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ORIGINAL PAPER

Mating system and evidence of multiple paternity in the Antarctic brooding sea urchin *Abatus agassizii*

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Abstract Broadcasting is the predominant spawning behavior among benthic marine invertebrates, mainly associated with planktotrophic and planktonic lecitotrophic development. Broadcasting allows genetic mixing that should contribute to increase the genetic diversity of a female clutch. Conversely, in brooding species characterized by protected development, oocytes are retained and only sperm is released, which is supposed to limit the number of males that contribute to a female clutch. This spermcasting behavior together with egg retention, unusually frequent among Antarctic marine invertebrates, putatively give brooders low dispersal capacities which may reduce genetic mixing and generate genetic and kinship structure at a small spatial scale. Like many other Antarctic marine benthic invertebrates, the irregular sea urchin *Abatus agassizii* is a spermcaster that broods its young. In this study, we assessed the genetic diversity among 66 adults using 6 polymorphic microsatellite loci and performed progeny array analyses in order to evaluate the number of mates per female as well as genetic structure at a small spatial scale. *A. agassizii* exhibited a polyandric system with 2–5 mates per female regardless of population density. Bayesian analyses suggested the absence of genetic structure along our 20-m transect, while relatedness among individuals did not differ from that expected under panmixia. Finally, we conclude that a limited number of males contribute to a female clutch, probably as a consequence of limited sperm dispersal and that movement of adults may be sufficient to avoid kinship structure in the population.

Keywords Parentage analysis · Polyandry · Spermcast · Vagility · Panmixia

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Introduction

Three main spawning behaviors have been defined in marine invertebrates (Shuster and Wade 2003; Havenhand and Styan 2009; Kamel et al. 2010). The most common is *broadcasting,* in which both gametes are released into the water column where fertilization occurs. Oocytes have a greater probability of being fertilized by gametes of a large number of males in this free-spawning mode (Johnson and Yund 2007). The second way is *copulatory behavior*, in which the sperm is released directly inside the female by reproductive structures or through copulatory or pseudocopulatory behavior (Slattery and Bosch 1993; Paterson et al. 2001; Voight and Feldheim 2009; Yue and Chang 2010). The third way of fertilization is the *spermcast*

modality (Pemberton et al. 2003; Bishop and Pemberton 2006), in which only male gametes are liberated which subsequently fertilize eggs that have been retained rather than spawned (Bishop 1996; Addison and Hart 2005; Marshall and Evans 2005; Bishop and Pemberton 2006; Johnson and Yund 2007; Serrao and Havenhand 2009; Kamel et al. 2010). In this case, fertilization occurs upon or inside females (Fell 1946; Schinner and McClintock 1993; David et al. 2005; Sewell and Hofmann 2011) which receive male gametes, potentially from multiple mates. The spermcast modality has been mainly described from sessile species with low dispersal capacity (Yund and McCartney 1994; Johnson and Yund 2004) and in species with sperm storage structures. When eggs are retained, the number of potential mates may be limited to the males in the vicinity, because of the rapid dilution and short longevity of sperm in sea water. The allele pool provided by matings is reduced in this scenario, decreasing the genetic diversity of the offspring. It is also expected that the number of mates should be modulated by population characteristics such as spatial distribution and density of males (Levitan et al. 1992; Levitan and Young 1995; Yund 2000; Levitan 2004; Marshall and Evans 2005; Avise et al. 2011). When the number of males per female (or the degree of multipaternity) increases, variance in male reproductive success will be reduced and therefore population effective size (Ne) should increase (Pearse and Anderson 2009). In the same manner, Ne should increase proportionally to the degree of population structure (Wright 1949).

Spermcast is not the most common spawning mode among marine invertebrates. However, it appears more frequently in species with extremely low density (e.g., deep sea) (Seibel et al. 2000; Mercier and Hamel 2008; Tsuruwaka and Shimada 2011) or that live in habitats characterized by turbulent flow or strong currents (Pennington 1985; Levitan and Petersen 1995; Levitan and Young 1995). Spermcast is also found in species lacking reproductive behavior such as aggregative behavior and synchronized spawning (Pemberton et al. 2003). It also appears to be a common reproductive behavior in the Southern Ocean, since brooding development is unusually common among Antarctic and Subantarctic marine invertebrates (Dell 1972; Dayton et al. 1974; Poulin et al. 2002; David et al. 2005; Pearse et al. 2009). Because most benthic marine invertebrates exhibit low vagility as adults, the larval stage is considered as the main dispersal stage during their life cycle. The persistence of larvae in the water column for days to months generally allows important connectivity among populations over large geographic scales (Palumbi 1994). In the case of brooders, juveniles are usually released in the neighborhood of the parents, where they initiate their benthic life. Consequently, this type of species should exhibit genetic structure at small geographic scales and also could generate kinship (i.e., family structure), which may lead to a loss of genetic diversity through inbreeding (Kalinowski and Hedrick 2001). According to genetic studies published so far on brooding species, differentiation and kinship structure may occur at small scales (Ledoux et al. 2012; Smilansky and Lasker 2014; Weber et al. 2015), but this is not a common pattern in brooding species; thus, genetic homogeneity can be found over large geographic distances such as 300–500 km (Helmuth et al. 1994; Arndt and Smith 1998; Boissin et al. 2008; Hunter and Halanych 2008; Neves et al. 2008; Hoffman et al. 2013; O'Hara et al. 2014; Boissin et al. 2015).

Although there have been advances in the evolutionary aspects of these unusually conspicuous developmental modes among benthic marine invertebrates in the Southern Ocean (Poulin et al. 2002; Pearse et al. 2009), the mating strategies remain to be explored. For instance, in brooding species little is known about (1) the number of mates per female in relation to their spatial distribution, (2) the consequence of the spermcast spawning mode on population genetic diversity and relatedness among offspring (Sarvesan 1969) and (3) the geographic scale of population structure (Gorospe and Karl 2013).

The genus *Abatus* Troschel, 1851 includes 11 irregular nominal sea urchin species, all endemic to the Subantarctic and Antarctic regions (David et al. 2005). Different aspects of this genus have been widely studied, such as the ecology (Magniez 1983; Mesphoulhé and David 1992; Poulin and Féral 1995), development (Schatt and Féral 1996; Gil et al. 2009), sexual and brooding cycles (Magniez 1983; Pearse and McClintock 1990; Schatt and Féral 1991; Gil et al. 2009), phylogenetic relationships (Díaz et al. 2012) and population structure (Carrea et al. 2016).

All *Abatus* species are brooders and probably direct developers, as described in *A. cordatus* from the Kerguelen Islands and *A. cavernosus* (Gil et al. 2009). These two species exhibit an annual reproductive cycle (Magniez 1983; Gil et al. 2009). The entire development process lasts 8–9 months and takes place inside four depressed petals located on the adult female test. Five stages characterize the direct development of these species: blastula, gastrula, post-gastrula, short-spined juveniles and finally long-spined juveniles, which are last developmental stage. Juveniles are then released on the bottom in the vicinity of the adults (Magniez 1983; Schatt 1985; Gil et al. 2009). *Abatus* species are distributed from the shoreline down to more than 3000 m depth (Pierrat et al. 2012), although they are mainly found on the upper part of the continental shelf. These species are deposit feeders that generally live grouped in dense patches on soft-bottom substrates ranging from muddy to sandy sediments.

By contrast, only very preliminary studies have been conducted so far on the mating system (Chenuil and Féral

2003; Chenuil et al. 2004) and on the consequences of brooding on the genetic diversity of *Abatus* populations (Poulin and Feral 1994; Díaz et al. 2012; Ledoux et al. 2012). Regarding the mating system, Chenuil et al. (2004) using dominant molecular markers (RAPD) found that more than one paternal genotype contributed to the progeny. Regarding differentiation, Ledoux et al. (2012) observed genetic structure at short distances (from meters to kilometers).

Abatus agassizii is only known from the South Shetland Islands and South Georgia (Díaz et al. 2012), but it has also been reported once in the eastern Weddell Sea (Van de Putte et al. 2012). Populations of *A. agassizii* are present in the vicinity of the Chilean Scientific Station "Prof. Julio Escudero" on King George Island South Shetland. They are found in patchy and dense aggregations (up to 17 ind. m^{-2}) in the shallow subtidal area (2–10 m depth) of the sheltered sector of Fildes Bay, buried in a medium to fine sandy sediment (Palma et al. 2007). Because of the easy access to King George Island and the logistic facilities provided by the Chilean Antarctic Institute (INACH), *A. agassizii* provides a good model to begin studying the consequences of brooding on the genetic diversity and mating system in *Abatus* and more generally in Antarctic brooding marine invertebrates.

The main objective of this study was to determine the mating system of *A. agassizii* through the estimation of the minimum number of mates necessary to explain the progeny array. Also, this study proposed to evaluate how population density modulates the mating system in this species and to detect potential kinship structure at a small geographic scale. Finally, this study represents a first step toward a comprehensive characterization of the relative impact of gene flow and genetic drift on the spatial pattern of genetic structure in the studied species.

Materials and methods

Field sampling method

The sampling was conducted by scuba-diving in Ardley Peninsula, Fildes Bay (62°12′50.5 S; 58°55′58 W) in King George Island (South Shetland Islands), during February, 2008. Sampling consisted of 19 quadrats of 0.5 m^2; each divided into cells of 100 cm^2, distributed in two areas separated by 6 m along a 20-m line transect (Fig. 1). The transect was installed between 8 and 10 m depth, crossing successively two areas with low and high density of sea urchins. Every quadrat was positioned on the substrate; all the individuals found inside the perimeter were collected and recorded. This sampling scheme allows mapping the exact position of every individual and measuring the linear distance between them.

Body size, sex ratio and brooding

Body size was estimated by measuring the anterior-posterior test length using a caliper (± 1 mm). Data were collected from individuals larger than 20 mm; immature individuals were excluded from the data analysis. Individuals were sexed based on the presence of four pronounced dorsal brood pouches on the female test (Schatt and Féral 1996; Gil et al. 2009). The sex ratio was estimated using 66 individuals; the statistical significance of the difference from the expected 1:1 ratio was determined through an exact test using 1000 iterations in RUNDOM PROJECT v1.1 (Jadwiszczack 2007).

Population density

The density of individuals (number of individuals per m^2) along the transect was estimated by the number of individuals in each quadrat. To determine whether the two chosen areas were significantly different from the null hypothesis (i.e., no differences in density between areas), we performed a permutation test among quadrats with 1000 iterations using RUNDOM PROJECT.

Molecular techniques

Genomic DNA was extracted from adult spine tissue and from whole juvenile individuals using the salting-out method (Aljanabi and Martinez 1997). All individuals were genotyped at 6 microsatellite loci, which were especially developed for this study (Table 1), using genomic enrichment and plasmid cloning methodology (Khasa et al. 2000).

Polymerase chain reaction (PCR) amplification mixtures (15 µl) contained 20–30 ng template DNA, 5 pmol each primer, 250 µM dNTPs, 1.25 µL 10 X PCR buffer and 0.2–0.5 U Taq DNA polymerase (Promega). Cycling conditions consisted of an initial denaturing step of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 s at the specific annealing temperature (Table 1), 1 min at 72 °C and a final elongation step at 72 °C for 5 min. The touchdown PCR protocol for ABag18t and ABag2t consisted of an initial denaturing step of 3 min at 94 °C, followed by 14 cycles of 1 min at 94 °C, 30 s annealing at 60 °C with decrease of 1° per cycle ending with 1 min at 72 °C, followed by 16 cycles of 1 min at 94 °C, 30 s at 47 °C and 1 min at 72 °C. Finally, we used 5 cycles of 1 min at 94 °C, 30 s with an annealing temperature of 55 °C and 1 min at 72 °C and a final elongation step at 72 °C for 5 min.

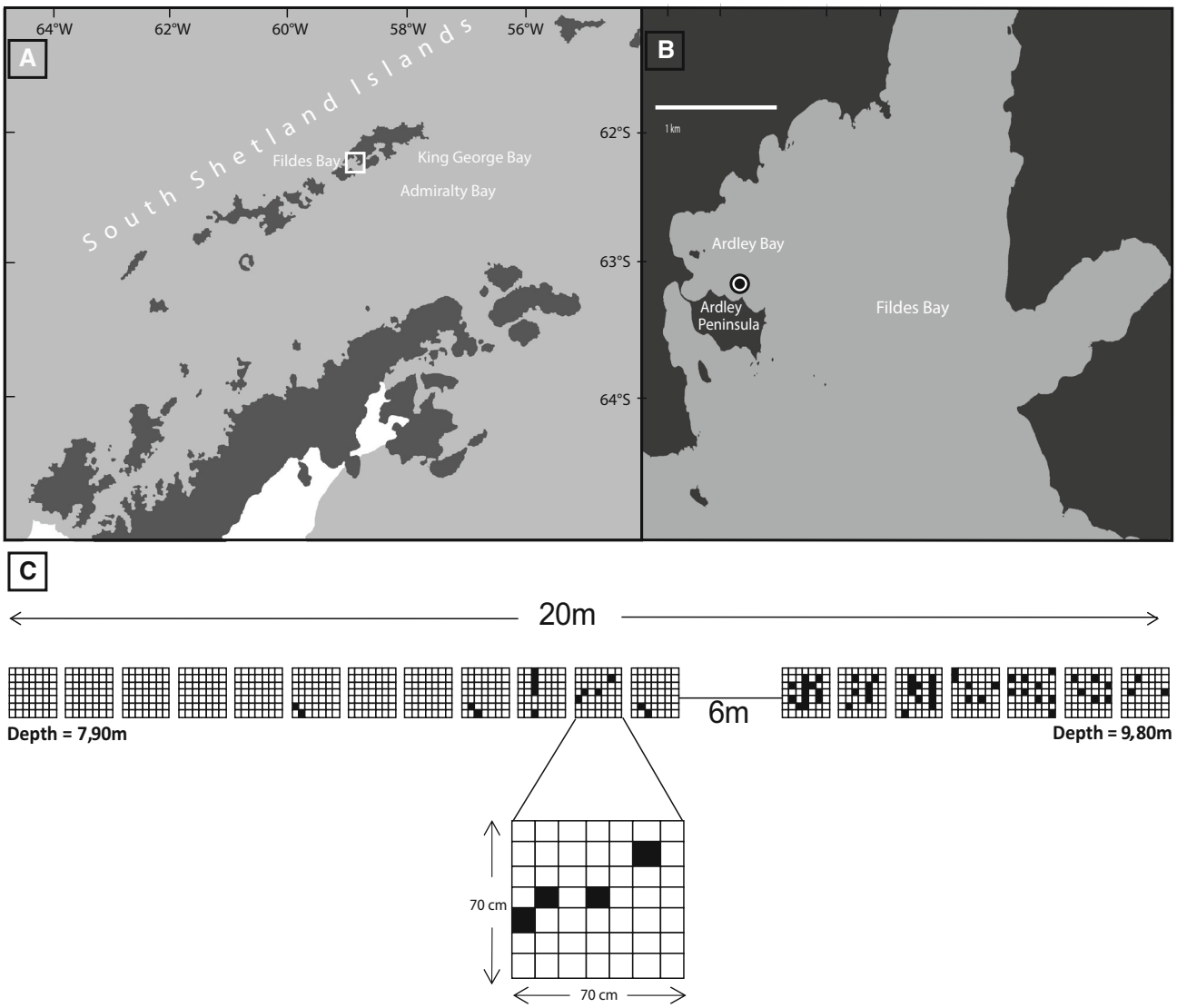

Fig. 1 Sampling site of *Abatus agassizii*. **A** Antarctic Peninsula and South Shetland Islands. **B** Zoom of the sampling site in Ardley Peninsula at Fildes Bay. **C** Diagram of sampling method showing the distribution of adults along the transect and its dimensions (Díaz et al. 2012)

PCR products were run in ABI-PRISM 3730xl Analyzer (Roy J. Carver Biotechnology Center) using the LIZ 500 Size Standard (Applied Biosystems); allele identification was performed using PEAK SCANNER software v.1 (Applied Biosystems).

Data analyses

Genetic diversity and Hardy–Weinberg equilibrium

The genetic diversity analyses were conducted on 66 adults. The presence of null alleles and scoring errors were checked with MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004). All non-amplifying genotypes were checked by re-amplification at least twice (Shaw and Sauer 2004). All loci were tested for linkage disequilibrium and for deviation from Hardy–Weinberg equilibrium (HWE) expectations by means of a permutation test implemented in the software GENETIX v.4.05.2. (Belkhir et al. 1996–09). The observed (*Ho*) and expected (*He*) heterozygosity and allele number (*Na*) were calculated with GENETIX.

Structure analyses

The parentage between adults in the transect was determined by the measure of the fraction of identical alleles shared by descent among individuals (i.e., relatedness coefficient) using the estimator of Konovalov & Heg (Konovalov and Heg 2008) implemented in the KINGROUP v2 software (Konovalov et al. 2004). The mean r

Table 1 Description of the microsatellite loci used in this study

Locus	Primer Sequence (5′–3′)	Repeat Motif (SSR)	Size range (bp)	T_m (°C)
ABag41	F: GAGGACAATTGTTCTCAGGA	$(TG)_{35}$	250	58.8
	R: TACTGGTAGTCGACTGCAAA			
ABag44	F: CCTTTTATCCACAATTGCATAATG	$(CA)_{20}$	162	51.5
	R: CTCAATGCTGAAGCCACTATT			
ABag2t	F: CTACCCACGCATAGATTGCA	$(CATA)_{17}$	319	Td
	R: AACGAATGTTCGCCCATAGG			
ABag18t	F: ACCGTGACTTAACCAGTGAA	$(CATA)_{20}$	173	Td
	R: CTGTATATATACACAACCTTATAAA			
ABag20t	F: CAAGCGCGAATACAAAGACA	$(GTAT)_4.(GTAT)_5$	166	60,5
	R: GCTGCATGTTGCCTTATTTG			
ABag22t	F: TAAGCCCTGCAGCAGCATCA	$(TAG)_{54}$	285	55,3
	R: CACCCTTTTTTTTCGGGTGCA			

T_m annealing temperature, T_d Touchdown PCR protocol

value is compared with its expected distribution under the hypothesis of no relatedness obtained by permutation procedure (Castric et al. 2002; Hoarau et al. 2005) across genotypes with 1000 iterations in IDENTIX v.1.1 (Belkhir et al. 2002) using Queller and Goodnight's estimator (Queller and Goodnight 1989). In order to evaluate possible kinship at the transect level, each pair of genotype was compared with their physical distance (in centimeters) performing an isolation by distance analysis using GENEPOP v.4.2 software (Raymond and Rousset 1995; Rousset 2008).

We tested the existence of genetic structure along the transect using the Bayesian approach implemented in the program STRUCTURE v.2.3.3. (Pritchard et al. 2000) that deduces the number of clusters (K) from the genotype data. Five runs of 1,000,000 iterations with a 500,000 burn-in were conducted for each value of K ranging from 1 (no population differentiation) to 4 (population differentiated in 4 genetic units). Because Structure may fail to detect subtle genetic structure (Waples and Gaggiotti 2006), the occurrence of spatial genetic structure was tested between the adults coming from the low and high density patches through permutation test on the F_{ST} value using GENETIX.

Mating system and multiple paternity level: mother/offspring

Since *A. agassizii* females brood their young, we assume that for each juvenile and at all loci, one allele came from the female and the second came from a male. Therefore, we assume that one parent genotype is known for the paternity analyses. The mating system was determined by the minimum number of males contributing to the genetic diversity of juveniles brooded by one female (i.e., multiple paternity level) (Shaw and Sauer 2004; Iwata et al. 2005; Voight and Feldheim 2009; Yue and Chang 2010). Five mothers from the low density area and 3 from the high density area were chosen from the sampled adults. The offspring of each female were removed from the dorsal brooding pouches and categorized into four developmental stages using an optical microscope. A single development stage was used for paternity analyses, to avoid mixing different reproductive events and thus overestimate the number of males involved in the mating event. Since we achieved better results in DNA quality and quantity, only long-spined juveniles (Schatt and Féral 1996) were genotyped. We performed this analysis in GERUD v2.0 (Jones 2005) which makes full use of multilocus data to determine the minimum number of males contributing to a progeny array and reconstructs those genotypes based on data from microsatellite markers (Jones 2001, 2005).

To evaluate the potential effect of the number of juveniles genotyped per mother on the minimum number of mates, we calculated Spearman's correlation coefficient of (r_S) (Zar 1984) using PAST 2.08 (Hammer et al. 2001). The effect of population density on female fertility and on the multiple paternity level was evaluated using PAST by performing a permutation test between low and high density areas, permuting (1) the total number of long-spined juveniles incubated offspring per brood, (2) the minimum number of mates and (3) the minimum number of mates corrected by rarefaction on the number of genotyped juveniles. Each observed value was compared with its expected distribution under the null hypothesis of no

difference obtained by permutation test with 1000 iterations because of the small size of the sample.

Results

Distribution of *A. agassizii* along the transect

We collected 160 individuals along the transect (42 males, 33 females, 85 immatures), from which 39 males and 27 females were employed for genetic analyses. The distribution of adults in the quadrats is described in Fig. 1. The contrasting density scenarios differed significantly ($p = 0.0001$), with an average of 1.6 ($\sigma^2 = 0.270$) and 8.6 ($\sigma^2 = 1.660$) individuals per quadrat in low and high density areas, respectively.

Genetic diversity and Hardy–Weinberg equilibrium in *A. agassizii*

The 66 individuals analyzed showed an average of 13 alleles per locus. Mean observed heterozygosity over all loci was 0.623. No linkage disequilibrium was observed among pairs of loci. Overall heterozygote deficiency ($F_{IS} = 0.124$) was highly significant ($p < 0.0001$), revealing a strong deviation from HWE. However, heterozygote deficiency was mainly due to the ABag44 locus, as F_{IS} decreased to 0.043 ($p = 0.02$) when this latter locus was excluded from the analysis (Table 2).

Relatedness and genetic structure

The mean relatedness coefficient in the population was -0.0159 and was not significantly different from the expectation under random mating ($p = 0.11$). Moreover, we did not find significant correlation between geographic distance and relatedness among adults ($p = 0.0823$). The Bayesian cluster analysis with STRUCTURE (Pritchard et al. 2000) found that the highest average log-likelihood value was associated with $K = 1$, suggesting a lack of population structure. Furthermore, no spatial genetic structure was detected between the adults coming from the low and high density patches through permutation test on F_{ST} ($p = 0.73$).

Mating system and level of multiple paternity

Multiple mates were detected for each of the 8 analyzed females, with a multiple paternity level ranging between 2 and 5 males per female (Table 3). However, we detected a strong relationship between the minimum number of mates and the number of genotyped juveniles ($r_S = 0.934$, $p = 0.007$). The fertility and the minimum number of mates were not significantly different in females from high and low population density areas, even when the minimum number of mates was corrected by rarefaction ($p = 0.61$).

Discussion

Genetic diversity

The overall level of genetic diversity found across loci in *Abatus agassizii* was moderate to low (*Ho* = 0.623 and *He* = 0.704) compared to other marine invertebrates (Cephalopod: *Loligo bleekeri Ho/He*: 0.868/0.895 (Iwata et al. 2005); Malacostraca: *Caridina ensifera Ho/He*: 0.785/0.900 (Yue and Chang 2010), *Eriocheir sinensis Ho/He*: 0.792/0.814 (Sui et al. 2009), but similar to *A. cordatus* from the Kerguelen Islands *Ho/He*: 0.5/0.57 (Ledoux et al. 2012). This low genetic diversity found in *A. agassizii* and *A. cordatus* could be related to their limited dispersion capacity, since both species are brooders and lack a planktonic dispersal stage. Low vagility and low connectivity between patches could then reduce effective

Table 2 Characterization of the genetic diversity of an *Abatus agassizii* population using 66 individuals

Locus	*n*	N_A	He	Ho	F_{IS}	Scoring error	Null allele frequencies (Oosterhout)
ABag41	61	26	0.915	0.8	0.046	Absence	0.0091
ABag44	38	10	0.839	0.327	0.543***	Presence	0.2506*
ABag2t	63	5	0.698	0.539	0.163**	Presence	0.0745
ABag18t	65	9	0.807	0.841	−0.051	Absence	−0.0344
ABag20t	63	3	0.134	0.111	0.146*	Absence	0.0524
ABag22t	65	24	0.942	0.831	0.011	Absence	0.0027
Total	66		0.723 ± 0.301	0.575 ± 0.305	0.124***		

n number of individuals, N_a number of alleles, H_E expected heterozygosity, H_O observed heterozygosity, F_{IS} Weir and Cockerham's (1984) estimation of F_{IS}; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; presence/absence of scoring error due to stuttering; null allele frequencies calculated by the Oosterhout algorithm (Van Oosterhout et al. 2006)

Table 3 Summary of main results obtained from multipaternity analyses

Mother	Brood size per mother	Long-spined juveniles	Offspring analyzed	Number of putative fathers
M2_LD	16	11	11	5
M5_LD	15	6	5	2
M7_LD	51	20	14	5
M15_LD	62	44	9	4
M16_LD	29	26	23	>6
M20_HD	23	10	9	3
M48_HD	31	16	11	4
M70_HD	10	5	5	2

LD low density area, *HD* high density area

population size, and therefore, genetic diversity measured at a local scale (Ledoux et al. 2012). We also detected a significant deviation from HWE associated with heterozygote deficit in the population ($F_{IS} = 0.124$; $p < 0.0001$). However, the heterozygote deficiencies observed in this study as well as Ledoux et al. (2012) did not seem to reflect the most common biological sources of heterozygote deficiencies (i.e., inbreeding and Wahlund effect) since the significant deviation from HWE did not appear in all loci. Alternatively, the presence of null alleles remains the main technical factor that generates heterozygote deficiency, as at some loci non-amplified alleles occur resulting in false homozygotes (Chapuis and Estoup 2007), as also reported for *A. cordatus* (Ledoux et al. 2012) and *Strongylocentrotus franciscanus* (McCartney et al. 2004). In this study, the locus Abag44 exhibited the highest frequency of null alleles, the most significant heterozygote deficiency and double null alleles in several individuals.

Mating system and level of multiple paternity

We found a polyandric system with a multiple paternity level from 2 to 5 mates. Multiple-paternal mating systems have been found in most marine invertebrates in which parentage analyses have been performed. In a review, Liu and Avise (2011) showed that the multiple paternity level is independent of clutch size, number of polymorphic markers and development mode, suggesting that direct/indirect development or broadcaster/spermcast spawning species do not differ in mate number. Multiple paternity levels estimated among different taxa of marine invertebrates are usually low, frequently related to ecological and natural history traits such as low population density (Liu and Avise 2011). However, in this study, we did not find a significant correlation between mate number and population density. Nevertheless, this result could be associated with our sampling scheme. Since paternity analyses were performed only on late juvenile stages, fertilization should have occurred at least 7–8 months earlier (Schatt and Féral 1996). Thus, population densities measured at sampling time could have drastically changed from the time of fertilization. *Abatus* is a deposit feeder that burrows a few centimeters below the surface of the sediment and obtains food particles by pushing sediment ahead (Poulin and Féral 1995; Thompson and Riddle 2005). Estimation in situ of the locomotion rate in *A. ingens* during 24-h periods showed prolonged periods of stationary time (16.7 h) and only 7.3 h of locomotion with a rate of 0.30 cm h^{-1} (Thompson and Riddle 2005). Assuming a similar locomotion rate for *A. agassizii*, individuals present in the sampling site at reproduction time could have moved several meters since they were fertilized. Additionally, some aggregative behavior could be involved during the reproductive period (Thompson and Riddle 2005), as shown in other echinoderms (Buchanan 1966; Campbell et al. 2001). Further studies on temporal variation of the distribution pattern will be necessary to test this hypothesis.

Spatial correlation between relatedness and geographic distance

Relatedness did not appear to be correlated with geographic distance (Mantel Test $p = 0.0823$), and the relatedness values did not differ from the expected under random mating (Belkhir et al. 2002; Ledoux et al. 2012) with a similar frequency of related ($r > 0$) and unrelated individuals ($r < 0$). Moreover, despite the low vagility of brooding-benthic marine invertebrates, absence of a larval stage and retention of eggs/young by females, we did not detect any genetic structure along our transect. These results suggest that adult and/or male gamete vagility (Grosberg 1991; Yund 1995) may be sufficient to permit panmixia at this small spatial scale and avoid kinship structure. The adult movement discussed above could

explain the absence of relationship of female fertility as well as mate number with adult density at the sampling time. Little is known about the mechanism by which the sperm reaches the female in *Abatus* species. The fact that they live buried in the sediment may imply that either the sperm diffuse through the sediment or a concerted exit behavior at the surface of the sediment. In polar species, high fertilization rates require sperm concentrations 1–2 fold higher than that of nonpolar species as in *Laternula elliptica*, *Nacella concinna* (Powell et al. 2001) and *Odontaster validus* (Grange et al. 2011). Sperm longevity was also found to be greater in polar species (Powell et al. 2001; Grange et al. 2007, 2011). Specific reproductive behavior and timing of spawning in Antarctic species are vital to enhance fertilization success in the polar marine environment (Powell et al. 2001) and should be efficient enough to lead to panmixia, as in *A. agassizii*.

Finally, this study represents a first step toward a comprehensive characterization of the relative impact of gene flow and genetic drift on the spatial pattern of genetic structure at a small spatial scale in the Antarctic brooding echinoid *Abatus agassizii*. The absence of relationship between female fertility and mate number and adult density suggests that the mobility of such organisms may have been underestimated. Nevertheless, the limited numbers of individuals sampled (66 adults for the spatial structure and 8 females for the progeny arrays) and microsatellite markers used (6) may limit the conclusions of different aspects of this study (e.g., detection of spatial structure, statistical power when comparing progeny arrays coming from low and high density areas). Future studies on the temporal differentiation between adults and immature individuals should improve the conclusions of the present study, as well as increase the spatial scale of the sampling scheme. These perspectives will allow us to determine the limits of panmictic units and evaluate the consequences of brooding behavior on the geographic scale of genetic differentiation.

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References

Addison JA, Hart MW (2005) Colonization, dispersal, and hybridization influence phylogeography of North Atlantic sea urchins (*Strongylocentrotus droebachiensis*). Evolution 59:532–543

Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 25:4692–4693

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol Ecol 7:1053–1064

Avise JC, Tatarenkov A, Liu JX (2011) Multiple mating and clutch size in invertebrate brooders versus pregnant vertebrates. Proc Natl Acad Sci USA 108:11512–11517. doi:10.1073/pnas.1109216108

Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (1996–09) GENETIX, logiciel sous Windows TM pour la génétique des populations. Montpellier, France

Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Notes 2:611–614

Bishop JDD (1996) Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. I Autoradiographic investigation of sperm movements in the female reproductive tract. Proc R Soc Lond B 263:369–376

Bishop JDD, Pemberton AJ (2006) The third way: spermcast mating in sessile marine invertebrates. Integr Comp Biol. doi:10.1093/icb/icj037

Boissin E, Hoareau TB, Féral JP, Chenuil A (2008) Extreme selfing rates in the cosmopolitan brittle star species complex *Amphipholis squamata*: data from progeny-array and heterozygote deficiency. Mar Ecol Prog Ser 361:151–159. doi:10.3354/meps07411

Boissin E, Egea E, Féral JP, Chenuil A (2015) Contrasting population genetic structures in *Amphipholis squamata*, a complex of brooding, self-reproducing sister species sharing life history traits. Mar Ecol Prog Ser 539:165–177. doi:10.3354/meps11480

Buchanan JB (1966) The biology of *Echinocardium cordatum* (Echinodermata: spatangoida) from different habitats. J Mar biol Assoc UK 46:97–114

Campbell AC, Coppard S, Tudor-Thomas R, D'Abreo D (2001) Escape and aggregation responses of three echinoderms to conspecific stimuli. Biol Bull Woods Hole 201:175–185

Carrea C, Burridge CP, King CK, Miller KJ (2016) Population structure and long-term decline in three species of heart urchins Abatus spp. near-shore in the Vestfold Hills region, East Antarctica. Mar Ecol Progress Series 545:227–238. doi:10.3354/meps11573

Castric V, Bernatchez L, Belkhir K, Bonhomme F (2002) Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus Fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity 89:27–35. doi:10.1038/sj.hdy.6800089

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Bio Evol 24:621–631

Chenuil A, Féral J-P (2003) Sequences of mitochondrial DNA suggest that *Echinocardium cordatum* is a complex of several sympatric or hybridizing species: a pilot study. In: Féral J-P, David B (eds) Echinoderm Research 2001, Proc. 6th Eur. Conf. Echinoderm, Banyuls-sur-mer, France,. Swets & Zeitlinger Publishers, Lisse, NL, pp 15–21

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

David B, Chone T, Mooi R, De Ridder C (2005) Antarctic echinoidea. Synopses of the Antarctic benthos, vol 10. Koeltz Scientific Books, Königstein

Dayton PK, Robilliard GA, Paine RT, Dayton LB (1974) Biological accommodation in the benthic community at McMurdo Sound, Antarctica. Ecol Monogr 44:105–128

Dell RK (1972) Antarctic Benthos. Adv Mar Biol 10:1–126

Díaz A, González-Wevar C, Maturana CS, Palma AT, Poulin E, Gerard K (2012) Restricted geographic distribution and low

genetic diversity of the brooding sea urchin *Abatus agassizii* (Spatangoidea: Schizasteridae) in the South Shetland Islands: A bridgehead population before the spread to the northern Antarctic Peninsula? Revista Chilena de Historia Natural 86:457–468

Fell HB (1946) The Embriology of the viviparous ophiuroid *Amphipholis squamata* Delle Chiaje. Trans R Soc N Z 75:419–464

Gil D, Zaixo HE, Tolonaso JE (2009) Brooding of the sub-Antarctic heart urchin, *Abatus cavernosus* (Spatangoida: Schizasteridae), in southern Patagonia. Mar Biol 156:1647–1657

Gorospe KD, Karl SA (2013) Genetic relatedness does not retain spatial pattern across multiple spatial scales: dispersal and colonization in the coral, *Pocillopora damicornis*. Mol Ecol 22:3721–3736. doi:10.1111/mec.12335

Grange LJ, Tyler PA, Peck LS (2007) Multi-year observations on the gametogenic ecology of the Antarctic seastar *Odontaster validus*. Mar Biol 153:15–23. doi:10.1007/s00227-007-0776-z

Grange LJ, Tyler PA, Peck LS (2011) Fertilization Success of the Circumpolar Antarctic Seastar *Odontaster validus* (Koehler, 1906): a diver-collected study. In: Pollock NW (ed), Portland, ME, p 140

Grosberg RK (1991) Sperm-Mediate gene flow and the genetic structure of a population of the colonial ascidian *Botryllus shlosseri*. Evolution 45:130–142

Hammer O, Harper DAT, Ryan PD (2001) PAST: paleontological Statistics software package for education and data analysis. Paleontología Electrónica 4:9

Havenhand JN, Styan CA (2009) Reproduction and Larvae/Spore types. In: Dürr S, Thomason JC (eds) *Biofouling*. Wiley-Blackwell, Oxford

Helmuth B, Veit RR, Holberton R (1994) Long-distance dispersal of a subantarctic brooding bivalve (*Gaimardia trapesina)* by kelp-rafting. Mar Biol 120:421–426

Hoarau G et al (2005) Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). Proceed Biol Sci R Soc 272:497–503. doi:10.1098/rspb.2004.2963

Hoffman JI, Clarke A, Clark MS, Peck LS (2013) Hierarchical population genetic structure in a direct developing antarctic marine invertebrate. PLoS ONE 8:e63954. doi:10.1371/journal.pone.0063954

Hunter RL, Halanych KM (2008) Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the drake passage in the Southern Ocean. J Hered 99:137–148. doi:10.1093/jhered/esm119

Iwata Y, Munehara H, Sakurai Y (2005) Dependence of paternity rates on alternative reproductive behaviors in the squid *Loligo bleekeri*. Mar Ecol Prog Ser 298:219–228

Jadwiszczack P (2007) Rundom projects: an application for randomization and bootstrap testing, version 1.1

Johnson SL, Yund PO (2004) Remarkable longevity of dilute sperm in a free-spawning colonial ascidian. Biol Bull 206:144–151

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

Jones AG (2001) GERUD 1.0: a computer program for the reconstruction of parental genotypes from progeny arrays using multilocus DNA data. Mol Ecol Notes 1:215–218

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. doi:10.1111/j.1471-8286.2005.01029.x

Kalinowski ST, Hedrick PW (2001) Inbreeding depression in captive bighorn sheep. Anim Conserv 4:319–324

Kamel SJ, Oyarzun FX, Grosberg RK (2010) Reproductive biology, family conflict, and size of offspring in marine invertebrates. Integr Comp Biol 50(4):619–629

Khasa PD, Newton CH, Rahman MH, Jaquish B, Dancik BP (2000) Isolation, characterization and inheritance of microsatellite loci in alpine larch and western larch. Genome 43:439–448

Konovalov DA, Heg D (2008) A maximum-likelihood relatedness estimator allowing for negative relatedness values. Mol Ecol Resour 8:256–263

Konovalov DA, Manning C, Henshaw MT (2004) KINGROUP: a program for pedirgree relationship reconstruction and kin group assignments using genetic markers. Mol Ecol Notes 4:779–782

Ledoux J-B et al (2012) Fine-scale spatial genetic structure in the brooding sea urchin *Abatus cordatus* suggests vulnerability of the Southern Ocean marine invertebrates facing global change. Polar Biol 35:611–623

Levitan DR (2004) Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. Am Nat 164:298–309

Levitan DR, Petersen C (1995) Sperm limitation in the sea. TREE 10:228–231

Levitan DR, Young CM (1995) Reproductive success in large populations: empirical measures and theoretical predictions of fertilization in the sea biscuit *Clypeaster rosaceus*. J Exp Mar Biol Ecol 190:221–241

Levitan DR, Sewell MA, Chia F (1992) How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. Ecology 73:248–254

Liu JX, Avise JC (2011) High degree of multiple paternity in the viviparous Shiner Perch, *Cymatogaster aggregata*, a fish with long-term female sperm storage. Mar Biol 158:893–901. doi:10.1007/s00227-010-1616-0

Magniez P (1983) Reproductive cycle of the brooding echinoid *Abatus cordatus* (Echinodermata) in Kerguelen (Antarctic Ocean): changes in the organ indices, biochemical composition and caloric content of the gonads. Mar Biol 74:55–64

Marshall DJ, Evans JP (2005) Does egg competition occur in marine broadcast-spawners? J Evol Biol 18:1244–1252

McCartney MA, Brayer K, Levitan DR (2004) Polymorphic microsatellite loci from the red urchin, *Strongylocentrotus franciscanus,* with comments on heterozygote deficit. Mol Ecol Notes 4:226–228

Mercier A, Hamel J-F (2008) Depth-related shift in life history strategies of a brooding and broadcasting deep-sea asteroid. Mar Biol 156:205–223. doi:10.1007/s00227-008-1077-x

Mesphoulhé P, David B (1992) Stratégie de croissance d'un oursin subantarctique: *Abatus cordatus* del îles Kerguelen. CR Acad Sci Paris 314:205–211

Neves EG, Andrade SC, da Silveira FL, Solferini VN (2008) Genetic variation and population structuring in two brooding coral species (*Siderastrea stellata* and *Siderastrea radians*) from Brazil. Genetica 132:243–254. doi:10.1007/s10709-007-9168-z

O'Hara TD, England PR, Gunasekera RM, Naughton KM (2014) Limited phylogeographic structure for five bathyal ophiuroids at continental scales. Deep Sea Res Part I 84:18–28. doi:10.1016/j.dsr.2013.09.009

Palma AT, Poulin E, Silva MG, San Martin RB, Muñoz CA, Díaz AD (2007) Antarctic shallow subtidal echinoderms: is the ecological success of broadcasters related to ice disturbance? Polar Biol 30:343–350

Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. Annu Rev Ecol Syst 25:547–572

Paterson IG, Partridge V, Buckland-Nicks J (2001) Multiple Paternity in Littorina obtusata (Gastropoda, Littorinidae) revealed by microsatellite analyses. Biol Bull 200:261–267

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

Pearse JS, McClintock JB (1990) A comparison of reproduction by the brooding spatangoid echinoids *Abatus shackletoni* and *A. nimrodi* in McMurdo Sound, Antarctica. Invertebr Reprod Dev 17:181–191

Pearse JS, Mooi R, Lockhart SJ, Brandt A (2009) Brooding and species diversity in the southern ocean: selection for brooders or speciation within brooding clades? Paper presented at the Smithsonian at the poles: contributions to international polar year science

Pemberton AJ, Hughes RN, Manríquez PH, Bishop JDD (2003) Efficient utilization of very dilute aquatic sperm: sperm competition may be more likely than sperm limitation when eggs are retained. Proc R Soc Lond B (Suppl) 270:S223–S226. doi:10.1098/rsbl.2003.0076

Pennington JT (1985) The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol Bull 169:417–430

Pierrat B, Saucède T, Festeau A, David B (2012) Antarctic, Sub-Antarctic and cold temperature echinoid database. ZooKeys 204:47–52

Poulin E, Feral J-P (1994) The fiction and the facts of Antarctic brood protecting: population genetics and evolution of schizasterid echinoids. In: David G, Féral & Roux (ed) Echinoderms through time. Balkema, Rotterdam, pp 837–844

Poulin E, Féral JP (1995) Pattern of spatial distribution of a brood-protecting schizasterid echinoid, *Abatus cordatus*, endemic to the Kerguelen Islands. Mar Ecol Prog Ser 118:179–186

Poulin E, Palma AT, Féral J-P (2002) Evolutionary versus ecological success in Antarctic benthic invertabrates. Trends Ecol Evol 17:218–222

Powell DK, Tyler PA, Peck LS (2001) Effect of sperm concentration and sperm ageing on fertilisation success in the Antarctic soft-shelled clam *Laternula elliptica* and the Antarctic limpet *Nacella concinna*. Mar Ecol Prog Ser 215:191–200

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure from multilocus genotype data. Genetics 155:945–959

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43(2):258–275

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population Genetics software for exact test and Ecumenicism. J Hered 86:248–249

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Sarvesan R (1969) Some observations on parental care in *Octopus dollfusi* Robson (Cephalopoda: Octopodidae). J Mar bio Ass India 11:203–205

Schatt PS (1985) DGeloppement et croissance embryonnaire de l'oursin incubant *Abatus cordatus* (Echinoidea: Spatangoidea). Univ, Paris VI

Schatt PS, Féral JP (1991) The brooding cycle of *Abatus cordatus* (Echinodermata: Spatangoida) at Kerguelen Islands. Polar Biol 11:283–292

Schatt PS, Féral J-P (1996) Completely direct development of *Abatus cordatus*, a brooding Schizasterid (Echinodermata: Echinoidea) from Kerguelen, With description of Perigastrulation, a hypothetical New Mode of Gastrulation. Biol Bull 190:24–44

Schinner GO, McClintock JB (1993) Structural characteristics of marsupial brood pouches of the antarctic sea urchins *Abatus nimrodi* and *Abatus shackletoni* (Echinoidea: Spatangoidea). J Morphol 216:79–93

Seibel BA, Hochberg FG, Carlini DB (2000) Life history of *Gonatus onyx* (Cephalopoda: Teuthoidea): deep-sea spawning and post-spawning egg care. Mar Biol 137:519–526

Serrao EA, Havenhand JN (2009) Fertilization strategies. In: Marine hard bottom communities: patterns, dynamics, patterns, and change. Springer, Heidelberg

Sewell MA, Hofmann GE (2011) Antarctic echinoids and climate change: a major impact on the brooding forms. Glob Change Biol 17:734–744. doi:10.1111/j.1365-2486.2010.02288.x

Shaw PW, Sauer WHH (2004) Multiple paternity and complex fertilisation dynamics in the squid *Loligo vulgaris* reynaudii. Mar Ecol Prog Ser 270:173–179

Shuster SM, Wade MJ (2003) Mating systems and mating strategies. Monographs in behavior and ecology. Princeton University Press, Princeton, p 533

Slattery M, Bosch I (1993) Mating behavior of a brooding Antarctic asteroid, *Neosmilaster georgianus*. Invertebr Reprod Dev 24:97–102

Smilansky V, Lasker HR (2014) Fine-scale genetic structure in the surface brooding Caribbean octocoral, *Antillogorgia elisabethae*. Mar Biol 161:853–861. doi:10.1007/s00227-013-2385-3

Sui L, Zhang F, Wang X, Bossier P, Sorgeloos P, Hänfling B (2009) Genetic diversity and population structure of the Chinese mitten crab *Eriocheir sinensis* in its native range. Mar Biol 156:1573–1583

Thompson BAW, Riddle MJ (2005) Bioturbation behaviour of the spatangoid urchin *Abatus ingens* in Antarctic marine sediments. Marine Ecology Progress Series 290

Tsuruwaka Y, Shimada E (2011) Rearing and spawning of the deep-sea fish *Malacocottus gibber* in the laboratory. Ichthyol Res 58:188–190

Van de Putte A, Youdjou N, Danis B (2012) The Antarctic biodiversity information facility. World Wide Web publication, available at http://www.biodiversity.aq

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO—CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Van Oosterhout C, Weetman D, Hutchinson WF (2006) Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. Mol Ecol Notes 6:255–256. doi:10.1111/j.1471-8286.2005.01082.x

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

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419–1439. doi:10.1111/j.1365-294X.2006.02890.x

Weber AA, Merigot B, Valiere S, Chenuil A (2015) Influence of the larval phase on connectivity: strong differences in the genetic structure of brooders and broadcasters in the *Ophioderma longicauda* species complex. Mol Ecol 24:6080–6094. doi:10.1111/mec.13456

Weir BS, Cockerham CC (1984) Estimating f-statistics for the analysis of population structure. Evolution 38(6):1358–1370

Wright S (1949) The genetical structure of populations. Ann Eugen 15:323–354

Yue GH, Chang A (2010) Molecular evidence for high frequency of multiple paternity in a freshwater shrimp species *Caridina ensifera*. PLoS ONE 5:e12721. doi:10.1371/journal.pone.0012721

Yund PO (1995) Gene flow via the dispersal of fertilizing sperm in a colonial ascidian (*Botryllus schlosseri)*: the effect of male density. Mar Biol 122:649–654

Yund PO (2000) How severe is sperm limitation in natural populations of marine free-spawners? TREE 15:10–13

Yund PO, McCartney MA (1994) Male reproductive success in sessile invertebrates: competition for fertilizations. Ecology 75:2151–2167

Zar JH (1984) Biostatistical analysis. Prentice-Hall Inc., Nueva York