When males and hermaphrodites coexist: a review of androdioecy in animals

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Synopsis Androdioecy (populations consisting of males and hermaphrodites) is a rare mating system in plants and animals: up to 50 plants and only 36 animals have been described as being androdioecious, with most of the latter being crustaceans. To date, a thorough comparative analysis of androdioecy in animals has not been undertaken. Herein we present such an analysis. Androdioecy has only been extensively surveyed in 2 animal taxa: the nematode *Caenorhabditis* and the clam shrimp *Eulimnadia*. The other major taxon having androdioecious species is the Cirripedia (barnacles), but there are only limited studies on androdioecy in this group. In animals, androdioecy is found either in species that have morphologically and ecologically distinct sexes (that is, hermaphrodites and small, "complemental" males) that are derived from hermaphroditic ancestors (that is, the barnacles) or in species that have similarly-sized males and hermaphrodites that have been derived from dioecious ancestors (the remaining androdioecious species). We suggest that the barnacles have evolved a sexual specialization in the form of these complemental males that can more efficiently use the constrained habitats that these barnacles often experience. For the remaining species, we suggest that androdioecy has evolved as a response to reproductive assurance in species that experience episodic low densities. Additionally, we hypothesize that the development of mechanisms allowing reproductive assurance in species with a number of sexually differentiated traits is most likely to result in androdioecy rather than gynodioecy (mixtures of females and hermaphrodites), and that these species may be developmentally constrained to stay androdioecious rather than being capable of evolving into populations solely consisting of efficient, self-compatible hermaphrodites. We conclude by suggesting several areas in need of further study to understand more completely the evolution and distribution of this interesting mating system in animals.

Introduction

Charnov and colleagues (1976) outlined the conditions under which hermaphroditism was thought likely to evolve. Specifically, they noted that when the fitness set relating the allocation of male and female gametes is convex, hermaphroditism should be advantageous. They suggested that this type of relationship should be found in low-mobility species, in species in which resources needed for male gamete production are offset from those needed for female gametes, and, in plants, where the costs associated with attracting pollinators are greater than the costs of producing gametes. Other situations in which hermaphroditism is thought likely to evolve are when population densities are low enough that finding mates is difficult (Baker 1955). Under these conditions, being a self-compatible hermaphrodite allows "reproductive assurance" because of the ability to produce offspring without another individual, and because a single hermaphroditic pair has a 100% chance of outcrossing whereas a single dioecious pair has only a 50% chance of outcrossing (that is, there is a 50% chance of a pair being of the same sex in dioecious populations). When the fitness set is concave and issues of reproductive assurance are unimportant, dioecy should be advantageous (Charnov 1982).

When environmental conditions change or when a species occupies a new habitat, selection may favor a transition from hermaphroditism to dioecy or vice versa (Fig. 1). During such a phase, 3 forms of transitionary mating systems are recognized (Charlesworth and Charlesworth 1978; Charnov 1982): gynodioecy (mixtures of females and hermaphrodites), androdioecy (mixtures of males and hermaphrodites), and trioecy (mixtures of females, males, and hermaphrodites). Of these mixed mating systems, trioecy is the rarest, followed by androdioecy, with gynodioecy being most common (Charlesworth and Charlesworth 1978; Charlesworth 1984; Jarne and Charlesworth
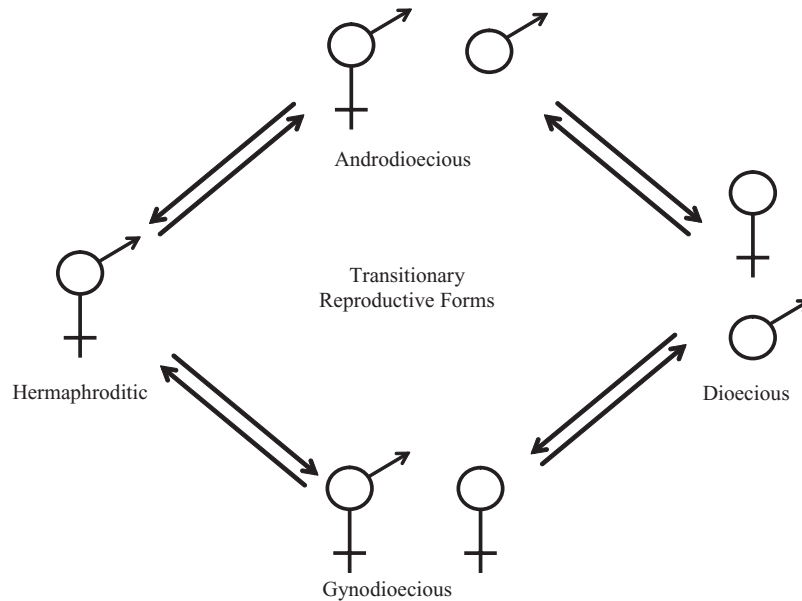
Fig. 1 Diagram of the evolutionary transition in reproductive systems from hermaphroditism to dioecy (or vice versa) and the two common transitionary reproductive systems: androdioecy (males + hermaphrodites) and gynodioecy (females + hermaphrodites).

1993; Pannell 2002). In this transitionary phase, a loss of 1 of the 2 sexes in a hermaphrodite (in transitions from hermaphroditism to dioecy) or the gain of the alternate sex in a single sex individual (in transitions from dioecy to hermaphroditism) leads to mixtures of the various mating types. In dioecious populations the invasion of a hermaphroditic mutant usually eliminates 1 of the 2 sexes, more commonly the males (Wolf and Takebayashi 2004). In hermaphroditic populations, the invasion of a single-sex mutant causes the remaining hermaphrodites to reallocate resources to the alternate sex, eventually leading to the evolution of dioecy as the hermaphrodites readjust their resource allocation to the increased presence of the single-sex morph (Charlesworth and Charlesworth 1978). Because invasions of hermaphrodites into dioecious systems usually drives 1 of the 2 sexes extinct and usually only a single single-sex morph is likely to spread through a hermaphroditic population at a time, having males, females, and hermaphrodites (that is, trioecy) is thought to be exceptionally unlikely (Charlesworth and Charlesworth 1978; Wolf and Takebayashi 2004). Thus, androdioecy and gynodioecy are viewed as the primary intermediate, transitionary stages between dioecy and hermaphroditism or vice versa (Fig. 1).

Interestingly, the evolution of androdioecy or gynodioecy as the transitionary phase is not equally likely, in either evolutionary scenario (that is, dioecy to hermaphroditism or vice versa). Because in most species, sperm (or pollen) is abundant relative to eggs (ovules), an all-male mutant has greater difficulty competing with hermaphrodites than an all-female mutant (Lloyd 1975; Charlesworth 1984; Pannell 2002; Wolf and Takebayashi 2004). This is especially true when hermaphrodites self-fertilize (Lloyd 1975; Charlesworth 1984). In the extreme case, males would be unable to produce offspring in an androdioecious population wherein all the hermaphrodites selfed. Thus, increasing levels of self-fertilization lead to decreased likelihoods of the evolution of androdioecy. The opposite is true for gynodioecy, wherein high levels of selfing would not compromise females because self-fertilizing hermaphrodites would still produce enough sperm (pollen) to allow effective outcrossing with females. Because rising levels of self fertilization commonly leads to inbreeding depression, females would be at an increasing advantage relative to selfing hermaphrodites when self-fertilization rates are high (Charlesworth and Charlesworth 1978). Thus, gynodioecy is predicted to be more common than androdioecy, which has been borne out in surveys of the 2 forms of reproduction (Charlesworth and Charlesworth 1978; Charlesworth 1984; Jarne and Charlesworth 1993; Barrett 1998, 2002).

Although androdioecy is thought to be unlikely to evolve, there are a handful of cases in which androdioecy has been described. The earliest species to be described as androdioecious were primarily plants. Charlesworth (1984) suggested that all 17 plant species that had been described to the date of publication were actually cryptically dioecious, with the "hermaphrodites" actually being functional females. However,

some of these species (for example, *Datisca glomerata*) were later verified as being truly androdioecious (Rieseberg and others 1992). In fact, in the most extensive review of androdioecy to date, Pannell (2002) outlines up to 50 plants and 10 animals that have been described as androdioecious. Of these 60 species, only 15 have been well documented as androdioecious (11 plants and 4 animals; Pannell 2002). As Jarne and Charlesworth (1993) point out, our understanding of the extent and importance of reproductive variants in the animal kingdom, including androdioecy, is considerably lagging that in the plant kingdom.

In this review, we will expand Pannell's (2002) treatment of androdioecy in animals by both expanding the range of animals discussed, and by going into more detail about the 2 androdioecious animal taxa that have been most extensively studied to date: nematodes in the genus *Caenorhabditis* and clam shrimp in the genus *Eulimnadia*. Additionally, we will discuss common life history and habitat features of these androdioecious animals and relate these features to those expected by the various models for the evolution of androdioecy. We will end by discussing promising future directions, and outline areas of research that should be pursued to understand more completely the maintenance of androdioecy in animal systems.

Overview of androdioecy in animal systems

Rhabditida nematodes

Rare instances of androdioecy are reported from 7 nematodes (Table 1) in the family Rhabditidae (LaMunyon and Ward 2002), and it appears that this peculiar mating system has independently evolved multiple times in this family (Fitch and Thomas 1997; Kiontke and others 2004). Two of these species are the more commonly studied *Caenorhabditis* species (see below), but the remaining 5 species are from a related genus, *Rhabditis* (also termed *Oscheius* or *Dolichorhabditis*). In all 7 of these soil-dwelling species, males are found at a frequency of <1%. In *Caenorhabditis,* males are known to be produced via non-disjunction of the sex chromosomes to produce XO males at a rate of ∼0.1% in *C. elegans* (Ward and Carrel 1979), a rate that "is comparable" in *C. briggsae* (McCulloch and Gems 2003). Estimations of these production rates in the remaining androdioecious nematode species have yet to be experimentally determined. Some have suggested that the low frequency of males indicates that males are unable to be selectively maintained but rather are only in these populations because of the constant low-level of muta-

tion (that is, they are at a mutation/selection balance; Chasnov and Chow 2002). Various phylogenetic analyses imply that androdioecious species have evolved from dioecious ancestors (Fitch and Thomas 1997; Azevedo and others 2000; Kiontke and others 2004). Very little comparable information about the 5 *Rhabditis* species has been reported.

Branchiopod crustaceans

Androdioecy has been described from 2 orders of branchiopod crustaceans (Table 1). The best studied of these are Spinicaudatan clam shrimp in the genus *Eulimnadia* (see below) that have hermaphrodites and males; the hermaphrodites can either self-fertilize or can mate with males but they cannot outcross with other hermaphrodites. This type of reproductive system has also been described in the Notostracan tadpole shrimp *Triops newberryi* (Sassaman 1989b), although the particulars of the reproductive system in this species have not been thoroughly investigated. Both shrimp occupy similar, ephemeral, aquatic habitats and thus are likely to experience a broad range of population densities as well as environmental conditions.

Cirriped crustraceans

Androdioecy has been established in 17 barnacle species, across 7 families (Table 1). The barnacles are sessile and often commensal organisms, living on the exterior surfaces of turtles, crabs, manatees and other marine organisms. The males of the androdioecious barnacles settle on or in depressions in the shell plates of the hermaphrodites, or in some cases even crawl inside the mantle (Foster 1983). Darwin (1851) was the first to document this mating system and he coined the term "complemental" males, which he distinguished from the miniature ("dwarf") males in dioecious species. The mode of sex determination in these species is uncertain. Two hypotheses have been proposed: (1) all larvae are potentially hermaphroditic, but those that settle in niches on large hermaphrodites do not grow to a size where female tissues may develop (that is, the substrate determines sex expression), or (2) the sexes are actually genetically fixed and will develop into each sex type regardless of environmental conditions. Each of these ideas may be valid in different species, given that complemental males have arisen separately in at least 7 instances in the Cirripedia (Foster 1983).

Crisp (1983) and Charnov (1987) both hypothesized that cirripedes stemmed from a hermaphroditic ancestor. However, this assessment was based purely on a historical perspective without tests of phylogeny

Table 1 Animal taxa for which androdioecy has been documented

Phylum	Class	Order	Family	Genus	Species
Nematoda	Secernentea	Rhabditida	Rhabditidae	*Caenorhabditis*	*elegans*
				Caenorhabditis	*briggsae*
				Rhabditis	*myriophila*
				Rhabditis	*sp.* (CEW1)
				Rhabditis	*sp.* (AF5)
				Rhabditis	*tipulae*
				Rhabditis	*guentheri*
Arthropoda (Crustacea)	Branchiopoda	Notostraca	Triopsidae	*Triops*	*newberryi*
		Spinicaudata	Limnadiidae	*Eulimnadia*	*texana*
				Eulimnadia	*africana*
				Eulimnadia	*agassizii*
				Eulimnadia	*antlei*
				Eulimnadia	*braueriana*
				Eulimnadia	*brasiliensis*
				Eulimnadia	*colombiensis*
				Eulimnadia	*cylindrova*
				Eulimnadia	*diversa*
				Eulimnadia	*feriensis*
				Eulimnadia	*follisimilis*
				Eulimnadia	sp. A
				Eulimnadia	sp. B
	Thecostraca (Cirripedia)	Thoracica	Heteralepadidae	*Paralepas*	*klepalae*
			Koleolepadidae	*Koleolepas*	*avis*
			Iblidae	*Ibla*	*quadrivalvis*
				Ibla	*cumingii*[1]
				Ibla	*idiotica*[1]
				Ibla	*pygmaea*
			Scalpellidae	*Scalpellum*	*vulgare*
				Scalpellum	*peronii*
				Scalpellum	*scalpellum*
				Scillaelepas	*arnaudi*
			Chelonibiidae	*Chelonibia*	*patula*
				Chelonibia	*testudinaria*
			Bathylasmatidae	*Bathylasma*	*alearum*
			Balanidae	*Balanus*	*galeatus*
				Balanus	*calceolus*
				Balanus	*merrilli*
				Balanus	*masignotus*
Chordata	Osteichthyes	Cyprinodontiformes	Aplocheilidae	*Rivulus*	*marmoratus*

See text for references.

[1] Foster (1978) suggests that these species may in fact be dioecious, although they were originally described as being androdioecious (Callan 1941; Batham 1945).

(Høeg 1995). Høeg (1995) tested this hypothesis using a phylogenetic analysis of the Cirripedia. He traced mating system transitions on the resulting tree. The resulting analysis revealed that the 2 outgroup lineages were dioecious. Additionally, within the Cirripedia, the Acrothoracica and the Rhizocephala exhibit dioecy. The Thoracica is the most derived lineage and exhibits the first transition into hermaphroditism (Høeg 1995).

The families within the Thoracica exhibit dioecy, hermaphroditism, and androdioecy, but the evolution of these sexual systems remains unclear. The Iblidae is the most basal family, diverging at the node where hermaphroditism is thought to have evolved. From this it may be argued that complemental males in this family (in a single species) could have evolved from a dioecious ancestor. In the remaining families, it is more parsimonious that the complemental males evolved secondarily from hermaphrodites (Høeg 1995). To address the evolution of mating systems adequately in this group a more robust phylogeny is required.

The barnacles described as androdioecious to date occur in various regions of the world and exhibit a variety of life histories. Darwin (1851) first noted this mating system in *Scalpellum vulgare* and *Ibla quadrivalvus*. In the genus *Scalpellum*, 2 more species are now known to have complemental males: *S. scalpellum* and *S. peronii*. Callan (1941) suggests the male sex in the androdioecious *Scalpellum* is environmentally determined due to an absence of sex chromosomes and due to the occurrence of oogonia in the males. Additionally, in the Scalpellidae, *Scillaelepas arnaudi* is androdioecious (Jones and Lander 1995). Within the genus *Ibla*, there is some confusion as to the number of androdioecious species. Callan (1941) uses *Ibla cumingii* as an example of androdioecy, and Batham (1945) originally described *I. idiotica* with complemental males. Alternatively Foster (1978) says that *I. cumingii* and *I. idiotica* are both dioecious species while *I. pygmaea* and *I. quadrivalis* are androdioecious. Some of this confusion may stem from authors often using the terms "hermaphrodite" and "female" interchangeably, as well as "dwarf males" and "complemental males."

The Balanidae contains 4 species with complemental males (Table 1). McLaughlin and Henry (1972) compared the morphology of these 4 Balanidae and found that while males are similar in the basic morphological plan, the degree of degeneration of a variety of structures differs among the species. Gomez (1975) showed that sex determination of hermaphrodites and males in *B. galeatus* is genetic, and he also documented a male to hermaphrodite sex ratio of 1 to 2.9.

Two species of *Chelonibia* are androdioecious, *C. patula* and *C. testudinaria,* and both are commensal barnacles. *Chelonibia patula* occurs along the eastern coast of the United States from Virginia to Florida, and are most commonly found on the carapace and legs of the blue crab (Crisp 1983). *Chelonibia patula* is protandrous, where male organs develop first and female organs develop later to produce a simultaneous hermaphrodite. The development of sex organs is size specific. Male organs arise when the organism reaches a basal diameter of ~2.5mm and become functional at 5 mm in basal diameter (Crisp 1983). Female organs begin development at about 4 mm in basal diameter and become mature at 7 mm in basal diameter, which is the point at which *C. patula* becomes a fully functional, simultaneous hermaphrodite. In this species, the male settles in the opercular region of the hermaphrodite and only reaches 2–4 mm in basal diameter. Because these males never grow beyond 4 mm and never have the opportunity to develop female function, Crisp (1983) argues that these are different from complemental males in other barnacles. Crisp (1983) terms them "apertural" males that have been developmentally arrested. Given time and opportunity these individuals can, in fact, develop into hermaphrodites.

Chelonibia testudinaria occurs worldwide as a commensal with sea turtles (Rawson and others 2003). In this species, complemental males settle in depressions in hermaphroditic shell plates. Zardus and Hadfield (2004) suggest these depressions may actually be specialized for the settlement of complemental males. It is unknown if these males can develop into full-grown hermaphrodites, but they can outgrow the depressions on the hermaphrodites, and it is likely they are actually protandrous. Zardus and Hadfield (2004) hypothesize that the small complemental males facilitate a higher rate of reproduction by producing sperm at a young age. *Chelonibia testudinaria* adheres to the outer scute of the turtle shell that is molted every year. Barnacles actually increase the drag on the sea turtle and accelerate this process. The smaller, complemental males may be selectively advantageous for 2 reasons: (1) their smaller size reduces the drag on the turtles, thus potentially lengthening the lives of the attached barnacles, and (2) speeding the rate of reproduction is useful when the life span of the barnacle is uncertain (Zardus and Hadfield 2004).

Koleolepas avis is the only species within its family to be described as androdioecious. It is known from southern Japan and lives as a symbiont around the pedal disks of sea anemones, which in turn occur on the gastropod shells used by hermit crabs (Yusa and others 2001). Yusa and colleagues (2001) found a positive relationship between the number of males and hermaphroditic body size, suggesting that larger hermaphrodites obtain more sperm. The mating group size is small in *K. avis*, but hermaphrodites typically settle within 50 mm of one another, which enables cross-fertilization (Yusa and others 2001).

Two other, less-studied androdioecious barnacles include *Bathylasma alearum,* where 83% of hermaphrodites were found with complemental males on their shells in a New Zealand population (Foster 1983), and *Paralepas klepalae,* where only 1 adult with a complemental male has been described from a population north of Madagascar (Kolbasov and Zevina 1999).

Osteichthyes fish

Within the vertebrates, only 1 animal is known to self-fertilize: *Rivulus marmoratus*, a marsh-dwelling fish ranging from Florida to Brazil (Harrington 1961). In Florida, *R. marmoratus* is known to occur in marshes above the intertidal zone in the burrows of the great land crab, and it often occurs in seasonal ponds (Taylor 2000). The hermaphrodites produce sperm and eggs in ovotestes and self-fertilize internally (Harrington 1961). Two types of males are known: primary and secondary. The primary males develop directly to males and produce sperm throughout their lives, while secondary males develop from the loss of ovarian tissue by hermaphrodites (Harrington 1967; Sakakura and Noakes 2000). These 2 types of males were found to be environmentally (temperature) induced, leading Harrington and Kallman (1967) to characterize them as ecophenotypes. Males are the only known agent of outcrossing, and thus hermaphrodites can either self-fertilize or mate with a male (Sakakura and Noakes 2000).

Males occur at very low levels (∼1%) in some populations in Florida. These populations have been shown to be completely homozygous (Turner and others 1990), suggesting high rates of selfing. However, 1 population of *R. marmoratus* in Belize has up to 25% males (Turner and others 1992), and it has a very high frequency of heterozygotes (Taylor and others 2001) suggesting high levels of outcrossing. In addition, Taylor and colleagues (2001) described another population in Honduras with males occurring at 2%, a population that has moderately low levels of heterozygosity, suggesting a mixture of selfing and outcrossing.

Although the most closely related species to *R. marmoratus* are dioecious, mitochondrial DNA analysis of *R. marmoratus* populations suggests that the highly outcrossing population in Belize is more derived than other all-hermaphroditic populations (Weibel and others 1999). This led Taylor and colleagues (2001) to suggest that outcrossing in the Belize population is not an ancestral condition, but is actually a phenotypically plastic condition. Thus the origin of androdioecy in *Rivulus marmoratus* remains unclear, and more investigations are needed to determine the evolutionary history of this interesting androdioecious species (Taylor and others 2001).

Two best known examples of androdioecy in animal systems

Nematodes in the genus Caenorhabditis

Caenorhabditis elegans is a tiny, bacteriophagous, soil-dwelling nematode, belonging to the family Rhabditidae, in the order Rhabditida (Table 1). It represents a very important model system for developmental biology, genetics, and behavior, especially because it has a very short life cycle, a small body with simple anatomical structures, and is easily reared in the laboratory (Riddle and others 1997). A great amount of information has been gathered on its life cycle, anatomy, development, genetics, and behavior in laboratory studies, using different strains and creating "ad hoc" mutants. Less is known about its ecology and behavior in natural environments.

Caenorhabditis elegans is a cosmopolitan species, with a worldwide distribution (Kader and Cote 1996; Hodgkin and Doniach 1997). It is typically found in soil containing fungal material, humus, and leaf litter. This habitat is probably heterogeneous in space and time, and is likely typified by fluctuations in food content, risk of predation, temperature, humidity, etc. (Riddle and others 1997). Embryogenesis occurs in 14 hours followed by a postembryonic developmental period (36 hours at 25°C) comprising 4 larval stages, L1-L4 (Riddle and others 1997). Adult life span varies between 12 and 17 days, at 20°C depending on the strain investigated (Gems and Riddle 2000a). If environmental conditions become harsh, a facultative diapause phase can be produced; the dauer larva forms at L2 and is specialized for dispersal (Riddle and Albert 1997).

Two sexes are present in *C. elegans*: sequential hermaphrodites (XX) and males (XO). Sex is determined by the ratio between sex chromosomes (X) and sets of autosomes (A): a X:A ratio of 0.75 or more leads to the development of hermaphrodites, a X:A ratio of 0.75 or less leads to the development of males (Nigon 1949). Self-fertile hermaphrodites are protrandrous: they are able to perform spermatogenesis during the last larval stage, L4 (Wood 1988), before irreversibly switching to oogenesis in the adult stage (Ward and Carrel 1979; Hodgkin 1988; Kimble and Ward 1988). The hermaphrodite-produced sperms are stored in the spermatheca and are only used for internal self-fertilization (Hodgkin and Barnes 1991; Hodgkin and Doniach 1997). Outcrossing with other hermaphrodites is not possible because the hermaphrodites lack specialized mating structures in the tail (copulatory bursa, fan with sensory rays and proctodeum with spicules) used by males for fertilization (Ward and Carrel 1979; Emmons and Sternberg 1997).

Males are produced from outcrossing (50%) or by spontaneous non-disjunction of the sex chromosomes during meiosis (Hodgkin and others 1979) at a frequency calculated between 0.1% (Ward and Carrel 1979; Chasnov and Chow 2002) and 0.2% (Hodgkin 1983; Hodgkin and Doniach 1997). Increased male production is found in a few strains containing active transposons, or is obtained in the laboratory

by heat-shock treatment or in *him* (high incidence of males) mutants.

Male sperm outcompetes hermaphroditic sperm in number, size, and motility (Ward and Carrel 1979; LaMunyon and Ward 1998; Singson and others 1999). This sperm competition results in suppression of self-fertilization and complete production of outcrossed progeny following copulation (Ward and Carrel 1979; Singson and others 1999). Hermaphrodites are sperm limited: spermatogenesis is restricted to the last larval stage, though males keep producing sperms throughout their adult life. Hermaphrodites mating with males actually increase, up to fourfold, the number of offspring produced (Hodgkin and Barnes 1991).

Mating efficiency is related to anatomical and behavioral features (Hodgkin 1983). Mating efficiency is density-dependent and is influenced by male age and hermaphrodite mobility. The mating efficiency of males in *C. elegans* is low, compared to the efficiency of males of dioecious species (Chasnov and Chow 2002; Stewart and Phillips 2002). For example in *C. remanei,* more sexual encounters are performed, and their duration is longer than in *C. elegans* (Chasnov and Chow 2002). In *C. elegans,* sperms are not transferred efficiently and sometimes can even be expelled by the hermaphrodite after sperm transfer has occurred (Ward and Carrel 1979). After the transfer occurs, however, utilization of hermaphroditic and male sperm is efficient: nearly every sperm fertilizes an oocyte.

The rate of outcrossing in nature is not well studied. Cutter and Payseur (2003) compared polymorphisms and recombination rates in populations of *Caenorhabditis elegans* and suggested that an outcrossing rate between 1 and 2% would best explain the patterns observed.

The common ancestor of *C. elegans* (androdioecious) and *C. remanei* (obligate outcrosser) was dioecious (Fitch and Thomas 1997). Molecular analyses suggest that *C. briggsae* (androdioecious) and *C. remanei* are sister species (Cutter and Payseur 2003; Kiontke and others 2004), an idea that is reinforced by hybridization experiments among *Caenorhabditis* species (Hill and L'Hernault 2001). Thus it appears that the most recent ancestor to both androdioecious *Caenorhabditis* species (*C. elegans* and *C. briggsae*) was dioecious (Kiontke and others 2004).

Although males seem to be both efficient at mating and increase reproductive output in hermaphrodites, there is little evidence suggesting that males are selectively maintained in *C. elegans* populations. Males are the only mechanism for outcrossing, and thus experiments seeking to detect a stable coexistence between males and selfing hermaphrodites have logically sought benefits to outcrossing. In one such study, Cutter and Payseur (2003) found no differences between the rate of deleterious mutations and the site substitution in obligate outcrossing and selfing *Caenorhabditis* species, suggesting no benefit to outcrossing. Similarly, no measurable inbreeding depression has been detected in *C. elegans* in laboratory experiments (Johnson and Wood 1982; Johnson and Hutchinson 1993; Chasnov and Chow 2002).

In fact Chasnov and Chow (2002) postulated that males are not selectively maintained by avoidance of inbreeding depression or any other selective advantage in *C. elegans* populations, but rather that males are at a mutation-selection balance (in this case, the mutations are via the non-disjunction of the sex chromosomes at a frequency of 1–2% of offspring produced) basically because there are no advantages to being male and because hermaphrodites have lost their ability to attract mates, and thus do not outcross. However, Simon and Sternberg (2002) found that hermaphrodites are not passive mating partners, but actively promote male mating behavior. Also Lipton and colleagues (2004) found that males are capable of detecting hermaphrodites which they can chemically detect near their food sources. Thus, the jury is still out on the ability of males to find and successfully fertilize hermaphrodites in *C. elegans*, and we are still unsure what factors (if any) selectively maintain males in natural *C. elegans* populations.

Unfortunately to date there are no available data on male frequency, outcrossing rate, and inbreeding depression in natural populations. All the data described above are the results of experiments conducted in laboratories where *C. elegans* was fed with *E. coli,* which is not a natural food item (Hodgkin and Doniach 1997). Thus, we do not have a sufficient understanding of the level of males in natural populations, nor of the selective factors that may favor outcrossing in this species.

Crustaceans in the genus Eulimnadia

Eulimnadia texana is a branchiopod crustacean within the order Spinicaudata and the family Limnadiidae (Table 1). Shrimp in this family generally inhabit temporary freshwater pools in habitats ranging from temperate forests in the United States, to tropical pools in Asia and South America, to desert flatlands in Australia (Belk 1989; Martin 1989; Yoon and Kim 1992; Roessler 1995; Smith and Wier 1999; Pereira and Garcia 2001). The common name of these shrimp derives from their "bivalved" carapace that is folded around their bodies. These are primitive crustaceans, having a series of unmodified, plate-like appendages (termed "phyllopod" appendages) that are used for

respiration, filter feeding, and limited locomotion. Given the nature of their environments, clam shrimp mature quickly, produce hundreds to thousands of eggs in several clutches, and then die within several weeks (Weeks and others 1997). The fertilized eggs (termed "cysts") are desiccation resistant and can lie dormant for decades (Clegg 2001).

In this androdioecious species, males coexist with hermaphrodites of 2 phenotypically similar but genetically different types: "amphigenic" and "monogenic" hermaphrodites. Sex initially was thought to be controlled by a single genetic locus (Sassaman and Weeks 1993), with a recessive allele coding for males (s) and a dominant allele coding for hermaphrodites (S). The homozygous dominants (SS) are termed "monogenic" hermaphrodites, the heterozygotes (Ss) are termed "amphigenic" hermaphrodites, and homozygous recessives (ss) are males (Sassaman and Weeks 1993). Subsequent studies have suggested that the sex determining mechanism may, in fact, be a set of linked genes (or possibly an entire chromosome with reduced crossing over), and that there may be numerous genes within this linkage group that encode sexual dimorphism (Weeks and others 2000, 2001a). Crossing over within this set of linked genes would be disadvantageous due to the production of intersexes with various mixtures of both sexes, which in most cases would prove inviable (Bull 1983).

The dynamics of the androdioecious mating system in *Eulimnadia texana* has been studied extensively. Males have a higher mortality rate than hermaphrodites, but this relationship is tempered by mating opportunities: increased male mating opportunities increases male lifespan, with the upper limit approaching that of hermaphrodites (Zucker and others 2001). Hermaphrodites produce primarily female gametes, with only a limited portion of the ovotestis devoted to sperm production (Zucker and others 1997). However, the amount of sperm produced is sufficient to fertilize all of the hermaphrodite's eggs if a male is unavailable to supply sperm (Weeks and others 2001b). Males can fertilize from 1 to 12 hermaphrodites per day, with this ability modified by relative male sex ratio and density; specifically, when males are rare and shrimp density is high (such that finding receptive hermaphrodites is maximized), male mating efficiency reaches its highest measured levels (Hollenbeck and others 2002). Inbreeding depression is high, ranging from 0.5–0.7, and negatively affects numerous aspects of the shrimp's biology (Weeks and others 1999, 2000, 2001b). Additionally, inbreeding depression is not reduced over several generations of inbreeding (Weeks 2004). This lack of purging of inbreeding depression may be due to associative overdominance, wherein 1 or more

deleterious recessive alleles is embedded within the large sex-determining linkage group and thus cannot be easily purged from the population by selection against these embedded alleles (Ohta 1971). Interestingly, although self-fertilization is common (inbreeding coefficients ranging from 0.20 to 0.97; Sassaman 1989a; Weeks and Zucker 1999), androdioecy is still maintained even though such self-fertilizations are expected to reduce male mating success to a degree predicted to make the maintenance of androdioecy unlikely (Lloyd 1975; Charlesworth 1984). All of the above measures have been made under laboratory environments, although early field experiments suggest that these lab results are similar to those found in controlled field experiments (Weeks and Bernhardt 2004).

An important aspect of male mating efficiency concerns mating behaviors. Males mate-guard hermaphrodites by using phyllopod appendages modified into claws (termed "claspers") used to attach the males to the hermaphrodite's carapace (Knoll 1995). Such physical pairing is necessary so that males can be in close proximity to the hermaphrodites when the latter transfer eggs from the ovotestes to the brood chamber. It is during this short period of time that hermaphrodites are most likely to outcross successfully (Weeks and others 2004). Such a narrow window of opportunity for mating selects for mate-guarding in a number of crustaceans (Jormalainen 1998). Hermaphrodites also behave in ways that increase the likelihood of outcrossing. Receptive hermaphrodites are attracted to males, swimming toward males when males are available (Hollenbeck and others 2002). Additionally, hermaphrodites will delay moving their eggs to their brood chambers when males are unavailable, suggesting that hermaphrodites can extend their "window of opportunity" for outcrossing when not in the presence of males (Zucker and others 2002).

Eulimnadia texana is the main animal system in which the stability of androdioecy has been examined by directly assessing their biology in the context of theoretical models for the maintenance of androdioecy. Because *E. texana* hermaphrodites cannot cross with one another (similar to *C. elegans*), previous models to evaluate the evolution of androdioecy (for example, Lloyd 1975; Charlesworth 1984) do not apply to these shrimp. Otto and colleagues (1993) recognized this shortcoming, and developed a population genetics model to examine the dynamics of *E. texana*'s mating system. This model attempts to predict the equilibrium frequencies of the 3 mating types in this system [males (u), monogenic (w), and amphigenic (v) hermaphrodites] based on information on 4 relevant parameters: α, relative male mating success; β, the

Table 2 Estimates of the 4 parameters of the Otto and colleagues (1993) model

Parameter	NM		AZ		Reference
	Low	High	Low	High	
$(1 - \sigma)$	0.8	0.87	0.79	0.91	Zucker and others (2001); Weeks and Bernhardt (2004)
β	1	1	1	1	Weeks and others (2001)
α	0.8	12.5	1.3	10.4	Weeks and others (2000); Hollenbeck and others (2002)
δ	0.47	0.53	0.54	0.63	Weeks and others (1999, 2000); Weeks and Bernhardt (2004)

Estimates are from 2 populations: one from Las Cruces, New Mexico (NM) and one Portal, Arizona (AZ). Where a range of estimates have been estimated, the highest and lowest estimates are presented.

proportion of eggs that are not fertilized by a male that are then self-fertilized by the hermaphrodite; $(1 - \sigma)$, relative viability of males to hermaphrodites; and δ, inbreeding depression experienced by selfed offspring. The model assumes that outcrossing rate is related to male frequency, u. The parameter α is defined by the fact that $\alpha \times u$ is the proportion of eggs that a hermaphrodite fertilizes with male sperm. While $\alpha \times u$ must lie strictly between 0 and 1 for all u, α need not. α is a function that includes several components of male-mating success: a) the number of encounters with hermaphrodites experienced by an average male during its reproductive lifetime, b) the probability of outcrossing per encounter, and c) the proportion of eggs fertilized using male sperm given that mating has occurred. The remaining proportion of eggs [that is, $(1 - \alpha u)$], are then available for selfing. The model allows for some proportion, $(1 - \beta)$, of these non-outcrossed eggs to remain unfertilized. This would occur if some eggs were "ear-marked" for outcrossing, or if the hermaphrodites were unable to produce enough sperm to fertilize all of their eggs in the absence of males. The model also incorporates the commonly observed difference in viability between the sexes in conchostracan shrimp; in many clam shrimp, males are found to have a higher mortality rate than females (Strenth 1977; Knoll 1995; Zucker and others 2001). In the Otto and colleagues (1993) model, this mortality difference is defined as $(1 - \sigma)$. Finally, the model provides for the frequently observed decrease in viability of offspring produced via self-fertilization (Holtsford and Ellstrand 1990; Agren and Schemske 1993; Dole and Ritland 1993; Eckert and Barrett 1994; Byers and Waller 1999), termed inbreeding depression (δ).

Using these 4 parameters, Otto and colleagues (1993) developed 3 complex equations to describe the equilibrium frequencies of males, monogenics, and amphigenics. They found 2 sets of equilibria: (a) fixation of monogenics ($w = 1.0$) and (b) a stable polymorphism of the 3 mating types. A stable polymorphism holds whenever the following is true: $\alpha(1 - \sigma) > 2\beta(1 - \delta)$. These 4 parameters

have been estimated from 2 separate populations of *E. texana* (Table 2). In these populations, a stable polymorphism is predicted in 3 of 4 comparisons; the single case against stability is under the lowest estimates of the 4 parameters in the New Mexico population. Although the Otto and colleagues (1993) model does predict stability of androdioecy in most instances, the predicted sex ratios from the model do not correspond with the observed sex ratios. In particular, natural proportions of the amphigenic hermaphrodites are well above even the highest predicted values in both populations examined: maximal predicted amphigenic proportions are 54% whereas the actual proportions of amphigenics are 70 and 63% in the New Mexico and Arizona populations respectively (Weeks and others 1999; Weeks and Bernhardt 2004). This lack of fit to the model suggests that the traditional within-population theoretical analysis (Lloyd 1975; Charlesworth 1984; Otto and others 1993) may not be appropriate for *E. texana*, and rather that a metapopulation model based on reproductive assurance may better describe the dynamics of this system (Pannell 1997, 2000, 2002).

Interestingly, although androdioecy is exceptionally rare in most plants and animals, it appears several times within the class Branchiopoda. Sassaman described androdioecy in the notostracan shrimp, *Triops newberryi* (Sassaman 1991), and in a second species of *Eulimnadia* (*E. antlei*; Sassaman 1990). Androdioecy has also been found in a total of 13 species of *Eulimnadia* (Table 1), with representatives from every continent except Antarctica (Weeks and others 2006). The total number of species in this genus is currently debated, with estimates ranging from 28 to 44 species (Baird 1849; Belk 1989; Martin 1989; Sassaman 1995; Brtek 1997). Nevertheless, androdioecy is the only known form of reproduction in this genus, and a phylogenetic analysis suggests that androdioecy is the ancestral mode of reproduction for this genus (Weeks and others 2006). The genus *Eulimnadia* is thought to have arisen in either the Paleogene (Chen and Shen 1981) or Cretaceous periods (Tasch and Shaffer 1964), but a biogeographic comparison suggests the genus arose up to 180 mya (Weeks and

others 2006). Thus, androdioecy appears to have persisted in this group for between 24 and 180 million years.

One additional clam shrimp within the family Limnadiidae, *Limnadia lenticularis*, also shows evidence of being androdioecious; it has virtually no males in its populations (Eder and others 2000) and is also almost completely homozygous at all loci so far examined (Tinti and Scanabissi 1996). These observations are similar to those reported for *E. texana*'s "monogenic" hermaphrodites (see above), and thus are consistent with an interpretation that this species is also androdioecious. If *L. lenticularis* is truly androdioecious, androdioecy would be the most common reproductive system in the family Limnadiidae, and could indicate that androdioecy has been a viable mode of reproduction in these crustaceans for ~280 million years (the inferred age of the family Limnadiidae, Orr and Briggs 1999). This would be a particularly surprising result given the rarity of this mating system in general, as well as its assumed evolutionary instability (Lloyd 1975; Charlesworth 1984). In fact, if androdioecy proves to be the ancestral reproductive mode within the Limnadiidae, the persistence of this "transitionary" mating system (Charlesworth 1984) would be in orders of magnitude greater than expected, making this family as anomalous to theories about the evolution of dioecy as the bdelloid rotifers are to theories about the evolution of sexual reproduction (Bell 1982).

Patterns evident among androdioecious animals

Several patterns emerge when comparing androdioecy among animal groups. First, androdioecy is exceptionally rare in animals (Pannell 2002). When it is found, it is only in 1 or a few related species, except for the genus *Eulimnadia* where it is the dominant mode of reproduction (Weeks and others 2006) and in the genera *Balanus, Ibla,* and *Rhabditis*, where it is found in 4, 4, and 5 species, respectively (Table 1). The genus *Balanus* has over 50 species (Pitombo 2004), and only 4 of these are androdioecious. It is unclear whether these 4 species are all closely related (indicating a single derivation of androdioecy) or if they represent 4 separate instances of the evolution of complemental males. A similar case is found in the nematode genus *Rhabditis* that has mixtures of dioecious and androdioecious species. In the genus *Ibla*, there are only 4 species (Foster 1978; Mizrahi and others 1998), but there is some controversy over whether all 4 species (and thus the genus) are androdioecious or if 2 species are dioecious and 2 are androdioecious (Table 1). In all

other taxa with androdioecy (with the exception of *Eulimnadia*), there is evidence for a rather recent derivation of androdioecy, which can be taken as evidence that androdioecy is generally a short-lived reproductive mode, as suggested by many theoreticians (Lloyd 1975; Charnov 1982; Charlesworth 1984; Pannell 2002). In contrast to the other animal taxa, the *Eulimnadia* appear to be exclusively androdioecious and phylogenetic inference suggests androdioecy evolved in the ancestor to this genus (Weeks and others 2006). Because *Eulimnadia* is an ancient lineage (as noted above) and contains dozens of species (Baird 1849; Sassaman 1995), it appears that androdioecy can, under certain circumstances, be long-lived and allow multiple speciation events (Weeks and others 2006).

A second pattern that emerges when considering all the androdioecious animals is that there are 2 distinct categories of androdioecy in animals: (1) androdioecious species with morphologically and ecologically distinct males (that is, complemental males) and hermaphrodites that have evolved androdioecy from hermaphroditic ancestors (that is, the barnacles) and (2) morphologically similar males and hermaphrodites (that is, largely similar in size, but with some lesser morphological differences) that have evolved androdioecy from dioecious ancestors (all other taxa; Table 1). These 2 categories suggest that there may be 2 main factors selecting for androdioecy in these various taxa. Within the barnacles, males and hermaphrodites are morphologically distinct, with males being much smaller than hermaphrodites. Barnacles are limited by space and time, occurring on small or ephemeral substrates. Therefore it is advantageous for small males to mature at an early age and for large hermaphrodites to make provisions for them (Charnov 1982, 1987; Crisp 1983). Overall it appears that the complemental males perform an ecologically unique function relative to the larger hermaphrodites by allowing greater outcrossing opportunities in a space-efficient and timely manner, and that this distinction may drive the evolution of androdioecy in these species. It is not completely clear what factors select for the maintenance of the hermaphrodites (that is, why the evolution of the complemental males hasn't driven a reallocation of resources in hermaphrodites to become functional females), so this aspect of the maintenance of androdioecy remains to be explored in these crustaceans.

In the second set of androdioecious species (morphologically similar sexes derived from dioecious ancestors), it is likely that these species have evolved androdioecy as a response to selective pressures for "reproductive assurance" (Baker 1955; Pannell 1997). It is probable that in these species, self-compatible

hermaphrodites can invade an otherwise dioecious population because such hermaphrodites can still produce offspring in low-density situations in which finding a mate can be problematic (Pannell 2002). The androdioecious Branchiopods, Nematodes, and *Rivulus* populations all appear to have life histories that suggest that these animals may periodically experience low-density habitats (for example, regularly colonizing new, ephemeral habitats).

Pannell (1997, 2000, 2002) has examined the maintenance of androdioecy in such scenarios and found that metapopulation models (in which species are subdivided into a series of smaller sub-populations that are dominated by migration to new sites and relatively common extinction of existing sites) can explain the maintenance of androdioecy. Pannell (2002) suggests that species in which metapopulation dynamics maintain androdioecy should be typified by having: (1) male sex ratios significantly less than 50%, (2) considerable among-population variation in the proportion of males, (3) hermaphrodites that self when densities are low but outcross when densities increase, (4) a genetic system in which maleness is dominant to hermaphroditism, and (5) male secondary sexual characters that allow greater male efficiency in the single-sexed individuals than in the hermaphrodites. *Rivulus* and *Eulimnadia* both have male sex ratios no higher than ~30% and have considerable among-population variation in proportion male (Sassaman 1989a; Turner and others 1992; Weeks and Zucker 1999), but as mentioned above, little is known about sex ratios in natural Rhabditida populations. Very little is known about outcrossing rates that vary with density in natural populations. However laboratory studies of male mating success found increased male mating with increased density in *E. texana* (Hollenbeck and others 2002) and in *C. elegans* (Chasnov and Chow 2002), with the caveat that high frequencies of males could lead to negative male-male interactions that tend to reduce male mating success in *C. elegans* (Gems and Riddle 2000b). In *C. elegans*, maleness is dominant (Wood 1988) whereas maleness is recessive in *E. texana* (Sassaman and Weeks 1993). The direction of dominance in *Rivulus* has not been described. In both *E. texana* and *C. elegans*, males have specialized grasping structures that allow pairing for sperm transfer; hermaphrodites do not have these grasping structures, which is consistent with Pannell's model. There does not appear to be any specific copulatory structures in male *Rivulus marmoratus*, but males are the only known agents of outcrossing in this species (Sakakura and Noakes 2000). Thus, in most cases the evidence is consistent with Pannell's metapopulation model for reproductive assurance, and hence if such metapopulation dynamics are responsible for the maintenance of androdioecy, then seeking within-population explanations for the maintenance of males and hermaphrodites (for example, Otto and others 1993; Weeks and others 1999, 2000, 2001a, 2001b; Chasnov and Chow 2002; Stewart and Phillips 2002; Weeks and Bernhardt 2004) should likely be replaced by experiments aimed at determining the importance of metapopulation dynamics in these androdioecious animal species.

The second important implication for androdioecious species that have evolved from dioecious ancestors is one of developmental constraints. In the nematodes, the branchiopods, and *Rivulus*, the only mode of outcrossing is via males, leaving hermaphrodites only capable of self-fertilization. If inbreeding depression is associated with selfing, which is commonly true (Charlesworth and Charlesworth 1987; Jarne and Charlesworth 1993; Byers and Waller 1999; Keller and Waller 2002), then the spread of hermaphrodites may be constrained by their inability to mate with one another. In dioecious species in which the sexes have specialized enough to be morphologically, physiologically, and/or behaviorally distinct, it may be quite difficult to develop a functional "intersex" (that is, hermaphrodite) because to do so requires just the right mix of specialized male and female components. Males of both *Eulimnadia* and *Caenorhabditis* have specialized morphological features (claspers and copulatory bursa, respectively) that allow males to pair with hermaphrodites. Because hermaphrodites lack these structures, they cannot pair for sperm exchange. In *E. texana*, we have found an intersex with hermaphroditic gonads, claspers, and male mating behavior, but lacking a brood chamber (Weeks and others, in press). This intersex produced malformed eggs that never hatched. This finding underscores the notion that dioecious animals may be developmentally constrained when under selection for reproductive assurance: they can either develop into selfing hermaphrodites or into androdioecious species (with a mixture of selfing and outcrossing), but the development of functioning, outcrossing hermaphrodites (that can efficiently perform both male and female functions) is rather unlikely. This idea is similar to the idea outlined in Pannell (2002), in which species in which males have specialized secondary sexual characters are likely to persist in otherwise hermaphroditic populations, but adds the notion that the further development of a truly functional hermaphrodite may be developmentally constrained by virtue of the likely evolutionary pathway by which a hermaphrodite would develop from a dioecious ancestor.

To elaborate this idea, let us consider a species that is evolving a self-compatible hermaphrodite in response to selective pressure to assure reproduction in low-density environments. Pannell (2002) has convincingly argued that in such a situation, a hermaphrodite should be selected to produce minimal sperm, only enough to fertilize its own eggs under low density conditions. If this is true, then it would make sense that a mutation in a female background that allowed the production of a small amount of sperm to be used to fertilize one's own eggs would be more parsimonious than a similar mutation in a male background that allocated most resources in the mutant to the production of eggs and only a small proportion toward sperm. Additionally, the production of a small amount of sperm in an otherwise female background would maximize the chances of having all the specialized phenotypes necessary to be a functioning female. In clam shrimp, eggs are much more difficult to produce than are sperm (Scanabissi and Mondini 2000, 2002), with eggs needing to be yolked, covered with a specialized egg shell, transferred to a specialized "brood chamber," and then buried into a specially dug hole (Zucker and others 2002). An egg-producing mutation in a male background would need to be accompanied by all of these additional changes to produce a viable hermaphrodite. On the other hand, the production of sperm in a female clam shrimp background merely requires that the ameboid sperms be released into the ovotestis where they can come into contact with and fertilize eggs. Thus it is likely that most transitions from dioecy toward hermaphroditism in animals with strongly differentiated sexes must progress through a female background, initially producing trioecy, but most likely quickly changing to androdioecy as the hermaphrodites (with the benefits of reproductive assurance) displace females (Wolf and Takebayashi 2004).

Once an androdioecious population has evolved from a dioecious ancestor, it can go 1 of 3 ways, assuming selective pressure for reproductive assurance continues. First, incremental changes in the various specialized male components may be "captured" in hermaphrodites to the point where the hermaphrodite can efficiently function both as a male and as a female, thus outcompeting males, which eventually results in the population becoming all hermaphroditic. Second, the population, under continued self-fertilization, may "purge" its genetic load, thereby eliminating inbreeding depression (Husband and Schemske 1996), that could in turn eliminate the need for outcrossing and thus provide a second mechanism in which the hermaphrodites can outcompete males. Third, if inbreeding depression cannot be purged (for a discussion of this see Byers and Waller 1999), and the ability of

hermaphrodites to develop all the required male phenotypes is constrained, then the population may maintain its "transitionary" androdioecious mating system. It is quite possible that *C. elegans* and *Rivulus marmoratus* correspond to the second of these 3 alternatives, and that males are being replaced by hermaphrodites across the range of these species. On the other hand, *E. texana* may match the third alternative: in *E. texana*, inbreeding depression is still quite substantial (Weeks and others 1999, 2000; Weeks and Bernhardt 2004) and has been shown to be unlikely to be purged (Weeks 2004). As noted above, the hermaphrodites continue to lack important male phenotypes (for example, claspers) that keep them from being successful at initiating outcrossing.

Promising future directions

In *Caenorhabditis*, more information about the ecology of the natural habitats and the population biology of these nematodes in the wild is paramount to understanding androdioecy. Ecologically, determining the significance of reproductive assurance is of importance, and could be evaluated by comparing the habitats of dioecious relative to androdioecious *Caenorhabditis*. For a population survey, it would be most informative to determine natural sex ratios and whether or not outcrossing is a significant component of natural reproduction. Additionally, it would be of interest to note whether inbreeding depression is important in natural populations. A greater understanding of the biology and ecology of the other androdioecious species in the family Rhabditidae would also be quite valuable.

For *Eulimnadia*, delineating the importance of reproductive assurance and the metapopulation processes outlined in Pannell's models (1997, 2000, 2002) would be useful. Additionally, a comparative study of dioecious and androdioecious clam shrimp in the family Limnadiidae could help to identify the important characteristics of the respective life histories that may correlate with these alternate reproductive modes. In Australia, dioecious *Limnadia* (sister genus to *Eulimnadia*; Baird 1849) exist in adjacent pools to androdioecious *Eulimnadia* (S.C.W., pers. obs.), and thus provide a unique opportunity to compare the habitats and life histories under similar conditions. Finally, the extent of androdioecy in the genus *Eulimnadia*, and whether any other genera in the family Limnadiidae have androdioecious species, would be useful for an understanding of how long-lived androdioecy may be in this group of crustaceans. At the moment, *Eulimnadia* appears to be the only animal group in which androdioecy is stable enough to

allow the taxon to maintain this reproductive mode for 24+ million years, despite having undergone multiple speciation events, with all species so far examined maintaining androdioecy (Weeks and others 2006). Thus, any theoretical or empirical studies that can explain this apparent anomaly would be fruitful to pursue.

In the other androdioecious taxa, understanding the specific mechanisms of sex determination would be informative. In the barnacles, a complete phylogeny of the Thoracica would be valuable. Such a phylogeny would allow a more complete understanding of the nature of the evolutionary development of androdioecy in *Balanus,* which would clarify whether any of the 4 androdioecious species were derived from an ancestral androdioecious species or if all 4 were independently (and thus recently) derived. A more thorough examination of the 4 species of *Ibla* would also be informative to note whether androdioecy is ancestral to the genus or a more recently derived character in a subset of these species. Additionally, resource allocation in the hermaphroditic barnacle species with complemental males should be thoroughly examined to note whether or not hermaphrodites associated with these males may be "cryptic females"; such an analysis of previously described androdioecious plants found that the hermaphrodites were functionally hermaphroditic but allocated virtually all their energy to the production of ovules (Charlesworth 1984). Much the same could be occurring in 1 or more of these androdioecious barnacles.

In *Rivulus marmoratus*, sampling a greater number of populations would be informative, as would a comparative survey of the habitat characteristics of the high- and low-male populations, to assess what factors may select for androdioecy. It would also be constructive to survey a wider array of populations with a variety of proportions of males in an attempt to determine whether males have evolved from hermaphrodites or if hermaphrodites have evolved from a dioecious precursor.

Our current knowledge of androdioecious mating systems provides an excellent foundation to explore the implications of this strategy in the evolution dioecy and hermaphroditism. We hope this review of androdioecious animals will help investigators to refine their own ideas as well as serve to encourage new research in this exciting area.

Acknowledgments

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