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# Developmental Plasticity in *Macrophiothrix* Brittlestars: Are Morphologically Convergent Larvae Also Convergently Plastic?

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**Abstract.** The pluteus larval forms of sea urchins (echinoids) and brittlestars (ophiuroids) use an internal skeleton to project arms that bear a long ciliated band used in swimming and feeding. The length of this ciliated band influences rates of maximum food clearance for larvae of both echinoderm classes and affects rates of growth and development in the plankton. Phylogenetic and morphological evidence, however, tend to support the view that the pluteus morphologies of the two classes are independently derived. Studies with echinoplutei have shown that investment in skeletal growth and ciliated band length changes in response to food conditions, with poorly fed larvae investing more in growth of the larval skeleton and arms either absolutely or in relation to other larval or developing postlarval structures. We present evidence for similar plasticity of skeletal growth in ophioplutei. We examined four species in the brittlestar genus *Macrophiothrix* that spanned a 3.8-fold range in egg size. Sibling larvae in 14 male-female crosses were reared with high (H) or low (L) food rations, and measurements were recorded for five skeletal arm rods and three non-arm body dimensions. The expression of adaptive plasticity (significantly longer arms in L *versus* H cultures on a given day) was apparent for most crosses in *M. koehleri,* the species with the smallest egg size. In the single cross for *M. longipeda,* larvae from L cultures had longer arms for their body length or stomach width than did larvae from H cultures. In these cases, plasticity was similar in timing, persistence, and magnitude to previously published results from echinoplutei. If internal skeletons are independently derived in the two classes, then plasticity in the expression of this homoplastic trait may itself be homoplastic.

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*Abbreviations:* AL, anterolateral (arm rod); BL, body length; BR, body rod; H, high food ration; L, low food ration; M, medium food ration; PD, posterodorsal (arm rod); PL, posterolateral (arm rod); PO, postoral (arm rod); SW, stomach width.

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## Introduction

Phenotypic plasticity allows many organisms to adaptively match trait expression to environmental conditions (Smith-Gill, 1983; Newman, 1992; Sultan, 2000; Wells and Pigliucci, 2000; Arsenault *et al.,* 2001). Despite the value of comparative methods for the study of adaptation (Huey, 1987; Harvey and Pagel, 1991) and calls for their increased use in research on plasticity (Doughty, 1995; Gotthard and Nylin, 1995), few studies have compared multiple populations or species that have known phylogenetic relationships to understand patterns in the relationship between plasticity and other functional characters (Pigliucci *et al.,* 1999; Rochet, 2000; Johnson, 2001; Van Buskirk, 2002; Hoffmann and Franco, 2003; Morey and Reznick, 2004).

Comparative methods allow evaluation of at least two kinds of hypotheses about the evolution of plasticity. The first involves cases in which a functional trait is shared among lineages by common descent. Closely related species then provide a common genetic background for assessing the evolution of correlated traits (Felsenstein, 1985; Huey, 1987). In such cases, are differences among species or populations in the degree of plasticity related to variation in ecological factors or other phenotypic traits (*e.g.,* Levitan, 2000; Morey and Reznick, 2004)? The converse question, less often explored, involves cases in which phylogenetic information suggests that similar traits in two or more taxa are independently derived (Strathmann and Eernisse, 1994; Day, 2002). When homoplasies have arisen from common

functional demands (by convergence or parallelism), then spatial or temporal variation in those demands could also select for similarities in the plastic expression of those traits (Hodin, 2000; Meinzer, 2003). In such cases, has plasticity in trait expression also evolved convergently?

A classic example of adaptive developmental plasticity involves the larval morphology of several species from the echinoderm class Echinoidea (Boidron-Metairon, 1988; Fenaux *et al.,* 1988). Echinoderm larvae collect food from suspension by using a ciliated band along the margins of the larval body (Hart, 1991). In the pluteus larva of echinoids, this ciliated band is elongated by the growth of several sets of arms supported by an internal skeleton. Echinoplutei express plasticity by developing arms that are absolutely longer, or longer relative to other body features, when reared under more limiting food conditions (Boidron-Metairon, 1988; Fenaux *et al.,* 1988; Strathmann *et al.,* 1992; Hart and Strathmann, 1994; Eckert, 1995; Miner, 2005; Sewell, 2005). Plasticity in skeletal growth is thought to be adaptive because (1) the rate at which food is cleared from suspension is correlated with ciliated band length (Hart and Strathmann, 1994), (2) the rate of development in nature or on natural diets is often food-limited (Paulay *et al.,* 1985; Fenaux *et al.,* 1994), (3) pre-metamorphic development in the plankton is associated with high *per diem* mortality risk (Rumrill, 1990), and (4) investment in arms appears to be traded off against investment in other larval or juvenile structures (Strathmann *et al.,* 1992; Miner, 2005).

Although echinoid larval form is a model example of tradeoffs governing the evolution of plasticity, little is known about the presence or degree of plasticity across closely related species or in related echinoderm classes (George, 1994; Strathmann *et al.,* 1994). Ophiuroids (brittlestars) are of special interest because they produce a feeding pluteus larva that is similar in structure and function to the echinoid pluteus. Pluteus-type larvae from these two classes show greater efficiency of particle capture than larvae from other echinoderm classes, which lack internal skeletons (Hart, 1996), suggesting that the larval skeleton provides similar functional benefits in the two classes. Phylogenetic and developmental evidence, however, supports the hypothesis that pluteus skeletons were gained independently in the two classes (Hendler, 1978; Smith *et al.,* 1993; Strathmann and Eernisse, 1994; Hotchkiss, 1995). If convergence had been driven by common functional roles of the skeleton in positioning a ciliated band for locomotion and feeding (Emlet, 1991), then variation in food conditions would also be predicted to result in convergence for plasticity of skeletal growth.

The species-rich brittlestar genus *Macrophiothrix* is common in certain Australian coral reef communities. Adults of many species co-occur on reefs, feed in similar ways, and introduce feeding pluteus larvae into nearby pelagic environments (Hoggett, 1991). Despite these similarities, species show remarkable variation in developmental traits, related to at least 60-fold variation in egg volume (unpubl. data). In echinoderms, egg size is correlated with many aspects of development and larval form and has been hypothesized to influence the degree of larval plasticity (Herrera *et al.,* 1996). Although previous reports have speculated about an evolutionary relationship between egg size and plasticity (Strathmann *et al.,* 1992; Hart and Strathmann, 1994; George, 1999), no published studies have compared the plasticity of close relatives that have different egg sizes and known phylogenetic relationships.

Evaluation of plasticity in a trait such as larval arm length presents at least three challenges. (1) Because arms grow during the larval phase and are resorbed near metamorphosis, arm length has no definable endpoint that can be plotted as a simple function of the environment (*i.e.,* as a reaction norm; Gotthard and Nylin, 1995; Dufty *et al.,* 2002). For traits under continuous development, differences between environments must be compared to some reference, typically age or size. (2) Arm length at a given age or size is determined both by the absolute amount of resource invested in growth and by its relative allocation to arms *versus* other structures. Because resources available for growth accumulate faster in well-fed cultures, treatment differences due to the first factor (resource gain) could mask treatment differences in the second factor (adaptive plasticity). The rates at which the two factors respond to food intake will determine when (if at all) during development plasticity is apparent. Studies of echinoid echinoderms have addressed this dilemma by measuring arm length relative to the size of the developing juvenile rudiment, so that plasticity is interpreted as involving a tradeoff between investment in larval and postlarval structures (Hart and Strathmann, 1994; Miner, 2005). In contrast, juvenile structures in ophioplutei become well-defined closer to metamorphosis, making such relative measures more difficult (Strathmann *et al.,* 1994). (3) Larval shape can change during development (McEdward, 1986; Hart and Scheibling, 1988), so even if arm size can be measured relative to body size, feeding treatments could differ in the stage of development for a given body size. These issues complicate the interpretation of data for inferring plasticity in a continuously developing trophic character.

To address whether ophiuroid larvae demonstrate plasticity of skeletal growth, and to examine the effect of egg size, we measured variation in larval form for four sympatric congeners in the genus *Macrophiothrix.* Adults of the four species co-occur on reefs, are similar in size and microhabitat, and produce feeding pluteus larvae. Egg volume varies 3.8-fold among these species and scales directly with growth and development rate, indicating that volume reflects energy content (Allen and Podolsky, unpubl. data). We measured larval form under different food concentra-

tions, and we standardized arm measurements relative to age and size, to address the following questions. (1) Do ophioplutei in *Macrophiothrix* show adaptive plasticity of skeletal growth? (2) Is the timing or magnitude of plastic expression comparable to what has been measured for echinoid larvae? (3) Does standardizing arm length relative to body size aid the detection of plasticity in a developing trait? (4) Do larvae from species with smaller eggs show greater plasticity than those from species with larger eggs?

## Materials and Methods

Adult brittlestars were collected from reefs near the Lizard Island Research Station (LIRS), Queensland, Australia, and maintained in covered tanks with flowing seawater. Spawning was induced in females by a combination of physical stresses (Selvakumaraswamy and Byrne, 2000; Allen and Podolsky, unpubl. data) and in males by injecting a trace volume of 0.5 *M* KCl into the body cavity. Average volumes of eggs of the four congeners—*Macrophiothrix koehleri* Clark, *M. lorioli* Clark, *M. longipeda* Lamarck, and *M. rhabdota* Clark—span a 3.8-fold range (Table 1). Larvae of the three species with smaller eggs require particulate food and 3 to 4 weeks to complete metamorphosis, whereas those of *M. rhabdota* are facultative feeders that develop faster and to larger size when reared with food, but can complete metamorphosis without feeding (Allen and Podolsky, unpubl. data). These four species occur as two pairs of close relatives (*longipeda* + *koehleri, lorioli* + *rhabdota;* Table 1) among at least eight other species within a strongly supported clade, as inferred from mitochondrial DNA sequences (Hart and Podolsky, 2005).

The bilaterally symmetric calcite larval skeleton includes a pair of short posterior body rods (BR) and four pairs of arm rods: posterolateral (PL), anterolateral (AL), postoral (PO), and posterodorsal (PD). PL and BR form a continuous axis (PL + BR), AL and PO both project from a common proximal position on PL + BR, and PD projects from a proximal position on AL (Fig. 1). The narrow band of cilia used to generate water currents for locomotion and feeding runs along the sides of each arm and between the arms in a continuous loop. Phytoplankton is concentrated from suspension by local reversals of ciliary beat (Hart, 1991). With regard to the measurements reported, at least three morphological features distinguish ophioplutei from echinoplutei: (1) arm growth is dominated by PL arms rather than being distributed more evenly among the four arm pairs; (2) all arm pairs are initiated within the first 24 to 48 h of development, rather than sequentially and gradually during development (McEdward, 1986); and (3) juvenile structures become distinct close to the time of metamorphosis, rather than as part of a well-defined juvenile rudiment formed throughout the latter half of development (Strathmann *et al.,* 1992). As a result, few developmental landmarks exist for comparing the growth of body structures, and we were limited to measuring arm growth in relation to larval body size rather than to the size of developing postlarval structures (*cf.* Strathmann *et al.,* 1992).

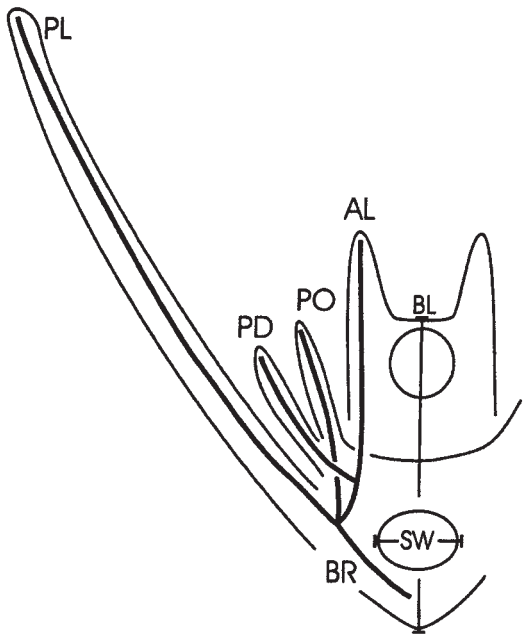


**Figure 1.** Diagram of left side of brittlestar larva (dorsal view) showing size measurements recorded. Thin curved lines represent soft tissue edges, and the five labeled thick lines are the main internal calcite skeletal rods. PL = posterolateral arm; AL = anterolateral arm; PD = posterodorsal arm; PO = postoral arm; SW = stomach width; BL = body length, measured from posterior end to tissue bridge between the AL arms (except for $koe_1$, where it was measured to the tissue bridge between PO arms).

**Table 1**

*Mean egg diameter and volume (assuming a sphere) and role of feeding during larval development* (Allen and Podolsky, unpubl. data) *among four* Macrophiothrix *species in this study*

| Species | Diameter (mm) | Volume (nl) | Feeding mode |
|---|---|---|---|
| *M. koehleri* | 0.147 | 1.66 | obligate |
| *M. longipeda* | 0.155 | 1.95 | obligate |
| *M. lorioli* | 0.166 | 2.40 | obligate |
| *M. rhabdota* | 0.230 | 6.37 | facultative |

The total genetic distance separating the two pairs of close relatives (*longipeda*+*koehleri* and *lorioli*+*rhabdota*) is 30% or 160%, respectively, greater than the distances separating sister species from each other (Hart and Podolsky, 2005).

Larvae were cultured at LIRS during short visits from October 1999 to February 2003, using established methods for fertilization and larval culture (Strathmann, 1987). Larvae were reared at densities of approximately 1 $ml^{-1}$ in 1-1 containers stirred gently by paddles at about 7 strokes $min^{-1}$ and held at ambient seawater temperatures (29 °C) in a water bath. Seawater was 0.45-$\mu$m filtered before use. In

feeding treatments, each container received a single type of alga, usually *Dunaliella tertiolecta* (CSIRO strain CS-175) but in a few cases *Isochrysis sp.* (both from DPI, Cairns, Australia). We assumed that regardless of food type, larvae fed a high ration would have better nutrition than those fed a low ration; in previous work, the nutritional quality of food types did not strongly affect the degree of larval plasticity (Klinzing and Pechenik, 2000). Algae were cultured at room temperature in autoclaved seawater enriched with a modified Guillard's f/2 medium (Florida Aqua Farms, Inc.) and were resuspended in fresh seawater before measurement and use.

Because larvae were reared for use in various other experiments, feeding levels and measurements differed somewhat among crosses reported here. Five crosses had larvae divided into cultures that received 10 (H), 1 (M), or 0.1 (L) algal cells per microliter (high, medium, and low, respectively). In nine other crosses, cultures received either 7.5 (H) or 0 (L) cells per microliter. Both H levels are well above saturating food concentrations, whereas both L levels are insufficient to complete development (Strathmann, 1971; Hart, 1996; Sewell, 2004). Nevertheless, even unfed larvae can express plasticity (Boidron-Metairon, 1988) and develop beyond the period when plasticity is expressed. In feeding treatments, food was added to containers daily, starting 24 h after fertilization, and water was changed every other day. Information was collected for six independent crosses of *M. koehleri,* five of *M. lorioli,* two of *M. longipeda,* and one of *M. rhabdota.* For convenience, cross IDs are abbreviated with the first three letters of the specific epithet and a number (*e.g., koe*$_4$). Details for all crosses are summarized in Table 2.

### *Larval measurement and analysis*

We removed 5 to 10 larvae from each container (Table 2) on a series of days (see Results) for measurements of larval morphology. Larvae were immobilized using a highly dilute formalin solution and mounted under a coverslip elevated by clay feet. Viewing the slides under magnification, we made camera lucida drawings of seven skeletal landmarks on the right side of each larva, and for some cultures, soft-tissue measurements of body length (BL, the posterior end to the tissue bridge between AL arms or PO arms) and stomach width (SW; see Fig. 1). Lengths of individual arm and body rods were reconstructed in three dimensions using the *x* and *y* coordinates from the drawings and *z* coordinates obtained from a rotary encoder coupled to the fine focus knob of the microscope (McEdward, 1985). The seven landmark coordinates allowed measurement of the lengths of five skeletal elements (PL, AL, PD, PO, BR) as described above. In two years we lacked the capacity to reconstruct larval measurements in three dimensions. For those cultures we recorded ocular micrometer measurements of the sum PL + BR, as well as of body length, which each lie within a plane of focus. Fed cultures of *M. rhabdota* began to metamorphose by day 5 and are not included in measurements.

We evaluated PL arms separately from the sum AL + PO + PD because (a) PL arms dominate skeletal growth;

**Table 2**

*Summary of conditions for crosses used in* Macrophiothrix *plasticity experiments, including food type, food levels, arms and body dimensions measured, number of containers maintained per treatment, and number of larvae measured per container at each time point*

| Species | Cross ID | Start date | Food type | Food level (cells $\mu l^{-1}$) H | M | L | Arm measures | Body measures | Number of containers | Measures (cont day)$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. koehleri* | *koe*$_1$ | 10.25.99 | D | 7.5 | | 0 | PL + BR | BL | 2 | 10 |
| | *koe*$_2$ | 12.17.00 | D | 7.5 | | 0 | PL + BR | BL,SW | 1 | 10 |
| | *koe*$_3$ | 12.20.00 | D | 7.5 | | 0 | PL + BR | BL,SW | 1 | 10 |
| | *koe*$_4$ | 12.10.01 | D | 10 | 1 | 0.1 | PL,AL,PO,PD | BR | 2–3 | 5 |
| | *koe*$_5$ | 12.24.01 | I | 10 | 1 | 0.1 | PL,AL,PO,PD | BR | 2 | 5 |
| | *koe*$_6$ | 01.20.02 | I | 10 | 1 | 0.1 | PL,AL,PO,PD | BR | 2 | 5 |
| *M. lorioli* | *lor*$_1$ | 11.29.00 | D | 7.5 | | 0 | PL + BR | BL,SW | 1 | 10 |
| | *lor*$_2$ | 12.08.00 | D | 7.5 | | 0 | PL + BR | BL,SW | 1 | 10 |
| | *lor*$_3$ | 12.09.00 | D | 7.5 | | 0 | PL + BR | BL,SW | 1 | 10 |
| | *lor*$_4$ | 11.30.99 | D | 7.5 | | 0 | PL + BR | BL | 1 | 10 |
| | *lor*$_5$ | 01.07.02 | I | 10 | 1 | 0.1 | PL,AL,PO,PD | BR | 2 | 5 |
| *M. longipeda* | *lon*$_1$ | 12.21.00 | D | 7.5 | | 0 | PL + BR | BL,SW | 2 | 5 |
| | *lon*$_2$ | 02.20.03 | D | 10 | 1 | 0.1 | PL,AL,PO,PD | BR,BL,SW | 2 | 5 |
| *M. rhabdota* | *rha*$_1$ | 02.21.02 | D, I | 7.5 | | 0 | PL,AL,PO,PD | BR,BL,SW | 2 | 5 |

Food types (only one type used per culture): D = *Dunaliella tertiolecta,* I = *Isochrysis* sp. Arms: PL = posterolateral, AL = anterolateral, PD = posterodorsal, PO = postoral, BR = body rod, BL = body length, SW = stomach width. For arm and body measurements, see Fig. 1.

(b) we had measurements of PL arms (or the sum PL + BR) for all cultures; and (c) they are the only arm pair retained even in other *Macrophiothrix* species that lack feeding development (*e.g., M. nereidina* and *M. belli;* Allen and Podolsky, unpubl. data), suggesting they may be under different selective pressures, given the dual roles of the ciliated band in swimming and feeding (Grunbaum and Strathmann, 2003).

We carried out three tests for plasticity while controlling separately for the effects of age and size. First, arm lengths on a given day were used to compare differences in the absolute growth of feeding structures. Second, arm lengths in proportion to other body features (BR, BL, and SW) were used to compare differences in relative allocation to feeding and nonfeeding structures, as described earlier. Third, the total length of the smaller arms (AL + PD + PO) in proportion to the length of the PL arm was used to compare differences in relative allocation to arms that are lost in nonfeeding species with the pair that is retained. For some crosses, only the size of skeletal elements was recorded (soft tissue had been dissolved away with dilute bleach); in these cases we did not have a useful body size measure, because BR did not increase significantly during the period of arm growth (see Results).

### *Statistical models*

For each male-female cross, we used linear mixed-models (SPSS, ver. 12.0) to assess absolute differences in larval arm lengths on individual measurement days, with food level as a fixed factor and container as a random factor (Boidron-Metairon, 1988; George, 1994; Eckert, 1995). We compared models with and without the random term and used the model that provided the better fit to the data using Akaike's information criterion (AIC) (Littell *et al.,* 1996). Because better-fed larvae always became absolutely larger after a week of development, the choice of when during development to stop statistical comparisons between treatments was arbitrary. As a result, it was unreasonable to apply traditional multiple comparison corrections to alpha, and sequential Bonferroni adjustments (Rice, 1989) would likewise be appropriate only for a fixed number of tests. We therefore report all tests based on $\alpha = 0.05$, acknowledging the potential for type I error across days. In a second analysis, for cultures where we had measures of body size (BL or SW), we used analysis of covariance (ANCOVA) to analyze the effect of food level on arm length while controlling for body size, and on the length of AL + PD + PO arms while controlling for PL length. For ANCOVAs, we analyzed only the periods when arm size and body size were both undergoing regular growth. In a small number of cases, data were transformed prior to analysis to meet normality assumptions.

For several cultures (*koe$_2$, koe$_3$, lor$_1$, lor$_2$, lor$_3$, lor$_4$, and lon$_1$;* Table 2), we had measurements for only one container per food level. As a result, we were forced to combine cultures as replicates for analyses (*koe$_2$-koe$_3$, lor$_1$-lor$_2$, and lor$_3$-lor$_4$; lon$_1$* had no paired culture and was dropped from the analysis). Because measurements for these paired cultures were taken at different times, we fit cubic *b*-splines (Schluter, 1988) to the container means for each food level, using cross as a covariate, to estimate patterns of growth. A "best-fit" cubic spline, as determined by adjusting the smoothing parameter $\lambda$ in Schulter's (1988) program, is a smooth but unconstrained function that best predicts the actual values as they are sequentially omitted during the fitting process. Differences between food levels were judged by non-overlap of 95% confidence intervals around splines, which provides a conservative estimator of significant differences between treatments (Schenker and Gentleman, 2001).

Tests of food level were one-tailed; our null hypothesis for evidence of plasticity was that high-fed cultures would show equal or greater absolute growth, given the greater level of energy intake (Strathmann, 1985). Under the alternative hypothesis of adaptive plasticity, arm lengths were predicted to be absolutely or relatively longer under poorer feeding conditions. We tested this directional prediction, considering only the two extreme food levels (*i.e.,* L > H), by performing one-tailed tests, using a *t*-statistic as calculated from $F$ ($F = t^2$) (Neter *et al.,* 1985). We use the term "plasticity" to refer only to cases in which differences between food levels were consistent with this adaptive prediction and were not just a trivial consequence of faster growth by well-fed larvae. For days when adaptive plasticity was detected, we calculated the magnitude of plasticity as the difference between L and H means divided by their average. We do not report data beyond day 10 for any cross, because larvae in high-fed cultures were always substantially larger, growth in most body features had already reached a plateau, and time constraints prevented our following most cultures through to metamorphosis.

## Results

In all four species, increases in posterolateral (PL) arms accounted for close to half of all skeletal growth (Fig. 2). Arm length reached a plateau in most crosses by about 10 days; *Macrophiothrix rhabdota* metamorphosed within 1 week, before this plateau (also observed by Allen and Podolsky, unpubl. data). Although we had planned to use body rod (BR) as a nontrophic character for measurement of body growth, BR length did not change significantly over the same interval (Fig. 3). BR increased by about 8% in two of the seven cultures for which we measured BR separately from PL (ANCOVA; *koe$_6$:* $F_{1,72} = 6.8$, $P = 0.11$; *lon$_2$:* $F_{1,55} = 4.8$, $P = 0.033$), and it decreased in a third culture by about 20% (*lor$_5$:* $F_{1,63} = 31.3$, $P < 0.001$). Food level

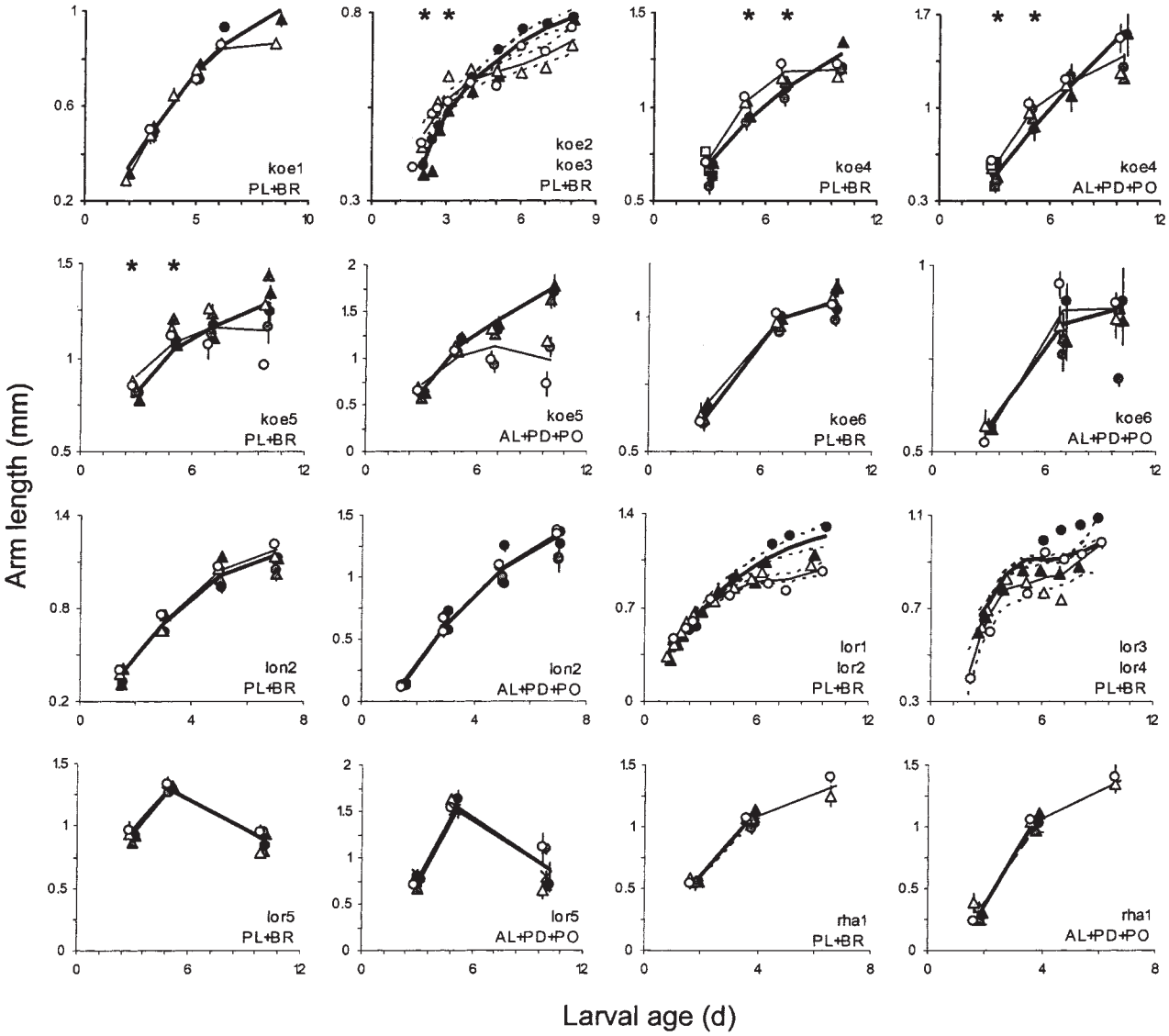


**Figure 2.** Changes in absolute arm length over time for cultures from high (H, black symbols) and low (L, white symbols) food treatments. Each point represents the mean ± SE per container for each measurement time; symbol shapes represent different containers or crosses. For comparison, points are included for medium food levels (M, grey symbols) when available, although these points were not included in the analysis. (For *Macrophiothrix rhadboda* only, grey symbols and the dashed regression line are for cultures fed *Isochrysis sp.* at high ration). Cultures are identified as in Table 2, and arm rods included in measurements were either the posterolaterals, including the short body rod (PL + BR) or the sum of the three other arms (AL + PD + PO), as indicated. In each graph, solid lines are cubic splines (Schluter, 1988) fitted to container means separately for H (thick line) and L (thin line) food treatments. In cases where replicate containers were analyzed within crosses, rejection of the null hypothesis in favor of the alternative ($H_a$: L > H) for a given measurement day (asterisk, $P < 0.01$) was based on a one-tailed test using mixed-model ANOVA. In cases where replicate crosses were combined for analysis, rejection of the null hypothesis was based on non-overlap of 95% confidence intervals (dotted lines), a conservative estimator of differences between treatments (Schenker and Gentleman, 2001).

did not have a significant effect on BR in any cross (ANCOVA, all $P > 0.05$). Because BR growth was trivial compared to PL growth, did not differ between food levels, and accounted for only 17% to 9% of the total length of PL + BR, for consistency we report analyses of PL + BR for all crosses. In the seven crosses for which we had separate measures of PL and BR, analyses of PL + BR and PL alone reached the same statistical conclusions.

In three of five comparisons involving *M. koehleri,* early growth rate of PL + BR was faster in low-fed (L) cultures

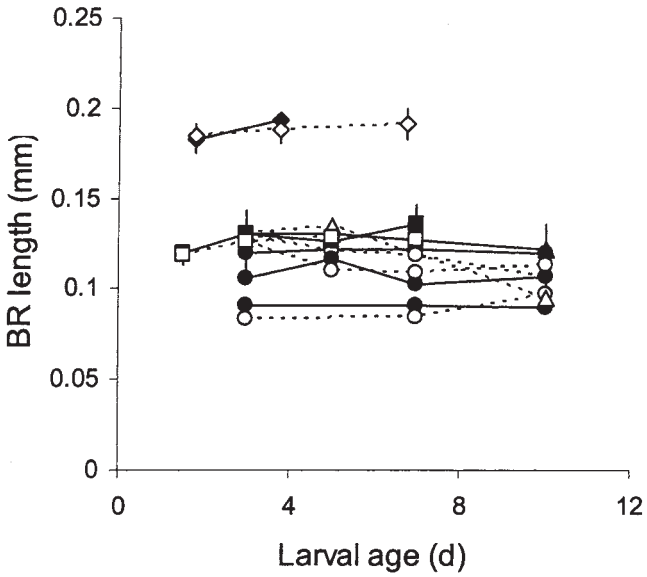


**Figure 3.** Changes in the length of the body rod (BR) over early development. Each point is the average ± SE for all individuals from a given male-female cross measured on a given day. Although we intended to use the size of the body rod as a measure of investment in and growth of a nontrophic structure, BR length remained static during the period of maximum arm growth. Solid symbols and lines refer to H cultures, open symbols and dashed lines refer to L cultures. Symbols: circle = *Macrophiothrix koehleri,* square = *M. longipeda,* triangle = *M. lorioli,* diamond = *M. rhabdota.*

than in high-fed (H) cultures—a pattern that is consistent with adaptive plasticity of arm growth under low food conditions (Fig. 2). This treatment difference in growth rates was rapidly reversed as development continued. In all other cultures, PL + BR growth was initially similar at the two food levels or was more rapid in H cultures throughout development. One of the six comparisons of the summed length of anterolateral, posterodorsal, and postoral arm rods (AL + PD + PO), again involving $koe_4$, showed evidence of significant adaptive plasticity (Fig. 2).

When treatment differences in PL + BR were analyzed controlling for body size, adaptive plasticity was detected in one ($lon_2$) of six comparisons for which we had measurements of BL ($F_{1,45} = 14.6$, $P < 0.001$; Fig. 4). Considering the two crosses ($lon_2$ and $rha_1$) with measures of both BL and the other three arm pairs, $lon_2$ again showed a significant effect of food level on the summed length AL + PD + PO ($F_{1,41.3} = 7.8$, $P = 0.008$; Fig. 4). Conclusions of analyses using stomach width (SW) as a size covariate were identical to those using BL, with significant effects of food level on PL + BR in the same comparisons (data not shown). None of six comparisons of AL + PD + PO length controlling for PL + BR length found that poorly fed larvae invested more in the three arm pairs that are lost in nonfeeding species than in the pair (PL) that is retained (ANCOVA, all $P > 0.05$).

In all four crosses where it was detected, significant plasticity was apparent as early as day 2 or 3 and was not apparent beyond day 7 (Fig. 2). Later in development, well-fed larvae had consistently grown arms that were significantly longer. Maximum plasticity of PL + BR, calculated as the difference between L and H means relative to their averages, was 16.6%, 9.0%, and 8.5% in $koe_2$-$koe_3$, $koe_4$, and $koe_5$, respectively. These values for PL + BR are probably underestimates of the magnitude of plasticity in PL arm growth, because BR did not contribute substantially to the growth of PL + BR and did not vary as a function of food level. If we assume conservatively that BR is about 10% of PL + BR (see Figs. 3 and 4), then these estimates of maximum plasticity in PL alone would be 18.4%, 10%, and 9.4%, respectively. In the single comparison ($koe_4$) that

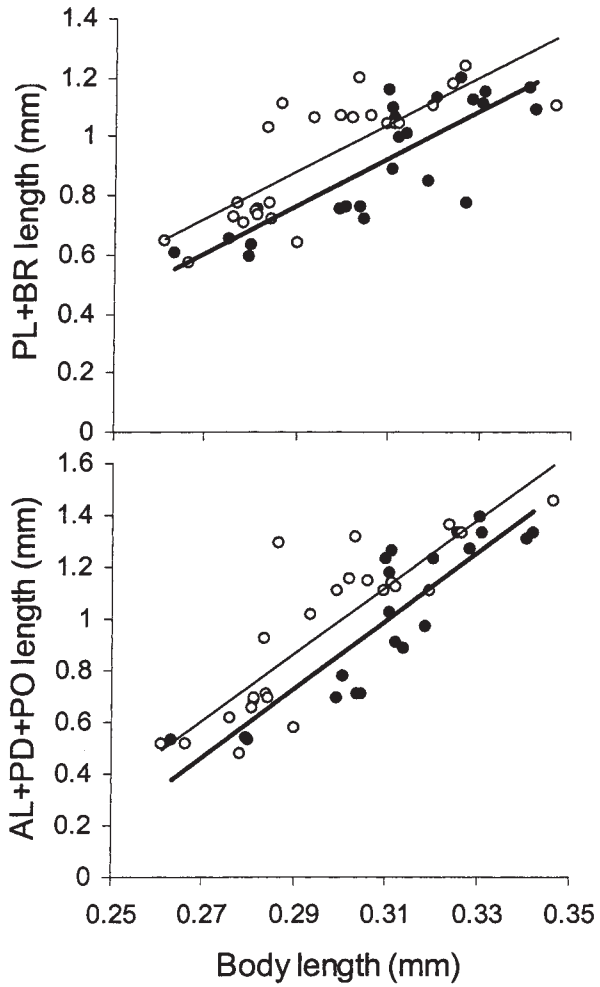


**Figure 4.** Changes in posterolateral arm-body rod length (PL + BR) and the length of the other three arm pairs (AL + PD + PO) relative to body size during development in culture $lon_2$ of *Macrophiothrix longipeda.* Data are individual measurements of larvae from H (solid symbols, thick line) and L (open symbols, thin line) food treatments. The statistical analysis controlled for the effects of container and measurement day (see text).

yielded significant plasticity in AL + PD + PO, the maximum plasticity expressed was 20%.

The need to pair cultures from distinct crosses as replicates in some cases may have resulted in a loss of our ability to resolve plasticity. For example, on one or two days, $lon_1$ (which lacked a comparable cross and thus was not analyzed), $lor_2$, and $lor_3$ individually showed evidence of plasticity—albeit under risk of pseudoreplication—not shared by the cultures with which they were paired for the analysis. Similarly, in $lor_1$ and $lor_3$ individually, PL + BR lengths were greater (controlling for BL) at low rations than at high rations.

## Discussion

Our results demonstrate plasticity of skeletal growth in ophiuroid larvae under conditions similar to those used in studies of echinoid larvae (Boidron-Metairon, 1988; Strathmann *et al.,* 1992; Eckert, 1995; Sewell, 2005). In particular, four of six crosses for *Macrophiothrix koehleri* and the single cross of *M. longipeda* showed patterns consistent with adaptive plasticity of investment in food-collecting arms. Patterns in the expression of plasticity indicate three areas for comparison with prior work. First, plasticity was most apparent early in development, before well-fed larvae grew absolutely larger, and was not detected after day 7. In crosses where plasticity was detected, low-fed cultures had absolutely greater arm lengths on no more than two measurement days. Because larvae of the three species with smaller eggs likely develop in the plankton over 3 to 4 weeks (based on laboratory culture; Allen and Podolsky, unpubl. data), and arm growth rapidly approaches a plateau, plasticity could be apparent for only a short period of development. *M. rhabdota* develops within this one-week window (Allen and Podolsky, unpubl. data), but plasticity was not detected in the cross we measured.

Second, we found some variation in the magnitude of the responses. On days when plasticity was detected, the average increase in the summed length of the posterolateral arm rod and the body rod (PL + BR) under food restriction was around 8%, and the maximum increases observed were 18% in PL and 20% in the single detection for the summed length of the anterolateral, posterodorsal, and postoral arm rods (AL + PD + PO) (Fig. 2). Because arm length is correlated with ciliated band length (McEdward, 1986), which is in turn related linearly to maximum clearance rate (Hart, 1991, 1996), the magnitudes of arm plasticity (PL and AL + PD + PO) provide a rough estimate of the gain in feeding capacity conferred by plasticity. Given the short period when plasticity is expressed and these relatively small magnitudes, absolute differences in arm length could contribute only a small percentage to improving food intake over the course of development. As in other studies, therefore, the ecological advantages of plasticity remain uncertain.

Third, we found variation in the expression of plasticity among crosses of *M. koehleri.* These differences could have resulted from variation in experimental conditions, although plasticity was detected in cultures reared during three different seasons. More likely, differences among crosses could reflect genetic variation for plasticity. We have recently found such variation in experiments with the echinoid *Lytechinus variegatus,* in which some genetic families expressed significant plasticity of arm length and others did not (unpubl. data). Variation among genetic families could result from differences in the magnitude of shifts in relative allocation or from the timing of such shifts, since plasticity is typically apparent for only a short period before it is apparently eclipsed by resource accumulation in well-fed larvae.

### *Homoplastic plasticity in larval skeletal growth?*

Recent phylogenetic evidence for echinoderm classes most strongly supports asteroids + ophiuroids as a sister group to echinoids + holothuroids (Smith *et al.,* 1993; Littlewood *et al.,* 1997; Janies, 2001). This topology implies that the internal larval skeletons exclusive to echinoids and ophiuroids were gained independently in each class or were lost in each of their sister classes. Embryological and structural differences—including the position and formation of skeletal rods, the crystalline structure of rods, and the association between rods and the formation of apical plates in echinoids—support a hypothesis of independent origins (Hendler, 1978; Strathmann, 1988; Hotchkiss, 1995; Smith, 1997). Similar functional roles in elongating the ciliated band to aid locomotion and feeding imply that larval skeletons in the two classes are a striking example of functional convergence (Strathmann and Eernisse, 1994). If these arguments about phylogeny and homology are correct, then the parallel evolution of plasticity in skeletal growth would be one of few examples in which adaptive plasticity for a homoplastic trait is itself homoplastic (Holloway *et al.,* 1997; Starnecker and Hazel, 1999).

To what degree is plasticity in the two classes similar in temporal pattern, magnitude, and consistency of expression? Although methodological details differ among studies, some parameters can be compared directly. First, the time window when plasticity was apparent is similar between our results and results gleaned from studies of echinoids. Plasticity of absolute arm length was detected on day 3 but not day 5 (when next measured) in *Encope michelini* (Eckert, 1995); on day 4 but not day 7 in *Lytechinus variegatus* (Boidron-Metairon, 1988); on day 6 but not days 4 or 8 in *Evechinus chloroticus* (Sewell, 2005); on day 3 but not day 4 in one study of *Dendraster excentricus* (Hart and Strathmann, 1994), and on days 10 and 13 but not days 6 or 16 in another study (Boidron-Metairon, 1988). Strathmann *et al.* (1992) reported statistically significant plasticity on

day 9 in *Paracentrotus lividus* but did not report tests for other days. As in our results, the signal of increased allocation to arm growth by poorly fed larvae was brief and apparently overcome by the effects of resource accumulation by well-fed larvae (Hart and Strathmann, 1994; Eckert, 1995; Sewell, 2005).

Second, the magnitude of plasticity in *M. koehleri* was also similar to reports for echinoid larvae. On the day or days that plasticity was detected, the maximum percent increase in arm length for low-fed relative to high-fed cultures, as determined from published data, was 10% in *Encope* (Eckert, 1995), 16% in *Lytechinus* (Boidron-Metairon, 1988), 8% (Hart and Strathmann, 1994) or 27% (Boidron-Metairon, 1988) in *Dendraster,* and 13% in *Paracentrotus* (Strathmann *et al.,* 1992), as compared with 9% to 17% (PL arms) and 20% (AL + PO + PD arms) for crosses in which plasticity was detected in this study.

Third, there is scant information in the echinoid literature concerning variation in plasticity among male-female crosses. Many prior studies have reported results for only a single cross (*e.g.,* Sewell, 2005), while others (Hart and Scheibling, 1988; Strathmann *et al.,* 1992; Eckert, 1995) may have used replicate crosses but did not report comparisons. Results from the two studies of *Dendraster excentricus* (Boidron-Metairon, 1988; Hart and Strathmann, 1994) show a larger difference in estimates of both timing and magnitude of plasticity than do comparisons between species (see data described above). The two *Dendraster* studies used foods of different quality and may have differed in other respects. In the absence of controlled comparisons, it is difficult to know whether the inter-cross variability we recorded is typical, but it is possible that negative results with single crosses have not been published in the past.

### *Plasticity and egg size*

Average egg volumes for *M. longipeda, M. lorioli,* and *M. rhabdota* are 17%, 44%, and 283% larger, respectively, than those of *M. koehleri.* As in other marine invertebrates, changes in egg size relate to a transition in larval dependence on food: the three smaller-egg species require food to complete metamorphosis, whereas *M. rhabdota* can complete metamorphosis without feeding. Species with greater dependence on feeding could therefore evolve greater capacity for plastic expression in feeding structures (Herrera *et al.,* 1996). Results from our interspecific comparisons are consistent with this hypothesis; evidence for adaptive plasticity was found for five of seven crosses from the two smaller-egg species, but none was found from analyses of the large egg species. Given phylogenetic relationships among these taxa, however, the two species with smaller eggs do not represent independent tests of the hypothesis.

### *Developmental plasticity across marine invertebrate taxa*

Studies of other echinoderm classes and more distant taxa indicate that larval plasticity could precede the evolution of internal skeletons. Unlike plutei, the bipinnaria larvae of asteroids lack internal skeletons, but under low food conditions the homologous ciliated band was lengthened by elaboration of body margins or by an increase in overall body size (Strathmann, 1989; George, 1994, 1999). In contrast, auricularia larvae of *Stichopus californicus,* which also lack an internal skeleton, showed no differences in body form between well-fed and poorly fed larvae (Strathmann *et al.,* 1994). Veliger larvae of *Crassostrea gigas* and *Crepidula fornicata* grown with a lower food ration showed increases of 9% and 18%, respectively, in the size of the food-collecting ciliated velum relative to the size of the shell (a structure that persists beyond larval development) (Strathmann *et al.,* 1993; Klinzing and Pechenik, 2000). Thus, plasticity in ciliated band length is a widespread response to variation in feeding conditions and may be achieved by different forms of growth. Analogous changes in the size of trophic structures have been observed in nonciliary suspension feeders (Lucas and Hunter, 1999; Repka *et al.,* 1999).

Given the need for planktonic larvae to cope with varying food supplies, what factors account for some apparent taxon differences in the level of plasticity? First, as already described, parental investment in the egg could influence plasticity by altering the dependence of development on food collection (Strathmann *et al.,* 1992). Reitzel and Heyland (2001), for example, reported greater plasticity in two sand dollars with relatively small eggs than in one with larger eggs, consistent with the evolutionary hypothesis and with our results. Second, Strathmann *et al.,* (1994) proposed that the ability to flexibly shift allocation could be of greater benefit for taxa that invested early in postlarval structures (*e.g.,* echinoids, asteroids, and molluscs) than for those that develop postlarval structures closer to the time of metamorphosis (*e.g.,* ophiuroids, holothuroids). Our results, however, reflect similarities between echinoplutei and ophioplutei in the timing, persistence, and magnitude of the absolute expression of plasticity. Likewise, Strathmann *et al.* (1994) and Strathmann (1996) briefly reported observations similar to ours with an ophiopluteus, as well as observations of faster accumulation of resources for postlarval structures in well-fed holothuroid larvae (Strathmann *et al.,* 1994). Third, ecological conditions are likely to affect the evolution of responses to food limitation. One might predict, for example, that taxa evolving under relatively constant but nutrient-poor planktonic feeding conditions would be selected to maximize growth of feeding structures that have little scope for plasticity, whereas those evolving under relatively rich but variable conditions would be selected for greater scope for plasticity. Although some of the lowest plasticity magnitudes recorded are for species from tropical or subtropical

waters (*e.g.,* this study; Boidron-Metairon, 1988; Eckert, 1995) and some of the highest are from cold temperate waters (*e.g.,* Boidron-Metairon, 1988; Hart and Scheibling, 1988), there are exceptions and the number of current examples is too small to adequately test this idea. Comparison between close relatives that occupy planktonic larval habitats with different patterns of productivity (Lessios, 1990) could provide a powerful test.

Our ability to quantitatively compare results among studies was complicated by variation in culture methods, measurements, statistical approaches, and genetic replication. Studies have used treatments with different food levels, larval densities, and feeding schedules—for example, using identical food additions at different frequencies *versus* different food additions at identical frequencies—that create variable patterns of food concentration as particles are grazed. Each of these factors affects the temporal variance in food supply, which can alter larval form independent of average feeding conditions (Miner and Vonesh, 2004). Studies have used different measurement schedules and statistics, making it difficult to compare, for example, the size of the window of plastic expression. Finally, studies have not consistently replicated results across male-female pairs. Acknowledging that studies ask different questions, we recommend that future research on plasticity in marine invertebrate larvae (1) minimize or control temporal variation in food levels within treatments, (2) establish in preliminary work which body size covariates change most consistently during growth and (3) use these as covariates in statistical analyses, and (4) replicate male-female crosses in controlled breeding designs to better estimate the contribution of genetic variance to plastic expression. Severe logistical constraints on the reliability of spawning and fertilizing *Macrophiothrix* females (Allen and Podolsky, unpubl. data) kept us from achieving some of these goals.

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