CRUSTACEAN SEXUAL BIOLOGY

Crustacean Sexual Biology

EDITED BY
RAYMOND T. BAUER AND
JOEL W. MARTIN

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PREFACE

Crustaceans are a diverse assemblage of animals that includes not only the well-known shrimps, lobsters, and crabs but also a variety of less familiar but biologically important groups. Inhabiting primarily aquatic habitats, crustaceans might be considered the marine equivalent of their overwhelmingly terrestrial brethren, the insects. However, knowledge about the sexual biology of crustaceans is slight in comparison to what is known for insects and many other animal groups. In spite of the importance of selection pressures related to successful mating on the morphology, behavior, life histories, and phylogeny of crustaceans, many aspects of sex attraction, mating behavior, and insemination remain poorly known. A review of the literature on crustacean reproduction revealed that there was no comprehensive treatment of crustacean sexual biology but that in recent years much new exciting work has been (and is being) done in this area. Accordingly, we organized a symposium titled "Sex Attraction, Mating Behavior, and Insemination in Crustacea," cosponsored by the Crustacean Society and the Invertebrate Zoology Division of the American Society of Zoologists, with travel

funds for participants provided by the Systematic Biology program of the National Science Foundation (Grant BSR 8806676). The symposium, which took place on December 30, 1988, served as a public forum and basis for this volume. We also solicited chapters from authorities on certain taxa or subject areas in order to round out the volume's coverage of crustacean sexual biology.

A series of entirely review articles on crustacean sexual biology, organized into chapters by taxa or by subject area, was not deemed desirable at this time, considering the lack of knowledge on the sexual habits and morphology of so many crustacean taxa. Instead, we asked our volume contributors, all active researchers in different areas of crustacean sexual biology, to strive to incorporate their recent research into a synthesis with past work in their field of interest. The chapters in this volume are loosely ordered from the topics of (1) sex attraction (bioluminescent displays in ostracods, chemical communication and sex recognition in crabs and amphipods), to (2) mating behavior and mating systems (stomatopods, isopods, anostracans, lobsters, brachyuran crabs, caridean shrimps), to (3) structure and function associated with insemination (penaeoid shrimps, rhizocephalan barnacles, isopods, copepods, remipedes, reptant decapods; chemical composition of decapod spermatophores; decapod sperm morphology). However, we have not formally arranged chapters into sections corresponding to these major subject areas because it is difficult in most cases to separate or to study these three aspects of sexual biology independently, given our present level of knowledge about crustacean reproduction. Thus there are varying degrees of overlap among these three topic areas in most chapters.

The details of sex attraction, mating behaviors, copulation, genitalia, spermatophores, and sperm structure are important not only for a basic understanding of processes and structures leading to insemination in crustaceans, but these basic data also serve as the raw material for proposing and testing hypotheses about various aspects of crustacean phylogeny and evolution. In several chapters, the question is asked "what is primitive or ancestral and what is derived or specialized" in terms of sexual morphologies or sexual systems. Other chapters deal with the evolution of mating systems and aspects of sexual selection, and one chapter deals specifically with sperm competition in crabs. Dramatic advances have been made in these areas of sexual biology by researchers using insect models, but development of the field is still in its infancy with regard to crustaceans. However, as with insect research, advances in these exciting theoretical areas must be preceded by basic studies on sex attraction, mating behavior, and functional morphology of copulation and insemination. We hope that the papers in this volume will stimulate the detailed work, couched in a broad evolutionary context, that is needed to heighten our understanding of crustacean sexual biology.

We owe a great deal of thanks to all the conscientious reviewers who contributed their time in making comments and suggestions on contributors' manuscripts. Our promise of anonymity prevents us from listing these reviewers individually. Our institutions (RTB: Center for Crustacean Research, University of Southwestern Louisiana; JWM: Natural History Museum of Los Angeles County) provided us with time, facilities, and travel funds associated with symposium and editorial activities. We thank Edward Lugenbeel of Columbia University

Press for assistance and advice at various points in the evolution of the volume. RTB thanks Adrian Wenner for helpful suggestions during the early stages of symposium and volume organization. Finally, we offer special thanks to Sue Martin and Lydia Bauer for supporting our efforts throughout this venture.

Raymond T. Bauer, Lafayette, Louisiana
Joel W. Martin, Los Angeles, California
November 1989

CRUSTACEAN SEXUAL BIOLOGY

ONE
Bioluminescent Displays, Courtship, and Reproduction in Ostracodes

JAMES G. MORIN and ANNE C. COHEN

Department of Biology,
University of California, Los Angeles, and
Division of Life Sciences,
Natural History Museum of Los Angeles County,
Los Angeles.

Abstract

This paper presents an overview of our recent work on courtship behavior in luminescent cypridinids and a summary of reproductive and mating patterns in ostracodes. The Ostracoda, as a group, is large, and there is much variation in their reproductive patterns, especially between the two major groups: the podocopans and the myodocopans. The review of the reproductive patterns includes a summary of their population biology, reproductive system morphology, sexual dimorphism, and mating behavior. Male cypridinids from over 50 species in the nominal genus *Vargula* use their bioluminescence in courtship to attract females for reproduction throughout the Caribbean. Males secrete species-specific luminescent displays as trains of light pulses above reef habitats in the early evening throughout the year. Details of the copulation are unknown. Species show high local diversity, high endemism, and limited geographic distribution. We propose that sexual selection on the luminescent courtship displays and poor dispersal are responsible for their apparent rapid speciation in the Caribbean.

OSTRACODES ARE generally small (most less than 3 mm), reproduce by copulation, have determinate growth, and have a fixed number of instars. The class is usually divided into two subclasses, the Myodocopa, with about 650 marine species, and the Podocopa, with about 7,000 species from marine, freshwater, and terrestrial habitats (table 1.1). Luminescence occurs only in some Cypridinidae within the Myodocopida, which is the focus of this paper, as well as in some Halocyprididae within the Halocyprida. Much is known about ostracode population biology, life history patterns, and reproductive morphology, but little is known about the actual copulation process and even less is known about precopulatory courtship. In this paper we (1) briefly summarize the reproductive patterns in ostracodes, especially myodocopids (see also Cohen & Morin 1990), (2) summarize the limited data available on precopulatory courtship not involving bioluminescence, (3) present an overview on our recent work on the precopulatory courtship patterns in one group of myodocopids, the cypridinids, where

TABLE 1.1. Classification and number of taxa of the Ostracoda, with emphasis on the Myodocopa.

SubCl	Myodocopa	~100 genera,	~600 spp
O	Myodocopida		
SubO	Myodocopina		
SuperF	Cypridinoidea		
F	Cypridinidae[a]	24 genera,	100 spp
SuperF	Cylindroleberidoidea		
F	Cylindroleberididae	27 genera,	100 spp
SuperF	Sarsielloidea		
F	Sarsiellidae	12 genera,	75 spp
F	Rutidermatidae	3 genera,	25 spp
F	Philomedidae	11 genera,	75 spp
O	Halocyprida		
SubO	Halocyprina		
SuperF	Halocypridoidea		
F	Halocyprididae[a]	7 genera,	150 spp
SuperF	Thaumatocypridoidea		
F	Thaumatocyprididae	3 genera,	10 spp
SubO	Cladocopina		
SuperF	Polycopoidea		
F	Polycopidae	4 genera,	40 spp
SubCl	Podocopa	~700 genera,	~7000 spp
O	Platycopida		
SubO	Platycopina		
SuperF	Cytherelloidea 1 family,	2 genera	
O	Podocopida		
SubO	Podocopina		
SuperF	Sigilloidea 1 family,	2 genera	
SuperF	Darwinuloidea 1 family,	2 genera	
SuperF	Bairdioidea 2 families,	11–15 genera	
SuperF	Cypridoidea 6 families,	164 genera	
SuperF	Cytheroidea 29 families,	500+ genera	

[a]Contains some luminescent species.

males use bioluminescence to attract females for mating, and (4) discuss the probable role of sexual selection in these mating systems.

REPRODUCTIVE PATTERNS

This section is a summary of a more extensive review of this topic by Cohen and Morin (1990). More specific references and information can be found in that paper.

Reproduction and Population Biology

Ostracodes generally reproduce sexually, but parthenogenesis is known in a few. Most Podocopa deposit eggs and most Myodocopa brood. Ostracode juveniles show gradual development, without metamorphosis, to a terminal adult stage. Depending on the taxon, there are 4–8 free-living juvenile instars. Total development time ranges from 16 days to over 3 years. Marine species tend to be perennial, and freshwater species tend to be annual. Seasonal reproduction is prevalent in species from habitats that are temporary, freshwater and/or subject to pronounced climatic changes. Nonseasonal reproduction is prevalent in at least a few species from less variable habitats (e.g., tropical, arctic, and antarctic waters). Resistant resting eggs occur in some species from habitats that are temporary or subject to severe winters. Sex ratios are biased toward adult females in many species.

Morphology Related to Reproduction

The male reproductive system, including the copulatory limbs, is usually paired. The copulatory limb (fig. 1.1A) is often large (figs. 1.1B, 1.2B, 1.2C, 1.2D), being as much as 35 percent of the body length (fig. 1.1B). Sperm are usually deposited in a seminal receptacle in the female (fig. 1.2E). Reproductive structures in females are generally paired, but the ovary-uterus system of some is separated from the vaginal-seminal receptacle system; in these cases mechanisms of sperm transfer are unknown. Myodocopids have the most simple reproductive system. In at least some male Cypridinidae, the gonads, vasa deferentia, seminal vesicles, and copulatory limbs are paired (fig. 1.2), but there is a single medial genital opening (penis) (fig. 1.1A). The copulatory limbs of myodocopids are taxonomically distinct (Cohen & Morin 1990) and may contribute to reproductive isolation. Copulatory organs of cypridinids show generic differences and, within some luminescent clades, specific differences as well. In addition, in some luminescent species, complex species-specific mating displays probably provide precopulatory reproductive isolation.

Sperm of some podocopids are among the largest in the animal kingdom, sometimes even longer than the animals themselves. Wingstrand (1988) described eight sperm types unique to higher ostracode taxa. Most myodocopids (Philomedidae, Rutidermatidae, Sarsiellidae, and Cypridinidae [which includes species that produce luminescent displays] but not Cylindroleberididae) have

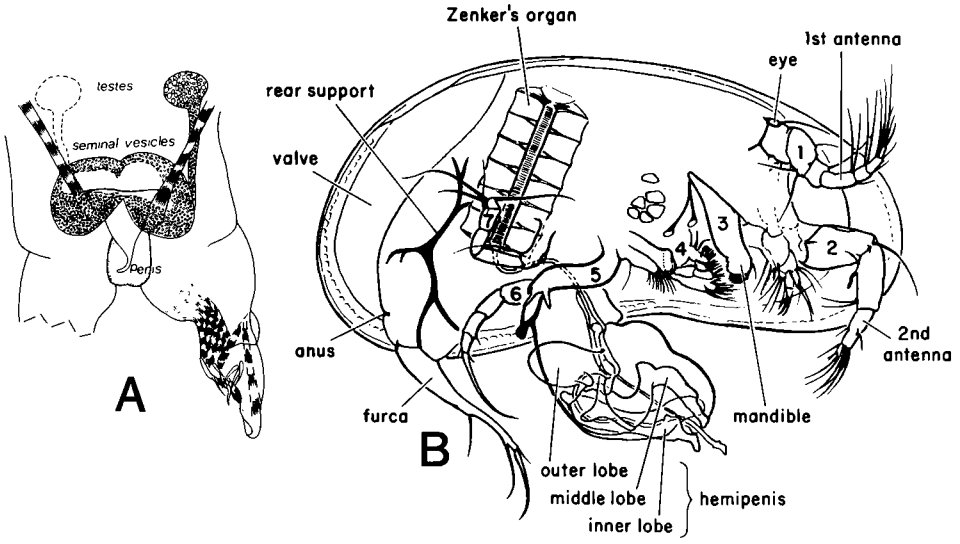

FIGURE 1.1. Male reproductive systems. A. Genitalia of *Spinacopia sandersi* (Myodocopa, Sarsiellidae): anterior view of testes, seminal vesicles, penis, and clasping limb (only left one shown). From Kornicker 1969. B. *Candona suburbana* (Podocopa, Cypridoidea) with right valve removed and left hemipenis fully erect, rotated, and in copulatory position. Outer face of hemipenis exposed to show the three lobes; limbs are numbered (1–7); sixth limb and palp of fifth limb are shown posterior to their position during copulation. Redrawn from McGregor and Kesling 1969a.

Sperm Type I (i.e., free mitochondria, a long crystalline perforatorium, and an enlarged and extended acrosomal vesicle) and a hardened spermatophore that is uniquely shaped by the external furrow on the female genital lobes during hardening (fig. 1.2E).

Most ostracodes exhibit sexual dimorphism. Sexual dimorphism generally correlates with courtship and mating, but corroborating behavioral observations are few. Lateral compound eyes, which occur only in the Myodocopida, are often larger and may have more ommatidia in males. Valve dimorphism is apparently associated with mating activities and/or brooding. Many males have some limbs specifically modified for clasping females, but clasping has been rarely observed. Suckerlike accessories occur on the first antennae of the Cypridinidae and some Cladocopina. Only in the cypridinid *Vargula hilgendorfii* have males been observed to use these suckers to grasp the female (Okada & Kato 1949). Sensory limbs (usually the first antennae) and swimming limbs (usually the second antennae) also may show dimorphism, being better developed in males. The sensory limbs may be used to find and/or court females. Finally, sexual dimorphism with uncertain function also occurs in the mandibles, fourth and fifth limbs, and furcae of many Myodocopida.

Mating Behavior

Copulation has been observed in only about 21 genera of podocopans and only three species of myodocopans. Six mating positions (two are shown in fig. 1.3)

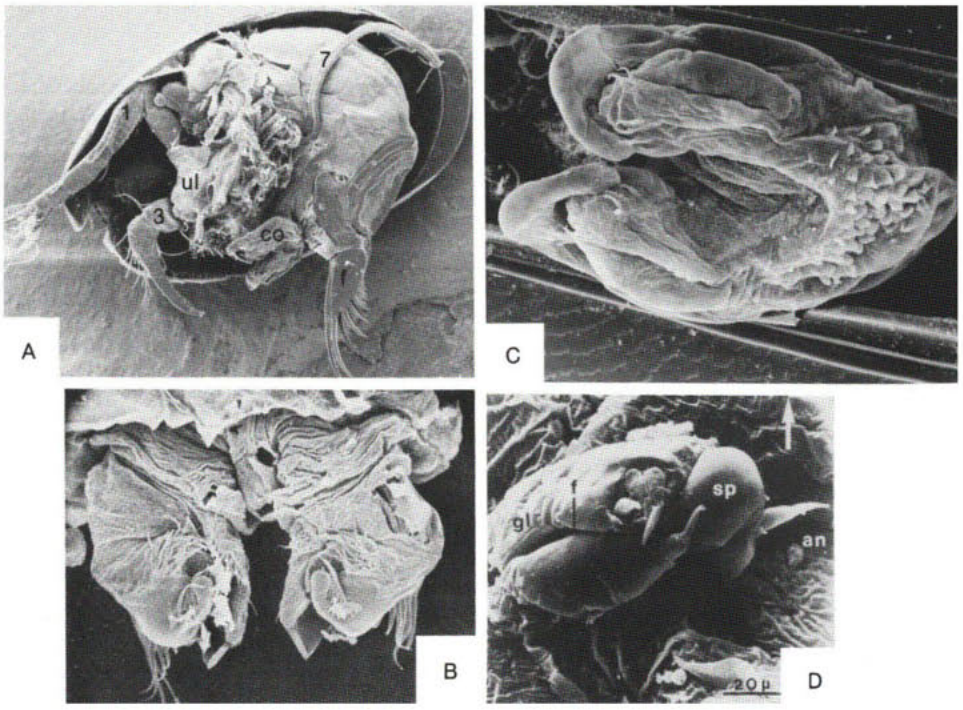

FIGURE 1.2. Scanning electronmicrographs of the reproductive limbs of cypridinid myodocopids. A. Whole lateral view of a male *Vargula kuna* (upward display type) with the left valve removed, limbs are numbered. co, copulatory limb; f, furca, ul, upper lip. B. Male copulatory limb of *Isocypridina quatuorsetae,* anterior view, from Kornicker 1975. C. Male copulatory limb of *V. contragula*. D. Female right genital lobe (gl) of *Vargula norvegica,* with spermatophore (sp) protruding from furrow (f), anterior direction shown by arrow. From Wingstrand 1988.

have been described (Cohen & Morin 1990). Copulation is rapid, usually no longer than a few seconds, but may take up to 30 mins (e.g., Okada & Kato 1949; McGregor & Kesling 1969b). Within the myodocopids mating has been observed in the cypridinid *Vargula hilgendorfii,* which copulated for more than 30 mins (Okada & Kato 1949), in the cypridinid *Cypridina dentata,* which copulated in minutes (Daniel & Jothinayagam 1979), and in two unidentified sarsiellids, which united for only a few seconds (Cohen personal observation). Behavioral observations suggest that females are monandrous with a single insemination, but a few are polyandrous, while males are polygynous, occasionally with multiple inseminations of one female (Cohen & Morin 1990).

Precopulatory courtship in which males locate or attract conspecific females must involve mechanical, chemical, or visual cues or any combination of these, if it occurs at all. Data on precopulatory behavior in the Ostracoda are sparse. In myodocopid males, structures used in searching for or attracting mates or both probably include the large and powerful second antennae used in swimming, the numerous and long sensory bristles on the first antennae, and the large eyes. We predict that precopulatory mechanical stimulation and chemical cues

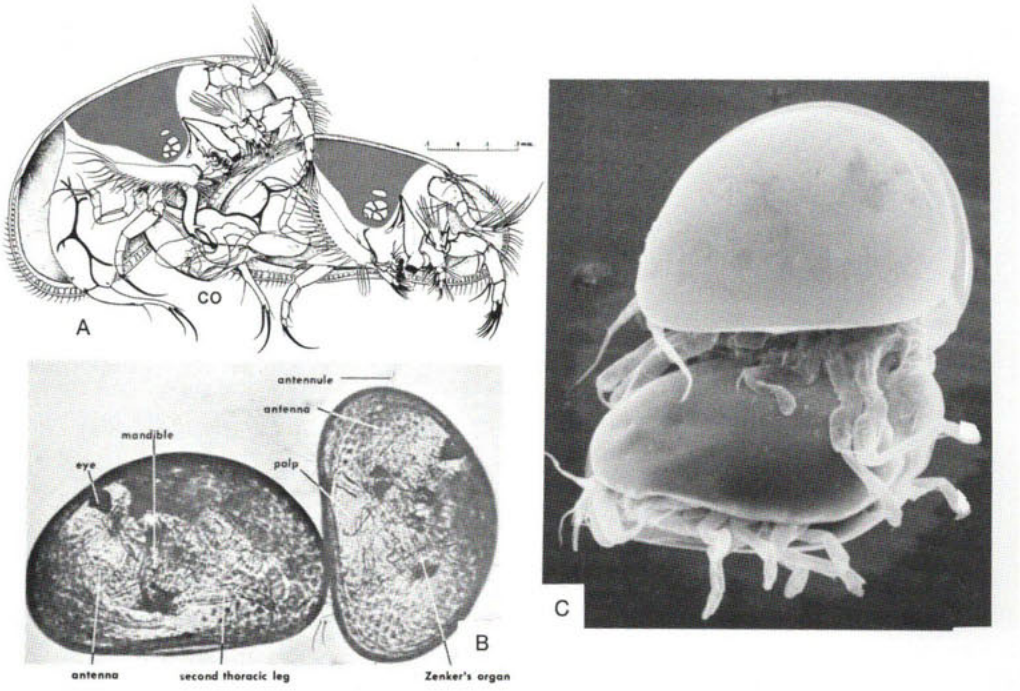

FIGURE 1.3. Copulation in some podocopids. A. *Candona suburbana* (a cypridoid) drawn with right valves removed and palp of right fifth limb of female withdrawn from proper position for clarity (see fig. 1.2B for identification of structures in male). CO, copulatory limb. B. *Cypria turneri* (a cypridoid) palps or claspers of male's fifth limbs released their hold on edges of female valves when killed so that the male slid and rotated slightly down on the female carapace. Both from McGregor & Kesling 1969b. C. Scanning electron micrograph of mating entocytherids (cytheroids). From Hart and Hart 1974.

(pheromones) will prove to be a common component of ostracode courtship. Luminescent courtship displays in some cypridinids clearly involve visual cues (see below). Wherever dimorphism occurs in the size or the number of ommatidia of the lateral eyes, visual cues are probably important in courtship.

BIOLUMINESCENCE AND COURTSHIP

Bioluminescence

Luminescence is known only from some species of halocyprids and cypridinids within the Myodocopa (Herring 1978), but both groups also contain many nonluminous species. Luminescent ostracodes are strictly marine and are known from all latitudes, oceans, and depths (to at least 3,400 m) and from pelagic, demersal, and benthic habitats. In general, luminescent halocyprids tend to be pelagic while cypridinids tend to be demersal or benthic. Chemically, halocyprid and cypridinid luminescence appear to be distinct from one another (P. Herring

personal communication), perhaps indicating an independent evolution of the two light-emitting systems. This possibility is further suggested by morphology: the light-emitting tissues in luminescent halocyprids are found widely distributed in the valves (Angel 1972), while luminescent cypridinids have a light organ in the upper lip (fig. 1.2A) from which luminescence is secreted outside the body (e.g., Morin 1986). Luminescent cypridinids have well-developed lateral eyes while the halocyprids are eyeless. Bioluminescence apparently serves as a mate attractant for some species of cypridinids, particularly within the greater Caribbean area (Morin 1986). In the Podocopa, none of which luminesce, relatively more is known about copulation events but little is known about precopulatory activities (courtship). The reverse is true for many of the luminescent myodocopans. Except for reports on the benthic/demersal *Vargula hilgendorfii* (Okada & Kato 1949) and the pelagic swarming *Cypridina dentata* (Daniel & Jothinayagam 1979), copulation has not been observed in luminescent species. Male swarming at the sea surface has been reported in a halocyprid (Angel 1972) and myodocopid (Daniel & Jothinayagam 1979). In fact, ostracodes are almost certainly responsible, at least in part, for the massive luminescent displays known to occur in the Indo-West Pacific (Kelly & Tett 1978; Herring & Horsman 1985); these are likely to be some sort of mating swarms. The waves of oceanic luminescence (Herring & Horsman 1985) could be visually coordinated entrainment phenomena similar to those observed in cypridinid reef displays (Morin 1986; see below). Since the early 1980s when the functions of luminescence were first precisely elucidated in cypridinids (Morin & Bermingham 1980), luminescent courtship displays have now been documented for over 50 species of cypridinids from the Caribbean (Morin 1986; Cohen & Morin 1986, 1989, in press; Morin & Cohen 1988).

Life Histories and Activity Patterns of Luminescent Cypridinids

Cypridinid species that produce luminescent courtship displays in the Caribbean Sea have fairly similar life histories and diel activity patterns. All appear to be primarily nonplanktonic species with distinct microhabitat preferences. Based on population and rearing data from a few of these species (Morin unpublished) and comparisons with other (some nonluminescent) cypridinids (Morin & Bermingham 1980; Morin 1986; Cohen & Morin 1986, 1989; Cohen 1983; Morin & Cohen 1988), Caribbean ostracodes with luminescent displays appear to share the following life history features: (1) males deposit a spermatophore that hardens in the genital furrow of the female (details of copulation are unknown); (2) sperm stored in the spermatophore fertilize the eggs as they are extruded and deposited in the brood pouch; (3) females brood from 12 to 48 eggs at a time; (4) there seems to be no brood overlap, and all embryos are of the same age and develop synchronously; (5) juveniles are released as benthic, crawl-away first stage instars, thus there is no planktonic stage anywhere in the life cycle; (6) new eggs are deposited in the brood pouch shortly after the previous brood is released (2–9 days in the nonluminescent *Skogsbergia lerneri* [Cohen 1983]); (7) embryonic development time is 2–4 weeks, and there are 5 juvenile instars and a terminal adult stage; (8) all juvenile and adult stages are capable of luminescing, but only

males produce mating signals; (9) sexual characteristics become evident by the last (A-1 = fifth) juvenile stage; (10) from limited data, sex ratios of broods appear to be about equal; (11) total development time of the 5 juvenile instars is 2–3.5 months; (12) there appears to be no seasonality in their life cycles, and all stages of the life cycle are present year round; (13) adult males appear to be able to copulate many times while females may be sexually receptive and attentive to male signals only after releasing a brood or for only a brief time after their terminal molt to adulthood, at which time they copulate for the only time during their life (see below); (14) more than one brood may be produced from sperm stored during a single copulation (3 broods have been so produced in *Skogsbergia lerneri* [Cohen 1983]); (15) adults live for at least 2–6 months, and philomedid myodocopids (nontropical) live up to 3 years (Elofson 1941); (16) sexual dimorphism in secondary characteristics is distinct in all species: males have smaller and more elongate valves but larger eyes than females, and males have suckers on their first antennae for grasping the female; (17) mating displays by males are restricted to a specific period of the early evening, most often from the end of twilight for about an hour; and (18) this is the only time that males are in the water column. As details of reproduction and life histories of the various species become known, some variations in these traits will probably be found, but the overall patterns should remain.

Luminescent Courtship Displays and Sexual Selection

Nearly every evening of the year across the entire Caribbean, toward the end of twilight, shallow water cypridinid males leave their benthic haunts, enter the water column, and become demersal plankters for about an hour as they attempt to attract and mate with females. Each species produces its own stereotyped luminescence that is spatially and temporally predictable (fig. 1.4). In all cases each luminescent pulse is a discrete extracellular secretion, from glands in the upper lip, that is left behind in a stationary position (relative to the surrounding water) while the male swims rapidly onward (Morin 1986). A series of such light pulses produces a species-specific train of glowing spots in the water column. Predation is minimal on the signalers during the displays, apparently because, by doing so, the predator puts itself in danger of being consumed by its own predators who cue in on the luminescent cloud that the ostracode produces when attacked (Morin 1986).

Intramale and male-female associations appear to be similar in all signaling species. If a fine meshed net is swept through any of these displays, there are often nonsignaling males of the same species caught with the signaler; these appear to be "silent" satellite males that assemble with the signaler presumably in an attempt to sneak a copulation should a female be attracted to the display. Shining a light along the path of the display often reveals several males within a few millimeters of one another and swimming rapidly together. Only rarely are females caught in our nets. These are invariably ovigerous females without broods. Similarly, plankton tows through the display area during the display period yield high numbers of males and very few females. Juvenile stages or brooding females are almost never caught above the bottom. Furthermore, tows

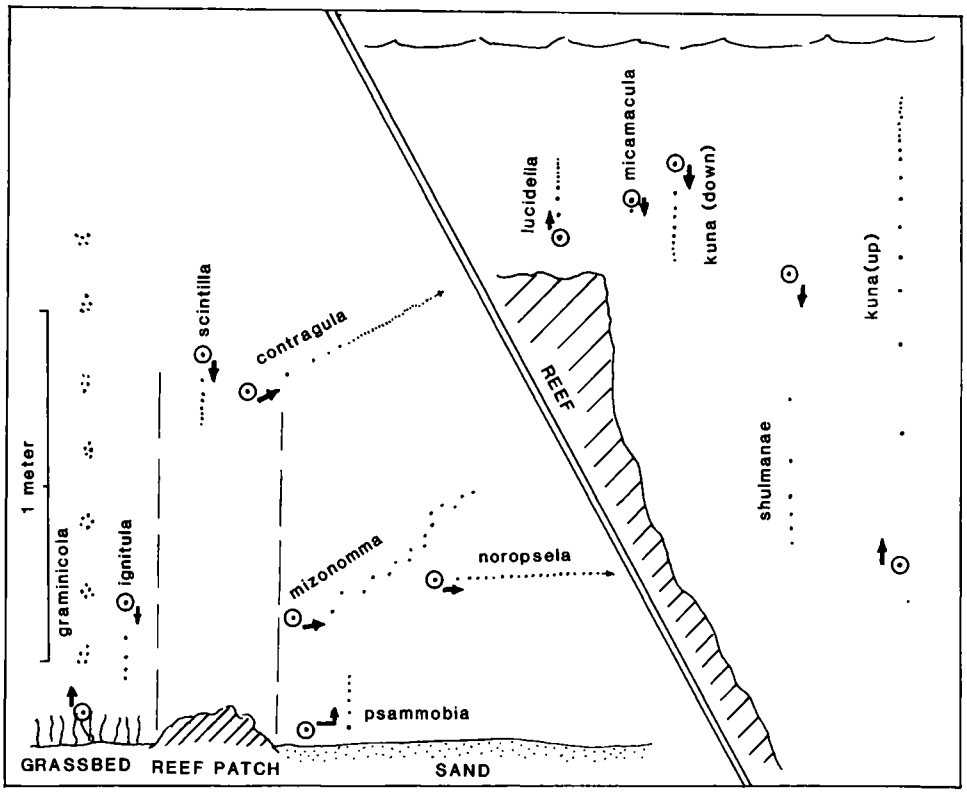

FIGURE 1.4. Spatial representation (with approximate position relative to a general habitat) of the courtship display pattern of 11 luminescent species of Caribbean cypridinid ostracodes in the nominal genus *Vargula* from Panama (see field key in Cohen & Morin 1989). Circled dot indicates first pulse in train; heavy arrow indicates direction of train; thin arrow indicates that the train continues (only in *V. noropsela* and *V. contragula*).

taken during the day or at other times of the night when they are not displaying collect few, if any, individuals of any instar or either sex. Thus during the period of luminescent displays within the water column there is a sex ratio that is strongly biased toward males.

During numerous hours of observations we have been unable to induce newly molted, virgin (nonbrooding) females to copulate with conspecific males in lighted (or unlighted) dishes in the lab, either during the day or night. They cannot be closely observed in the dark in the field. Thus, despite numerous attempts, copulation has not been observed in any of these species. We surmise that, unless conditions are appropriate (i.e., it is dark enough, the proper luminescent pattern exists, they are in the water column and not on the bottom, the female is receptive, etc.), individuals generally do not copulate.

Nonetheless, based on what is known about the life cycle of these and other related species (as indicated above) combined with the information about display activities (see below), we conclude that males are actively competing with

numerous other males for copulations with females, which are only sexually receptive for a short time following their final molt to adulthood. In those species that produce luminescent displays, it is during this particular display period that males enter the courtship arena of the water column above their daytime haunts and call, using their species-specific codes, for newly molted virgin females. Once impregnated, the female is no longer sexually receptive, returns to the benthos (presumably after only a few minutes in the water column to mate), and begins the brooding process. From this one impregnation she can produce at least one brood and perhaps several. We further hypothesize that the hardened spermatophore in these and many other myodocopids may serve as a "mating plug" to prevent subsequent male impregnations. Thus she may mate only once in her life (however, at this time we lack direct evidence for this). Regardless of the number of broods produced per mating, the net effect is to skew the operational sex ratio of receptive individuals toward males, even though the actual sex ratio of the population may be about equal (i.e., the number of receptive [=all] males is about equal to the number of receptive females *plus* unreceptive females). The more broods produced by a female from a single mating, the greater will be the operational bias toward males. The operational sex ratio may be skewed further toward males if, as appears to be the case, the relatively few receptive (virgin) females are in the water column only long enough to mate, while the much more numerous and always receptive males are in the water column signaling for a much longer time (e.g., about an hour). Since we rarely find females in the vicinity of the displays, this suggests to us that females are in the water column for only a very short period of time *and* that they mate once or, at most, only a few times during their lives. A major effect of this kind of mating system is to increase the competition among males for copulations and increase the variance among males for copulation success; some males may mate often, while others seldom or never. Each female, on the other hand, would appear to have ready access to several males displaying above her. Thus variance among females for copulations should be small (all will be mated) and each has the *potential* to choose among several males. Whether females do exert a choice is not yet known, but the possibility is there. All of this adds up to a classic situation of sexual selection: many males are competing for few (choosy?) females and at least part of that competition is expressed via their luminescent displays. The basic components of this mating system are analogous to the well-known sexual selection-driven systems in many insects (see, e.g., Thornhill & Alcock 1983: Kaneshiro 1983), amphibians (e.g., Gerhardt 1982), birds (e.g., Mortin 1975), leks of various sorts (e.g., Bradbury and Gibson 1983), and other animals.

From a consideration of the biological patterns of cypridinids given above and an analysis of the luminescent displays, we hypothesize that the mating systems of these luminescent ostracodes have evolved as a result of sexual selection (both intrasexual and epigamic). Cypridinids that produce luminescent displays show the following traits that are considered evidence for sexual selection (see, e.g., Bateson 1983; Thornhill & Alcock 1983; Partridge & Halliday 1984): (1) apparent polygyny (males attempt to mate repeatedly while females do so once [or infrequently]), (2) sexual dimorphism, (3) differential investment

and success in mating (males invest more in mating and show a greater variance in mating success than females), (4) differential investment in parental care (males invest little while female investment is significant), (5) the production of conspicuous mate attraction signals with particular locations and times for the displays, and (6) an operational sex ratio skewed toward males. We further hypothesize that sexual selection along with the poor dispersal capabilities at all life stages of each species has been an important factor in the sexual isolation and rapid speciation that has apparently occurred within this group in the Caribbean.

Variations in the Luminescent Display Patterns

Three major display patterns have been observed and recorded from Caribbean reef systems (Morin 1986): (1) *vertical shortening displays*, (2) *even lateral displays*, and (3) *rapid flashing displays* (fig. 1.4).

Vertical Shortening Displays. The mating display of *Vargula lucidella* from the San Blas Islands of Panama (Cohen & Morin 1989) is representative of the vertical shortening display (fig. 1.4). If the moon is not above the horizon at sunset, displays commence 44 mins after sunset (predictably within 2 min of this time), near the very end of twilight, and *only* over the shallow reef crests associated with the corals *Millepora complanata* and *Acropora palmata* at water depths of 0–2 m. The display period lasts for 45–60 mins and then ceases. Each display proceeds upward from within 5–25 cm of the reef. The display train, which is secreted by one male swimming rapidly (ca. 4–7 cm/sec), consists of a row of 4–5 (occasionally up to 7) bright points of light, each of which lasts for about 3–4 sec, secreted progressively closer together. The initial 2 pulses are about 6 cm apart and the last two about 4.5 cm apart. Occasionally this initial shortening phase is followed by a "trill" of 7–15 dim pulses, which each last about 1 sec and are spaced about 1 cm apart. The total train lasts 9–12 secs and is about 25 cm in total length (or 35 cm if there is a trill). Densities of trains over the reef are often quite high, up to about 5 displays/m^2. Displays entrain with one another so that they coincide temporally on about a 30–45 sec cycle. That is, the light from one signaling male will stimulate neighboring males to commence signaling at the same time, and they terminate at about the same time. Thus a wave of luminescent trains sweeps over the reef, remains for 10–15 secs, and is then followed by a 20–30 sec period of darkness before the next wave commences.

About two-thirds of the displaying species from the Caribbean show specific patterns that are variations of this vertical shortening display type (Morin 1986). Examples of extreme variations in vertical displays in species from the San Blas Islands of Panama (fig 1.4) include: (1) downward (e.g., *Vargula ignitula*) versus upward displays, (2) very long trains (up to 2.5 m) with widely spaced pulses (e.g., *V. kuna*) to very short trains (ca. 2–6 cm) with few, close pulses (e.g., *V. micamacula*), (3) long duration pulses (up to 11 sec) (e.g., *V. scintilla*) to short (2–3 sec) (e.g., *Vargula ignitula*), (4) many pulses per train (e.g., *V. kuna*) to few (e.g., *V. micamacula*), (5) strong entrainment between displaying males (e.g., *V. mica-*

macula) to very little (e.g., *Vargula psammobia*); (6) long display periods (e.g. *V. psammobia*) to quite short (e.g., *Vargula kuna*). All of these specific behavioral characters can be used to identify and separate the species (Cohen & Morin 1989).

Even Lateral Displays. The mating display of *Vargula noropsela* from the San Blas Islands of Panama (Cohen & Morin 1989) is representative of the even lateral display (fig 1.4). These displays generally occur 30–100 cm above the edges of the sand-coral interfaces in spur and groove regions of coral slopes at water depths of 4–22 m. Displays commence 35–45 mins postsunset and continue for about 45 mins. The displays are lateral, either horizontal to the sand slope or slightly oblique to it. The display train from a male swimming about 3.5 cm/sec is usually many meters long, continues for many seconds, and consists of hundreds of evenly spaced (about 2 cm) pulses, each with a duration of about 2–3 secs. As new pulses are produced and older ones (5 to 10 pulses behind) fade, it gives a visual effect reminiscent of the vapor trail of a jet. Frequently 2 to 4 or more other males will begin to signal beside (about 2 cm) and in register with the initiator and then gradually diverge from him in the horizontal plane. In addition, "silent" males also often accompany the signaling males. Densities may be locally high but are generally low (6–8 displays at any one time per sand groove [ca. 3 by 8–10 m]), and there is usually weak cycling between a minute or so of displays followed by several minutes of no activity. About one-quarter of the displaying species from the Caribbean show variations of this even lateral display type (Morin 1986). Most of the species of ostracodes that produce even lateral displays are found near and display along horizontal types of habitat relief (edges of sand patches or grooves being the most common). Species with even lateral displays are found in only one of the major clades within the luminescent signaling species in the Caribbean (Cohen & Morin, in press; see below).

Rapid Flashing Displays. The mating display of *Vargula graminicola* from the San Blas Islands of Panama (Cohen & Morin 1986) is representative of the rapid flashing display (fig. 1.4). These displays occur over seagrass beds at water depths of 3–10 m. Displays commence about 45 min after sunset and continue most of the night. These remarkable displays are a series of rapid *group* flashes placed vertically upward. Each display begins within the grass bed itself as a single bright flash with a duration of about 1 sec. The second and succeeding pulses are accompanied by 2 to 40 flashes from other *non*-silent males in a pack, swimming within a few mm of each other and the leader. Swim speeds are rapid, about 10 cm/sec (or about 60 body lengths per second!). Each cluster of pulses is 15–25 cm above the previous ones, all have a duration of about 1 sec, there are about 8–12 pulse clusters per train, and each train may be as long as 2.5 m. Display densities can be very high, up to about 20 displays/m^2, and there is loose entrainment at about 60 sec intervals. The effect is dramatic: huge numbers of tight clusters of brief flashes repeatedly pulsing upward toward the sea surface in approximate synchrony with other clusters across vast expanses of grass beds. About 10 percent of the signaling species are "flashers," and most

areas of the western Caribbean have one or two species that show variations of this rapid flashing. Major variations occur in the duration of the flash (some are as short as 0.250 sec), number of flashes per train, number of males signaling, and degree of entrainment. Species occur in a variety of habitats ranging from grass beds to coral reefs. Flashers are restricted to one clade within the luminescent signalers in the Caribbean (Cohen & Morin in press; see below).

Systematics and Biogeography of Ostracodes with Luminescent Displays

All of the signaling species we have discovered in the Caribbean belong to the nominal genus *Vargula*. However, our preliminary cladistic analysis, based on morphology, indicates that the genus is polyphyletic (Cohen & Morin, in press). The analysis also indicates that all the Caribbean species belong to 2 distinct monophyletic groups (clades A [with about 44 species] and F [with about 11 species]) that are distantly related. Vertical shortening displays occur in both clades while even lateral displays occur only in clade A and rapid flashing displays only in clade F. These differences infer that vertical shortening displays are the more ancestral state and evolved before the two clades diverged, while the other two signal types are more derived and evolved subsequent to the divergence in each of the two branches.

Each of the more than 50 species of signaling ostracodes we have discovered shows a high degree of habitat specificity and an apparent restricted distribution. Habitat specificity may include particular depth requirements, a minimal average amount of water movement, water clarity, substrate type (sand, coral, rubble, pavement, etc.), and particular biotic associates (e.g., particular taxa such as seagrasses, specific gorgonians or corals, etc.). The net effect of these various requirements is a highly predictable distribution of a given species within a reef system. Thus on a reef slope, for example, 4 or 5 species may co-occur, but only 2 or 3 will be displaying over the same localized reef area, and these species generally have distinctively different display patterns (e.g., upward versus downward displays, long versus short trains). Sibling species usually show very different signal patterns and/or habitat distributions (Morin & Cohen 1988; Cohen & Morin 1989).

All of the species we have found in the Caribbean appear to have limited geographic distributions; endemism is high. All species on one island or coastal area appear to be distinct in both morphology and display pattern, not only from each other but also from all other geographic locations (Cohen & Morin in press). This high local diversity and limited distribution phenomenon is likely due to isolating mechanisms produced by sexual selection of particular luminescent signals, probably by both intrasexual selection between males and epigamic selection by female choice, *and* the low dispersal characteristics of the group. Current data suggest that dispersal distances of about 50 km or less appear to be sufficient to allow gene flow, while distances of 250 km or more block it (i.e., at that distance local speciation via sexual selection exceeds rates of dispersal). The only areas where at least some species overlap apparently occurs is between (1) Curaçao and Bonaire (separated by ca. 45 km of open sea) and (2) three Belize sites and Roatan (sites with either contiguous reefs or that are within 50 km of

the nearest contiguous reef). On the other hand, the Belize/Roatan area is contiguous with the San Blas area of Panama, but there is no species overlap. These areas are separated by 1500 km of coast, much of which is sand and without reef development (most of coastal Nicaragua and part of Honduras and Costa Rica). The sand and absence of reef probably act as major dispersal barriers.

How widespread around the world are these luminescent courtship displays in ostracodes? Luminescent cypridinids, but not the clades we have discovered in the Caribbean, are known from around the world, shallow to deep, boreal to equatorial. In order to detect the displays, one must be in the proper *underwater* habitat in the *dark* at the *precise time* of signaling; this is probably why the displays went unnoticed for so long. Once we knew where and when to look, we discovered signaling ostracodes on every major reef system we examined in the Caribbean; they are abundant and ubiquitous (Cohen & Morin, in press). However, based on previous and recent nocturnal underwater observations by the senior author (Morin, unpublished), we have been unable to locate any similar luminescent signaling patterns by ostracodes from: Bermuda; Cape Cod, Massachusetts; the Gulf of Aqaba in the Red Sea; Lizard, Heron, and One Tree islands on the Great Barrier Reef; Banda Islands, Indonesia; Negros and Mactan islands, Philippines; Canton Island in the Phoenix Group; Leigh, New Zealand; Isla Uvas on the Pacific coast of Panama; southern Baja, Mexico; Catalina Island and Malibu, California; and San Juan Islands, Washington. Luminescent ostracodes were found, but no signals have been seen, from the Great Barrier Reef and California sites. This lack of apparent signaling patterns elsewhere around the globe indicates either: (1) signaling is restricted to the Caribbean and has not evolved (or was lost) elsewhere or (2) it exists elsewhere, but we have not found it because (a) the species use a different luminescent code, undetected by us; (b) they display at other times of the night than the early evenings when we searched; or (c) there are restricted lunar or seasonal times for signaling, which we missed. In either case, it is clear that the types of luminescent displays described above appear to be restricted to the Caribbean. The localized nature of this phenomenon provides an interesting arena for determining more about the way that sexual selection has shaped the evolution of this intriguing group of ostracodes.

The general lessons to be learned regarding courtship and reproduction in ostracodes from our observations of luminescent sexual displays in cypridinids are that courtship activities (1) may be subtle and difficult to detect, (2) may occur on a very precise diel, lunar, or seasonal timetable and over a very short time period, and (3) may only occur in restricted habitat situations or conditions and that (4) individual interactions may occur very rapidly. If all of these phenomena are operating for a given species, they will hinder observations of the events that lead up to insemination. We can be thankful for those few species that seem not to mind the restrictive dishes, bright microscope lights, and voyeuristic scientists; those few have given us the little information we now have about copulation.

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TWO

Intrinsic Factors Mediating Pheromone Communication in the Blue Crab, Callinectes sapidus

RICHARD A. GLEESON

The Whitney Laboratory,
University of Florida,
St. Augustine.

Abstract

A review of pheromone communication and its role in the reproductive behavior of *Callinectes sapidus* is presented. A pheromone found in the urine of pubertal females evokes a specific courtship display and cradle-carry behavior in male crabs. Males detect this pheromone via the aesthetasc sensilla on the outer flagellum of the antennules. The display-inducing pheromone appears to be absent in females that have completed the pubertal molt. Renewed release can occur in females initiating ecdysis subsequent to the pubertal molt; however, such a molt is an extremely rare event. Pubertal females appear to be preferentially attracted to waterborne odors from males. This ability to detect and locate prospective mates using chemical cues may be an important mechanism for assuring successful coupling. With eyestalk ligation/ablation a significant number of male crabs exhibit spontaneous display behavior. This effect is attributed to the loss of a hormonal factor of eyestalk origin that acts to modulate the activity of CNS "centers" controlling courtship behavior. A model for the activation and modulation of courtship behavior in the male is proposed.

THE BLUE crab, *Callinectes sapidus*, a member of the swimming crab family Portunidae, is distributed along the coastal regions of the western Atlantic Ocean from Nova Scotia to northern Argentina (Williams 1974). The female of this species will normally mate only once during her life. This copulation occurs immediately following her "maturity" or "pubertal" molt, which is also her final molt, since she enters terminal anecdysis after reaching maturity (Van Engel 1958). During the penultimate instar, the female undergoes dramatic morphological changes that prepare her for reproduction and are expressed at the time of the pubertal molt. Most prominent among these changes are the development of seminal receptacles (spermathecae) for the reception and storage of spermatophores and a transformation of the abdomen in preparation for spawning. Females in the proecdysial stage of the penultimate instar are herein referred to as "pubertal females."

Courtship and mating in *Callinectes sapidus* involve a sequence of behaviors that extends over a period of several days. Male crabs are observed to attend or to guard pubertal females that are within approximately 10 days of molting. When such a female is first encountered, the male will frequently exhibit a distinctive courtship-display behavior (Teytaud 1971). This display is characterized by an extension of the chelae toward a lateral position, extension of the walking legs (second to fourth pereiopods) such that the male is elevated high off the bottom, and rhythmic lateral waving of the swimming appendages (fifth pereiopods), which are rotated to an anterodorsal position above the carapace (fig. 2.1A). In addition, there is often an increase in the amount of water pumped through the branchial chambers; this increased pumping is associated with forward extension of the maxillipeds and rapid waving of the flagellum on each maxilliped. As a result there is a strong flow of water projected forward from the male that may be a significant component of the communication elicited by the display, e.g., by transporting chemical information to the female. The male orients the display toward the female and slowly approaches her. In response, the female either may remain passive or move toward the male, maneuvering to a position under him. The male then embraces the female and, using his first pair of walking legs, holds her beneath him in what is termed the cradle-carry position (fig. 2.1B). Laboratory observations suggest that the courtship display by the male is not an essential prelude to coupling. Males will sometimes grasp and cradle-carry females that are in close proximity without displaying at all. Furthermore, pubertal females will frequently initiate the cradle-carry themselves by approaching and repeatedly bumping against nondisplaying males. A pair of crabs may remain coupled for several days until the female undergoes her pubertal molt. At this time she is released; however, the male continues to stand over her. At the completion of the molt the pair once again assumes the cradle-carry position.

Mating is generally initiated by the female within a few minutes to an hour after she molts. With the assistance of the male, she positions herself upside-down beneath him and extends her abdomen, allowing the male to insert his paired copulatory appendages into her genital pores (fig. 2.1C). Spermatophores are deposited within the spermathecae over a 5–12 hr period after which the cradle-carry position is reestablished and maintained for two or more days. The

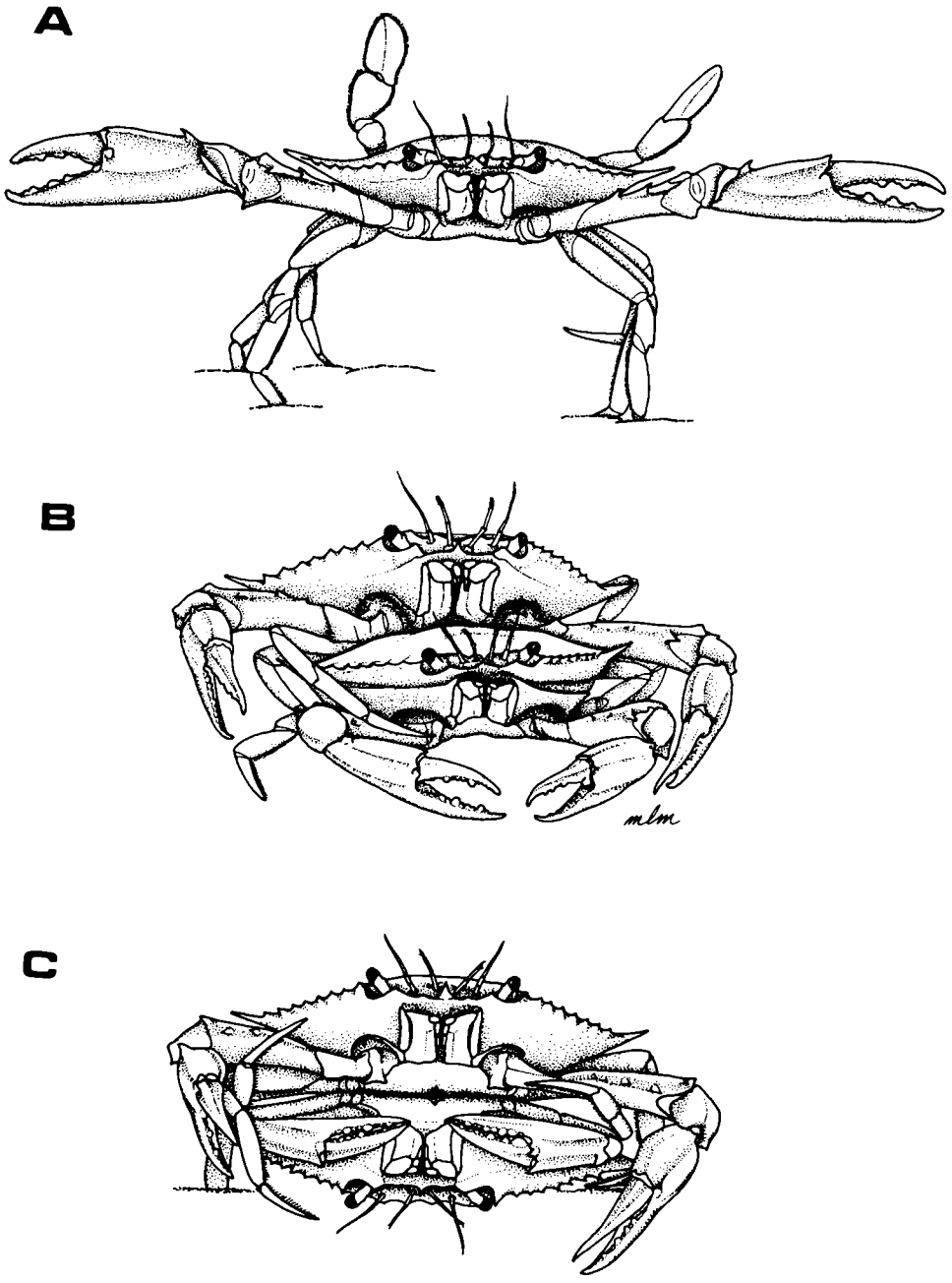

FIGURE 2.1. A. Male *Callinectes sapidus* in the courtship-display stance. B. Pubertal female in the cradle-carry position beneath a male. The female depicted is in the initial phase of her pubertal molt. C. Pair of crabs copulating. The recently molted female is upside-down beneath the male. Modified from Carr 1988.

sperm stored in the spermathecae during copulation remain viable for at least a year and are used to fertilize the two or more spawnings that the female undergoes during her lifetime (Hard 1942; Van Engel 1958).

EVIDENCE FOR PHEROMONE RELEASE BY FEMALES

Studies were performed to determine if courtship behavior (i.e., display and cradle-carry behavior) on the part of the male is triggered by a pheromone released by the pubertal female (Gleeson 1980). Water from a tank containing either pubertal females or control animals was introduced to a second tank containing "test-males." Two control groups were used: (1) mature females that were well past the pubertal molt and (2) male crabs that were of the same size and molt stage as the pubertal females. With the introduction of water from tanks containing control animals, little change in test-male behavior was observed. In contrast, when water from tanks containing pubertal females was presented to the males, a significant number of test-males began to exhibit courtship display behavior and/or attempted to cradle-carry other test-males. These findings clearly demonstrated that pubertal females release a chemical signal into the water that triggers the specific courtship behavior of the male.

The importance of visual cues in this behavior was investigated in experiments comparing the responses of males when only visual information versus visual plus chemical information from a pubertal female was available (Gleeson 1980). In this study a pubertal female was placed in a glass cylinder that was positioned within a tank containing several test-males. This allowed visual contact between the males and the female, but there was no exchange of water between the cylinder containing the female and the tank containing the males. Male activity was observed over a 10 min period. Water was then introduced into the cylinder from which it overflowed into the tank, and male behavior was again observed for five minutes. Visual cues alone did not elicit courtship behavior in males. Only with the overflow of water from the cylinder did males begin to exhibit courtship activity. The display behavior did not appear to be selectively directed toward the female; displaying males would readily orient toward other test-males and attempt to place them in a cradle-carry. These results suggested that the pheromone signal is of primary importance in initiating the male's courtship behavior and that visual cues are secondarily used to orient this behavior toward any crab in the immediate vicinity.

The urine of pubertal females was examined as a potential route of pheromone release to the environment (Gleeson 1980). For these experiments, urine samples were collected from the antennal gland ducts of pubertal females plus appropriate control animals. The two types of control animals described above were utilized as control-urine sources. Aliquots (0.1 ml) of each type of urine were sequentially introduced into tanks containing test-males and the stimulation of any courtship behavior noted over a five-minute observation period. Control urines elicited no reactions in test-males; however, urine from pubertal females stimulated courtship behavior in 15 of the 42 males tested, with a mean latency for onset of the behavior being approximately one minute. These results

showed that the pheromone is indeed present in the urine of pubertal females and, consequently, may be an important route of release.

In recent experiments we have begun to examine the release of pheromone from females that have completed the pubertal molt but have not copulated (unmated, post–pubertal-molt females [UPPM-females]). Although under normal conditions, females mate immediately after the pubertal molt, laboratory observations reveal that they will copulate as late as two to four weeks after this molt and are cradle-carried immediately following copulation (Teytaud 1971; personal observation). This would suggest that the UPPM-female continues to release the pheromone until she copulates. To explore this, experiments were performed in which the induction of male display behavior was examined. Water from a tank containing UPPM-females (from 1–10 days postmolt) was introduced to tanks containing single test-male crabs, and the induction of display was monitored. Water presented under identical conditions from a tank containing pubertal females served as a benchmark for comparison of display frequency. Remarkably, of the 172 males tested, none exhibited courtship-display activity when exposed to water from tanks containing UPPM-females. In contrast, when 91 of these males were exposed to water from tanks containing pubertal females, 35 (39 percent) displayed. These findings were corroborated in tests using urine collected from UPPM-females (6–8 days postmolt). In bioassay tests with this urine, no display behavior was induced in six males, all of which consistently exhibited display activity when exposed to pubertal-female urine. Also in agreement with these results is the observation that males and UPPM-females will generally copulate without preamble when placed together in the same tank (personal observation).

Although courtship behavior in test-males can be triggered by urine collected from females immediately following the pubertal molt (Gleeson 1980), the present findings suggest that UPPM-females either do not continue to produce the display pheromone or do not release it at an effective concentration for display induction. Since males are observed to cradle-carry females after copulations that occur well past the pubertal molt, these findings also raise the possibility that the display and cradle-carry behaviors are mediated by two distinct pheromone signals. Further studies to test the induction of cradle-carry behavior by urine from UPPM-females should provide insight into these questions.

Although pheromone production/release appears to decline dramatically in UPPM-females, there is evidence that it can be released anew if the female initiates another molt. Ecdysis subsequent to the pubertal molt in the female of *Callinectes sapidus* is an extremely rare occurrence (see Olmi 1984). Although there are reports in the literature describing mature females in proecdysis, no accounts have definitively shown the release of pheromone at this time or documented the successful completion of such a molt (Hard 1942; Abbe 1974; Olmi 1984; Skinner 1985). An opportunity to examine this arose in April 1986, when a mature female in proecdysis was obtained from the St. Johns River in Florida and taken to the Whitney Laboratory for observation. When placed in a tank containing a mature male, the female approached the male and attempted to position herself beneath him. The male immediately responded by cradle-carrying the female; this position was maintained for over two hours before the

female was removed and placed in a separate tank. There were no attempts to copulate during this observation period. Urine obtained from the female was bioassayed with four males, each tested individually. All four males responded strongly to the urine with courtship displays. After seven days in the laboratory, the female successfully molted, and when she was placed together with a male, copulation was initiated. The pair was immediately separated, however, and subsequent inspection of the spermathecae revealed that the female had not copulated previously.

These observations show that pheromone release can accompany a molt occurring after that of puberty. Interestingly, Truitt (in Hard 1942) has observed that females that do not copulate after the pubertal molt will initiate a second molt. If such a relationship does in fact exist, it would suggest that copulation is linked to the mechanism of terminal anecdysis in the female and may, therefore, represent a means by which females can enhance their opportunities for copulation in the event that mating is unsuccessful following the pubertal molt. This is an intriguing question that is certainly worthy of further investigation.

PHEROMONE RECEPTION IN MALES

The location of chemoreceptors mediating pheromone detection by the male was determined in a series of ablation experiments (Gleeson 1980, 1982). These studies centered on the antennules (first antennae), which are the olfactory organs in decapod crustaceans (Ache 1985). In *Callinectes sapidus* the antennule is biramous, with an inner and outer flagellum; olfactory sensilla (aesthetascs) are situated in a prominent tuft on the ventral face of the outer flagellum (fig. 2.2). To localize pheromone-sensitive chemoreceptors, various ablations of the antennules were performed in groups of male crabs and their responses to the pheromone (i.e., water from tanks containing pubertal females) compared to those of appropriate control animals. Initial experiments revealed that with bilateral removal of both flagella from the antennules, males would no longer respond to the pheromone. This effect was also observed with bilateral ablation of only the outer flagellum, whereas removal of the inner flagella had no effect on pheromone detection. These results indicated that chemosensory structures important for pheromone detection are associated with the outer flagellum of the antennule (Gleeson 1980).

Experiments then focused on the aesthetasc tuft region of the outer flagellum as a possible site of pheromone-sensitive chemoreceptors. Scanning electron microscopy studies revealed that the tuft is comprised of approximately 700 aesthetasc sensilla that originate from grooves situated distally on the ventral faces of most flagellar segments. The tuft is divided into distinct mesial and lateral halves on each segment by a central region of cuticle that has no sensilla (fig. 2.3). A second type of sensillium, which is only found on the mesial side of the tuft, arises from sockets located proximal to the aesthetasc row of each flagellar segment. From one to four of these sensilla, termed asymmetric sensilla, are found on most segments; they project across the tuft, terminating within the lateral half of the tuft (fig. 2.3). Based on these morphological findings, three

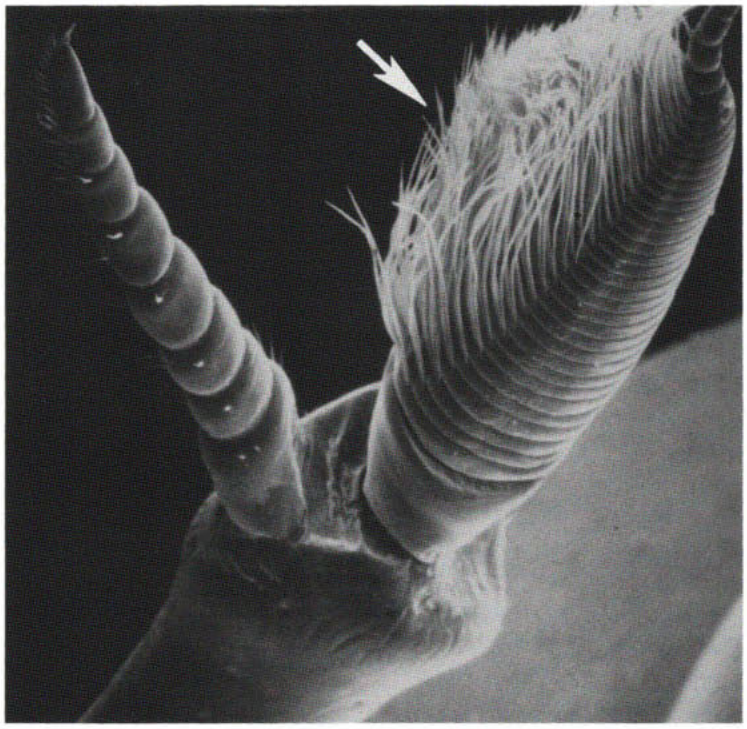

FIGURE 2.2. Scanning electron micrograph showing the inner and outer flagella of an antennule from *Callinectes sapidus* (lateral view). The tuft of aesthetasc sensilla on the outer flagellum is indicated by the arrow. The length of the outer flagellum is approximately 2 mm.

different types of lesions were bilaterally performed on the antennules of test-male crabs: (1) using fine-tipped forceps, all of the sensilla were removed from the lateral half of the tuft; (2) all sensilla were similarly removed from the mesial half of the tuft; and (3) using micro-dissecting scissors, the entire tuft was cut, resulting in the ablation of all but the basal portion of each aesthetasc. Behavioral assays demonstrated an approximately 20 percent decrease (relative to a sham control group) in the incidence of responses to the pheromone for males receiving lesions in either the mesial or lateral half of the tuft. With the entire tuft lesioned, however, there was a profound loss of response to the pheromone (Gleeson 1982). These findings are consistent with the notion that the aesthetascs are the chemosensory structures mediating pheromone detection in the male crab and suggest that the asymmetric sensilla do not play a role in this detection.

CHEMICAL PROPERTIES OF THE FEMALE PHEROMONE

The chemical nature of the pheromone of pubertal females was investigated using male crabs in a bioassay system that allowed testing small volumes of material for pheromone activity (Gleeson, Adams & Smith 1984). Urine was used

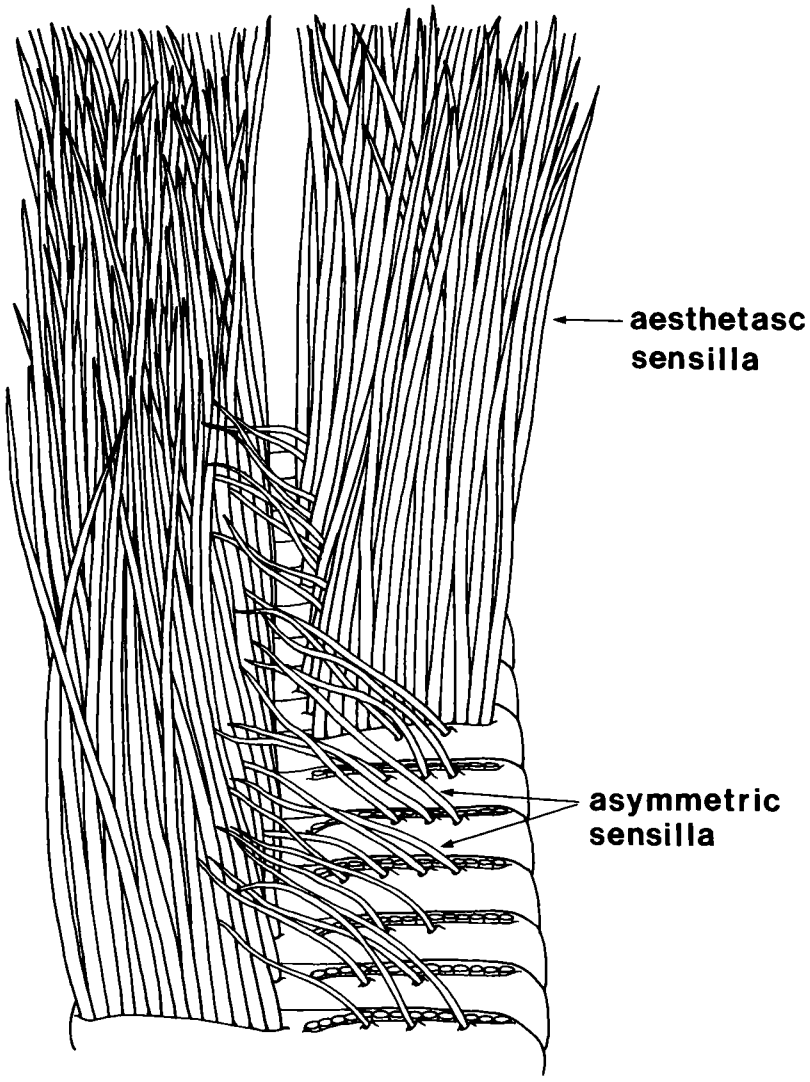

FIGURE 2.3. Portion of the outer flagellum (ventral view) of the antennule in which the aesthetasc sensilla have been removed from several segments to reveal the asymmetric sensilla. These sensilla are found only on the mesial side of the aesthetasc tuft and project laterally.

as a source of the pheromone and served as a benchmark in each bioassay for quantifying the relative activity of fractions derived from the urine. Initial studies showed that the urine can be heated to 95°C for five minutes without significant loss of activity and can be stored at −20°C for extended periods of time; it can also be lyophilized to dryness and reconstituted in water with no loss of biological activity. Sephadex gel filtration using columns calibrated with peptide molecular weight markers have indicated a molecular weight for the pheromone of between 300 and 600 daltons. Partial purification of the pheromone

can be achieved by sequential ultrafiltration of the urine; since the pheromone will pass through an Amicon YC-2 filter but is retained by a YM-05 filter (molecular weight cutoffs of 1000 and 500, respectively), substances greater than 1000 and less than 500 daltons can be removed from the pheromone fraction. Further purification of the pheromone has been accomplished with high-pressure liquid chromatography using a Whatman reverse-phase preparative column (M9-ODS-3, 70:30 methanol-water solvent mixture). This procedure yields a fraction with pheromone activity equivalent to that of the urine itself and containing three components as detected by ultraviolet absorbance at 220 nm and confirmed by thin-layer chromatography (Gleeson, Adams & Smith 1984).

The potential role of the molting hormone, crustecdysone, as a pheromone in decapod crustaceans was proposed by Kittredge, Terry & Takahashi. (1971). In that study crustecdysone was reported to induce precopulatory behavior in males of *Pachygrapsus crassipes, Cancer antennarius,* and *C. anthonyi.* Moreover, the authors noted an apparent lack in species specificity of the pheromone released by various crabs, e.g., courtship behavior in *C. antennarius* appeared to be stimulated by *P. crassipes.* Based on these findings it was concluded that either crustecdysone is the sex pheromone for these species or that it has a structure sufficiently similar to the pheromones to mimic their actions. They subsequently proposed that the molting hormone served as a substrate for the evolution of sex pheromone communication, which initially involved leakage of

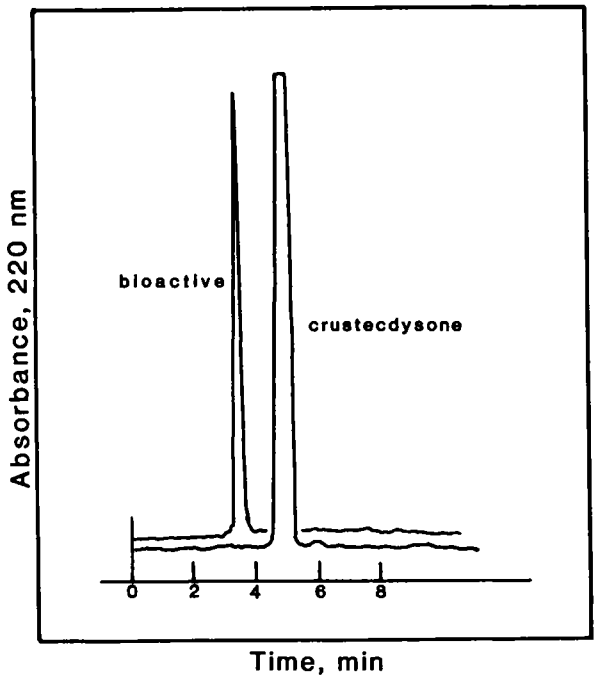

FIGURE 2.4. HPLC traces comparing the retention times for crustecdysone and the partially purified pheromone derived from the urine of pubertal *Callinectes sapidus* females. From Gleeson, Adams, & Smith 1984.