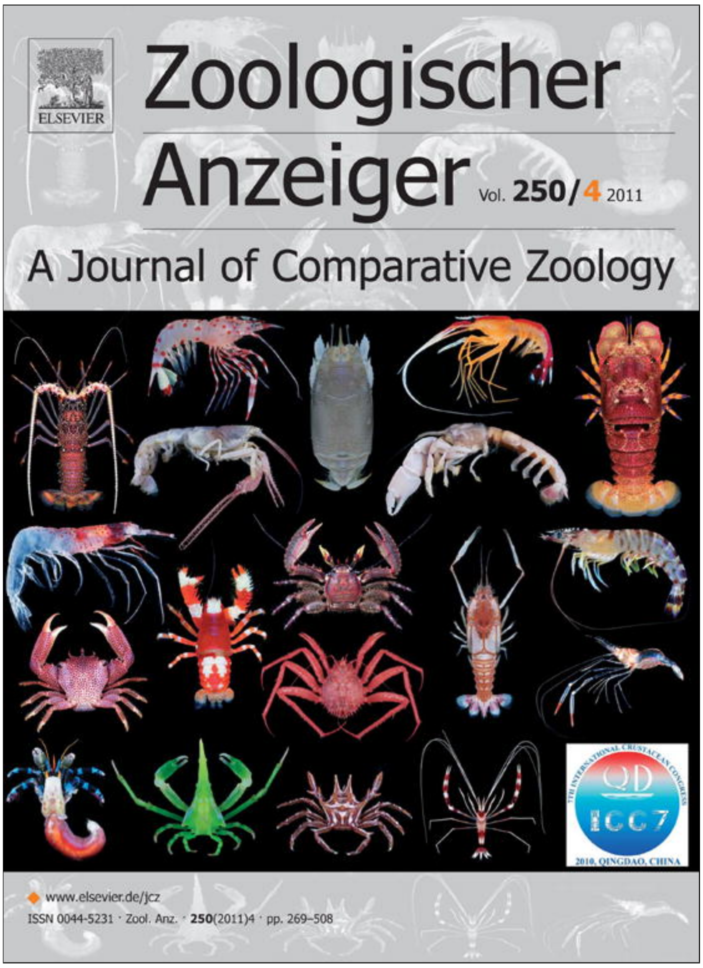
ELSEVIER
Zoologischer
Anzeiger Vol. 250/4 2011
A Journal of Comparative Zoology
17TH INTERNATIONAL CRUSTACEAN CONGRESS
QD
ICC7
2010, QINGDAO, CHINA
www.elsevier.de/jcz
ISSN 0044-5231 · Zool. Anz. · 250(2011)4 · pp. 269–508

Available online at www.sciencedirect.com
ScienceDirect
Zoologischer Anzeiger 250 (2011) 378–406
Zoologischer Anzeiger
www.elsevier.de/jcz

A hypothesis about the origin of sperm storage in the Eubrachyura, the effects of seminal receptacle structure on mating strategies and the evolution of crab diversity: How did a race to be first become a race to be last?

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Received 1 July 2010; received in revised form 13 June 2011; accepted 13 June 2011
Corresponding Editor: S. De Grave.

Abstract

The origins and evolution of sperm storage in Brachyura are enigmatic: sperm is either stored in seminal receptacles, accessible via the vulvae on the sixth thoracic sternite, or in spermathecae at the border between the seventh and eighth sternites. Crabs with spermathecae are collectively referred to as "podotremes" while crabs with seminal receptacles belong to the Eubrachyura. The position of gonopores is the primary basis for subdividing the Eurachyura into the Heterotremata (female vulvae + males with coxal gonopores) and Thoracotremata (female vulvae + males with sternal gonopores). We present a hypothesis about the evolution of seminal receptacles in eubrachyuran female crabs and argue that the sternal gonopore has been internalized into chitin-lined seminal receptacles and the vulva is in fact a secondary aperture. The loss of some or all of the ancestral chitinous seminal receptacle lining was linked to ventral migration of the oviduct connection. Male and female strategies are to maximize gamete fertilization. The most important variable for females is sperm supply, enhanced by long-term storage made possible by the seminal receptacle. To maximize their fertilization rates males must adapt to the structure of the seminal receptacle to ensure that their sperm are close to the oviduct entrance. The major evolutionary impetus for female mating strategies was derived from the consequences of better sperm conservation and the structure of the seminal receptacle. The advantages were all to the females because their promiscuity and sperm storage allowed them to produce more genetically variable offspring, thereby enhancing variation upon which natural selection could act. We extend our arguments to Brachyura as a whole and offer a unifying explanation of the evolution of seminal receptacles, comparing them with the spermathecae found in "Podotremata": they were independent solutions to the same problem: maintaining sperm supply during evolutionary carcinization.

Explanation of eubrachyuran mating strategies requires analysis of the mating–moulting link, indeterminate vs. determinate growth format and seminal receptacle structure. Two alternatives for each of these characters means that there are eight possible outcomes. Six of these outcomes have been realized, which we term Portunoid, Majoid, Eriphoid, Xanthoid, Cancroid, and Grapsoid–Ocypodoid strategies, respectively. Mapping these characters on to a workable phylogeny (wherein some changes to the seminal receptacle + moulting–mating links are assumed to have occurred more than once) produces the following relationships: Portunoids + Majoids are a sister group to the rest of the Eubrachyura, which fall into two sister groups, Eriphoids + Xanthoids and Cancroids + Grapsoid–Ocypodoids and the "Podotremata" is sister group to all the Eubrachyura.

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doi:10.1016/j.jcz.2011.06.002

We conclude that what began as a race to be the first to mate was turned on its head to become a race to be last, by the evolutionary changes to the seminal receptacle. Eubrachyuran females were advantaged by greater reproductive autonomy, more opportunity to mate with other males, resulting in more genetically variable progeny and leading to the evolution of much greater taxonomic diversity compared to "podotremes".

Keywords: Crustacea; Decapoda; Brachyura; Podotremata; Carzinization; Copulation; Evolution; Fertilization; Growth format; Mate guarding; Moulting; Spermatheca; Seminal receptacle; Sperm competition; Sperm transfer; Sperm storage

1. Introduction

Evolving from schooling natant ancestors, the first problem faced by the more solitary bottom-living reptant decapods was finding a mate of the opposite sex. It is not possible to hide a whole school in a crevice or under a stone! No longer was there a large number of males with which females could mate so sperm was in short supply and there was a premium on efficient use and more importantly on sperm storage. In most Decapoda the location of spermatophore storage by the female is not connected to the ovary. The spermatophores may be simply attached to the ventral surface of the female or placed in an external chamber of some kind, which affords sperm some protection until they are used to fertilize the ova. However, with mating linked to moulting females always shed any unused sperm when they moulted, a habit of little consequence to a natant, but one with serious repercussions for a reptant. The female gonopores open in the coxal article of the third pereopods and ripe eggs are extruded into a chamber, sometimes formed by curling the abdomen beneath the thorax, where the attached spermatophores burst open releasing immotile sperm that fertilize them. Fertilization is external and normally involves sperm from only one male. This is the ancestral condition. After fertilization in the chamber the sticky eggs adhere to the endopods of the pleopods. However, spermatophores in Brachyura are placed in sites where they are better protected and sometimes conserved, even during female moulting.

Three terms have been used to refer to sperm storage sites: "thelycum", "spermatheca" and "seminal receptacle". These terms have been clearly defined by Tavares and Secretan (1993). They defined a "spermatheca" as an intersegmental invagination between two adjacent sternites while a "thelycum" is an invagination of the surface of a single sternite (there is some dispute about the number of sternites involved, but this does not materially alter the function). Both are kinds of seminal receptacles *sensu lato*, but neither include the case where there is a direct connection to the oviduct. Thelyca and spermathecae are protected chambers where spermatophores are stored, but are not sites of fertilization. Bauer (1994) takes a different view about the use of these terms, so as to accommodate the structures found in penaeid shrimps, but this does not result in useful clarification of decapod reproductive terminology as a whole. Here we use "seminal receptacle" in the sense of Diesel (1991) and Tavares and Secretan (1993) because this is where fertilization occurs. Guinot and Tavares (2001) point out that paired spermathecae, not connected to the oviduct, are a synapomorphy that distinguishes the podotreme crabs from the Eubrachyura having seminal receptacles. Many authors (e.g. Jensen et al., 1996; Sainte-Marie and Sainte-Marie, 1999; Jennings et al., 2000; Gardner and Williams, 2002) have used the term "spermatheca" instead of "seminal receptacle", but this only serves to obscure the important difference about where fertilization actually occurs. Indeed we have been guilty of this ourselves (López Greco et al., 1999; Brockerhoff and McLay, 2005a)!

Mating in Brachyura is either linked to female moulting (ancestral condition) or occurs during the intermoult when both sexes have hard exoskeletons (derived condition). Crabs belonging to the Cancridae and most of the Portunidae mate when the female is soft, immediately after moulting, while those belonging to the Majidae (the first mating may be with soft females), Hymenosomatidae, Varunidae and Ocypodidae mate when both sexes are hard shelled (Hines, 1991; Turner et al., 2003; Yamaguchi, 2001; Brockerhoff and McLay, 2005b; Sainte-Marie et al., 2008; Van Den Brink and McLay, 2009). Hartnoll (1969) produced the first synthesis of mating behaviour in Brachyura, later contributing an important analysis of moulting–mating–brood production patterns amongst Crustacea, which high-lighted the fact that in Brachyura there are effectively no constraints on growth-reproduction patterns (Hartnoll, 1985). The most valuable and up-to-date discussion of crab reproduction is by Jivoff et al. (2007), who summarized what is known about the blue crab, *Callinectes sapidus* Rathbun, 1896, and compared it with a range of other species.

Neither eggs nor sperm can be assumed to be infinite in supply and so they must be used efficiently so as to maximize the number of zygotes. Here we present a hypothesis to explain the origin of the seminal receptacle and how it might have evolved given the limited supply of gametes. Such a hypothesis has particular relevance to the origin of the Eubrachyura because sternal female vulvae + seminal receptacles are a synapomorphy of this group while sternal spermathecae + spermathecae are a synapomorphy of the podotreme crabs (Guinot and Tavares, 2001). Using reproductive characters we examine sperm storage and reproductive strategies in Eubrachyura and indirectly we consider the question of "Podotremata" monophyly or paraphyly. In an earlier attempt to analyse crab reproduction Hartnoll (1968b) examined the complete reproductive tract, concentrating on the structural mechanisms for closing the vagina and the presence or absence of opercula closing the vulva. However, we believe that a better evolutionary answer is to be obtained by examining the structure of the seminal receptacle. The chief value of the seminal receptacle is that it gives the female a place to store sperm, as well as autonomy and control over her reproduction (González-Gurriarán et al., 1998; Rorandelli et al., 2008). Any hypothesis that can explain the origin of seminal receptacles must also provide an explanation of the variety of reproductive behaviours seen in Brachyura. Our approach to this problem is to first provide an evolutionary mechanism that could have favoured the changes to the female sternal structure and then to employ variation in this mechanism to analyse and explain reproductive behaviour. Reproduction is a partnership between females and males, but it is our view that in the evolution of eubrachyuran crabs, the role of female structure has been much neglected. Characterization of brachyuran mating strategies has traditionally only looked at them from the male point of view, asking why *males* had different mating behaviours (e.g. Christy, 1987), but we intend to argue here that we have been looking at this problem from the wrong point of view, thereby obscuring the really important part: *female* reproductive morphology and autonomy.

We take on the challenge of synthesizing a hypothesis that offers an evolutionary explanation that unites variation in the structure of the seminal receptacle with the link between mating and moulting (soft shell vs. hard shell), the growth format (indeterminate vs. determinate growth), the mating behaviour (mate attraction, courtship and guarding) leading to a phylogeny of growth and reproduction in Brachyura. We attempt to identify the critical elements of crab reproduction, explain how they originated and what have been the consequences of this evolution. Was Hartnoll (1985, p. 121) correct when he stated that there are "no constraints" on brachyuran reproductive patterns?

Our method is to begin with a hypothesis, use it to identify the important characters to look for, gather the evidence and then evaluate the hypothesis within a phylogenetic framework governed by the need to find a parsimonius explanation of the adaptations. It is not our aim to provide a new tree showing phylogenetic relationships, but instead to provide an estimate of the minimum number of times that variations in seminal receptacle structure, growth format and the mating–moulting link occurred. The explanation of such reproductive adaptations is to be found in the ancestry of these crabs. If it is possible to explain the two patterns of sperm storage in Brachyura, then we will have an evolutionary explanation of the origin of these two groups, because seminal receptacles and spermathecae are the basis of the division. The central question that we attempt to answer can be paraphrased: "Why did a race to mate first become a race to mate last?" For names and allocation of species to families, we follow Ng et al. (2008).

2. Female strategy

2.1. Seminal receptacle structure and fertilization in eubrachyuran crabs

In eubrachyuran crabs seminal receptacles are extensible sperm storage chambers and an important part of the female reproductive tract. They consist of a highly elastic mesodermal dorsal part, which is connected to the ovary, and an ectodermal ventral part connected to the vagina and vulva. The vagina is normally a closed tube, but can be opened by lateral muscles anchored to the sternite to allow copulation and egg-laying (Hartnoll, 1968b; Diesel, 1991). The dorsal part consists of an outer flexible epithelium of connective tissue and an inner layer of glandular epithelium. This epithelium is highly stratified in *Chionoecetes opilio*, *Portunus pelagicus*, *Portunus sanguinolentus*, *Leurocyclus tuberculosus*, *Libinia spinosa* and *Maja squinado* (Ryan, 1967a; Bawab and El-Sherief, 1988; Lanteigne et al., 1996; González-Gurriarán et al., 1998; Sainte-Marie and Sainte-Marie, 1998; González-Pisani and López Greco, 2007; Rotllant et al., 2007; Sal Moyano et al., 2010; González-Pisani et al., 2007a; González-Pisani, 2011). Hartnoll (1968b) also reported this pattern in *Carcinus maenas*, *Hyas araneu*s and *Hyas coarctatus*. Recently Lautenschlager et al. (2010) found that both *Uca ecuadorensis* and *Uca tangeri* had multi-layered glandular epithelium. In some of these species the epithelial lining can be shed into the receptacle and spermatophores have been reported in the receptacle. In three pinnotherids, *Pinotheres pisum*, *Pinnotheres pectunculi* and *Nepinnotheres pinnotheres*, the dorsal area of the receptacle is lined with glandular multi-layered tissue, but all sperm were free and no spermatophores were present (Becker et al., 2011). However, it is not highly stratified in *Neohelice granulata*, *Ocypode quadrata*, *Uca cf. forcipata*, *Ucides cordatus*, *Metopograpsus messor* (Forskal, 1775) (Lee and Yamazaki, 1990; Anilkumar et al., 1996; López Greco et al., 1999, 2009; Sant'Anna et al., 2007; Lautenschlager et al., 2010). Except for *U. forcipata*, spermatophores have not been reported in the receptacle of these species, perhaps because they rapidly burst upon entrance. Whereas in the first group of species with stratified epithelium, which can shed the inner layer, sperm may be stored for long periods, in the second group with unstratified epithelium and no shedding layer, sperm may only be stored for short periods because the females have more opportunities to mate (see Lautenschlager et al., 2010). Sperm maintenance is a cost borne only by females unless males provision their spermatophores. The ventral part of the seminal receptacle is lined with chitin and shed when the crab moults. The dorsal part of the receptacle is capable of considerable expansion to accommodate sperm transferred during copulation. The secretory activity of the mesodermal epithelium may be partially involved in sperm maintenance (Anilkumar et al., 1996) and dehiscence of spermatophores (Diesel, 1989; Anilkumar et al., 1999). The size and degree of secretory activity of the seminal receptacle are closely correlated to the ovarian cycle (Subramoniam, 1993).

Detailed descriptions of the histology of the tissues making up the receptacles have been described in several species: e.g. Portunidae: *C. maenas* (Hartnoll, 1968b), *C. sapidus* (Johnson, 1980), *P. sanguinolentus* (Ryan, 1967a); Cancridae: *Metacarcinus magister* (Jensen et al., 1996); Oregoniidae: *C. opilio* (Beninger et al., 1988; Sainte-Marie and Sainte-Marie, 1998), *H. araneus* and *H. coarctatus* (Hartnoll, 1968b); Inachidae: *Inachus phalangium* (Diesel, 1989); Varunidae: *N. granulata* (López Greco et al., 1999); Ocypodidae: *O. quadrata* (López Greco et al., 2009); and Ucididae: *U. cordatus* (Sant'Anna et al., 2007). Ontogenetic changes in the shape of the seminal receptacles during the reproductive cycle have been described for *P. pelagicus* by Bawab and El-Sherief (1988, see Fig. 7 and Table 1).

The dorsal and ventral parts of the seminal receptacle in some species are separated by an epithelial sheet as in *C. sapidus* (Johnson, 1980) orby and iris-like diaphragm the velum in *I. phalangium*, *I. communissimus*, *Pisa tetraodon* and *Maja crispata* (Diesel, 1991) and *L. spinosa* (Sal Moyano et al., 2010), which is assumed to be able to contract thereby dividing the receptacle into a dorsal sperm storage chamber and a ventral fertilization chamber (Diesel, 1991). Some folds are found in the ecto-mesodermic transition in *L. tuberculosus* (Gonzalez-Pisani and Lopez-Greco, unpubl.) and in *O. quadrata* (López Greco et al., 2009). The seminal receptacle in studied species of *Uca* and *Pinnotheres* is not divided into two halves (Lautenschlager et al., 2010; Becker et al., 2011 respectively) but nevertheless, like most crabs which have a ventrally entering oviduct, the dorsal part lined with glandular epithelium is primarily for sperm storage, while the cuticle-lined ventral region is where gametes meet. A more complicated internal seminal receptacle structure has been reported in the freshwater crab *Potamon fluviatile* (Brandis et al., 1999) although exactly how this works remains to be established.

When mated females are dissected the seminal receptacles are usually evident as prominent balloon-like sacs in the skeleton of the sixth sternite segment. The oviduct enters the receptacle either dorsally, opposite the vaginal entrance, or ventrally, adjacent to the vaginal entrance, Fig. 1. These have been termed "dorsal-type seminal receptacles" and "ventral-type seminal receptacles" by Diesel (1991), but we have now established that there are intermediate types where the oviduct connects to the seminal receptacle somewhere in between these extremes. The importance of the seminal receptacle-oviduct connection lies in the fact that this is the place that provides the first opportunity for fertilization to occur and it alters the proportion of the receptacle volume that is available for trans-moult sperm storage.

Strictly speaking the only event that takes place in the seminal receptacle is that one or more sperm become attached to the oocyte envelope, while the acrosomal reaction and penetration of the oocyte by the male DNA actually occurs in the abdominal brood chamber. Combination with female DNA is probably not completed until the eggs become attached to the pleopods, which could be 60–90 min later (Hinsch, 1971). Herein we use "fertilization" to refer to only the first step: the attachment of sperm to the oocyte. Given the short residence time of eggs in the seminal receptacle (see below) only a small part of the process is strictly speaking internal. The arms of the stellate spermatozoa barely have enough time to grab hold of the egg as it passes by. Regardless of when the egg is fertilized most of the process of fertilization actually occurs in the abdominal chamber.

At the time of mating sperm are transferred packaged up in spermatophores to the female seminal receptacle, accompanied by varied amounts of seminal fluids. Sperm must be released and dispersed in the receptacle for fertilization to occur. In *Libinia emarginata* Leach, 1815 free sperm appear "soon after copulation" (Hinsch, 1986, 1991), but in *I. phalangium* some spermatophores remain intact 2 or 3 months later (Diesel, 1989). Jennings et al. (2000) could not find any spermatophores in the receptacles of recently mated *Hemiplax hirtipes* (as *Macrophthalmus*). Anilkumar et al. (1999) closely followed the dissolution of spermatophores in *M. messor* and found that they remain intact for the first 24 h and dissolution did not begin until 24–36 h after copulation and was complete by 72 h. The length of the delay between mating and spermatophore breakdown is very relevant to the duration of male post-copulatory mate guarding and when the female lays her eggs.

The dynamics of fertilization in crabs have only been studied in *I. phalangium*. Diesel (1989, Fig 8; 1990, Fig. 1) describes the process of fertilization: the seminal receptacle in *I. phalangium* is divided into a dorsal storage chamber and a ventral fertilization chamber by a muscular velum that may control the amount of sperm used. Eggs are moved by muscular contractions of the oviduct walls towards the fertilization chamber where they encounter the sperm, and after fertilization, the eggs are passed on to the vagina whose muscular contractions expel them into the abdominal incubation chamber. We can estimate the amount of time that *I. phalangium* eggs spend in the fertilization chamber from the observation that an average brood of 688 eggs (SD = 496) is laid in 1–2 h (see Diesel, 1988): since there are two receptacles through which the eggs can pass this means that eggs spend on average approximately 3600/344 to 7200/344 s or 10.5–21 s in the receptacle. Brood size and female size are not correlated in this species, but even at the maximum brood size reported (2744 eggs) the residence time would be 3600/1372 to 7200/1372 or 2.6–5.2 s per egg, which should be ample time for immotile sperm to contact the eggs. In most crabs large females carry more eggs than small females so residence times would be expected to be shorter. Depending on female size, *Hemigrapsus sanguineus* lays from 3700 to 43,000 eggs (McDermott, 1998) within 3 h (Anderson and Epifanio, 2010) giving residence times ranging from 5.8 to 0.5 s per egg. In some portunids which have large broods, the residence time may only be 0.0206–0.0072s per egg (fecundity

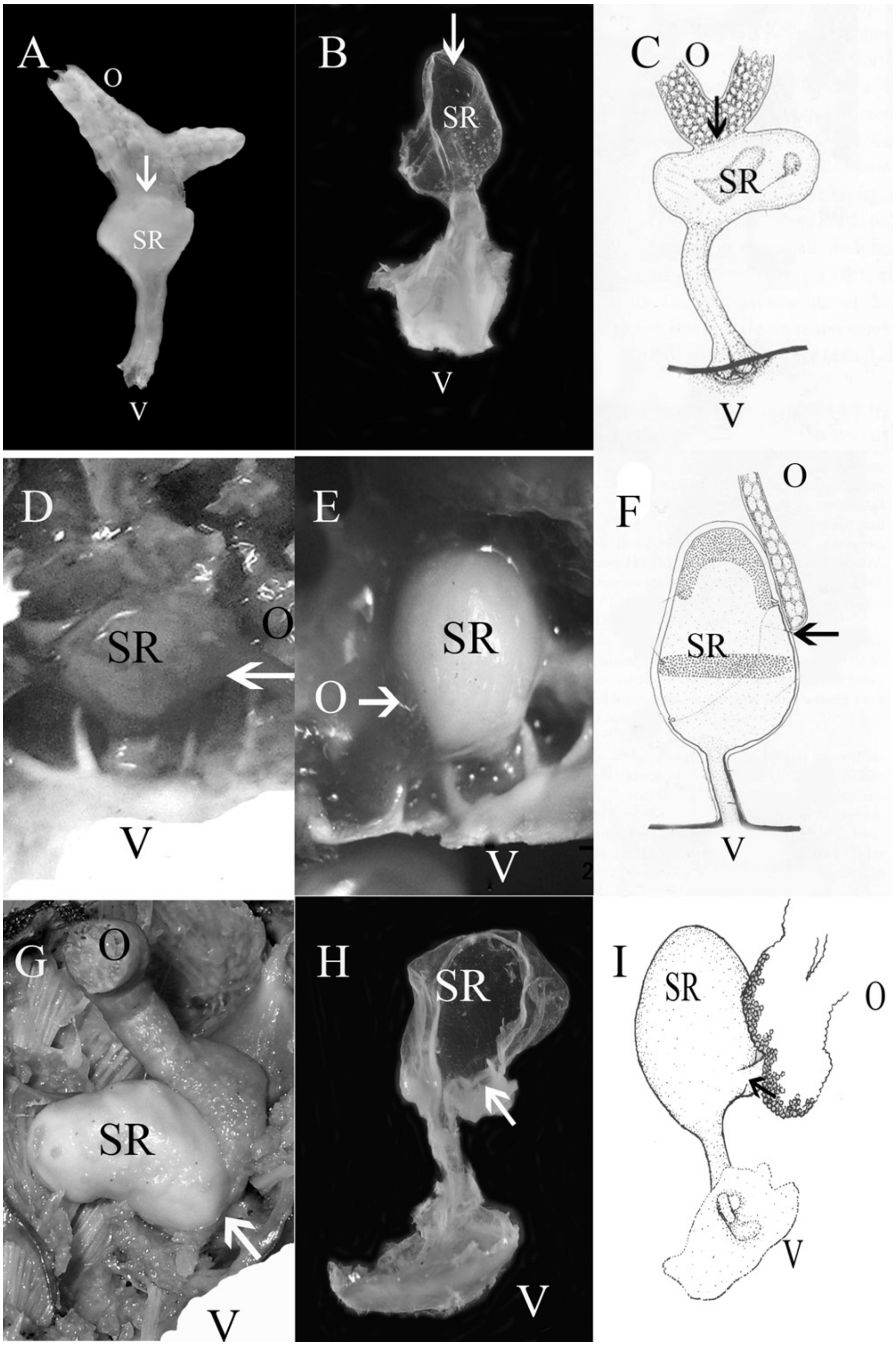

Fig. 1. Variation in seminal receptacle structure in the Eubrachyura: A–C are examples of the "dorsal type"; D–F are "intermediate type"; and G–I are the "ventral type". All figures are oriented so that the vulva (V) is at the bottom, and the connection of the ovary (O) to the seminal receptacle (SR) is shown by an arrow. (A) *Trichopeltarion fantasticum* Richardson and Dell, 1964 (Atelecyclidae) ovigerous female 33.5 mm × 25.8 mm, 100 m, off Banks Peninsula, New Zealand (part of the ovary is attached); (B) *Pilumnus lumpinus* Bennett, 1964 (Pilumnidae) mature female 22.4 mm × 17.0 mm, intertidal Kaikoura Peninsula, New Zealand (note receptacle has been torn open where the oviduct connects); (C) *L. depurator* (Portunidae) after Diesel (1991, Fig. 9.2B), mature female newly moulted but containing old sperm residue; (D) *Leurocyclus tuberculosus* (Inachoididae) North Patagonia, Argentina, 10 m depth, female CW > 42 mm; (E) *Libinia spinosa* (Epialtidae) modified after Sal Moyano et al. (2010, Fig. 2); (F) *Portunus sanguinolentus*, after Diesel (1991, Fig 9.2C), shows sperm layering; (G) *Libidoclaea granaria* H. Milne Edwards and Lucas, 1842 (Epialtidae) Coquimbo, Chile, 15 m depth, female CW = 40 mm. (H) *Platymera gaudichaudii* H. Milne Edwards, 1837 (Calappidae) mature female 55.8 mm × 31.9 mm, April 4, 1940, coll. J. Garth, sta. 1120–1140, off

700,000–2,000,000 eggs) for *C. sapidus* (Van Engel, 1958) or 0.006–0.0027s per egg (fecundity 900,000–2,000,000 eggs) for *P. sanguinolentus* (Ryan, 1967a). In these cases sperm would need to be free of spermatophore packaging and very concentrated (not diluted by gel or other media) if all the eggs are to encounter sperm. The extent to which females could control the fertilization rate and amount of sperm used is unknown. The exact nature of fertilization in ventral receptacles where sperm is not layered (as in *I. phalangium*) has not been established and it is also not known for species with dorsal receptacles. Indeed for the crabs with dorsal receptacles it is unclear how eggs are propelled through the receptacle towards the vagina and what the effects of sperm plugs are on fertilization.

Three different factors could explain variation in whether or not sperm are stored in spermatophores or in a mixture of both: fecundity, oviduct connection to the receptacle, and presence/absence of a descamative layer. Egg residence time is very short in highly fecund species so sperm would need to be free whereas in low fecundity species egg residence time may be several orders of magnitude longer and spermatophore bursting could be precipitated by arrival of the eggs. In species with dorsal receptacles eggs must traverse the receptacle, thus having longer exposure time to sperm whereas in species with ventral receptacles eggs only pass across the small gap between the oviduct entrance and vaginal exit point. Between these two extremes are cases where the fertilization opportunity is intermediate. Finally, the dependence of stored sperm on nutrition supplied by the female, via a shedding layer, will be reflected in whether they are free (fed by the female) or packaged (provisioned by the male). Sperm survival during storage should be inversely linked to in the frequency of copulation.

To summarize: seminal receptacles are extensible chambers consisting of an elastic mesodermal dorsal part and a ventral ectodermal part (lined with chitin) connected to the vagina. The dorsal part is lined with glandular epithelium that may be stratified, and can be partially shed into the receptacle. In these species sperm may be stored for long periods. In some species the dorsal and ventral parts are separated by a sheet of muscular tissue, the velum, so that separate storage and fertilization chambers are formed. Seminal receptacle structure varies in relation to the distance between the entrance of the oviduct and the vaginal exit, the extremes of which give rise to so-called "dorsal" and "ventral" seminal receptacles, but intermediate oviduct connections are possible. Sperm can be transferred to the receptacles either packaged in spermatophores or free in seminal fluid, which can result in sperm layering. Rendezvous of the sperm and egg occurs in the ventral part of the receptacle and given the wide range of fecundity, the residence time of eggs as they pass from the ovary to the vulva, can vary from ~20 s to only 0.0027s, or 5 orders of magnitude. There are three main factors which determine whether sperm are stored in spermatophores or whether they are free: fecundity, oviduct connection to the receptacle, and the presence or the absence of a descamative layer. Differences in sperm survival during storage should be inversely linked to frequency of copulation.

2.2. Evolution of seminal receptacles in the Eubrachyura

We present a hypothesis about the origin of seminal receptacles in the Eubrachyura. Firstly we outline an evolutionary pathway for the seminal receptacle and secondly we present arguments for the selective forces that may have driven this evolutionary process in females. At the same time we build up a picture of the hypothetical ancestor, which we compare and evaluate against extant crabs later. Central to our hypothesis is the assumption that sperm are in short supply and hence there is a need for sperm conservation (see below). Consequential upon this is the importance of sperm competition, which results from the fact that females can mate with multiple partners, they can store viable sperm for long periods and there is a delay between copulation and fertilization. Regardless of whether sperm competition is present, the strategy for both males and females is to maximize gamete fertilization. The impact of any changes to structures and/or reproductive behaviours must therefore be measured in terms of the probability of fertilization of gametes produced by individuals.

Evolution of the seminal receptacles involved four morphological changes:

(1) Shift of the female gonopore from a coxal to a sternal position;
(2) Origin of the sperm storage receptacle or chamber externally;
(3) Loss of the seminal receptacle chitinous lining internally; and
(4) Migration of the oviduct connection with the seminal receptacle from a dorsal towards a ventral position. These last two changes appear to have been linked.

The primary selective force generating these evolutionary changes was sperm conservation and the advantages to females of reproductive autonomy, accompanying carcinization (evolution of a crab-like body). The migration of the oviduct connection ventrally lead to changes in reproductive strategies of both sexes, but it is suggested that chiefly it resulted in females being able to mate with more males (polyandry) and for them to store more sperm.

San Nicolas Is, California, Allan Hancock Foundation (note receptacle has been torn open); (I) *Ocypode quadrata*, after López Greco et al. (2009, Fig. 1B). Note: seminal receptacles in (D, E, and G) are shown *in situ*, while the others are shown isolated and in profile.

2.3. Shift of the female gonopore from a coxal to a sternal position

The ancestral state for the female gonopores in Decapoda is their presence in the coxae of the third pereopods and the defining feature of the Eubrachyura is for them to be embedded in the sternum (as vulvae) corresponding to the third perepods (Guinot, 1979). There are no crabs (extant or fossil) showing transitional stages between coxal and sternal gonopores in either females or males (Guinot, 1979; Tavares, 1992). Recent work by González-Pisani et al. (2007b) and González-Pisani (2011) on the sexual differentiation of *L. tuberculosus* and *L. spinosa* has shown that the first ontological step is the differentiation of the vulva, followed by the invagination of the ectoderm to form the vagina and the seminal receptacle. At this stage the ovary is not developed so there is no connection with the vagina or receptacle. Nearing the puberty moult, ovary differentiation (without contact with the ectodermal section of the reproductive tract) occurs nearing the puberty moult and the ovary connects to the vagina at the time of the puberty moult. If the first step in the evolution of the female tract was the ectodermal invagination to form a chamber then it is not difficult to imagine how the oviducal connection with the sternal chamber, rather than the pereopod coxa, came about (see below).

We hypothesize that the shift of the gonopore to the sternum was a consequence of carcinization. The main changes in body shape during carcinization were for the cephalothorax to become progressively wider and shorter with the result that the gonopores were much further apart. With coxal female gonopores and a narrow sternum in both sexes, males could use their "gonopods", such as they were, to deposit spermatophores around each gonopore and achieve fertilization, but as shortening and widening of the cephalothorax continued, the limited ability to widen the space between male gonopods, due to the narrow male abdomen, meant that spermatophores could no longer be deposited close to the gonopore. Sternal female gonopores were favoured because of limitations in the male, a change that could have happened gradually or suddenly by a hox-mutation in the females. Males were already adapted to transferring spermatophores to a central position on the sternum so no sudden change is required on their part. Carcinization of the female abdomen meant that it became shorter and wider and folded under the thorax to create a fertilization/brood cavity, but while the male abdomen also became shorter, and folded, it remained narrow as in the ancestor. So initially males could only deposit spermatophores around sternal gonopores. Another advantage of sternal openings in the female was the need to deposit eggs into the brood chamber where external fertilization occurred. The ancestral condition was a larger curved abdomen forming a fertilization chamber whose integrity was improved by deposition of glial strands to constrain the ova. The answer to this age-old problem on the origin of crab gonopores (see Guinot and Quenette, 2005 for the most exhaustive discussion) may well lie in further study of the ontogeny of the female sternum and reproductive tract.

2.4. Origin of the seminal receptacle

In the ancestral condition males deposited spermatophores that adhered to the chitinous surface around the female gonopore just as their ancestors had done. Sperm supply was enhanced by the evolution of an open receptacle resulting from an invagination of the genital area on sternite six that provided greater protection to spermatophores deposited therein. We term this transitional stage a "sternal cavity" (avoiding use of the term "thelycum", which does not involve close connection to the gonopore) where it was still necessary for the male to attach his spermatophores to the surface. Up to this point fertilization was external. Closure of this cavity created the vulva leading to the seminal receptacle wherein spermatophores were no longer attached to the chitinous surface because males no longer had direct access to the area surrounding the gonopore (Fig. 2). From this point on fertilization was initiated internally and spermatophores were transported in seminal fluid. We follow Guinot (1979) and use "vulva" for this new female opening because it is now a conduit for both sperm and fertilized eggs whereas the "gonopore" is only a conduit for unfertilized eggs.

In order to introduce sperm into the seminal receptacle, males required tubular gonopods to overcome the resistance created by having to inflate or displace contents of the seminal receptacle. There may have only been a few changes to their paired gonopods, which had previously transferred sperm to the sternal surface and which may have been already tubulated, with a long second gonopod, as is found in extant podotreme crabs for example. A long second gonopod is also still found amongst many Eubrachyura and the very short gonopod (second gonopod $\ll$ half first gonopod) has probably evolved several times (see Section 3 below). The final stage involved evolution of the vagina, a short cuticularized tube whose opening and closing was controlled by muscles attached to the wall and skeletal apodemes (see Hartnoll, 1968b). This made the seminal receptacle more isolated, adding another level of protection for the sperm and providing a means by which females could gain some control over mating. Independently, females in several families also evolved opercula and other impediments to copulation. The evolution of male gonopods (in all their variations) alongside the female seminal receptacle is a subject well beyond the scope of this paper and here we only need to assume that males had evolved intromittent organs capable of introducing spermatophores into the receptacle. For this to occur the essential requirement is a tubular first gonopod and a second gonopod able to assist the passage of sperm from the male vas deferens to the female storage organ.

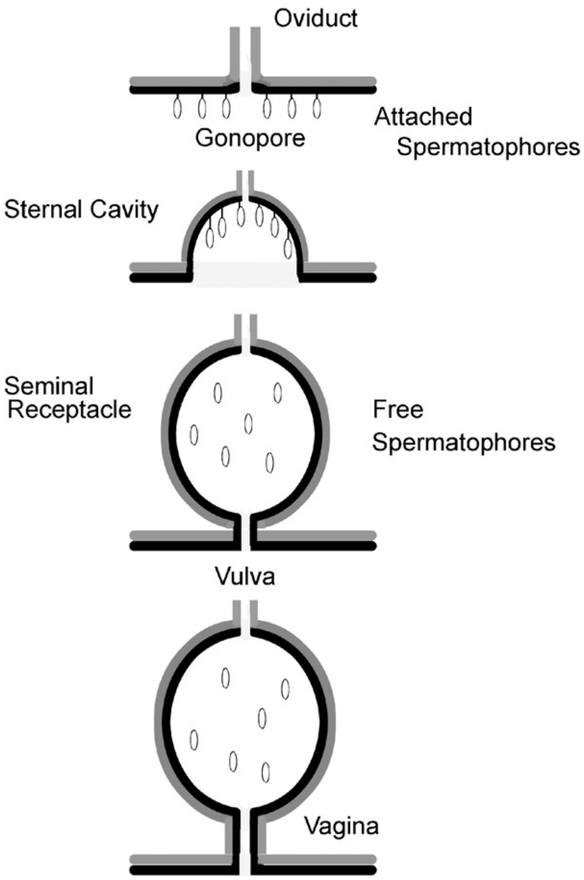

Fig. 2. Stages in the origin of the seminal receptacles of the Eubrachyura. A transverse section (dorso-ventral axis is vertical) through one of the two receptacles in thoracic segment 6 is shown: the black layer is chitinous exoskeleton, the underlying grey layer is mesoderm and spermatophores are shown diagrammatically. Four stages are shown beginning with the oviduct opening in the sternal gonopore (top) through to the vagina opening in the vulva (bottom) in sternite 6.

2.5. Lining of the seminal receptacle and origin of the ventral seminal receptacle

The chitinous lining of the seminal receptacle was continuous with the exterior and part of the exoskeleton, so it was shed at moulting, and the site where the oviduct entered was roughly dorsal in its location. Two separate evolutionary pathways can be hypothesized from this ancestral condition leading to the range of types of receptacle as seen in extant species: in the dorsal type the chitin-free part came about as a result of symmetrical growth of the mesoderm surrounding the dorsal oviduct entrance, allowing more spermatophores to be stored, with the proportionate amount of chitin lining the receptacle decreasing because its volume increased (Fig. 3 left-hand pathway). This type of receptacle retained the ancestral position of the oviduct. An oviduct that enters more ventrally could have resulted from asymmetrical mesodermal growth and chitin loss, beginning near the oviduct entrance and proceeding ventrally towards the vagina (Fig. 3 right-hand pathway). The migration of the oviduct ventrally resulted from loss of chitin and increased receptacle volume. Both of these pathways were advantageous for the female because she was able to accumulate sperm from multiple matings and trans-moult sperm retention: females could reproduce without re-mating. Now the seminal receptacle had the dual roles of being the site where fertilization was initiated as well as the site of sperm storage.

Retention of sperm depends upon the shape of the seminal receptacle because the amount is proportional to how much of the seminal receptacle is lined by mesoderm. Consider a dorso-ventral axis (x) running through the vagina, the centre of the receptacle and the oviduct: in a tubular shaped receptacle each unit on the axis measures the same volume, so that the relationship between sperm retention and amount of mesodermal lining is linear and positive, but if the receptacle is bubble shaped (circular) then the relationship is non-linear because the volume per axis unit tails off towards the dorsal and ventral limits (Fig. 4). The slope of this relationship is always positive (advantageous for the female) and initially it increases more rapidly, but over most of the axis the slope remains about the same and only begins to decrease as the ventral limit is approached. We assume that when moulting occurs the contents of the part of the seminal receptacle lined by chitin is lost, while the rest is retained. As more chitinous lining was lost, more sperm were retained (Fig. 4). The gain in sperm retention begins to tail off once the ventral quarter of the receptacle is reached, after which only small gains in reproductive security are made from continued reduction in the lining. The slope of this line measures the fitness gain for the female that results from increasing the area of mesodermal lining.

Given that the seminal receptacle is full, the relationship between the volume of sperm retained (v) and distance along the central axis of a spherical seminal receptacle of diameter (D) is:

$$v = \pi \left[\left(\frac{D}{2} \right) x^2 - \left(\frac{x^3}{3} \right) \right]$$

and

$$\frac{dv}{dx} = \pi(Dx - x^2).$$

As v increases the proportion of the seminal receptacle used for storage ($v/(0.167\,\pi D^3)$) increases at the expense of the part used for fertilization ($1 - \{v/(0.167\,\pi D^3)\}$). Fundamentally, there has to be a trade-off in volume between storage and fertilization. This evolutionary mechanism provides a unified explanation for what we believe to be the dual origin of extant seminal receptacles. The more mesodermal lining that was gained the more sperm was retained, increasing the potential level of sperm competition. Reduction of the chitinous lining by either route (see Fig. 3) would also make the wall of the receptacle more flexi-

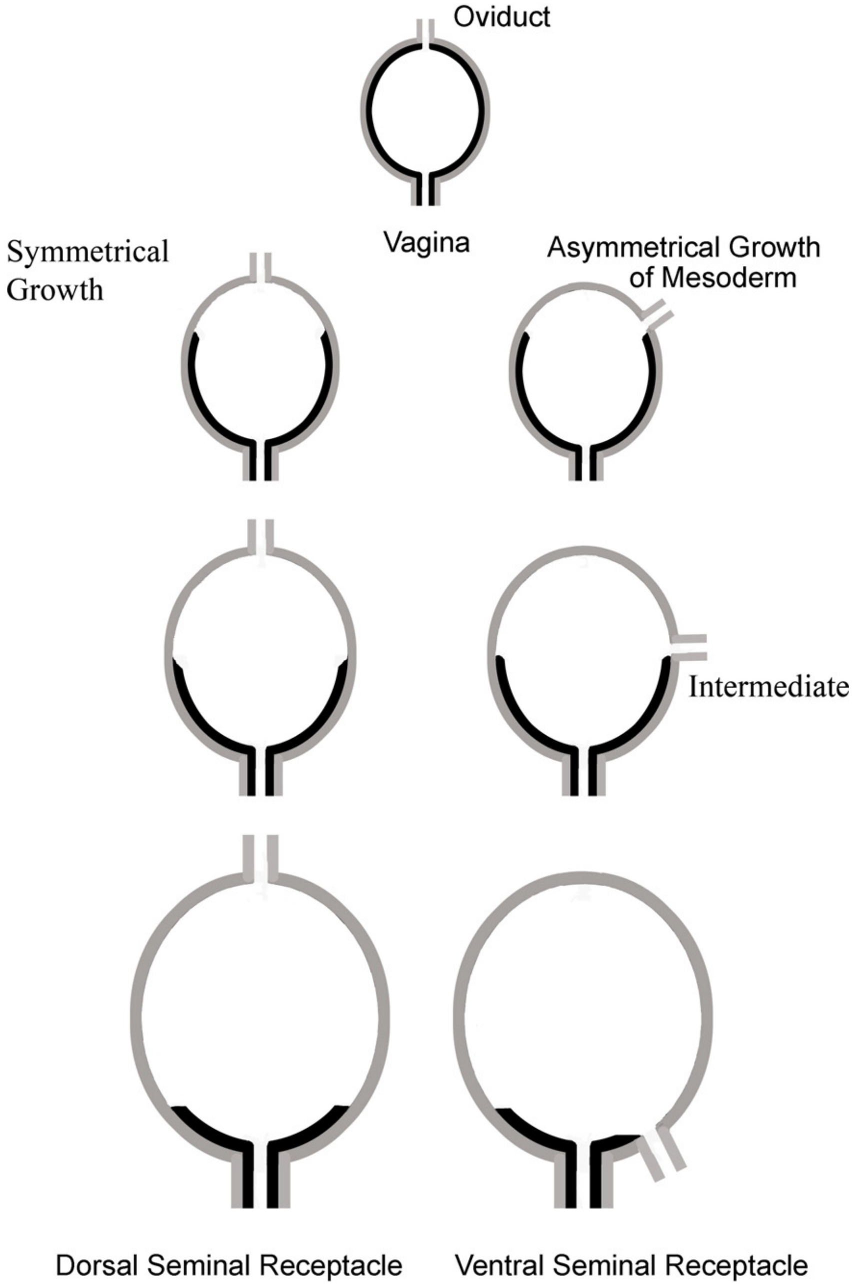

Fig. 3. Evolutionary relationships between the three types of seminal receptacles (dorsal, intermediate and ventral): this figure uses the same format as in Fig. 2. Two separate pathways show stages in the evolution of the dorsal-type seminal receptacle by symmetric growth of mesodermal wall (left-hand side) and stages in the evolution of the ventral-type seminal receptacle (right-hand side) by reduction in the chitinous lining (black layer), accompanied by asymmetrical mesodermal (grey layer) growth. Growth on the left side of the oviduct entrance and shrinkage on the right side accompany the loss of chitin. The combined effect is to shift the oviduct from a dorsal through intermediate to a ventral position. Note that the seminal receptacle shows an evolutionary increase in diameter on both pathways.

ble and expandable so that more sperm could be stored. Having the sperm in contact with the secretory epithelium lining may have allowed the female to provide nutrients that increased sperm longevity, thus enhancing the sperm supply. When mating was linked to moulting (the assumed ancestral condition) the intervals between sperm replenishment could have been longer, especially as females aged and became larger.

2.6. Trans-moult sperm retention

The conservation of sperm through trans-moult sperm retention is an important part of the hypothesis. Most decapods have external fertilization and only ephemeral sperm storage with males simply sticking their spermatophores to the female exoskeleton (Sainte-Marie, 2007). They lack any internalized storage organ and even those

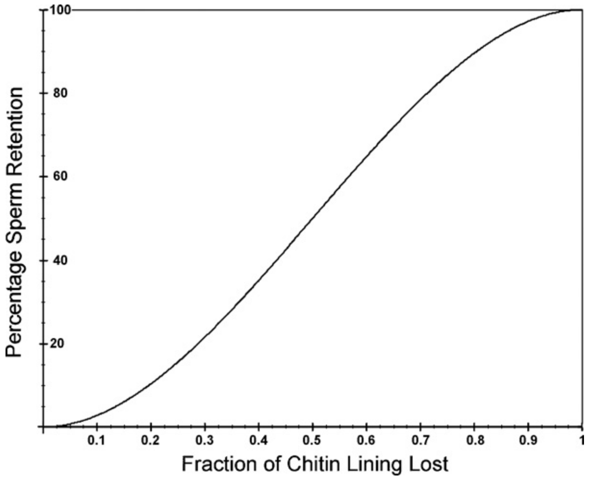

Fig. 4. Sperm retention during moulting in relation to loss of chitinous lining of the seminal receptacle. Chitin loss is measured as linear distance along the dorso-ventral diameter D. As chitin is lost more of the surface becomes mesodermal and consequently more sperm is retained at moulting. The ancestral condition is at the origin while the derived dorsal and ventral types are at some point along the x-axis depending upon the amount of chitinous lining that remains. When $x = 1.0$ the receptacle has no chitinous lining at all and sperm retention is 100% and sperm volume = $0.167\,\pi D^3$.

which do provide some protection for the sperm (some shrimps, crayfishes, lobsters and podotreme crabs), shed any unused sperm when they moult. Trans-moult sperm retention is only possible in eubrachyurans because they store sperm internally and do not shed the entire contents when they moult. Fertilization is at or near the storage site. The need for a female to mate again to fertilize her eggs is reduced if sperm are conserved and stored. Trans-moult retention of viable sperm has been demonstrated for 14 species from 11 families of eubrachyurans: *C. sapidus* (Portunidae) (Jivoff et al., 2007); *M. magister* (Cancridae) (Jensen et al., 1996); *Telmessus cheiragonus* (Cheiragonidae) (Kamio et al., 2002, 2003; Nagao and Munehara, 2007); *Pilumnus vespertilio* (Fabricius, 1793) (Pilumnidae) (Kyomo, 2001); *Menippe mercenaria* (Say, 1818) (Cheung, 1968), *Pseudocarcinus gigas* (Lamarck, 1818) (Gardner and Williams, 2002) (Menippidae); *Austinixia gorei* (Manning and Felder, 1989) (Pinnotheridae) (McDermott, 2006); *Gaetice depressus* (De Haan, 1835) (Fukui, 1990), *Hemigrapsus crenulatus* (Clark, 1987), *N. granulata* (López Greco et al., 1999) (Varunidae); *Candidiopotamon rathbunae* (De Man, 1914) (Potamidae) (Liu and Li, 2000); *Aratus pisonii* (Sesarmidae) (Hartnoll, 1965); *Uca pugnax* (Smith, 1870) (Ocypodidae) (Greenspan, 1982). There seems to be no known cases where trans-moult sperm retention has been shown to be absent in eubrachyurans, except perhaps in the case of *Dyspanopeus sayi* (Smith, 1869) (see Swartz, 1978, as *Panopeus sayi*).

At the time of moulting the contents of the seminal receptacle are divided into the portion to be discarded and that to be retained. Which particular portion of sperm falls into will depend upon mating order, how sperm are stored and the disposition of lining. In general if there is no mixing, the sperm most likely to be lost belong to the last ejaculate. The amount of sperm retained during the moult depends upon how much of the seminal receptacle is lined with chitin. Only a few studies have attempted to measure the extent of the lining and these suggest that crabs with a dorsal-type receptacle have a larger chitinous surface than crabs with the ventral type. In the dorsal category, with approximately 50% lining, we have *C. sapidus* (Johnson, 1980), *Portunus sanguinolentis* (Ryan, 1967a), *P. pelagicus* (Bawab and El-Sherief, 1988) and *Liocarcinus depurator* (Diesel, 1991), while in the ventral category, with approximately 20% lining, is *M. magister* (Jensen et al., 1996), *H. coarctatus* (Hartnoll, 1968b) and *P. tetraodon* (Diesel, 1991). Three species of pinnotherids, *Pinnotheres pisum*, *P. pectunculi* and *N. pinnotheres*, all have about 30% cuticular lining of ventral type receptacles (Becker et al., 2011). *U. cordatus* is reported as having no chitinous lining (Sant'Anna et al., 2007) so that it would be the same as a species that had determinate growth (e.g. *I. phalangium*) and did not moult after reaching maturity because moulting would not remove any sperm. However, *U. cordatus* would continue to grow and maximize sperm conservation. Lautenschlager et al. (2010) found some variation in the seminal receptacle lining in three other Ocypodoid species of *Uca*: *U. tangeri* only had about 10% cuticular lining near the vaginal entrance, while *U. ecuadorensis* and *U. cf. forcipata* had about 75% cuticle lining. Clearly there is the possibility of wide variation of the amount of sperm retained at moulting within a genus. There are as yet no studies of the exact relationship is between sperm retention and percentage cuticle lining of the receptacle. Histological sections of the receptacles of newly moulted females, with known mating history, are needed to measure exactly how much sperm remains after moulting.

The other aspect of sperm conservation is sperm viability. Trans-moult sperm retention can be tested when a female can produce another brood of viable eggs without access to a male, which is also a test for sperm viability so all of the 14 species listed above have been shown to be able to store viable sperm over at least one moult, which in some cases means between years. Sperm remain viable for at least 4 years in *P. gigas* (Gardner and Williams, 2002). However, Paul (1984) found that stored sperm of *Chionoectes bairdi* Rathbun, 1893 had a limited shelf-life and was not viable after 2 years. The possibility that females may nurture sperm by secreting sustenance through the wall of the seminal receptacles warrants more investigation. If sperm were in short supply one would predict that sperm nurture would be favoured by natural selection.

It is clear that internalized sperm storage provides an opportunity to store sperm, which can be retained even though moulting occurs, because some of the seminal receptacle wall is not lined with chitin. The few studies available suggest that crabs with a dorsal receptacle have about 50% chitinous lining while those with a ventral receptacle only have about 20% lining. Studies of 14 species of crabs in 11 families

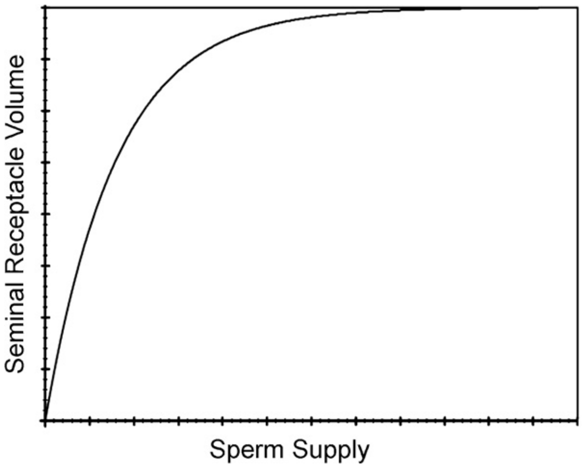

Fig. 5. Relationship between seminal receptacle volume (SV) and sperm supply (s).

have shown that trans-moult sperm retention is possible and it remains viable. Sperm storage for up to 4 years has been demonstrated although a shelf-life of only 1–2 years may be more typical for crabs.

2.7. Maximizing sperm supply

Maximizing the sperm supply is the area in which there is conflicting male and female interests. Males need to fertilize as many eggs as possible whereas females just need sperm to initiate egg development and where it comes from is of little consequence. What eventuates is a shared outcome of both the male and the female strategies.

For females we assume that egg fertilization probability depends upon sperm supply, which is approximately the same as number of copulations gained. The relationship between seminal receptacle volume (SV) and sperm supply is non-linear and asymptotic (Fig. 5):

$$\mathrm{SV} = D(1 - e^{(-s/D)})$$

where D = diameter of the seminal receptacle and s = number of copulations.

When sperm supply is low fertilization probability is also low, but even a small increase in sperm supply rapidly accelerates the probability of egg fertilization because of anisogamy. However, as the quantity of stored sperm increases the slope of the curve approaches zero as an asymptote is reached. The slope and asymptote of the curve depend in part on the maximum volume of the seminal receptacle ($0.167\,\pi D^3$), ejaculate size and number of copulations (delivering standard ejaculates). For species with trans-moult sperm retention new sperm must added to old sperm hence the asymptotic relationship. The female strategy must be to maximize the probability of egg fertilization by maintaining a viable sperm supply and keeping the seminal receptacle as full as possible.

For males we assume that the most important factor is to deposit sperm in the place where it will fertilize the most eggs (see below) and not to waste sperm in fruitless copulations. Faced with the possibility of sperm competition and a limited supply of sperm it is the male who should be selective, not the female.

3. Male strategy

3.1. Sperm supply

Is it really necessary for males and females to conserve sperm? Since sperm are smaller than eggs they are often assumed to be available in more than adequate supply, hence leading to the assumption that populations are limited by the supply of eggs. For a number of reasons, however, this assumption may not be justified. Whereas a great deal about egg supply is known almost nothing is known about sperm supply in crab populations (Sainte-Marie, 2007). Females of different species can package up their yolk production into eggs of different sizes and males can package up the sperm production into spermatophores of different sizes. Males can also adjust ejaculate size according to female numbers (sex ratio) or situation (e.g. presence of male competitors) so as to maximize their reproductive success. Some examples of recent studies demonstrating that males adjust sperm transferred according to female "quality" (size) are: *Chionoectes opilio* (Rondeau and Sainte-Marie, 2001); *C. sapidus* (Jivoff, 2003); *Hemigrapsus sexdentatus* (Brockerhoff and McLay, 2005a); *Paralithodes brevipes* (A. Milne-Edwards and Lucas, 1841) (Sato et al., 2005); *Hapalogaster dentata* (De Haan, 1849) (Sato and Goshima, 2007). [Note that the latter two species do not have internal sperm storage.] There have been few studies of sperm supply in decapod populations, but the deleterious consequences of an inadequate supply have been investigated in some male-biased crustacean fisheries, e.g. *M. magister* (Hankin et al., 1989, 1997), *C. sapidus* (Kendall et al., 2001, 2002; Hines et al., 2003), *C. opilio* (Sainte-Marie et al., 2008), *M. squinado* (González-Gurriarán et al., 1998), *P. gigas* (Gardner and Williams, 2002), and *Jasus edwardsii* (Hutton, 1875) (Achelata) (MacDiarmid and Butler, 1999). Large decadal swings in the sex ratio of mature *C. opilio* can produce a limited supply of primiparous females (reproducing for the first time) in some years, resulting in highly promiscuous mating and the accumulation of large sperm stores, while in other years they greatly outnumber males, resulting in sperm limitation (Sainte-Marie et al., 2002). There is unfortunately no clear idea of what constitutes a "sufficient" sperm supply for an exploited crab stock of any species and even less about what the critical value of the sperm/egg ratio might be (Hines et al., 2003). It seems reasonable to assume that for non-migratory species, which are probably the majority, sperm is likely to be in short supply because sparse distribution in complex habitats makes mate encounters rare. The cases discussed above reinforce the idea that crabs need more than chance to find each other with the consequence that sperm transferred should be sperm preserved.

The most detailed analysis of sperm dynamics in seminal receptacles is by Hines et al. (2003) for *C. sapidus*. Seminal receptacle weight over a period of 7 years at one site and a conceptual model was developed to assess the potential for sperm limitation in this species, including post-mating sperm regeneration time in males. Assuming that they mate only once in their lifetime at the pubertal moult, females at most sites had enough sperm to fertilize the expected maximum number of broods, except in the heavily fished population of Lower Chesapeake Bay, although females at all sites may run out of sperm as they age and have reduced hatching success. Estimated sperm:egg ratios over a lifetime are around 20:1 to 30:1, but it is as yet unclear what the minimum ratio might be for sustainability or full fertility (Hines et al., 2003). Another careful analysis of sperm supply is for the snow crab, *C. opilio*, incorporating demographic factors involving recruitment of primiparous crabs, indicating the importance of the interaction of sperm allocation by males, and the degree of promiscuity of females (Sainte-Marie et al., 2002). *C. opilio* can have post-pubertal mating, however, which is more like the norm for crabs with determinate growth.

There is thus some evidence that crab populations are not necessarily egg-limited, but we know very little about sperm supply compared to egg production. There is some evidence that male crabs can adjust ejaculate size according to female "quality". Even in the most intensively studied species, like *C. sapidus* and *C. opilio,* the critical value of the sperm/egg ratio remains unknown. This ratio is likely to be different for each species and dependant upon the combination of growth format, the mating–moulting link and seminal receptacle structure.

3.2. Male strategy

It is assumed that male fertilization success is dependent upon where in the circular seminal receptacle they deposit their sperm. It is also assumed that both receptacles are filled equally by a male and that the female passes the same number of ova through each seminal receptacle at fertilization. We measure sperm location in terms of receptacle structure, using proximity of the oviduct entrance to the vagina (C) as the independent variable: the maximum distance $C_{\max} = (1.0)(\pi/2D)$, where D is the diameter of the receptacle, half way $C_{0.5} = (0.5)(\pi/2D)$ and as they get closer the distance approaches zero and $C_0 = (0)(\pi/2D)$. For the purposes of discussion we assume sperm layering, or more exactly, we ignore any effects of sperm mixing. It is assumed here that sperm displacement is the main mechanism of sperm competition. Decapod sperm are aflagellate and immotile (Krol et al., 1992).

Although there are an infinite number of possibilities, we model four cases ($C_{1.0}$, $C_{0.75}$, $C_{0.5}$ and $C_{0.25}$) differing in the position of the oviduct entrance into the seminal receptacle. Around each of these entrance points we assume that the probability of a sperm fertilizing an egg (SFP) is distributed according to a normal distribution (Fig. 6).

$$\mathrm{SFP} = \left(2\pi v \exp\frac{(C - \mathrm{OE})^2}{v}\right)^{-0.5}$$

where v = variance of the distribution and OE is the oviduct entrance point measured along the wall of the seminal receptacle (i.e. has values from 0 to $\pi/2D$).

When the distance between the oviduct and vagina is maximal (dorsal type receptacle), sperm that are deposited first into an empty seminal receptacle will have the highest priority, favouring the first male to mate. For sperm closest to the oviduct the probability of paternity by the first male will approach 1.0 and those at increasing distance ($C < \pi/2D$) from the oviduct will have markedly reduced chances to fertilize eggs. Eggs entering the dorsal side of the receptacle still have to "run the gauntlet", through other sperm in order for them to be laid, so there may be a chance that some will have different paternities if the female has had more than one mate. The rate at which eggs are released will influence which sperm get to fertilize them. Conversely when the distance is minimal (ventral type receptacle), sperm that is deposited last will have the best chance to encounter and fertilize the ova, favouring the last male to mate. For this ventral type receptacle the ova do not have to "run the gauntlet" because they are close to the vagina. In between these extremes are a range of possible intermediate oviduct positions where being neither the first nor the last male to mate would be optimal. The assumed evolutionary force driving ventral migration of the oviduct is increasingly mixed paternity and more stored sperm. The need for more studies of paternity in crabs is discussed below.

If the two extremes of dorsal vs. ventral locations are not the only evolutionary stable states, what is the selective force that stops the inevitable once initiated ventral slide? If sperm displacement is the only mechanism of sperm competition in crabs then the hypothesis developed thus far offers no explanation for intermediate states (see Fig. 6), because the slope of the curve in Fig. 4 is always positive and results in female gains in fitness. An alternative, but more complex hypothesis, still invoking sperm layering, could be that the female adapts to the male gonopods, which have evolved to place sperm at a certain distance from the vulva. This is more complex because the distance that sperm is delivered into the receptacle is measured linearly along the diameter (D), while the position of the oviduct is measured along the curved surface of the sphere $\pi/2D$. This balance or compromise idea holds that the female loses chitinous lining so as to increase sperm retention (her advantage), but opposing male evolution of gonopod structure is headed in the opposite direction because the male gains an advantage by placing his sperm deeper in the receptacle closest to the oviduct. This idea would predict that there may be gonopod differences in species where the female has an intermediate seminal receptacle, but additional behavioural characters would be required to make it

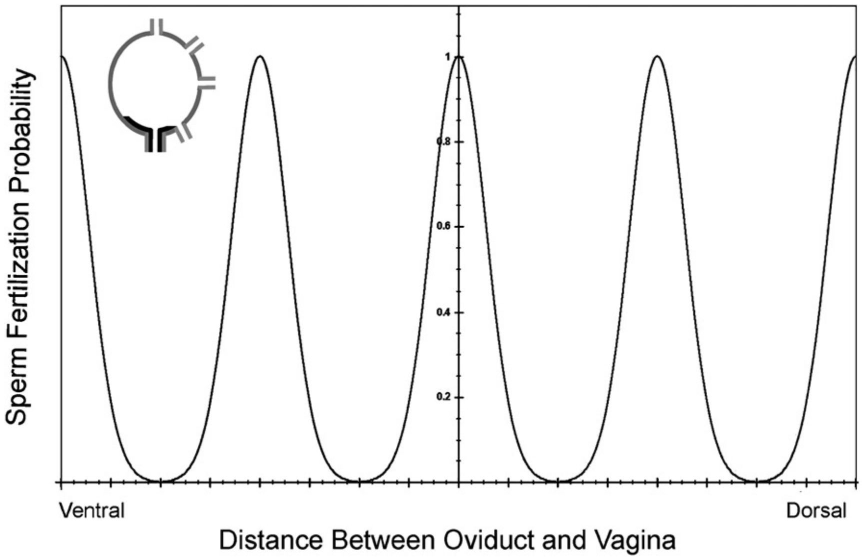

Fig. 6. Relationship between sperm fertilization probability and distance between oviduct entrance and vagina, as measured along the curved wall of the receptacle (i.e. C in the equation). A diagram of the seminal receptacle (top left) shows four evolutionary stages in ventral migration of the oviduct and the graph shows the distribution of fertilization probabilities around the oviduct entrance for each of those stages. Strictly speaking these probabilities are normally distributed only where a vertical or horizontal axis passes through the duct ($C = 0$, 0.5 and 1.0) while in both the dorsal and the ventral 90° quarters in between the distribution is distorted by the curvature. The oviduct cannot be totally ventral because then it would compete for access with the vagina so C only approaches 0 but cannot reach it. A spherical shape is assumed and distance = $\pi/2D$ is half of the perimeter where D is the diameter of the seminal receptacle. Note that the independent variable here is $\pi/2D$ in relation to the x-axis in Fig. 4 which measures linear distance from 0 to D.

work. The female gains advantage by conserving sperm while the male gains advantage by improving the probability of his paternity. A conspicuous variation in crab gonopods is whether the second gonopod (G2) is longer or shorter than the first (G1). Ventral-type receptacles are sometimes associated with short G2's (e.g. Varunidae, Ocypodidae, but not in the Cancridae and many other heterotreme families) while dorsal-type receptacles are associated with long G2's (e.g. Portunidae). The analysis of seminal receptacle and gonopod structure is complex and beyond our present purpose, but should be the subject of future research. For example in the Goneplacidae and Bythograeidae one finds a few genera with short G2 while most have long G2 (Castro, 2007; McLay, 2007), but nothing is known about their seminal recepatcles.

Other factors that result in sperm mixing are nevertheless involved so that the same genetic advantage for females may result and under some circumstances deliver a superior level of variation in paternity to that delivered by multiple partners and sperm layering. Some recent work on the inachid spider crab *I. phalangium* suggests that males transfer both intact spermatophores and free sperm (Rorandelli et al., 2008). Some sperm is released from spermatophores by sharp spines in the ejaculatory canal of the male first gonopod rupturing them as they are delivered into the female. The result is a mixture of free and packaged sperm that can be mixed with the contents of the seminal receptacle, thereby making the exact position of the oviduct less critical. We term this mechanism of sperm competition "sperm infiltration" as opposed to "sperm layering". The critical information that would help resolve this problem is paternity variation in crab broods (see Section 4 below).

4. The evidence

4.1. Variation of seminal receptacle structure in the Eubrachyura

The present hypothesis aims to explain variation in seminal receptacle structure, primarily how the "dorsal" and "ventral" types evolved. A summary of seminal receptacle variation amongst eubrachyurans from published and unpublished work is given in Tables 1–3. The species are grouped into "dorsal", "ventral" and "intermediate" according to where the oviduct enters the seminal receptacle. Of the 117 species 77 (65.8%) have a ventral oviduct entrance, while 34 (29%) have a dorsal entrance, with 6 (5.2%) species intermediate. Generally speaking species belonging to the same family also have the same kind of seminal receptacle. These data are a first approximation to describing receptacle variation in crabs but more attention needs to be paid to widening the range of families and within some families to see if other species therein do have the same type, especially the Xanthoids. Our data includes representatives of only 31 (37%) out of the 83 eubrachyuran families (see Ng et al., 2008).

Table 1. Summary of characteristics of the seminal receptacles of the Eubrachyura. Receptacles are classed as "dorsal" if the oviduct is opposite the vagina, "ventral" if the oviduct is beside the vagina and "intermediate" if it lies in between. Where a "?" is used it indicates that we have made an assumption based on taxonomic association, resulting in a prediction that needs verification in some cases. A blank indicates that nothing is known for that species. The source reference is indicated while data for other species are reported here for the first time as "Author (unpub.)".

Species	Family	Oviduct entrance into receptacle	Reference
Callinectes bocourti A. Milne-Edwards, 1879	Portunidae	Dorsal	López Greco (unpub.)
Callinectes ornatus Ordway, 1863	Portunidae	Dorsal	López Greco (unpub.)
Callinectes sapidus Rathbun, 1896	Portunidae	Dorsal	Johnson (1980)
Ovalipes catharus (White and Doubleday, 1843)	Portunidae	Dorsal	McLay (unpub.)
Portunus sanguinolentus (Herbst, 1783)	Portunidae	Intermediate	Ryan (1967a)
Portunus pelagicus (Linnaeus, 1758)	Portunidae	Dorsal	Bawab and El-Sherief (1988)
Carcinus maenas (Linnaeus, 1758)	Portunidae	Dorsal	Hartnoll (1968b)
Charybdis hellerii (A. Milne-Edwards, 1867)	Portunidae	Dorsal	López Greco (unpub.)
Liocarcinus depurator (Linnaeus, 1758)	Portunidae	Dorsal	Diesel (1991)
Ashtoret lunaris (Forskål, 1775)	Matutidae	?Dorsal	Perez and Bellwood (1989)
Erimacrus isenbeckii (Brandt, 1848)	Cheiragonidae	Dorsal	Nagao et al. (1996)
Telmessus cheiragonus (Tilesius, 1812)	Cheiragonidae	Dorsal	Nagao and Munehara (2001)
Trichopeltarion fantasticum Richardson and Dell, 1964	Trichopeltariidae	Dorsal	McLay (Fig. 1A, herein)
Pilumnus hirtellus (Linnaeus, 1761)	Pilumnidae	Dorsal	Diesel (1991)
Pilumnus lumpinus Bennett, 1964	Pilumnidae	Dorsal	McLay (Fig. 1B, herein)
Pilumnus novaezelandiae Filhol, 1885	Pilumnidae	Dorsal	McLay (unpub.)
Cycloxanthops novemdentatus (Lockington, 1877)	Xanthidae	Dorsal	Knudsen (1960)
Paraxanthias taylori (Simpson, 1861)	Xanthidae	Dorsal	Knudsen (1960)
Metacarcinus gracilis (Dana, 1852)	Cancridae	Ventral	Orensanz et al. (1995)
Metacarcinus magister (Dana, 1852)	Cancridae	Ventral	Jensen et al. (1996)
Romaleon setosus (Molina, 1782)	Cancridae	Ventral	López Greco (unpub.)
Neommatocarcinus huttoni (Filhol, 1885)	Goneplacidae	Ventral	McLay (unpub.)
Pycnoplax meridionalis (Rathbun, 1923)	Goneplacidae	Ventral	McLay (unpub.)
Corystes cassivelaunus (Pennant, 1777)	Corystidae	Ventral	Diesel (1991) and Hartnoll (1968a)
Calappa granulata (Linnaeus, 1758)	Calappidae	Ventral	Cano (1891)
Platymera gaudichaudii H. Milne Edwards, 1837	Calappidae	Ventral	McLay (Fig. 1H, herein)
Hepatus pudibundus (Herbst, 1785)	Aethridae	Ventral	López Greco (unpub.)
Heterozius rotundifrons A. Milne-Edwards, 1867	Belliidae	Ventral	McLay (unpub.)
Paragalene longicrura (Nardo, 1868)	Progeryonidae	Ventral	Cano (1891)
Geryon fenneri (Manning and Holthuis, 1984)	Geryonidae	?Ventral	Hinsch (1988)
Ilia nucleus (Linnaeus, 1758)	Leucosiidae	Ventral	Cano (1891)
Randallia ornata (Randall, 1840)	Leucosiidae	Ventral	McLay (unpub.)
Derilambrus angulifrons (Latreille, 1825)	Parthenopidae	Ventral	Cano (1891)
Macrocoleoma trispìnosum (Latreille, 1825)	Majidae	Intermediate	López Greco (unpub.)
Maja crispata Risso, 1827	Majidae	Ventral	Cano (1891)
Maja squinado (Herbst, 1788)	Majidae	Ventral	Diesel (1991)
Maja brachydactyla Balss, 1922	Majidae	Ventral	Rotllant et al. (2007)
Microphrys bicornutus (Latreille, 1825)	Majidae	Intermediate	López Greco (unpub.)
Mithraculus forceps (A. Milne-Edwards, 1875)	Majidae	Intermediate	López Greco (unpub.)
Mithrax hispidus (Herbst, 1790)	Majidae	Ventral	López Greco (unpub.)
Mithrax verrucosus H. Milne Edwards, 1832	Majidae	Ventral	López Greco (unpub.)
Jacquinotia edwardsi (Jacquinot, in Jacquinot and Lucas, 1853)	Majidae	Ventral	McLay (1988)
Inachus communissimus Rizza, 1839	Inachidae	Ventral	Diesel (1991)
Inachus dorsettensis (Pennant, 1777)	Inachidae	Ventral	Cano (1891)
Inachus phalangium (Fabricius, 1775)	Inachidae	Ventral	(Diesel, 1988, 1989, 1990, 1991)
Macropodia rostrata (Linnaeus, 1761)	Inachidae	Ventral	Diesel (1991)
Libidoclaea granaria (H. Milne Edwards and Lucas, 1842)	Epialtidae	Ventral	López Greco (unpub.)
Libinia spinosa (H. Milne Edwards and Lucas, 1843)	Epialtidae	Intermediate	González-Pisani and López Greco (2007)
Pisa tetraodon (Pennant, 1777)	Epialtidae	Ventral	Diesel (1991)

Table 1 (*Continued*)

Species	Family	Oviduct entrance into receptacle	Reference
Pisa armata (Latreille, 1803)	Epialtidae	Ventral	Diesel (1991)
Taliepus dentatus (H. Milne Edwards, 1834)	Epialtidae	Ventral	López Greco (unpub.)
Chionoecetes opilio (Krøyer, 1838)	Oregoniidae	Ventral	Sainte-Marie and Sainte-Marie (1998)
Hyas araneus (Linnaeus, 1758)	Oregoniidae	Ventral	Hartnoll (1968b)
Hyas coarctatus Leach, 1816	Oregoniidae	Ventral	Hartnoll (1968b)
Leurocyclus tuberculosus (H. Milne Edwards and Lucas, 1843)	Inachoididae	Intermediate	González-Pisani and López Greco (2007)
Halicarcinus cookii Filhol, 1885	Hymenosomatidae	Ventral	Van Den Brink and McLay (2009)
Halicarcinus innominatus Richardson, 1949	Hymenosomatidae	Ventral	Dunnington (1999)
Halicarcinus varius (Dana, 1851)	Hymenosomatidae	Ventral	Hosie (2004)
Cardisoma guanhumi (Latreille, 1825)	Gecarcinidae	Ventral	López Greco (unpub.)
Austrohelice crassa (Dana, 1851)	Varunidae	Ventral	Brockerhoff and McLay, 2005b
Cyclograpsus lavauxi H. Milne Edwards, 1853	Varunidae	Ventral	Brockerhoff and McLay, 2005b
Hemigrapsus crenulatus (H. Milne Edwards, 1837)	Varunidae	Ventral	Brockerhoff and McLay, 2005b
Hemigrapsus sexdentatus (H. Milne Edwards, 1837)	Varunidae	Ventral	Brockerhoff and McLay, 2005b
Neohelice granulata (Dana, 1851)	Varunidae	Ventral	López Greco et al. (1999)
Eriocheir sinensis (H. Milne Edwards, 1853)	Varunidae	Ventral	(Lee and Yamazaki, 1990)
Aratus pisonii (H. Milne Edwards, 1837)	Sesarmidae	Ventral/dorsal[a]	Hartnoll (1965); López Greco (unpub.)
Metopaulias depressus (Rathbun, 1896)	Sesarmidae	Ventral	Hartnoll (1964)
Sesarma bidentatum (Benedict, 1892)	Sesarmidae	Ventral	Hartnoll (1964)
Sesarma verleyi (Rathbun, 1914)	Sesarmidae	Ventral	Hartnoll (1964)
Spiralothelphusa hydrodroma (Herbst, 1794)	Parathelphusidae	Ventral	Anilkumar and Adiyodi (1977)
Potamon fluviatile (Herbst, 1785)	Potamidae	Ventral	Brandis et al. (1999)
Scopimera globosa (De Haan, 1835)	Dotillidae	Ventral	Koga et al. (1993)
Hemiplax hirtipes (Hombron and Jacquinot, 1846)	Ocypodidae	Ventral	Jennings et al. (2000)
Uca ecuadorensis Maccagno, 1928	Ocpodidae	Ventral	Lautenschlager et al. (2010)
Uca cf. forcipata (Adams and White, 1849)	Ocypodidae	Ventral	Lautenschlager et al. (2010)
Uca lactea (De Haan, 1835)	Ocypodidae	Ventral	Murai et al. (1987)
Uca tangeri (Eydoux, 1835)	Ocyodidae	Ventral	Lautenschlager et al. (2010)
Ocypode quadrata (Fabricius, 1787)	Ocypodidae	Ventral	López Greco et al. (2009)
Ucides cordatus (Linnaeus, 1763)	Ucididae	Ventral	Sant'Anna et al. (2007)
Pinnotheres pectunculi Hesse, 1872	Pinnotheridae	Ventral	Becker et al. (2011)
Pinnotheres pisum (Linnaeus, 1767)	Pinnotheridae	Ventral	Becker et al. (2011)
Nepinnotheres pinnotheres (Linnaeus, 1758)	Pinnotheridae	Ventral	Becker et al. (2011)
Tunicotheres moseri (Rathbun, 1918)	Pinnotheridae	Ventral	López Greco (unpub.)

[a] *Aratus pisoni* was reported by Hartnoll (1965) as having a ventral receptacle, but our observations suggest that it is dorsal and therefore different to the rest of the sesarmids. Further work is required to sort out this conflict.

4.2. The link between moulting and mating, growth format and seminal receptacle structure

Asakura (2009) reviewed decapod mating systems and, with a focus on the life styles and male–female relations, resolved them into nine types. Here we focus on the fundamental reproductive variables to argue that we can explain the evolution of the variety of mating systems in simpler terms. Our hypothesis holds that there are only three important variables that affect crab mating strategies: (1) whether mating and moulting are linked; (2) whether they have indeterminate or determinate growth; and (3) the structure of the seminal receptacle. For the first two variables there are only two alternatives: mating and moulting linked/not-linked and growth is either indeterminate/determinate. These latter two terms need clear understanding: indeterminate growth involves indefinite continuation of moulting after puberty so that the number of instars is dependent upon survivorship; determinate growth is a consequence of moulting ceasing at or soon after the pubertal moult. Hartnoll (1985) noted that only one mature instar occurs in the Leucosiidae (see also Schembri, 1982, 1983), Corystidae, Majidae, Oregoniidae, Inachidae, and most Hymenosomatidae (except in *Hymenosoma orbiculare* Desmarest, 1823). The number of post-pubertal instars in the Portunidae and Matutidae is discussed below (see also Table 3). For the seminal receptacle

Table 2. Link between mating, moulting and seminal receptacle structure for crabs with indeterminate growth. Receptacle structure is presented as a dichotomy, but those with the intermediate type are [bracketed].

Female mates when	Dorsal seminal receptacle	Ventral seminal receptacle
Soft shell	Cheiragonidae: *Erimacrus isenbeckii*, *Telmessus cheiragonus* Menippidae: ?*Menippe mercenaria* Eriphiidae: ?*Eriphia smithii*	Cancridae: *Metacarcinus gracilis*, *M. magister*, *Romaleon setosus* Progeryonidae: ?*Paragalene longicrura* Geryonidae: *Chaceon maritae*, *Geryon fenneri*, *G. quinquedens* Belliidae: *Heterozius rotundifrons*
Hard shell	Pilumnidae: *Pilumnus lumpinus*, *P. novaezelandiae*, ?*P. hirtellus*, ?*P. vespertilio.* Panopeidae: ?*Lophopanopeus diegensis*, *L. Leucomanus*, *Neopanope sayi* Xanthidae: *Cycloxanthops novemdentatus*, ?*Paraxanthias taylori*, ?*Xantho incisus* Platyxanthidae: ?*Platyxanthus patagonicus*, ?*Homalaspsis plana*	Aethridae: *Hepatus pudibundus* Calappidae: ?*Calappa granulata* Potamidae: *Potamon fluviatile*, *Candidiopotamon rathbunae* Parathelphusidae: ?*Spirothelphusa hydrodroma* Grapsidae: *Goniopsis cruentata*, *Grapsus grapsus* Varunidae: *Cyclograpsus lavauxi*, ?*C. punctatus*, *Hemigrapsus crenulatus*, *H. sexdentatus*, *Austrohelice crassa*, *Neohelice granulata*, *Eriocheir sinensis* Sesarmidae: *Metopaulius depressus*, *Sesarma bidentatum*, *S. verleyi*, [?*Aratus pisoni*] Gecarcinidae: *Cardisoma gaunhumi*, *Gecarcinus lateralis*, *Johngarthia malpilensis* Dotillidae: ?*Scopimera globosa* Ocypodidae: *Uca lactea*, *Ocypode quadrata* Macrophthalmidae: *Hemiplax hirtipes* Ucididae: *Ucides cordatus*

? indicates species assigned to a seminal receptacle type by association with known members of the same group, but this assumption needs confirmation.

we recognize three states: dorsal/ventral/intermediate, but for the purposes of summarizing the data we combine ventral and intermediate receptacles. Accepting for the moment that there is a dichotomy (rather than trichotomy or polychotomy) of receptacle types, we can identify eight possible strategies. A summary of what is known about realized crab mating strategies is presented for indeterminate growers (Table 2) and for determinate growers (Table 3), a total of 88 species belonging to 35 families. The main reason for separating these two growth formats is that for the former group moulting after sexual maturity results in the female shedding part or all of the contents of the seminal receptacle, but species with determinate growth have no way of removing sperm from the seminal receptacle because they cannot moult, so after puberty females continue to accumulate sperm by mating.

Examples of all four possible strategies are found in crabs with indeterminate growth (Table 2). The growth format for the deep-sea geryonid crabs has until recently been uncertain, but Pezzuto and Sant'Ana (2009) showed that for *Chaceon* and *Geryon* the data on population size structure and maturation size indicates that they have indeterminate growth. By far the majority of crabs that have an indeterminate format (20 vs. 7 families) have a ventral seminal receptacle and of these the majority (16 vs. 4 families) have lost the link between mating and moulting. Many of the 25 species of crabs in which males use sperm plugs to block the female vulvae or vagina belong to families which have soft-shell mating and indeterminate growth: Cancridae: *Cancer borealis* Stimpson, 1859, *C. irroratus* Say, 1817, *C. pagurus*, *Metacarcinus gracilis*, *M. magister*, *Romaleon polyodon* (Poeppig, 1836) (as *Cancer setosus*); Geryonidae: *Chaceon fenneri* (as *Geryon*), *Geryon longipes* (A. Milne-Edwards, 1882); Goneplacidae: *Entricoplax vestita* (De Haan, 1835), *Psopheticus stridulans* Wood-Mason, 1892, *Pycnoplax meridionalis*, *P. victoriensis* (Rathbun, 1923); Cheiragonidae: *Erimacrus isenbeckii*, *T. cheiragonus* and Eriphidae: *Eriphia smithii* MacLeay, 1838 (Table 2) (Tomikawa and Watanabe, 1990; Castro, 2007; Doi et al., 2007; Asakura, 2009). The other 10 species belong to Portunidae (*C. sapidus*, *C. maenas*, *L. depurator*, *L. holsatus* (as *Macropipus*), *Macropipus tuberculatus* (Roux, 1830), *Necora puber* (Linneaus, 1767) (as *Macropipus*), *Ovalipes ocellatus* (Herbst, 1799), *P. pelagicus*, *P. sanguinolentus*), but these have determinate growth (see Table 3). An exception may be freshwater crabs of the family Potamidae: *P. fluviatile* is reported as having a "sperm plug" (although occupying the entrance to the seminal receptacle rather than the vagina) and

Table 3. Link between mating, moulting and seminal receptacle structure for crabs with determinate growth. Receptacle structure is presented as a dichotomy, but those with the intermediate type are grouped with the ventral type [bracketed]. Where it is known, the number of mature instars is shown "(x)" after each species.

Female mates when	Dorsal seminal receptacle	Ventral seminal receptacle
Pubertal soft/hard	Matutidae: *Ashtoret lunaris*? Portunidae: *Callinectes sapidus* (1), *Carcinus maenas* (6), *Charybdis hellerii* (2), *Charybdis natator* (1), *Liocarcinus depurator*, *Necora puber*, *Ovalipes catharus* (4–5)), *Portunus pelagicus* (2), [*P. sanguinolentus* (2)], *Scylla olivacea* (1), *Scylla serrata* (1)	Majidae: *Jacquinotia edwardsi* Oregoniidae: *Chionoecetes bairdi* (1), *C. opilio* (1), *Hyas araneus* (1), *H. coarctatus* (1)
Hard	Portunidae: *Thalamita picta* (1), *T. prymna* (1), *T. sima* (?2)	Corystidae: *Corystes cassivelaunus* (1) Leucosiidae: *Ebalia tuberosa* (1), *Ilia nucleus*, *Philyra laevis*, *P. scabriuscula* Epialtidae: *Libinia emarginata* (1), *L. spinosa* (1), ?*Libidoclaea granaria*, *Pisa armata*, *P. tetraodon*,?*Taliepus dentatus* Majidae: [*Macrocoeloma trispinosus*], *Maja squinado* (1), ?*M. crispata* (1), [*Microphrys bicornutus*], [*Mithraculus forceps*], ?*Eurynome aspersa* (1), ?*E. spinosa*(1) Inachidae: *Inachus dorsettensis* (1), *Inachus phalangium* (1), *Inachus communissimus* (1), ?*Macropodia longirostris* (1), *M. rostrata*(1) Inachoididae: [*Leurocyclus tuberculosus* (1)] Hymenosomatidae: *Halicarcinus cookii* (1), *H. innominatus* (1), *H. varius* (1), *Hymenosoma orbiculare* (>1).

? indicates species assigned to a seminal receptacle type by association with known members of the same group, but this assumption needs confirmation. Some part of the information needed to classify the species is missing or uncertain.

also has a complex seminal receptacle meaning that the fertilization mechanism involved requires further study (Brandis et al., 1999).

Crabs with determinate growth show a similar range of strategies (Table 3) although less certain. The majority of crabs have a ventral receptacle (9 vs. 3 families) and females mating when hard shelled (8 vs. 4 families) and, as in crabs with indeterminate growth, they too have lost the link between mating and moulting. There are two possible mating outcomes: females may mate at the pubertal moult and subsequently when they are hard shelled (some Majidae and Oregoniidae) or they do not mate when soft, but only later when hard and when their ovaries have developed. The majority of these crabs fall into this last strategy (Corystidae, Leucosiidae, Epialtidae, some Majidae, Inachidae, Inachoididae and Hymenosomatidae). In fact, the latter two outcomes could be combined because hard-female mating is the main way that sperm is transferred, but this would mask the possibility that there may be two different mechanisms of mate attraction being used (growth-linked and ovarian-linked) (see Jones and Hartnoll, 1997). Hymenosomatid females can mate precociously in the penultimate instar, which is hard-shell mating, because it precedes the pubertal moult (Van Den Brink and McLay, 2010). Newly moulted females are not attractive to males. In the epialtid *L. spinosa* both males and females, in a population at Mar del Plata, Argentina, undergo the pubertal moult over a wide size range, but in females the size of morphological and gonad maturity is roughly the same, while in males gonad maturity is attained prior to morphological maturity, allowing them to mate precociously (Sal Moyano et al., 2011).

Portunids and matutids are similar in life style, both being burrowers/swimmers, and they have the same growth format. In the Matutidae, the moon crab *Ashtoret lunaris* (Forskål, 1775) has determinate growth and males grow much larger than females (Bellwood and Perez, 1989). Males are attracted to pre-pubescent females and guard them until they moult, then mate while the female is soft, and guard her until she is hardened (Perez and Bellwood, 1989; see also http://www.wildsingapore.com/wildfacts/crustacea/crab/matutidae/lunaris.htm for recent photos of guarding in the moon crab). In the Portunidae *Thalamita* species (*T. picta* Stimpson, 1858, *T. prymna* (Herbst, 1803), *T. sima* (H. Milne Edwards, 1834)) seem to have independently evolved hard shell female mating: newly moulted pubertal female *Thalamita picta* are not attractive to males who, unlike other portunids, do not use sperm plugs (Norman, 1996; Norman et al., 1997, 1999). Whether portunids have indeterminate or determinate growth has long been controversial (Ryan, 1967b; Fielder and Eales, 1972; Hartnoll, 1985; Havens and McConaugha, 1990; Haddon and Wear, 1993; Moser et al., 2002; Smith and Chang, 2007). The main advantage of determinate growth is that it allows females to invest all their energy into first growth and then reproduction. Here we take the view that growth is determinate, but some species may have more than one mature instar (see Table 3 for variation between species). Some crabs may moult when mature, but that does not mean that they all do. The case of the deep-sea portunid *Bathynectes piperitus* is instructive: epibiosis on different sized crabs suggests that this species does not have a terminal moult because, while most of the larger crabs had some epibionts (small crabs had none) there were large numbers in the larger size classes that had none, indicating that they had recently moulted (Abello et al., 1990). Approximately 4 moults would be required to reach maximum size assuming a 15% increment per moult after female maturity.

The greatest number of crabs with intermediate receptacles are found amongst those with determinate growth: *L. tuberculosus* (Inachoididae) and in the Majidae three out of seven species are intermediate (*Macrocoeloma trispinosum*, *Microphrys bicornutus*, *Mithraculus forceps*) while the rest are ventral. More data are required in order to obtain a more accurate picture of variation within these Majoid families.

A sample of 117 species from around a third of the eubrachyuran families shows that 65.8% have ventral seminal receptacles, 29% dorsal and 5.2% intermediate receptacles. The reproductive strategies of these crabs can be analysed in relation to growth format (indeterminate/determinate), seminal receptacle structure (ranging from dorsal to ventral oviduct connections) and the link between mating and moulting (soft shell/hard shell mating). With alternate states for 3 variables there 8 possible combinations and eubrachyurans provide examples of each of these, confirming the opinion of Hartnoll (1985) that in crabs "anything is possible". However, some combinations are more successful than others. In crabs with indeterminate growth, those with a dorsal receptacle mostly have hard shell mating (Xanthoids) as do those with a ventral receptacle (Grapsoids–Ocypodoids), while in crabs with determinate growth those with a dorsal receptacle mostly have soft shell mating (Portunoids) whereas those with a ventral receptacle mostly have hard shell mating (Majoids). Whether or not Portunoids have determinate growth, with a fixed number of post-pubertal instars, remains an open question. The greatest number of species with receptacles intermediate between dorsal and ventral ones are found amongst the Majoids (Epialtidae and Majidae).

4.3. Evolution of mating and growth strategies

On the basis of the frequency of mating opportunities (number of females × receptivity), Wickler and Seibt (1981) resolved crustacean mating systems into three kinds: monogamy (low frequency), female guarding (intermediate) and pure searching (high frequency of mating opportunities). The ancestral eubrachyuran probably had all the generalized features of other reptant decapod crustacean mating strategies as typified by gonochoristic carideans: mate attraction via the moulting hormone crustecdysone (+ a pheromone indicating gender), guarding of receptive females, soft shell mating, spawning quickly following mating, and external fertilization (see Bauer, 2004, p. 145). These features provided few, if any, chances for multiple partners and the possibility of sperm competition. Various degrees of male mate guarding seems to be an ancestral character for at least the reptant Decapoda.

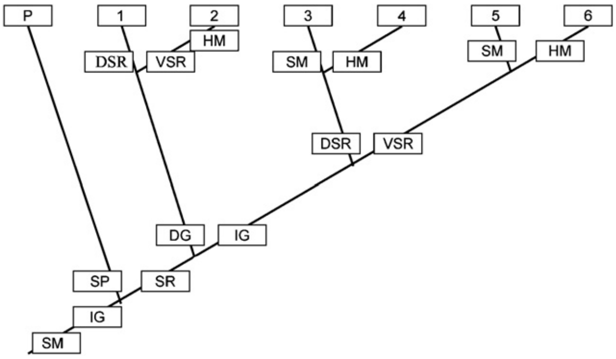

Fig. 7. A phylogeny of growth, mating and sperm storage characters of the Eubrachyura. The crabs are divided into 6 groups: (1) Portunoids; (2) Majoids; (3) Eriphioids; (4) Xanthoids; (5) Cancroids; and (6) Grapsoid–Ocypodoids. The outgroup is the Podotremata (P). Data for the strategies and composition of the groups are summarized in Table 4. Shared characters are marked by labelled boxes: Growth characters are: IG – indeterminate growth, DG – determinate growth; Mating characters are: SM – soft shell mating, HM – hard shell mating, Sperm storage characters are: SP – spermatheca, SR – seminal receptacle; Oviduct connection: DSR – dorsal seminal receptacle, VSR – ventral seminal receptacle.

An analysis based on alternate states of 3 characters (growth format, receptacle structure and mating–moulting link) results in $2^3 = 8$ possible combinations, or strategies, that can be arranged to produce 6 possible trees depending upon the order in which the characters changed. In crabs with indeterminate growth there are 4 possible combinations, and there are examples of each of them, but in crabs with determinate growth there is only good evidence for 2 of the possible 4 strategies having evolved (see Table 4 footnote) so that overall there are only 6 strategies. A tree has been chosen as a working hypothesis that best explains reproductive evolution within the Eubrachyura. This tree (Fig. 7) assumes that the character hierarchy was growth format – seminal receptacle structure – mating–moulting link.

The first step on the path to eubrachyurans was sperm storage (Fig. 7). This was not possible until the seminal receptacle, and later, trans-moult sperm retention, evolved, allowing the storage of sperm from more than one male and delayed, internal fertilization. The second step was the evolution of determinate growth which amounts to an ontogenetic change from simultaneous investment in growth + reproduction (indeterminate growth) to sequential investment (determinate growth), first using all the energy for growth, and then for reproduction. Crabs with indetermi-

Table 4. Summary of the differences between the four major mating strategies used by eubrachyurans (note that not all families are included because of lack of data). The families belonging to each group are listed in the footnote below [IG = indeterminate growth; DG = determinate growth; SM = soft shell mating; HM = hard shell mating; PSR = Portunoid seminal receptacle; DSR = dorsal seminal receptacle; VSR = ventral seminal receptacle].

Elements of mating strategy	Group (1): Portunoids DG + SM + PSR	Group (2): Majoids DG + HM + VSR	Group (3): Eriphioids IG + SM + DSR	Group (4): Xanthoids IG + HM + DSR	Group (5): Cancroids IG + SM + VSR	Group (6): Grapsoid-Ocypodoids IG + HM + VSR
Mate attraction	Ecdysone and ?ovarian signal	Ecdysone and ovarian signal	Ecdysone and ?ovarian signal	Ovarian signal	Ecdysone and ?ovarian signal	Ovarian signal
Courtship display[a]	Present in Portunidae	None	None	None	None	Present in Varunidae, Dotillidae, Ocypodidae, Macrophthalmidae
Pre-copulatory guarding[b]	Prolonged	Prolonged	Prolonged	Brief or absent	Prolonged	Brief or absent
Post-copulatory guarding[b]	Prolonged	Prolonged	Prolonged	Brief or absent.	Prolonged	Brief or absent
Sperm plug	Present in Portunidae	None	Present in Cheiragonidae, Eriphiidae	None	Present in Cancridae, Geryonidae, Goneplacidae	Present in Gecarcinidae and Potamidae
Hinged opercula in vulvae	None	Only in Corystidae	Only in Atelecyclidae	None	Only in Belliidae	Present in Grapsoidea, Ocypodoidea
Sperm competition	Limited	Common	Limited	?Common	Limited	Very common

[a] We define “courtship” as male behavioural displays, directed towards receptive females, advertising sexual services. If accepted mate-guarding usually follows. Group (1) Portunoids: Portunidae. Group (2) Majoids: Corystidae, Leucosiidae, Majidae, Oregoniidae, Epialtidae, Inachidae, Inachoididae, Hymenosomatidae. Group (3) Eriphioids: Atelecyclidae, Cheiragonidae, Menippidae, Eriphiidae, Platyxanthidae. Group (4) Xanthoid: Panopeidae, Pilumnidae, Xanthidae. Group (5) Cancroids: Cancridae, Geryonidae, Goneplacidae, Progeryonidae, Belliidae, Calappidae. Group (6) Grapsoid-Ocypodoids: Sesarmidae, Gecarcinidae, Grapsidae, Varunidae, Dotillidae, Ocypodidae, Ucididae, Parathelphusidae, Potamidae.

[b] We use “guarding” to mean that the male carries the female around by grasping one of her pereopods or it may mean that the male covers the female with his body and cages her within his pereopods. Also includes males that guard females in their burrows or guard at the burrow entrance.

nate growth simply retained the ancestral state. Within the determinate growth group two paths evolved as a result of changes to the seminal receptacle: the Portunoids (group 1) (see Table 4), evolved a seminal receptacle from growth of the dorsal wall, but otherwise retained ancestral characters, while in the Majoids (group 2) a version of the ventral seminal receptacle evolved. This determinate growth group is the sister group of all the other crabs that retained indeterminate growth. The remaining crabs form two groups, one evolving the ventral receptacle (Cancroids + Grapsoid–Ocypodoids) and the other retaining the ancestral dorsal receptacle (Eriphoids + Xanthoids). Within each of these groups hard-shell mating evolved independently. According to this view Eriphoids are defined by ancestral characters and Cancroids are only one step away by having a ventral receptacle. It is necessary to hypothesize that the ventral receptacle evolved twice and hard shell mating evolved three times (see Fig. 7). Any acceptable phylogenetic hypothesis about crab reproduction, based on these characters, is not going to meet the criterion of maximum parsimony because only three characters are used and there are 6 terminal groups. More characters additional to those listed in Table 4 could be used, but these do not help because they simply reflect the three main characters and therefore do not really add any more information to the analysis.

It is suggested that these crabs with determinate growth form a monophyletic group which involved a change to the ecdysteroid-regulated moult system (see Smith and Chang, 2007, p. 216). Some of the Portunoids have retained the ancestral state and have several mature instars: at one extreme there is *C. maenas* and *Ovalipes catharus* which continue growing through 5–6 instars, while at the other extreme *Callinecetes sapidus* and *Scylla serrata* (Forskål, 1775) with only one instar; however all instars can produce multiple broods. Multiple mature instars do provide the opportunity to replenish sperm supplies. In the *Callinectes* and *Carcinus* mating system, males search for and defend pre-pubertal females, carrying them around for several days prior to moulting, and then after copulation continue guarding until the female exoskeleton hardened (Berrill and Arsenault, 1982; Jivoff et al., 2007). In the Portunidae intermoult hard-shell mating may have evolved independently in *Thalamita* (Norman, 1996; Norman et al., 1997, 1999). Majoids have only one mature instar (see Table 3). Some of these species arrive at the pubertal moult with ripe ovaries (e.g. *C. opilio*, *H. coarctatus*, *Halicarcinus* spp.), but others (e.g. *Inachus dorsettensis*, *I. phalangium, M. squinado*, *M. brachydactyla*, *L. tuberculosus*) do not have any ovarian development until after the pubertal moult. So only the latter species can be said to have truly severed the link between growth and reproduction. Determinate growth in Majoids could be regarded as an extreme form of sperm conservation; however, the cost of this strategy is loss of the ability to repair damage to the exoskeleton and replace autotomized limbs. Majoids are sperm accumulators because they can also mate during the final instar.

We might expect that symbiotic crabs living in a restricted space, such as pinnotherids and cryptochirids, might also have independently evolved determinate growth because it is not in the interests of the crab to kill the host by growing too large. Pinnotherids have ventral seminal receptacles (Becker et al., 2011) live in shellfish and holothurian hosts and have post-pubertal moulting, but it is unclear whether there is a finite number of instars (Watanabe and Henmi, 2009). Where the crab remained resident a determinate growth format would predict that crab size would be related to host size except that in larger hosts it would plateau out. Data are ambiguous for a sample of eight species of pea crabs (mostly *Pinnotheres* spp.) living in these kinds of hosts (Atkins, 1926; Jones, 1977; Bierbaum and Ferson, 1986; Soong, 1997; Hamel et al., 1999; Hsueh, 2001; Asama and Yamaoka, 2009; Saeedi and Ardalan, 2010). There is usually no relationship in male pinnotherids between crab- and host-size because they are not permanent residents and small size, resulting from determinate growth, may be a prerequisite for a roaming life. Given the hazards of limb loss that may result from such a life style, however, determinate growth would come with a serious price tag. Other cases provide clear support for indeterminate growth: a study of *Arcotheres* sp. living in the bivalve *Barbatia virescens* (Reeve, 1844) provides unequivocal evidence of moulting by mature female and male crabs as well as a strong linear relationship with host size for females that does not plateau, but no relationship for males (Watanabe and Henmi, 2009). This pinnotherid clearly has indeterminate growth. Another pinnotherid with indeterminate growth is *Dissodactylus primitivus* Bouvier, 1917 that lives on spatangoid sea urchins (Pohle and Telford, 1982). Another family where determinate growth might be expected to have evolved is in the Cryptochiridae, which live in galls and cavities in corals. The male *Hapalocarcinus marsupialis* Stimpson, 1859 never reaches the same size as females because if they did they would be unable to enter the gall containing the captive female, but females may have several mature instars (Kotb and Hartnoll, 2002; Potts, 1915). Determinate growth may be a sexually dimorphic life history feature having evolved only in males.

Hard shell mating evolved once in the group with determinate growth (Majoids) and twice in the group with indeterminate growth (Xanthoids + Grapsoid–Ocypodoids). With mating linked to moulting one female usually only mates with one male who fertilizes all her eggs until she moults again, but when the female mates during the intermoult she can have several mates for each brood until she moults again and thereafter sperm retention elevates the level of sperm competition (Thompson and McLay, 2005). Compared to their reptant ancestors the evolution of hard-shell mating as well as a ventral seminal receptacle was a suite of changes that made these eubrachyurans unique and successful. They no longer needed male protection when they mated (i.e. post-moult), and they could mate with several males thereby increasing the genetic diversity of their offspring, indeed one might say that they became truly emancipated!

In order to do this they had to evolve a new chemical signal, indicating mature ovaries, to attract males since moult hormones were no longer involved. These ancestors radiated into the great diversity of crabs that we see today.

Courtship behaviour amongst eubrachyurans appears to have evolved in only two groups (Table 4): the Portunoid group and the Grapsoid–Ocypodoid group, although it is restricted to the Varunidae, Dotillidae, Ocypodidae and Macrophthalmidae and is probably the result of convergent evolution (Kitaura et al., 2002). It is widespread amongst other decapods and therefore it has evolved in different forms many times.

Mate attraction in crabs is mostly based on chemical signals emanating from females. These signals advertise imminent moulting or release of a brood of larvae. Urine-carried moulting hormone, ecdysone, ovarian signals (indicating readiness to spawn) or brood signals (indicating readiness to hatch), alert males in the vicinity to mating opportunities. The latter two signals are necessary when mating is not linked to moulting (see Table 4). Contrary to the common assumption that female crabs are always able to mate, many females are not attractive to males and so do not get any copulations (Brockerhoff and McLay, 2005b). Most, if not all, female crabs only have a short period of attractiveness when they are soft shelled or when they are hard-shelled and ready to spawn, signified by having mobile vulvar opercula for example, but outside this window they are ignored by males. Portunid and Cancroid males attend and guard females about to moult (e.g. species of *Callinectes* and *Cancer*, which have soft-shell mating, but dorsal and ventral receptacles respectively), but males in the Majoid group attend and guard females about to release their offspring. The only exceptions are the Xanthoids and Grapsoid–Ocypodoids where guarding is brief or non-existent. The only difference between these latter two groups is that they have dorsal and ventral receptacles, respectively.

Vaginal sperm plugs are a device used by males to minimize the number of partners a female can have. In minimizing sperm competition, almost all the advantage of the plug is to the male, not the female. Given that plugs are found in a small number of crabs belonging to six families (Portunidae, Cheiragonidae, Eriphiidae, Cancridae, Geryonidae and Goneplacidae) a likely scenario is that they evolved independently three times: in Portunoids, Eriphoids and Cancroids. Female vulva defence is in the form of hinged opercula which have evolved independently in Corystidae, Atelecyclidae as well as in the families of Grapsoidea and Ocypodoidea.

In summary, ancestral eubrachyuran reproductive characters included soft-shell mating, ecdysone male attraction and mate guarding. How these were incorporated into their reproductive strategies depended upon evolution of the seminal receptacles and hard-shell mating. There are eight combinations of growth, mating–moulting link and seminal receptacle structure (each with two alternative states) of which there are many examples of six of these strategies. The other two strategies may be represented by families not yet incorporated into our analysis because of lack of data. A working hypothesis, using the character hierarchy of growth format – receptacle structure – mating/moulting link, results in a tree that identifies the 6 groups as (1) Portunoids, (2) Majoids, (3) Eriphoids, (4) Xanthoids, (5) Cancroids and (6) Grapsoid–Ocypodoids. Vaginal sperm plugs deposited by males seem to have evolved independently three times in Portunoids, Eriphoids and Cancroids. The evolution of hard shell mating and ventral seminal receptacles were key innovations that contributed to eubrachyuran success.

4.4. Promiscuity and paternity

One key component of our hypothesis about the evolution of seminal receptacles is the benefit that females obtain-from having multiple males fertilizing their eggs. It is suggested that polyandry is selected for because it increases genetic diversity amongst progeny thereby raising fitness and it is assumed that there are no reliable pre-copulatory cues as to which male is genetically the best (Jennions and Petrie, 2000; Zeh and Zeh, 2001). Maternal genes would be combined with a greater array of paternal genes. This implies, however, a conflict between males and females. While it may be advantageous for females to combine their eggs with the sperm of as many males as possible this implies a great disadvantage for males for whom the best outcome is for their sperm (limited in quantity) to be the only ones that fertilize the eggs. The ideal situation for females is to have these sperm randomly mixed and for their donors to have equal shares of paternity, but this is far from ideal for a male. In order for a male to maximize his paternity he must try and ensure that his sperm are the only ones in the seminal receptacle or at least the only ones with access to the eggs. Except for primiparous females this is an almost impossible task if females can store sperm from previous mates, possibly over more than one mating season. The only tools available to the male are mate guarding and sperm displacement. A suggested female tactic to beat these male tactics is to release a substance into the seminal receptacle that causes spermatophore dehiscence, thereby promoting sperm mixing (Jennings et al., 2000). The two sexes have opposing strategies: for females the aim is to have as much sperm from as many males as possible, while for males the aim is to have his sperm occupy as much receptacle space as possible. The result is sexually antagonistic coevolution (Arnqvist and Nilsson, 2000).

For females to be promiscuous they must have prolonged periods when they are attractive to males. Storing sperm after spawning means that all crabs which survive to another breeding season must be promiscuous because it is unlikely they will mate again with the same male. Apart from paternity studies using microsatellite DNA (see below) our knowledge of promiscuity amongst female crabs comes from examining seminal receptacle contents of mated females or from observing the number of partners a female mates with. The first is the only practical method for crabs in the wild while the second can only be done with captive crabs, but may result in laboratory artifacts. The receptacles of wild caught *I. phalangium* have up to 8 sperm packets (Diesel, 1988, 1990); in *C. opilio* 3–5 sperm packets have been found (Urbani et al., 1998); and in *P. gigas* (Menippidae) X-ray examination reveal up to 3 ejaculates (Gardner et al., 1998). Mating observations show that *M. magister* (as *Cancer*) females mate up to 9 times (Jensen et al., 1996), while multiple partners have also been observed for *M. gracilis* (as *Cancer*) (Orensanz et al., 1995) and *C. borealis* (Elner et al., 1985). Like *P. gigas*, other menippid females such as *M. mercenaria* and *M. adina* Williams and Felder, 1986 can also have 2–3 mates (Wilber, 1989). Captive *D. sayi* (as *Panopeus*) females had on average at least two partners (Swartz, 1978). In the laboratory *Heterozius rotundifrons* (Belliidae) females can have several mates because they remain attractive to males longer than the period of post-copulatory guarding by males (Thompson and McLay, 2005). In fiddler crabs (*Uca* spp.) promiscuity is well known (Nakasone et al., 1983; Henmi et al., 1993; Nakasone and Murai, 1998). In *H. hirtipes* (as *Macrophthalmus*) (Macrophthalmidae) Jennings et al. (2000) found that under laboratory conditions females mated an average of 7 ± 2.5 times (maximum 19 times). The average duration of the period of operculum mobility during the intermoult was 11.35 + 1.35 days and females are only attractive to males at this time. Seminal receptacle weights of laboratory-mated and field-mated females in relation to CW showed good correspondence and calibration suggested that the number of mates in the field may well be similar to the number observed in captive crabs (i.e. about 7). Brockerhoff and McLay (2005b, Table 3) summarize data about multiple mating in Grapsoids and show that promiscuity is widespread amongst these crabs (*A. pisonii*, *Austrohelice crassa* (as *Helice*), *Cyclograpsus lavauxi*, *H. crenulatus*, *H. sexdentatus*, *H. oregonensis* (Dana, 1851), *Pachygrapsus crassipes* Randall, 1840, *P. transversus* (Gibbes, 1850), *Armases cinereum* (Bosc, 1802) (as *Sesarma*), *Sesarma reticulatum* (Say, 1817)). Clearly both storage and mating with multiple partners contribute to the diversity of sperm conserved by female crabs: copulation is one thing but fertilization and paternity is another.

There have been surprisingly few studies of paternity in eubrachyurans and these investigations often only involve small numbers of ovigerous females. The technique involves sampling female microsatellite DNA from multiple loci, comparing it with that obtained from fertilized eggs and assuming that any alleles present in the eggs that are not of maternal origin must have come from males (see e.g. Gosselin et al., 2005). The number of alleles equals the number of males who fertilized at least some of the eggs, although it does not necessarily equal the number copulation partners, which could be more. Given small sample sizes it is much easier to rule out genetic monogamy by finding extra microsatellite alleles, than it is to rule out multiple paternity where absolute certainty would only be given by 100% sampling (McKeown and Shaw, 2008). Studies of the layered contents of seminal receptacles, based on a total 91 females in the snow crab (*Chioneocetes opilio*), showed that 88% had mated with two to six males, but the proportion of broods expressing multiple paternity was very small (about 4%) because sperm from one male probably had precedence over other sperm (Urbani et al., 1998). In such studies it is not clear whether all the layers of sperm were present at the time of fertilization or whether some were obtained later while the females were ovigerous. McKeown and Shaw (2008) did not find any mixed paternity broods amongst 18 ovigerous *Cancer pagurus*, which is probably not surprising given the Cancroid mating behaviour (see Table 4). Definitive estimates of the frequency of multiple paternity broods in eubrachyurans are still to be made. Unfortunately, there are no estimates available for species in the Xanthoid and Grapsoid–Ocypodoid groups (see Table 4) where sperm mixing is more likely. Paternity needs to be particularly investigated especially in those species where sperm in the seminal receptacle is neither layered nor packaged in spermatophores so that there is a real chance of ejaculates from different males being mixed (see e.g. Jennings et al., 2000 on the macrophthalmid *H. hirtipes* (as *Macrophthalmus*)).

4.5. Implications of the seminal receptacle hypothesis in the phylogeny of Brachyura

Sperm storage by females in Brachyura is either in spermathecae ("podotremes") or in seminal receptacles (Eubrachyura) (see Fig. 7). Since the podotreme sperm storage is separate from the oviduct the gametes must meet outside the body of the female, in the abdominal chamber, so that fertilization is external. One essential question is how the release of the sperm from the spermathecae (whose entrances are often sealed by hardened ejaculate, see for, e.g. the Dromiidae, McLay, 1999, p. 455; 2001, p. 96) is coordinated with the release of the eggs through the gonopores. Eubrachyuran seminal receptacles are connected to the oviduct, meaning that fertilization is initiated internally (Guinot, 1977). However, most of the fertilization process actually occurs under the abdomen in both cases and they are not as different as they first appear. Eubrachyuran eggs do not have to be only fertilized internally because in *U. tangeri* (Eydoux, 1835) mature eggs from the ovaries and sperm from the seminal receptacle can be mixed in seawater and still result in successful fertilization (Medina, 1992). Thus there is no essential difference in the biochemical conditions required for fertilization and, of course, both undergo embryonic development in sea water.

The chief evolutionary advance made by the Eubrachyura was reproductive autonomy: females no longer needed an attendant male to reproduce. The possibility that seminal receptacles somehow evolved from spermathecae seems unlikely because this alternative means of sperm storage had no connection with the oviduct. Even if the spermatheca, as in dromiids such as *Lauridromia intermedia* (Laurie, 1906), where the entrance is near the coxae of the third perepods, were considered as a possible ancestral state, there seems little utility in hypothesizing that the oviduct somehow became linked to the spermatheca aperture because that would mean that sperm would still be stored some distance posteriorly in sacs between sternites seven and eight. Too many radical changes need to be assumed in order to derive seminal receptacles from the spermathecae of podotreme crabs that would have had to "capture the oviducts" in order to combine sperm storage and fertilization (Hartnoll, 1979; Guinot and Quenette, 2005). We agree with Guinot and Quenette (2005, p. 332) who stated that "the pathways of change for the female condition are unknown, and there is no evidence for a transition between the podotreme and eubrachyuran organization". A simpler hypothesis holds that the most important change was the connection of the oviduct to a sternal chamber and the consequent elaboration of seminal receptacles. The advantage offered by seminal receptacles was trans-moult sperm retention: podotremes have to mate after every moult because the spermathecal contents are lost (McLay, 1982).

The argument that "Podotremata" are paraphyletic and that Cyclodorippoida are the sister group of Eubrachyura (Ahyong et al., 2007; Scholtz and McLay, 2009) results in a number of difficulties. Regardless of which of the podotreme groups are regarded as sister to the Eubrachyura the hypothetical ancestor would have had spermathecae. So not only the origin of seminal receptacles needs to be explained, but the loss of the spermathecae must be explained. The alternative is that the "Podotremata" is indeed monophyletic (all having spermathecae) and that Eubrachyura shared a common ancestor with them, which had no means of sperm storage (see discussion in Tavares, 2003). Regarding reproduction, the most parsimonious evolutionary scenario is that these two groups found two different solutions to the same problem of sperm storage: spermathecae and seminal receptacles. The common ancestor had already begun to evolve carcinization, but at least some of the crab-like features shared by podotremes and eubrachyurans are the result of parallel evolution.

There can be little doubt that Eubrachyura is a monophyletic group as both the morphology (Guinot and Tavares, 2001) and molecular evidence (Ahyong et al., 2007) agree on this point, but what is missing is how this came about. It seems that simply establishing who belongs in which family, super-family, etc., really avoids the most important question needing an answer: an explanation of how the two crab groups evolved and what were the selective forces that brought this about. We suggest that the seminal receptacles were the evolutionary arena where the selective forces operated. This is unfortunately only half the story of Brachyura because very little is known about the function of spermathecae and the mechanics of mating and fertilization in podotreme crabs, other than the fact that they store sperm in a different way and have no trans-moult sperm retention (McLay, 2001; Guinot and Quenette, 2005). Were the spermathecae the evolutionary arena for these crabs as well and if so then how did they evolve? The position of the spermathecal apertures has a much more radical effect on the structure of the sternum of podotremes than does the position of the seminal receptacle openings (i.e. vulvae) on the sternum of eubrachyurans. With the posterior thoracic sternites almost sub-dorsal, as a result of the use of the last two pairs of legs for camouflage carrying behaviour, the spermathecal openings had to move anteriorly in order for the male to be able to transfer sperm to the spermathecae (Tavares and Franco, 2004).

Guinot and Tavares (2001, Fig. 16) suggest a pre-Jurassic (or early Jurassic) origin for the Eubrachyurans from a common ancestor with "Podotremata". Podotreme fossils are known from the early Jurassic while the oldest eubrachyurans are Cretaceous. The earliest known crab with sternal female vulvae was described by Feldmann et al. (2008) from Montana: *Componocancer roberti* Feldmann et al., 2008 (Componocancridae) from shale strata of Albian, Early Cretaceous age. It already has a well carcinized body and the vulvae are quite large and close to the median body axis. This suggests that the Eubrachyura may have a much earlier origin than has been suggested.

5. Brachyuran diversity

If having multiple males fertilizing a female's progeny adds to the genetic diversity of the brood, then we might predict that taxonomic diversity would be higher in eubrachyurans, which can store sperm, than in "podotremes". Of the approximately 6800 species of Brachyura only approximately 380 (in 10 families) are "podotremes" while 6420 (in 83 families) belong to the Eubrachyura (Ng et al., 2008). A contributing factor to this almost 17-fold difference in diversity is the greater genetic variation that resulted from storage of sperm from multiple partners and trans-moult sperm retention. The Tertiary "crab-explosion" (Schweitzer and Feldmann, 2010) was the result of greater genetic variation in progeny of eubrachyuran females compared to "podotremes" because more variation results in faster evolution.

6. Conclusions

1. The essential stages in the evolution of the seminal receptacles in Eubrachyura were linked to carcinization (evolution of the crab-like body form) and include the appearance of sternal gonopores and development of an external chitin-lined sperm storage chamber around the gonopore, which opened into the chamber dorsally. These changes meant that the site of fertilization changed from external to internal and the gonopore became internalized. The vulvae are secondary openings which are conduits for both sperm and eggs.
2. Two groups within the Eubrachyura differ in the derivation of their seminal receptacles: one group increased storage capacity by symmetrical growth of mesoderm around the oviduct entrance giving rise to dorsal-type receptacles;

in the second group asymmetrical growth meant that the chitinous lining was reduced and accompanied by ventral migration of the oviduct connection (original gonopore) towards the vagina giving rise to ventral-type receptacles.

3. To deposit sperm in a storage chamber, males needed tubular first gonopods working in concert with the second gonopods, which propelled the sperm into the seminal receptacle. The aim of males must be to deposit their sperm as close as possible to where the oviduct enters the seminal receptacle.
4. Reduction of the chitinous lining allowed trans-moult sperm retention and accumulation of sperm from multiple partners.
5. In a sample of 117 species from 31 families of eubrachyurans only 29% have a dorsal receptacle but 65.8% have a ventral receptacle, with 5.2% having intermediate connections.
6. The hypothesis proposed to explain evolution of the seminal receptacles is based on the assumption that only three characters, affecting crab mating strategies, are important: (a) whether growth is indeterminate or determinate, (b) the linkage between mating and moulting and (c) the structure of the seminal receptacle. In evolutionary terms, these variables were probably linked rather than independent. Of the eight possible character combinations six are realized: (a) Portunoids with determinate growth (DG) + soft-shell mating (SM) + dorsal seminal receptacles (DSR); (b) Majoids that have DG + hard-shell mating (HM) + ventral seminal receptacles (VSR); (c) Eriphioids that have indeterminate growth (IG) + SM + DSR; (d) Xanthoids that have IG + HM + DSR; (e) Cancroids that have IG + SM + VSR; and (f) Grapsoid–Ocypodoids that have IG + HM + VSR. Sperm competition is probably more common in the Majoids, Xanthoids and Grapsoid–Ocypodoids than in the other groups.
7. The phylogenetic status of these characters is that indeterminate growth and soft-shell mating are ancestral to all Brachyura, seminal receptacles are an apomorphy of the Eubrachyura, dorsal seminal receptacles essentially retain the ancestral state while ventral seminal receptacles and hard-shell mating are derived. A race to be first became a race to be last because females evolved seminal receptacles to store sperm, and the male who came last fertilized the most offspring. Hard-shell mating and ventral seminal receptacles evolved more than once.
8. Spermathecae and seminal receptacles are independent solutions to the same problem – sperm conservation and storage. They are strong apomorphies of their respective groups: “Podotremata” and the Eubrachyura which shared a common ancestor. Some crab-like characters evolved independently in these two groups.
9. Very important items on future research agendas must be sperm storage and sperm mixing in the seminal receptacle, as well as sperm loss at moulting and detailed studies of brood paternity. We need a model of sperm storage (packaged as spermatophores or free) and the dynamics of fertilization, with estimates of the parameter values involved. In addition we need to analyse variation in the structure of the male gonopods and better understand how sperm is transferred to females with different seminal receptacles.

Acknowledgements

We would like to thank Peter Ng, National University of Singapore and Richard Hartnoll, Port Erin, University of Liverpool for their helpful advice as well as all the people who have worked on crab moulting, mating and reproduction whose work we have drawn together in this paper. In particular we have gained many valuable insights into crab behaviour from the work of Annette Brockerhoff. We are grateful to Thomas Steinke who translated our rough sketches into precise mathematical formulae and Matt Walters who took the some of the seminal receptacle photos. We also thank Rudi Diesel who planted the seeds of many of the ideas presented here, but we take responsibility for what these seeds have blossomed into. Peter Castro, California State Polytechnic University, made many helpful suggestions that improved the presentation of our ideas.

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