EVOLUTION OF POECILOGONY FROM PLANKTOTROPHY: CRYPTIC SPECIATION, PHYLOGEOGRAPHY, AND LARVAL DEVELOPMENT IN THE GASTROPOD GENUS ALDERIA

RYAN A. ELLINGSON AND PATRICK J. KRUG
Department of Biological Sciences, California State University, 5151 State University Drive, Los Angeles, California 90032-8201
E-mail: pkrug@calstatela.edu

Abstract.—Poecilogony, a rare phenomenon in marine invertebrates, occurs when alternative larval morphs differing in dispersal potential or trophic mode are produced from a single genome. Because both poecilogony and cryptic species are prevalent among sea slugs in the suborder Sacoglossa (Gastropoda: Opisthobranchia), molecular data are needed to confirm cases of variable development and to place them in a phylogenetic context. The nominal species Alderia modesta produces long-lived, feeding larvae throughout the North Atlantic and Pacific, but in California it can also produce short-lived larvae that metamorphose without feeding. We collected morphological, developmental, and molecular data for Alderia from 17 sites spanning the eastern and western Pacific and North Atlantic. Estuaries south of Bodega Harbor, California, contained a cryptic species (hereafter Alderia sp.) with variable development, sister to the strictly planktotrophic A. modesta. The smaller Alderia sp. seasonally toggled between planktotrophy and lecithotrophy, with some individuals differing in development but sharing mitochondrial DNA haplotypes. The sibling species overlapped in Tomales Bay, California, but showed no evidence of hybridization; laboratory mating trials suggest postzygotic isolation has arisen. Intra- and interspecific divergence times were estimated using a molecular clock calibrated with geminate sacoglossans. Speciation occurred about 4.1 million years ago during a major marine radiation in the eastern Pacific, when large inland embayments in California may have isolated ancestral populations. Atlantic and Pacific A. modesta diverged about 1.7 million years ago, suggesting trans-Arctic gene flow was interrupted by Pleistocene glaciation. Both Alderia species showed evidence of late Pleistocene population expansion, but the southern Alderia sp. likely experienced a more pronounced bottleneck. Reduced body size may have incurred selection against obligate planktotrophy in Alderia sp. by limiting fecundity in the face of high larval mortality rates in warm months. Alternatively, poecilogony may be an adaptive response to seasonal opening of estuaries, facilitating dispersal by long-lived larvae. An improved understanding of the forces controlling seasonal shifts in development in Alderia sp. may yield insight into the evolutionary forces promoting transitions to nonfeeding larvae.

Key words.—Cryptic species, larval development, lecithotrophy, phylogeography, planktotrophy, poecilogony, sacoglossan, trans-Arctic exchange.

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Marine ecosystems have fewer morphologically distinguishable species than terrestrial habitats, and many marine species have cosmopolitan distributions (May 1994). This may result from the considerable dispersal ability of ocean-dwelling organisms, since pelagic adults and planktonic larvae face few geographical obstacles to large-scale transport (Strathmann 1990; Palumbi 1992; Pechenik 1999). However, molecular studies of marine organisms frequently uncover subdivided populations and cryptic species, suggesting genetic divergence may commonly occur at small spatial scales or with minimal morphological diversification (Palmer et al. 1990; Palumbi 1992; Knowlton 1993, 2000; Lee 2000; Dawson and Jacobs 2001; McGovern and Hellberg 2003; Lee and O’Foighil 2004; Tarjuelo et al. 2004; Collin 2005; Meyer et al. 2005). Where biological lines are sharply drawn in what appears to be an easily traversed oceanic milieu, we may gain insight into the forces that promote divergence, maintain boundaries between species, and drive the adaptive evolution of marine life histories.

Development mode strongly affects patterns of phylogeography and macromutation in marine invertebrates (Arndt and Smith 1998; Collin 2000; Jeffery and Emlet 2003). Planktotrophic species produce feeding larvae with high dispersal potential, resulting in panmictic populations and extended ranges (Caley et al. 1996; Todd 1998; Bohonak 1999; Pechenik 1999). An abbreviated planktonic period tends to decrease a species’ range and geological longevity while increasing local adaptation and the likelihood of speciation events (Hansen 1978; Vermeij 1982; Jablonski 1986; Palumbi 1995; Sanford et al. 2003). Understanding transitions from feeding to nonfeeding modes of larval development is an outstanding goal of evolutionary biology (Strathmann 1978; Raff 1987; Hadfield et al. 1995; Havenhand 1995; Hart et al. 1997; Duda and Palumbi 1999; Hart 2000; McEdward 2000; Jeffery et al. 2003). However, the selective forces that drive the evolution of nonfeeding development remain opaque, partly for lack of robust phylogenies paired with relevant ecological and developmental data for the species involved (Havenhand 1995; Wray 1995; McEdward 2000). Small body size has been hypothesized to favor brooding or direct development (Strathmann and Strathmann 1982; Byrne and Cerra 1996), but there is little theory accounting for why pelagic lecithotrophy should be more adaptive than planktotrophy (Chia 1974).

Insight into life-history evolution may come from organisms that exhibit poecilogony, a stable reproductive polymorphism in which larvae of a single species can develop by alternative pathways (Giard 1905; Chia et al. 1996). Molecular data often reveal that putative cases of poecilogony are cryptic species differing in development, however (Hoagland and Robertson 1988; Boucheñet 1989). Convincing examples of variable development are limited to polychaete worms in the family Spionidae (Levin 1984; Gibson et al. 1999; Morgan et al. 1999; Schultze et al. 2000; Gibson and
TABLE 1. Populations of *Alderia modesta* sampled for development mode, morphology, and genetic analyses. Slugs from Russia, Belgium, Vancouver, and Humbolt were obtained as ethanol-preserved specimens. Unless otherwise noted, all populations are in California.

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude, longitude</th>
<th>Date sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doel, Belgium (DB)</td>
<td>51°19'N, 4°16'E</td>
<td>Aug. 2005</td>
</tr>
<tr>
<td>Amurskii Bay, Russia (RS)</td>
<td>43°09'N, 131°54'E</td>
<td>June 2004</td>
</tr>
<tr>
<td>Kodiak, Alaska (AK)</td>
<td>57°35'50''N, 152°28'17''W</td>
<td>June 2005</td>
</tr>
<tr>
<td>Vancouver, British Columbia, Canada (BC)</td>
<td>49°18'21''N, 123°09'32''W</td>
<td>July 2003</td>
</tr>
<tr>
<td>Tillamook, Oregon (TI)</td>
<td>45°26'55''N, 123°50'57''W</td>
<td>March 2005</td>
</tr>
<tr>
<td>Humboldt (HU)</td>
<td>40°44'31''N, 124°12'59''W</td>
<td>February 2004</td>
</tr>
<tr>
<td>Bodega Harbor (BH)</td>
<td>38°18'58''N, 123°03'24''W</td>
<td>September 2003, September 2004</td>
</tr>
<tr>
<td>Walker Creek (WC)</td>
<td>38°13'46''N, 122°54'56''W</td>
<td>September 2004</td>
</tr>
<tr>
<td>Cow Landing (CL)</td>
<td>38°11'02''N, 122°54'40''W</td>
<td>September 2004</td>
</tr>
<tr>
<td>South Tomales Bay (TB)</td>
<td>38°06'53''N, 122°51'08''W</td>
<td>September 2003, September 2004</td>
</tr>
<tr>
<td>San Francisco Bay (SF)</td>
<td>37°52'57''N, 122°31'00''W</td>
<td>September 2003–August 2004 (5×)</td>
</tr>
<tr>
<td>Morro Bay (MB)</td>
<td>35°20'39''N, 120°50'35''W</td>
<td>September 2002</td>
</tr>
<tr>
<td>Santa Barbara (SB)</td>
<td>34°24'01''N, 119°32'07''W</td>
<td>July 1999, February 2000</td>
</tr>
<tr>
<td>Los Angeles Harbor (LA)</td>
<td>33°42'48''N, 118°17'02''W</td>
<td>January 2004–August 2004 (4×)</td>
</tr>
<tr>
<td>Newport Bay (NB)</td>
<td>33°37'16''N, 117°53'32''W</td>
<td>June 2003–August 2004 (6×)</td>
</tr>
</tbody>
</table>

Gibson 2004) and herbivorous sea slugs in the gastropod suborder Sacoglossa (West et al. 1984; Clark 1994; Krug 1998). The adaptive value of expressing multiple dispersal strategies from one genotype is well documented for adult morphs of terrestrial insects (Denno et al. 1980; Harrison 1980; Langelotto and Denno 2001); it is therefore perplexing that poecilogony is so rare among marine invertebrates. Equally puzzling are the phylogenetic constraints that may limit this phenomenon to polychaetes and opisthobranch molusks.

Sacoglossan sea slugs are suctorial feeders that specialize on coenocytic or large-celled, filamentous algae (Jensen 1983, 1996; Trowbridge 2002). Adults feed, mate, and deposit eggs on their algal host and are generally unable to switch hosts after settlement (Jensen 1989; Trowbridge 1991; Trowbridge and Todd 2000). Furthermore, planktonic larvae preferentially metamorphose in response to host-specific chemical cues (West et al. 1984; Krug and Manzi 1999; Trowbridge and Todd 2000; Krug 2001). Host acceptance via selective metamorphosis may thus determine both assortative mating and ecological specialization in these marine herbivores, as in some insect host races (Feder et al. 1994; Caillaud and Via 2000). In addition to their host specificity, sacoglossans display a high degree of reproductive plasticity, with many independent transitions to nonfeeding larval development and several documented cases of poecilogony (West et al. 1984; Clark 1994; Jensen 1996; Krug 1998). Such developmental flexibility may facilitate the selective pressures behind, and consequences of, alternative larval morphs (Krug and Zimmer 2004).

The sacoglossan *Alderia modesta* is found in temperate estuaries throughout the Northern Hemisphere with its obligate hosts, yellow-green algae in the genus *Vaucheria* (Hartog 1959; Trowbridge 1993). Atlantic populations of the monotypic genus *Alderia* are planktotrophic, as are Pacific populations from Monterey, California, north to Canada, and from Russia (Hand and Steinberg 1955; Hartog 1959; Seeleman 1967; Gibson and Chia 1995; Chernyshew and Chaban 2005). However, the nominal taxon *A. modesta* exhibits poecilogony in southern California, where adults produce either planktotrophic larvae with a month-long maturation period, or lecithotrophic larvae competent to settle upon or before hatching (Krug 1998, 2001). In this life-history trade-off, lecithotrophy limits adult fecundity by an order of magnitude and doubles benthic development time, but greatly reduces the planktonic period and hence the mortality rate of larvae. Although prior evidence indicated this was a case of variable development, the northern limit of poecilogony was not established, nor was the evolutionary relationship between strictly planktotrophic and southern, poecilognous populations delineated.

The geographically restricted expression of poecilogony in an otherwise planktotrophic taxon provided the opportunity to explore an evolutionary origin of variable development. We used molecular, developmental, and morphological characters to assess the global phylogeography of the genus *Alderia*. Sequences from two mitochondrial loci were used to test for genetic breaks between strictly planktotrophic versus poecilognous populations, and to examine population structure in the eastern Pacific. A molecular clock was calibrated with gernimate sacoglossans to date major divergence events between and within lineages. Our data support a model of cryptic speciation via peripheral isolation, accompanied by a rare life-history shift to environmentally cued changes in development mode. We discuss features of adult and larval ecology that may have favored seasonal lecithotrophy in southern estuaries.

**Materials and Methods**

**Study System and Population Sampling**

The genus *Alderia* was described from Ireland (Thompson 1844; Allman 1845), and the sole recognized species, *A. modesta*, from Sweden (Lovén 1844). Specimens superficially resembling *A. modesta* were collected between 1999 and 2005 from 15 sites along the northeastern Pacific coast of the United States and Canada (Table 1). Slugs were collected at low tide from exposed patches of *Vaucheria* species, examined morphologically under a dissecting microscope, and placed individually in petri dishes of seawater for egg mass depo-
sition. Development mode was typed one to two days later according to egg size within the first deposited clutch (Krug 1998). Typed adults were frozen at −80°C or stored in 95% ethanol prior to DNA extraction. Specimens were collected and preserved from the western Pacific in Amurskii Bay, Sea of Japan, Russia, in June 2004, and from the Atlantic in the Scheldt estuary, Doel, Belgium, in August 2005 by colleagues (see Acknowledgments).

No closely related genus exists in the temperate eastern Pacific, and the phylogenetic position of Alderia is controversial (Evans 1953; Gascoigne 1976; Jensen 1996). The Atlantic genus Limapontia was united with Alderia in the family Limapontiidae based on distinctively sabot-shaped radular teeth (Jensen 1996), and was closely related to Alderia in a preliminary molecular phylogeny of the Limapontioidea (R. A. Ellingson and P. J. Krug, unpubl. data). For outgroup comparisons, L. depressa was therefore collected from Galway Bay, Republic of Ireland (53°13′N, 08°54′E) in August 2005.

**DNA Extraction, Amplification, and Sequencing**

Genomic DNA was extracted from whole frozen slugs using the QIAamp DNA Mini Kit (Qiagen, Inc., Valencia, CA) and stored in extraction buffer at −20°C prior to amplification. Polymerase chain reactions (PCR) were used to amplify a 710-base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene, using primers LCO1490 and HCO2198 (Folmer et al. 1994), and a 480-bp fragment of the mitochondrial 16S rRNA (large ribosomal subunit) gene using primers 16Sar-5′ and 16Sbr-3′ (Palumbi 1996). A negative control (no template) was included in each run. Amplification used a reaction volume of 50 μl containing 1× Promega (Madison, WI) PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100), 2.5 mM MgCl2, 0.2 mM dNTPs, 0.2 μM of each primer, 1 U Promega Taq DNA Polymerase and 50–200 ng of template DNA. To amplify the COI fragment, the following thermal cycler profile was used: denaturation at 94°C for 2 min followed by 35 cycles of (94°C for 30 sec, 50°C for 45 sec, and 72°C for 60 sec). For 16S, the thermal cycler profile began with denaturation at 94°C for 2 min followed by 40 cycles of (94°C for 30 sec, 50°C for 30 sec, and 72°C for 60 sec), followed by an extension step at 72°C for 7 min.

Polymerase chain reaction products were visualized by electrophoresis on a 1% agarose gel, and were purified with the Wizard SV Gel and PCR Clean-Up System (Promega). Both strands of purified products were directly cycle-sequenced in both directions using the amplification primers and Big Dye Terminator 3.1 Cycle Sequencing chemistry, and electrophoresed on an ABI 3100 Avant Capillary Sequencer (Applied Biosystems, Foster City, CA). Sequencing both strands and resequencing of unique haplotypes revealed no evidence of PCR error. Sequences used for this study have been deposited in GenBank (accession numbers DQ364252–DQ364426).

**Phylogenetic Analyses**

To determine phylogenetic relationships and population structure, COI sequences were amplified from specimens of Alderia from the eastern Pacific (n = 233), western Pacific (n = 3), and Atlantic (n = 7). Suitable sequence data was obtained from all specimens for 480 bp of COI, corresponding to positions 139 to 618 of the complete Aplysia californica COI gene (GenBank accession number AY569552), and aligned with SeqScape 2.1.1 software (Applied Biosystems). A 450-bp fragment of the 16S gene was sequenced from a subset of specimens (n = 49), resulting in 12 haplotypes from *A. modesta* (six each from the Atlantic and Pacific), and eight haplotypes from *Alderia* sp. (see Results). All 16S sequences were aligned in ClustalX 1.83 (Thompson et al. 1997), with adjustments made by eye using three models of gastropod 16S secondary structure (Lydeard et al. 2000; Medina and Walsh 2000; Valdés 2003). Amino acid substitutions, mean base composition, and transition/transversion (s/v) ratio were determined in Mega 3.0 (Kumar et al. 2004).

A neighbor-joining (NJ) tree of all eastern Pacific COI haplotypes was constructed using PAUP* 4.0b10 (Swofford 2001). For NJ analysis, the Tamura-Nei model of sequence evolution was used (Tamura and Nei 1993), and robustness of the topology was assessed with 2000 bootstrap replicates. Subsequent analyses included Atlantic and western Pacific specimens, and used both COI and 16S sequence data from 49 individuals to determine (1) interspecific relationships and (2) intraspecific divergence between ocean basins. A partition-homogeneity test as implemented in PAUP* indicated no significant conflict between COI and 16S datasets; we therefore performed Bayesian and parsimony analyses on the combined data (Cunningham 1997; Wiens 1998). For Bayesian analysis, COI and 16S datasets were partitioned so that a separate substitution model could be applied to each. The best-fit model for the COI dataset selected using the Akaike information criterion as implemented in Modeltest 3.7 (Posada and Crandall 1998) was TrN + Γ, with gamma distribution shape parameter of 0.1397. For 16S, the best-fit model was HKY + I + Γ, with gamma shape 0.2932. Bayesian analysis used the metropolis-coupling Markov chain Monte Carlo method as implemented in MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). The analysis was run for 3,000,000 generations with a tree saved every 1000 generations; a consensus tree was generated in PAUP* after a burn-in period of 300. For parsimony analysis, the maximum number of trees saved was limited to 5000 due to computational time constraints, and bootstrap values (1000 replicates) were obtained using the “fast” stepwise-addition option.

**Within-Clade Genetic Structure and Expansion Estimates**

Genetic structures of the two major COI clades from the eastern Pacific were characterized in Arlequin 2.000 (Schneider et al. 2000). Haplotype diversity (H), nucleotide diversity (π), and mismatch distributions were calculated for each clade, hereafter referred to as *A. modesta* and *Alderia* sp. (see Results). The proportion of genetic variation partitioned among estuaries was estimated by analysis of molecular variance (AMOVA; Excoffier et al. 1992), using both Slatkin’s (1991, 1995) linearized *F*<sub>ST</sub> and *Φ*<sub>ST</sub> values. Sites within Tomales Bay (south Tomales Bay, Cow Landing, and Walker Creek) were not significantly different in preliminary AMOVA analysis and, being located in the same estuary, were
grouped results for subsequent analyses. Based on Modeltest results, the best-fit model of sequence evolution for each clade was used to calculate $D_{ST}$ distance matrices and $\pi$ (A. modesta, Tamura-Nei; Alderia sp., Tamura three-parameter) (Tamura 1992; Tamura and Nei 1993). Tajima’s $D$ and Fu’s $F_{S}$ were calculated by comparing observed values against a null distribution based on 1000 simulations to determine significance levels for each test statistic. Tajima’s $D$ compares the number of segregating sites with the number of pairwise differences in a population, and should be near zero for an equilibrium population; negative values indicate a selective sweep or population expansion. Large negative values of Fu’s $F_{S}$ suggest population expansion (Fu 1997; Excoffier and Schneider 1999).

Parameters $\tau$ (time since expansion, expressed in units of mutation rate), $\theta_0$ (population size before expansion), and $\theta_1$ (population size after expansion) were estimated from mismatch distributions using a generalized nonlinear least-squares approach (Schneider and Excoffier 1999). Confidence intervals were determined by a parametric bootstrap procedure, assuming a sudden expansion model. The validity of the model was tested using the sum of square deviations (SSD) between observed and expected mismatch distributions as a test statistic. The SSD distribution was generated via coalescent simulations of expansion using the estimated parameters $\tau$, $\theta_0$, and $\theta_1$; the P-value was the proportion of times the difference between simulated and expected mismatch distribution exceeded the observed SSD (Schneider and Excoffier 1999). To express expansion time in years, values of $\tau$ were multiplied by the mutational time scale (generation time/2u, where $u$ is the number of substitutions per year for the fragment of interest) (Rogers 1995; Excoffier and Schneider 1999). The substitution rate per nucleotide site was calculated from our COI data, and then multiplied by the length of the COI fragment used in mismatch analyses, yielding a rate of $2.62 \times 10^{-5}$ substitutions/yr. Based on larval period and laboraory rearing data, we estimated generation times (egg-to-egg period) of two months for $A. modesta$, versus one month for Alderia sp. (averaging the generation time from a lecithotrophic larva, 2.5 weeks, and a planktotrophic larva, 5.5 weeks).

**Divergence Estimates**

We calibrated a molecular clock using the only well-established gerniate sacoglossans. Specimens of the Caribbean Elysia (= Tridachia) crispata were collected December 2004 from Bocas del Toro, Panama, with permission of the Panamanian government; preserved specimens of E. (= Tridachillia) diomedea from the eastern Pacific were obtained from the Los Angeles County Museum of Natural History. Any gerniate may have been isolated prior to final closure of the Panamanian seaway (Knowlton and Weigt 1998; Marko 2002). However, E. crispata and E. diomedea live in shallow mangrove fringe, probably the last habitat shundered by the Isthmus; we therefore used a minimum divergence time of 3.1 million years ago (MYA) for rate calibration (Coates and Obando 1996). Plots of s/v ratio against genetic distance indicated third positions of COI were saturating at distances greater than 15% (mean ratio = 1.5); substitution rate was thus determined for the first codon position of COI, for which log-likelihood ratio tests could not reject molecular clock assumptions. Based on Modeltest, the TrNe model was used to calculate first position distances (Tamura and Nei 1993; Huisenenbeck and Crandall 1997; Posada and Crandall 1998). To correct for intraclade variance, net nucleotide divergences were calculated as $\rho_{AB}^{(nei)} = \rho_{AB} - 0.5(\rho_{A} + \rho_{B})$, where $\rho_{A}$ and $\rho_{B}$ are mean intraclade distances, and $\rho_{AB}$ is the mean interclade distance (Nei and Li 1979; Edwards and Beerli 2000). The rate determined with E. crispata–E. diomedea was used to estimate divergence times for (1) $A. modesta$ versus Alderia sp., and (2) Pacific versus Atlantic populations of $A. modesta$.

**Copulatory Behavior and Reproductive Isolation**

Reproductive isolation between slugs was assessed by monitoring mating behavior and egg mass production between pairs in the laboratory. Because field-caught Alderia store sperm for weeks, virgin slugs were reared by individually metamorphosing lecithotrophic larvae of Alderia sp. from San Diego parents on filaments of Vaucheria longicaulis. Virgin slugs were raised in isolation for three to four weeks, by which time all were reproductively mature; control slugs raised in pairs began reproducing within two weeks of metamorphosis.Virgin $A. modesta$ were not available, due to difficulties in culturing their long-lived planktotrophic larvae. Laboratory-reared virgins ($n = 7$) were paired with individual specimens of the northern clade from Bodega Harbor, and observed under a dissecting microscope over 6 h. The members of each pair were then isolated and monitored for egg production for 48 h. Controls were paired virgin Alderia sp. put together at the same time as the northern-southern pairs.

**Size and Fecundity Measurements**

For wet weight determinations, freshly collected slugs were first blotted dry with a paper towel and weighed to the nearest 0.1 mg, then returned to individual dishes for egg laying. Size differences between northern and southern species (see Results), and between sites within each species, were tested with a nested ANOVA using site as a random effect nested within species as a fixed effect (Sokal and Rohlf 1995). For fecundity measurements, the first egg mass deposited by each preweighed adult was maintained in a petri dish with 4 ml of 0.22 µm filtered seawater until hatching (three days for planktotrophic, six days for lecithotrophic eggs), and larvae per clutch were counted under a dissecting microscope. Relationship between size and fecundity was determined by regressing egg number for the first clutch deposited after collection on adult weight (Krug 2001). Regressions were run for three populations of $A. modesta$ and for two populations of Alderia sp. (one for each larval morph). Mean fecundity was measured for an additional two populations of $A. modesta$ and five populations of Alderia sp.

**RESULTS**

**Cryptic Species and Variable Development in Alderia**

In a survey of estuaries along the northeastern Pacific coastline, specimens of $A. modesta$ from Bodega Harbor, Cal-
FIG. 1. Seasonal distribution of development modes exhibited by sea slugs in the genus *Alderia* along the northeastern Pacific coast. Data are the proportion of adult slugs at each site that produced planktotrophic (black) or lecithotrophic (white) larvae, with sample size given on each pie graph. Sampling dates are in Table 1; summer sampling was conducted from June through September, winter sampling from November through February.

California, northward laid only planktotrophic eggs; in contrast, south of Bodega, slugs produced mostly lecithotrophic clutches in summer and both development modes in winter (Fig. 1). Concordant morphological differences were evident: slugs from Bodega north had a smooth yellow dorsum dotted with brown speckles, whereas south of Tomales Bay, most had a dark background color with a raised dorsal hump, split down the midline by a band of yellow (Krug et al. 2007). Both morphotypes were present within Tomales Bay. In planktotrophic egg masses from northern slugs, eggs were coiled into a spiral, a plesiomorphic character in the superfamily Limapontioidea. In contrast, both planktotrophic and lecithotrophic clutches of southern slugs lacked any spiral, with eggs haphazardly arranged inside the egg mass (Krug et al. 2007).

To determine whether there were genetic differences across
the Bodega Harbor breakpoint, a portion of the mitochondrial COI gene was sequenced from slugs previously scored for development mode of their larvae. The mean percent base composition was 37T:23A:21C:19G. This fragment was highly polymorphic, yielding 146 unique haplotypes with 155 variable positions (21 first, 2 second, 132 third codon position). Nearly all substitutions were synonymous, excepting four haplotypes that had one conservative amino acid substitution compared to all others, each at a different site.

A neighbor-joining (NJ) tree of eastern Pacific COI haplotypes showed a genetic divide between populations north and south of Bodega Harbor, concordant with developmental and morphological differences (Fig. 2). The COI haplotypes formed two reciprocally monophyletic clades with 100% bootstrap support. Intraclade divergence was a maximum of 5% but the two clades were 18–24% different from each other (Tamura-Nei distance), exceeding the interspecific divergence between many sacoglossans (R. A. Ellingson and P. J. Krug, unpubl. data). Further, there were fixed differences between clades at 27 positions (Krug et al. 2007). Based on differences in morphology, development, and DNA sequence data, the southern clade represents a cryptic species hereafter referred to as *Alderia* sp.

Within *Alderia* sp. there was no concordance between development and tree topology, and 12 different haplotypes were shared by specimens that produced different types of larvae (Fig. 2). The southern *Alderia* sp. is thus poecilogonous, expressing alternative larval developmental modes within a single evolutionarily independent lineage. There were two weakly supported intraspecific clades that did not correlate with geographic origin of haplotypes. Northern populations were morphologically consistent with the original description of *A. modesta*, which is planktotrophic in all known Atlantic and Pacific populations.

The more broadly distributed *A. modesta* had higher haplotype and nucleotide diversities and more segregating sites than its geographically restricted congener (Table 2). The northern species *A. modesta* also had a significantly higher mean number of differences between haplotypes (Fig. 3 and results of a permutation test, \( P < 0.0005 \)). In contrast, *Alderia* sp. had fewer pairwise differences and a common haplotype shared throughout its range (Fig. 3). Tajima’s \( D \) and Fu’s \( F_S \) were significantly negative for both species, suggesting recent expansion (Table 3), and a model of sudden population expansion could not be rejected for either species (\( A. modesta, P = 0.89; Alderia sp., P = 0.95 \)). Parameters \( \tau \) and \( \theta_0 \), estimated from mismatch distributions, suggested *A. modesta* began expanding at an earlier date and from a population of larger size (Table 3). The mutational time scale calculated for each species placed these expansions at the end of the Pleistocene.

**Phylogeography of Alderia spp**

Based on \( F_{ST} \) values, a significant percentage of the total genetic variance was distributed among estuaries in *Alderia* sp. (AMOVA: \( P < 0.05 \)). All significant pairwise \( F_{ST} \) comparisons involved Morro Bay, the most geographically isolated site (Table 4). Only Morro Bay contained multiple private haplotypes shared by four or more individuals. In *Alderia* sp., no among-estuary comparisons were significant based on \( \Phi_{ST} \). No population structure was evident in Pacific *A. modesta* by either \( F_{ST} \) or \( \Phi_{ST} \).

We subsequently included specimens of *A. modesta* from the Atlantic and western Pacific in a broader phylogeographic analysis. In addition to COI, a 450-bp fragment of the more conserved 16S gene was sequenced from a subset of specimens. In both Bayesian and parsimony trees based on combined COI and 16S sequence data, *A. modesta* from the North Pacific and Atlantic formed a clade that was reciprocally monophyletic with *Alderia* sp. (Fig. 4). The sister species of *Alderia* were a mean 20.6% divergent in COI (Tamura-Nei distance) and 3.1% in 16S (uncorrected distance). Within *A. modesta*, Pacific and Atlantic populations were also reciprocally monophyletic and substantially divergent (11.0% in COI, 1.3% in 16S). Three haplotypes from the western Pacific (Sea of Japan) fell within the larger *A. modesta* clade, but did not group together. Within *Alderia* sp., bootstrap values and posterior probabilities supported a clade composed primarily of haplotypes from southern California (Fig. 4).

A gennate pair of elysiid sacoglossans yielded a rate of 1.57% change per million years for the first codon position of COI. Based on this rate, *Alderia* species diverged about 4.1 MYA, in the Miocene, whereas Atlantic and Pacific populations of *A. modesta* diverged about 1.7 MYA, in the early Pleistocene.

**Overlap Zone**

To refine the range limits of *Alderia* spp., five sites were sampled between San Francisco Bay and Bodega Harbor in 2003–2004 (Fig. 5). Individuals were typed by morphology, development, and mitochondrial DNA analysis. In both summers, only *A. modesta* was found in Bodega Harbor, and the southern end of Tomales Bay contained only *Alderia* sp. The two species co-existed in central (Cow Landing site) and northern (Walker Creek site) Tomales Bay (Fig. 5). A few *A. modesta* were found in San Francisco Bay in December 2003 and August 2004. Genetic analysis confirmed that all slugs (\( n = 80 \)) from mixed populations were correctly typed by morphology (haplotypes included in Fig. 2). There was no evidence of mitochondrial DNA introgression in either direction where the two species coexisted. The host alga at the south Tomales Bay site (nominally *V. longicaulis*) was similar in appearance to *Vaucheria* from southern California, forming dense, discrete patches with a mosslike texture. The alga in San Francisco, the rest of Tomales Bay, and Bodega Harbor had a more filamentous morphology not typically observed in southern California.

**Copulatory Behavior and Reproductive Isolation**

To assess reproductive isolation between the sibling species, laboratory-reared virgin *Alderia* sp. (\( n = 7 \)) were paired with individual *A. modesta* from Bodega and observed for 6 h. All Bodega slugs reacted to contact with a virgin *Alderia* sp. by immediately extending the penis; at least three were observed to inseminate a virgin, based on flow of sperm through the translucent penis. All virgin slugs extended their penis upon contact with a Bodega slug and one aberrantly discharged sperm into the seawater, but none were observed
Fig. 2. Phylogenetic relationships among slugs in the genus *Alderia* from the northeastern Pacific, based on COI sequences. A neighbor-joining (NJ) tree was derived from all nucleotide sites; branches with bootstrap support ≥70 are bold. Unmarked haplotypes were obtained from adults producing planktotrophic larvae, those labeled with a dark circle from adults producing lecithotrophic larvae, and those marked with a triangle were shared by adults that produced larvae differing in development mode.
to inseminate their partner even after prolonged contact (>10 min). Only one of seven inseminated virgins produced egg masses over the next 48 h, whereas all paired control virgins reciprocally inseminated each other and produced fertilized clutches. One former virgin produced two fertilized clutches that developed normally; however, it was subsequently found that Alderia sp. can self-fertilize, leaving the parentage of offspring from the virgin slug ambiguous (N. Smolensky and P. J. Krug, unpubl. data).

Size and Fecundity

Overall, specimens of A. modesta were significantly larger than Alderia sp. (Fig. 6, Table 5). This size difference initially drew attention to rare individuals of A. modesta in San Francisco Bay. Within each species, there was highly significant variation in size between sites (Table 5). Specimens from Coos Bay, Oregon were larger than slugs from all Californian sites (Fig. 6, and results of a post-hoc Scheffé test: P < 0.05). At Cow Landing, specimens of A. modesta were significantly larger than sympatric Alderia sp. (post-hoc Scheffé test: P < 0.05). Size did not consistently discriminate between species, however; A. modesta from Walker Creek 2004 were not significantly larger than Alderia sp. from that or any other site (Fig. 6). Interannual differences were found at San Francisco, where the mean size in Aug 2004 was significantly larger than in Aug 2003 (post-hoc Scheffé test: P < 0.05). Within Alderia sp., there was no increase in size with latitude, and the smallest specimens came from south Tomales Bay.

Fecundity (eggs per clutch) was positively related to size in both species and, in Alderia sp., for both development modes (Table 6). The mean fecundity of A. modesta ranged from 267 ± 34 to 702 ± 98 planktotrophic eggs per clutch (n = 5 populations), whereas specimens of Alderia sp. produced 166 ± 15 and 311 ± 25 planktotrophic eggs per clutch in two populations. The mean number of lecithotrophic eggs per clutch ranged from 32 ± 2 to 325 ± 71 (n = 5 populations), but the maximum value was atypically high; four of the five populations had a mean fecundity of less than 120 lecithotrophic eggs per clutch.

Discussion

Cryptic Speciation and Phylogeography in Alderia

Based on molecular, morphological, and developmental differences, most specimens previously regarded as A. modesta south of Bodega Harbor, California, comprised a cryptic species (Dayrat 2005; DeSalle et al. 2005). The sibling species can be differentiated by fixed nucleotide differences in the COI gene, allozymes at the PGI locus, and dorsal morphology (Krug et al. 2007). Whereas A. modesta is exclusively planktotrophic, the pereicogonous Alderia sp. expresses either planktotrophy or lecithotrophy depending on the season; specimens differing in development cannot be distinguished by any other criteria, share COI haplotypes, and switch development mode under laboratory conditions (Krug 1998, 2007).

The Californian endemic Alderia sp., likely evolved at the southeastern range limit of a broadly distributed ancestor. An emerging paradigm for macroevolution of intertidal gastropods is speciation via peripheral isolation. Intertidal organisms are effectively confined to one-dimensional strips of shoreline, and may be susceptible to transient isolation caused by climate change (Valentine and Jablonski 1983; Reid 1990). For instance, Pleistocene conditions forced many taxa into refugia in the Southern California Bight, followed by Holocene expansion northward (Hellberg 1994; Marko 1998; Dawson et al. 2001; Hellberg et al. 2001; Marko et al. 2003; Jacobs et al. 2004). However, in no previous case has a major life-history shift been potentially linked to transient isolation at the edge of a species’ distribution in the eastern Pacific. As lecithotrophy is expressed by the southern species primarily in summer months, it is likely an adaptation to warm conditions. The data for Alderia sp. are consistent with speciation by peripheral isolation from a planktotrophic ancestor. Lecithotrophy is an apomorphy of the southern species, as planktotrophy is pleisiomorphic in the Limapontiidae (Jensen 1996). Whereas A. modesta retained the ancestral development mode, Alderia sp. incorporated an extraordinary degree of flexibility into its life history, seasonally expressing alternative larval morphs and bet-hedging dispersal strategies (Krug 2001).

In the absence of paleontological data for shell-less molluscs, molecular methods may permit reconstruction of their evolutionary history. A molecular clock, calibrated with geminates sacoglossans, suggested a coalescence time of 4.1 MYA for A. modesta–Alderia sp. In the late Miocene and early Pliocene, coastal geomorphology and high productivity in the eastern Pacific contributed to radiations of many marine taxa (Jacobs et al. 2004). High sea level also created isolated estuaries in the Central Valley of California, providing opportunities for allopatric speciation among wetland organisms such as Alderia (Hall 2002; Jacobs et al. 2004).

Pleistocene climate change likely severed trans-Arctic gene flow in A. modesta. The molluscan fauna of the North

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**Table 2. Molecular diversity in Alderia species from the northeastern Pacific.** Sequence data for COI were collected for A. modesta and its cryptic congener, Alderia sp., and analyzed in Arlequin 2.000. “Unique” haplotypes were sampled only once in the study. Haplotype and nucleotide diversities are given ± variance.

<table>
<thead>
<tr>
<th></th>
<th>A. modesta</th>
<th>Alderia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (no. of individuals)</td>
<td>104</td>
<td>129</td>
</tr>
<tr>
<td>k (no. of haplotypes)</td>
<td>84</td>
<td>62</td>
</tr>
<tr>
<td>% unique haplotypes</td>
<td>94.0%</td>
<td>74.2%</td>
</tr>
<tr>
<td>No. of segregating sites (S)</td>
<td>103</td>
<td>72</td>
</tr>
<tr>
<td>Haplotype diversity (H)</td>
<td>0.9860 ± 0.0062</td>
<td>0.9119 ± 0.0217</td>
</tr>
<tr>
<td>Nucleotide diversity (π)</td>
<td>0.0209 ± 0.0107</td>
<td>0.0141 ± 0.0074</td>
</tr>
<tr>
<td>Mean no. of pairwise differences (95% CI)</td>
<td>9.68 (5.86–12.29)</td>
<td>6.66 (3.29–10.28)</td>
</tr>
</tbody>
</table>
EVOLUTION OF VARIABLE LARVAL DEVELOPMENT

FIG. 3. Histogram showing the distribution of pairwise differences between COI haplotypes for (A) *Alderia modesta*, ranging from Kodiak, Alaska, south to San Francisco, California, and (B) *Alderia* sp., ranging from Walker Creek, California, south to San Diego.

TABLE 3. Comparative estimated population expansion parameters for *Alderia* species, based on mismatch distributions of COI sequences from the northeastern Pacific. Time since expansion, $\tau$, is given in units of the mutational time scale, or as $t$ in 1000 years (KY; Excoffier and Schneider 1999). Calculations were based on $u = 2.62 \times 10^{-5}$ substitutions per year and generation times of two months (*A. modesta*) versus one month (*Alderia* sp.). Raw values of $\theta_0$ were divided by $2u$ to estimate population size at expansion (given as no. of individuals), and after expansion ($\theta_1$, given as 1000 individuals). The 95% confidence intervals around $t$, $\theta_0$, and $\theta_1$ are given in the corresponding units. *$P < 0.05$; ***$P < 0.0001$.

<table>
<thead>
<tr>
<th></th>
<th>A. modesta</th>
<th>Alderia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tajima’s $D$</td>
<td>-1.67**</td>
<td>-1.58*</td>
</tr>
<tr>
<td>Fu’s $F_S$</td>
<td>-24.49***</td>
<td>-24.92**</td>
</tr>
<tr>
<td>$\tau$ (KY)</td>
<td>11.76</td>
<td>11.23</td>
</tr>
<tr>
<td>95% CI</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>$\theta_0$ (individuals)</td>
<td>24,000</td>
<td>40</td>
</tr>
<tr>
<td>95% CI</td>
<td>18–59</td>
<td>6–27</td>
</tr>
<tr>
<td>$\theta_1$ (1000 individuals)</td>
<td>405</td>
<td>207</td>
</tr>
<tr>
<td>95% CI</td>
<td>0–72,000</td>
<td>0–100,000</td>
</tr>
<tr>
<td>$\theta_1$</td>
<td>220–1710</td>
<td>100–830</td>
</tr>
</tbody>
</table>

Atlantic and Pacific evolved independently for about 100 million years until the opening of the Bering Strait, as early as 5.5 MYA or as recently as 3.1 MYA (Marincovich and Gladenkov 2001). Formation of the Strait initiated a trans-Arctic migration lasting until the late Pliocene. In this asymmetric invasion, molluscs of Pacific origin disproportionately colonized the North Atlantic by an 8:1 ratio (Vermeij 1991). Species that participated in the trans-Arctic exchange show either genetic differences suggesting prolonged isolation (Grant and Stahl 1988; McDonald and Koehn 1988; Zaslavskaya et al. 1992; Collins et al. 1996), or low intraspecific variation indicating recent gene flow (Graves and Dizon 1989; Palumbi and Wilson 1990; Palumbi and Kessing 1991). A divergence time of 1.7 MYA for Atlantic and Pacific *A. modesta* suggests early Pleistocene glaciation removed suitable habitat from the Arctic, and conditions have since remained unfavorable for larval transport between basins. Atlantic and Pacific slugs have not diverged in morphology or development, perhaps due to ecological similarity of their habitats (Schneider et al. 1999); however, they represent evolutionarily independent lineages separated by Quaternary climate change. Speciation predated the trans-Arctic exchange, indicating a Pacific origin for the genus *Alderia*. 
Both species show evidence of recent population expansion, the parameters of which were estimated from mismatch data. Assuming Atlantic and Pacific A. modesta diverged 1.7 MYA, we derived a substitution rate of 5.47% per site per million years for COI, or \( u = 2.62 \times 10^{-5} \) substitutions per year for a 480-bp fragment. The average generation time of A. modesta was estimated as twice that of Alderia sp., due to obligate planktotrophy and colder temperatures during larval maturation. Dividing their respective generation times by 2\( u \) gave a mutational time scale of 3180 for A. modesta and 1580 for Alderia sp.; multiplying by \( \tau \) converts this parameter to years since expansion (Rogers 1995). From these estimates, Alderia sp. began expanding about 18,000 years ago (95% CI: 6000–27,000), whereas A. modesta expanded about 37,000 years ago (95% CI: 18,000–59,000). Values of \( \theta_0 \) were also higher for A. modesta (\( \theta_0 = 24,000 \) slugs) than Alderia sp. (\( \theta_0 = 40 \) slugs), but the confidence intervals for both were too broad (95% CI: 0–100,000) to draw conclusions about pre-expansion population sizes. However, the higher haplotype diversity and mean number of pairwise differences in A. modesta are also consistent with a larger pre-expansion population. The ability of long-lived larvae to disperse across the Pacific may have allowed the cold-adapted A. modesta to migrate between viable patches during glacial maxima, escaping population crashes associated with regional glaciation (Warner et al. 1982; Marko 2004; Hickerson and Cunningham 2005).

In contrast, low sea levels and a concomitant loss of estuarine habitat across central California may have restricted Alderia sp. to refugia in southern California throughout the Pleistocene; reduced nucleotide and haplotype diversities, the single common COI haplotype, and estimates of \( \theta_0 \) all suggest Alderia sp. experienced a more dramatic bottleneck than its congener (Grant and Bowen 1998; Caudill and Bucklin 2004). A selective sweep, the alternative basis for reduced diversity, is unlikely given the predominance of silent substitutions in our COI dataset. The expansion of Alderia sp. dates to the beginning of the Holocene, when higher sea levels may have allowed it to recolonize estuaries as they reformed across central California, eventually coming into secondary contact with its sister species (e.g., Marko 1998).

Both Alderia species are specialists on Vaucheria species, which grow only in protected back bays separated by uninhabitable stretches of open coast. The fragmented nature of their estuarine habitat was expected to inhibit gene flow. However, no phylogeographic structure was evident in A. modesta within the Pacific, despite its considerable range; this strictly planktotrophic species was panmictic in the northeastern Pacific, and haplotypes from the Sea of Japan grouped within the eastern Pacific clade. The dispersal potential of long-lived, feeding larvae is evidently realized in A. modesta, genetically homogenizing distant estuaries. Within Alderia sp., \( F_{ST} \) results indicated reduced gene flow between Morro Bay and three other estuaries. This could result from the geographically isolated nature of Morro Bay, and also from the limited dispersal potential of lecithotrophic larvae produced by Alderia sp. for much of the year (Krug 2001; Krug and Zimmer 2000, 2004). Although \( F_{ST} \) analysis of the COI data found no phylogeographic structure in Alderia sp., a clade with primarily southern distribution was evident in phylogenetic studies combining COI and 16S data.

**Present Distribution of the Sister Species**

Historical processes do not explain the current biogeography of Alderia. The range endpoints of Alderia species fall neither at Point Conception, a major faunal transition zone, nor in central California, where some coastal taxa have phylogeographic breaks (Valentine 1966; Burtons 1998; Wares et al. 2001; Dawson 2001; Sotka et al. 2004). The northern limit of Alderia sp. was Bodega, and A. modesta was rare south of Tomales Bay. Evidence from diverse taxa suggests gene flow may be interrupted between Bodega and sites to the south of Point Reyes, California. In the supralittoral copepod *Tigriopus californicus*, a northern clade with reduced genetic diversity ranges from Alaska to Bodega, suggesting *T. californicus* expanded from refugia south of Point Reyes (Edmards 2001). The intertidal crab *Petrolisthes cinctipes* has a genetic discontinuity between Bodega and the mouth of Tomales Bay, only 10 km distant (R. Toonen and R. Grosberg, unpubl. data). In the urchin *Strongylocentrotus franciscanus*, only Bodega is differentiated from other populations (Moberg and Burton 2000). The concordant break at Bodega across diverse taxa may reflect (1) historical processes such as contracting ranges during glaciation events; (2) differences in the abiotic environment imposing divergent selection on either side of Bodega, affecting the distribution and frequency of linked loci; (3) unusual hydrographic features that affect patterns of dispersal around the mouth of Tomales Bay; or some combination thereof. Further study of the factors controlling the demographics of Alderia species around Point Reyes may shed light on why this area produces phylogeographic patterns in diverse invertebrates.

Although larvae of Alderia species can disperse hundreds

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### Table 4. Pairwise differences among estuaries in Alderia sp.

<table>
<thead>
<tr>
<th></th>
<th>Tomales Bay (n = 29)</th>
<th>San Francisco (n = 32)</th>
<th>Morro Bay (n = 26)</th>
<th>Los Angeles (n = 14)</th>
<th>Newport Bay (n = 14)</th>
<th>San Diego (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB</td>
<td>0.013 ± 0.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>–0.000</td>
<td>0.015 ± 0.008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>0.037*</td>
<td>0.036*</td>
<td>0.012 ± 0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.024</td>
<td>0.010</td>
<td>0.082**</td>
<td>0.018 ± 0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB</td>
<td>–0.002</td>
<td>0.001</td>
<td>0.017</td>
<td>0.049</td>
<td>0.014 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>–0.008</td>
<td>–0.011</td>
<td>0.034</td>
<td>0.019</td>
<td>–0.014</td>
<td>0.015 ± 0.008</td>
</tr>
</tbody>
</table>

Significant pairwise differences were determined by AMOVA in Arlequin with 10,000 permutations of the data. *P < 0.05, **P < 0.01.
of km, they were partitioned over spatial scales of less than 15 km in Tomales Bay. Distributions of *Alderia* species may reflect those of the host algae if larvae only settle in areas where a preferred species of *Vaucheria* grows. Proximal populations can remain differentiated due to larval habitat choice behavior, an emerging mechanism by which genotypes segregate without extrinsic barriers to gene flow (Appelbaum et al. 2002; Gilg and Hilbish 2000, 2003a,b; Hilbish et al. 2003; Baird et al. 2003; Bierne et al. 2003). The sister species could also express divergent host-specific adaptations limiting their ability to use different *Vaucheria* species but few studies have tested whether local coadaptation occurs in marine herbivores and host algae (Sotka 2003; Sotka et al. 2003). Alternatively, a gradient in the physical environment may limit where *Alderia* species live if they differ in physiological tolerance (Theison 1978; Koehn et al. 1980; Schmidt and Rand 2001; Bierne et al. 2002; Brind’Amour et al. 2002; Riginos et al. 2002).

Fig. 4. Bayesian tree showing phylogenetic relationships among *Alderia* species from the Pacific and Atlantic, based on combined COI and 16S mtDNA haplotypes. Bayesian posterior probabilities (above branches), and parsimony bootstrap values based on 1000 replicates (below branches), are shown for clades that received $\geq80\%$ support in both analyses. Haplotypes from the western Pacific are indicated with asterisks. Collection sites: Doel, Belgium (DB); Sea of Japan, Russia (RS); Kodiak Island, Alaska (AK); Vancouver, British Columbia (BC); Coos Bay, Oregon (CB); Bodega Harbor, California (BH); Tomales Bay (TB); San Francisco (SF); Morro Bay (MB); Los Angeles (LA); Newport Bay (NB); and San Diego (SD).
There was no evidence of hybridization or introgression where *Alderia* species were sympatric, yet both species attempted to inseminate each other in laboratory crosses. There is no premating isolation between *Alderia* species; slugs mate year-round via hypodermic insemination, injecting sperm anywhere on the recipient’s body (Hand and Steinberg 1955; Angeloni 2003). Divergence in sperm chemoattractants or gamete recognition proteins may promote prezygotic isolation (Vacquier 1998; Lyon and Vacquier 1999; Swanson and Vacquier 2002; Zigler et al. 2003; Riffell et al. 2004), but this has not been tested in a marine organism with internal fertilization. Postzygotic isolation between *Alderia* species was implicated by the failure of most laboratory crosses despite insemination, and could be a pleiotropic by-product of the different genetic programs that direct larval development in the sister species.

**Evolution of Poecilogony**

Transitions between planktotrophy and lecithotrophy have occurred frequently in the evolutionary history of many taxa and can substantially alter the population structure and macroevolutionary fate of a lineage (Raff 1987; Jeffery and Swalla 1992; Hadfield et al. 1995; Hart et al. 1997; Duda and Palumbi 1999; Hart 2000; McEdward 2000; Jeffery et al. 2003, Collin 2004). However, for any given species the se-
selective forces that favored the evolution of nonfeeding larvae remain unclear. Due to the rarity of poecilogony, studies contrasting different development modes usually employ interspecific comparisons (e.g., Villinski et al. 2002), which may be confounded by other differences between species. As a case of variable development, *Alderia* sp. should be a model system with which to study developmental evolution from ecological and biochemical perspectives.

There are few confirmed examples of poecilogony among marine invertebrates, despite the adaptive value of dispersal polymorphisms (Giesel 1976; Denno et al. 1980; Harrison 1980; McPeek and Holt 1992; Toonen and Pawlik 1994; Chia et al. 1996; Hopper 1999; Langellotto and Denno 2001). Most claims of poecilogony were resolved as cryptic species differing in development upon detailed molecular or morphological study (Hoagland and Robertson 1988; Hirano and Hirano 1992; Sisson 2002). In molluscs, multiple larval types within a species have been confirmed by breeding studies or molecular data for three sacoglossans: *Elysia chlorotica* (West et al. 1984), *Alderia* sp. (Krug 1998; present study), and *Costasiella ocellifera* (R. A. Ellingson and P. J. Krug, unpubl. data). Among polychaetes, examples include *Boccardia proboscidea* (Gibson et al. 1999; Gibson and Gibson 2004), *Streblospio benedicti* (Levin 1984; Schulze et al. 2000), and *Pygospio elegans* (Morgan et al. 1999). Identifying the selective forces favoring poecilogony, and the phylogenetic constraints that limit it to a few taxa, may substantially advance our understanding of marine life-history evolution.

In southern California, slugs produced mostly lecithotrophic clutches from May through September, with a varying proportion expressing planktotrophy the rest of the year. Furthermore, some adults switch from lecithotrophic to planktotrophic clutch production upon transport to the laboratory (Krug 2007). Development in *Alderia* sp. is thus to some extent phenotypically plastic. Populations of the poecilogonous polychaete *S. benedicti* did not change development seasonally, nor did individuals ever change the trophic mode of their offspring (Levin and Huggett 1990; Levin and Creed 1991; Levin and Bridges 1994). The adaptive value of seasonal polyphenism has been studied for morphological characters (Brakefield and French 1999; Nijhout 1999), diapause strategies (Shapiro 1976), and sex determination (Conover and Heins 1987), but has not been described for larval development in a marine animal.

**Table 5.** Results of a nested ANOVA on weights of *Alderia* sp. and *A. modesta* from sites in California and Oregon, with site as a random effect nested within species as a fixed effect.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Type</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>fixed</td>
<td>1</td>
<td>16,493.25</td>
<td>16,493.25</td>
<td>24.50</td>
<td>0.0005</td>
</tr>
<tr>
<td>Site(species)</td>
<td>random</td>
<td>10</td>
<td>7011.50</td>
<td>701.15</td>
<td>42.16</td>
<td>0.0000</td>
</tr>
<tr>
<td>Species</td>
<td>fixed</td>
<td>1</td>
<td>6015.15</td>
<td>6015.15</td>
<td>8.93</td>
<td>0.0136</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>318</td>
<td>5288.61</td>
<td>16.63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The same factors that favored lecithotrophy in the ancestor of *Alderia* sp. could favor its present-day expression during warm months. Understanding the ecology of *Alderia* sp. may thus pinpoint the selective pressures behind the evolution of nonfeeding development. Lecithotrophy could be an adaptation to decrease larval mortality, either from offshore advection during upwelling or from a seasonal reduction in phytoplankton abundance. However, the transition away from planktotrophy occurs in May and June in southern California, after the peak in upwelling and when phytoplankton abundances are high (Bakun and Nelson 1977; Reid et al. 1985; P. J. Krug, unpubl. data). The environmental parameter best correlated with lecithotrophy is freshwater input from winter rain and spring snowmelt. Historically, estuaries in southern California were closed by sand berms when runoff ceased in summer and fall; lecithotrophy may thus be an adaptive response to seasonal habitat closure. Runoff could then flush planktotrophic larvae out of back bays and allow alongshore transport in winter and spring, facilitating dispersal. Alternatively, lecithotrophy could allow exploitation of stable adult food resources if *Vaucheria* is less seasonal in the south, which could be tested with monthly field surveys of host algal abundance.

Size and fecundity differences between *Alderia* species suggest another hypothesis for the evolution of poecilogony, integrating biochemical with ecological constraints. The southern species *Alderia* sp. is smaller than its congener even where they coexist, and produces fewer larvae. Due to high planktonic mortality (Morgan 1995), there may be a threshold clutch size below which no long-lived larvae are likely to survive, leading to selection against obligate planktotrophy in *Alderia* sp. If *Vaucheria* species are of lower nutritional value during the summer in southern waters, *Alderia* sp. may be unable to produce enough planktotrophic offspring to offset larval mortality. The adult energy budget and odds of larval survivorship could thus interactively drive evolution of development mode. Quantifying the caloric content of *Vaucheria* spp. should indicate whether host value fluctuates with latitude or over the course of a year. Presently being described (Krug et al. 2007), *Alderia* sp. is an excellent model organism with which to explore the factors controlling expression of alternative developmental pathways, providing insight into how adult and larval ecology interactively shape life-history evolution.

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