 fin-clips (including 1192 new recruits) following a modified HotSHOT method (36), and subsequently stored the DNA at  $-20^{\circ}\text{C}$ . We then screened the DNA at 22 polymorphic microsatellite loci (20). We controlled for human errors and sample contamination by using negative controls during PCR and electrophoresis. We also controlled for genotype errors by reamplifying and reanalyzing a sample of 789 individuals with the same typing software (GeneMapper v3.7). The error rate was  $\sim 2\%$ . Genetic diversity metrics such as allelic richness, average expected and observed heterozygosities ( $H_s$  and  $H_o$ , respectively), and the departure from Hardy-Weinberg equilibrium fixation index or inbreeding coefficient ( $F_{IS}$ ) were estimated by using the R package HIERFSTAT (37).

Work was carried out under ethics approval A1643 from James Cook University.

**Pedigree and Family Reconstructions.** Pedigree reconstruction was carried out using the genotype data from the parentage analysis. We first identified the individuals sampled multiple times over the years by using the Excel macro GenAlex v6.5 (38) to compare multilocus genotypes from the 2003, 2005, 2007, 2009, 2011, and 2013 samples. Juvenile fish were assigned to parental pairs, using software FaMoz (39). This software is based on the calculation of log of the odds ratio of putative parent-offspring pairs being true and determines critical thresholds to accept or reject assignments by simulating true and false parent-offspring pairs. FaMoz does not require a priori information of the proportion of candidate parents in the sample to determine critical logarithm of odds (LOD) thresholds (40). Overlapping generations were assumed for the pedigree reconstruction to accommodate the biology of the clownfish species (i.e., reproduction frequency and lifetime), such that two individuals within a cohort were defined as a potential parent for any individual sampled the same year or the following years (Fig. 1A). We only retained offspring-parent-pair assignments when genotypes were matched to fish living in the same anemone or in an anemone in close proximity within the same period (for more details, see ref. 20). Because of the presence of overlapping generations in this system, we rejected all assignments to single parents to avoid possible false assignments to other related individuals that were not true parents. We accepted offspring-parent-pair couples with LOD scores  $>30$ .

We tested all of the possible parent-couple-offspring links between the different periods (Fig. 1A). First, we compared individuals sampled in the same year and hypothesized that two adults from same or nearby anemone can be potential parents of subadults and new recruits, and two adults from same or nearby anemone can be potential parents of other adults (i.e., the dataset of offspring to test in FaMoz is exactly the same as the dataset of parents). Second, we compared individuals sampled in two different years and hypothesized that two individuals from adult or subadult categories living in same or nearby anemone and sampled at a specific period can be the parents of adults, subadults, and new recruits sampled at the following years, and two individuals from adult or subadult categories sampled in two

consecutive sampling years can be the parents of adults, subadults, and new recruits sampled the periods after. We measured the local reproductive success for each individual as the sum of self-recruiting offspring produced from 2003 to 2013.

**Pedigree and Dispersal Analyses.** Pedigree summary statistics estimating pairwise relatedness between all individuals and maximal depth pedigree were obtained by using the R package pedantic (41). We constructed the pedigree containing only those individuals that were informative (i.e., a member of a family from Kimbe Island). Then, we analyzed separately the structure and depth of each family that was present in the pedigree. The families were plotted as family trees using the R package KINSHIP2 (42). Mothers are represented with a circle, males and subadults with a square, and new recruits with a diamond. The same individual can be both a father and a mother because of the sex changes through the life of the clownfish. This hermaphroditism has no effect on the pedigree reconstruction. We used as a representative symbol a circle within a square for the individuals for which we detected sex change during the studied period.

Parentage analyses combined with sampling site information allowed us to identify offspring dispersal from the natal anemone to the anemone of settlement. We evaluated whether descendants preferentially returned to the same anemone species (*H. magnifica* or *S. gigantea*) as their founder ancestors or not.  $\chi^2$  test was used to evaluate whether the anemone species of offspring was independent of the anemone species occupied by parents. Then, we tested whether the local reproductive success of offspring was linked by the parental anemone species to evaluate a potential effect of parental environment by using Student's *t* test. Statistical analyses were performed using R version 3.0.2 (43), and their significance was interpreted with  $P = 0.05$ .

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