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LETTER

Strong habitat and weak genetic effects shape the lifetime reproductive success in a wild clownfish population

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Abstract

The relative contributions of environmental, maternal and additive genetic factors to the Lifetime reproductive success (LRS) determine whether species can adapt to rapid environmental change. Yet to date, studies quantifying LRS across multiple generations in marine species in the wild are non-existent. Here we used 10-year pedigrees resolved for a wild orange clownfish population from Kimbe Island (PNG) and a quantitative genetic linear mixed model approach to quantify the additive genetic, maternal and environmental contributions to variation in LRS for the self-recruiting portion of the population. We found that the habitat of the breeder, including the anemone species and geographic location, made the greatest contribution to LRS. There were low to negligible contributions of genetic and maternal factors equating with low heritability and evolvability. Our findings imply that our population will be susceptible to short-term, small-scale changes in habitat structure and may have limited capacity to adapt to these changes.

Keywords

Adaptation, additive genetic variation, environmental effects, evolvability, heritability, maternal effects, multi-generational pedigree, selection.

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INTRODUCTION

Lifetime reproductive success (LRS) – the number of successful offspring an individual contributes to the next generation – is a critical variable underpinning ecological and evolutionary responses to the environment (Clutton-Brock 1988). Several factors including different environmental, parental, and additive genetic effects can influence LRS (Hendry *et al*. 2018). If LRS is exclusively a phenotypic response to the conditions experienced by individuals, populations will be severely impacted by rapid environmental change and there is no prospect of adaptive microevolution. Maternally transmitted responses to environmental conditions can mediate the effect of those changes on the next generation (Mousseau & Fox 1998). However, it is the additive genetic variation in LRS that determines the rate of adaptation of a population to the environmental demand (Fisher 1930). To date, few studies have distinguished these relative contributions to LRS over multiple generations in wild populations (Hendry *et al*. 2018). This situation is changing as long-term, individual-based ecological studies with multi-generational pedigrees provide the necessary longitudinal information to quantify the different components of LRS (Pemberton 2008). Such studies are imperative as we seek to understand the ability of species to withstand or adapt to accelerating climate change (Munday *et al*. 2017).

Intergenerational responses to selection are a product of the interplay between evolutionary and ecological mechanisms that ultimately shape inherited variation in fitness-related traits. Environmentally driven mechanisms (e.g. phenotypic plasticity, genetic assimilation) can facilitate (Ghalambor *et al*. 2007; Danchin *et al*. 2019) or constrain the microevolutionary response to selection (Pujol *et al*. 2018). However, in the absence of genetic variation in LRS, these mechanisms will likely have little effect on a negligible rate of adaptive evolution. In quantifying additive genetic variation, it is important to distinguish between *heritability* and *evolvability* (Wheelwright *et al*. 2014). Narrow sense heritability is widely used as a measurement of the population potential to respond to selection (Mousseau & Roff 1987). It is the additive genetic variance standardised by the total phenotypic variance. Low heritability values can either reflect low additive genetic variance or large environmental, or residual effects. Evolvability is the mean-standardised additive genetic variance (Houle 1992). Environmental or maternal effects do not affect it, which makes it a more appropriate metric in the comparison of evolutionary potential between traits, populations and species. When measured for a surrogate of fitness like LRS, it

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estimates the expected proportional change per generation in population mean fitness given a unit strength of selection. Heritability for LRS reveals whether its additive genetic variance represents a non-trivial proportion of its total variance in the actual environmental context of a given wild population. Together, evolvability and heritability inform us about how much environmental change a wild population can withstand on the basis of its evolutionary potential.

The few ($n = 15$) long-term, individual-based studies that have quantified additive genetic variation, heritability and evolvability of LRS in wild populations have all focused on terrestrial species (Postma 2014; Hendry *et al.* 2018; Table S1). They have largely confirmed low additive genetic variation and evolvability (*c.* 0.08) for LRS, which nevertheless reflects some evolutionary potential (Hendry *et al.* 2018). Whether marine fish and terrestrial species reveal the same patterns remains unknown to date. Until recently, quantifying LRS in marine organisms with a pelagic larval stage has been considered impossible because of the difficulties in following the fate of offspring from one generation to the next. However, there is increasing evidence of some degree of natal philopatry or self-recruitment in local marine populations (Jones *et al.* 2009). Genetic parentage analysis is making it possible to assign a significant proportion of offspring to their parents (Planes *et al.* 2009) and construct multigenerational pedigrees for the offspring that return to their natal population (Salles *et al.* 2016). As in most quantitative genetic studies of LRS, it is impossible to measure the recruitment of marine fish dispersing juveniles at other locations. The regional component of LRS, which would inform us on fitness variation beyond the local scale is impossible to obtain. Measuring the local component of LRS in marine fish is nevertheless an opportunity as in any other species because it estimates the relative contribution of local fish to the population self-recruitment and replenishment.

For coral reef fishes, quantifying environmental and genetic components of LRS and assessing its evolvability in wild populations is of great contemporary importance. Between 30 to 50% of the world's coral reefs have been lost and those remaining are considered highly vulnerable (van Hooidonk *et al.* 2016). The rapid loss of suitable habitat is widely acknowledged to be contributing to a decline in reef fish populations and biodiversity (Jones *et al.* 2004). The potential for adaptation of reef fish is uncertain and near future environmental conditions predicted under climate change should have a dramatic effect on their reproductive success (Munday *et al.* 2013). To date, environmental, maternal and additive genetic contributions to LRS in wild coral reef fish populations have not been assessed. However, recent work establishing high levels of natal philopatry in some coral reef fishes (Jones *et al.* 2005) that allowed for resolving their pedigree across multiple generations (Salles *et al.* 2016) opened the way for quantifying the local component of LRS for the first time in coral reef fishes.

Here, we focus on the entire local population of the orange clownfish *Amphiprion percula* at Kimbe Island, Papua New Guinea where each year ~ half the juveniles successfully recruiting are progeny of local breeding pairs (Salles *et al.* 2016). We use multi-generational pedigrees of up to 5 generations obtained from biennial DNA sampling over 10 years (Salles *et al.* 2016) and apply a quantitative genetic linear mixed model approach (Kruuk & Hill 2008) to quantify the additive genetic, maternal and environmental components of variation in LRS for the self-recruiting portion of the population. Habitat effects were quantified by examining this local LRS for individuals resident in two different anemone species and from different geographic locations around the island. By integrating habitat data with the pedigree information in a quantitative genetic generalised linear mixed model, we were able to assess the relative contribution of additive genetic, maternal and habitat effects to local LRS. We also calculated the evolvability and heritability of local LRS to evaluate its evolutionary potential to respond to selection at the scale of the Kimbe island population.

MATERIAL AND METHODS

Study population and data collection

A natural population of orange clownfish (*A. percula*) living in the reef surrounding Kimbe Island (Fig. 1a; 5°12'22.56'' S, 150°22'35.58'' E), West New Britain Province of Papua New Guinea, was surveyed every second year from 2003 to 2013. Here, *A. percula* lives in a mutualistic association with one of two host sea anemone species, *Heteractis magnifica* (Fig. 1b) and *Stichodactyla gigantea* (Fig. 1c). We geographically located and tagged a total of 310 anemones (176 *H. magnifica* and 134 *S. gigantea*) that were occupied by *A. percula* on the entire reef surrounding the island.

These two anemone species are remarkably different in terms of the micro-habitat they provide, including a wide range of shapes, sizes, depth distributions and surrounding substrata (Dunn 1981). Although we did not directly measure these variables (other than depth), the combination of host anemone species (*H. magnifica* or *S. gigantea*) with one of the three geographical areas covering the entire reef around the island (northern, western or eastern areas, Fig. 1a) where it is located describes a 'habitat' variable for each fish that encompasses a suite of biotic and abiotic environmental conditions. These geographical areas correspond to the different coasts of the island that reflect possible environmental effects of the geographic location. In total, the combination of the two host anemone species with the three geographical areas allowed us to describe six different habitats.

Clownfish within one anemone live in group of typically three to five individuals in the Kimbe Island population. The size-based dominance hierarchy in *Amphiprion* allows us to determine the reproductive status of each individual (Fricke 1979). The female is the largest, the male is the second largest, and the non-breeders rank progressively lower in the hierarchy as they decrease in size. If the single female adult of a group dies, then the male changes sex to female, and the largest non-breeder from the anemone becomes sexually mature as male. 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 *c.* 7 days of paternal care into larvae that spend *c.* 10 days in the pelagic environment (Berumen *et al.* 2010) before settling

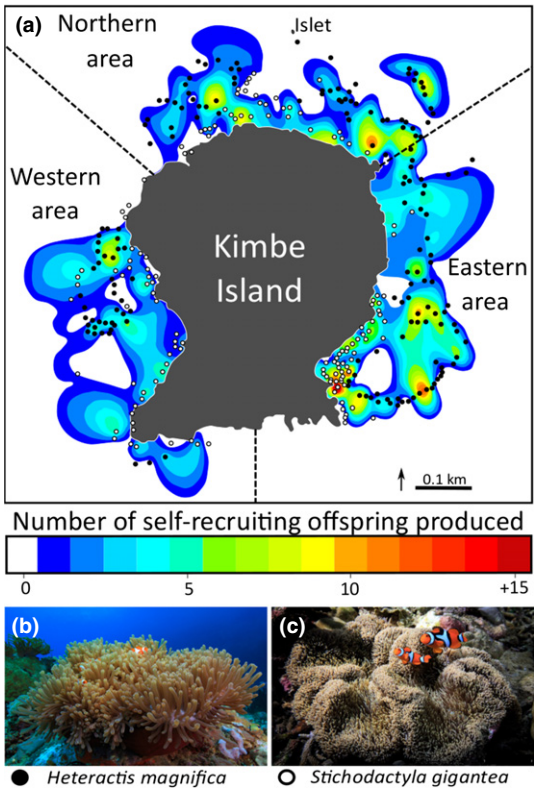

Figure 1 Variation in the total number of offspring orange clownfish produced on each anemone around Kimbe Island between 2003 and 2013. (a) The studied region was divided into three areas (northern, eastern and western areas). Colors correspond to the variation in the total number of juveniles locally self-recruited that were produced on each anemone (varying from 0 to 27) over a 10-year period. The expected value is interpolated from those around it (using default algorithms implemented in *Origin* software). Dots correspond respectively to the location of the two host anemones species: (b) *Heteractis magnifica* (black dots) and (c) *Stichodactyla gigantea* (white dots). Photos by Tane Sinclair-Taylor.

to an anemone, either at their natal location (Kimbe Island) or elsewhere (Planes *et al*. 2009).

Divers captured fish by using hand nets. Individuals were measured *in situ* using calipers, fin-clipped (size > 35 mm) or collected whole (size < 35 mm) for genetic analysis and then released back on the same anemone. Small pieces of fin tissue were preserved in 95% ethanol in 2-mL vials. 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 non-breeders. We extracted DNA from all samples and genotyped them at 22 polymorphic microsatellite loci (Bonin *et al*. 2015). Then, we identified the individuals sampled multiple times over the years by using the Excel macro GenAlex v6.5 (Peakall & Smouse 2012) to compare multilocus genotypes from 2003, 2005, 2007, 2009, 2011 and 2013. Individuals were in average sampled 2.88 ± 0.04 times (mean ± SE) over the six surveys (1% of individuals persisted over the 10-yr period, Salles *et al*. 2016). The 2-yr sampling scheme precluded calculating a precise measurement of the age of individuals, in particular for fish sampled in 2003 during the first sampling period, wherein age was unknown. The total duration of this long-term survey did not allow us to obtain many replicated measurements within individuals before and after sex change (n = 41 individuals). Estimating sex-dependent additive genetic variance is precluded in this case because some effects cannot be disentangled as the clownfish only changes sex in one direction (from male to female). This change is always associated with a change of sexual partner and with an increase in female body size, which we expect to generate a confounding effect between a female condition and its genetic quality. We therefore did not consider sex in our model as a result of data and analytical limitations.

Pedigree used for quantitative genetic analysis

Pedigree reconstruction was conducted by assigning juvenile fish to parental pairs on the basis of their multilocus genotypes (Salles *et al*. 2016). We used the software FaMoz (Gerber *et al*. 2003). This approach is based on the calculation of LOD scores (Log of the odd-ratio comparison) for any potential parentage relationship. It determined critical thresholds to accept or reject assignments by simulating true and false parent–offspring pairs. Further details on parentage analyses and pedigree reconstruction are given in Salles et al. (2016). We kept assignments to known parental pairs, but rejected assignments to single adults. In the context of overlapping generations, we used the year of first sampling and the anemone of each parental couple as information to avoid possible false assignments. As a result, sibship links could not be confused with parental links. The same individual can be related to its offspring with either a paternal or maternal link because of sex changes. We identified the mother and the father based on the size of the two parents on the year of first capture. The original population pedigree includes 2927 clownfish over five generations, including 121 families, 987 paternal, 987 maternal, 1809 full-sib, 412 maternal half-sibs, 248 paternal half-sib, 135 maternal grandmothers, 135 maternal grandfathers, 278 paternal grandmothers, 278 paternal grandfathers and 218 cousins (Salles *et al*. 2016). For this study, we excluded from the original pedigree the 1192 individuals that were removed from the habitat at the juvenile stage (size < 35 mm, 10–458 days old). The final pedigree used for this study includes 1735 individuals from five generations (Fig. S1). We used the R package 'pedantics' (Morrissey & Wilson 2010) to assess the power of the resolved pedigree to detect significant quantitative genetic parameters (Fig. S1).

LRS: the individual contribution to self-recruitment

LRS, which when measured at the scale of the local population is also the contribution of an individual to self-recruitment, corresponds to the total number of offspring produced during its lifetime and recruiting into Kimbe Island (e.g. the local breeder population). We used biennial measurements of their reproductive success (using field-data from 2003, 2005, 2007, 2009, 2011 and 2013) to compare LRS between individuals because some fish were still alive at the end of sampling and others might have already reproduced before the first sampling year. The LRS corresponds here to the total number of descendants produced on a biennial basis that successfully

recruited into Kimbe Island population, which provided us with repeated measures over the period of the survey from 2003 to 2013. In the Supplementary information, we present results from an alternative approach based on the De-lifing method (DL). The calculation of DL takes into account the temporal variation in the population growth and estimates the contribution of every clownfish to biennial changes in population size through both reproduction and survival (Coulson et al. 2006). Statistical problems with the use of DL, potentially leading to precision issues, have been pointed out (Dupont *et al.* 2017). DL was used in two of the 15 studies where the genetic variation of fitness was quantified in wild populations (Table S1), which limits our ability to discuss its properties. We therefore also provided DL results in this study in the supplementary section.

Quantitative genetic generalised linear mixed model approach

Similarities between relatives living in contrasted micro-habitats allowed us to evaluate simultaneously the genetic and habitat components of LRS. Repeated 'records' on individuals made it possible to estimate permanent environmental effects, and therefore account for intra-individual and unmeasured environmental trait variation across time. Permanent environmental effects also account for a part of non-additive genetic effects (Wilson *et al.* 2010). LRS variance was partitioned into six random effects: Additive genetic (V_A), Maternal (V_M), Natal Habitat (V_{NH}), Resident Habitat (V_{RH}), Permanent Environment (V_{PE}) and Residual (V_R) variances by using the 'animal model' quantitative genetic approach (Kruuk 2004). This Linear Mixed Model (LMM) approach uses pedigree information to extract the additive genetic component. This approach is more powerful than traditional analyses (*e.g.* parent–offspring regressions) because it takes into account every relationship link in a pedigree. Maternal variance was modelled using the mother's identity as a random effect, allowing maternal effects to include both genetic and environmental maternal effects. Permanent environmental effects were modelled by including the identity of individuals as a random effect. The LRS variance is the sum of six variance components:

$$V_{LRS} = V_A + V_M + V_{NH} + V_{RH} + V_{PE} + V_R \quad (1)$$

Quantitative genetic models were computed as univariate GLMMs using the 'MCMCglmm' package (Hadfield 2010) in R version 3.5.1 (R.Core.Team 2018), with LRS as a Poisson response variable. Using this Bayesian framework facilitates parameter estimation for non-Gaussian traits. We used parameter expanded priors for all analyses ($V = 1$, $nu = 0.02$), which are often referred to as 'non informative' priors although such denomination can be debated, as we wanted posterior estimates to be determined from the data and not from the priors (Morrissey *et al.* 2014). We ran model MCMC chains over 1 000 000 iterations with initial burning of 10 000 iterations and a thinning of 1000 iterations. Historically, the Deviance Information Criterion (DIC) was often used to compare models and assess the significance of the random variance components in this type of approach. However, it is becoming less commonly used since it was recognised as an inappropriate tool for model comparisons of the same type than quantitative genetic GLMM analyses (Spiegelhalter *et al.* 2014). Effects of variance components were considered statistically supported if their posterior distributions did not overlap zero (Wilson *et al.* 2010).

Variance components

Variance components were estimated as the mode of the posterior distributions established on the MCMC sample and we reported lower and upper limits of the 95% credible intervals. For the six variance components, we calculated their relative contribution to the sum of all variance components, thereby expressing their effects as percentages of the total phenotypic variance (V_{LRS}). As a result, we obtained standard narrow sense heritability estimates for LRS (h^2) by applying the basic formula ($h^2 = V_A/V_P$), and similarly maternal effects by estimating the proportion of total phenotypic variance explained by the maternal variance ($m^2 = V_M/V_P$). Evolvability (I_A) of LRS is the additive genetic variance divided by the squared mean of the LRS (Houle 1992). Analyses assumed a Poisson distribution and provided parameter estimates on a statistically convenient latent scale for non-Gaussian traits. We therefore back-transformed these estimates onto the observed data scale to improve our inferences by using the 'QGlmm' package (de Villemereuil *et al.* 2016). We used the function 'QGparams' to estimate additive components such as V_A and h^2, and 'QGicc' to estimate broader sense components such as V_M and m^2, V_{NH}, V_{RH}, V_{PE} and V_R. Although parameter estimates transformed back on the data-scale are expected to be upward biased, their ratio is reliable, and hence the estimators derived from their relative proportions such as h^2. It is necessary to point out two specific aspects of this back transformation on the observed data scale. First, V_R is estimated on the basis of the additive over-dispersion term in the nonlinear model and its value cannot be interpreted similarly to the usual residual variance term estimated by classical quantitative genetic linear mixed models. Second, the sum of the variance components estimated on the data scale are not additive and therefore not expected to sum up to the value of the phenotypic variance calculated directly on the raw data. For the sake of clarity and comparison, we present the results on the latent scale and the observed data scale. We calculated the 95% credibility intervals from the posterior distributions of observed parameters for all the variance components and other estimates expressed on their basis by using the 'HDInterval' package (Meredith & Kruschke 2016).

RESULTS

Habitats dominantly shape the Lifetime Reproductive Success in the clownfish population

Biennial estimates of the Lifetime Reproductive Success (LRS) measured inside the area of the Kimbe island population ranged from 0 to 13, with a phenotypic variance $V_{LRS} = 1.31$ and an average value of 0.54 ± 0.05 (mean ± SE) offspring per individual for a two-year period. Because clownfish live in strong association with their

anemone, we were able to identify and geo-locate the precise position and habitat where breeders contributed more to the local replenishment of the population (Fig. 1a). Breeders that produced more self-recruiting offspring lived in Kimbe Island's eastern area and mostly in *S. gigantea* anemones. Our analysis also revealed fish that did not contribute at all to the local replenishment of the population over the 10-year monitoring period. These fish represented 25% of the pairs of local breeders and were located in 48 *H. magnifica* and 30 *S. gigantea* of the 310 anemones monitored in both deep and shallow waters (Fig. 1a).

Quantitative genetic generalised linear mixed models on the latent and the observed data scale gave very close results. Our results on the scale of observed data showed that Natal Habitat and Resident Habitat explained, respectively, 19.1 and 5.7% of the variance in local LRS, furthermore, residual and permanent environment explained, respectively, 41.0 and 31.0%, whereas additive genetic effects and maternal effects were very weak and explained 1.3 and 1.9% respectively (Fig. 2a, variances on observed data-scale). Similar results were obtained for DL (see Supporting Information for more details).

Low evolvability and low heritability for LRS

The modes of the posterior distributions estimating the additive genetic variance for LRS, expressed on the latent and observed data-scales, were extremely small (Table 1). On the observed data scale, we found $V_A = 0.030$ ($CI_{95\%}$ 4.94×10^{-4} to 0.060). This could be linked to the statistical power of our pedigree (Fig. S1). Our model nevertheless placed fairly restricted bands on the 95% credible intervals (Table 1). Credible intervals did not overlap the zero but were close. The extent to which these very low values of genetic estimates are not null must therefore be considered with caution. LRS evolvability estimated on the observed data scale, which evaluated the micro-evolutionary change of the number of self-recruiting offspring that can be reached by the population, was equal to 0.103 ($CI_{95\%}$ 1.661×10^{-3} to 0.511). In other words, 0.103 additional juveniles were added to the average number of juveniles originating and recruiting in the population per generation. The heritability estimate expressed on the observed data-scale was $h^2 = 0.013$ ($CI_{95\%}$ 4.951×10^{-5} to 1.227×10^{-2}) for LRS (Table 1). We can therefore estimate the maximum response (R) to selection (S), expected in theory, in the presence of strong selection pressures acting on the Kimbe Island orange clownfish population by using the Breeder's equation $R = h^2 \times S$ (Lush 2008). The low to negligible value of the LRS heritability means that the maximum genetic change of the population average LRS would never exceed ~ 0.020 offspring per generation. Similar results were obtained for DL (see Supplementary Information for more details).

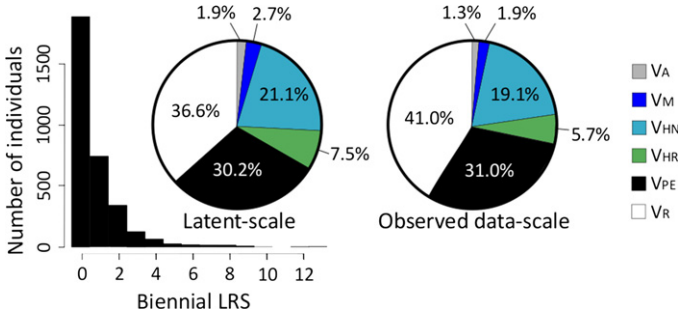

Figure 2 Sources of variation in the biennial estimate of the local Lifetime Reproductive Success (LRS) of the Kimbe Island orange clownfish. Distribution of the biennial estimate of the LRS (histograms). Variance components on both latent-scale and observed data-scale (pie charts) for the biennial estimate of the LRS explained by Additive genetic (V_A), Maternal (V_M), Natal Habitat (V_{NH}), Resident Habitat (V_{RH}), Permanent Environmental (V_{PE}) and Residual (V_R) variances. These proportions were calculated from the values of the posterior modes of a quantitative genetics generalised linear mixed model analysis (for details see Table 1).

Weak maternal effects for LRS

Our analysis detected maternal variance for the LRS but it was also extremely small to the extent that it might be considered as null (Table 1): $V_M = 0.019$ ($CI_{95\%}$ 2.966×10^{-5} to 2.044×10^{-2}). It made very little contribution to the total variance in LRS ($m^2 = 1.9\%$, expressed on observed data-scale, Fig. 2). The habitat occupied by the mother (Natal Habitat) had a stronger effect on LRS than the mother herself. The relative contribution of individuals to the population replenishment was indeed influenced by the Natal Habitat to an extent of 19.1% for LRS. Similar results were obtained for DL (see Supplementary Information for more details).

Table 1 Sources of variation in Lifetime Reproductive Success (LRS) for the Kimbe Island orange clownfish

	LRS Latent scale	LRS Observed data-scale
V_A (CI)	0.046 (1.381×10^{-3} to 0.146)	0.030 (4.94×10^{-4} to 0.060)
V_M (CI)	0.067 (2.000×10^{-3} to 0.211)	0.046 (8.822×10^{-3} to 0.287)
V_{NH} (CI)	0.516 (0.015 to 1.529)	0.450 (0.126 to 1.524)
V_{RH} (CI)	0.184 (0.264 to 0.473)	0.135 (0.038 to 0.457)
V_{PE} (CI)	0.737 (0.496 to 0.952)	0.726 (0.203 to 2.460)
V_R (CI)	0.894 (0.717 to 1.105)	0.963 (0.270 to 3.264)
V_{LRS} (CI)	2.44 (1.71 to 3.65)	2.35 (0.65 to 8.05)
h^2 (CI)	0.019 (6.827×10^{-4} to 0.057)	0.013 (4.951×10^{-5} to 1.227×10^{-2})
m^2 (CI)	0.027 (9.157×10^{-4} to 0.083)	0.019 (2.966×10^{-5} to 2.044×10^{-2})
I_A (CI)	0.154 (4.643×10^{-4} to 0.492)	0.103 (1.661×10^{-3} to 0.511)

Here we reported variance component estimates quantified by using the animal model approach: Additive genetic (V_A), Maternal (V_M), Natal Habitat (V_{NH}), Resident Habitat (V_{RH}), Permanent Environmental (V_{PE}) and Residual (V_R) Variances. We also report size effects as proportions of explained phenotypic variance: narrow-sense heritability (h^2), maternal effects (m^2) and the mean standardised additive genetic variance: evolvability (I_A) for biennial LRS. Measures are expressed on a latent-scale (direct *MCMCglmm* R results) and the observed data-scale (*QGglmm* R back-transformation). 95% credible intervals (CI) are reported for each estimate.

DISCUSSION

Strong habitat and weak genetic effects on LRS

Our study revealed that biennial LRS in the Kimbe Island orange clownfish population quantified over five generations was largely explained by ecological factors. Host anemone species, depth and geographical location explained *c*. 25%, and permanent environmental effects *c*. 30% of its variation, with only weak maternal (1.9%) and additive genetic effects (1.3%). Intrinsic biological characteristics of anemone species (e.g. size, shape and toxicity) affecting the life-history traits of their resident clownfish might explain strong habitat effects (Chausson *et al*. 2018). In addition, the higher toxicity of *S. gigantea* (Nedosyko *et al*. 2014) might provide better protection against predators of eggs attached to the host anemone, but this hypothesis remains to be tested. The geographical location also appears to be important. Most successful individuals were close to the land in shallow water on *S. gigantea* and in deeper lagoons for *H. magnifica*, which might promote greater local retention of larvae. Mechanisms responsible for geographical differences in LRS around Kimbe Island remain unknown (Berumen *et al*. 2010). Some breeders likely have a different reproductive success beyond the sampled population, through dispersers rather than self-recruiters. Inside the Kimbe island clownfish population, weak genetic effects on LRS indicate a low to negligible rate of adaptive evolution in progress and raise concern about the ability of this population to adapt to rapid climate change.

Susceptibility to habitat change

Habitat largely drove LRS variation. Individuals that happen to settle on particular anemones and places do well. The dependence on habitat quality of LRS inside the Kimbe island population suggests this species will be extremely susceptible to habitat degradation over ecological time scales. Detrimental direct and indirect anthropogenic impacts on reef anemone habitats are already affecting numerous clownfish species (Saenz-Agudelo *et al*. 2011). *S. gigantea* anemones located in shallow waters are likely to be disproportionately more impacted by increasing water temperatures and irradiance (Hobbs *et al*. 2013). If these habitats are differentially impacted, this will affect clownfish contributions to the local replenishment of the population and compromise population persistence.

Low to negligible evolutionary potential

Our findings provide the first empirical support for a wild marine population to Fisher's fundamental theorem of selection that additive genetic variance in fitness is depleted under selection and tends towards zero in a population reaching evolutionary equilibrium (Gustafsson 1986). Although normal and expected, low heritability and evolvability in LRS is concerning giving the increasing rate of environmental change. The low to negligible scope for adaptive evolution (estimated by evolvability) and the low to negligible genetic potential for responding to selection (estimated by heritability) may not be a problem for gradual environmental change. At this rate, it would take around at least 10 generations for the population average LRS to increase by one juvenile, which highlights the stability of the demographic rate of self-recruitment in this population. Our results therefore support the hypothesis that the population is at an evolutionary equilibrium (no genetic changes) in the context of environmental stability over the timescale of the survey.

Connectivity as a plausible cause

Our findings were at first surprising because immigration accounts for on average 44% of the juvenile recruitment (Salles *et al*. 2016). Average dispersal distance in Kimbe Bay is between 10 and 20 km, providing substantial connectivity among adjacent reefs and potential dispersal of up to 100 km (Pinsky *et al*. 2017). The associated gene flow would be expected to bring new genetic variants and thereby increase genetic variation (Facon *et al*. 2008). Under such scenario, selection for self-recruitment, and thereby against migrants, has to be strong to keep the population at evolutionary equilibrium. An alternative scenario is that homogenisation by gene flow results in most immigrants sharing a similar genetic background. As a result, no new genetic variants are brought in the population by gene flow and low genetic variation is maintained (Pujol *et al*. 2010). Low genetic variation for LRS implies that evolution by selection at the local scale is extremely limited in its current state. However, this does not imply a dead end for adaptive evolution in this clownfish population because several mechanisms can provide adaptive evolutionary potential over the long term (Pujol *et al*. 2018).

Slight but probably negligible maternal genetic effect

Additional adaptive evolutionary potential can in theory be provided to a population by maternal effects (Räsänen & Kruuk 2007). In the Kimbe Island orange clownfish, we found that maternal effects explained 1.9% of the LRS variance, which is quite small, even if it was more than additive genetic effects. Maternal effects therefore increased slightly the low to negligible rate of LRS change by adaptive evolution. One must note that this increase is nearly negligible. It is likely that this small value reflects the genetic component of the maternal effect. The habitat of birth, which is also the maternal habitat, probably encompassed a non-negligible part of the maternal environmental effects (Germain & Gilbert 2014). There is growing awareness that maternal environmental effects can contribute to adaptation in natural populations, especially when maternal and offspring environments are positively correlated (Shama 2015). It might even buy some time for adaptive evolution through slow genetic change to occur (Levis & Pfenning 2016).

Towards a wider sample of contemporary rates of adaptive evolution in the wild

In our study, LRS estimated local fish contribution to the local population replenishment. As with most wild population pedigrees, this excludes the dispersal fitness because the amount of offspring that successfully dispersed somewhere

else is unknown (Kruuk *et al*. 2000). Its genetic variation evaluates the rate of adaptive evolution inside the Kimbe island population. While there are no comparable data from marine systems, 15 studies conducted on terrestrial vertebrates have also estimated the additive genetic variation and the heritability of LRS (Table S1). The number of estimates of maternal effects on LRS variation are extremely rare (McFarlane *et al*. 2014 and references therein). A majority of these studies similarly found low to negligible contributions of additive genetic effects in the wild. Marine fish, mammals and birds revealed the same patterns. There is little evidence of additive genetic variance for LRS in studies conducted in the wild. This situation is less clear for maternal effects, partly because studies remain scarce. Very low but significant additive and maternal genetic variations for fitness indicate that there was some genetic change over the course of the survey but very limited potential for short-term adaptive evolution.

A wider use of LRS does not preclude caution

LRS studies in wild populations with a known pedigree have multiplied during the last thirty years. Although useful insights were gained, one must note that the use of LRS as a surrogate for fitness is not well suited for iteroparous species (Grafen 1988). This is particularly true when population growth varies between reproductive seasons (Coulson *et al.,* 2006). Furthermore, although quantitative genetic approaches decomposing the genetic and environmental components of LRS are theoretically correct, they might provide imprecise parameter estimates in the wild because temporal and spatial environmental effects (e.g. condition-dependent LRS, cohort effects) might not have been adequately corrected for. Caution must therefore be taken when interpreting the additive genetic variance of LRS as adaptive evolutionary rate or evolutionary potential in wild populations where it might in fact reflect other ecological parameters. Our quantitative genetic model explained a large amount of environmental variation in LRS in the Kimbe island clownfish population, which supports the reliability of our genetic estimates.

CONCLUSION

The major outcome of this study is that the heterogeneity of the habitat of the Kimbe Island orange clownfish had a profound influence on the individual contribution to the local population replenishment over five generations. This finding implies that habitat ecology is crucial for this clownfish population. In terms of future persistence, expected changes in habitat quality and configuration over relatively short time scales might affect the ability of fish to self-recruit. This ability harbored low to negligible additive genetic and maternal genetic variation. As a consequence, this population potential for rapid evolutionary change of LRS by selection, and therefore its rate of adaptive evolution, can be considered negligible in the current state of the population. This finding, which is in line with other studies on the topic, stresses the importance of environmental mechanisms (e.g. plasticity) that have the potential to enable rapid adaptive responses. From the perspective of management, our results caution against hoping for local adaptive evolutionary responses and lend support to focusing conservation efforts on maintaining habitat quality.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

AUTHORS' CONTRIBUTIONS

GPJ, SLRT, and SP designed the research program; OCS, BP, GRA, and MLB contributed new reagents/analytic tools; OCS and BP analyzed data; OCS, BP and SP wrote the manuscript and all authors contributed substantially to revise the paper.

DATA AVAILABILITY STATEMENT

R programming protocols, script and codes, metadata and Rdata files to call upon any given parameter estimates from this study can be obtained on the Zenodo repository: https://doi.org/10.5281/zenodo.3476529

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