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Genetic analysis of sexual reproduction in the dendrophylliid coral *Balanophyllia elegans*

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Abstract The ahermatypic scleractinian *Balanophyllia elegans* has served as a model of limited larval dispersal in an aclonal species. However, other species from the same family (Dendrophylliidae) produce larvae asexually, and closely connected polyps of *B. elegans*, potentially the result of asexual reproduction, are commonly observed in the field. Here, we use a combination of laboratory experiments, controlled crosses, and genetic surveys of field-collected individuals to demonstrate (1) marker allozymes are inherited in accordance with Mendel's laws, (2) polyps that are connected in the field are not genetically identical, and (3) laboratory manipulation of a single polyp can produce a second polyp on the aboral side of the original, but not adjacent to the original. In combination, these results suggest that the larvae of *B. elegans* result from mictic sexual reproduction, and that connected polyps result not from asexual budding but rather from the fusion of genetically distinct individuals.

Introduction

Numerous anthozoan species produce larvae that are genetically identical to their broodparent (Ottaway and Kirby 1975; Black and Johnson 1979; Stoddart 1983; Ayre and Resing 1986; Edmands 1995). Production of such ameiotic larvae confuses the usual way in which biologists distinguish asexual investment in reproduction (via fission, budding, fragmentation, or a variety of other means; see Highsmith 1982; Harrison and Wallace 1990) from sexual investment in reproduction (via production of gametes and larvae). This ability to distinguish between asexual and sexual modes of reproduction is critical to a mechanistic understanding of genetic population structure (Ayre 1984; Hoffman 1986; Grosberg 1991; Edmands and Potts 1997; Coffroth and Lasker 1998), reproductive strategies (McFadden 1991, 1997; Ayre and Duffy 1994), and social interactions (Grosberg 1988) between sessile marine invertebrates.

The solitary cup coral *Balanophyllia elegans* Verrill 1864 occurs along the Pacific coast of North America between the intertidal and 500 m depth. This species has served as a model for studying colonial versus solitary strategies (Bruno and Witman 1996) and interactions between benthic invertebrates and algae (Coyer et al. 1993). The larvae of this ahermatypic coral are not planktonic but rather crawl along the substrate, resulting in extremely limited dispersal (Gerrodette 1981) and extensive genetic subdivision over its broad geographic range (Hellberg 1994, 1995). The combination of this limited larval dispersal and apparently strictly aclonal reproduction possessed by *B. elegans* is a rarity among scleractinians, which otherwise exhibit a great diversity of reproductive modes (Jackson 1986; Carlon 1999). Studies of *B. elegans* thus offer the potential to disentangle the consequences of limited larval dispersal and clonal reproduction, traits that are generally correlated among scleractinians (Jackson 1986). However, whether *B. elegans* is truly aclonal, or might be capable of asexual reproduction via ameiotic larvae or other means, has not been established conclusively.

Histological work suggests that the larvae of *B. elegans* result from sexual reproduction (Fadlallah and Pearse 1982). The sex ratio appears to be 1:1, and presumptive females bear oocytes and embryos throughout the year. *B. elegans* also exhibits no evidence of

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inbreeding at small spatial scales (Hellberg 1994). With regard to the potential for *B. elegans* to reproduce asexually, however, these data are not conclusive. Some anthozoans in which histological studies had found normal gonads were later determined to produce asexual larvae (see Edmands 1995), thus cautioning against equating the production of gametes or larvae with sexual investment. Analysis of field populations to detect asexual reproduction can also produce errors, as inbreeding coefficients utilize departures of *heterozygote* frequencies from expectations, not *genotype* frequencies. Finding populations near Hardy–Weinberg equilibrium need not imply that asexual reproduction is not a significant, even the predominant, mode of reproduction (McFadden 1997).

A few lines of evidence suggest that the mode of reproduction in *B. elegans* deserves scrutiny. First, ameiotic larvae are known from two species of *Tubastrea* (Ayre and Resing 1986), a genus in the same family (Dendrophylliidae) as *Balanophyllia*. Aside from their phylogenetic affinity, both *Balanophyllia* and *Tubastrea* brood their larvae, as do all dendrophylliids (Fadlallah 1983a). In addition, although budding has not been reported in *B. elegans*, this mode of asexual reproduction occurs widely elsewhere within its dendrophylliid clade (Zibrowius 1985; Cairns 1988).

In addition, social interactions between polyps of *B. elegans* often appear more similar to those between clonemates within clonal species than between genetically distinct individuals. Individuals whose tentacles touch do not interact aggressively (Chadwick 1991), unlike the case for nonclonemates in aggressive species. Such tentacle touching is probably very common in the field (Fig. 1A), where *B. elegans* can occur at high densities (>500 per square meter; Fadlallah 1983b). In some cases, different polyps appear so closely joined as to raise the possibility that they are physiologically connected (Fig. 1B, C). In these instances, living tissue from different polyps appears confluent, with no necrotic tissue or any other indication of antagonism appearing where the tissues meet.

Connected polyps could be produced by the fusion of polyps that settled very close together. Such fusion is well documented between clonemates and close kin in clonal organisms (Grosberg 1988) but has been thought rare among aclonal animals (Jackson 1986; but see Kingsley et al. 1989). Presumably, fusion is restricted due to the cost of losing resources (and thereby fitness) to unrelated conspecifics. Limited dispersal should mitigate such costs to some extent by increasing the likelihood of fusing with kin. Costs would be eliminated altogether if larvae were asexual and (genetically identical) broodmates fused.

Here, we use laboratory experiments and genetic analyses of field samples to assess the potential for asexual reproduction in *B. elegans*. Comparisons of multi-locus genotypes of lab-reared larvae and their parents demonstrate that (1) larvae are not genetically identical to either parent, (2) allozyme markers are inherited in Mendelian fashion, and (3) marker loci are not genetically linked. We demonstrate that although some damage to adult polyps can induce second polyps to form asexually, these do not form in the manner in which polyps are connected in the field. Finally, genetic analysis of polyps connected in the field suggests such joined pairs do not result from clonal reproduction. Together, our results suggest that reproduction in *B. elegans* is purely sexual, and that this species can serve as a model for the causes and consequences of limited dispersal in aclonal, sessile marine invertebrates.

Materials and methods

Laboratory breeding experiments

We established two types of breeding treatments. "Crosses" of two corals were established to compare parent genotypes to offspring genotypes. "Isolations" of single corals were established to determine (1) how long females deprived of fertilization opportunities could continue to release larvae and (2) whether isolated corals would release asexual larvae. For both treatments, corals were placed in 100-ml jars (Ball) and maintained in a coldroom at 8–12°C. Forty-two potential breeding pairs were established on 8 October 1990. Eighteen additional individuals were also isolated at that time. Paired individuals had been collected from two localities: Pacific Grove, California (by M.E.H. on 7 October 1990) and just north of Lighthouse Point in Santa Cruz, California (by J.S. Pearse in April 1990). Ten of the potential crosses were between Pacific Grove and Santa Cruz individuals, 4 between Santa Cruz individuals, and the remaining 28 between Pacific Grove individuals. Four of the isolated individuals were from Santa Cruz. Two additional

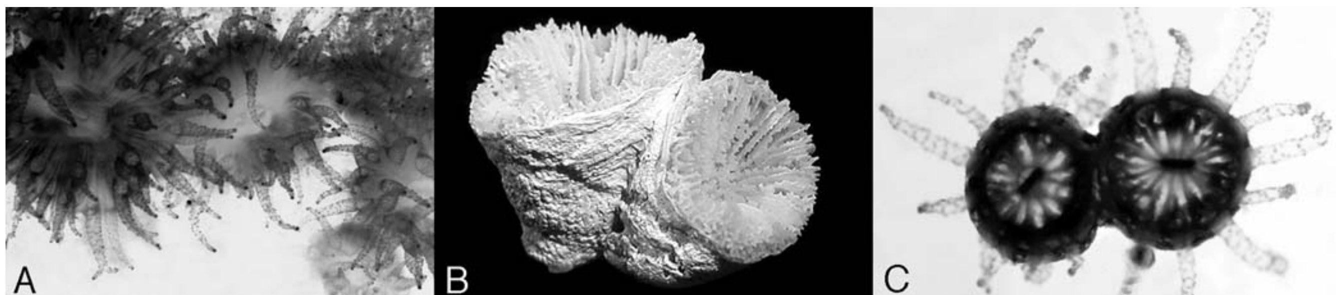

Fig. 1A–C. *Balanophyllia elegans*. Photographs showing close connections between polyps. **A** Adults off Nanaimo, British Columbia at 10 m depth. Note that adult in *center* has tentacles touching those of four other polyps. (Negative image to enhance contrast.) **B** Skeletons of two joined adults. **C** Fused juveniles produced in laboratory crosses

crosses were made on 12 December 1990 from corals collected at Monterey, California on 6 December 1990. Nine individuals were also isolated at that time.

Corals were fed 3-day-old *Artemia* larvae once a week. Seawater was replaced twice a week and the presence of any crawling larvae or settled juveniles was noted at that time. All juveniles were harvested from bowls between 8 and 16 August 1991; however, storage error resulted in their destruction. Subsequently produced juveniles were saved at –80°C for genetic analyses, beginning 14 October 1992 and continuing over the next year.

Genetic survey of joined individuals from natural populations

Samples were collected from 18 localities spanning over 3,000 km along the Pacific coast of North America: Hoya Pass (HOY), McInnes Island (MCI), Broughton Strait (BRS), Nanaimo (NAN), and Bamfield (BAM), British Columbia; Tatoosh Island (TAT), Washington; Cape Arago (CAR), Oregon; Trinidad (TRN), Caspar (CSP), Bodega Bay (BOD), Santa Cruz (CRZ), Monterey (MON), San Simeon (SIM), Goleta (GOL), East Anacapa Island (EAN), and San Diego (PTL), California; and Punta Banda (PTB) and Isla San Geronimo (ISG), Baja California (see Hellberg 1994 for details). At each locality, corals were gathered from within each of three to eight patches (circumscribed by a circle with a radius of 33 cm). Sampled patches were separated by at least 4 m. Most patches were between 5 and 20 m depth. Only adult corals (with a volume >150 mm^3, Fadlallah 1983b) were sampled. Corals were removed whole from rock substrate with a chisel. Two or more individual corals that were close to each other often came off together. If such individuals were connected by living (orange) tissue extending from the base up at least one-quarter of their height, they were noted as joined and then separated with a razor. All samples were then frozen in liquid nitrogen. Samples were stored at –80°C.

Electrophoresis

We characterized seven-locus genotypes for all pairs of joined individuals collected in the field. All samples were run on 12% (w/v) starch gels. Enzyme stains and electrophoretic conditions were modified from Selander et al. (1971). Three loci – (hexokinase (*Hk*, EC 2.7.1.1), phosphoglucomutase (*Pgm*, EC 2.7.5.1), and phosphoglucose isomerase (*Pgi*, EC 5.3.1.9) – were assayed using the pH 8.0 Tris-citrate buffer system of Selander et al. (1971). Four milliliters of 2-mercaptoethanol was added to 300 ml of starch before pouring to improve the resolution of *Pgi* bands. An additional polymorphic locus – mannose-phosphate isomerase (*Mpi*, EC 5.3.1.8) – was encountered late in the study and was assayed only for adults and larvae in the crossing experiment. Four other loci – triosephosphate isomerase (*Tpi*, EC 5.3.1.1), peptidase (*Pep*, EC 3.4.11/13., leucyl alanine substrate), aspartate aminotransferase (*Aat*, EC 2.6.1.1), and leucine aminopeptidase (*Lap*, EC 3.4.11.1/2) – were assayed for all individuals using the dilute pH 8.0 Tris-citrate buffer system of Ward and Beardmore (1977).

Genetic analysis

We used LINKAGE-1, version 3.50 (Suiter et al. 1983), to test whether offspring from a cross departed from Mendelian expectations, and to determine whether the eight allozyme markers were genetically linked to each other. This software program calculates χ^2 values associated with tabulated filial genotypes descended from known biparental genotypes, assuming a null hypothesis of independent assortment; significant differences indicate possible linkage between two or more loci. If two or more loci are indeed linked, then these loci should be significantly linked for all laboratory crosses. Of the 21 crosses established that turned out to contain one male and one female, we analyzed only the 12 crosses in which ten or more larvae were successfully genotyped; analysis of smaller broods would be too weak to reliably reveal patterns of inheritance or linkage. For all statistical analyses, significance values were Bonferroni corrected as appropriate for multiple simultaneous tests (Miller 1980).

We used two genetic analyses to assess the possibility that *B. elegans* reproduces asexually in the field. First, using genotypes from all individuals (not only joined ones) from all patches (Hellberg 1994), we compared observed genotypic diversity to values expected under purely sexual reproduction, using the BIOSYS package (Swofford and Selander 1981). A second set of tests makes use of the fact that sexual reproduction can conceivably increase or decrease heterozygosity at a particular locus but should always decrease genotypic diversity relative to purely sexual reproduction. Decreased genotypic diversity can be estimated with several measures when using allozyme markers; we calculated the two considered to be the most reliable (Johnson and Threlfall 1987; Uthicke et al. 1998; Ayre and Hughes 2000).

The first of these, N_{go}:N_i, estimates the minimum contribution of sexual reproduction to the population, where N_{go} is the number of genotypes observed in the population and N_i is the sample size (Johnson and Threlfall 1987). This ratio can underestimate sexual input because it ignores repeated sexual production of the same genotype. The second measure, N^*:N_i, estimates the maximum input of sexual reproduction (Johnson and Threlfall 1987). N^* estimates the number of identical genotypes expected from sexual reproduction. The calculation of N^* is detailed in Uthicke et al. (1998). Briefly, the expected frequency of each unique multi-locus genotype was estimated by the product of each of the frequencies of the single-locus genotype classes. The upper 95% confidence limit value of the expected frequency estimate was determined by applying the formula of Hald (1952) and then multiplied by the sample size minus one to obtain the maximum number of individuals of each genotype expected under purely sexual reproduction. If the number of observed individuals with identical genotypes exceeded the 95% confidence limit of sexually produced individuals, the additional individuals were considered to be asexually produced. If the number of observed individuals with identical genotypes was below the 95% confidence limit, they were considered to be entirely the result of sexual reproduction. The summation of the frequencies of each multi-locus genotype estimates the overall contribution of sexual recruits to the population.

We assessed whether fused individuals were more closely related to each other than were randomly sampled individuals from the same patch using Relatedness 5.0.8 (Goodnight 2001). This program calculates relatedness of individuals based on identity-by-descent (Queller and Goodnight 1989). Relatedness (r) can range from $+1$ (identical alleles at all loci) to –1 (different alleles at all loci). Only patches with 16 or more individuals and three or more fused pairs were analyzed. We determined r for each fused pair or, when more than 2 individuals were fused, for all pairwise combinations within a fused group. A single mean for fused pairs/groups from within a patch (r_{fused}) was then determined. This r_{fused} value was then compared to the mean relatedness for all individuals (fused and nonfused) on the patch (r_{patch}). If r_{fused} fell within the 95% confidence limits of r_{patch}, then fused individuals were not significantly more (or less) closely related to each other than were randomly sampled individuals from within the same patch.

Inducibility of polyp formation in the laboratory

To test whether joined individuals might result from budding or regeneration following damage to the skeleton, undamaged single polyps were cut in one of two ways: longitudinally (perpendicular to the oral disc), or transversely (parallel to the oral disc). Longitudinal cuts removed an arc or chip from the side of the coral that amounted to 10–20% of the area of the oral disc (Fig. 2). Similar damage might result from boulders thrown by surge in the field. Live corals with such moderately damaged skeletons can be seen in the field. Transverse cuts were made near the base of the polyp, parallel to the oral disc (Fig. 2). Transverse cuts were repeated, moving incrementally closer to the oral disc, until living tissue totaling ≥10% of the area of the oral disc was exposed. Similar

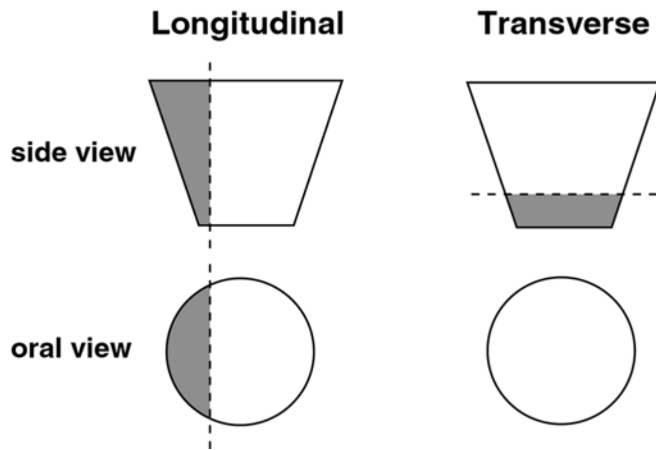

Fig. 2. Cuts (*dashed lines*) made to remove portions of polyps (*shaded*) from *B. elegans* for study of subsequent polyp formation

damage would result in dislodgment and death in the field (Fadlallah 1983b).

Cuts were made in late May/early June 1991. Polyps were maintained separately in jars in the same manner as the breeding corals (see above). Damaged polyps were inspected under a dissecting microscope 4 months later (early October 1991). Individuals scored as having induced polyps had developed a second mouth and ring of tentacles in the formerly damaged region.

Results

Production of larvae

Of the 27 individual corals maintained in isolation, 14 produced larvae in the interval before the first harvest of larvae (8–10 months) and 13 did not. This is consistent with the 50:50 sex ratio determined histologically for *Balanophyllia elegans* by Fadlallah and Pearse (1982), assuming that all corals producing larvae were females and all that did not were males. Presumed females released between 2 and 22 planulae during this first season. However, no single coral that had produced planulae during its first 8–10 months of isolation (i.e., up until August 1991) produced any larvae subsequently. Most larvae were released between December and March, with the peak release period shifting about a month later each year for the 3 years of close observation (data not shown), consistent with previous reports of Fadlallah and Pearse (1982).

Of the 44 pairs of corals, 7 released no larvae before August 1991 nor any thereafter; these were assumed to be male:male pairings. Of the 35 pairs that produced planulae in the first season, 16 did not release any larvae in the subsequent 59 months of observation and were considered female:female pairings. Twenty pairs produced larvae throughout the observation period and were assumed to be male:female pairs. In addition, 2 pairs produced no larvae during the period before the first harvesting but produced larvae thereafter.

The number of larvae that settled and were subsequently collected for genetic analysis varied substantially among bowls (range: 3–50, mean = 15.1, SE = 13.1). The four crosses between Santa Cruz and Pacific Grove individuals produced more larvae on average (mean = 30.75, SD = 17.2) than did crosses in which both parents came from the same population (mean = 11.2, SD = 8.8, *t*-test $P = 0.003$), despite low gene flow between these populations ($Nm = 1.16$, Table 5 in Hellberg 1994; this value is almost certainly an overestimate, as gene flow and drift are unlikely to have come to equilibrium).

Transmission genetics and linkage of allozyme loci

A few harvested larvae could not be successfully genotyped, so the average number of larvae analyzed for the 12 pairs for which there were sufficient larvae (mean = 19.8) was slightly smaller than the number of larvae collected from these same pairs (mean = 21.2). Only 1 of the 12 analyzed crosses yielded offspring genotypes that deviated significantly ($P < 0.05$) from Hardy–Weinberg expectations (see Appendix): an *ab*×*ab* cross at the *Lap* locus (cross 7) produced an excess of *aa* homozygotes and a deficit of *bb* homozygotes. The only other *ab*×*ab* cross for *Lap* (cross 6) showed no such deviation. None of the alleles were genetically linked (data not shown, $P > 0.05$). These results demonstrate that the eight allozyme markers are unlinked and inherited in a Mendelian fashion, indicating the offspring from our laboratory crosses were produced through sexual reproduction. The independence of the allozyme markers, combined with their high heterozygosity (about 0.3 averaged over all populations, Hellberg 1994), should permit powerful tests for asexual reproduction.

Genetic analysis of joined corals

Mean observed heterozygosity across loci within populations was lower than Hardy–Weinberg expectations for all populations except Bodega Bay and Monterey (Table 1), consistent with previous analyses documenting subdivision between patches within populations (Hellberg 1994). Significant departures ($P < 0.05$) from Hardy–Weinberg expectations at individual loci were detected in only three instances, all heterozygote deficits at the *Aat* locus from northern populations (Nanaimo, Tatoosh, and Cape Arago; data not shown).

Estimated minimum sexual reproductive input (N_{go}:N_i) ranged from 0.47 to 0.94 (Table 1). The two lowest ratios occurred at Monterey (0.47) and Hoya Pass (0.51). N_{go}:N_i was recalculated separately for each of the eight patches within these two populations. For Monterey, N_{go}:N_i ranged from 0.62 to 0.88, with all but one patch (no. 4) ≥0.75. For Hoya Pass, N_{go}:N_i ranged from 0.75 to 0.88. For both populations, the low overall N_{go}:N_i ratio (lower than that at any patch considered individually) can be attributed to a single common genotype occurring across different patches within each population. The most common genotype in Monterey

Table 1. *Balanophyllia elegans*. Summary measures describing genetic variability from 18 locations sampled along the west coast of North America, listed in order from north to south. Standard errors for mean heterozygosities indicated in parentheses. Site abbreviations are provided in the text. H_o/H_e Observed/expected heterozygosity; N_i sample size; N_{go} number of observed genotypes; N^* number of expected identical genotypes

Site	Number of polymorphic loci (of 7 surveyed)	Mean no. of alleles per polymorphic locus	Mean heterozygosity					
			H_o	H_e	N_i	N_{go}	N_{go}:N_i	N^*
HOY	6	3.3	0.267 (0.075)	0.267 (0.072)	128	65	0.51	127.8
MCI	7	4.0	0.316 (0.065)	0.341 (0.066)	128	94	0.73	128.0
BRS	6	3.3	0.286 (0.098)	0.300 (0.098)	72	50	0.69	72.0
NAN	5	3.4	0.274 (0.080)	0.307 (0.086)	126	73	0.58	126.0
BAM	7	4.1	0.320 (0.078)	0.328 (0.081)	128	102	0.80	128.0
TAT	6	5.3	0.266 (0.085)	0.267 (0.078)	128	77	0.60	128.0
CAR	6	4.2	0.317 (0.095)	0.366 (0.086)	60	48	0.80	60.0
TRN	6	5.0	0.369 (0.083)	0.387 (0.086)	128	113	0.88	128.0
CSP	7	5.1	0.366 (0.084)	0.379 (0.083)	68	62	0.91	68.0
BOD	7	4.4	0.425 (0.101)	0.410 (0.088)	128	118	0.92	128.0
CRZ	7	4.3	0.357 (0.078)	0.402 (0.092)	128	116	0.91	128.0
MON	7	3.9	0.230 (0.092)	0.226 (0.082)	128	60	0.47	127.0
SIM	6	4.8	0.234 (0.068)	0.259 (0.075)	128	73	0.57	128.0
GOL	5	4.8	0.258 (0.095)	0.283 (0.102)	128	77	0.60	128.0
EAN	6	4.5	0.300 (0.084)	0.304 (0.079)	128	88	0.69	128.0
PTL	7	4.9	0.374 (0.072)	0.415 (0.079)	128	110	0.86	128.0
PTB	6	6.0	0.330 (0.104)	0.347 (0.102)	48	45	0.94	48.0
ISG	6	3.7	0.205 (0.065)	0.222 (0.073)	46	29	0.63	46.0

occurred in 22 of 128 sampled individuals. For the patch (no. 6) with the N_{go}:N_i ratio of 0.62, this genotype was found in 6 of the 16 sampled individuals. The most common genotype in Hoya Pass occurred in 12 of 128 sampled individuals and was distributed roughly equally across all patches. Overall, only 6 fused pairs (of 219 pairwise comparisons) shared identical genotypes.

The estimated maximum input from sexual reproduction (N^*:N_i) was 1 (purely sexual reproduction) for all but three populations: Hoya Pass, Monterey, and East Anacapa Island; these three populations had a N^*:N_i ratio of 0.99. N^*:N_i was recalculated for patches within each population and each patch had a ratio of 1, except for a single patch from Monterey, which had a N^*:N_i value of 0.97. Not surprisingly, this was the same patch (no. 6) with the lowest N_{go}:N_i of 0.62. As with N_{go}:N_i, the slight deviation from unity for N^*:N_i for Monterey, Hoya Pass, and East Anacapa Island populations can also be attributed to a single common genotype occurring across different patches within the population. The six individuals with identical genotypes from Monterey patch number 4 were not joined. None of the Monterey marker allozymes deviated from Hardy–Weinberg expectations and observed heterozygosity closely matched expected heterozygosity (Table 1), so sexual production of these six individuals seems likely. Together, the results from both the laboratory crosses and the population genetic survey are strong evidence that the usual mode of reproduction in *B. elegans* is sexual.

A total of 144 fused groups were observed on 63 patches with ≥16 total individuals. Fused groups comprised 2–4 individuals (mean = 2.2). Only 24 patches had ≥3 fused groups. Fused individuals were not more closely related to each other than to other individuals from their patch, and some fused groups were significantly less related to each other than were randomly sampled individuals (Table 2). To increase the power of our analysis, we also analyzed patches with a minimum of 2 fused groups per patch (41 patches) and 1 fused group per patch (63 patches). We also compared relatedness of fused groups on a patch as independent measures to the mean relatedness of the patch (144 groups). The relatedness for fused individuals (r_{fused}) generally fell within the 95% confidence limits of the relatedness of randomly sampled individuals (r_{patch}); r_{fused} was significantly *less* than r_{patch} nearly as often as it was greater than r_{patch} (Table 2).

Inducibility of polyp formation

Seven of eight individuals that were cut transversely generated a new polyp in the region of tissue exposed by the cut (opposite the original oral disc). None of ten

Table 2. *B. elegans*. Mean relatedness of fused individuals (r_{fused}) compared to relatedness of all individuals in the same patch (r_{patch}). Fused groups significantly more related than random are indicated by $r_{fused} > r_{patch}$. *All* treats each group of fused individuals as independent measurements

Minimum number of fused pairs	$r_{fused} > r_{patch}$	$r_{fused} = r_{patch}$	$r_{fused} < r_{patch}$
3	1	23	0
2	5	35	1
1	11	45	7
All	39	76	29

individuals that received longitudinal cuts generated a new mouth or new tentacles. The two treatments differed significantly (sign test, $P < 0.05$).

Discussion

Because *Balanophyllia elegans* affixes permanently to hard substrate at settlement, joined polyps could conceivably form in only two ways: either new polyps bud from existing polyps, or young individuals (larvae or small juveniles) that settle close to or touching each other subsequently join. If budding occurs, the joined polyps should be genetically identical. If individuals join as they grow, joined polyps could display a range of relatedness, with the closest being clonemates produced asexually by the same mother. Our data show that joined polyps of *B. elegans* are highly unlikely to result from any form of asexual reproduction because (1) individuals found joined in the field rarely share identical genotypes, (2) new polyps could not be induced to form off of existing polyps in a position where they might be expected to survive in the wild in this species (although they were induced in another position), and (3) allozyme transmission genetics were in accord with Mendelian expectations.

Past genetic studies have revealed extensive clonal reproduction in some anthozoan species that invest in the formation of gametes (Stoddart 1984; Ayre 1984; Billingham and Ayre 1997; Coffroth and Lasker 1998). This was not the case here. Genetic indices of clonal reproduction indicate that essentially all individuals from the 18 populations surveyed resulted from sexual reproduction (Table 1), consistent with previous histological work (Fadlallah and Pearse 1982). Furthermore, in the few instances when individuals at the same locality shared identical allozyme genotypes, those individuals either occurred on different rocks (and thus could not have resulted from asexual budding, as *B. elegans* cannot reattach), or occurred at such low frequency that, given levels of allozyme variability, their appearance was in accordance with chance.

Cnidarian polyps generally show strong regenerative abilities (see summary in Hyman 1940). Kawaguti (1937; cited in Hyman 1940) previously noted the ability of cut coral polyps to regenerate at ends opposite the oral surface, as seen here in *B. elegans*. The regenerative abilities of solitary corals, especially those in the family Fungiidae, appear to be correlated with the ability to replicate asexually by fragmentation (Yamashiro and Nishihira 1998). However, no *B. elegans* damaged at its oral end showed any sign of generating a new polyp, consistent with this species' inability to reproduce by budding.

Many dendrophyllid corals can reproduce asexually (Fadlallah 1983a; Cairns 1988). Most notably, Zibrowius (1985) reported peripheral bud shedding as the dominant mode of reproduction in two species of *Balanophyllia*. However, most of these solitary corals that can bud asexually are free-living forms that live in deep water (>100 m) on soft sediment. Such asexual buds need not attach firmly to the substrate to survive. In contrast, *B. elegans* inhabits high scour conditions, where detachment from hard substrate soon results in death (Fadlallah 1983b).

Ameiotic larvae might seem a more reliable means of asexual reproduction for a solitary coral inhabiting high energy waters, but again our results suggest *B. elegans* does not employ this reproductive mode. The Mendelian inheritance of allozyme markers we observed in *B. elegans* stands in contrast to patterns reported for three other scleractinian species. Larval genotypes of *Pocillopora damicornis* (Stoddart 1983) and two *Tubastrea* species (Ayre and Resing 1986) were identical to maternal genotypes (paternity was unknown), suggesting larvae were produced asexually. Unlike *B. elegans*, these species appear to be hermaphrodites. Two other hermaphroditic species (*Acropora palifera* and *Seriatopora hystrix*) brood larvae that, like *B. elegans*, differ in their allozyme genotypes when compared to their broodmother (Ayre and Resing 1986), as do the larvae and broodparents of other anthozoans compared using dominant genetic markers (Brazeau et al. 1998; Barki et al. 2000).

Our genetic analyses indicate that individuals whose tissues touch and whose skeletons are fused are not clonemates. Fusion has been reported for solitary corals that were clonemates in *Fungia scutaria*; nonclonemates of this species neither fused nor rejected each other (Jokiel and Bigger 1994). Fusion between aclonal marine invertebrates has been considered rare (Jackson 1986) but has been observed previously (Kingsley et al. 1989). Among clonal invertebrates, allorecognition systems may serve to prevent the somatic overthrow of one genotype by germ cells of another (Buss 1982; Stoner et al. 1999). In *B. elegans*, such costs of somatic cell parasitism could be mitigated or eliminated by (1) the limited dispersal of larvae, such that fusion occurs solely between close kin (Grosberg and Quinn 1986), and (2) the anatomy of polyps and the nature of the ''fusion,'' which may not permit movement of cells between joined individuals (see Bishop and Sommerfeldt 1999). Although we did find that fused individuals are no more related to each other than are randomly sampled individuals from the same population of origin (Table 2), it may be that all *B. elegans* within an aggregation the size of our patches are sufficiently closely related to avoid severe fitness costs from fusion.

Previous genetic work on *B. elegans* (Hellberg 1994, 1995) confirmed that directly observed limited larval dispersal (Gerrodette 1981) must be the norm in this species. The results of the genetic and experimental analyses presented here demonstrate that most (and likely all) reproduction in *B. elegans* is strictly sexual. This confirms earlier histological work by Fadlallah and Pearse (1982) and justifies further work on *B. elegans* as a means of teasing apart the benefits of limited dispersal and clonal reproduction.

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Appendix

Hardy–Weinberg frequencies

Observed and expected Hardy–Weinberg frequencies are given for informative crosses at each of eight loci in 12 laboratory crosses, with different crosses indicated by different letters. The only significant departure ($P<0.05$) from Hardy–Weinberg expectations after Bonferroni corrections for multiple simultaneous tests was for *Lap* in cross A (shown in bold).

Locus	Cross	Parental genotypes	Offspring	Observed (expected)		Chi-square	*P*
Hk	B	*ff*×*fs*	40	*ff* = 26 (20)	*fs* = 14 (20)	3.600	0.058
	C	*fs*×*ss*	34	*fs* = 15 (17)	*ss* = 19 (17)	0.471	0.493
	D	*ff*×*fs*	15	*ff* = 8 (7.5)	*fs* = 7 (7.5)	0.067	0.796
	G	*fs*×*ff*	14	*ff* = 7 (7)	*fs* = 7 (7)	0.000	1.000
Pgm	A	*ff*×*fs*	48	*ff* = 28 (24)	*fs* = 20 (24)	1.333	0.248
	D	*fs*×*fs*	15	*ff* = 5 (3.75)	*fs* = 5 (7.5)	1.667	0.435
				ss = 5 (3.75)			
	E	*sx*×*fs*	15	*fs* = 3 (3.75)	*fx* = 1 (3.75)	7.133	0.068
				ss = 8 (3.75)	*sx* = 3 (3.75)		
	F	*fx*×*fx*	15	*ff* = 5 (3.75)	*fx* = 6 (7.5)	0.733	0.693
				xx = 4 (3.75)			
	G	*fs*×*ff*	14	*ff* = 5 (7)	*fs* = 9 (7)	1.143	0.285
	H	*ff*×*fs*	13	*ff* = 7 (6.5)	*fs* = 6 (6.5)	0.077	0.782
	I	*ff*×*fs*	13	*ff* = 7 (6.5)	*fs* = 6 (6.5)	0.077	0.782
	J	*fs*×*fs*	11	*ff* = 2 (2.75)	*fs* = 5 (5.5)	0.818	0.664
				ss = 4 (2.75)			
	K	*fs*×*ff*	10	*ff* = 5 (5)	*fs* = 5 (5)	0.000	1.000
	L	*fs*×*ff*	10	*ff* = 7 (5)	*fs* = 3 (5)	1.600	0.206
Pgi	B	*ps*×*fs*	40	*fp* = 12 (10)	*fs* = 9 (10)	1.800	0.615
				ps = 7 (10)	*ss* = 12 (10)		
	C	*fs*×*ff*	34	*ff* = 20 (17)	*fs* = 14 (17)	1.059	0.303
	D	*ss*×*fs*	15	*fs* = 8 (7.5)	*ss* = 7 (7.5)	0.067	0.796
	E	*ss*×*fs*	15	*fs* = 9 (7.5)	*ss* = 6 (7.5)	0.600	0.439
	G	*ss*×*fs*	14	*fs* = 9 (7)	*ss* = 5 (7)	1.143	0.285
	H	*fs*×*ss*	13	*fs* = 7 (6.5)	*ss* = 6 (6.5)	0.077	0.782
	L	*fs*×*ss*	10	*fs* = 6 (5)	*ss* = 4 (5)	0.400	0.527
Tpi	G	*ff*×*fm*	14	*ff* = 8 (7)	*fm* = 6 (7)	0.286	0.593
	J	*ff*×*fm*	11	*ff* = 8 (5.5)	*fm* = 3 (5.5)	2.273	0.132
Pep	A	*fs*×*ff*	48	*ff* = 26 (24)	*fs* = 22 (24)	0.333	0.564
	B	*ff*×*fs*	40	*ff* = 24 (20)	*fs* = 16 (20)	1.600	0.206
	C	*fs*×*ff*	34	*ff* = 22 (17)	*fs* = 12 (17)	2.941	0.086
	F	*ff*×*fs*	15	*ff* = 9 (7.5)	*fs* = 6 (7.5)	0.600	0.439
	G	*fs*×*fs*	14	*ff* = 4 (3.5)	*fs* = 6 (7)	0.286	0.867
				ss = 4 (3.5)			
	H	*fs*×*ff*	13	*ff* = 4 (6.5)	*fs* = 9 (6.5)	1.923	0.166
	J	*fs*×*ff*	11	*ff* = 5 (5.5)	*fs* = 6 (5.5)	0.091	0.763
Aat	B	*ac*×*cc*	40	*ac* = 20 (20)	*cc* = 20 (20)	0.000	1.000
	D	*cc*×*ac*	15	*ac* = 5 (7.5)	*cc* = 10 (7.5)	1.667	0.197
	G	*ac*×*ac*	14	*aa* = 4 (3.5)	*ac* = 6 (7)	0.286	0.867
				cc = 4 (3.5)			
	I	*cs*×*cc*	13	*cc* = 9 (6.5)	*cc* = 4 (6.5)	1.923	0.166
	J	*ac*×*cc*	11	*ac* = 5 (5.5)	*cc* = 6 (5.5)	0.910	0.763
Lap	A	*ab*×*ab*	48	*aa* = 25 (12)	*ab* = 20 (24)	21.500	**0.000**
				bb = 3 (12)			
	B	*bc*×*bc*	40	*bb* = 11 (10)	*bc* = 17 (20)	0.950	0.622
				cc = 12 (10)			
	C	*ac*×*ac*	34	*aa* = 2 (8.5)	*ac* = 24 (17)	7.882	0.019
				cc = 8 (8.5)			
	D	*cc*×*ac*	15	*ac* = 8 (7.5)	*cc* = 7 (7.5)	0.067	0.796
	E	*ac*×*bc*	15	*ab* = 2 (3.75)	*ac* = 7 (3.75)	3.933	0.269
				bc = 3 (3.75)	*cc* = 3 (3.75)		
	F	*cc*×*bc*	15	*bc* = 7 (7.5)	*cc* = 8 (7.5)	0.067	0.796

Appendix (Contd.)

Locus	Cross	Parental genotypes	Offspring	Observed (expected)		Chi-square	*P*
	G	*cc*×*ac*	14	*ac* = 7 (7)	*cc* = 7 (7)	0.000	1.000
	H	*ab*×*ab*	13	*aa* = 2 (3.25) *bb* = 3 (3.25)	*ab* = 8 (6.5)	0.846	0.655
	I	*cc*×*ab*	13	*ac* = 7 (6.5)	*bc* = 6 (6.5)	0.077	0.782
	J	*ab*×*bc*	11	*ab* = 1 (2.75) *bb* = 3 (2.75)	*ac* = 4 (2.75) *bc* = 3 (2.75)	1.727	0.631
	K	*cc*×*ab*	10	*ac* = 3 (5)	*bc* = 7 (5)	1.600	0.206
	L	*ac*×*bc*	10	*ab* = 3 (2.5) *bc* = 2 (2.5)	*ac* = 2 (2.5) *cc* = 3 (2.5)	0.400	0.940
Mpi	A	*ss*×*ms*	48	*ms* = 16 (24)	*ss* = 32 (24)	5.333	0.021
	C	*ss*×*ms*	34	*ms* = 19 (17)	*ss* = 15 (17)	0.471	0.493
	E	*ms*×*ss*	15	*ms* = 5 (7.5)	*ss* = 10 (7.5)	1.667	0.197
	F	*sx*×*ms*	15	*ms* = 3 (3.75) *ss* = 5 (3.75)	*mx* = 3 (3.75) *sx* = 4 (3.75)	0.733	0.865
	H	*fs*×*ms*	13	*fm* = 4 (3.25) *ms* = 3 (3.25)	*fs* = 2 (3.25) *ss* = 4 (3.25)	0.846	0.838
	I	*ms*×*ss*	13	*ms* = 1 (6.5)	*ss* = 12 (6.5)	9.308	0.002
	K	*ms*×*ms*	10	*mm* = 5 (2.5) *ss* = 1 (2.5)	*ms* = 4 (5)	3.600	0.165

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