Dimorphic development in *Streblospio benedicti*: genetic analysis of morphological differences between larval types

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ABSTRACT The marine polychaete *Streblospio benedicti* exhibits two distinct larval types, making it a model for the study of developmental evolution. Females produce either large eggs or small ones, which develop into distinct lecithotrophic or planktotrophic larvae with concomitant morphological and life-history differences. Here, we investigate the inheritance of key morphological traits that distinguish the larval types. We used genetic crosses to establish the influence of maternal and zygotic differences on larval phenotypes. We found a large maternal effect on larval size and the number of larval chaetae, while the number and length of these chaetae were also strongly influenced by zygotic genotype. Interestingly, the distribution of larval phenotypes produced by these crosses suggests traits intermediate to the two parental types should not be uncommon. Yet, despite gene flow between the types in natural populations, such intermediates are rarely found in nature, suggesting that selection may be maintaining distinct larval modes.

KEY WORDS: *Streblospio benedicti*, poecilogony, quantitative genetics, life-history evolution

Introduction

The evolution of development couples molecular and cytological processes with ecological and population-genetic phenomena such as dispersal and life-history strategies. Benthic marine animals are particularly well suited to studies of developmental evolution in an ecological context, because while lineages exhibit a broad diversity of adult forms, their pelagic larvae fit loosely within one of two suites of correlated life-history characteristics (Thorson, 1950): planktotrophic larvae are small, obligately feeding larvae that spend days to months developing in the plankton before reaching metamorphic competence; lecithotrophic larvae generally lack complex feeding structures and are able to metamorphose and settle to the benthos soon after their release, provisioned only by maternal yolk (Strathmann, 1985).

How and why this developmental dichotomy occurs has been a subject of longstanding debate and inquiry (Marshall and Morgan, 2011; McEdward, 2000; Moran and McAlister, 2009; Pechenik, 1999; Vance, 1973). While divergent developmental modes are common across pairs of sister species (Strathmann, 1978; Wray and Raff, 1991), most species exhibit only one mode, precluding genetic analysis of differences between offspring morphs and confounding larval mode with all other interspecific differences. Little is known about how, at the population level, suites of life history traits related to larval mode vary and evolve.

The phenomenon of poecilogony, whereby a single species produces distinct types of embryos and thus two distinct life-history strategies, offers a route toward answering these questions (Knott and McHugh, 2012). Poecilogony provides a microevolutionary model for the pervasive macroevolutionary pattern of larval life-history evolution, enabling forward genetic dissection of developmental and life-history traits and their genetic correlations. Here we use the polychaete *Streblospio benedicti*, the best characterized case of poecilogony from a genetic perspective, to explore the genetic basis for several characters that distinguish planktotrophic and lecithotrophic larvae.

*S. benedicti* is a common spionid polychaete that inhabits muddy estuarine sediments. Adults live in the sediment and dispersal is primarily, if not exclusively, achieved in the larval phase. Females produce either clutches of tens of lecithotrophic embryos, each ~200 μm diameter, or clutches of hundreds of planktotrophic embryos of, each ~100 μm diameter (Bridges, 1993; Levin, 1984; Levin and Bridges, 1994; McCain, 2008). Classical quantitative genetic studies indicate that poecilogony is a heritable polymorphism in *S. benedicti*, and that larval mode is unaffected by large differences in temperature, day length, and food availability (Levin, 1986; Levin and Bridges, 1994; Levin and Creed, 1986; Levin et al., 1991). In particular, Levin et al., (1991) used a reciprocal mating design between adults of the two larval modes and found a large additive contribution for many reproductive and larval traits associated with...
Females of both offspring types can co-occur in small spatial areas, although generally populations tend to consist of only one offspring type (Zakas and Wares, 2012). Both mitochondrial and nuclear loci indicate recent gene flow between the two morphs (Rockman, 2012; Schulze et al., 2000; Zakas and Wares, 2012). The rare occurrence of contrasting suites of heritable larval traits within a single species provides an opportunity to determine the genetic components of phenotypic variance associated with life history.

Here we use experimental crosses to disentangle the roles of zygotic genetic effects and maternal effects. We use a mating design with replicate families of reciprocal crosses to identify the genetic components of phenotypic variation associated with the two larval types. We extend our crosses to F₂ and four classes of backcross, and we describe, for the first time, larval morphological variation within genetically segregating families in S. benedicti.

We focus our study on a few key morphological differences between the two larval types. First, alternative types of S. benedicti larvae differ dramatically in size as a result of their difference in egg volume. We ask whether the anterior-posterior axis length of a larva depends only on the oocyte cytoplasm or whether it is influenced as well by the larval genome. Second, we focus on a striking morphological difference between the two types: the larval chaetae. Chaetae are epidermal structures made of β-chitin in a protein matrix, and are ancestral characters of annelids and name-defining for polychaetes (Hausen, 2005). Sponid larvae typically have many long larval chaetae protruding from two chaetal sacs on either side of the first segment. Though lecithotrophic larvae develop chaetal sacs, the larval chaetae themselves are absent (Gibson et al., 2010, and Fig. 1). Planktotrophic larvae spend a long period developing in the plankton where predation mortality is large and chaetae can confer an advantage by deterring predation (Pennington and Chia, 1984; Pernet et al., 2002; Vaughn and Allen, 2010). In lecithotrophic larvae, these chaetae may be unnecessary or disadvantageous.

**Results**

Egg size, estimated as the average of two orthogonal measurements of the diameter, falls into three discrete classes. The eggs of Bayonne planktotrophs averaged 102 μm, with a standard deviation of 7.5 μm (n=8), those of Long Beach lecithotrophs averaged 205.1 ± 4.9 μm, (n=8) and the F₁ eggs averaged 137.1 ± 5.4 μm (n=30). The planktotrophic and lecithotrophic eggs thus differ in volume by a factor of 8, and the F₂ larvae differ in size as a result of their difference in egg volume. We ask whether the anterior-posterior axis length of a larva depends only on the oocyte cytoplasm or whether it is influenced as well by the larval genome. Second, we focus on a striking morphological difference between the two types: the larval chaetae. Chaetae are epidermal structures made of β-chitin in a protein matrix, and are ancestral characters of annelids and name-defining for polychaetes (Hausen, 2005). Sponid larvae typically have many long larval chaetae protruding from two chaetal sacs on either side of the first segment. Though lecithotrophic larvae develop chaetal sacs, the larval chaetae themselves are absent (Gibson et al., 2010, and Fig. 1). Planktotrophic larvae spend a long period developing in the plankton where predation mortality is large and chaetae can confer an advantage by deterring predation (Pennington and Chia, 1984; Pernet et al., 2002; Vaughn and Allen, 2010). In lecithotrophic larvae, these chaetae may be unnecessary or disadvantageous.

**Inheritance of larval body length at release, measured as**

<table>
<thead>
<tr>
<th>Cross Name</th>
<th>Female Parent</th>
<th>Male Parent</th>
<th>Larvae Sampled</th>
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</thead>
<tbody>
<tr>
<td>BB1</td>
<td>B</td>
<td>B</td>
<td>55</td>
</tr>
<tr>
<td>BB2</td>
<td>B</td>
<td>B</td>
<td>73</td>
</tr>
<tr>
<td>BL1</td>
<td>B*</td>
<td>L</td>
<td>58</td>
</tr>
<tr>
<td>BL2</td>
<td>B*</td>
<td>L</td>
<td>58</td>
</tr>
<tr>
<td>BL3</td>
<td>B*</td>
<td>L</td>
<td>89</td>
</tr>
<tr>
<td>F1</td>
<td>B*</td>
<td>L</td>
<td>27</td>
</tr>
<tr>
<td>BL4</td>
<td>B</td>
<td>L</td>
<td>45</td>
</tr>
<tr>
<td>BCB1</td>
<td>B</td>
<td>F₁</td>
<td>104</td>
</tr>
<tr>
<td>BCB2</td>
<td>B</td>
<td>F₁</td>
<td>40</td>
</tr>
<tr>
<td>BCB3</td>
<td>F₁</td>
<td>B*</td>
<td>38</td>
</tr>
<tr>
<td>F2-1</td>
<td>F₁</td>
<td>F₁</td>
<td>34</td>
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<tr>
<td>F2-2</td>
<td>F₁</td>
<td>F₁</td>
<td>29</td>
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<tr>
<td>F2-3</td>
<td>F₁</td>
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<td>F2-4</td>
<td>F₁</td>
<td>F₁</td>
<td>23</td>
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<tr>
<td>BCL1</td>
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<tr>
<td>BCL2</td>
<td>F₁</td>
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</tr>
<tr>
<td>LB1</td>
<td>L</td>
<td>B*</td>
<td>12</td>
</tr>
<tr>
<td>LB2</td>
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<td>B</td>
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</tr>
<tr>
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<td>L</td>
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<td>B*</td>
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<td>LB5</td>
<td>L</td>
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<td>F₁</td>
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<tr>
<td>LL1</td>
<td>L</td>
<td>L</td>
<td>16</td>
</tr>
<tr>
<td>LL2</td>
<td>L</td>
<td>L</td>
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</tr>
<tr>
<td>LL3</td>
<td>L</td>
<td>L</td>
<td>13</td>
</tr>
<tr>
<td>LL4</td>
<td>L</td>
<td>L</td>
<td>21</td>
</tr>
</tbody>
</table>

B. Bayonne; L. Long Beach. The asterisks indicate Bayonne individuals that are full siblings, and the crosses indicate F₁ individuals that are full siblings. Superscripts a, b, and c indicate that the same male was used in multiple crosses.

*Fig. 1. S. benedicti larvae upon release from the maternal brood pouches. All are shown at the same magnification. (A,B) DIC images of parental (wild-type) larvae. (A) A Long Beach lecithotrophic larva (cross type LxL). (B) A Bayonne planktotrophic larva (cross type BxB). (C,D) Brightfield views of representative F₁ larvae. The larva in (C) has no chaetae originating from the chaetal sac, similar to the LxL larva in (A). Scale bar, 100 μm.*

**COEFFICIENTS FOR ANALYSIS OF LINE-CROSS MEANS**

<table>
<thead>
<tr>
<th>Cross Class</th>
<th>$\theta_H$</th>
<th>$\theta_D$</th>
<th>$\theta_S$</th>
<th>$\theta_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B x B (Bayonne, BB)</td>
<td>-1 1 1 -1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B x F₁ (Backcross₁ₕ)</td>
<td>-1 1 ½ 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B x L (F₁ₕ)</td>
<td>-1 0 1 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁ x B (Backcross₁ₕ)</td>
<td>0 ½ 0 1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁ x F₁ (F₁)</td>
<td>0 0 0 1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁ x L (Backcross₁ₕ)</td>
<td>0 ½ 0 1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x B (F₁ₕ)</td>
<td>1 0 1 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x F₁ (Backcross₁ₕ)</td>
<td>1 ½ 0 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x L (Long Beach, LL)</td>
<td>1 -1 -1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each cross class, the first named line is the mother. Refer to Materials and Methods for an explanation of the coefficients and models.
However, maternal effects are also pronounced. This is clear from contrasts between reciprocal crosses that differ only in egg size ($F_{1B}$ vs $F_{1LB}$, Backcross$_{BF1}$ vs Backcross$_{F1B}$, Backcross$_{FL1}$ vs Backcross$_{F1L}$). Segregation variance is visible in these data, as more variation in chaetae number is present in the backcross and $F_2$ classes due to genetic variation, as discussed below. Variation in penetrance is also evident in chaetae number: within $F_{1LB}$ families all larvae are identical in their Bayonne genetic content, but only 30% are lacking chaetae. Our full model, incorporating additive and dominance maternal and zygotic effects, is favored by the data, with additive zygotic effects the largest (Table 4).

We modeled the length of the longest measured chaeta for each larva, excluding zero-chaetae animals from consideration. We found no evidence of maternal effects, but there is strong support for additive zygotic effects (Table 5; $p < 10^{-5}$). Thus our data suggest that chaetal length is inherited zygotically, but that the penetrance of chaetal growth (i.e., the presence or absence of chaetae) depends on maternal effects as well.

Table 3

<table>
<thead>
<tr>
<th>Model</th>
<th>DF</th>
<th>AIC</th>
<th>BIC</th>
<th>logLik</th>
<th>Chisq</th>
<th>DF (Pr(Chisq))</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>3</td>
<td>-1513.7</td>
<td>-1498.8</td>
<td>759.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu + \alpha_M$</td>
<td>4</td>
<td>-1569.1</td>
<td>-1549.3</td>
<td>788.54</td>
<td>57.43</td>
<td>$1 &lt; 10^{-5}$</td>
</tr>
<tr>
<td>$\mu + \alpha_M + \alpha_Z$</td>
<td>5</td>
<td>-1567.0</td>
<td>-1542.3</td>
<td>788.52</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$\mu + \alpha_M + \alpha_Z + \delta_Z$</td>
<td>6</td>
<td>-1579.9</td>
<td>-1550.2</td>
<td>795.94</td>
<td>14.85</td>
<td>$1 &lt; 10^{-5}$</td>
</tr>
<tr>
<td>$\mu + \alpha_M + \alpha_Z + \delta_Z + \delta_M$</td>
<td>7</td>
<td>-1578.2</td>
<td>-1543.6</td>
<td>796.13</td>
<td>0.36</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Interaction Model

$\mu + \alpha_M + \alpha_Z + (\alpha_M \alpha_Z)$ | 6  | -1577.3 | -1547.7 | 794.67 |

Larval gut length ($\log(\mu)$)

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>6.299</td>
<td>0.019</td>
</tr>
<tr>
<td>$\alpha_M$</td>
<td>0.016</td>
<td>0.024</td>
</tr>
<tr>
<td>$\alpha_Z$</td>
<td>-0.013</td>
<td>0.039</td>
</tr>
<tr>
<td>$\delta_Z$</td>
<td>0.089</td>
<td>0.021</td>
</tr>
<tr>
<td>$\delta_M$</td>
<td>0.024</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Larval phenotype distributions depend on maternal and zygotic constitution. Each row represents a class of cross. The crosses are grouped into three sets by Egg Type: small eggs, $F_1$ eggs, and large eggs. Within each egg type category, crosses are ordered by Bayonne Gene Content. The cross classes also vary in the proportion of individuals that are heterozygous for alleles from the two populations, as shown in the Heterozygosity column. The number (n) of individual larvae measured for each phenotype is reported above each histogram.
Moreover, conditional on chaetal presence, number and length are correlated. In Fig. 3A, we show the correlation between the residual variation in chaetal number and the residual variation in chaetal length, after the effects of $\alpha_m$, $\alpha_z$, $\delta_m$, and family have been accounted for. This correlation ($r = 0.49$, $p < 10^{-10}$) may be due to the segregation of genetic factors within families or to common microenvironmental effects influencing both traits in individual larvae. Tentative evidence for genetic correlation comes from the generally higher correlation among the residuals of $F$-backcross populations: the effective number of factors (independently segregating genes of equivalent effect size) accounting for this amount of segregation variance (2,679 $\mu^2$) can be estimated by

$$n_e = \left(\mu_{BB}.\mu_{LL}\right)^2/(8\sigma_s^2)$$

where BB and LL are the parental (Bayonne and Long Beach) crosses. Here, $\mu_{BB}$ is unknown, because the lecithotrophic larvae lack chaetae. We can use an alternative estimator for the parental phenotypic difference: twice the additive effect of Bayonne alleles, as estimated from our linear model. That estimate gives a predicted Bayonne chaetal length of 202.3 $\mu$m and a predicted Long Beach chaetal length of 47.5 $\mu$m (i.e., if Long Beach larvae had chaetae; though theoretical, this is the chaetal length the model predicts for a Long Beach genome in a Bayonne egg). We conclude that, conditional on the presence of chaetae, chaetal length is influenced by 1.12 additive genetic factors.

### Discussion

The three larval developmental traits we measured differ in their underlying genetic architecture. Larval gut length is almost entirely maternally determined with a small, non-additive zygotic contribution. The presence of larval chaetae and their number involve substantial effects of both egg type and larval genome. Remarkably, the length of the chaetae — in larvae that have chaetae — appears to depend exclusively on additive zygotic effects. The genetic contribution to chaetae number and length is also correlated within families; larvae with long chaetae tend to have many (>10) while larvae with short chaetae only have a few (1-5).

We find that larvae originating from the same

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**TABLE 4**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$ Mean</td>
<td>0.509</td>
<td>0.103</td>
</tr>
<tr>
<td>$\alpha_m$ Maternal Additive</td>
<td>-0.464</td>
<td>0.099</td>
</tr>
<tr>
<td>$\alpha_z$ Zygotic Additive</td>
<td>2.126</td>
<td>0.203</td>
</tr>
<tr>
<td>$\delta_m$ Maternal Dominance</td>
<td>0.538</td>
<td>0.117</td>
</tr>
<tr>
<td>$\delta_z$ Zygotic Dominance</td>
<td>0.856</td>
<td>0.161</td>
</tr>
</tbody>
</table>

**TABLE 5**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$ Mean</td>
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<tr>
<td>$\alpha_m$ Maternal Additive</td>
<td>7.916</td>
<td>6.691</td>
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<tr>
<td>$\alpha_z$ Zygotic Additive</td>
<td>77.377</td>
<td>16.996</td>
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<tr>
<td>$\delta_m$ Maternal Dominance</td>
<td>-3.120</td>
<td>10.013</td>
</tr>
<tr>
<td>$\delta_z$ Zygotic Dominance</td>
<td>12.175</td>
<td>12.546</td>
</tr>
</tbody>
</table>

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Fig. 3. The length and number of larval chaetae are correlated within families. (A) The distribution of residual chaetae length and number for all larvae. (B) Mean and confidence intervals of the correlation among residuals within each class of cross. The correlation coefficients are generally higher in populations in which two genotypes are segregating at a locus (backcrosses, red) or three genotypes ($F_2$, green) than for populations that are not segregating (parentals and $F_i$, black). The LxL cross is not shown because it lacks larval chaetae.
egg class are generally of the same size at release, as assessed by body length measured along the gut. $F_1$ mothers produce larvae that are intermediate in size to the two parental lines (Fig. 2). Although lecithotrophic gut development is delayed by about a day relative to planktotrophs (Pernet and McHugh, 2010), the gut is fully formed before release in both larval morphs. Small differences in gut length within egg-size classes relate to features of the zygotic genome, but the direction of this zygotic effect is not consistent across egg size classes. This may be due to greater variability in the larval stage, and thus size, at which lecithotrophic females released their larvae (results not shown). As larvae age, they increase the number of setigers and begin to develop adult features such as branchiae and palps. We observed that the same lecithotrophic mother could release subsequent broods at earlier or later stages, although larvae within a release were at the same developmental stage. This difference in developmental stage at release could be due to microenvironmental differences between clutches (such as longer days or temperature variation), random chance, or a response to physical perturbance. While lecithotrophic mothers showed variation in larval sizes across clutches, larvae from $F_1$ mothers tended to have variability in larval size within a clutch, which was especially noticeable in the $F_2$'s (Fig. 1 C-D). Planktotrophic mothers, on the other hand, released larvae at the same stage consistently.

We observe that a large egg does not necessarily result in a “phenotypically lecithotrophic” offspring lacking chaetae. While the size of a larva is almost entirely maternally specified, there is variation in the penetrance of chaetal development. Development of the larval chaetae begins a few days after fertilization, consistent with an important role for zygotic gene activity, and the chaetae are fully formed by the time the larvae are released. While chaetal sacs occur in all larvae, the presence and number of chaetoblasts, the cells responsible for generating chaetae, are not known for each morph. Approximately 70% of $F_{1,6}$ larvae develop some chaetae, though these larvae all carry one copy each of the Long Beach and Bayonne genomes. Further, ~23% of Backcross, $F_{1,6}$ larvae, which are homozygous for Long Beach alleles across 50% of the genome and heterozygous at the other 50%, also develop chaetae. Additionally, our estimate of the additive effect of Bayonne alleles on chaetal length suggests that a larva with a pure Long Beach genome in a small egg would produce moderate-length chaetae. This indicates that a switch in both maternal specification and zygotic gene action has to occur to produce a phenotypically lecithotrophic larva from a planktotrophic background. This pattern is particularly interesting in the context of life-history evolution from small to large egged larvae that has been described in most marine invertebrate groups. It demonstrates that simply increasing maternal allocation to egg size is not sufficient to produce a phenotypically lecithotrophic larva.

Previous work in *S. benedicti* showed that many of the traits associated with life history strategy are highly heritable. Levin *et al.*, (1991) estimated heritability from crosses between populations of planktotrophic and lecithotrophic parents within Bogue Sound, NC. While our phenotypes also show a strong genetic component, our results are not directly comparable to theirs as the only overlapping measurement is presence of larval chaetae. However, Levin *et al.*, (1991) measured a different phenotype: proportion of families within a cross that had chaetae present, whereas we measured variation in chaetae number within a family. They found that the presence of chaetae is highly heritable (93.9%), but they did not recover a significant maternal effect for chaetal presence.

*S. benedicti* evolved poecilogony from an ancestor with a single life-history strategy, most likely planktotrophy (Mahon *et al.*, 2009). Lecithotrophy could have evolved through the accumulation of small-effect changes throughout the genome, but our results suggest that only one or a few genes (genomic regions) of large effect can account for major reduction in the length of larval chaetae. A complete accounting of this trait’s evolution requires genetic dissection of the maternal effects, which awaits analysis of $F_2$ and subsequent generations.

Reconciling the phenotypic results from $F_1$ and $F_2$ populations with the persistence of poecilogony is challenging. Our results show that random mating in *S. benedicti* would result in heterogeneous populations that include intermediate larval phenotypes, which are absent from most natural populations (with rare exceptions: Levin and Huggett, 1990). Our study and others (Levin *et al.*, 1991; Schulze *et al.*, 2000) show that planktotrophic and lecithotrophic worms can freely mate in the lab. Further, gene flow is occurring in natural populations (Zakas and Wares, 2012). The ultimate explanation may involve ecologically structured metapopulation dynamics and directional gene flow (Zakas and Hall, 2012), and discovery of the causal genes for lecithotrophy will facilitate our dissection of its ecological genetic basis. In the meantime, we show that a fraction of $F_1$ larvae are indistinguishable in gut length and chaetal traits from their pure-morph parents, with the variable penetrance of chaetal growth playing a central role. In $Lx$ crosses (lecithotrophic mothers and planktotrophic fathers), ~30% of $F_{1,6}$ larvae are phenotypically similar to LL larvae. Assuming these individuals are subject to the same selection pressures as LL larvae, this could account for gene flow in the face of strong selection against intermediate phenotypes. $F_{1,6}$ larvae (planktotrophic mothers and lecithotrophic fathers) have less phenotypic overlap with BB larvae, but some overlap occurs. Occasionally, $F_1$ larval migrants could provide the gene flow required to homogenize the genomic background among populations while strong selection preserves differentiation at the loci directly responsible for larval phenotypes. Genetic analysis offers the prospect of eventually discovering those loci.

**Materials and Methods**

**Crosses**

Individuals derive from two populations. Lecithotrophic animals from Long Beach, CA, and planktotrophic animals from Bayonne, NJ, have been maintained under common conditions in the lab. The Long Beach larvae are lecithotrophic in the sense that they need not feed prior to settlement. However, they are facultatively planktotrophic, eating when fed (Pernet and McHugh, 2006; and personal observation). Both Bayonne and Long Beach have been sampled multiple times in previous years and the offspring mode recovered is consistent (Rockman, 2012; Zakas and Wares, 2012). Larvae from wild-collected adults of these populations were collected and planktotrophic larvae were fed an excess of *Dunaliella salina* algae until settlement. Lecithotrophic offspring were collected and transferred immediately to new sediment. Juveniles and adults were kept individually in wells of 6-well cell-culture plates with artificial seawater (Instant Ocean, 28 ppt in milliQ water) and ~1 ml defaunated mud slurry, which completely covers the bottom of the well. The mud averages 0.5 g dry weight per ml of slurry. The mud, collected from a tidal mudflat in Newark Bay, New Jersey, was sieved through 250μm mesh, frozen at ~80°C, heated to 55°C, and then returned to room temperature for use. Water and mud were changed bi-
weekly. Virgin females were placed with reproductive males (determined by
the presence of spermatophores in the well) and checked daily for broods.
egg size was measured for a representative female of each type by remov-
ing oocytes or early embryos from brood pouches and imaging them on
a slide at 20x magnification as described below for the larvae. Crosses were
performed in both directions to assess maternal effects. Animals from a
single F2 family were used as parents in the backcross and F2 families
to minimize genetic variation. Each class of cross is represented by larvae
from one to five full-sib families as listed in Table 1.

Measurements
Larvae were collected and measured at release from maternal brood
pouch, prior to larval feeding, and imaged on a Zeiss Axio Imager A2
microscope with achromatic 10x and 20x dry objectives. Because larvae
were measured prior to feeding, differences in phenotypes are due strictly
to maternal and zygotic developmental factors. Pictures were taken of live
larvae, which were slightly compressed under a coverslip on a depression
slide coated with 400 μl of 0.2% agarose in artificial seawater, which limited
their movement and allowed for image capture (Fig. 1). Some larvae were
fixed prior to measurement. These were relaxed in 7% MgCl2 and trans-
ferred to 4% formaldehyde in seawater overnight, followed by transfer to
PBS. Comparative measurements between fixed and live larvae showed
 fixation did not have an effect on larval size or chaetal measurements.
Measurements of larval gut length, chaetal length, and number of chae-
tae per chaetal sac were recorded in ImageJ (Schneider et al., 2012). To
account for possible variation due to focal plane, three pictures of each
larva were taken in different focal planes so that replicate measurements
of each trait could be averaged.

Gut length was measured by tracing the dorsal edge of the gut in ImageJ.
We averaged three replicate measurements (focal planes) for each larva,
and we log-transformed these averages to generate a normally-distributed
variable. The gut length dataset includes observations for 1,041 larvae.
The number of larval chaetae was determined by counting chaetae on one
side of each of 1,070 larvae. The length of the longest observed chaeta
was studied in a dataset including only the 674 larvae that have chaetae.

Fitting models of inheritance and estimating maternal and zygotic
effects
With data on nine distinct classes of cross between lecithotrophic
Long Beach animals (L) and planktotrophic Bayonne animals (B), we can
separately estimate the effects of maternal and zygotic differences between
the two populations on each phenotype. Our approach to this line-cross
dataset follows the line-cross mean analysis laid out by Lynch (1991) and
references therein, and described in Lynch and Walsh (1998, chapter 9),
with modifications to incorporate maternal effects. Briefly, we model the
mean larval phenotype of each class of cross as a function of the fraction
of its genome that derives from the Bayonne population, the fraction of
the animals in the line-cross that are expected to be heterozygous for alleles
that differ between B and L, and the type of egg that the larva develops
from (Fig. 2).

To isolate line-cross means from family-level effects (e.g., shared
environment), we used mixed-effect linear models, incorporating family
as a random effect. Family includes maternal effects that are unique to
individual females. We estimate the composite additive effect of zygotic
gene action, \(\alpha_y\), by regressing phenotype on each family’s Bayonne gene
content (the source index, \(\theta_S\)), using the F2 population as a reference point.
In this formulation, \(\theta_S = 0\) in the F2 and other populations that carry equal
proportions of Bayonne and Long Beach alleles, -1 in pure Long Beach
animals, 1 in pure Bayonne animals, and -0.5 and 0.5 in each backcross.
We extend this regression approach to estimate dominance effects, \(\delta_P\).
The expected fraction of heterozygotes in each population, relative to the
F2 population, is the variable \(\theta_D\) (the hybridity index). In this formulation, F2
and backcross populations, which are expected to be 1/2 heterozygous,
have a hybridity index of 0, while F1s (completely heterozygous) have an
index of 1 and the parental populations have an index of -1.

To estimate maternal effects that are due to egg type, we modeled an
additive maternal effect, \(\alpha_M\). Larvae derived from large eggs carry an \(\alpha_M\)
coefficient (which we term \(\theta_M\)) of 1, F, eggs 0, and small eggs -1. To ac-
commodate nonadditively (i.e., F, eggs conferring maternal effects that are
not perfectly intermediate to large and small eggs), we also considered a
dominant maternal effect, \(\delta_M\). Its coefficient (\(\theta_M\)) is 1 in F-derived eggs,
and 0 in large and small eggs. Coefficients for the nine cross class line
means are shown in Table 2 and schematized in Fig. 2.

We examined nested models incorporating the random effect of family
only (null) and successively adding the additive and dominant maternal and
zygotic effects, \(\alpha_y\), \(\alpha_M\), \(\delta_P\), and \(\delta_M\) in the order of their anticipated effect size.
The complete model for a measured phenotype of \(y\) larva in family \(i\) is

\[ y_i = \mu + \alpha_y \theta_y + \alpha_M \theta_M + \delta_P \theta_P + \delta_M \theta_M + \text{family} \text{, random} + \text{error.} \]

All analyses were performed in R (R Core Development Team, 2011),
version 2.14.1. Models for the chaetal length and log gut length pheno-
types were fit by REML using the R function \texttt{lmer} (Bates et al., 2011). The
chaetal number phenotype includes a large number of observations of
zero chaetae, and the data are not conducive to analysis with Gaussian
assumptions about error. We instead fit a Poisson generalized linear mixed-
effect model using the \texttt{glm} function, proceeding otherwise as above.
Model fit was tested by comparing log likelihoods with a chi-square test.
We also report alternative model-fit criteria (AIC and BIC), which support
identical conclusions.

The maternal effects are themselves heritable, as both Bayonne
planktotrophs and Long Beach lecithotrophs breed true in the common
environment in the lab (and as documented by Levin et al., 1991). How-
ever, our crosses do not allow segregation of egg-size or other maternal
effects, as we observed larvae only through F1 and backcross generations.
As a result, maternal effects are correlated with zygotic effects; across
the three egg size classes, larvae derived from planktotrophic eggs have
more Bayonne genes than larvae derived from lecithotrophic eggs (see
Fig. 2). Consequently, we can only compare zygotic and maternal effects
when both terms are simultaneously included in our models. Reported
effect estimates therefore derive from the full model, even in cases where
individual terms did not achieve formal significance.

We estimated the effective number of segregating factors affecting lar-
val chaeta length using Lande’s (1981) modifications of the Castle-Wright
estimator (Wright, 1968), adapted to the peculiarities of the phenotype as
described in the results.

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