Asexual reproduction and anterior regeneration under high and low temperatures in the sponge associate Polydora colonia (Polychaeta: Spionidae)

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Regeneration in polychaetes is an important process because of its role in recovery after injury and in asexual reproduction via architomy. This study examined architomy and regeneration in the spionid worm, *Polydora colonia* (Moore 1907) a symbiont of sponges. Based on collections of *P. colonia* from Long Island, New York, prevalence of architomy was 24% (188 out of 780 worms) with the highest prevalence recorded during the summer and early fall and the lowest prevalence during late fall and winter. Morphogenesis during regeneration of *P. colonia* was studied with light and scanning electron microscopy at two different temperatures. Worms regenerated faster under high temperatures (24°C), whereas it took more than twice as long to regenerate under low temperatures (14°C). Morphogenesis during anterior regeneration included the formation of a blastema from which a maximum of eight anterior segments regenerated. At high temperatures, palp buds and initial segments were observed to form by day 2 and 1–2 major spines were observed in the fifth segment by day 8. This is the first report of asexual reproduction in the field for the genus *Polydora* and the results indicate that temperature plays a role in regeneration.

Introduction

The life history of some polychaete worms in the family Spionidae has been studied in detail, particularly in terms of reproduction and larval development. Sexual reproduction is the main mode of reproduction in the spionids (>450 species; Rouse and Pleijel 2001), leading to planktotrophic, lecithotrophic, or adelphophagic larvae (see Blake and Arnofsky 1999; Blake 2006). In contrast, asexual reproduction in spionids is rare, having been found in only 13 species ($<3\%$ of spionids) from the genus *Pygospio* and members of the *Polydora* complex. There are two modes of asexual reproduction found within spionids: architomy and paratomy. Architomy is the most common type and involves fragmentation of the worm followed by regeneration of posterior and anterior ends to form new individuals (Blake and Arnofsky 1999; Lopez et al. 2001; Tinoco-Orta and Caceres-Martinez 2003; Lindsay et al. 2007). Architomy has been reported under laboratory conditions for *Amphipolydora vestalis* (Gibson and Paterson 2003), *Dipolydora caullery* (Mesnil 1896), *Dipolydora socialis* (Schmarda 1861), and *Pygospio elegans* (Claparede 1863). *Pygospio elegans*, is a model spionid used in the study of architomy because of its cosmopolitan distribution, its ease of inducing fragmentation, and its ability to track

formation of specific structures during morphogenesis. Paratomy is less common in spionids (occurring only in members of the genus *Polydorella* and one species of *Polydora*) and involves budding (production of stolons) from a parental stock worm and subsequent fragmentation (Blake and Arnofsky 1999; Williams 2004).

The factors responsible for initiating asexual reproduction are not fully known. Rasmussen (1953) and Armitage (1979) reported that *P. elegans* from Denmark and California showed higher prevalence of asexual reproduction as temperature increased. However, Anger (1984) showed that abiotic factors such as temperature and salinity did not correlate with increased frequency of asexual reproduction in Baltic Sea populations. Blake and Arnofsky (1999) suggested that these differences might be due to different experimental methods, based on the fact that Armitage (1979) conducted long-term field studies and Anger (1984) completed controlled laboratory experiments. Wilson (1985) reported a density-dependent response, where increased food levels correlated with increased prevalence of fragmentation during architomy in *P. elegans*. Although laboratory studies on architomy have been completed, few studies have quantified prevalence of architomy in the field

(Rasmussen 1953; Armitage 1979) and no field studies of architomy have been conducted on any member of the genus *Polydora*.

Regeneration is the process by which an organism replaces a lost body part. Like most polychaetes, spionid worms are capable of regenerating lost body parts, and this process plays a critical role in survivorship following sublethal predation (Zajac 1985; Hentschel and Harper 2006; Lindsay et al. 2007) and can also aid in recovery from injuries due to physical disturbances (Lindsay 2010). Regeneration following injury and asexual reproduction has been shown to follow similar morphogenic patterns (Gibson and Paterson 2003) and may be under the same genetic controls (Bely and Wray 2001). However, recent evidence indicates that despite strong similarities on a superficial level, the processes may be developmentally distinct with different evolutionary trajectories (Zattara and Bely 2011). Members of the Spionidae vary in their ability to regenerate anterior regions (Bely 2010) but species that are capable of anterior regeneration have been reported to follow very similar morphogenic pathways (Gibson and Harvey 2000; Gibson and Paterson 2003; Lindsay et al. 2008). Posterior regeneration has been found in all spionids studied and nearly all polychaetes; this ability appears to be so widespread because it is similar to regular adult growth by segment addition (Stock 1964; Bely 2006).

Lindsay et al. (2007) showed that the rate at which segments were formed during anterior regeneration in two spionids was directly related to the extent of tissue loss. In particular, specimens of *D. quadrilobata* that lost five segments took longer to regenerate than specimens losing the first segment (peristomium) only, indicating that the region of ablation impacts regeneration. Similarly *Dipolydora commensalis* (Andrews 1891) is capable of regenerating anterior and posterior ends and feeding appendages (palps) form earlier on worms cut at the 5th setiger versus the 15th setiger (Dualan and Williams, 2011). Loss of palps has also been shown to impact feeding behavior and regeneration in polydorids (Zajac 1985, 1995; Lindsay and Woodin 1992, 1995, 1996; Hentschel and Harper 2006). Zajac (1985, 1995) examined sublethal predation on *P. cornuta* and found that worms with both palps removed took significantly longer to regenerate than those with one intact palp. Similarly, Hentschel and Harper (2006) found that loss of both palps in *P. cornuta* resulted in a significant decrease in the regular growth rate (RGR), whereas the loss of one palp had virtually no effect on its RGR. Only a few other experimental studies on regeneration in polydorids exist, including those of Abeloos (1950, 1954) working on *P. ciliata* (Johnston 1838) and *D. flava* (Claparède 1870), Thouveny (1958) working on *D. flava*, Stock (1964) working on *D. caulleryi*

(Mesnil 1897), *D. flava*, and *D. socialis*, and Tinoco-Orta and Caceres-Martinez (2003) working on *Polydora* sp.

Polydora colonia (Moore 1907) is a small polydorid worm that has been reported from various regions of the Atlantic Ocean and the Mediterranean Sea. The species is cryptogenic and appears to have been introduced to various regions (Neves and Rocha 2008; Occhipinti-Ambrogi et al. 2010; David and Williams in review). *Polydora colonia* is typically found associated with sponges but has also been reported from algae (Blake 1971; Aguirre et al. 1986). The biology of *P. colonia* is virtually unknown except for taxonomic studies, although it has recently been found to be a predator of host sponges and to exhibit adelphophagy (David and Williams in review). Previously, sexual reproduction was the only mode of reproduction that has been reported for this species (Hartman 1945). However, asexual reproduction via architomy was noted in specimens of this species from New York and Massachusetts (David and Williams in review). The objectives of this study were to document: (1) asexual reproduction in the field, (2) morphogenesis during regeneration, and (3) impact of temperature on anterior regeneration.

Materials and methods

Field studies on architomy

Field collections of the red-beard sponge *Microciona prolifera* (Ellis and Solander 1786) were made during 2007–2010 on the east coast of the United States at the Town of Hempstead East Marina, Point Lookout, New York (40°35′37.71″ N, 73°35′07.09″ W). Specimens were collected during the months of September and October 2007; September, October, and November 2008; January, March, and October 2009; June–November 2010. Sponges were removed from the side of the docks and transported in buckets filled with unfiltered seawater (salinity: 33‰). For removal of worms, *M. prolifera* was immersed in 7% $MgCl_2$ to anesthetize *P. colonia* inside its burrows. The sponge was then returned to seawater and examined with an Olympus SZX12 dissecting microscope. Metal probes were used to dislodge the burrow from the sponge and worms were forced out of the burrows with a stream of seawater from a 1-mm diameter pipette. For field studies on asexual reproduction, 60 worms were extracted from sponges for each month collected ($n = 780$ worms). Worms were then examined for evidence of architomy by classifying each specimen into the following categories: (1) complete (C), worms complete with no evidence of regeneration; (2) anterior regenerates (AR), worms with regeneration of anterior chaetigers; (3) posterior regenerates (PR), worms with regeneration of posterior chaetigers; and (4) anterior/

posterior regenerates (AP), worms with anterior and posterior regenerations (i.e., middle chaetiger fragments with regeneration of both ends). These estimates of asexual reproduction via architomy based on field specimens are considered conservative because some specimens that were farther along in regeneration were likely scored as complete (C). Pearson's chi-square was used to determine if the frequency of architomy was independent of months sampled and a *post hoc* test (simultaneous test procedure) was used to determine if there was a seasonal difference in frequency of architomy between months.

Effect of temperature on anterior regeneration

Worms collected in the field as described above were isolated upon extraction from *M. prolifera* and placed in 60×15 mm Petri dishes with 10 mL of seawater (5 worms/dish). Worms were kept at 24°C with artificial seawater at 33‰ for 1 day before ablation. In order to control for size, only complete and non-regenerating worms with 28–31 chaetigers were used in this experiment (representing adult worms; David and Williams in review). Twenty worms were ablated between chaetigers 14–16 using microscalpels and the size of the posterior end was measured using a dissecting scope and Image J software calibrated with an ocular micrometer. All anterior and posterior fragments survived the ablation. Worms were then placed in an incubator at 14°C. Another 20 worms were ablated between chaetigers 14–16 and maintained at 24°C. Regenerating worms were examined daily using an Olympus CX31 compound microscope and the seawater in Petri dishes was changed every 2 days. Anterior regeneration was followed until regeneration was completed, as indicated by the formation of 5th chaetiger spines. After regeneration was completed, the size of the anterior end of the worms from both treatments was measured and the number of anterior chaetigers regenerated was recorded. A student *t*-test was used to determine if there was a significant difference in size between regenerating worms reared at low (14°C) and high (24°C) temperatures.

Morphogenesis during regeneration

Light and scanning electron microscopy (SEM) was used to document anterior regeneration in isolated adult worms ($n = 20$) that were cut at the 14–16 chaetiger range and maintained at 24°C. Posterior regeneration was followed until the appearance of the first boat hook (a type of modified notochaetae found in some polydorids, see Blake 1979); posterior fragments were not isolated for SEM studies. Specimens during early regeneration were fixed in 3% glutaraldehyde for 24 h. Worms were then rinsed in 0.1 M

phosphate buffers three times, 30 min each. After rinsing, worms were dehydrated in an ascending EtOH series (70%, 75%, 80%, 85%, 90%, 95%) for 10 min each and in 100% EtOH three times for 15 min each. Specimens were critical point dried (Samdri-795 Critical Point Dryer), mounted on an aluminum stub with adhesive sticky tape and coated with gold (EMS-550 Sputter Coater). Specimens were observed using a Hitachi 2460N SEM.

Results

Field studies on architomy

Total prevalence of anterior (AR), posterior (PR), and anterior-posterior (AP) regeneration in the field for *P. colonia* over the course of 4 years in the months sampled was 24% (188 of 780 worms). More than half (14% of the total sample) of regenerating worms were regenerating anterior ends only, whereas 7% were regenerating posterior ends and 3% were regenerating both anterior and posterior ends simultaneously (Figure 1(a)). Of all years sampled, frequency of architomy did not support the null hypothesis of an equal distribution between months (Pearson's chi-square; $\chi^2 = 86.84$, df $= 6$, $p < 0.0005$) (Figure 1(b) and (c)). *Post hoc* tests indicated that there were no significant differences in percentage of worms regenerating in summer (July) and autumn (September and October), whereas there were significantly more worms regenerating in July, September, and October than in winter months (December, January, and March). There was no significant differences in percentage of worms regenerating between November and winter months (Figure 1(c)). Worms showed a trend of decreasing frequency of architomy from fall to winter: 36% to 26% from September to October in 2007, 30% to 1% from September to December in 2008, and 32% to 20% from September to November in 2010. The lowest frequency was found in December with only 1% of the worms regenerating. The highest frequency of regeneration (62%) was found in the one summer month sampled (July 2010) (Figure 1(b)).

Mean number of anterior chaetigers regenerated was 5 ± 2.2 ($n = 107$) and maximum number of anterior chaetigers regenerated was 8; mean number of posterior chaetigers regenerated was 9 ± 3.8 ($n = 56$) and maximum number of posterior chaetigers regenerated was 15. Mean numbers of anterior and posterior chaetigers regenerated simultaneously were 7 ± 1.6 and 6 ± 2.7 ($n = 25$), respectively; maximum numbers of anterior and posterior chaetigers regenerated simultaneously were 8 and 16, respectively.

In one sponge branch collected on October 2010, 12% (12 of 100) of the worms showed abnormalities with two anterior ends (7–10 setigers) and fully
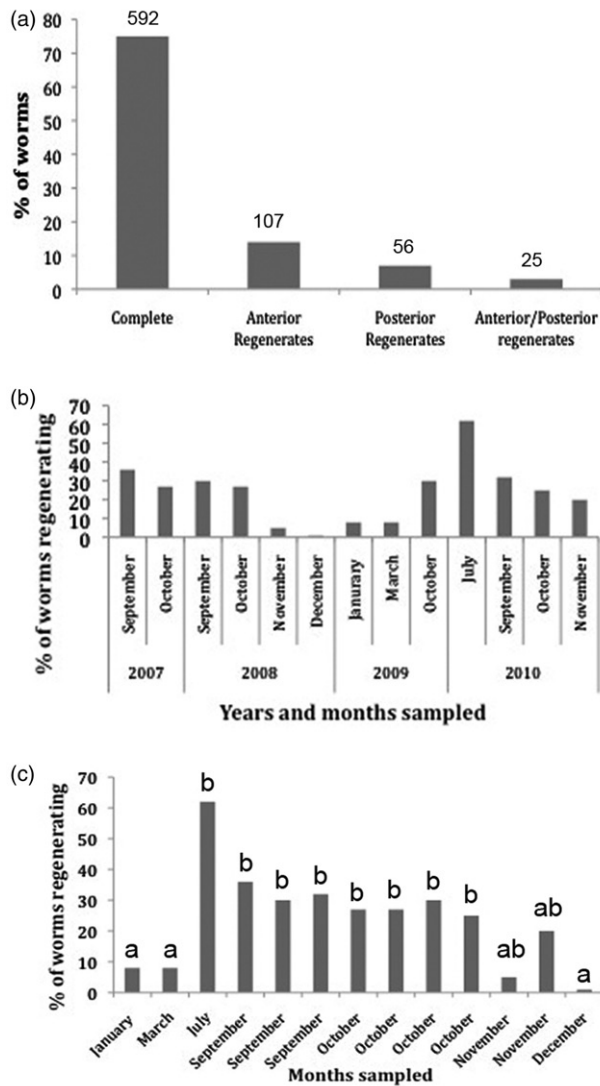
Figure 1. (a) Overall prevalence (%) of regeneration types during architomy in *P. colonia*; including complete, non-regenerating worms, anterior, posterior, and anterior–posterior regenerates sampled from 2007 to 2010; numbers above bars represent total number sampled. (b) Prevalence (%) of architomy in *P. colonia* sampled on selected months from 2007 to 2010 from Point Lookout, New York. Percentage of worms regenerating anterior, posterior, and anterior–posterior regions per month ($n = 780$ worms; 60 worms per month). (c) Prevalence (%) of architomy in *P. colonia* on selected months. Different letters above bars represent frequency of architomy that are significantly different according to the simultaneous test procedure ($\chi^2 = 12.59$, df = 6).

regenerated palps (Figure 2(a)). Three of the 12 abnormally regenerating worms were ovigerous (Figure 2(b)).

Effect of temperature on regeneration in P. colonia

Worms under low and high water temperatures regenerated a maximum of eight chaetigers and
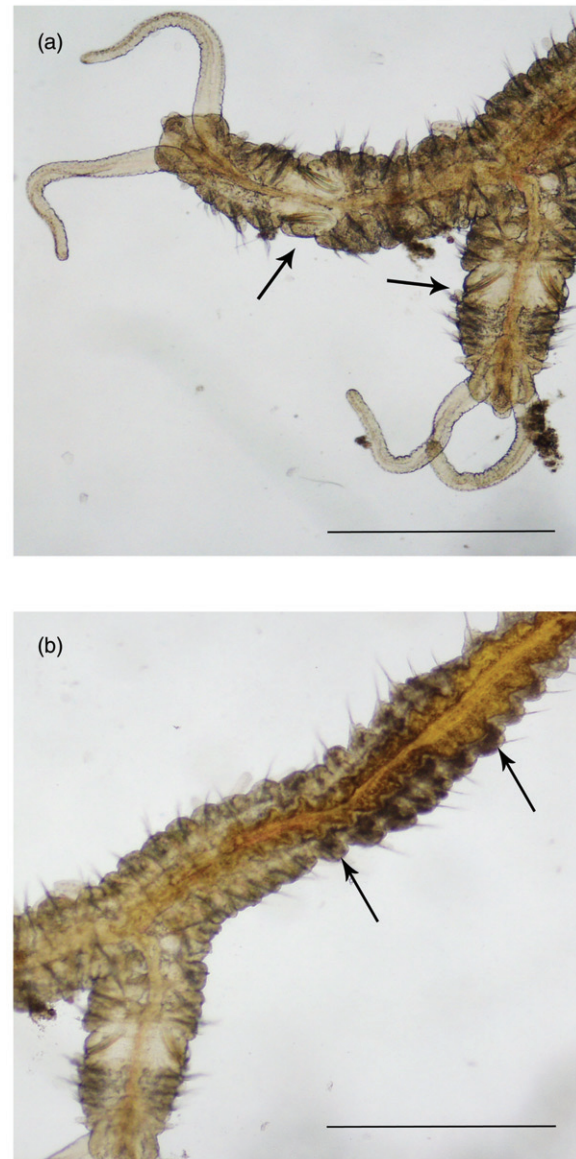
Figure 2. Abnormalities in *P. colonia*: (a) individual with two anterior regions fully regenerated with well-developed 5th setiger (arrows) and (b) middle region of the two-headed specimen showing an enlarged gut and ovigerous segments 15–21 (arrows) (scale: 500 μm).

100% in each group successfully regenerated anterior structures, with no abnormalities. Mean size of posterior fragments following ablation from low and high temperature treatments was 0.70 mm ± 0.19 ($n = 20$) and 0.77 mm ± 0.18 ($n = 20$), respectively. There was no significant difference in size of the posterior fragments (*t*-test 1.21, $p = 0.24$). Mean size of anterior tissue regenerated at completion of regeneration in high-temperature specimens was 0.4 mm ± 0.09 ($n = 20$) and 0.39 mm ± 0.07 ($n = 20$) in low temperature specimens; there was no significant difference in the sizes (*t*-test 0.06, $p = 0.70$).

Table 1. Summary of morphogenesis during regeneration at 24°C in *P. colonia*.

	Day							
Structure	1	2	3	4	5	6	7	8
Anterior blastema		+						
Prostomium			*	**	**	**	**	**
Caruncle							*	**
Palps			*	**	**	**	**	**
Chaetigers			+					
(no.)			1–2	4–5	5–6	6–7	8	8
5th chaetiger spine								+
(no.)								1–2
Posterior blastema		+						
Chaetigers			+					
(no.)			1–2	2–3	2–3	4–5	5–6	5–6
Boat hooks								+
(no.)								1
Pygidium			+					

Notes: + = structure recognizable, * = structure visible as a bud or rudiment, ** = structure well-developed but smaller than original worm.

Mean regeneration rate under both experimental conditions was significantly different (*t*-test 25.55, $p = 0.004$). Anterior regeneration under low temperature was completed in 15.9 days ± 1.2 ($n = 20$) and at high temperature was completed in 7.5 days ± 0.76 ($n = 20$). Morphogenesis followed the same sequence for both experimental conditions. However, there was an average lag time of 6.7 days ± 0.47 ($n = 20$) before the blastema became visible in low temperature specimens as opposed to only 2 days for the high temperature specimens (Table 1).

Morphogenesis during anterior regeneration in *P. colonia*

Morphogenesis was recorded for worms cultured at 24°C. During anterior regeneration, all major structures appeared 8 days following ablation (Table 1). On day 1, the wound at the site of ablation healed via constriction of tissue until a smooth surface replaced the cut tissue (Figures 3(a) and 4(a)). On day 2, a transparent blastema formed after the wound healed (Figures 3(b) and 4(b)). On day 3, multiple structures formed: the blastema extended forward and the anterior tip formed the early prostomium and a pair of rounded palp buds also formed (Figures 3(c) and 4(c)). Day 3 was characterized by the appearance of the mouth cleft and the addition of segments that appeared on the elongated blastema (Figure 4(d)). On day 4, palp buds extended, the mouth opening formed (Figures 3(d) and 4(e)), and segments became defined. Days 5 and 6 were characterized by elongated palps, addition of segments (6–7 segments with chaetae in at least 3) and extension of the gut from the mouth

(Figure 3(e)). By day 7 or 8 regeneration was completed and was characterized by fully formed palps with food grooves, a caruncle and chaetae present on all eight regenerated segments (Figures 3(f) and 4(f)). Enlargement of the 5th chaetiger occurred between days 6 and 7 with the 5th chaetiger spines visible on day 8.

Early morphogenesis during posterior regeneration followed a similar pattern as anterior regeneration. On day 1, the site of ablation on the anterior fragments had healed. Day 2 was characterized by the formation of a posterior blastema. The early pygidium was formed on day 3 and segments continued to be added until day 7; the maximum number of posterior segments regenerated was six. The first boat hook observed was regenerated on day 8, after all six segments were fully defined with chaetae.

Discussion

This study shows that *P. colonia* commonly reproduces asexually via architomy in the field and when ablated in the laboratory follows a similar pattern of regeneration. The frequency of architomy in the field is dependent upon the months sampled and the increased prevalence of regenerating worms in July suggests a relationship between increasing temperature and increased frequency of architomy. Similarly, Rasmussen (1953) reported increased prevalence of asexual reproduction with increasing spring temperatures in *P. elegans*. However, as noted by Blake and Arnofsky (1999), field data may not necessarily reflect the variables associated with asexual reproduction since other studies showed neither salinity nor
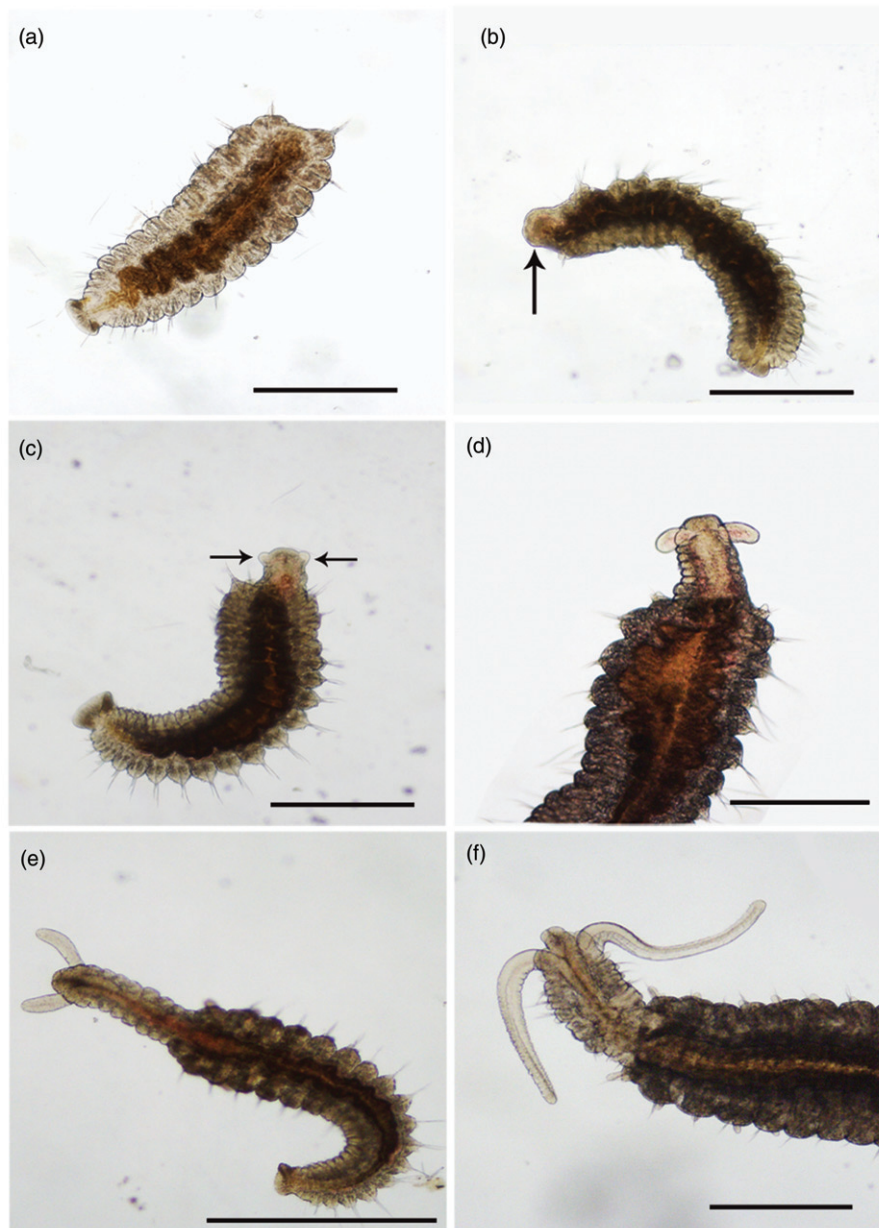
Figure 3. Light microscopy images of anterior regeneration in *P. colonia* at 24°C: (a) original posterior fragment 24 h after ablation, (b) formation of a transparent blastema (arrow) at day 2 of regeneration, (c) formation of the prostomium and emergence of a pair of palp buds (arrows) at day 3 of regeneration, (d) appearance of early segments and palps by day 4, (e) differentiation of segments and further extension of palps by day 5, and (f) completion of regeneration (days 6–8) with setae present in all segments and fully formed prostomium with caruncle (scale: 500 μm).

temperature associated with asexual reproduction (e.g., Anger 1984). It should be noted that the prevalence of regeneration in the field may be higher than reported here since it was in some cases difficult to differentiate regenerating worms from complete worms, especially those with posterior regeneration.

Martin and Britayev (1998) and Lopez et al. (2001) suggested that asexual reproduction could be considered as an adaptation allowing symbiotic polychaetes to attain high densities after colonization of host substrate and thereby outcompete other symbionts.

This may explain why the highest numbers of regenerating *P. colonia* specimens coincided with the appearance of *M. prolifera*. *Polydora colonia* is the dominant spionid on *M. prolifera* and can attain densities as high as 7.9 worms/mm^3 (David and Williams in review). Additional symbionts of *M. prolifera* compete with *P. colonia*, including amphipods and other polychaetes (Long 1968; Biernbaum 1981; David and Williams in review). Asexual reproduction could also be a strategy for minimizing costs of parasitism (McCurdy 2001) and *P. colonia* has been
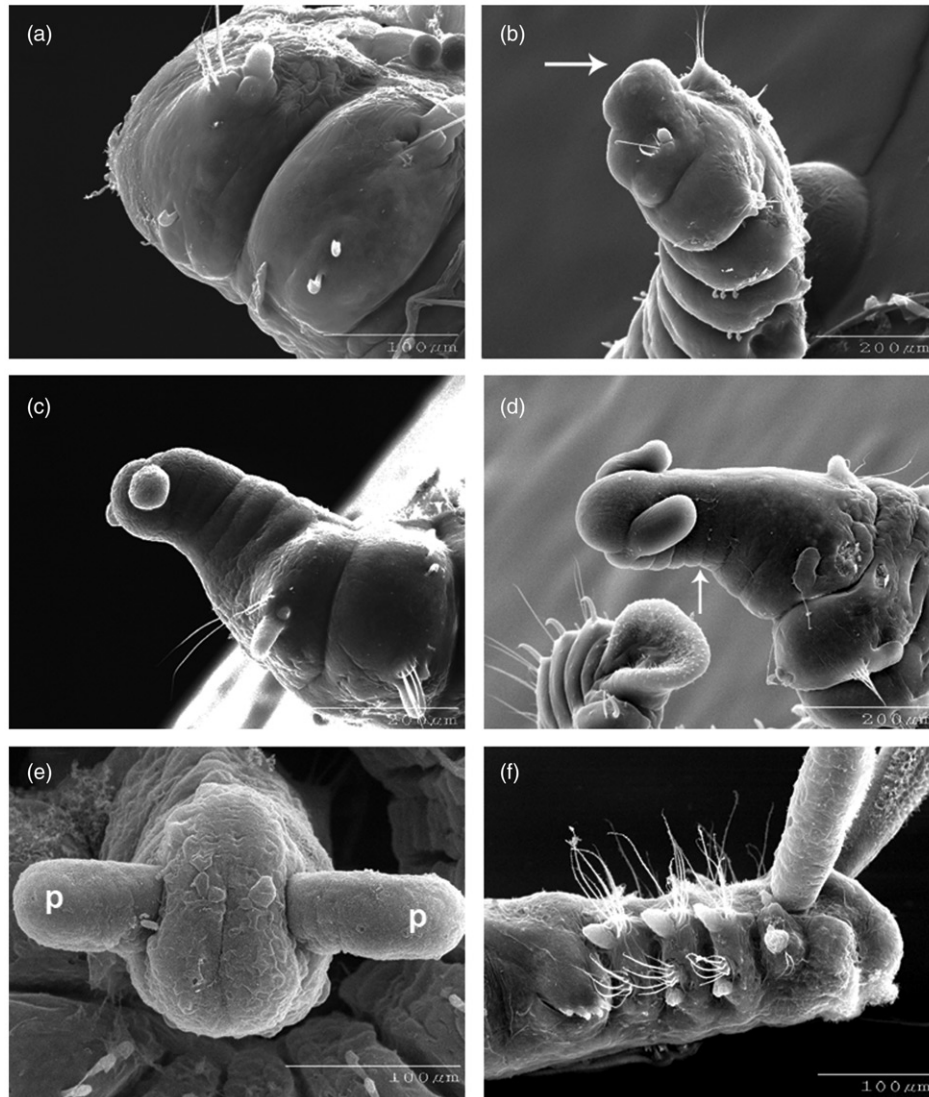
Figure 4. SEM images of anterior regeneration in *P. colonia* at 24°C: (a) smooth posterior region 24 h after ablation, (b) blastema formation (arrow) by day 2, (c) extension of blastema with formation of palp buds by day 3, (d) palps elongated with differentiation of segments observable on the ventral region (arrow) by day 4, (e) frontal view of prostomium and elongated palps (p), and (f) a completely regenerated specimen with conspicuous 5th setiger formed along with fully differentiated setigers, prostomium, mouth, and palps (days 6–8) (scale: a, e, f = 100 µm; b–d = 200 µm).

found as a host for an endoparasitic copepod of the family Monstrilloidae (David and Williams in review).

Morphogenesis during anterior regeneration in *P. colonia* followed a similar pattern as other spionids with the formation of an anterior blastema and subsequent formation of the prostomium, mouth, palps, chaetigers, caruncle, and modified spines (Gibson and Harvey 2000; Gibson and Paterson 2003; Lindsay et al. 2007). *Polydora colonia* showed an upper limit of 8 anterior chaetigers that can be regenerated following loss of 14–16 chaetigers in the lab. In addition, all field specimens that exhibited anterior regeneration had a maximum of eight regenerating chaetigers. Other polydorids have been shown to regenerate a fixed number of chaetigers after

anterior tissue loss. Specifically, *D. caulleryi* and *D. quadrilobata* regenerated a maximum of 10 chaetigers, *P. ciliata* regenerated a maximum of 9 chaetigers, and *D. flava* and *D. socialis* regenerated a maximum of 8 chaetigers (Abeloos 1950, 1954; Stock 1964; Lindsay and Jackson 2007).

Morphogenesis during posterior regeneration in *P. colonia* included the formation of a posterior blastema, pygidium, chaetigers, and boat hooks. Since the boat hooks are formed last, individuals lacking or regenerating posterior ends can appear to lack boat hooks, potentially leading to taxonomic confusion with *Polydora spongicola*. *Polydora spongicola* is morphologically similar to *P. colonia*; however, it lacks boat hooks and has four eyes in adults (adults

of *P. colonia* always have boat hooks when fully regenerated and lack eyes). The late regeneration of boat hooks may also explain some of the variation in hook forms that were observed in the field previously (see David and Williams in review).

During regeneration, abnormalities can occur both naturally and during experimental studies. Stock (1964) showed abnormalities occurring in *D. caulleryi* that included segmental and branchial abnormalities, lateral heads, axially twisted and bent regenerates, and double palps. Gibson and Harvey (2000) documented a "spontaneous asexual event", where *P. elegans* regenerated two head regions. Dualan and Williams (2011) documented *D. commensalis* regenerating a lateral head and posterior region from the same segment and regeneration of four palps. In contrast to the above studies that were completed in the laboratory, the abnormalities found in this study were from the field and all had two anterior ends fully regenerated. The 12 specimens were observed in the same sponge branch and some specimens had ovigerous segments indicating that the abnormal specimens were able to complete sexual reproduction.

The abnormalities found in *P. colonia* could be the result of architomy "gone wrong" (Gibson and Harvey 2000) or perhaps regeneration following a predation event. Regeneration is a feature of architomy and an adaptation to sublethal predation (Lindsay et al. 2007, 2008) and worms cut diagonally across two segments can result in the formation of two blastemas. Spionids that are subjected to sublethal predation are usually non-symbiotic species, inhabiting soft sediment benthic communities where they can suffer damage by browsing predators (Zajac 1985, 1995; Lindsay and Woodin 1992; Lindsay et al. 2008; Lindsay 2010). In contrast, *P. colonia* is a small symbiotic species that lives on sponges that are known to deter browsing fish predators through chemical and mechanical defenses (e.g., Hill et al. 2005; Huang et al. 2008). Some fish may attempt to prey on larger fauna associated with the sponge (especially *M. prolifera* that can harbor diverse assemblages of invertebrates) and in the process damage some specimens of *P. colonia*. There could be invertebrate associates of sponges that prey on *P. colonia*; however, predation on spionids by invertebrate predators is poorly known (see Michaelis and Vennemann 2005, for an example). Thus, we consider it to be unlikely that predators account for the relatively high prevalence of regeneration observed in the field, especially those with both anterior and posterior regions regenerating that would need to represent predation events on both ends simultaneously, an unlikely occurrence. The anterior–posterior regenerate ratio of 2:1 (Figure 1(a)) may be due to the fact that estimation of posterior regenerates was conservative since posterior regeneration is more difficult to discern than anterior regeneration.

Stock (1964) similarly found anterior regenerates to outnumber middle fragments and posterior regenerates in other spionids.

Regeneration is a post-embryonic developmental process and the mechanism underlying this process has been shown to be similar to embryogenesis and asexual reproduction via fission. Specific genes involved in regeneration include homeobox genes, engrailed (*en*) and orthodenticle (*otd/Otx*) that also have specialized roles during regeneration in other phyla with the gene *en* involved in segmentation and *Otx* involved in head patterning (Finkelstein et al. 1990; Bruce and Shankland 1998). Bely and Wray (2001) used *in situ* hybridization on the oligochaete *Pristina leidyi* to show that three homologs of *orthodenticle* and *engrailed* were expressed during regeneration. Specifically, *Pl-en* was expressed during nervous system development and *Pl-Otx1* and *Pl-Otx2* were expressed during anterior wall development and development of the foregut. However, *orthodenticle* and *engrailed* homologs have not been identified in polychaete regeneration. The regenerative abilities and ease of care of *P. colonia* make this species a potential model organism to investigate genetic control in both architomy and regeneration.

Polydora colonia regenerated faster at higher temperatures (24°C) and it took more than twice as long for specimens to regenerate at temperatures 10°C lower. When ablated at the 5th setiger, *D. quadrilobata* and *P. elegans* also regenerated major structures at a similar rate (15 days) to *P. colonia* under the same temperature (14°C) (Lindsay et al. 2007). However, the blastema took 3 days to form in *D. quadrilobata* and *P. elegans*, whereas it took 6–7 days before *P. colonia* formed a blastema. The reason for this difference may be partly due to the site of ablation since *P. colonia* was cut more posteriorly.

The longer time frame for regeneration at lower temperatures may explain why there were low numbers of worms regenerating in nature during colder months relative to summer and early fall. Energy allocation is an important aspect of regeneration, and regenerating body parts involves trade-offs that may be influenced by nutrient reserves (Bely 2010; Lawrence 2010). Under low temperature, when regeneration takes longer, it may not be advantageous to allocate energy toward asexual reproduction via architomic division. Rather, it could be more advantageous to allocate energy when asexual reproduction can be completed faster (i.e. summer/early fall) and colonization can occur faster when energy costs are less. Further studies on *P. colonia* can include testing the effect of food limitation on prevalence of architomy. Also, since previous studies have suggested that asexual reproduction may be an adaptation for colonization (Lopez et al. 2001), future research could focus on the effect of worm density on the host sponge and examine the

prevalence of asexual reproduction and sexual reproduction as a sponge branch becomes colonized. Such studies could have important implications because *P. colonia* has been noted as an introduced species in the Mediterranean (Neves and Rocha 2008; Occhipinti-Ambrogi et al. 2010; David and Williams in review), and asexual reproduction is a trait that is thought to facilitate invasion success in other species (e.g., Karako et al. 2002; Hayes and Barry 2008; Bock et al. 2011).

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