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Long-term storage lipids and developmental evolution in echinoderms

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ABSTRACT

Question: How is maternal investment of energy storage lipids linked to the evolution of development for echinoderms with larval phases?

Hypotheses: Egg nutrients sustain development to the exotrophic larval stage in echinoderms with feeding (planktotrophic) larvae and to the exotrophic juvenile stage in species with non-feeding (lecithotrophic) larvae. Whereas planktotrophic echinoderm development requires egg lipid reserves that are readily metabolized, lipids suitable for long-term energy storage might be more appropriate fuels for lecithotrophic development.

Organisms: We considered closely related asteroid and ophiuroid species that possess a range of egg sizes and represent three modes of larval development (planktotrophy, planktonic lecithotrophy, benthic lecithotrophy).

Methods: We used Iatroscan TLC-FID to quantify maternal investment of lipids on a per egg basis for each species and focused on egg content of the two dominant classes of energy storage lipid, triacylglycerol (TAG) and diacylglycerol ether (DAGE).

Results: Energetic lipids in the small eggs of echinoderms with feeding larvae are primarily TAG, a class of short-term storage lipids. DAGE, which is metabolized more slowly than TAG, dominates the large eggs of echinoderms with non-feeding larvae. Increased deposition of DAGE lipids in the eggs of planktotrophic species may facilitate the transition to lecithotrophy.

Keywords: developmental mode, diacylglycerol ether, echinoderm, evolution, Iatroscan, lipid, triacylglycerol.

INTRODUCTION

Most echinoderms have a dichotomous life history and initially develop through a larval stage before metamorphosing into a juvenile that must grow and develop further before reaching reproductive maturity (McEdward and Miner, 2001; Raff and Byrne, 2006). Echinoderm larvae may possess feeding structures and a digestive system (planktotrophy) or lack the ability to feed, being sustained by the endogenous reserves supplied in the form of egg yolk (lecithotrophy). Planktotrophic larvae exist and feed in the plankton, whereas lecithotrophic larvae

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may be planktonic, benthic or develop via external or internal brood protection. Within the Echinodermata, there is good evidence that planktotrophic development is the ancestral state from which lecithotrophic development has repeatedly evolved (McEdward and Miner, 2001; Hart, 2002; Raff and Byrne, 2006).

The evolution of lecithotrophic development is clearly tied to the emergence of new strategies of maternal investment (Sewell and Young, 1997; Byrne *et al.*, 1999a, 1999b, 2003; Villinski *et al.*, 2002; Falkner *et al.*, 2006; Prowse *et al.*, 2008). With the exception of brooding species that provide an extra-embryonic source of nutrients (reviewed by Gillespie and McClintock, 2007), each echinoderm egg must contain, at a minimum, all the energetic and structural components required to form an exotrophic larva (planktotrophy) or exotrophic juvenile (lecithotrophy). Early feeding larvae are smaller, less complex systems than feeding juveniles and so require far less material for their construction. Consequently, echinoderms with planktotrophic larvae produce smaller eggs and far more of them than lecithotrophic developers of the same body size. Feeding and non-feeding modes of development therefore represent two different approaches to the trade-off between egg size and fecundity (Strathmann, 1985; McEdward and Miner, 2006).

Although the evolution of lecithotrophic development in echinoderms from ancestral species with planktotrophic larvae has come at the expense of greater fecundity, there are two primary benefits that might offset this cost. First, planktotrophic larvae must accumulate the materials required to construct a juvenile by feeding in the plankton and have a longer developmental time and greater mortality risk (e.g. due to predation, starvation, and disease) (Lamare and Barker, 1999). The construction of lecithotrophic larvae that rapidly reach metamorphic competence might therefore increase the number of juveniles produced by a female parent. This has long been recognized in models of egg size evolution that calculate reproductive fitness as a function of the number of metamorphs produced (e.g. Vance, 1973; McEdward, 1997; Levitan, 2000; McEdward and Miner, 2003). Second, lecithotrophic larval development has been shown to lead to the production of larger and/or better provisioned juveniles (Hoegh-Guldberg and Emlet, 1997; T.A.A. Prowse *et al.*, unpublished data). In particular, the large eggs of echinoderms with lecithotrophic development are dominated by energy storage lipids that are not exhausted by larvae, but persist to fuel juvenile development until mouth formation and beyond (Emlet and Hoegh-Guldberg, 1997; Byrne and Cerra, 2000; Villinski *et al.*, 2002; Byrne *et al.*, 2003). Juveniles with larger lipid reserves are presumably more likely to survive to sexual maturity, although this fact is not incorporated into models of egg size evolution (Levitan, 2000).

To investigate how maternal provisioning is linked to the evolution of non-feeding development, we investigated egg lipid profiles for species from two echinoderm classes, the Asteroidea and Ophiuroidea. From the Asteroidea we included seven asterinids that exhibit three different modes of development: planktotrophy (*Patiriella regularis* and *Meridiastra mortenseni*), planktonic lecithotrophy (*Stegnaster inflatus*, *Meridiastra oriens*, *M. calcar*, and *M. gunnii*), and benthic lecithotrophy (*Parvulastra exigua*). Phylogenetic relationships are known for these asterinids and *S. inflatus*, *P. exigua*, and the *Meridiastra* genus represent three independent evolutionary transitions to lecithotrophic development (Byrne, 2006). We also included *Coscinasterias muricata*, an asteriid sea star with planktotrophic larvae. From the Ophiuroidea, we examined two species from the genus *Ophionereis: O. fasciata*, which has ancestral-type planktotrophic development, and *O. schayeri*, which has lecithotrophic larvae (Selvakumaraswamy and Byrne, 2000; Falkner *et al.*, 2006). The planktotrophic developer *Ophiactis resiliens* was also included. Since the evolution of non-feeding development is associated with increased maternal provisioning of energy storage lipids, we focused our attention on

triacylglycerol (TAG) and diacylglycerol ether (DAGE), the two dominant energetic lipid classes present in the eggs of these species.

TAG is the most common energy storage lipid present in biological organisms (Allen, 1976), and consists of a glycerol molecule esterified with three fatty acids and is usually considered a short-term energy store (Lee *et al.*, 1971; Lee and Patton, 1989). Although not as common as TAG, DAGE lipids are prevalent in the tissues of some marine fauna, including the ovaries of asteroids (Karnovsky and Brumm, 1955; Oudejans and van der Sluis, 1979; Falk-Petersen and Sargent, 1982; Hayashi and Kishimura, 1997), the livers of sharks (Kayama *et al.*, 1971; Deprez *et al.*, 1990; reviewed by Phleger, 1998), and the digestive gland and integument of pteropod molluscs (Phleger *et al.*, 1997; Kattner *et al.*, 1998; Falk-Petersen *et al.*, 2001; Boër *et al.*, 2005). DAGE differs from TAG by having an ether bond at the first carbon of the glycerol backbone. This ether linkage is resistant to hydrolysis by lipases (Malins and Robisch, 1974; Taguchi and Armarego, 1997; Sato *et al.*, 2002) and is cleaved by only one known enzyme, alkylglycerol monooxygenase (Koetting *et al.*, 1987). In sharks and rodents, the ether bond in DAGE is cleaved far less readily than the corresponding bond in glycerol ethers with no acyl linkages (Snyder *et al.*, 1973; Malins and Robisch, 1974). Furthermore, the ester bonds in DAGE are turned over more slowly than those in TAG in these taxa (Malins and Robisch, 1974; Sato *et al.*, 2002). In contrast to TAG, therefore, the stable nature of DAGE lipids apparently renders them suitable for long-term energy storage (Sargent, 1989; Kattner *et al.*, 1998; Boër *et al.*, 2005, 2006, 2007).

Here we demonstrate convergent evolution of egg lipid profiles associated with the transition from planktotrophic to lecithotrophic development in two echinoderm classes. While TAG is the dominant egg energetic lipid in echinoderms with planktotrophic development, species with derived lecithotrophic development provision their eggs with an abundance of DAGE. This finding corrects previous assessments of the dominant energetic lipid class present in the large eggs of echinoderms with non-feeding development (Villinski *et al.*, 2002; Falkner *et al.*, 2006; Prowse *et al.*, 2008).

METHODS

Echinoderm species were collected during their reproductive season (Byrne, 1992; Selvakumaraswamy and Byrne, 2000) from multiple locations in Australia and New Zealand (Table 1). Asteroid eggs were induced to spawn from ovaries *in vivo* or *in vitro* with a solution of $1–3 \times 10^{-5}$ M 1-methyladenine (1-MA) in 1-μm filtered sea water. Ophiuroid eggs were obtained by heat-shocking both male and female specimens in a single container and so were predominantly fertilized. The eggs were rinsed with filtered sea water and egg diameters measured for *Stegnaster inflatus* using an ocular micrometer. All other egg diameters reported were sourced from previously reported values (Table 1). For all species except *O. schayeri*, replicate egg samples ($n = 2–3$ per species) were collected in microcentrifuge tubes for lipid analysis. Sample tubes were centrifuged briefly, excess sea water was removed, and samples stored at −80°C until analysis. Egg numbers placed in each tube ranged from 500 to 700 per replicate for planktotrophic developers and from 15 to 250 per replicate for lecithotrophic developers.

Lipid was extracted from frozen egg samples using the procedure of Sewell (2005) and the minor modifications of Prowse *et al.* (2008). Extracted lipids were re-dissolved in a known volume of chloroform (10–150 μl). Lipid classes were identified and quantified using an Iatroscan Mark V^{new} thin-layer chromatography-flame ionization detection (TLC-FID) system. In short, 1 μl of each sample lipid solution was spotted on to replicate silica

Table 1. Species sampled, mode of development, and egg diameter

Class	Order	Family	Species	Mode	Egg diameter (μm)
Asteroidea	Valvatida	Asterinidae	*Patiriella regularis*	Pt	165[a]
			Meridiastra mortenseni	Pt	239[a]
			Stegnaster inflatus	PL	398*
			Meridiastra oriens	PL	399[a]
			Meridiastra calcar	PL	415[a]
			Meridiastra gunnii	PL	431[a]
			Parvulastra exigua	BL	384[a]
	Forcipulatida	Asteriidae	*Coscinasterias muricata*	Pt	150[b]
Ophiuroidea	Ophiurida	Ophionereididae	*Ophionereis fasciata*	Pt	103[c]
			Ophionereis schayeri	PL	248[c]
		Ophiactidae	*Ophiactis resiliens*	Pt	83[d]

Note: Egg diameters are derived from the sources stated. Mode of development abbreviations are as follows: Pt, planktotrophic; PL, planktonic lecithotrophic; BL, benthic lecithotrophic.

* Current study. [a] Prowse *et al.* (2008); [b] Barker (1978); [c] Falkner *et al.* (2006); [d] Selvakumaraswamy and Byrne (2000).

Chromarods ($n = 2$). The Chromarods were conditioned over saturated $CaCl_2$ for 5 min and then developed for 35 min in a non-polar solvent system of hexane–diethyl ether (96 : 4 v/v). This solvent system has previously been used to separate major energetic lipids present in marine organisms, including DAGE and TAG (Phleger *et al.*, 1997; Nelson *et al.*, 1999; Nichols *et al.*, 2001). After drying the Chromarods for 5 min at 70°C, they were analysed via the flame ionization detector of the Iatroscan.

Lipid class quantification was achieved by applying calibration curves produced for individual lipid classes on each rack of 10 Chromarods used. Lipid standards were the same as those used by Prowse *et al.* (2008) and represented four energetic lipid classes (aliphatic hydrocarbon, wax ester, methyl ester, and TAG) as well as two structural lipid classes (sterol and phospholipid). As there is no commercially available authenticated lipid standard for DAGE, we assumed that this lipid class could be quantified using the same standard curve as TAG. This assumption is justified because the flame ionization detector is essentially a carbon counter (Holm, 1999). The change in effective carbon number resulting from the substitution of one ether bond in DAGE for one ester bond in TAG is negligible for molecules of this size (Jorgensen *et al.*, 1990). To confirm the presence or absence of TAG and DAGE in egg samples, we spiked some Chromarods with small amounts of TAG standard or shark liver oil (extracted from Good Health 1000-mg capsules), which contains 25% DAGE by weight.

RESULTS

The non-polar solvent system used for thin-layer chromatography clearly separated the DAGE and TAG lipid peaks (Fig. 1). Spiking samples with shark liver oil containing DAGE either produced or enlarged a lipid peak with a slightly greater R_f than TAG as expected. Similarly, spiking samples with a TAG standard produced or enlarged a lipid peak with a smaller R_f than DAGE (Fig. 1b, d).

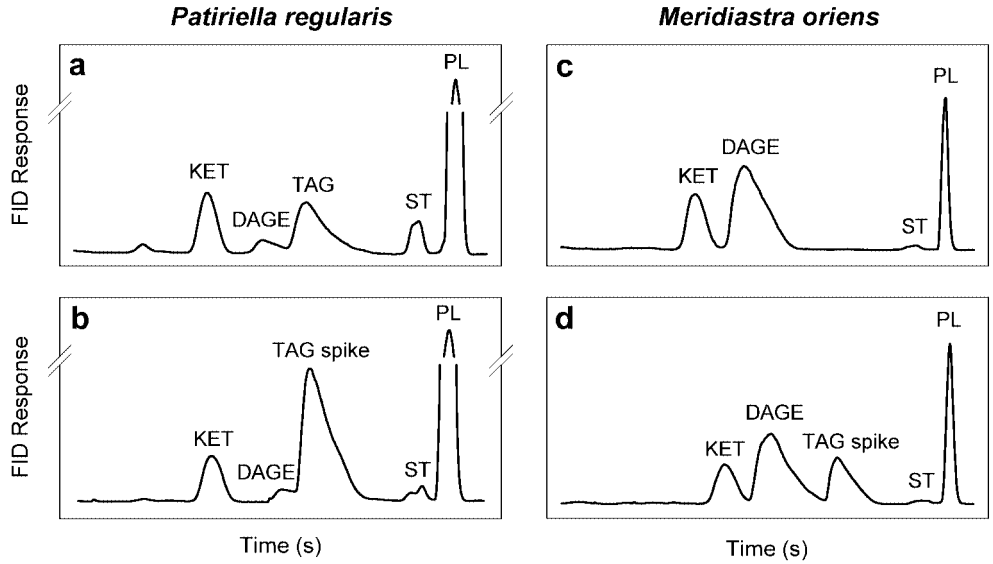

Fig. 1. Representative TLC/FID chromatograms of egg lipid extracts analysed according to the text. (a) Eggs of *Patiriella regularis*, which has planktotrophic larvae: the two major classes of energetic lipid are DAGE and TAG. (b) Spiking lipid extracted from *P. regularis* eggs with a TAG standard (tripalmitin) enlarged the TAG peak confirming its identity. (c) Eggs of *Meridiastra oriens*, which has lecithotrophic larvae: DAGE dominates this egg and TAG is not detected. (d) Spiking lipid extracted from *M. oriens* eggs with a TAG standard creates a TAG peak to the right of the dominant DAGE peak. KET, ketone internal standard; DAGE, diacylglycerol ether; TAG, triacylglycerol; ST, sterol; PL, phospholipid.

The small eggs of species with planktotrophic development were dominated by the structural lipids (sterol and phospholipid), whereas energetic lipids comprised 23.3–39.5% of total egg lipid. In all the species with planktotrophic development, egg energetic lipids were dominated by TAG (range 81.1–100% of energetic lipids) not DAGE. DAGE lipids were present in the eggs of the three sea stars with planktotrophic development (*Coscinasterias muricata*, *Patiriella regularis*, and *Meridiastra mortenseni*) (Fig. 2a). For these species, the DAGE-to-TAG ratio was 0.01, 0.23, and 0.47, respectively. DAGE was not detected, however, in the eggs of the ophiuroids *Ophiactis resiliens* or *Ophionereis fasciata* with planktotrophic larvae (Fig. 2a). The remaining three energetic lipids quantified (aliphatic hydrocarbon, wax ester, and methyl ester) were either undetectable or only present at trace levels in the eggs of the echinoderms with planktotrophic development.

In contrast, the large eggs of species with lecithotrophic development were dominated by energetic lipids (67.1–77.9% of total egg lipid). DAGE, not TAG, was the dominant energetic lipid in these species (range 52.7–97.9% of total energetic lipid). Of the asterinids with lecithotrophic development, TAG was undetectable in the eggs of *Meridiastra oriens* and *M. calcar* but present at low levels in the eggs of *Stegnaster inflatus*, *M. gunnii*, and *Parvulastra exigua* (Fig. 2b). For the latter three species, the DAGE-to-TAG ratio was 5.94, 34.0, and 23.0, respectively. Both DAGE and TAG were important components of the eggs of the ophiuroid *Ophionereis schayeri*, which exhibited a DAGE-to-TAG ratio of 1.13. As

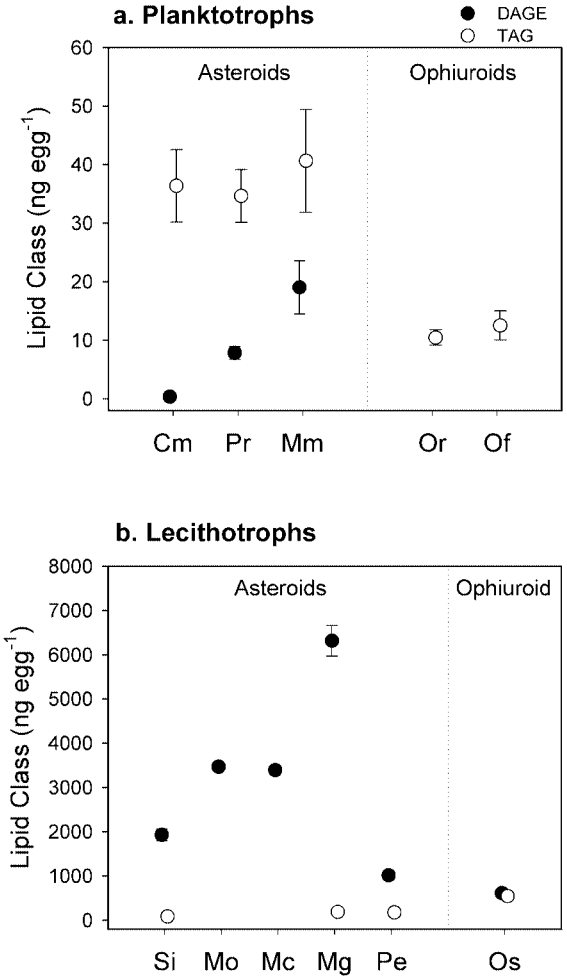

Fig. 2. Mean egg content (±standard error) of DAGE and TAG (ng per egg) for echinoderms with (a) planktotrophic development and (b) lecithotrophic development. Symbols represent lipid class: DAGE, solid symbols; TAG, open symbols. Cm, *Coscinasterias muricata*; Pr, *Patiriella regularis*; Mm, *Meridiastra mortenseni*; Or, *Ophiactis resiliens*; Of, *Ophionereis fasciata*; Si, *Stegnaster inflatus*; Mo, *M. oriens*; Mc, *M. calcar*; Mg, *M. gunni*; Pe, *Parvulastra exigua*; Os, *Ophionereis schayeri*.

for the species with planktotrophic development, egg contents of aliphatic hydrocarbon, wax ester, and methyl ester were either below the detection limit or only present in trace amounts.

DISCUSSION

The first naturally occurring glycerol ethers were isolated from two sea stars with planktotrophic larvae, *Asterias rubens* and *Astropecten aurantiacus* (Dorée, 1909; Kossel and Edlbacher, 1915; Bergmann and Stansbury, 1943). Subsequent studies demonstrated that DAGE is present in the ovaries of asteroid species with both planktotrophic and lecithotrophic larvae

(Table 2 and references therein) and these lipids were assumed to be located at least partially within oocytes (Falk-Petersen and Sargent, 1982). Surprisingly, then, DAGE lipids were not detected in the eggs of several asterinids, including those investigated here (Villinski *et al.*, 2002; Prowse *et al.*, 2008). Although asterinids with non-feeding larvae were suggested to provision their eggs with an abundance of wax ester (Villinski *et al.*, 2002), we recently used the Iatroscan TLC-FID system and the three-stage development recommended by Parrish (1999) to demonstrate that wax ester, which appears on the first chromatogram, is not a dominant egg lipid in these species (Prowse *et al.*, 2008). Although large lipid peaks on the second chromatogram aligned with a TAG standard, our subsequent research revealed that the solvent system used for the second development of Parrish (1999) was too polar to clearly separate the DAGE and TAG lipid classes. The current study corrects our previous assessments of asteroid and ophiuroid eggs (Falkner *et al.*, 2006; Prowse *et al.*, 2008). By using a simple one-stage development that clearly separates wax ester, DAGE, and TAG, we show that DAGE lipids are deposited in the eggs of these echinoderms. By quantifying lipids on a per egg basis for echinoderm species from two classes and encompassing three modes of development, we may now place these results in an evolutionary context.

Patiriella regularis and *Meridiastra mortenseni* develop through ancestral-type feeding bipinnaria and brachiolaria larval stages before undergoing metamorphosis and are representative of the ancestral mode of development for the Asterinidae (Byrne, 2006). TAG was the dominant energetic lipid in the eggs of these species (Fig. 2a). In contrast, the eggs of asterinids with derived lecithotrophic development are dominated by DAGE (Fig. 2b). This trend is also evident in the ophiuroid genus *Ophionereis*, in which TAG was abundant and DAGE not detected in the eggs of *O. fasciata*, which has feeding ophioplutei, whereas DAGE and TAG content were similar in the eggs of *O. schayeri*, which has lecithotrophic larvae. Falkner *et al.* (2006) previously used TLC-FID to quantify egg lipid classes of *O. schayeri* and noted a double TAG peak, but it now appears that the first of these actually represents DAGE and the second is a TAG peak. Furthermore, large wax ester reserves previously identified in the eggs of the lecithotrophic echinoids *Heliocidaris erythrogramma* and *Holopneustes purpurescens* (Villinski *et al.*, 2002) are also probably DAGE lipids (M. Byrne *et al.*, unpublished data).

We therefore propose that the evolution of non-feeding development in the Echinodermata has involved increased oogenic deposition of DAGE (Figs. 1, 2). Available lipid class data for other asteroid species supports this hypothesis, since calculated ratios of DAGE to TAG in ovarian tissue are greater for species with non-feeding larvae (Table 2). Further investigation of egg lipid profiles in echinoderm clades that exhibit both feeding and non-feeding developmental modes could provide an empirical test of our hypothesis. For example, the asteriid *Coscinasterias muricata* produces eggs in which TAG is the dominant energetic lipid (current study). We predict, however, that the large eggs produced by *Uniophora granifera*, a related asteriid with lecithotrophic development (Marshall and Keough, 2003b), are dominated by DAGE lipids.

Why are DAGE lipids important for lecithotrophic larvae?

Given that TAG is the most ubiquitous energy storage lipid present in animals (Allen, 1976), the high egg DAGE content of asteroids and ophiuroids with non-feeding larvae requires an explanation. There are three non-mutually exclusive reasons why such a maternal provisioning strategy may suit this developmental mode.

Table 2. Ovarian contents of DAGE and TAG lipid classes in asteroids with planktotrophic and lecithotrophic larvae, obtained from the stated sources

Order	Family	Species	Mode	Units	DAGE	TAG	DAGE-to-TAG ratio
Forcipulatida	Asteriidae	*Asterias rubens*[a]	Pt	mg per 100 mg of individual	235	340	0.69
		Asterias amurensis[b]	Pt	% of total lipid	8.55	30.9	0.28
Valvatida	Asterinidae	*Asterina pectinifera*[c]	Pt	mg in total extract	0	500	0
Paxillosida	Goniopectinidae	*Ctenodiscus crispatus*[d]	PL	% of total lipid	60	6	10.0
Velatida	Pterasteridae	*Solaster paxillatus*[b]	PL	% of total lipid	86.0	1.98	43.4
		Pteraster militaris[d]	PL/EBL	% of total lipid	66	10	6.6

Note: Data for *C. crispatus* were available over the reproductive cycle of this species but are shown here for the reproductive peak in February. Mode of development abbreviations are as follows: Pt, planktotroph; PL, planktonic lecithotroph; EBL, externally brooded lecithotroph.

[a] Oudejans and van der Sluis (1979); [b] Hayashi and Kishimura (1997); [c] Okabe and Noma (1974); [d] Falk-Petersen and Sargent (1982).

1. *Long-term nutrition*. DAGE lipids consist of a glycerol backbone together with one ether-linked fatty alcohol and two esterified fatty acids. In fish and rodents, ether and ester linkages within a DAGE molecule are more resistant to enzymatic degradation than identical bonds present in other lipid species (Snyder *et al.*, 1973; Malins and Robisch, 1974; Sato *et al.*, 2002). The apparent stability of DAGE in these taxa is likely due to the chemical properties of this lipid class. Specifically, the polarity of DAGE molecules may be suboptimal for interaction with the active sites of alkylglycerol monooxygenase and water-soluble lipases (Lee and Patton, 1989). Although it is not known whether these results may be generalized to other taxa, the relative stability of DAGE may render this lipid class suitable for long-term energy storage (Sargent, 1989; Kattner *et al.*, 1998). In support of this notion, larvae of the pteropod *Clione limacina* store both TAG and DAGE within lipid droplets and TAG is used for short-term energy needs such as fuelling growth, whereas DAGE is used for long-term needs like over-wintering and starvation (Boër *et al.*, 2005, 2006, 2007). In echinoderms with planktotrophic development, TAG reserves initially present in the egg are rapidly metabolized during the construction of a feeding larva (Yasumasu *et al.*, 1984; Sewell, 2005; Meyer *et al.*, 2007; Byrne *et al.*, 2008b; Prowse *et al.*, 2008). On the other hand, echinoderms with lecithotrophic larvae require that the energetic reserves be laid down in their eggs to persist through larval development and metamorphosis to fuel the perimetamorphic period (Hoegh-Guldberg and Emlet, 1997; Byrne and Cerra, 2000; Byrne *et al.*, 2003). DAGE lipids may therefore be a more suitable long-term energy store for the development of echinoderms with lecithotrophic development.

2. *Buoyancy.* DAGE is inherently more positively buoyant than TAG (Lewis, 1970; Malins and Barone, 1970) and is suggested to play a buoyancy role in several marine groups, including pteropod molluscs (Phleger *et al.*, 1997, 2001) and sharks (Malins, 1968; Malins and Barone, 1970; Wetherbee and Nichols, 2000). An increase in the ratio of DAGE to TAG present in the eggs of echinoderms could potentially have two benefits: (i) if eggs float in sea water, fertilization rates might be maximized if fertilization occurs at the two-dimensional water surface rather than within the three-dimensional water column (Harrison and Wallace, 1990; Sewell and Levitan, 1992); (ii) even if eggs do not float, greater larval buoyancy might improve the ability of non-feeding larvae to remain in the water column and thus facilitate dispersal.

3. *Disease resistance.* Glycerol ethers with methoxy substitutions possess anti-bacterial and anti-fungal properties and may help prevent microbial infections (reviewed by Hallgren, 1983). A similar role in disease resistance is proposed for DAGE by Boër *et al.* (2007), who noted that the tissues of *C. limacina* that lack DAGE-rich lipid droplets are the first to succumb to infection. If some level of disease resistance is conferred by DAGE, this benefit would be more relevant for non-feeding than feeding larvae, since egg storage lipids persist significantly longer during non-feeding development (Emlet and Hoegh-Guldberg, 1997; Prowse *et al.*, 2008; T.A.A. Prowse *et al.*, unpublished data).

While the third hypothesis is an intriguing one, it is weakly supported at present due to the lack of concrete evidence showing that DAGE helps fight microbial infections. Furthermore, DAGE lipids in *C. limacina* and echinoderm eggs and larvae are stored in discrete, intracellular droplets, whereas it is likely that anti-bacterial and anti-fungal properties would be most usefully expressed at cell surfaces. Of the remaining two hypotheses, we favour the first because selection favouring the production of more buoyant eggs and larvae is unlikely to account for the high egg DAGE content of echinoderms with non-feeding larvae. Of the six species with lecithotrophic larvae investigated here, only *Meridiastra gunnii* produces eggs that float in sea water. The eggs of *P. exigua*, which are

DAGE-rich but also have a high protein content (Prowse *et al.*, 2008), are actually negatively buoyant and develop into benthic 'tripod' larvae (Byrne, 1995). Therefore, while the increased buoyancy afforded by DAGE may enhance the fertilization success or dispersal in some species, it is likely that the primary function of DAGE in the large eggs of echinoderms with non-feeding larvae is as a long-term energy reserve for both larval and juvenile development. This adds to a growing body of literature suggesting that post-metamorphic effects can have a strong influence on the optimum strategy of maternal investment (e.g. Emlet and Hoegh-Guldberg, 1997; Marshall and Keough, 2003a, 2008; Marshall *et al.*, 2003; Phillips, 2006; Byrne *et al.*, 2008a).

Convergent evolution of DAGE-rich eggs

The occurrence of DAGE-rich eggs in echinoderms with non-feeding larvae is a clear example of convergent evolution because the pattern holds in multiple clades. Given the paucity of this lipid class in marine food webs (Sargent, 1989), echinoderms with lecithotrophic development can presumably synthesize DAGE *de novo* (Oudejans and van der Sluis, 1979; Falk-Petersen and Sargent, 1982). Convergent evolution of egg lipid profiles in echinoderms with non-feeding larvae may therefore have involved (1) elaboration on an ancestral programme of DAGE synthesis and subsequent oogenic deposition of this lipid class, or (2) evolution of the ability to synthesize DAGE followed by its elaboration and incorporation into oogenesis. The first hypothesis is more parsimonious and probably true for the Asteroidea for two reasons: first, the asteriid sea star *Asterias forbesi* can synthesize DAGE despite exhibiting ancestral-type planktotrophic development (Snyder *et al.*, 1969), and second, all sea stars with feeding larvae so far examined contain measurable DAGE in their eggs or ovaries (current study, Table 2). Although we did not detect DAGE in the small eggs of two ophiuroids with feeding larvae (Fig. 2), this lipid class may have been present at concentrations below the detection limit of the TLC/FID technique (Nelson *et al.*, 1999).

Of the planktotrophic developing echinoderms included in this study, the asterinid *Patiriella regularis* and *Meridiastra mortenseni* produce the largest eggs (165 and 239 μm, respectively) with the highest DAGE-to-TAG ratios (0.23 and 0.47). This suggests that echinoderms with relatively large eggs and feeding larvae may benefit from adding a quotient of DAGE to the egg. Whereas TAG is rapidly depleted during early development, additional DAGE reserves could persist to serve as a buffer against a spatially and temporally variable food supply (Sargent, 1989; Boër *et al.*, 2005, 2006). Assuming that egg lipid profiles have a heritable component (Levin *et al.*, 1991; Miles *et al.*, 2007), improvements to the nutritional condition of feeding larvae brought about by increased maternal provisioning of DAGE could facilitate the transition to a large, DAGE-rich egg. In fact, as the egg lipid profile of the ancestral asterinid may have resembled that of *P. regularis* and *M. mortenseni* (i.e. had a significant DAGE component), this mechanism might help explain the repeated evolution of non-feeding development in the Asterinidae (Byrne, 2006). It is clear that the fitness consequences of different maternal investment strategies now need to be properly incorporated into models of egg size evolution (Byrne *et al.*, 2008b).

Given that the echinoderms with non-feeding development examined here apparently biosynthesize fatty alcohols *de novo* during DAGE synthesis, it is surprising that they deposit little or no wax ester in their eggs (Falk-Petersen and Sargent, 1982). The biochemistry of DAGE and wax ester is similar, since both consist of fatty acids and fatty alcohols and are more buoyant and metabolized more slowly than TAG (Lewis, 1970; Sargent, 1976, 1989). Like

DAGE, wax ester appears to play roles in buoyancy and long-term energy storage, particularly in polar and deep-sea zooplankton (Lee *et al.*, 1971, 2006; Sargent *et al.*, 1981) and in the eggs of tropical reef corals (Lee and Patton, 1989; Arai *et al.*, 1993). Hermatypic corals apparently convert large portions of the TAG derived from their symbiotic zooxanthellae into wax ester, so coral-reef ecosystems may be unusually rich in wax ester (Benson *et al.*, 1978; Lee and Patton, 1989). Interestingly, the ophiuroid *Ophiarthrum elegans*, which has non-feeding larvae, was sampled from the Great Barrier Reef and shown to produce eggs dominated by wax ester (Falkner, 2007). It may be that wax ester performs the same reproductive function as DAGE in echinoderms inhabiting environments where dietary sources of wax ester are available. Alternatively, the relative importance of DAGE and wax ester in the maternal provisioning strategies of echinoderms with non-feeding larvae may differ between lineages.

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