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ORIGINAL PAPER

Environmental regulation of individual body size contributes to geographic variation in clonal life cycle expression

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Abstract

Clonal behavior has been hypothesized to provide an escape from allometric metabolic scaling that limits the maximum mass achieved by a single individual. Here, we demonstrate the capacity of a wide-spread, non-native sea anemone to buffer its colony biomass accumulation rate across environments by modulating ramet body size through environmentally dependent growth, fission, and catabolism. In 2015, thermal reaction norms for growth and fission behavior were constructed using clonal lines of the sea anemone *Diadumene lineata*. In 2018, variation in growth patterns under a factorial cross of temperature level and oxygen availability was examined to test the hypothesis that individual ramet size is regulated by oxygen limitation in accordance with optimal size theory. Across a wide range of temperatures, colonies accumulated a similar amount of biomass despite a radical shift from unitary to clonal growth, supporting fission as a mechanism to buffer growth rates over a range of conditions. Individual body size appears to be regulated by the environment with increased temperature and reduced oxygen modifying fission and mass-specific growth patterns, leading to the production of smaller-bodied ramets in warm conditions. However, whether anemones in common garden conditions reduce individual body size through catabolism or fission depends on the region of origin and may relate to differences in seasonal temperature patterns among coastlines, which influence the energetic benefits of fission rate plasticity.

Introduction

Despite the relative simplicity of the cnidarian body plan, an astounding diversity of life cycle and growth patterns is achieved through variation in the timing and extent of investment in clonal growth (Fautin 2002; Geller et al. 2005). While many hypotheses have been put forth to explain an adaptive advantage of clonality under specific ecological conditions (see Francis 1979, 1988; Jackson 1977; Sebens 1979, 1987; Hughes 1987,1989), traits that influence fission rate (Ferretti and Géraudie 1998; Geller et al. 2005), and how selection acts on these traits (Reitzel et al. 2011), remain poorly understood. Many studies have looked for interspecific patterns, comparing the distribution of clonal and aclonal taxa with ecological factors to illuminate putative selective pressures (e.g., Chia 1976; Sebens 1979; Jackson 1985; Jackson and Coates 1986; Francis 1988). Where intraspecific comparisons have been made, there appears to be ample variation among genotypes in many aspects of growth, physiology, and fission behavior (e.g., Shick et al. 1979; Sebens 1980; Ayre 1985; McManus et al. 1997; Edmunds 2007; Reitzel et al. 2013), suggesting that traits

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governing clonality are labile. Phylogenetic comparisons in anemones suggest that clonality has evolved independently several times (Geller and Walton 2001). Consequently, life cycle traits are expected to respond readily to selection in this group of cnidarians. However, our knowledge of the mechanistic pathways underlying fission behavior in cnidarians remains rudimentary despite more than a century of interest in the lives of clonal marine animals (but see Mire and Venable 1999; Geller et al. 2005; Reitzel et al. 2011). To evaluate hypotheses about which demographic and environmental factors favor the evolution of clonality, more information is needed about the nature of variation in clonal behavior among genotypes and how traits are shaped by the environment (McManus et al. 1997).

In both unitary and clonal animals, body size is a fundamental property that governs metabolic rate and reproductive potential, as well as ecological relationships (e.g., competition and predation risk). Thus, it is expected to be a key target of selection. Theories of metabolic scaling suggest that smaller-bodied individuals with large respiratory surface area to biomass ratios are more energetically efficient under diffusion limiting conditions than large-bodied individuals (e.g., Atkinson 1994; Kooijman 2010; Glazier 2014). Similar logic has been used to explain the observation that for a diverse array of taxa, warmer conditions lead to smaller adult body sizes, even for species where reproductive fitness is positively correlated with body size (Kingsolver and Huey 2008; but see Audzijonyte et al. 2018). The risk of oxygen limitation is particularly important in aquatic organisms where the strong negative correlation between temperature and dissolved oxygen availability acts as a fundamental constraint on body shape, size, and performance (Pörtner 2001; Forster et al. 2012; Horne et al. 2015).

Under conditions that limit individual body size (e.g., hypoxia), clonal animals can have an energetic advantage. Genets (the collective term for a group of descendants produced through an asexual process) that are capable of dividing biomass into smaller units (known as ramets) can grow indefinitely (i.e., iso- versus allometric growth), where unitary animals typically reach a maximum size and cease somatic growth (Cancino and Rodger 1985; Sebens 1987). Accumulating evidence suggests that the link between ramet body size and metabolic performance may be critical for explaining the evolution of clonal and colonial life histories (Burgess et al. 2017). However, the degree to which clonal animals can regulate growth and fission rates to achieve energetically optimal ramet sizes is not well understood.

The role of fission in body size regulation is difficult to characterize, as metabolic, growth, and fission rates often vary with the environment (e.g., Johnson and Shick 1977; Buss and Blackstone 1991; Geller et al. 2005; Reitzel et al. 2013). Such effects may be an unavoidable consequence of physical or chemical properties, leading to non-adaptive variation in the expressed phenotype across environments (Gotthard and Nylin 1995). Alternatively, growth and fission rate plasticity may be adaptive, allowing genetically identical clones to express a locally optimal body size, and investment in asexual reproduction, across a range of environmental conditions (Edmunds 2007). Indeed, for species that live in fluctuating environments, body size plasticity through shrinkage (e.g., Levitan 1988; Chomsky et al. 2004) or a variable fission rate (Ryan 2018) may be essential for tracking changing size optima through time.

To better understand the role of temperature and oxygen availability in regulating individual body size and shaping clonal life histories, we examined growth, fission, and body size patterns produced by the clonal sea anemone *Diadumene lineata* (Verrill) under a variety of experimental conditions. We also explored variation in these patterns among individuals collected from different parts of the extant range that differ in seasonal temperature patterns to look for evidence of variation in asexual behavior that can inform predictions about the role of thermal regime on the evolution of life cycle plasticity. Phenotypic plasticity can be described as a "reaction norm," or a linear relationship between an environmental gradient and a phenotype produced by a particular genotype (Bradshaw 1965). Small differences in the curvature or slope of reaction norms can lead to large differences in expressed phenotypic patterns, particularly in fluctuating environments. We used this conceptual framework to understand how individuals may modulate growth patterns to match energetically optimal patterns dictated by the local environment and discuss the evolutionary implications of variation in reaction norms that govern clonal behavior.

Diadumene lineata is a small-bodied, clonal sea anemone that occurs in the high intertidal zone. The species likely originated in East Asia, but has been spread around the world through anthropogenic activity for more than 100 years (Uchida 1932; Cohen and Carlton 1995). Like many nonnative species, *D. lineata* persists under a broad range of abiotic conditions and occurs across a range of habitat types. The success of this species has been attributed, in part, to a prolific schedule of binary fission (Uchida 1932). Fission rate is also known to increase with temperature (Miyawaki 1952; Minasian 1979) which may allow individuals to modulate body size and reproductive effort in response to novel or fluctuating conditions and likely structures seasonal and geographic patterns of sexual and clonal reproduction across its distribution (Ryan 2018; Ryan and Miller 2019).

Seasonal and geographic temperature patterns vary across the species' North American range (Table 1), which may impose differential selection on life cycle expression. Highly seasonal temperature patterns across the Atlantic coast mirror conditions along the species' native distribution in Japan. Nearshore waters of the Gulf of Mexico are similarly seasonal, but on average much warmer than the

Table 1 Approximate seasonal and latitudinal patterns in nearshore sea surface temperature across the known range of *Diadumene lineata* in Japan and the United States

Coast	Known latitudinal range of species	Seasonal range of monthly mean water temperature	Latitudinal range of mean annual water temperature
Pacific Japan (native)	Hokkaido to Oita	10 to 20 °C	9 to 25 °C
Atlantic US	Cobscook Bay, ME to Cape Canaveral, FL	13 to 20 °C	9 to 25 °C
Gulf of Mexico US	Corpus Christi, TX to Tampa Bay, FL	12 to 17 °C	22 to 24 °C
Pacific US	Vancouver, BC to San Diego, CA	2 to 5 °C	9 to 17 °C

Mean sea surface temperature ranges approximated for 2014–2017 using data from NOAA National Data Buoy Center https://www.ndbc.noaa.gov/). Species occurrence based on WoRMS data base (marinespecies.org) and personal observation

Atlantic coast. Across the Pacific coast, there are smaller seasonal fluctuations and cooler average sea surface temperatures. As residents of the high intertidal zone, these anemones can also experience large daily temperature fluctuations through tidal cycles, though the species is often found in tidal pools, under rocks, or embedded among co-occurring organisms that can help buffer short-term abiotic fluctuations. We expected highly seasonal environments to favor genotypes that rapidly increased fission rate and reduce ramet body size under high temperatures as such a mechanism would allow genets to track predictable changes in the optimal body size on the scale of weeks to months. Conversely, for anemones in environments where daily temperature fluctuations were larger than seasonal fluctuations (i.e., Pacific US), we expected fission to be less responsive to temperature, as engaging in fission in response to short-term peaks in temperature could cause ramets to be perpetually below the optimal body size for the environment and, thus, be maladaptive.

In this study, a series of experiments examine both the short-term (4 week) and longer-term (12 week) responses of *D. lineata* to environmental manipulation. The short-term manipulation of temperature and oxygen helps illuminate mechanisms of environmental body size regulation, whereas the longer-term manipulation demonstrates the variation in growth and fission patterns that emerge from such regulation. We also explore geographic variation in the shape of reaction norms of *D. lineata* using individuals collected from the Atlantic, Gulf, and Pacific coasts of the US, which vary substantially in their seasonal temperature regimes. We discuss our findings in the context of potential local adaptation, although we remain conservative in these conclusions as patterns of genetic differentiation within and among sites are currently unknown. Specifically, we ask:

1. What are the general shapes of the thermal reaction norms of fission rate, individual body size, and clonal biomass accumulation in this species?
2. How do temperature, oxygen, and coastline of origin contribute to ramet body size regulation via changes in fission and growth rate?
3. Does basal metabolic rate differ among coastlines of origin?
4. Are reaction norm differences among anemones from different coastlines consistent with the expected effects of seasonality?

Methods

Experiment 1: characterizing reaction norms across five levels of temperature

Producing clonal replicates

Twenty individual anemones were collected from each of the three field sites across the US Atlantic and Gulf coasts [Nahant, MA (Atlantic, 42 °N); St. Simon, GA (Atlantic, 31 °N); St. Teresa, FL (Gulf, 30 °)] in January 2013. Within sites, individual anemones were collected from points spaced one to two meters apart in an effort to represent the range of genetic diversity available at each site. No clonal replicates were knowingly included in the initial collection; however, the individuals used to initiate lab cultures reflected the genetic structures of the field populations, which were unknown. Single-strand conformation polymorphism data from a single locus performed on individuals haphazardly collected from sites along the Atlantic and Pacific coasts suggest that most sites host multiple genotypes, and that repeated genotypes are common within, but not among sites (Ting and Geller 2000). Given the geographic distance among sites, we are confident that the sample of individuals included in the experiment contained multiple genotypes; however, none of our conclusions requires this assumption.

Collected individuals were used to establish isolated clonal lines under common garden conditions that exposed them to a seasonally adjusted range of temperatures between 15 and 29 °C, mimicking field measured conditions from St. Teresa, FL (see Ryan 2018). Between March and September 2015, clonal lines were kept at 20 °C and fed *Artemia* nauplii (Brine Shrimp Direct, Ogden, UT, USA) two times per week.

Temperature treatments

In September of 2015, individual ramets were randomly selected from each of the twelve genets (FL: 5, GA: 4, MA: 3) that had sufficiently large clonal populations. Individual anemones from each putatively distinct genotype were measured for pedal disk area, then isolated in a 50 ml Falcon tube of artificial seawater (Instant Ocean; salinity 32 ppt) and randomly assigned to one of the five temperature chambers (including three refrigerators (see Ryan 2018), a bench top incubator (PR205075G Thermo Scientific, Waltham, MA, USA), and a climate control chamber (I-36VL Percival Scientific, Perry, IA, USA). Chambers differed in make and size (ranging from an interior volume of 0.05 to 0.85 cubic meters), so they were left dark to standardize light conditions and prevent algal fouling. This species is not known to harbor photosymbionts and often occurs under rocks in total darkness and, therefore, does not require light for growth or nutrition. All the chambers maintained static cultures within 1 °C of the target temperature. The water in each tube was exchanged and anemones fed to repletion on 3-day-old *Artemia* nauplii (Brine Shrimp Direct, Ogden, UT, USA) twice per week. Five temperature treatments were used (6, 9, 14, 21.5, and 29 °C) spanning the range of average monthly water temperatures experienced by this species on the east coast of North America. Because the experiment required five temperature levels, we were unable to use more than one environmental chamber per temperature treatment. Thus, chamber and temperature treatment level are unfortunately confounded. However, as anemones were isolated in sealed vials within chambers, we have no a priori reason to suspect systematic bias due to chamber identity.

Each genet was initially represented by 1–4 ramets in each temperature condition, depending on replicate availability. Eight of the 12 genets had at least two replicate anemones assigned to each treatment. Replicate ramets were limited for the other four genets such that some temperature treatments only had one anemone assigned (see Table S1). Of these genets, only two were retained in analysis (see "Results"). The probability of survival of each genet in each temperature treatment was calculated as the number of initial replicates still represented by at least one ramet at the end of the experiment (week 12) divided by the initial number of genotypic replicates in each treatment. Fission rate was quantified as the number of daughter clones produced by each individual over the experimental period. Body size was measured by tracing the outline of the attached pedal disk onto a sheet of acetate, scanning the drawings into a computer and using Image J software (Rasband 1997) to calculate the area of the pedal disk (mm^2). Initial pedal disk area was used to estimate dry mass using the regression measured in Ryan (2018). All individuals were then rinsed in freshwater to remove extraneous salt, separated into pre-tared foil boats and dried at 70 °C for 72 h. Dried tissue (μg) was weighed using a microbalance. Final tissue density was calculated as dry mass per pedal disk area ($\mu g/mm^2$). Change in colony weight was calculated as the difference between final and estimated initial colony dry weight. Mean individual mass was calculated as the mean dry mass (μg) of all ramets within a replicate.

Analysis

The individuals used were biased toward those genets that had enough ramets available. Mortality during the experiment left both GA and MA with complete final data for only two and one genets, respectively (see details in Table S1). Thus, no attempt was made to characterize variation among sites of origin in this experiment. Genet survival, fission rate, and change in colony dry weight were genet-level traits, so genet ID was used as a random factor in all models (Table 3). Ramet body size and tissue density were calculated as ramet-level traits, so both genet ID and replicate ID within genet were used as random factors to account for variation among ramets derived from independent cultures of clonal replicates derived from the same genet. No genotype by treatment interactions was considered.

The effect of temperature on the probability of genet survival was estimated with generalized linear mixed model (GLMER) with a logit-linked binomial distribution. The effect of temperature on the number of clonal descendants (fission rate) was analyzed with GLMER using a negative binomial distribution. The effects of temperature on change in colony dry weight, ramet dry weight, and tissue density were analyzed with GLMERs using log-linked Gaussian error distributions. In all cases, temperature was initially fitted with the highest order polynomial supported by the levels of temperature (4th degree) to elucidate the shape of the relationship. Model selection using AICc was then used to find the best-fit model using the dredge function in the R (R Core Team 2014) package MuMIn (Barton 2018). The model with the lowest AICc value was chosen, except where a model with fewer parameters had a similar AICc value (dAICc < 2) (see model details in Supplement 1). The significance of the contribution of each retained parameter in the best fit model was evaluated using the type II Anova function in the R package car (Fox and Weisberg 2011).

All analyses were done in R ver. 3.5.1 (R Core Team 2014).

Experiment 2A: characterizing growth and fission variation among individuals collected from the Pacific, Gulf, and Atlantic coasts of the United States

Anemone collection

Between June and November 2017, *Diadumene lineata* individuals were collected from ten sites in the species' US

range (Table 2). At each site, from one to twenty individuals were collected from each of five 0.25 m^2 quadrats along a 25 m transect through the high intertidal zone, parallel to the water line, except at ESL where quadrats were placed at 5 m intervals along a floating dock. This resulted in the collection of between 19 and 100 individuals per site. The number collected from each quadrat at each site varied, as this species has a patchy distribution. For the experiments, samples were drawn randomly from anemones available from each site without respect to quadrat of origin. To reduce the effects of prior environmental variation, collected individuals were maintained separately in the laboratory in 50 ml tubes of artificial seawater at 30ppt, 15 °C temperature, on a 12:12 h light:dark cycle from the time of collection until use in the experiment, a period ranging from one to 6 months. Anemones were fed weekly with Microvert liquid invertebrate food (Kent Marine, Franklin, WI, USA) until the experiment began. Size variation naturally occurred among populations and persisted in collected samples until the start of the experiment (Table 2). However, within site of origin, the mean initial size of individuals assigned to each treatment group did not differ significantly when compared with ANOVA (see results).

Experimental design

In December 2017, 20–25 individuals were randomly selected from among the common garden-maintained cultures from each site and were assigned to one of the four treatment conditions representing a factorial cross of temperature (15° and 25° C) by dissolved oxygen level (50% and 100% of normoxia). As above, no clonal replicates were knowingly included in the experiment; however, the underlying genetic structure of these populations was unknown. Individuals used to initiate lab cultures reflected a random sample of the genetic structures of the field populations. For the purposes of this experiment, each individual was treated as an independent replicate; however, we were careful to avoid drawing conclusions with regard to the role of genetic diversity underlying the observed variation in phenotypes.

Each individual was wet weighed, photographed for pedal area measurements (see method in Experiment 1), and then placed individually into a tube with 15 ml of artificial seawater (30 ppt). Live anemones in tubes of seawater were shipped with ice packs from the University of Alabama at Birmingham (UAB; Birmingham, AL, USA) to the Marine Biological Association of the United Kingdom (MBA; Plymouth, UK) and arrived within 36 h of shipment. Upon arrival, 20 anemones from each site (except CFP, where only 19 individuals were available) were transferred individually into the wells of twenty 12-well plates (Corning Inc., NY USA) ($N=5$ per treatment) filled with filtered natural seawater (salinity 30 ppt), which were then divided among four, 4 L sealable plastic tanks fitted with air stones. Remaining anemones were set aside for experiment 2B (see below).

To facilitate water exchange with a surrounding tank, holes were pre-drilled into the lids of the 12-well plates and then were lined with a fine mesh to prevent anemones from escaping or moving among wells. The ability for water to exchange freely between the tank and each well was confirmed by observing the ability of food dye to diffuse easily across the mesh when a prototype plate was submerged in water. The plastic tanks, each containing five plates, were then set in water baths to control their temperature. All anemones were maintained at approximately 10 °C and aerated for 2 weeks to allow them to acclimate to the growth chambers with minimal mass change and no fission. On January 2, 2018 a factorial cross of temperature and oxygen

Table 2 *Diadumene lineata* collection sites for geographical comparison. Individuals collected from sites in Washington were pooled to achieve sufficient replication from this region

Coast	Site ID	Site	Collection date	Collector	Median initial wet weight (g)
Atlantic	ESL	VIMS Eastern Shore Laboratory, Wachaprague, VA	Sep. 2017	SAKH, GB	0.006
Atlantic	**STS**	St. Simon Island, GA	Nov. 2017	WHR	0.033
Gulf	**WAK**	Wakulla beach, FL	Nov. 2017	WHR	0.020
Gulf	**ESP**	Eastpoint, FL	Nov. 2017	WHR	0.021
Gulf	SGI	St. George Island, FL	Nov. 2017	WHR	0.009
Gulf	CFP	Copano fishing pier, Rockville TX	Jul. 2017	WHR	0.001
Pacific	**ROK**	Morro Bay, CA	Nov. 2017	WHR	0.038
Pacific	**AZO**	Azevedo Pond, Elkhorn Slough, CA	Sep. 2017	SAKH, GB	0.017
Pacific	**BRK**	Berkeley, CA	Nov. 2017	WHR	0.050
Pacific	**WAS**	Pooled from Grey's Harbor, WA & Willapa Bay, WA	Jun. 2017	WHR	0.011

No individuals from sites north of Virginia on the Atlantic coast could be acquired at the time of the experiment. Bold site ID denotes use in both experiments 2A and 2B. Median initial wet weight (g) was calculated for all replicates assigned to experiment 2A

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level manipulations was initiated. Five individuals from each site, randomly positioned across plates, were subjected to each of the four treatments. The temperature of the room was raised to 15 °C and then submersible aquarium heaters were added to water baths surrounding half of the plates, raising their temperature to 25 °C over the course of two days. Temperature was monitored at 5-min intervals with Hobo loggers (Onset, Bourne, MA, USA) in each plastic tank and by daily checks with an infrared thermometer. To manipulate the availability of dissolved oxygen, half of the tanks were aerated with ambient air fed from outside the building (100% ambient oxygen), the remaining tanks were aerated with a pre-mixed gas of 10.5% oxygen and 89.5% nitrogen (BOC, Plymouth, UK), equivalent to 50% of the ambient oxygen treatments. Because pre-mixed gas was used, oxygen level was not monitored during the experiment. While maintaining an independent environmental manipulation for each replicate plate would have been ideal for statistical independence, it would have required a prohibitively large volume of mixed gas to run the experiment. Thus, each treatment consisted of one treatment tank that housed replicate plates.

Through the duration of the experiment, anemones were fed to repletion on a diet of 2-day-old *Artemia* nauplii (Brine Shrimp Direct, Ogden, UT, USA) every other day. Plates were removed from treatment tanks, the water in wells discarded (with care not to dislodge individuals), and a 3 ml aliquot of a well-stirred culture of nauplii was pipetted into each well. Plates were then returned to treatment tanks. This feeding protocol resulted in an 18% water replacement in treatment tanks per week.

After 4 weeks, anemones were returned to 15 ml tubes of filtered seawater and shipped back to UAB on ice where all anemones were wet weighed, photographed for pedal area, and then dried at 72 °C for 72 h and dry weighed. Fission rate, change in colony mass, change in mass-specific growth rate, and mean individual mass were calculated for each genet as in Experiment 1.

Analysis

Variation in initial size among treatments and site of origin was assessed using a two-way ANOVA. Since initial size varied among sites of origin, initial body size was considered in all full models. Initial models also used site as a random variable to account for variation within coastline. However, in no case did including site as a random variable improve the fit, thus it was dropped for all analyses. In all cases, an initial model containing all predictor variables (temperature, oxygen, and coastline of origin) and interactions as well as a polynomial series of the natural log of initial wet weight was constructed using GLMER in the lme4 package (Bates et al. 2015) for R (R Core Team 2014). To determine the shape of the relationship with initial size, the initial model used the highest order polynomial supported by available degrees of freedom (typically 5°) (see supplement 2 for details). Stepwise model selection using AICc was then used to determine the best fit models (Table 3) as described for experiment 1. The probability of genet survival and probability of fission were modeled with binomial distributions. The mass-specific change in mass, calculated as the natural log of the final wet weight minus the natural log of initial wet weight, was normally distributed. The total change in colony mass, calculated as the final wet weight minus the initial wet weight, was also modeled to provide a visualization of biomass change patterns. But, given the highly leptokurtic distribution of this metric, statistical inferences are best drawn from mass-specific analyses above. The final body size of individual anemones (grams wet weight) was modeled with

Table 3 Overview of experiments and related analyses performed. Model structure reflects best-fit model resulting from model selection procedures. The predictor initial weight is the natural log transformed wet weight (g) of individual anemones at the start of the experiment

Exp	Response variable	Model structure[a]	Error distribution	Link function
1	Genet survival	~ 1 + (1|genetID)	Binomial	logit
	Number of clonal descendants	~ temp + (1|genetID)	Negative binomial	log
	Δ colony dry mass	~ temp + temp^2 + temp^3 + (1|genetID)	Gaussian	log
	Ramet dry weight	~ temp + temp^2 + temp^3 + (1|genetID/rep)	Gaussian	log
	Tissue density	~ temp + (1|genetID/rep)	Gaussian	log
2A	Genet survival	~ oxygen	Binomial	logit
	Occurrence of fission	~ initial weight * (coast + oxygen) + temp	Binomial	logit
	Mass-specific **Δ** colony wet weight	~ initial weight * (temp + oxygen)	Gaussian	Identity
	Δ colony wet weight	~ temp * initial weight + initial weight^2 + initial weight^3 + initial weight^4	Gaussian	Identity
	Individual wet weight	~ initial weight + temperature + (1|genetID)	Gaussian	log
2B	**Δ** wet weight	~ initial weight + coast	Gaussian	log

[a]Model structures are displayed following the syntax of the R package lme4 (Bates et al. 2015)

a log-linked gaussian distribution. Genet ID was included as a random factor to account for the non-independence of ramets produced by each genet. Analysis of variance tables was constructed for each model to aid in interpreting the contribution of each predictor. Goodness of fit values for all models were calculated with the r.squaredGLMM function in the R package MuMIn or rsquared function in the R package piecewiseSEM (Lefcheck 2018) for GLM(M)s.

Experiment 2B: estimating relative differences in basal metabolic rate through starvation

Experimental design

Thirty-three individuals representing seven sites of origin on three coastlines [Pacific: 4 sites ($n=5$), Gulf: 2 sites ($n=4$), Atlantic: 1 site ($n=5$); Table 1] were used to measure loss of body mass through starvation. Once at the MBA, these individuals were kept sequestered at 15 °C in individual tubes with 14 mL of artificial seawater, leaving a small headspace of air in each tube. This temperature was chosen to minimize the likelihood of fission which would complicate the interpretation of the results. Approximately, once per week, tubes were agitated gently, but no food was provided. After 4 weeks, anemones were returned to UAB on ice and were weighed and measured as in Experiment 2A.

Analysis

Weight loss through starvation was calculated as the final wet weight (g) minus initial wet weight. To test whether the rate of biomass catabolism depended on initial body size, weight loss was regressed on the natural log of initial wet mass with log-linked gaussian linear regression (GLM) (Table 3). Coastline of origin was treated as a fixed factor to evaluate differences in starvation-induced shrinkage as a proxy for basal metabolic rate (Sebens 1981).

Results

Experiment 1: characterizing reaction norms across five levels of temperature

Of the 125 individuals originally included in the experiment, 23 died soon after being moved into the experiment. This transplant mortality was heavily concentrated among two genets, which were both removed from all subsequent analyses (see Table S1). Among the remaining ten genets (FL:5, GA:3, MA: 3), mortality was low during the experiment; the average probability of replicate survival was 0.93. Genet identity was the major factor contributing to variance in survival (conditional $r^2=0.23$). Temperature was not retained as a significant predictor of mortality in the best-fit binomial GLMM (Fig. 1a, see analysis details in S1). For two genets, all replicates in 29 °C died, precluding the construction of growth and fission reaction norms. Thus, only the eight genets with complete data (FL: 5, GA: 2, MA: 2) were used in subsequent analyses.

The number of clonal descendants produced ranged from one (no fission) to 13 ramets and showed a significant, monotonic increase with temperature (GLMM, χ^2 $(1,73)=67.75$, $p<0.001$; Fig. 1b). All surviving colonies accumulated biomass over the 12-week experiment. Change in colony dry weight (mass accumulation) across temperature was best described by a third order polynomial (GLMM, χ^2 $(3,71)=66934$, $p<0.001$; Fig. 1c). Mass accumulation was lowest at low temperatures and highest at intermediate temperatures, peaking in the 14 °C treatment. Mass accumulation remained intermediate to high across the warmer temperatures despite a rapid increase in fission; though, many genets showed a dip in mass accumulation at 21.5 °C relative to 14 and 29 °C. At the end of the experiment, individual ramet dry mass also differed significantly across temperatures, which was best described by a third-order polynomial (GLMM, χ^2 $(3,168)=51.89$, $p<0.001$; Fig. 1d). Ramet body size was unimodal, peaking at 14 °C. Individuals in the coldest treatment grew, but stayed small without dividing, whereas individuals in warmer treatments (≥ 21.5 °C) accumulated colony mass through the production of daughter clones which were smaller bodied than the founding individual. Tissue density showed a significant, monotonic decline with increasing temperature (GLMM, χ^2 $(1,169)=17.11$, $p<0.001$; Figure S1). See Supplement 1 for model selection details and parameter estimates for all analyses above.

Experiment 2A: characterizing growth and fission variation among genotypes collected from the Pacific, Gulf, and Atlantic coasts of the United States

Of the 199 individuals included in the initial experiment, data from 5 individuals were excluded from analysis. Two individuals from CFP were too small for initial wet weights to be measured confidently (< 0.0001 g). Two individuals from WAS were an order of magnitude smaller any others from the site (> 1.9 standard deviation units below the mean). One individual from WAS was an order of magnitude larger than any others from the site (> 2.5 standard deviation units above the mean). These replicates were removed to prevent statistical estimations from being extrapolated over a size range for which not all treatment levels were represented. Statistical inferences were not altered by the inclusion or exclusion of these data.

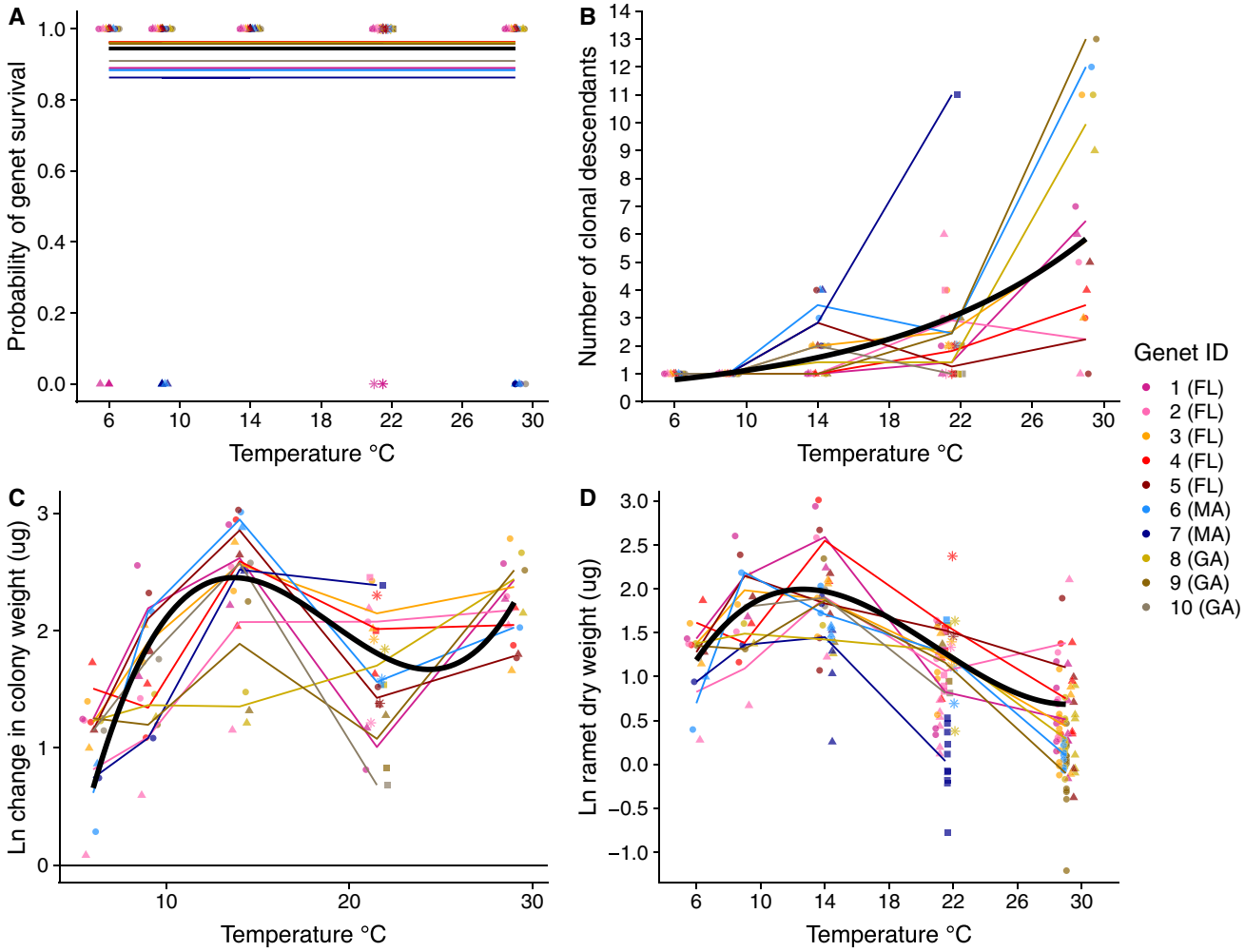

Fig. 1 Experiment 1: reaction norms for four variables across five levels of temperature for 10 genotypes of *Diadumene lineata* grown for 12 weeks in common garden conditions (experiment 1), including **a** probability of genet survival, **b** the number of clonal descendants produced, **c** the natural log of the change in estimated colony dry mass, and **d** ramet body size [ln dry mass (μg)]. Colored lines show average pattern for each genet, black lines indicate the best-fit linear regression model. Points show either genet replicate-level (**a**–**c**) or ramet-level (**d**) values. Different point shapes of the same color designate independent replicates of a genet within temperature level. Genets 7 and 10 were excluded from statistical analysis as all replicates raised at 29 °C died. Points horizontally jittered to reduce overplotting

There was variation in initial body size (the natural log of wet weight) among sites of origin (two-way ANOVA, $F(9,154) = 29.79$, $p < 0.001$), but no systematic variation among assigned treatment levels (Two-way ANOVA, $F(3,154) = 0.862$, $p = 0.46$). There was also no significant difference in initial size between treatment by site of origin (Two-way ANOVA, $F(27, 154) = 0.52$, $p = 0.52$). The median initial wet weight was highest for Pacific coast individuals, followed by Atlantic and Gulf Coast individuals (0.020, 0.018, 0.012 g, respectively) See Table 2 for the median initial wet weights by site for individuals used in experiments 2A.

Over 4 weeks, survival was high (94%) among the 194 individuals included in the experimental analysis. Exposure to low oxygen conditions significantly reduced individual survival to 90% compared to 98% of individuals in high oxygen conditions (GLM, $r^2 = 0.18$, χ^2 (1,193) = 6.00, $p = 0.014$; Table S2). The lowest genet survival rate (83%) occurred for anemones of Pacific origin experiencing both high temperature and low oxygen conditions, though neither temperature nor coastline of origin was retained as significant predictors in the best-fit GLM model. Likewise, initial body size was not retained in the final model. Site of origin as a random factor was removed through model selection.

Among the individuals that survived, the probability of undergoing fission was influenced by initial body size, coastline of origin, temperature, and oxygen treatments (GLM, $r^2 = 0.49$, Fig. 2). As expected, high temperature

significantly increased the probability of fission (GLM, χ^2 (1, 173) = 47.77, $p < 0.001$). Coastline of origin had a significant effect (GLM, χ^2 (2, 173) = 17.95, $p < 0.001$); Gulf Coast individuals had the highest probability of dividing across all treatments, followed by the Atlantic, then Pacific individuals. The same order was reflected in the mean number of clonal descendants produced across treatments (Gulf: 1.40 ± 0.08 se, Atlantic: 1.32 ± 0.10 se, Pacific: 1.08 ± 0.03 se). The influence of initial body size differed among coastline (GLM, initial size × coastline χ^2 (2, 173) = 6.74, $p = 0.034$). The probability of fission declined with initial body size for Pacific genets and increased with body size for Gulf genets. Initial body size showed no average effect for Atlantic genets, partly due to the significant interactive effect between oxygen and initial size. In all cases, exposure to low oxygen increased the probability of fission at large initial body sizes relative to the high oxygen treatment (GLM, initial size × oxygen χ^2 (1, 173) = 4.89, $p = 0.027$) (see analysis details Supplement 2).

Mass-specific weight change, or the growth rate per unit initial biomass, shows a clear monotonic decline with initial body size (GLM $r^2 = 0.60$, F(1, 176) = 258.46, $p < 0.001$; Fig. 3a). High temperature reduced average growth (GLM, $F(1, 176) = 6.58$, $p = 0.011$) and caused a marginally significantly steeper slope in the decline of growth with body size (GLM, initial size × temperature $F(1, 176) = 3.78$, $p = 0.053$). Oxygen level did not change the average mass-specific weight change (GLM, $F(1,176) = 0.115$, $p = 0.115$), but did have a significant interaction with initial body size (GLM, initial size × oxygen $F(1, 176) = 5.06$, $p = 0.026$). Individuals exposed to low oxygen showed a trend of dampened growth among small individuals where growth rates were highest, but did not alter the threshold size above which anemones lost mass. Coastline of origin was not retained in the best-fit model suggesting that all regions of origin showed similar treatment responses (see analysis details in Supplement 2).

When the raw change in colony wet weight was plotted against initial wet weight (Fig. 3b), both the energetic benefit of optimal size and the high cost of being too large are evident. The best-fit model describing the change in colony wet weight is a forth degree polynomial (GLM, $r^2 = 0.67$), which demonstrates the peak in growth at an intermediate initial size, and precipitous loss of mass for larger individuals (Model details are provided in supplement 2; however, the influence of temperature and oxygen is best understood from the patterns of mass-specific growth described above).

The final individual wet weight (ramet size) was influenced by initial size and temperature (GLM, $r^2 = 0.56$, Fig. 3c), and varied among genets within coastline (variance = 0.18, sd = 0.43). Final size was significantly, positively correlated with initial wet weight (GLM, χ^2 (1, 223) = 59.49, $p < 0.001$); though, the slope of the relationship was consistently less than one suggesting a tendency for body size to converge on a similar size within treatment over time regardless of initial size. High temperature led to significantly smaller body sizes on average (GLM, χ^2 (1, 223) = 14.49, $p < 0.001$) (median wet weight: 0.010 vs. 0.022 g in low temperature treatment). The rank order in body size among coastlines persisted (median wet weight: 0.021, 0.015, 0.009 g for Pacific, Atlantic, Gulf individuals, respectively), but coastline of origin was not retained as an explanatory variable in the best-fit model (see supplement 2). Likewise, there was little effect of oxygen treatment and this factor was not retained in the final model. Interestingly, ramets from Gulf Coast genets tended to become smaller through fission, whereas Pacific Coast ramets tended to shrink through catabolism without undergoing fission (Fig. 4). Atlantic Coast genets showed a mix of individual shrinkage and fission to reduce body size. In most cases, fission produced two similarly sized daughter clones (i.e.,

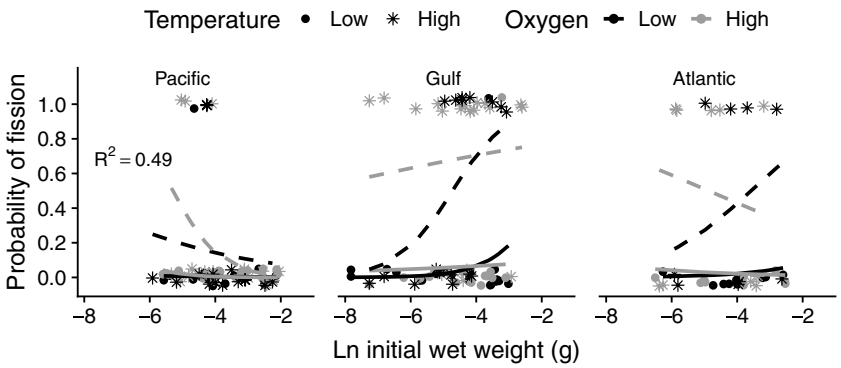

Fig. 2 Experiment 2A: the probability that *Diadumene lineata* individuals engaged in fission over 4 weeks in treatment conditions (experiment 2A) depending on initial body size and coastline of origin (panels). Lines reflect the best-fit linear model for individuals exposed to a temperature of either 15 °C (solid line, dots) or 25 °C (dashed line, stars), and an oxygen level of either 50% (gray) or 100% (black) of normal. Points are vertically jittered to reduce overplotting

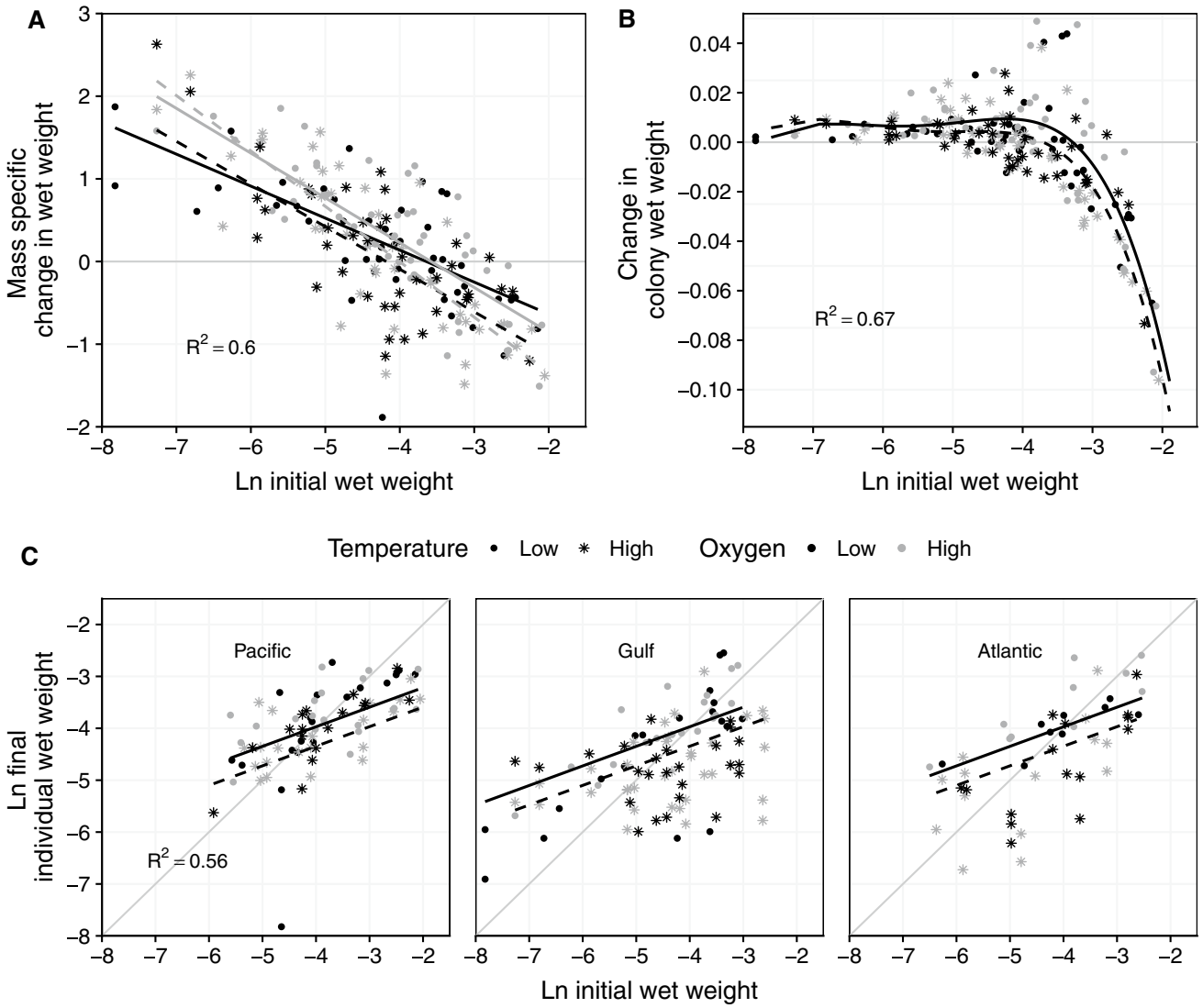

Fig. 3 Experiment 2A: the mass-specific change in wet weight (**a**), change in total colony wet weight (g) (**b**), and ramet body size [ln wet weight (g)], **c** as a function of initial body size (ln wet weight (g) of *Diadumene lineata* individuals after 4 weeks in treatment conditions (experiment 2A). Lines reflect the best-fit linear model for individuals exposed to a temperature of either 15 °C (solid line, dots) or 25 °C (dashed line, stars), and an oxygen level of either 50% (gray) or 100% (black) of normal. Model fit does not differ statistically between oxygen levels for (**b**, **c**). **c** Coastline of origin. The light gray line in each panel denotes a zero-change isocline

binary fission), though pronounced asymmetry in final ramet size was observed particularly among Gulf individuals with large initial sizes (Fig. 4). In one case, a Pacific Coast individual produced a pedal lacerate.

Experiment 2B: estimating relative differences in basal metabolic rate among coastlines of origin through starvation

All individuals survived the duration of the experiment and none underwent fission. All individuals lost weight over 4 weeks, but the rate of weight loss, which is inversely proportional to resting metabolic rate, was significantly influenced by both initial body size and coastline of origin (GLM, $r^2 = 0.93$, Fig. 5). The natural log of mass loss increased significantly with the natural log of initial body size (GLM, $F(1, 22) = 288.29$, $p < 0.001$). Individuals from the Gulf and Atlantic coasts lost significantly more mass than those from the Pacific coast (GLM, $F(2, 22) = 5.99$, $p = 0.008$), but showed a similar slope (see Supplement 3 for statistical details). Because there was no complete overlap in initial body size distribution available from the coasts, inferences about the performance of very large or very small individuals are limited in this data set.

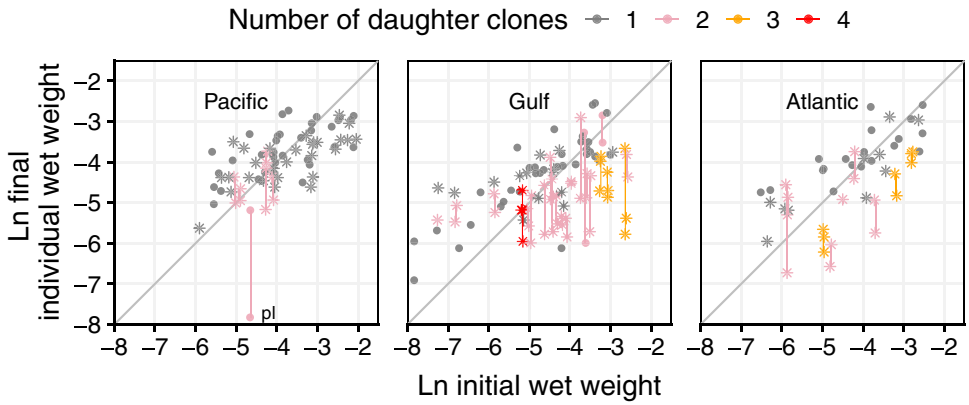

Fig. 4 Experiment 2A: final versus initial size [ln wet weight (g)] of *Diadumene lineata* ramets after 4 weeks depending on fission activity (colors), treatment temperature (15 vs 25 °C shown by dots vs. stars), and coastline of origin (panels). Lines connect ramets produced by the same genet. Points below the zero-change isocline (gray line) show ramets that are smaller than the initial individual, either due to fission or shrinkage. One case of pedal laceration was observed (pl)

Discussion

Thermal growth curves for most ectothermic animals take the shape of a left skewed distribution, with a gradual increase to a peak followed by a rapid decline in performance (Schulte et al. 2011). The first experiment shows that the thermal reaction norms constructed for *Diadumene lineata* over 12 weeks were different. They revealed the capacity of a clonal animal to maintain high biomass accumulation rates across a wide range of temperatures while modulating the size of ramets through changes in fission and growth. The second set of experiments showed that individual body size is regulated by the abiotic environment via changes in the slope of size-dependent growth curves. Over 4 weeks, individual growth, catabolism, and fission all contributed to the pattern of individuals converging toward environment-specific body sizes. Together these results support the hypothesis that life cycle plasticity in clonal animals can stabilize growth across variable environments and encourages more exploration into how reaction norms for clonal behavior are shaped by local environmental patterns.

The interaction of temperature and dissolved oxygen with metabolic rate is complex. As temperature increases, metabolic rate increases, which increases the demand for oxygen. At the same time, higher temperatures reduce the capacity for water to hold dissolved oxygen. Thus, both a direct reduction in oxygen input and temperature increase can increase the risk of oxygen limitation. Metabolic demand for oxygen also increases with body size. Thus, the optimal body size for avoiding oxygen limitation depends on both water temperature and rate that oxygen flows into the environment.

In the longer term experiment, fission rate increased linearly while individual ramet size was unimodal over a broad range of temperatures. There was a transition from large and unitary to small-bodied and clonal as temperature increased, though the exact slopes and inflection points appeared to vary among genets. Notably, the rate of total colony mass accumulation was similar for many genets between 14° and 29 °C despite the major transition in growth pattern. Over the 12 weeks of the experiment, fission served to stabilize tissue growth rates across a span of temperatures over which oxygen consumption could reasonably be expected to triple ($Q_{10} \sim 2$; Sassaman and Mangum 1970). The reduction

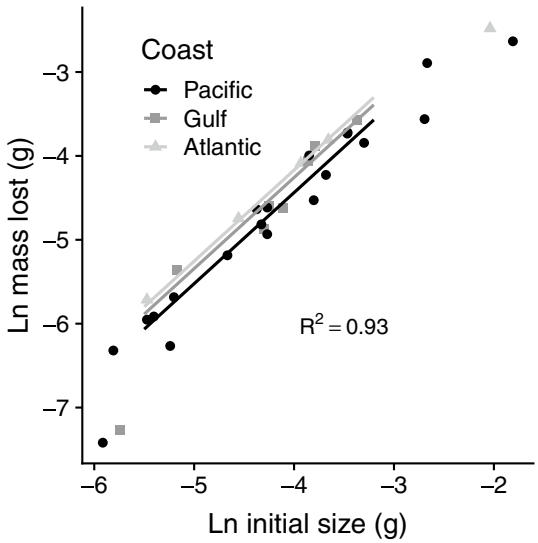

Fig. 5 Experiment 2B: the relationship between initial mass and mass lost [ln wet weight (g)] through starvation over 4 weeks at 15 °C (experiment 2B). Weight loss through starvation is inversely proportional to resting metabolic rate. Lines reflect best fit linear model. Point shade and shape reflect coastline of origin. Regions of the initial size spectrum that did not have representatives from all three coasts available (i.e., regions beyond the best fit lines) were excluded from statistical analysis

observed for many genets in biomass accumulation at 21.5 °C may suggest that asymmetry in the energetic costs and benefits of fission may lead to uneven growth across temperatures.

In the four-week experiment (2A), fission increased with temperature but showed a complicated relationship with body size, oxygen level and coastline of origin. Fission appeared to be stimulated when anemones were too large for the abiotic conditions, resulting in lost mass. However, fission also occured when anemones were growing rapidly, so were presumably well suited to the environment. Both patterns are consistent with oxygen limitation acting as a cue for fission. These alternate roles for fission behavior may help account for the observed interaction between oxygen treatment and body size in predicting the likelihood of fission in high temperature conditions.

Overall, Gulf Coast anemones showed the highest propensity toward fission, consistent with previous observations of high fission rates and small body sizes for Gulf versus Atlantic genets of the species under a seasonal temperature cycle (Ryan 2018). The role of fission in growth also varied among coastlines. For Gulf Coast genets, fission occurred most frequently where individuals were initially large bodied and played a role in reducing ramet size. Despite being initially larger bodied on average, Pacific Coast genets rarely engaged in fission during the 4 week experiment. Pacific ramets that ended up smaller than the initial size mostly did so through catabolism rather than fission, similarly to how unitary anemones perform under high temperatures (Chomsky et al. 2004). Along with evidence of lower basal metabolic rates in Pacific genets, these results are consistent with patterns of reduced temperature sensitivity found for other organisms evolving under weak versus strong seasonal temperature fluctuation (Baumann and Conover 2011).

Recent theory suggests that non-fluctuating environments favor thermal performance curves that match optima based on mean temperature, whereas strongly seasonal environments tend to favor higher resting metabolic rates and strategies that minimize the risk of stress during summer high temperatures due to asymmetry in the energetic cost of being warmer rather than cooler than optimal (Amarasekare and Johnson 2017). Because body size influences metabolic rate, traits that modulate body size (such as growth and fission) are expected to be more responsive to temperature in seasonal environments, where the energetic cost of environmental mismatch is likely much higher (Scranton and Amarasekare 2017).

In anemones, an increased risk of exposure to heat-related hypoxia in predictably varying environments may drive the evolution of fission rates that are more responsive to temperature. Gulf anemones are at the highest risk of predictable periods of persistent hypoxia due to high mean water temperatures coupled with strong seasonal fluctuations. In contrast, Pacific anemones, which may encounter bouts of high temperature and hypoxia during low tide (Helmuth et al. 2002), do not experience sustained and predictable exposure to warm, hypoxic water to the same degree. As a consequence, Pacific anemones may use fission primarily to maintain colony growth through the production of uniformly sized ramet whereas Gulf and Atlantic populations use fission for both growth and as a means to rapidly modulate body size to avoid hypoxic stress during seasonal flux. As a point of comparison, the larger-bodied clonal species, *Anthopleura elegantissima*, occur across a similar latitudinal distribution on the Pacific coast of the US. Fission in this species has been described primarily as a mechanism of asexual growth driven by food availability, rather than as a mechanism for modulating body size through temperature cycles (Sebens 1980, 1982). However, we currently lack both the local-scale temperature data and depth of sampling for *D. lineata* to evaluate the merits of this explanation.

Genets from all three coastlines showed size-dependent growth, where mass-specific growth rate declined steeply with increased body size resulting in a unimodal pattern of total colony mass change. As predicted, the energetic cost of being smaller than optimal (reduced growth rate) is much lower than the cost of being too large (mass loss), perhaps favoring fission behavior when the risk of hypoxia is high. Optimal size theory extends the predictions of metabolic scaling theory to suggest that the body size that maximizes energetic efficiency decreases monotonically from cold to warm conditions (Sebens 2002, Kingsolver and Huey 2008 Forster et al. 2011; Sheridan and Bickford 2011). Consistent with these predictions, a combination of fission, growth, and shrinkage behavior led ramets to converge toward an environment-specific body size. The size onto which ramets converged differed between temperature treatments and mirrored the body size that led to the highest colony biomass accumulation, consistent with regulation of growth and body size via allometric metabolic scaling (reviewed by Glazier 2014). These results all support the existence of energetic advantage for genets that can modulate the size of ramets produced in variable environments. Thus, the patterns we observed suggest a degree of adaptation in the shape of the underlying reaction norms that govern fission and growth behavior in *D. lineata*. However, there is little known about how such plasticity is expressed under field conditions in spatially or temporally heterogenous environments. Here, we have interpreted patterns through the lens of oxygen limitation, but there are likely many additional effects of temperature (e.g., changes in gene expression, tissue damage, etc.) influencing growth and fission in ways that we have not yet investigated. Likewise, there is much to explore about the role of invasion dynamics and habitat filtering in the geographic patterns observed for this species, whose range has been expanded through human activity to encompass three

coastlines with very different climatic patterns. Differences in the invasion history of non-native populations across the US could provide an alternate explanation for the observed variation among coastlines. Future work elucidating patterns of genetic diversity and connectivity within and among sites will likely provide critically needed context for interpreting the eco-evolutionary significance of such variation.

These findings also contribute to a growing pool of observations in need of synthesis on how environmental parameters influence asexual reproduction in sea anemones. For example, several authors have concluded that asexual reproduction is a hallmark of growth in favorable conditions, such as reporting increases in asexual behavior with increased food consumption (*Diadumene lineata*: Minasian 1979; *Nematostella vectensis*: Reitzel et al. 2007), or in temperatures associated with high survival and growth rates (*Metridium senile (L.)*: Glon et al. 2019). While others have suggested fission to be a stress response, for example during starvation (*Anthopleura elegantissima*: Sebens 1980,1982; *Exaptasia diaphana*: Bedgood et al. in revision). There are a number of complications preventing a general explanation for asexual reproduction in anemones, chief among them being the diversity of processes included in that description, including binary fission, transverse fission, and pedal laceration (Fautin 2002). There is intriguing variation even within *D. lineata*, where some populations in the native range are known to asexually reproduce by pedal laceration rather than binary fission (Atoda 1973). In our experiment, only one out of 194 individuals considered from across the US invaded range displayed pedal laceration. The factors favoring this alternate asexual mode are unknown, and its apparent rarity in invasive populations (it is previously undocumented outside of the native range) remains unexplained. There is also some evidence that species differ in their capacity for physiological acclimation (Zamer and Mangum 1979), though this has not been explored widely. Another factor limiting a unified understanding is the tendency to measure environmental performance across a subset of conditions (e.g., two temperature treatments), using a narrow range of potential body sizes. As is well appreciated in the plasticity literature, describing the trend of a non-linear process depends heavily on which treatment levels are included (Murren et al. 2014). The results of our study emphasize the complexity of the relationship between growth and the abiotic environment, particularly for clonal organisms. It appears that fission can increase in both favorable *and* stressful conditions. Reaction norm experiments are logistically challenging, but necessary for understanding the true shape of environmental performance curves. Likewise, concepts of metabolic scaling provide a strong framework for integrating information about food and oxygen availability, temperature, body size, and shape, but require a lot of data. Moreover, to understand the evolutionary basis of clonal life cycles, such physiological knowledge needs to be combined with ecological and demographic data to capture the multifaceted fitness effects of life cycle variation. Studies such as this one, however, suggest that there are strong unifying mechanisms waiting to be characterized underneath the overwhelming reproductive diversity in anemones.

In summary, the adaptive value of clonality depends on the degree to which the lifetime fitness of a genet is increased by dividing its mass into multiple units as opposed to retaining all biomass in a unitary body. For long-lived organisms in fluctuating environments, such as *D. lineata*, the ability to manipulate body size through fission seems to offer an obvious advantage. The fitness value of fission behavior must, however, be integrated across the environments a genet experiences, which may be linked in different sequences, or on different time scales, all of which influence the energetic costs and benefits of a given body size. Other size-dependent phenomena are combined with metabolic scaling dynamics to shape the adaptive landscape on which organisms must "decide" if and when they should divide. Because sexual maturity and gamete production depend on body size (Ryan and Miller 2019), fitness defined as the production of sexual offspring might be very different between a single large or many small anemones. We currently lack data to compare genet-level gamete or offspring production of replicates raised in different temperature conditions, though this comparison is necessary to fully appreciate the effects of temperature-dependent fission behavior on sexual fitness. Much future work is needed to fully understand how and why species maintain clonal life cycles. But, with this study we add to the collection of tantalizing observations that have long made this species a promising model for the eco-evolutionary forces driving life cycle evolution.

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Data availability Data generated during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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