Marine Biology (2019) 166:38
https://doi.org/10.1007/s00227-019-3487-3

ORIGINAL PAPER

Genetic evidence for alloparental care and frequent multiple paternity in the brooding sea star (*Leptasterias* sp.)

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Received: 6 October 2018 / Accepted: 2 February 2019

Abstract

Echinoderms form an abundant and ecologically important group of marine animals, and they are found in nearly every marine environment, from shallow tropical waters to deep polar benthos and even in the pelagic zone. They exhibit a wide diversity of reproductive strategies that range from broadcasting millions of gametes, with no parental care, to internal brooding of a few embryos for several weeks. While many echinoderm species have become model systems for studies of community ecology, evolutionary genetics, and development biology, very little is known about the distribution of mating and reproductive success in natural populations. In this study, we examined patterns of genetic maternity and paternity in the six-rayed sea star *Leptasterias* sp., an important predator of many intertidal communities and a species that exhibits maternal care of embryos. We used next-generation sequencing to rapidly develop informative microsatellite markers for this species, and used these markers to genotype 439 juveniles across 15 broods collected from the intertidal in Fogarty Creek, Oregon, USA. Our data show an unambiguous pattern of multiple paternity in all but one clutch examined, with some broods showing some of the highest levels of polyandry reported for a marine invertebrate. Moreover, we detected two cases of mixed maternity in which a female sea star carried another mother's offspring mixed with her own. Alloparental care by females is rare, and since female *Leptasterias* do not eat during the 40–60 days brooding period, this expensive behavior may provide a useful system for examining the evolutionary costs and benefits of parental care in dynamic intertidal environments.

Introduction

Parental investment in the form of nest guarding or brooding of developing embryos occurs in many aquatic taxa, besides mammals, including crustaceans (Toonen 2004; Baggio et al. 2011; Jense and Bentzen 2012), pycnogonids (Barreto and Avise 2010, 2011; Burris 2011), polychaete annelids (Wilson 1991; Hess 1993), molluscs (Dupont et al. 2006; Voight and Feldheim 2009), echinoderms (Chenuil et al. 2004; Gillespie and McClintock 2007), and bony fishes (Avise and Liu 2010; Coleman and Jones 2011). Species vary in the modality of care with regards to where embryos are placed, and in which parent attends to the brood. Postzygotic parental care is widely regarded to be costly to the attending parent because of energy expenditure, susceptibility to predation, and reduced future mating opportunities (Royle et al. 2012).

Because of these costs, uniparental care of embryos is theoretically expected to be performed when the parent has high confidence in its genetic contribution to the brood or clutch. Consistent with this prediction, males of some species have been shown to adjust parental efforts according to the level of recognized cuckoldry or female promiscuity (Neff 2003; Mehlis et al. 2010). In certain groups with prolonged paternal care, such as sea spiders (Pycnogonida; Barreto and Avise 2008, 2010, 2011) and syngnathid fishes (McCoy et al. 2001; Jones et al. 2001a), specialized mating behaviors have allowed males to guarantee genetic paternity of all progeny they carry. However, in many other species with paternal care, genetic analyses have revealed striking patterns of cuckoldry and alloparental care (i.e., care of embryos unrelated to the guardian). For instance, in most

Responsible Editor: M. Byrne.

Reviewed by undisclosed experts.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00227-019-3487-3) contains supplementary material, which is available to authorized users.

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Published online: 13 February 2019

species of fish with exclusive paternal care examined with molecular markers, single broods are routinely detected to contain embryos sired by multiple males, with the guardian male sometimes siring only a small fraction of the progeny (Avise et al. 2002; Coleman and Jones 2011). The offspring of multiple males were also mixed in broods carried by individual males of an intertidal snail, despite high metabolic costs of care (Kamel and Grosberg 2012). Hypotheses for the ultimate persistence of seemingly expensive alloparental care by males vary depending on taxa, and include the presence of alternative mating tactics, such as sneaker males (Jones et al. 2001b), attraction of additional mates due to parental abilities (Unger and Sargent 1988; Porter et al. 2002), and brood parasitism (Philipp and Gross 1994; DeWoody et al. 2000).

Conversely, females generally have stronger control of timing and placement of eggs. This is true not only in species with internal fertilization, but also in those with external fertilization, wherein eggs are released into a brood pouch or cavity before being fertilized. Accordingly, in species with uniparental care by females, genetic assessments have shown almost invariably that caring females are successful in guaranteeing maternity of their entire attended brood. For instance, no evidence of alloparental care by females (i.e., broods with mixed maternity) has been reported in invertebrate species, including decapod crustaceans (Toonen 2004; Gosselin et al. 2005; Vulstek et al. 2013), corals (Lasker et al. 2008), and bivalves (Ferguson et al. 2013), all of which are fertilized externally. A few exceptions have been observed in female mouthbrooding African cichlids, in which 14–65% of broods examined contained at least a few fry that were unrelated to the guardian female (Kellogg et al. 1998; Sefc et al. 2012). These cases have been hypothesized to be caused by accidental mixing when there is high density of females guarding late-stage swimming fry.

Behavioral observations in the brooding sea star *Leptasterias* spp. (Class Asteroidea) point to a system with an unusual combination of reproductive traits. The genus contains multiple complexes of cryptic species that differ in distribution, and their taxonomic assignments are still being debated (Foltz et al. 2008; Melroy et al. 2017), but all are known to be lecithotrophic brooders (McEdward and Miner 2001). While most echinoderms are broadcast spawners, female *Leptasterias* releases and holds unfertilized eggs on her oral side, where these are then fertilized with sperm recently released by males into the water column (Chia 1966). While brooding is relatively rare in echinoderms (Gillespie and McClintock 2007), spermcasting, in which only males release gametes into the water, is commonly found in sessile invertebrates (Bishop 2006), such as barnacles (Barazandeh et al. 2013; Plough et al. 2014), mussels (Wacker et al. 2018), and colonial ascidians (Johnson and Yund 2007). Single broods in *Leptasterias* sp. contain 50–2000 embryos (Chia 1966; Menge 1974), and are protected underneath the mother for ~ 40–60 days, until the young have developed a functioning mouth and tube feet (Chia 1966). While brooding, the female does not feed (Chia 1966; Menge 1974). Moreover, Chia (1966) observed, via experimental manipulations, that 'orphaned' embryo masses were sometimes picked up by non-brooding individuals ($n = 9$), with some of these then spawning their own eggs into the adopted mass. These observations, under low-density laboratory conditions, suggest the presence of alloparental care by female *Leptasterias*, but no investigations have reported this unusual behavior in nature.

In this study, we develop and use genetic markers to examine patterns of paternity and maternity in wild-collected broods in a species of *Leptasterias* from the central Oregon coast. Based on recent phylogenetic analyses (Foltz et al. 2008), this species is either the same or a closely related sister to those studied by Chia (1966) and Menge (1974) from the Salish Sea, Washington. To avoid possible conflict with the ongoing taxonomic debate, we will refer to this local species simply by genus in this study. Our aims are to assess (1) whether single broods carried by each female are sired by more than one male, and if so, (2) what is the range and variation of such multiple mating by the females, and (3) whether broods with mixed maternity occur in the wild.

Materials and methods

Sample collection

Sea stars were collected during two low tides on April 26 (transect 1) and May 21 (transect 2), 2017, from Fogarty Creek, Oregon (44.8364°N/124.0586°W). On each collection day, we followed a 5-m transect perpendicular to the shore, searched for brooding individuals, and then collected these as well as all adults seen within 1 m on either side of the transect. We also haphazardly collected additional adult individuals from throughout the transect, for a total of 74 adult individuals, 19 of which carried broods. Broods were carefully removed from the guardian adult using a small spatula and stored in 95% ethanol; from adults, a ~ 5-mm tissue sample was excised from the arm and stored in ethanol. In the laboratory, the number of progeny in each brood was counted under a dissecting microscope, and the developmental stage of each brood was categorized as either 'eggs' or 'juveniles', with the latter category assigned when arms were visible on individual progeny. In total, we collected 13 broods with juvenile sea stars and 6 broods still in the egg stage.

Illumina sequencing and computational processing

Genomic DNA was isolated from tube feet tissue from a single adult individual using a phenol:chloroform protocol (Sambrook and Russell 2010), and then treated with RNase A. The integrity of the DNA was checked on a 1% agarose gel, and the concentration quantified with a Qubit Fluorometer (Thermofisher). A single DNA library using Wafergen Biosystems was prepared from 500 ng of genomic DNA, and the library was size-selected with a BluePippin system (Sage Science) in the range of 400–900 bp, with median of 600 bp. The library was sequenced at Oregon State University's Center for Genome Research and Biocomputing in the Illumina MiSeq platform as 2 × 300 bp paired-end reads.

We examined reads with FastQC (Andrews 2010) and cleaned them with *cutadapt* (Martin 2011) by removing Illumina adapters, trimming off end base pairs with Phred score below 20, and retaining only reads with minimum length of 70 bp after quality trimming. Since a large fraction of the fragments in our library was shorter than twice the read length, we generated longer sequences by merging overlapping read pairs with the script FLASH (Magoc and Salzberg 2011) with following parameters: -m 15 -M 200 -z -t 3. This merging step increases the range of read lengths and the opportunity to find long microsatellite loci with sufficient flanking region for primer design. Merged reads (i.e., contigs) were converted from *fastq* into *fasta* for subsequent processing. We used the program MISA (Beier et al. 2017) to screen contigs for microsatellite loci, retaining only loci with minimum number of repeats of 10 for dinucleotides, 8 for trinucleotides, 8 for tetranucleotides, and 6 for pentanucleotides. Contigs identified to have these microsatellite loci were then input into MSATCOMMANDER (Faircloth 2008), which was used to design PCR primers for amplicons in the range of 100–400 bp.

Microsatellite marker development and characterization

We selected 50 primer pairs for screening and added an M13(–29) tail (5′-CACGACGTTGTAAAACGAC-3′) to the 5′ end of each forward primer. These primer pairs, as well as a standalone M13(–29) primer, were synthesized by Integrated DNA Technologies (IDT). Primers were tested in 12.5-µl PCR reactions containing 1 µl of genomic DNA, 1 × PCR buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.5 U AmpliTaq DNA Polymerase (Applied Biosystems), 0.05 µM of the M13-tailed locus-specific forward primer, 0.5 µM of the locus-specific reverse primer, and 0.5 µM of the M13 primer. PCR cycling parameters consisted of initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 15 s, annealing temperature for 30 s, and 72 °C for 30 s, with a final extension step at 72 °C for 15 min. Four annealing temperatures were tested: 50 °C, 54 °C, 58 °C, and 62 °C. PCR products were resolved on a 2% agarose gel and checked for the predicted size. Primer pairs were then screened for polymorphism in a panel of 16 adult individuals. Some primer pairs were further optimized by varying concentration of Mg^+ up to 2.5 mM. Loci that showed fewer than four alleles discernible on an agarose gel were excluded from additional screening.

A total of 16 polymorphic loci were chosen for a final step of screening in which 48 adult sea stars from our sample were genotyped via capillary electrophoresis in an ABI 3730 DNA Analyzer. For this step, PCR reactions were set up as above, but the M13 primer was labeled with four different fluorescent dyes (Applied Biosystems), which allows pooling of PCR products for electrophoresis. Electrophoretic data from the capillary instrument were scored in Geneious v10.4, and the program GenePop (Raymond and Rousset 1995) was used to estimate population-wide allele frequencies, to test for deviations from Hardy–Weinberg equilibrium, and test for linkage disequilibrium (Supplementary Table 1). After this final screening stage, eight loci showed no deviation from Hardy–Weinberg equilibrium or evidence of linkage (Supplementary Table 1). The five loci chosen for parentage analysis in this population (*Lepta27*, *Lepta28*, *Lepta40*, *Lepta42*, and *Lepta47*) were picked from this shortlist of eight because they had high allelic variation while maintaining the lowest expected proportion of null alleles, based on the method of Brookfield (1996) (Supplementary Table 2).

We genotyped the remaining 26 adult individuals at the five chosen loci, for a total of 74 sea stars, and used the population estimates of allele frequencies to calculate probabilities of parentage exclusion following (Jamieson and Taylor 1997). In addition, we estimated genotyping error rate for each locus by repeating PCR and electrophoresis for all 74 adult samples (Table 1). Finally, we examined levels of genotypic diversity in this population by quantifying heterozygosity and the inbreeding coefficient F_{is} (Weir and Cockerham 1984) using GenePop, as well as by estimating genetic relatedness among adults using ML-RELATE (Kalinowski et al. 2006).

Genetic parentage analysis

Each brood mass was removed from ethanol and briefly soaked in a petri dish containing deionized water, where offspring were separated from each other as needed using fine tweezers. Individual offspring were then transferred to 0.2-ml PCR tubes containing 80 µl of lysis buffer (10 mM Tris–HCl pH 8.0, 50 mM KCl, 0.5% Tween-20, 250 µg/ml proteinase K). Tubes were incubated at 55 °C for 3 h, then heated to 95 °C for 15 min to inactivate the proteinase. Samples were centrifuged at ~ 2400×*g* for 5 min to pellet cellular

Table 1 Features of five microsatellite loci used in parentage analysis of broods of the sea star *Leptasterias* sp.

Locus	Repeat motif	H_e	H_o	No. of alleles	Allele size range (bp)	Prob. exclusion[a]	Genotyping error rate[b]	5′ dye used
Lepta27	$(AATC)_{11}$	0.67	0.70	9	332–384	0.454	0	PET
Lepta28	$(AGAT)_{10}$	0.86	0.82	17	366–442	0.729	0.007	VIC
Lepta40	$(AATC)_{27}$	0.96	0.93	50	288–620	0.913	0.041	NED
Lepta42	$(ACTC)_{13}$	0.81	0.73	13	159–215	0.647	0.035	PET
Lepta47	$(AATG)_{15}$	0.89	0.78	16	173–241	0.773	0.012	VIC

Population-level statistics (columns 3–7) were estimated from a sample of 74 adult sea stars. Additional features including primer sequences for these and 11 other loci can be found on Supplementary Table 1

H_e expected heterozygosity, H_o observed heterozygosity

[a]Probability of genetic exclusion under the assumption that one parent is known, based on Jamieson and Taylor (1997); the combined probability of exclusion is 0.998

[b]Genotyping error rate was estimated directly by repeating PCR, electrophoresis, and scoring of all 74 adult individuals

debris, and supernatant was transferred to new PCR tubes and stored at − 20 °C until genetic analyses.

DNA from progeny was diluted threefold with molecular grade water and 1 µl used for PCR at each of the five loci (as above). Before electrophoresis, PCR products from each progeny were pooled at the following ratios: 4:2:4:8:1 for loci *Lepta27*, *Lepta28*, *Lepta40*, *Lepta42*, and *Lepta47*, respectively, followed by dilution with 61 µl of deionized water. These dilution factors were determined preliminarily by testing each locus at different dilutions and selecting the volume that resulted in peaks with 300–10,000 relative fluorescent units (rfu). Fragments were then resolved on an ABI 3730 DNA Analyzer as above. Only progeny that were successfully genotyped in at least three loci were retained for further analyses. The number of offspring assayed varied depending on brood size, with up to 55 from large broods. Fifteen of the 19 broods collected from adult *Leptasterias* were ultimately examined. The four broods not included had egg masses that became brittle during storage, and we were not able to separate individual eggs without destroying multiple eggs and mixing their tissue.

For each brood, we first assessed maternity by visually comparing the multilocus genotype of each progeny to that of the guardian mother; maternity was assigned when mother and offspring share an allele in each locus. Any offspring that showed mismatches in one or more loci were excluded from that female's brood and analyzed as a separate cohort of 'unknown' maternity. The program GERUD2.0 (Jones 2005) was used to confirm maternal genotypes assigned manually, and to deduce possible maternal genotypes in broods of 'unknown' maternity. In the latter circumstance, reconstructed maternal genotypes were compared to those of other collected adult individuals.

We examined the degree of multiple paternity in each brood via three methods. We estimated the minimum number of sires by simple allele counting. For this, we tallied the number of paternal alleles in each locus, and, for the most polymorphic locus, divided the number of alleles by two and rounded to the nearest integer. This method is conservative, since it assumes that each contributing sire is heterozygous at the assessed locus. We then used GERUD2.0 to estimate the minimum number of sires. After subtracting the maternal genotype, this program estimates the minimum number of sires and attempts to deduce their multilocus genotypes by incorporating empirical population allele frequencies. Analyses with GERUD2.0 were performed with each brood separately.

Analyses of paternity with GERUD2.0 were not possible with certain broods because this program only estimates up to six sires in a brood. In addition, GERUD2.0 does not incorporate genotyping error rates or accommodate missing data, which can reduce the size of data sets. We, hence, performed a final analysis with the likelihood-based program COLONY (Jones and Wang 2010), which estimates the most likely number of contributing fathers, accepts entries with missing genotypes, and incorporates our marker-specific error rate estimates. By simultaneously considering all individuals in our sample (i.e., genotyped progeny and adults), COLONY reconstructs sibships among progeny, assigns paternity and maternity among sampled adults when possible, and deduces parental genotypes contributing to each offspring, placing confidence levels on such assignments using likelihood. The output from COLONY allows us to estimate the most likely number of sires, as well as to obtain confidence levels for maternity assignment of each offspring in the entire data set. Our analysis with COLONY included all 74 sampled adults and 439 progeny across all broods.

Sire evenness

In broods with multiple paternity, we used the index of sire evenness E to better reflect the proportion of

fertilization by each sire within a brood. For this analysis, we used the paternity results from COLONY and included only broods for which at least 10 progeny were genotyped.

E was calculated following (Schmoll et al. 2007), as follows:

$$E = \left(-\sum_{i=1}^{S} p_i \times \ln p_i \right) / \ln S.$$

In this equation, *S* is the total number of sires in a brood and p_i is the proportion of progeny sired by the *i*th sire. The numerator formula calculates a sire diversity index based on the Shannon–Wiener Index of diversity, while the denominator calculates the maximum sire diversity if paternity is distributed evenly given the number of sires in the brood. Therefore, *E* ranges from 0 to 1, with high values reflecting even distribution of paternity among sires.

Results

Microsatellite discovery and genetic diversity

The Illumina MiSeq run generated over 30 million read pairs, and 97% of them passed quality and length filters. Sequence data were deposited in the NCBI Sequence Read Archive under BioProject PRJNA515106 and accession SRR8441838. Merging of overlapping paired reads resulted in 19.6 million contigs ranging from 310 to 560 bp. MISA detected 20,235 dinucleotides, 104,098 trinucleotides, 22,212 tetranucleotides, and 14,086 pentanucleotides, and MSATCOMMANDER designed PCR primers for 5402 loci, from which the 50 tested loci were selected. When examined across 74 adults from the same locality, the five loci selected exhibited high polymorphism, with an average of 21 alleles per marker. These loci combined provide a very high probability of paternity exclusion (0.998, Table 1). While our study relies on five loci, other loci from this set (Supplementary Table 1) may become useful for different purposes and/or in different populations.

Genotypic diversity among adults was high. No pair of sampled adults shared identical multilocus genotypes across the five loci, and observed heterozygosity ranged from 0.70 to 0.93 across loci. Levels of inbreeding were likely low, with overall $F_{is} = 0.0571$ and low relatedness coefficients. The latter metric showed a distribution that was highly skewed towards $r = 0$ and only 8.07% ($n = 436$) of all pairwise relationships ($n = 5402$) had $r \geq 0.25$ (mean = 0.059; median = 0; Supplementary Fig. 1).

Patterns of maternity

A total of 439 offspring were successfully genotyped in at least three loci and included in our analyses of maternity and paternity. Based on their multilocus genotypes, all progeny in 13 of the 15 broods ($n = 406$ of 439) were consistent with being genetic offspring of the guardian female, since each of these offspring shared an allele with their guardian in all loci amplified. These maternity assignments were confirmed by GERUD and COLONY.

In the broods carried by females M3 and M10, a subset of embryos was assigned to their guardian as above (7 progeny from M3, 39 from M10). However, several other progeny showed allelic mismatches to their guardian in two or more loci, and were, hence, excluded as their genetic offspring. The excluded cohort in each of these broods (26 progeny from M3, and 7 from M10) were analyzed as separate broods, with the assumption that each had a single dam of 'unknown' genotype. These cohorts were re-labeled as M3c2 and M10c2, respectively. GERUD reconstructed a single putative maternal multilocus genotype for M3c2, and this genotype was an identical match to that of one of the collected non-brooding adults (individual FC29), found along the same transect as M3. Assuming Hardy–Weinberg equilibrium, the probability that this exact multilocus match is spurious (i.e., not indicative of maternity) is very low (1.8×10^{-8}, Table 2, Supplementary Table 2). COLONY also assigned maternity of all 26 progeny from cohort M3c2 to candidate individual FC29, with high confidence (*probability* = 1.00), even though the analysis was run with maternity 'unknown' for that subset of offspring. Based on maximum likelihood estimates from ML-RELATE, dams M3 and FC29 are unrelated ($r = 0$). For cohort M10c2, GERUD confirmed that the cohort was consistent with having a single mother, but the program was not able to deduce a maternal genotype with confidence, likely because only 7 offspring are available for inference. COLONY did not assign maternity of any of the 7 offspring to any of the candidate collected adults. Nonetheless, COLONY results were concordant with GERUD's, in that a single unknown mother was likely dam of the full cohort, but again her genotype could not be deduced with confidence.

Multiple mating by females and sire evenness

Our analyses revealed unambiguous genetic evidence for multiple mating by individual females. Subtraction of the maternal alleles from each brood allowed for estimates of the number of sires contributing to progeny arrays using allele counting, GERUD, and COLONY. Among the 15 cohorts carried by their genetic mother (including M3c1 and M10c1), all but one showed evidence of multiple paternity. One of the cohorts carried by an alloparental mother

Table 2 Genotypic description of a brood with multiple maternity

Individual	*Lepta27*	*Lepta28*	*Lepta40*	*Lepta47*	No. of mismatching loci
M3 (guardian)	**344/376**	**382/434**	**388/452**	**173/185**	
Progeny consistent with M3					
M3–3	**344/344**	398/**434**	**388**/396	**173**/181	0
M3–7	**344/344**	**382**/398	380/**452**	**185/185**	0
M3–10	**344/376**	398/**434**	**452**/572	**173**/181	0
M3–14	**344/376**	**382**/398	**388**/572	**173**/181	0
M3–19	**376/376**	**382**/398	380/**388**	**173**/181	0
M3–34	**344/376**	**382**/398	380/**388**	**173**/181	0
M3–36	**344/376**	**382**/398	**452**/572	**173**/181	0
Progeny excluded					
M3–1	364/368	398/418	444/568	**185**/189	3
M3–4	**344/376**	398/414	444/592	189/213	3
M3–5	364/**376**	398/414	444/568	189/213	3
M3–6	364/368	398/414	380/568	**185**/189	3
M3–8	364/368	398/398	444/612	177/189	4
M3–9	**376/376**	398/414	0/0	201/213	2
M3–11	364/368	398/418	380/612	**185**/189	3
M3–12	**376/376**	398/398	420/444	189/221	3
M3–13	364/368	398/410	444/612	**173**/189	3
M3–15	364/368	398/398	444/612	**173**/213	3
M3–17	**376/376**	398/414	444/584	189/213	3
M3–18	364/**376**	414/418	444/564	189/193	3
M3–20	**344**/364	382/398	380/380	189/189	3
M3–22	**344/376**	398/410	380/380	189/189	3
M3–23	364/368	398/398	444/612	181/213	4
M3–24	364/**376**	398/414	384/444	**173**/189	2
M3–26	364/368	414/418	444/612	**173**/213	3
M3–27	364/368	398/414	384/444	189/213	4
M3–28	364/368	398/398	0/0	**173**/189	2
M3–29	364/368	398/414	444/568	**185**/189	3
M3–30	364/368	410/414	444/612	**173**/189	3
M3–31	**344**/364	398/398	380/380	189/189	3
M3–37	0/0	398/398	356/380	**173**/189	2
M3–38	364/368	398/414	0/0	**173**/189	2
M3–39	364/368	0/0	444/568	**185**/189	2
M3–41	368/**376**	398/398	444/612	**185**/189	2
Assigned maternity					Probability of identity
FC29	364/376	398/414	380/444	189/213	1.8×10^{-8}

Shown are genotypes for female M3 and for 33 progeny sampled from the brood she carried. Bold faced alleles are consistent with those from the putative mother M3. Underlined genotypes depict progeny mismatches to female M3. Also shown is the genotype of the adult individual who was assigned maternity of offspring excluded as M3's biological progeny. This genotype was deduced by GERUD2.0, and matched to collected individual FC29. The probability of genetic identity of this match was calculated from empirical population allele frequencies, assuming random mating and Hardy–Weinberg equilibrium. Genotypes written "0/0" denote missing data

(M3c2) also had alleles from multiple fathers, after accounting for the deduced dam's genotype. Because the genotype of the mother of M10c2 was not successfully deduced above, the number of sires in that cohort was not estimated with confidence. Although the estimated number of sires varied depending on method, ranging from 1 to 38, all three methods agreed that the brood by dam M15 was the only one composed entirely of full sibs (Table 3). Hence, 15 of the 17 cohorts produced by individual females were sired by two or more males, indicating frequent polyandry.

Table 3 Summary of genetic paternity analyses of offspring carried by 15 *Leptasterias* sp. females

Dam ID	Stage	No. in brood	No. genotyped	Number of sires[a]		
				Allele counting	GERUD	COLONY
M2	Juveniles	38	32	8	6 +	20
M3	Eggs	69	7	2	2	2
FC29 (carried by M3)	Eggs	–	26	5	5	15
M4	Juveniles	6	5	2	2	4
M5	Juveniles	7	6	3	3	4
M7	Juveniles	90	55	10	6 +	38
M9	Juveniles	12	10	4	4	10
M10	Juveniles	49	39	8	6 +	25
Unknown (carried by M10)	Juveniles	–	7	–	2	4
M11	Eggs	91	44	13	6 +	31
M12	Juveniles	61	29	5	6	13
M13	Juveniles	133	52	6	6 +	12
M14	Juveniles	12	12	4	5	9
M15	Juveniles	9	9	1	1	1
M16	Juveniles	78	44	8	6 +	19
M17	Juveniles	155	46	4	5	8
M18	Juveniles	19	16	3	3	5

[a]Both allele counting and GERUD methods estimate the minimum number of sires, while COLONY estimates the most likely number

As mentioned above, estimates of minimum number of sires using GERUD were limited in several broods because the program does not continue computation if it has detected that more than six sires have already been counted. Even with the most conservative approach of allele counting, however, eight broods had ≥ 5 sires, and two had ≥ 10 sires, with a mean (± standard deviation) of 5.4 ± 3.2 (Table 3). Numbers from COLONY were consistently higher (12.9 ± 10.6; Table 3), which is expected since this algorithm estimates most likely number of sires.

Twelve broods with 10 or more progeny genotyped were assessed for sire evenness. In all broods, several fathers deduced by COLONY sired a single offspring. Sire evenness across these broods ranged from 0.56 to 1 (Fig. 1). The brood with $E = 1$ was carried by dam M9, in which each of the 10 progeny analyzed was deduced to be the genetic offspring of a different father. Of these 12 broods, the highest proportion sired by a single father occurred in brood carried by dam M18, in which one father sired 75% of the 16 offspring genotyped. We tested whether the levels of sire evenness were randomly distributed among broods collected on different transects. Sire evenness E for the six broods collected along transect 1 (mean $E = 0.97$) was significantly higher than E for broods from transect 2 (mean $E = 0.79$) (Mann–Whitney U test, $U = 33$, $n_1 = 6$, $n_2 = 6$, $P = 0.015$; Fig. 1). While our sample size is small for this analysis (only six broods per transect), the total

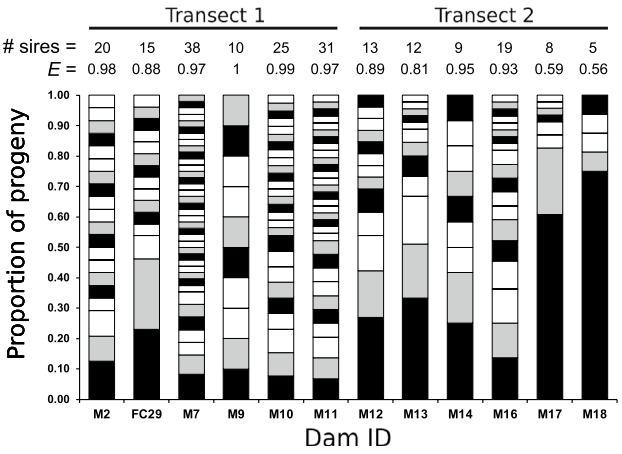

Fig. 1 Relative contribution of fathers to broods with multiple paternity. With each brood, each section reflects a different sire's proportion of genetic progeny, but section colors and patterns are repeated for ease of visualization and do not identify the same father. The number of sires was estimated in COLONY (Jones and Wang 2010). Sire evenness E was calculated by the method in Schmoll et al. (2007). Shown are only broods in which at least 10 progeny were genotyped

number of offspring was similar between the two sets (206 and 199, Table 3).

None of the candidate (sampled) individuals included in the COLONY analysis were assigned paternity of any

offspring. While COLONY uses likelihood to attempt to reconstruct paternal genotypes from progeny arrays, many broods contained fathers that sired single offspring, which provides insufficient information for accurate reconstruction of father multilocus genotypes. Because of such low-confidence multilocus reconstructions, we made no attempt to track multiple mating events by males across broods.

Discussion

Here, we report on the genetic mating system of a species of *Leptasterias*, an important predator of intertidal communities (Menge 1972; Gravem and Morgan 2017). The microsatellite markers we developed are the first codominant markers developed for this species, and will be useful for studies of genetic kinship, dispersal, and population structure. Our study is among the few to examine a genetic mating system in this phylum, likely due to the relative rarity of embryo brooding in shallow water echinoderms (Gillespie and McClintock 2007). The observational laboratory study by Chia (1966) suggested that spermcasting in this species provides the opportunity for polygamy in both sexes; nevertheless, only with the use of genetic markers can we unambiguously quantify reproductive success of breeding adults (Birkhead and Møller 1992; Avise et al. 2002).

Frequent polyandry

Our genetic markers readily detected multiple paternity in all but one sampled brood (94.1%), demonstrating that polyandry is frequent in this species. This was true even when only a few offspring were collected and genotyped (Table 3). For instance, dams M4 and M5 carried broods with, respectively, 6 and 7 juveniles at the time of collection, and simple allele counting revealed that a minimum of two and three sires contributed to the fertilization of these broods (Table 3). Considering the range of brood sizes (Chia 1966; Menge 1974) and the advanced stage of the juveniles in our collection, we suspect that the small number of embryos in these broods simply reflects the remaining individuals from previously larger and well-mixed clutches, instead of actually being very small clutches with multiple fathers. Therefore, the number of sires per brood in our study may actually be an underestimate, and future sampling of more intact clutches may reveal a more accurate population-wide measure of polyandry.

In addition to high frequency, the magnitude of multiple paternity per brood in *Leptasterias* is among the highest ever reported for a marine invertebrate. We detected a mean number of sires per brood of 12.9 (range 1–38), a value that is comparable to species with extreme promiscuity, such as the colonial ascidian *Botryllus schlosseri* (mean, range 11.7, 4–15; Johnson and Yund 2007), the freshwater mussel *Margaritifera margaritifera* (11, 1–32; Wacker et al. 2018), and the intertidal snail *Littorina saxatilis* (19.3, 15–23; Panova et al. 2010). The only other brooding echinoderms in which genetic parentage was explicitly examined were polar sea urchins in the genus *Abatus* (Chenuil et al. 2004; Maturana et al. 2016). These also showed frequent polyandry albeit at much lower levels (2–5 sires per brood). The presence of multiple paternity in *Leptasterias* broods is not surprising given the mode of fertilization via spermcasting. The unusually high number of sires observed, however, suggests that mating in this species occurs in patches with high density of individuals. We argue that high levels of female multiple mating in *Leptasterias* provide opportunity for genetic 'bet hedging', which is a proposed benefit of polyandry thought to occur in species in which females are unable to assess the quality of potential mates (Jennions and Petrie 2000; Yasui 2001). Because fertilization is external and utilizes sperm cast into the water instead of via copulation, females in this species likely have a limited role in controlling whose sperm contribute to fertilization of their brood. Bet hedging, hence, provides indirect benefits to the female by increasing the likelihood of mating with a high-quality male and reducing that of genetic incompatibilities. Similarly, mating with a diversity of males lowers the amount of inbreeding within a brood (Stockley et al. 1993), and this may be particularly important in this system due to the lack of pelagic dispersal. Finally, bet hedging may also benefit the female through the higher genetic diversity of her offspring, which improves the chances that some of will survive in fluctuating environments (Watson 1991; Yasui 1998), such as the rocky intertidal.

Variation in paternity evenness

Our analysis of sire evenness among broods found a significantly lower evenness (higher skewness) in the later sampling transect compared to the earlier. This suggests that the distribution of paternity among embryos in a brood may vary at small scales, either temporally (the two transects were separated by 25 days) or among microhabitats. At the time of collection, we did not quantify differences in habitat structure between the transects, as our primary goal of having two transects was to avoid sampling from the same group of individuals. We, hence, cannot test for possible ecological correlates for this difference in sire evenness. Nevertheless, our results provide initial evidence that there is ecological or demographic patchiness that affects reproductive success of mating sea stars. For instance, we hypothesize that habitat complexity, such as size and number of crevices or the presence of mussel bed habitat, may determine relative proximity of males and females at the time of mating, and hence change the relative contribution of certain sperm to

the brood. Alternatively, since broods with higher sire skewness were sampled at the later time point, the observed pattern could reflect differential mortality among half-sibs in a brood that may occur during development, possibly as a result of fitness differences among them. These results warrant new studies with targeted sampling designs.

Alloparental care

Perhaps our most striking result was the detection of two broods with mixed maternity, in which the guardian female was brooding another mother's offspring along with her own. During sample collection and processing, we did not observe any features that could alert us to the presence of mixed clutches, such as different developmental stages. The fact that we found two mixed broods out of only 15 analyzed suggests that this phenomenon is relatively common in this species, despite being theoretically unexpected. To our knowledge, only a few cases of alloparental care by females in aquatic species have ever been reported, with some in whales (Gero et al. 2009), which have complex social structures, and in two species of mouthbrooding cichlids (Kellogg et al. 1998; Sefc et al. 2012). In contrast, this behavior is relatively common in species with male-only brood care, especially bony fishes, wherein the mode of fertilization has allowed the evolution of alternative male mating behaviors such as 'sneakers' or 'satellites' (Wisenden 1999; Avise et al. 2002; Mackiewicz et al. 2005).

The mode of maternal care in *Leptasterias* likely increases opportunities for brood mixing, while viviparity found in several other asteroids (Byrne 1996; Keever et al. 2013; Puritz et al. 2012) should prevent this phenomenon from occurring in those species. While it has not been quantified directly, brooding behavior in *Leptasterias* is likely very costly for females since they do not eat during embryo development (40–60 days; Chia 1966; Menge 1974). The female deposits her eggs underneath her oral side and protects them with her oral tube feet, while the distal tube feet remain attached to the rocky substrate, forming a "puckered" position (Menge 1974). Chia (1966) showed that an unattended egg or embryo mass is readily picked up by non-brooding adults, suggesting the existence of chemical and tactile cues for this active behavior. We hypothesize that mixed broods in this sea star occur when females carrying their brood pick up unattended embryos left on the substrate after their original guardian was dislodged by wave or surge action. This might occur in patches with high density of brooding females, such that brood pick up may happen accidentally, or when a female lost part of her own brood and picked up the wrong one during recovery. The latter mechanism is consistent, for example, with the brood from dam M3, which contained only 7 of her genetic offspring but 26 from another mother (Table 3). This hypothesis can be tested with laboratory manipulations, and also in the field by comparing the frequency of mixed maternity broods between sites varying in, for instance, sea star density and wave exposure; we predict that sites with less protection from wave shock will provide more opportunities for brood abandonment and subsequent mixing. Adoption of abandoned broods may also be predicted to occur in populations in which genetic relatives remain spatially close together, such that an individual is more likely to adopt a brood from a kin. Our current findings are not consistent with this prediction, since relatedness among sampled adults was very low and the two dams of mixed brood M3 were genetically unrelated. However, a proper test of this hypothesis should examine multiple populations, encompassing variation in levels of relatedness and frequency of alloparental care. Regardless of mechanism, alloparental care in female *Leptasterias* is an intriguing phenomenon because it raises the question of why rejection mechanisms have not evolved given the perceived energetic costs to the mother.

Alternatively, if *Leptasterias* embryos are targets of predation, brood mixing may provide a benefit of dilution to a female's genetic clutch. In this case, unattended embryos would be actively adopted by an unrelated female that already has or is ready to spawn her own eggs. Testing this hypothesis, therefore, requires quantifying the balance between rates of embryo predation and costs of adoption.

Conclusions

Prolonged parental care behaviors can substantially increase the costs associated with successful reproduction. These behaviors provide excellent opportunities for examining the relationship between ecology and mating systems. In a dynamic environment such as the rocky intertidal, parental brooding of embryos requires exposure to predators as well as to desiccation, rapid temperature fluctuations, and wave shock. Here, we demonstrated that eggs of the brooding intertidal sea star *Leptasterias* can be successfully fertilized by upwards of 30 or more males in a single clutch. High levels of multiple paternity are expected to lower the variance in mating success among males, which has been shown to increase overall genetic variation and effective population size (Sugg and Chesser 1994; Pearse and Anderson 2009). Our estimates of within-population genotypic diversity and relatedness suggest that this species can maintain largely outbred populations, despite the lack of a pelagic dispersal stage. This contrasts sharply with the extremely low genetic diversity documented in self-fertilizing viviparous asteroids that give rise directly to crawl-away juveniles (Keever et al. 2013; Puritz et al. 2012). Effective population size and genetic diversity, hence, may vary among taxa depending on specific mode of development and parental care (Ostrovsky

et al. 2016). Nevertheless, future studies should examine levels of genetic variation among multiple populations and test for the degree of differentiation among them. Finally, this system may be an excellent model for examining the influence of demographic and ecological parameters on the maintenance of alloparental care.

Acknowledgements We thank Sarah Gravem for helpful comments on the manuscript, and Mark Dasenko for help with Illumina sequencing.

Funding This research was supported by Oregon State University funds to F. S. B. and SURE Science internship funds to K. K. B.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.ukprojects fastqc. Accessed 22 Aug 2018

Avise JC, Liu JX (2010) Multiple mating and its relationship to alternative modes of gestation in male-pregnant versus female-pregnant fish species. Proc Natl Acad Sci USA 107:18915–18920

Avise J, Jones A, Walker D, DeWoody J (2002) Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. Annu Rev Genet 36:19–45

Baggio RA, Pil MW, Boeger WA et al (2011) Genetic evidence for multiple paternity in the mangrove land crab *Ucides cordatus* (Decapoda: Ocypodidae). Mar Biol Res 7:520–524. https://doi.org/10.1080/17451000.2010.528771

Barazandeh M, Davis CS, Neufeld CJ et al (2013) Something Darwin didn't know about barnacles: spermcast mating in a common stalked species. Proc R Soc B 280:20122919. https://doi.org/10.1111/mec.12009

Barreto FS, Avise JC (2008) Polygynandry and sexual size dimorphism in the sea spider *Ammothea hilgendorfi* (Pycnogonida: Ammotheidae), a marine arthropod with brood-carrying males. Mol Ecol 17:4164–4175. https://doi.org/10.1111/j.1365-294X.2008.03895.x

Barreto FS, Avise JC (2010) Quantitative measures of sexual selection reveal no evidence for sex-role reversal in a sea spider with prolonged paternal care. Proc R Soc B 277:2951–2956. https://doi.org/10.1098/rspb.2010.0311

Barreto FS, Avise JC (2011) The genetic mating system of a sea spider with male-biased sexual size dimorphism: evidence for paternity skew despite random mating success. Behav Ecol Sociobiol 65:1595–1604. https://doi.org/10.1007/s00265-011-1170-x

Beier S, Thiel T, Münch T et al (2017) MISA-web: a web server for microsatellite prediction. Bioinformatics 33:2583–2585. https://doi.org/10.1093/bioinformatics/btx198

Birkhead TR, Møller A (1992) Sperm competition in birds: evolutionary causes and consequences. Academic Press, New York

Bishop JDD (2006) The third way: spermcast mating in sessile marine invertebrates. Integr Comp Biol 46:398–406. https://doi.org/10.1093/icb/icj037

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5:453–455

Burris ZP (2011) The polygamous mating system of the sea spider *Achelia simplissima*. Invertebr Reprod Dev 55:162–167. https://doi.org/10.1080/07924259.2011.557555

Byrne M (1996) Viviparity and intragonadal cannibalism in the diminutive sea stars *Patiriella vivipara* and *P. parvivipara* (family Asterinidae). Mar Biol 125:551–567

Chenuil A, Gault A, Feral J (2004) Paternity analysis in the Antarctic brooding sea urchin *Abatus nimrodi*. A pilot study. Polar Biol 27:177–182

Chia F-S (1966) Brooding behavior of a six-rayed starfish, *Leptasterias hexactis*. Biol Bull 130:304–315. https://doi.org/10.2307/1539738

Coleman SW, Jones AG (2011) Patterns of multiple paternity and maternity in fishes. Biol J Linn Soc 103:735–760

DeWoody JA, Fletcher DE, Wilkins SD, Avise JC (2000) Parentage and nest guarding in the tessellated darter (*Etheostoma olmstedi*) assayed by microsatellite markers (Perciformes: Percidae). Copeia 2000:740–747. https://doi.org/10.1643/0045-8511(2000)000%5b0740:pangit%5d2.0.co;2

Dupont L, Richard J, Paulet YM et al (2006) Gregariousness and protandry promote reproductive insurance in the invasive gastropod *Crepidula fornicata*: evidence from assignment of larval paternity. Mol Ecol 15:3009–3021. https://doi.org/10.1111/j.1365-294X.2006.02988.x

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94. https://doi.org/10.1111/j.1471-8286.2007.01884.x

Ferguson CD, Blum MJ, Raymer ML et al (2013) Population structure, multiple paternity, and long-distance transport of spermatozoa in the freshwater mussel *Lampsilis cardium* (Bivalvia:Unionidae). Freshw Sci 32:267–282. https://doi.org/10.1899/12-028.1

Foltz DW, Nguyen AT, Kiger JR, Mah CL (2008) Pleistocene speciation of sister taxa in a North Pacific clade of brooding sea stars (*Leptasterias*). Mar Biol 154:593–602. https://doi.org/10.1007/s00227-008-0952-9

Gero S, Engelhaupt D, Rendell L, Whitehead H (2009) Who cares? Between-group variation in alloparental caregiving in sperm whales. Behav Ecol 20:838–843. https://doi.org/10.1093/beheco/arp068

Gillespie JM, McClintock JB (2007) Brooding in echinoderms: how can modern experimental techniques add to our historical perspective? J Exp Mar Biol Ecol 342:191–201. https://doi.org/10.1016/j.jembe.2006.10.055

Gosselin T, Sainte-Marie B, Bernatchez L (2005) Geographic variation of multiple paternity in the American lobster, *Homarus americanus*. Mol Ecol 14:1517–1525

Gravem SA, Morgan SG (2017) Shifts in intertidal zonation and refuge use by prey after mass mortalities of two predators. Ecology 98:1006–1015. https://doi.org/10.1002/ecy.1672

Hess HC (1993) The evolution of parental care in brooding spirorbid polychaetes: the effect of scaling constraints. Am Nat 141:577–596. https://doi.org/10.1086/285492

Jamieson A, Taylor SC (1997) Comparisons of three probability formulae for parentage exclusion. Anim Genet 28:397–400

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev Camb Philos Soc 75:21–64. https://doi.org/10.1017/S0006323199005423

Jense PC, Bentzen P (2012) A molecular dissection of the mating system of the dungeness crab, *Metacarcinus magister* (Brachyura: Cancridae). J Crustac Biol 32:443–456. https://doi.org/10.1163/193724012X626458

Johnson S, Yund P (2007) Variation in multiple paternity in natural populations of a free-spawning marine invertebrate. Mol Ecol 16:3253–3262

Jones AG (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Mol Ecol Notes 5:708–711. https://doi.org/10.1111/j.1471-8286.2005.01029.x

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555. https://doi.org/10.1111/j.1755-0998.2009.02787.x

Jones AG, Walker D, Avise JC (2001a) Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. Proc R Soc B 268:2531–2535. https://doi.org/10.1098/rspb.2001.1841

Jones AG, Walker D, Kvarnemo C et al (2001b) How cuckoldry can decrease the opportunity for sexual selection: data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. Proc Natl Acad Sci USA 98:9151–9156. https://doi.org/10.1073/pnas.171310198

Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes 6:576–579

Kamel SJ, Grosberg RK (2012) Exclusive male care despite extreme female promiscuity and low paternity in a marine snail. Ecol Lett 15:1167–1173. https://doi.org/10.1111/j.1461-0248.2012.01841.x

Keever CC, Puritz JB, Addison JA et al (2013) Shallow gene pools in the high intertidal: extreme loss of genetic diversity in viviparous sea stars (*Parvulastra*). Biol Lett 9:20130551

Kellogg KA, Markert JA, Stauffer JR, Kocher TD (1998) Intraspecific brood mixing and reduced polyandry in a maternal mouthbrooding cichlid. Behav Ecol 9:309–312. https://doi.org/10.1093/beheco/9.3.309

Lasker H, Gutiérrez-Rodríguez C, Bala K et al (2008) Male reproductive success during spawning events of the octocoral *Pseudopterogorgia elisabethae*. Mar Ecol Prog Ser 367:153–161

Mackiewicz M, Porter BA, Dakin EE, Avise JC (2005) Cuckoldry rates in the Molly Miller (*Scartella cristata*; blenniidae), a hole-nesting marine fish with alternative reproductive tactics. Mar Biol 148:213–221. https://doi.org/10.1007/s00227-005-0010-9

Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal 17:10–12. https://doi.org/10.14806/ej.17.1.200

Maturana CS, Gérard K, Díaz A et al (2016) Mating system and evidence of multiple paternity in the Antarctic brooding sea urchin *Abatus agassizii*. Polar Biol 40:787–797. https://doi.org/10.1007/s00300-016-2001-3

McCoy EE, Jones AG, Avise JC (2001) The genetic mating system and tests for cuckoldry in a pipefish species in which males fertilize eggs and brood offspring externally. Mol Ecol 10:1793–1800

McEdward LR, Miner BG (2001) Larval and life-cycle patterns in echinoderms. Can J Zool 79:1125–1170. https://doi.org/10.1139/cjz-79-7-1125

Mehlis M, Bakker TCM, Engqvist L, Frommen JG (2010) To eat or not to eat: egg-based assessment of paternity triggers fine-tuned decisions about filial cannibalism. Proc Biol Sci B 277:2627–2635. https://doi.org/10.1098/rspb.2010.0234

Melroy LM, Smith RJ, Cohen CS (2017) Phylogeography of direct-developing sea stars in the genus *Leptasterias* in relation to San Francisco Bay outflow in central California. Mar Biol 164:1–14. https://doi.org/10.1007/s00227-017-3184-z

Menge BA (1972) Competition for food between two intertidal starfish species and its effect on body size and feeding. Ecology 53:635–644. https://doi.org/10.2307/1934777

Menge BA (1974) Effect of wave action and competition on brooding and reproductive effort in the seastar, *Leptasterias hexactis*. Ecology 55:84–93. https://doi.org/10.2307/1934620

Neff BD (2003) Decisions about parental care in response to perceived paternity. Nature 422:716–719. https://doi.org/10.1038/nature01528

Ostrovsky AN, Lidgard S, Gordon DP et al (2016) Matrotrophy and placentation in invertebrates: a new paradigm. Biol Rev 91:673–711

Panova M, Boström J, Hofving T et al (2010) Extreme female promiscuity in a non-social invertebrate species. PLoS One 5:e9640. https://doi.org/10.1371/journal.pone.0009640.t001

Pearse DE, Anderson EC (2009) Multiple paternity increases effective population size. Mol Ecol 18:3124–3127. https://doi.org/10.1111/j.1365-294X.2009.04268.x

Philipp DP, Gross MR (1994) Genetic evidence for cuckoldry in bluegill *Lepomis macrochirus*. Mol Ecol 3:563–569. https://doi.org/10.1111/j.1365-294x.1994.tb00087.x

Plough LV, Moran A, Marko P (2014) Density drives polyandry and relatedness influences paternal success in the Pacific gooseneck barnacle, *Pollicipes elegans*. BMC Evol Biol 14:81. https://doi.org/10.1186/1471-2148-14-81

Porter B, Fiumera A, Avise J (2002) Egg mimicry and allopaternal care: two mate-attracting tactics by which nesting striped darter (*Etheostoma virgatum*) males enhance reproductive success. Behav Ecol Sociobiol 51:350–359. https://doi.org/10.1007/s00265-002-0456-4

Puritz JB, Keever CC, Addison JA et al (2012) Extraordinarily rapid life-history divergence between *Cryptasterina* sea star species. Proc R Soc B 279:3914–3922

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Royle N, Smiseth P, Kölliker M (2012) The evolution of parental care. Oxford University Press, Oxford

Sambrook J, Russell DW (2010) Purification of nucleic acids by extraction with phenol:chloroform. Cold Spring Harb Protoc. https://doi.org/10.1101/pdb.prot4455

Schmoll T, Schurr FM, Winkel W et al (2007) Polyandry in coal tits *Parus ater*: fitness consequences of putting eggs into multiple genetic baskets. J Evol Biol 20:1115–1125. https://doi.org/10.1111/j.1420-9101.2006.01288.x

Sefc KM, Hermann CM, Taborsky B, Kolbmüller S (2012) Brood mixing and reduced polyandry in a maternally mouthbrooding cichlid with elevated among-breeder relatedness. Mol Ecol 21:2805–2815. https://doi.org/10.1111/j.1365-294X.2012.05573.x

Stockley P, Searle JB, Macdonald DW, Jones CS (1993) Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. Proc Biol Sci B 254:173–179. https://doi.org/10.1098/rspb.1993.0143

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. Genetics 137:1147–1155

Toonen R (2004) Genetic evidence of multiple paternity of broods in the intertidal crab *Petrolisthes cinctipes*. Mar Ecol Prog Ser 270:259–263

Unger L, Sargent R (1988) Allopaternal care in the fathead minnow, *Pimephales promelas*: females prefer males with eggs. Behav Ecol Sociobiol 23:27–32

Voight J, Feldheim K (2009) Microsatellite inheritance and multiple paternity in the deep-sea octopus *Graneledone boreopacifica* (Mollusca: Cephalopoda). Invertebr Biol 128:26–30

Vulstek SC, Tallmon DA, Linderoth TP, Guyon JR (2013) Spatiotemporal population genetic structure and mating system of red king crab (*Paralithodes camtschaticus*) in Alaska. J Crustac Biol 33:691–701. https://doi.org/10.1163/1937240X-00002173

Wacker S, Larsen BM, Jakobsen P, Karlsson S (2018) High levels of multiple paternity in a spermcast mating freshwater mussel. Ecol Evol 14:1803–1809. https://doi.org/10.1002/ece3.4201

Watson PJ (1991) Multiple paternity as genetic bet-hedging in female sierra dome spiders, *Linyphia litigiosa* (Linyphiidae). Anim Behav 41:343–360. https://doi.org/10.1016/S0003-3472(05)80486-5

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370

Wilson WH (1991) Sexual reproductive modes in polychaetes: classification and diversity. Bull Mar Sci 48:500–516

Wisenden BD (1999) Alloparental care in fishes. Rev Fish Biol Fish 9:45–70

Yasui Y (1998) The "genetic benefits" of female multiple mating reconsidered. Trends Ecol Evol 13:246–250. https://doi.org/10.1016/S0169-5347(98)01383-4

Yasui Y (2001) Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. Ecol Res 16:605–616. https://doi.org/10.1046/j.1440-1703.2001.00423.x