The Varied Ways of Being Male and Female

LUKAS SCHÄRER*

Evolutionary Biology, Zoological Institute, University of Basel, Basel, Switzerland

SUMMARY

Our understanding of sexual reproduction is mainly informed by research on gonochorists (i.e., species with separate sexes), including insects, birds, and mammals. But the male and female sexes are not two types of individuals; they actually represent two different reproductive strategies, and in many organisms, these two strategies are distributed among individuals in a population in a variety of ways. For example, sequential hermaphrodites (or sex-changers) exhibit one strategy early in life and later switch to the other, while simultaneous hermaphrodites exhibit both strategies at the same time. There are also many intermediate sexual systems that mix gonochorists and hermaphrodites in the same species and within many organismal groups, shifts occur between these sexual systems. A fascinating collection of six articles in this special issue on *Hermaphroditism & Sex Determination* impressively documents some important challenges to our understanding of sex determination, and the specification of male and female reproductive function when these need to occur within the same individual rather than in two separate individuals. Moreover, hermaphroditism changes how we need to think about reproductive allocation to sexual functions, how such allocation can be specified, as well as how the sexual system affects sexual conflict and the resulting antagonistic coevolution. Our understanding of sexual reproduction will profit greatly from exploring the varied ways of being male and female.

``We [should] dare to leave our gonochoristic islands and learn to swim in this vast sea of sexual diversity.''

*Corresponding author:
    Evolutionary Biology
    Zoological Institute
    University of Basel
    Vesalgasse 1
    4051 Basel, Switzerland.
    E-mail: lukas.scharer@unibas.ch

INTRODUCTION

When zoologists talk about sexual reproduction, this usually invokes images of an eager, armed, or ornamented male seeing off rival males or wooing often drabber, albeit choosy, females into mating with him. Much of what we know about sexual reproduction on the molecular level is dominated by studies on gonochoristic (separate-sexed) organisms, such as insects, birds, and mammals (including, of course, our own species), in which the above scenario is largely vindicated. What clearly emerges from the stimulating collection of six articles in this Special Issue, which highlight a range of hermaphroditic animals, is the striking extent to which these generic images of sexual reproduction do not do justice to the varied ways in which extant organisms exhibit the male and female sexual strategies. The hermaphroditic organisms covered in these six articles span all major subdivisions of the metazoans (the animals), including the early branching non-bilaterian metazoans (cnidarians), the Lophotrochozoa (flatworms and molluscs), the Ecdysozoa (nematodes), and the Deuterostomia (ascidians and fishes) (Philippe et al., 2009; Egger et al., 2015). In fact, hermaphroditism is even more widespread, and actually occurs in about two thirds of all animal phyla (Jarne and Auld, 2006).

To better understand this diversity, we should first unpack the term "sexual reproduction," and then clarify what we actually mean by "male" and "female," or more generally, what we mean by "the sexes." In eukaryotes, sexual reproduction involves the fusion of two haploid gametes to

form a diploid zygote, resulting in a genetically recombined individual. But importantly, and perhaps paradoxically, sexual reproduction does not require the sexes. Indeed, many sexually reproducing eukaryotes contribute an equal "dowry" to the sexual union—that is, the fusing gametes are equal in size, a state called isogamy (from the Greek iso-"equal' and gamos "marriage"); these organisms, for example, include yeasts, green algae, and brown algae (Lehtonen et al., 2016). What we tend to think of as the sexes arose from this isogamous state during the evolution of anisogamy (from the Greek aniso- "unequal"), where the emerging male and female sexual strategies involve, by definition, the production of many small or few large gametes, respectively (Parker et al., 1972). Although this process led to the emergence of the "male" and "female" sexual strategies, whether or not it led to the evolution of males and females (i.e., gonochoristic individuals that only produce one type of gamete throughout their life), as is often assumed in models (Lessells et al., 2009; Parker, 2011), is currently unclear. The evolution of anisogamy also may have led to the evolution of other sexual systems, such as sequential or simultaneous hermaphroditism (Schärer et al., 2014; Schärer and Ramm, 2016). Moreover, given that anisogamy has evolved separately in, for example, plants, animals, and green algae (Kirk, 2006), the male and female sexual strategies actually represent convergent phenomena (Schärer and Ramm, 2016), and the ancestral sexual systems might well have differed in the various incarnations. While only a single origin of anisogamy likely occurred at the base of the metazoans, even the basal metazoans show a striking variety of sexual systems.

What clearly emerges from the sexual diversity among metazoans is that the distribution of the sexes among individuals within a population can occur in a variety of ways, and that striking phylogenetic patterns underlie how they are distributed (Ghiselin, 1969; Jarne and Auld, 2006; Eppley and Jesson, 2008). Some organismal groups are either only gonochorists or hermaphrodites, while others show more variability. Striking differences in the manifestation of the sexes can be garnered from the organisms covered in this Special Issue, with sometimes high variability even among closely related species (cnidarians); some being almost exclusively hermaphroditic (flatworms and ascidians); others being largely gonochoristic (nematodes and fishes); while yet others show very strong phylogenetic patterns within certain subgroups (molluscs). Of note, the otherwise well-studied insects, birds, and mammals are strikingly absent here—with not a single species among these groups showing hermaphroditism (for details on a supposedly hermaphroditic scale insect, however, see Gardner and Ross, 2011). What has driven the emergence of these phylogenetic patterns is currently unclear, but the kinds of molecular developmental studies on sex determination and the specification of male and female reproductive function in these organisms presented here in this Special Issue will certainly be crucial to shed light on the processes involved.

The six articles in this Special Issue address three central topics that are important in the sex life of a hermaphrodite, although they do so to very different extents and depths, with some articles focussed on a single topic, whereas others touching on all three. The first topic deals with the developmental machinery that allows for the specification of both male and female sex functions within the same individual (either sequentially or simultaneously). The possibility of dual sexuality naturally precludes well-known mechanisms in gonochorists, such as genetic sex determination via sex chromosomes that drive circulating sex hormones that affect the sexual fate of the entire body (as, for example, occurs in our own species). As indicated in the articles, we are beginning to gain a reasonably good understanding of these processes in a few hermaphroditic organisms. The second topic deals with the important question of sex allocation, that is, how an individual determines the energy expenditure toward the male versus female sex function. In gonochorists, sex allocation concerns the sex ratio of the offspring, while in hermaphrodites it involves an individual's decision about the timing and direction of sex change (in sequential hermaphrodites) or the relative effort spent on male versus female function (in simultaneous hermaphrodites). Sex allocation research is a very successful branch of evolutionary biology (West et al., 2000), and given its central importance for thinking about sex, I briefly outline some of the most relevant considerations in the next paragraph. The third topic deals with the question of how individuals interact with each other during competition, courtship, mating, and fertilization, given that all individuals can be both male and female (and may make sex allocation decisions accordingly). The sexual roles of gonochoristic males and females are quite well-defined; by contrast, hermaphrodites exhibit fascinating additional options, as each individual can either try to play the male, the female, or both sexual roles during a sexual interaction, leading to some intriguing sexual conflicts between social and sexual partners (Charnov, 1979; Michiels, 1998). Many fascinating sexual adaptations, morphologies, and behaviors in hermaphrodites probably need to be seen in this light (Schärer et al., 2014).

As mentioned above, a central consideration underpinning anisogamous sexual reproduction is the economics of sex allocation (Charnov, 1982; Schärer, 2009; West, 2009). Each zygote results from the fusion of one sperm and one egg. Although the two sexes usually make a highly unequal material contributions, their nuclear genetic contribution to the zygote is necessarily equal—except in some unusual genetic systems (Normark, 2009). In other words, every offspring has exactly one father and exactly one mother, a truism sometimes called the Fisher condition (Houston and McNamara, 2005), which applies to all sexual systems. The seemingly simple Fisher condition has far-reaching consequences, given that the fitness obtained via the male and female sexual strategies must necessarily be exactly equal, which, under commonly made assumptions of random mating and large population size, means that the investment into male and female reproduction should be exactly equal as well (Düsing, 1884; Fisher, 1930; Charnov, 1982). While many biological and ecological situations exist under which these assumptions are broken—and where more careful and differentiated arguments based on sex

allocation theory are required to arrive at the correct predictions—the Fisher condition rules must still be obeyed. I do not intend to review sex allocation theory here, in part because the articles on flatworms and molluscs in this collection cover several relevant aspects (for more detailed reviews on gonochorists, sequential hermaphrodites, and simultaneous hermaphrodites, see Charnov, 1982; Hardy, 2002; Munday et al., 2006; Schärer, 2009; West, 2009). Instead, I simply point out that any decision about the investment into male or female reproductive function needs to be viewed in the context of sex allocation, and that the overproduction of one sex function necessarily alters the fitness pay-offs for the other sex function, leading to frequency-dependent selection and co-evolutionary feedbacks. In other words, one individual's sex allocation decision will tend to alter the pay-offs that other individuals can expect from their own sex allocation decisions. Thus, sex determination and sexual interaction both need to be considered in light of the Fisher condition and sex allocation theory.

In the following, I briefly highlight and comment on a few—admittedly haphazardly chosen—findings covered in the accompanying articles of this Special Issue. The nature of these findings differs considerably as a result of the articles' theses, available techniques, and life history strategies utilized by the different organismal groups. I also try to add, where appropriate, some broader considerations about what might potentially constrain the sexual systems within each group. Finally, I provide a brief outlook for future work in this expanding field.

CNIDARIANS

Siebert and Juliano (2016) provide a beautifully illustrated introduction to the striking diversity of sexual phenomena among the cnidarians, which show multiple shifts between sexual systems in many taxa. In a few cases, this diversity quite well understood at the molecular level, due to a number of well-established model systems, including hydrozoans (e.g. *Hydra magnipapillata* and *Clythia hemisphaerica*) and anthozoans (*Nematostella vectensis*).

One fascinating feature of cnidarians—which also applies to a number of other hermaphroditic groups, including plants, flatworms, ascidians, and bryozoans—is that the process of asexual proliferation involved in coloniality, budding, fission, and/or fragmentation can make it difficult to clearly conceptualize the "individual" (from Latin *in-* "not" and *dividuus* "divisible"). This clonal lifestyle calls into question our usual definitions, such as hermaphroditism being "a sexual system where individuals usually exhibit both the male and female sexual strategy, either at different times of their lives (sequential hermaphroditism) or at the same time (simultaneous hermaphroditism)" (Schärer and Ramm, 2016). Should our definitions of the sexual system depend on whether the asexually produced asexual ramets of a specific sexually produced genet stay in physical contact with each other (as in coloniality) as opposed to whether they separate after budding new polyps or zooids

and live "individually" afterward? Similarly, how is one to think about the "individual" medusae produced by a colony, given that they are genetically identical (except for mutations that have arisen since the single-celled zygote)? Moreover, distinguishing sequential from simultaneous hermaphroditism in such cases can be difficult, given that some of these asexually produced ramets show negligible aging and can, therefore, exist "individually" for a long time (Martínez, 1998) and may even change sex (see below).

Biologically speaking, there is no real problem if generally used terminology happens not to readily apply to a particular organismal group. After all, terms are always coined with specific organisms in mind, and such terms can, therefore, become ambiguous if one shifts to other organisms. But such shifts can certainly lead to communication problems if asked to translate findings among different fields, especially since people that work on more canonical systems often appear to show scant awareness of the existing diversity (see the Outlook section below). Instead, researchers must aim at defining terms very clearly, use them consistently, and recognize that all definitions probably gloss over some complexities. For example, I was initially confused by the fact that Siebert and Juliano (2016) talk about ramets when they refer to solitary cnidarians like *N. vectensis* in Figure 3A, until I became aware that there are in fact some populations of *N. vectensis* that have asexual reproduction by transverse fissioning (Hand and Uhlinger, 1992); thus, these solitary polyps are not necessarily sexually produced genets, as which I had erroneously seen them, but can also represent ramets of a given genet. So, the discovery (or in this case my realization) of asexual reproduction may influence the definition of the sexual system.

A number of experimental approaches have allowed impressive insights into these cnidarian systems, especially in freshwater hydrozoans, such as *H. magnipapillata*. Aggregation experiments with dissociated cells from multiple donors; grafting experiments allowing transplantation of tissues from donors to recipients (note that also natural chimeras can form in histocompatible colonies in some colonial hydroids, as occurs in tunicates); and experiments taking advantage of the fact that some cell populations can be killed off by a temperature shock before or after transplantation have all provided details attesting to the sexual potential of different cell populations. Insights stemming from such approaches include the existence of separate populations of male and female germ-line determining cell populations; the observation that the male stem cells are dominant over female stem cells in the gonochoristic and sequentially hermaphroditic species; and an understanding that the germ cells, not the surrounding somatic cells, are responsible for sex determination—although the latter may influence where the gonads form. Extending such experimental approaches to other organismal groups would be of interest, and, based on my understanding of the literature, similar experiments should be feasible in both flatworms and tunicates.

Other experiments revealed that in the hydrozoan *C. hemisphaerica*, sex is somewhat temperature-dependent

in that hermaphroditic colonies produce more female medusae at higher temperatures, though it is unclear to which degree such temperature effects might depend on the colony genotype, as only two hermaphrodite colonies were tested (Carré and Carré, 2000). It seems likely that the male- and female-determining germ cells have different temperature optima or that some inhibitive effects are temperature-dependent (Siebert and Juliano, 2016). And although rare, even medusae can be hermaphroditic: in *Clythia* species, for example, hermaphroditic medusae were observed when male and female medusae were kept in the laboratory for extended periods or when medusae were kept in extended isolation, as some initially female medusae became hermaphroditic, either simultaneously carrying both male and female gonads, or having hermaphroditic gonads, then later switching entirely to the male sex (Carré and Carré, 2000).

Unfortunately, we currently have a poor understanding of the fitness consequences of the observed variation in sexual systems, a fact that is actually acknowledged by the authors in their Perspectives section. I think that some cnidarian groups might be particularly suited to such investigations, such as corals, which have a long history of studies on sex allocation (e.g., Hall and Hughes, 1996) and fertilization biology (e.g., Lotterhos and Levitan, 2010). Other approaches would be to investigate the intraspecific variability in sexual forms in local populations, ideally including information about the relative frequencies of different sexual types and how that may vary seasonally. Currently, the available data often seem to rely on only a few laboratory strains, making these sexual strategies difficult to understand in an individual versus population sex allocation context.

FLATWORMS

Ramm (2016) provides a nice overview of the sexual diversity of flatworms, which are morphologically extremely variable and inhabit a striking range of ecological niches. A large number of quite well-studied genetic model organisms belong to this group of animals, ranging from the free-living planarian and *Macrostomum* spp. flatworms, to a number of parasitic groups that are relevant in the agricultural and medical context, such as schistosomes and tapeworms, because they cause considerable morbidity in both livestock and humans.

Much of the diversity and the often dazzlingly complex life cycles in parasitic flatworms are probably due to the neoblasts (Buss, 1987), pluripotent stem cells that represent the only proliferating cell population from which all differentiated cells directly emerge (Baguña et al., 1989; Ladurner et al., 2008; Wagner et al., 2011) (note that these stem cells are sometimes also called totipotent, but I consider it useful to distinguish that pluripotent cells can generate all cell types if they reside in the appropriate somatic cellular environment, whereas totipotent cells can generate all cell types from single isolated cells, such as in many plants). Neoblasts are also responsible for the impressive regenerative capacity of flatworms, and the many forms of asexual proliferation—ranging from fragmentation, over architomy, paratomy, budding, to highly complex asexual formation of zooids, proglottids, scolices, and a multitude of iterated larval stages. With very few exceptions (e.g., Gruber-Vodicka et al., 2011), however, these asexual phenomena are usually embedded in life histories that include sexual reproduction (Buss, 1987), which in some cases can be induced by external stimuli (e.g., Kobayashi et al., 1999).

Flatworms are predominantly hermaphroditic, with only a single gonochoristic group—the trematode family Schistosomatidae—showing considerable diversification. Other cases of gonochorism are scattered throughout the phylum, and are usually represented by small groups or single species at the tips of the phylogenies, surrounded by hermaphroditic ancestors. Thus, while shifts to gonochorism have occurred many times, these are either evolutionarily short-lived and/or tend to not diversify (note that the two alternatives could potentially be distinguished based on branch lengths in molecular phylogenies). This rarity of gonochorism in flatworms is puzzling, and seems to imply some fundamental constraints in their evolution—which apparently do not affect the schistosomes. A plausible explanation for why schistosomes appear to be an exception might be that they are actually less "separate-sexed" that initially meets the eye: the (much thinner) female lives in near-permanent association inside of a male's gynaecophoric canal (Neves et al., 2005), and receives from the male both food and other substances that are needed for proper female development. The tight functional integration of male and female schistosomes—proposed to have been selected in the context of a division of labor between the sexes, with the large males providing anchoring and motility while the thin females being capable of reaching very thin blood vessels (Basch, 1990; Després and Maurice, 1995) —may have relaxed the putative constraint in this family, allowing it time to diversify.

A useful feature of *Macrostomum* spp. flatworms is their transparency, which permits direct observation of many internal structures, such as the testes, ovaries, received sperm, and genital morphology, and permits whole-mount in situ hybridization assays (Arbore et al., 2015). This feature has enabled researchers to obtain quantitative estimates of reproductive allocation patterns, thus permitting tests of assumptions and predictions of sex allocation theory; indeed, these small flatworms are one of the best-studied hermaphrodites in this field (Schärer, 2009; Janicke et al., 2013; Schärer and Pen, 2013; Sekii et al., 2013).

Finally, intraspecific contrasts between sexuals and asexuals (e.g., planarians) (Wang et al., 2010; Chong et al., 2011), between males and females (e.g., schistosomes) (Verjovski-Almeida et al., 2003), or between individuals in different social environments (e.g., *Macrostomum lignano*) (Ramm et al., unpublished information) allow powerful approaches to identify genes involved in the different reproductive functions—particularly if such contrasts have independently arisen multiple times in different lineages. In combination with the establishment of RNA interference

techniques in a number of species, this has permitted to accelerate the discovery of genetic factors involved in flatworm reproduction.

MOLLUSCS

Koene (2016) provides an excellent entry-point about how to think about hermaphrodite reproduction, incorporating all three major topics discussed in the Introduction section. In terms of behavior and neurobiology snails are probably the best-understood simultaneous hermaphrodites, as there are many common land snails, such as the garden snail, *Cornu aspersum*, and several freshwater snails, including *Lymnaea stagnalis*, *Physa acuta*, and *Biomphalaria glabrata*, that have been studied in this context for decades. Yet, molluscs are arguably lagging somewhat behind some of the other hermaphroditic models, particularly in terms of detailed understanding of sex determination and the specification of male and female reproductive function at the molecular level. No well-established genetic model organisms exist in this group (although there is progress on several fronts)—a point that is mostly responsible for the current lack in molecular details. I will, therefore, highlight the behavioral aspects of hermaphroditism here, some of which involve molecular details of the effects of seminal fluids, for which, as far as hermaphroditic animals are concerned, research on molluscs is clearly leading the way.

Many studies have documented how the motivation to mate in the male and/or female sex role can vary as a function of a snail's physiological state, including as a result of sexual isolation (e.g., Van Duivenboden and Ter Maat, 1985; Dillon et al., 2011). In one case, sexual motivation has been experimentally shown to be mediated by a neurological signal about the fill grade of the seminal vesicle —the organ that contains produced sperm before it is transferred to a mating partner—by surgically cutting the relevant nerve (De Boer et al., 1997).

Elegant work by Koene and Schulenburg (2005) has clarified the biological function of a truly enigmatic behavior in land snails, namely the shooting of an elaborate, calcareous "love dart" through the skin of the mating partner before sperm transfer. In the garden snail, this behavior plays a role in delivering manipulating substances, so called allohormones (Koene and ter Maat, 2001), into the mating partner. Allohormones cause conformational changes in the female genital system (Koene and Chase, 1998), and in turn cause a greater proportion of the dart-shooter's sperm to be channelled into the sperm storage organ rather than into an organ where sperm is digested and destroyed (Koene, 2006). Moreover, successful dart-shooting was experimentally confirmed to lead to a higher paternity share, when a sperm donor was aided by the researchers by manually injecting the partner with a homogenate of the dart gland mucus using a syringe (Chase and Blanchard, 2006). Progress is being made in identifying the molecular players in these interactions (Koene, 2016; see also Stewart et al., 2016).

In more recent work, Koene and coworkers have elucidated the effects of specific, transferred seminal fluid substances on the mating behavior and subsequent sperm donation patterns of the sperm recipient in *L. stagnalis* (Nakadera et al., 2014). Interestingly, these effects were proposed to result from a sexual conflict between the mating partners over the optimal sex allocation of their partner (Nakadera et al., 2014; Schärer, 2014; Schärer and Ramm, 2016).

NEMATODES

The article by Ellis (2016), with its detailed schemes of molecular interactions involved in organogenesis and sex determination, makes clear that the best-studied case of hermaphroditism, from a molecular perspective, occurs in a number of nematode species, and thus in members of a phylum that is otherwise predominantly gonochoristic (Jarne and Auld, 2006). A somewhat unusual system of hermaphroditism is used by these nematodes, one in which individuals are either self-fertilizing protandrous sequential hermaphrodites (producing first sperm, which hang around long enough to fertilize the later-produced eggs) or outcrossing males that mate with these hermaphrodites. This sexual system can be seen as a form of androdioecy, although that is often defined as including the presence of both males and simultaneous hermaphrodites in the same population, in which both types might be able to outcross with other hermaphrodites (Pannell, 2002; Weeks et al., 2006).

Producing hermaphrodites in a normally gonochoristic species may require a switch from regulation that concerns the fate of the whole organism—such as the presence or absence of the male hormone HER-1 driving overall male or female development, respectively, in gonochoristic *Caenorhabditis* species—to a kind of regulation that addresses only specific parts of the body. This is achieved in *Caenorhabditis elegans* by translational regulation of mRNAs in the germ cells: a somatically female individual is locally induced to go through spermatogenesis early in life through the action of two factors, FOG-2 and GLD-1, that prevent the translation of *tra-2* mRNA, which is required for oogenesis. Interestingly, spermatogenesis in the related species *Caenorhabditis briggsae* is also induced by blocking TRA-2 activity, but the way in which this is achieved is mechanistically different, supporting multiple independent evolutionary origins of this sexual system in rhabditid nematodes (see also Fig. 1 in Ellis, 2016). By regulating the presence of translational regulators, individuals can modulate the time point at which a switch from spermatogenesis to oogenesis occurs, allowing some level of flexibility in this decision (Ellis, 2016). Similar localized processes are likely required to regulate sex allocation decisions in simultaneous hermaphrodites (Schärer, 2009), and possibly also in sequential hermaphrodites (see below).

Being able to perform the male function requires not only the ability to produce sperm, but also the follow-through to achieve sperm activation and capacitation. The activating

compounds seem to be added to the seminal fluid by the male *Caenorhabditis* nematodes during ejaculation, suggesting that the sperm activators are male derived. Consequently, while some gonochoristic *Caenorhabditis* species can be induced to become hermaphrodites that produce sperm (by knocking down *tra-2*), this does not automatically lead to self-fertility (Baldi et al., 2009; Wei et al., 2014). Thus, hermaphroditic species must have evolved ways to express the sperm activators in the female reproductive organs, namely the spermatheca; indeed, different methods are utilized among the different species, mirroring the outcome with respect to the activation of spermatogenesis (Ellis, 2016).

This nematode form of androdioecy (i.e., self-fertilizing sequential hermaphroditism with rare production of males) provides many of the benefits of self-fertilizing simultaneous hermaphroditism, such as being able to reproduce in isolation. The multiple independent transitions to this sexual system among rhabditid nematodes imply substantial selective pressures to obtain and maintain this system. This should not be so surprising, given that the ecology of these worms seems to involve the exploitation of temporally abundant food sources (Kiontke et al., 2011). What is striking, however, is that no case has been reported in which these or other nematodes have transitioned to outcrossing simultaneous hermaphroditism—which, in combination with facultative selfing, would combine the best of both worlds. Arguably, full hermaphroditic function would require the ability to both donate sperm to, and receive sperm from, a mating partner. Given that the hermaphrodite (and female) worms have two gonadal arms, one could hypothesize that a worm could dedicate one arm each to build the male or female sex organs, respectively.

Detailed knowledge of the ontogeny of the somatic gonads in *C. elegans* suggests that simultaneous hermaphroditism might be difficult to achieve: in the female, the anterior and posterior somatic gonad arms are largely built by the progeny of the Z1 and Z4 cells, respectively (although each cell also contributes a few of their progeny to the spermatheca of the alternate gonad arm, and both contribute to the syncytial spermatheca−uterine valve). In contrast, progeny of both the Z1 and Z4 cells are essentially contributing to all of the formed structures in the single-male somatic gonad, suggesting that a functional male system cannot be built exclusively by either progeny of the Z1 or Z4 cells (Kimble and Hirsh, 1979). The highly rigid development in these nematodes may thus represent a developmental constraint that blocks the evolution of fully functional simultaneous hermaphrodites in this group.

TUNICATES

In their beautifully illustrated, article Rodriguez et al. (2016) emphasize the features of a single species of tunicate, the cosmopolitan colonial ascidian, *Botryllus schlosseri*, which has been used as a laboratory model for many decades and to which we owe a lot of our knowledge of tunicates. Several other tunicates have also been studied in considerable detail, including the solitary ascidian *Ciona intestinalis*, which is mainly studied in the context of fertilization biology, embryology, metamorphosis, and neurobiology (Dehal et al., 2002); the colonial ascidian *Diplosoma listerianum*, which is used as a model for sexual selection in sperm-cast mating simultaneous hermaphrodites (e.g., Bishop, 1996; Hammerschmidt et al., 2011); and the appendicularian *Oikopleura dioica* (Denoeud et al., 2010), which not only has an unusually small genome for a metazoan ($\sim$70 megabases) but is also unusual among tunicates in having separate sexes.

The life cycle of *B. schlosseri* involves regular alternation between sexual and asexual reproduction, which has a number of interesting consequences. While *B. schlosseri* can clearly be considered a simultaneous hermaphrodite, the timing of male and female reproduction within the sexual cycle is actually somewhat temporally separated, with the testes forming first and ovaries only later. Moreover, the timing of sperm release and sperm receipt is also temporally separated—possibly to avoid selfing (Gasparini et al., 2015)—which allows the use of male- and female-functioning colonies in experimental setups (Yund, 1998) and permits the identification of genes involved in male and female function (Rodriguez et al., 2016). As for *Macrostomum* spp. flatworms, the transparency of the *B. schlosseri* colonies, when viewed through a glass slide on which they are grown, allows for quantification of testis size and thus male allocation, which has been used to test core assumptions and predictions of sex allocation theory (Yund and McCartney, 1994; Yund et al., 1997; Yund, 1998), both under controlled laboratory conditions and in the field.

The combination of asexual budding and sexual reproduction requires de novo establishment of germ cells for gonad development in each newly formed bud, which is affected by germs cells that migrate from existing zooids into the new buds, following a gradient of sphingosine-1-phosphate (Rodriguez et al., 2016), among other substances. Such de novo germ cell establishment can also lead to fascinating germ-line competition between genetically distinct colonies during so-called colony fusion events (Stoner et al., 1999). When lateral growth results in contact between two neighboring colonies (or if a colony encounters itself after growing around, for example, a seagrass leaf), the colonies either fuse to form a larger colony or reject the fusion and remain as separate colonies. Fusion occurs when the two colonies share at least one allele at the highly polymorphic *Fu/HC* histocompatibility locus (which is inevitable when the same colony meets, but may also happen between unrelated colonies). Upon fusion, the different fates of the germ line and soma can lead to an intriguing outcome, with one colony "winning" the germ line and the other "winning" the soma, in which case the somatic winner has essentially lost any way of contributing to the next generation and can thus be considered reproductively dead, albeit somatically alive (Stoner et al., 1999). Such colony fusions might allow the study of whether specific sex allocation patterns are determined by the somatic environment or the stem cells.

FISHES

As outlined by Liu et al. (2016), hermaphroditism is widespread among fishes, with estimates ranging from 7 to 9 orders and 20 to 27 families—although in terms of absolute numbers, only about 2% of fish species are thought to be hermaphroditic (Sadovy de Mitcheson and Liu, 2008; Avise and Mank, 2009). The observed phylogenetic distribution clearly reveals that hermaphroditism has independently arisen many times among fishes. Interestingly, nearly all known hermaphroditic fishes inhabit marine environments; by comparison, freshwater species make up around 40% of all known fish species (Cohen, 1970), and include some enormously diverse and well-studied groups, such as the cyprinids, characids, and cichlids (Sadovy de Mitcheson and Liu, 2008). Remarkable exceptions to this pattern are species among the swamp eels (Synbranchidae), several of which are protogynous hermaphrodites (Sadovy de Mitcheson and Liu, 2008; Liu et al., 2016), and also a perciform sea bass, *Lates calcarifer*, which has been shown to be protandrous (Sadovy de Mitcheson and Liu, 2008; Liu et al., 2016)—although that species is actually catadromous, spending much time in freshwater, but eventually reproducing in the marine environment. Nevertheless, why hermaphroditism apparently evolved relatively easily in marine fishes, but not in freshwater fishes, is of considerable interest with respect to the identification of the relevant constraints involved; the exceptions might represent useful test cases to evaluate the potential constraints.

Experimental hormone manipulations have identified which hormones are ultimately responsible for gonadal sex change in sequentially hermaphroditic fishes, both in vivo and in vitro (summarized in Liu et al., 2016). Specifically, the circulating androgen testosterone is either enzymatically converted to 17β-estradiol (by aromatase) or 11-ketotesterone (by 11β-hydroxylase and 11β-hydroxysteroid dehydrogenase), leading to production of oocytes or sperm, respectively, in the gonads. The challenge currently lies in identifying how the sensory input perceived by an individual fish is translated into the necessary gene-regulatory changes that then affect these hormonal changes. Liu et al. (2016) provide a detailed summary of the relevant research, mostly in *Thalassoma bifasciatum*, which has already uncovered a number of players from known sex determination and neuroendocrine signalling pathways used in response to changes in the (social) environment in other vertebrates. The authors also present testable hypotheses and a clear roadmap for future work to understand the different sex-changing behaviors linked to social hierarchies.

In my view, an additional challenge is to explore how not only the qualitative regulation of (complete) sex change is controlled in sequential hermaphrodites, but also how the fine-tuned quantitative adjustments involved in sex allocation decisions of simultaneously hermaphroditic fishes are affected. Simultaneous hermaphroditism occurs in at least four orders and in about one dozen fish families (Sadovy de Mitcheson and Liu, 2008; Avise and Mank, 2009), and it co-occurs with sequential hermaphroditism within several taxa, such as the Gobiidae, Serraninae, Muraenidae, and in some cases even within the same species, such as *Serranus fasciatus* (Petersen, 1990) and *Lythrypnus dalli* (St. Mary, 2000). In such cases, drastic shifts that systemically affect the whole body via circulating hormones are difficult to imagine, as gonads will often have to produce both gametes simultaneously, presumably necessitating a more fine-tuned and/or local regulation.

Finally, a major challenge for this field appears to be a lack of established laboratory model species in which sex change can be studied in behavioral, physiological, and molecular detail—especially in the context of functional and experimental studies. The highly complex social environments of many of these sex-changing species will likely be difficult to replicate in the laboratory, although even the field-oriented studies conducted to date may be biased in that they tend to explore only a subset of the possible social environments. For example, many *T. bifasciatum* studies have focused on small populations of a few dozen individuals living on small patch reefs (e.g., Warner and Swearer, 1991), whereas its sexual system is highly dependent on reef size (Warner and Hoffman, 1980a). In large, contiguous reefs, populations of tens of thousands of individuals can exist (Warner, 1995), for which the sexual system transitions into a state that is nearly gonochoristic (i.e., close to a 50:50 sex ratio among group-spawning individuals) and where territorial defence and pair-spawning becomes a less economical strategy (Warner and Hoffman, 1980b), so sex-switching becomes rather rare, dropping from 12% to less than 1% of the population (Warner and Hoffman, 1980a). Given the relative abundance of contiguous versus patch reef habitats, most *T. bifasciatum* individuals likely live and reproduce in fairly large populations (Warner, 1995).

OUTLOOK

Much of the literature on the evolution of sexual reproduction confounds the phenomena of male and female sexual strategies with the phenomenon of males and females, and many people who work on gonochoristic organisms—which, of course, match the general terminology—may assume that the other kinds of organisms are either weird, rare, or both (see also Aanen et al., 2016). The six articles in this Special Issue clearly show that other sexual systems are widespread—a pattern that only becomes more true if one also considers, fungi, plants, algae, and the staggering diversity of single-celled and multi-cellular eukaryotes (Keeling et al., 2005).

The emergence of -omics tools and massively parallel sequencing approaches has led to a welcome democratization to the detailed study of molecular mechanisms in "non-model" organisms. I anticipate that we will learn a lot about the molecular underpinnings of sexual reproduction over the coming years, and from a much broader range of organismal groups than hitherto possible. However, -omics alone will not suffice, as a detailed understanding also requires establishment of techniques that permit

functional tests of candidate factors. Several authors here express the hope that powerful genome editing techniques will pave the way toward functional experimentation—although such transgenic approaches will hopefully remain restricted to laboratory for the foreseeable future.

In summary, a deeper understanding of the evolution of anisogamy and its consequences for sexual reproduction now urgently requires that we dare to leave our gonochoristic islands and learn to swim in the vast sea of sexual diversity. Exciting times are ahead.

ACKNOWLEDGMENTS

REFERENCES

Aanen D, Beekman M, Kokko H. 2016. Weird sex: The underappreciated diversity of sexual reproduction. Phil Trans R Soc Lond B 371:20160262.

Arbore R, Sekii K, Beisel C, Ladurner P, Berezikov E, Schärer L. 2015. Positional RNA-seq identifies candidate genes for phenotypic engineering of sexual traits in *Macrostomum lignano*. Front Zool 12:14.

Avise JC, Mank JE. 2009. Evolutionary perspectives on hermaphroditism in fishes. Sex Dev 3:152−163.

Baguña J, Saló E, Auladell C. 1989. Regeneration and pattern formation in planarians. III. Evidence that the neoblasts are totipotent stem cells and the source of blastema cells. Development 107:77−86.

Baldi C, Cho S, Ellis RE. 2009. Mutations in two independent pathways are sufficient to create hermaphroditic nematodes. Science 326:1002−1005.

Basch PF. 1990. Why do schistosomes have separate sexes? Parasitol Today 6:160−163.

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

Buss LW. 1987. The evolution of individuality. Princeton, New Jersey: Princeton University Press.

Carré D, Carré C. 2000. Origin of germ cells, sex determination, and sex inversion in medusae of the genus *Clytia* (Hydrozoa, Leptomedusae): The influence of temperature. J Exp Zool 287:233−242.

Charnov EL. 1979. Simultaneous hermaphroditism and sexual selection. Proc Natl Acad Sci USA 76:2480−2484.

Charnov EL. 1982. The theory of sex allocation. Princeton, NJ, USA: Princeton University Press.

Chase R, Blanchard KC. 2006. The snail's love-dart delivers mucus to increase paternity. Proc R Soc Lond B 273:1471−1475.

Chong T, Stary JM, Wang Y, Newmark PA. 2011. Molecular markers to characterize the hermaphroditic reproductive system of the planarian *Schmidtea mediterranea*. BMC Dev Biol 11:69.

Cohen DM. 1970. How many recent fishes are there? Proc Calif Acad Sci 38:341−346.

De Boer PACM, Jansen RF, Koene JM, Ter Maat A. 1997. Nervous control of male sexual drive in the hermaphroditic snail *Lymnaea stagnalis*. J Exp Biol 200:941−951.

Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, Davidson B, Di Gregorio A, Gelpke M, Goodstein DM, Harafuji N, Hastings K, Ho I, Hotta K, Huang W, Kawashima T, Lemaire P, Martinez D, Meinertzhagen IA, Necula S, Nonaka M, Putnam N, Rash S, Saiga H, Satake M, Terry A, Yamada L, Wang HG, Awazu S, Azumi K, Boore J, Branno M, Chin-Bow S, DeSantis R, Doyle S, Francino P, Keys DN, Haga S, Hayashi H, Hino K, Imai KS, Inaba K, Kano S, Kobayashi K, Kobayashi M, Lee BI, Makabe KW, Manohar C, Matassi G, Medina M, Mochizuki Y, Mount S, Morishita T, Miura S, Nakayama A, Nishizaka S, Nomoto H, Ohta F, Oishi K, Rigoutsos I, Sano M, Sasaki A, Sasakura Y, Shoguchi E, Shin-i T, Spagnuolo A, Stainier D, Suzuki MM, Tassy O, Takatori N, Tokuoka M, Yagi K, Yoshizaki F, Wada S, Zhang C, Hyatt PD, Larimer F, Detter C, Doggett N, Glavina T, Hawkins T, Richardson P, Lucas S, Kohara Y, Levine M, Satoh N, Rokhsar DS. 2002. The draft genome of *Ciona intestinalis*: Insights into chordate and vertebrate origins. Science 298:2157−2167.

Denoeud F, Henriet S, Mungpakdee S, Aury J, Da Silva C, Brinkmann H, Mikhaleva J, Olsen L, Jubin C, Cañestro C, Bouquet J, Danks G, Poulain J, Campsteijn C, Adamski M, Cross I, Yadetie F, Muffato M, Louis A, Butcher S, Tsagkogeorga G, Konrad A, Singh S, Jensen M, Huynh Cong E, Eikeseth-Otteraa H, Noel B, Anthouard V, Porcel B, Kachouri-Lafond R, Nishino A, Ugolini M, Chourrout P, Nishida H, Aasland R, Huzurbazar S, Westhof E, Delsuc F, Lehrach H, Reinhardt R, Weissenbach J, Roy S, Artiguenave F, Postlethwait J, Manak J, Thompson E, Jaillon O, Du Pasquier L, Boudinot P, Liberles D, Volff J, Philippe H, Lenhard B, Roest Crollius H, Wincker P, Chourrout D. 2010. Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. Science 330:1381−1385.

Després L, Maurice S. 1995. The evolution of dimorphism and separate sexes in schistosomes. Proc R Soc Lond B 262:175−180.

Dillon RT, Jr., Wethington AR, Lydeard C. 2011. The evolution of reproductive isolation in a simultaneous hermaphrodite, the freshwater snail *Physa*. BMC Evol Biol 11:144.

Düsing C. 1884. Die Regulierung des Geschlechtsverhältnisses. Jena Z Naturwiss 17:593−940.

Egger B, Lapraz F, Tomiczek B, Müller S, Dessimoz C, Girstmair J, Škunca N, Rawlinson KA, Cameron CB, Beli E, Todaro MA, Gammoudi M, Noreña C, Telford MJ. 2015. A transcriptomic-phylogenomic analysis of the evolutionary relationships of flatworms. Curr Biol 25:1347−1353.

Ellis RE. 2016. The persistence of memory''—Hermaphroditism in nematodes. Mol Reprod Dev [Epub ahead of print], DOI: 10.1002/mrd.22668

Eppley SM, Jesson LK. 2008. Moving to mate: The evolution of separate and combined sexes in multicellular organisms. J Evol Biol 21:727−736.

Fisher RA. 1930. The genetical theory of natural selection. Oxford, UK: Oxford University Press.

Gardner A, Ross L. 2011. The evolution of hermaphroditism by an infectious male-derived cell lineage: An inclusive-fitness analysis. Am Nat 178:191−201.

Gasparini F, Manni L, Cima F, Zaniolo G, Burighel P, Caicci F, Franchi N, Schiavon F, Rigon F, Campagna D, Ballarin L. 2015. Sexual and asexual reproduction in the colonial ascidian Botryllus schlosseri. Genesis 53:105−120.

Ghiselin MT. 1969. The evolution of hermaphroditism among animals. Q Rev Biol 44:189−208.

Gruber-Vodicka HR, Dirks U, Leischa N, Baranyi C, Stoecker K, Bulgheresi S, Heindl NR, Horn M, Lott C, Loy A, Wagner M, Ott J. 2011. Paracatenula, an ancient symbiosis between thiotrophic Alphaproteobacteria and catenulid flatworms. Proc Natl Acad Sci USA 108:12078−12083.

Hall VR, Hughes TP. 1996. Reproductive strategies of modular organisms: Comparative studies of reef-building corals. Ecology 77:950−963.

Hammerschmidt K, Pemberton AJ, Michiels NK, Bishop JDD. 2011. Differential maternal allocation following mixed insemination contributes to variation in oocyte size in a sea squirt. Mar Ecol Prog Ser 422:123−128.

Hand C, Uhlinger KR. 1992. The culture, sexual and asexual reproduction, and growth of the sea-anemone Nematostella vectensis. Biol Bull 182:169−176.

Hardy ICW, editor. 2002. Sex ratios: Concepts and research methods. Cambridge: Cambridge University Press.

Houston AI, McNamara JM. 2005. John Maynard Smith and the importance of consistency in evolutionary game theory. Biol Philos 20:933−950.

Janicke T, Marie-Orleach L, De Mulder K, Berezikov E, Ladurner P, Vizoso DB, Schärer L. 2013. Sex allocation adjustment to mating group size in a simultaneous hermaphrodite. Evolution 67:3233−3242.

Jarne P, Auld JR. 2006. Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals. Evolution 60:1816−1824.

Keeling PJ, Burger G, Durnford DG, Lang BF, Lee RW, Pearlman RE, Roger AJ, Gray MW. 2005. The tree of eukaryotes. Trends Ecol Evol 20:670−676.

Kimble J, Hirsh D. 1979. The postembryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans. Dev Biol 70:396−417.

Kiontke KC, Félix M-A, Ailion M, Rockman MV, Braendle C, Pénigault J-B, Fitch DHA. 2011. A phylogeny and molecular barcodes for Caenorhabditis, with numerous new species from rotting fruits. BMC Evol Biol 11:339.

Kirk DL. 2006. Oogamy: Inventing the sexes. Curr Biol 16:1028−1030.

Kobayashi K, Koyanagi R, Matsumoto M, Hoshi M. 1999. Switching from asexual to sexual reproduction in the planarian Dugesia ryukyuensis. Invertebr Reprod Dev 36:153−158.

Koene JM. 2006. Tales of two snails: Sexual selection and sexual conflict in Lymnaea stagnalis and Helix aspersa. Integr Comp Biol 46:419−429.

Koene JM. 2016. Sex determination and gender expression: Reproductive investment in snails. Mol Reprod Dev [Epub ahead of print], DOI: 10.1002/mrd.22662

Koene JM, Chase R. 1998. Changes in the reproductive system of the snail Helix aspersa caused by mucus from the love dart. J Exp Biol 201:2313−2319.

Koene JM, Schulenburg H. 2005. Shooting darts: Co-evolution and counter-adaptation in hermaphroditic snails. BMC Evol Biol 5:25.

Koene JM, ter Maat A. 2001. ''Allohormones'': A class of bioactive substances favoured by sexual selection. J Comp Physiol A 187:323−326.

Ladurner P, Egger B, De Mulder K, Pfister D, Kuales G, Salvenmoser W, Schärer L. 2008. The stem cell system of the basal flatworm Macrostomum lignano. In: Bosch TCG, editor. Stem cells: From Hydra to man. Berlin: Springer. pp 75−94.

Lehtonen J, Kokko H, Parker GA. 2016. What do isogamous organisms teach us about sex and the two sexes? Phil Trans R Soc Lond B 371:20150532.

Lessells CM, Snook RR, Hosken DJ. 2009. The evolutionary origin and maintenance of sperm: Selection for a small, motile gamete mating type. In: Birkhead TR, Hosken DJ, Pitnick S, editors. Sperm biology: An evolutionary perspective. Burlington, MA, USA: Academic Press. pp 69−149.

Liu H, Todd EV, Lokman PM, Lamm MS, Godwin JR, Gemmell NJ. 2016. Sexual plasticity: A fishy tale. Mol Reprod Dev [Epub ahead of print], DOI: 10.1002/mrd.22691

Lotterhos KE, Levitan DR. 2010. Gamete release and spawning behavior in broadcast spawning marine invertebrates. In: Leonard JL, Córdoba-Aguilar A, editors. The evolution of primary sexual characters in animals. Oxford: Oxford University Press. pp 99−120.

Martínez DE. 1998. Mortality patterns suggest lack of senescence in *Hydra*. Exp Gerontol 33:217−225.

Michiels NK. 1998. Mating conflicts and sperm competition in simultaneous hermaphrodites. In: Birkhead TR, Møller AP, editors. Sperm competition and sexual selection. London, England: Academic Press. pp 219−254.

Munday PL, Buston PM, Warner RR. 2006. Diversity and flexibility of sex-change strategies in animals. Trends Ecol Evol 21:89−95.

Nakadera Y, Swart EM, Hoffer JNA, den Boon O, Ellers J, Koene JM. 2014. Receipt of seminal fluid proteins causes reduction of male investment in a simultaneous hermaphrodite. Curr Biol 24:859−862.

Neves RH, Biolchini CD, Machado-Silva JR, Carvalho JJ, Branquinho TB, Lenzi HL, Hulstijn M, Gomes DC. 2005. A new description of the reproductive system of *Schistosoma mansoni* (Trematoda: Schistosomatidae) analyzed by confocal laser scanning microscopy. Parasitol Res 95:43−49.

Normark BB. 2009. Unusual gametic and genetic systems. In: Birkhead TR, Hosken DJ, Pitnick S, editors. Sperm biology: An evolutionary perspective. Burlington, MA, USA: Academic Press. pp 507−538.

Pannell JR. 2002. What is functional androdioecy? Funct Ecol 16:862−865.

Parker GA. 2011. The origin and maintenance of two sexes (anisogamy), and their gamete sizes by gamete competition. In: Togashi T, Cox PA, editors. The evolution of anisogamy: A fundamental phenomenon underlying sexual selection. Cambridge: Cambridge University Press. pp 17−74.

Parker GA, Smith VGF, Baker RR. 1972. Origin and evolution of gamete dimorphism and male-female phenomenon. J Theor Biol 36:529−553.

Petersen CW. 1990. The relationships among population density, individual size, mating tactics, and reproductive success in a hermaphroditic fish, *Serranus fasciatus*. Behaviour 113:57−80.

Philippe H, Derelle R, Lopez P, Pick K, Borchiellini C, Boury-Esnault N, Vacelet J, Renard E, Houliston E, Quéinnec E, Silva CD, Wincker P, Guyader HL, Leys S, Jackson DJ, Schreiber F, Erpenbeck D, Morgenstern B, Wörheide G, Manuel M. 2009. Phylogenomics revives traditional views on deep animal relationships. Curr Biol 19:706−712.

Ramm SA. 2016. Exploring the sexual diversity of flatworms: Ecology, evolution, and the molecular biology of reproduction. Mol Reprod Dev [Epub ahead of print], DOI: 10.1002/mrd.22669

Rodriguez D, Kassmer SH, De Tomaso AW. 2016. Gonad development and hermaphroditism in the ascidian *Botryllus schlosseri*. Mol Reprod Dev [Epub ahead of print], DOI: 10.1002/mrd.22661

Sadovy de Mitcheson Y, Liu M. 2008. Functional hermaphroditism in teleosts. Fish Fisheries 9:1−43.

Schärer L. 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. Evolution 63:1377−1405.

Schärer L. 2014. Evolution: Don't be so butch, dear! Curr Biol 24: R311−R313.

Schärer L, Janicke T, Ramm SA. 2014. Sexual conflict in hermaphrodites. Cold Spring Harb Perspect Biol DOI: 10.1101/cshperspect.a017673

Schärer L, Pen I. 2013. Sex allocation and investment into pre- and postcopulatory traits in simultaneous hermaphrodites: The role of polyandry and local sperm competition. Phil Trans R Soc Lond B 368:20120052.

Schärer L, Ramm SA. 2016. Hermaphrodites. In: Kliman R, editor. Encyclopedia of evolutionary biology. Oxford: Elsevier. pp 212−224.

Sekii K, Vizoso DB, Kuales G, De Mulder K, Ladurner P, Schärer L. 2013. Phenotypic engineering of sperm-production rate confirms evolutionary predictions of sperm competition theory. Proc R Soc Lond B 280:20122711.

Siebert S, Juliano CE. 2016. Sex, polyps, and medusae: Determination and maintenance of sex in cnidarians. Mol Reprod Dev [Epub ahead of print], DOI: 10.1002/mrd.22690

St. Mary CM. 2000. Sex allocation in *Lythrypnus* (Gobiidae): variations on a hermaphrodite theme. Env Biol Fish 58:321−333.

Stewart MJ, Wang T, Koene JM, Storey KB, Cummins SF. 2016. A "love" dart allohormone identified in the mucous glands of hermaphroditic land snails. J Biol Chem 291:7938−7950.

Stoner DS, Rinkevich B, Weissman IL. 1999. Heritable germ line and somatic cell lineage competitions in chimeric colonial protochordates. Proc Natl Acad Sci USA 96: 9148−9153.

Van Duivenboden YA, Ter Maat A. 1985. Masculinity and receptivity in the hermaphrodite pond snail, *Lymnaea stagnalis*. Anim Behav 33:885−891.

Verjovski-Almeida S, DeMarco R, Martins EAL, Guimaraes PEM, Ojopi EPB, Paquola ACM, Piazza JP, Nishiyama MY, Kitajima JP, Adamson RE, Ashton PD, Bonaldo MF, Coulson PS, Dillon GP, Farias LP, Gregorio SP, Ho PL, Leite RA, Malaquias LCC, Marques RCP, Miyasato PA, Nascimento A, Ohlweiler FP, Reis EM, Ribeiro MA, Sa RG, Stukart GC, Soares MB, Gargioni C, Kawano T, Rodrigues V, Madeira A, Wilson RA, Menck CFM, Setubal JC, Leite LCC, Dias-Neto E. 2003. Transcriptome analysis of the acoelomate human parasite *Schistosoma mansoni*. Nature Genet 35:148−157.

Wagner DE, Wang IE, Reddien PW. 2011. Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. Science 332:811−816.

Wang Y, Stary JM, Wilhelm JE, Newmark PA. 2010. A functional genomic screen in planarians identifies novel regulators of germ cell development. Genes Dev 24:2081−2092.

Warner RR. 1995. Large mating aggregations and daily long-distance spawning migration in the bluehead wrasse, *Thalassoma bifasciatum*. Env Biol Fish 44:337−345.

Warner RR, Hoffman SG. 1980a. Local population size as a determinant of mating system and sexual composition in two tropical marine fishes (*Thalassoma* spp.). Evolution 34:508−518.

Warner RR, Hoffman SG. 1980b. Population density and the economics of territorial defense in a coral reef fish. Ecology 61:772−780.

Warner RR, Swearer SE. 1991. Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). Biol Bull 181:199−204.

Weeks SC, Benvenuto C, Reed SK. 2006. When males and hermaphrodites coexist: A review of androdioecy in animals. Integr Comp Biol 46:449−464.

Wei Q, Zhao Y, Guo Y, Stomel J, Stires R, Ellis RE. 2014. Co-option of alternate sperm activation programs in the evolution of self-fertile nematodes. Nat Commun 5:5888.

West SA. 2009. Sex allocation. Princeton, NJ, USA: Princeton University Press.

West SA, Herre EA, Sheldon BC. 2000. The benefit of allocating sex. Science 290:288−290.

Yund PO. 1998. The effect of sperm competition on male gain curves in a colonial marine invertebrate. Ecology 79:328−339.

Yund PO, Marcum Y, Stewart-Savage J. 1997. Life-history variation in a colonial ascidian: Broad-sense heritabilities and trade-offs in allocation to asexual growth and male and female reproduction. Biol Bull 192:290−299.

Yund PO, McCartney MA. 1994. Male reproductive success in sessile invertebrates: Competition for fertilizations. Ecology 75:2151−2167.

MRD