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Sex determination in *Bonellia viridis* (Echiura: Bonelliidae): population dynamics and evolution

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In the echiuran worm *Bonellia viridis* Rolando, the vast majority of sexually undifferentiated larvae metamorphose into dwarf males that live inside the female when exposed to females, but differentiate into females when developing in the absence of females. By means of a spatially explicit, individual-based model we examine how this specific form of environmental sex determination (ESD) affects dynamics of *Bonellia* populations and investigate the selective advantage of ESD over the more widespread genotypic sex determination (GSD). Population dynamics of *Bonellia* appear rather simple and not too sensitive to parameter changes around their measured values, or to changes in distribution and sizes of inhabitable patches. Starting even from low sizes, populations soon attain equilibrium densities. Explored aspects of population dynamics indicate an advantage of ESD over GSD. Moreover, simulated invasibility experiments show that while the maternal inheritance scenario allows for fixation of GSD under some limited conditions, both the classical and proportional inheritance scenarios always lead to fixation of ESD in the population. We also show that only the ability of ESD larvae to adapt their ultimate sex both in competition for empty burrows and for mating within females gives them a competitive edge over nonadaptive response to feminising and/or masculinising signals and generally leads to fixation of ESD by small step evolution. The original hypothesis of Charnov and Bull thus needs to be refined in the sense that along with females forming an unpredictable resource for males, empty burrows are an unpredictable resource for females.

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Essentially, there are two mechanisms that produce males and females in sexually reproducing species. In most of them, sex is determined at or before conception, as in male or female heterogamy. This mechanism is often referred to as genotypic (or syngamic) sex determination (GSD). In a much lower number of species, environmental (or metagamic) sex determination (ESD) causes offspring to become male or female in response to some environmental factor during development, i.e. later than at conception (Charnov and Bull 1977). The best studied examples of ESD come from reptiles (Bull 1980, Deeming and Ferguson 1988, Johnston et al. 1995).

Other examples are known in fish (Conover and Heins 1987, Römer and Beisenherz 1996), crustaceans (Naylor et al. 1988, Becheikh et al. 1998), nematodes (Anya 1976), and even plants (Charnov and Bull 1977).

A classical example of ESD among marine invertebrates is the echiuran worm *Bonellia viridis* Rolando (Echiura: Bonelliidae) (Baltzer 1931, Dawydoff 1959, Pilger 1978, Jaccarini et al. 1983). In this species, the vast majority of sexually undifferentiated larvae metamorphose into males when exposed to females, but differentiate into females when developing in the absence of females. The existence of ESD in *B. viridis* was disputed

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by Wilczynski (1960, 1968) who argued for and presented evidence supporting normal GSD in this animal. However, Leutert (1974) re-examined the case, refuted Wilczynski's evidence, and confirmed ESD in this species. Later on, Jaccarini et al. (1983) repeated and extended some of the classical experiments on sex determination in *B. viridis* to exclude interactions between larvae (a confounding factor in previous studies), and showed conclusively that sex determination is not fixed at fertilisation but is the result of an interaction between genetic and environmental factors. However, although it is now established that ESD does occur in *B. viridis*, its selective advantage has not been fully clarified yet. No previous study has explicitly investigated the evolutionary significance of ESD in this species, beyond a short verbal argument provided in Charnov and Bull's (1977) influential paper.

In this paper, we develop a population dynamic model of *B. viridis*, parameterise it with empirical data obtained from experiments as well as observations of this species in nature and in aquarium culture, and address two main questions: (i) how does ESD affect population dynamics of *B. viridis* and (ii) why is ESD maintained in *B. viridis* and what evolutionary mechanisms are likely to underlie this unique form of sex determination.

Methods

The species

Bonellia viridis is a common northeastern Atlantic and Mediterranean marine invertebrate that lives on hard bottoms in the infralittoral zone (Stephen and Edmonds 1972), and is renowned for its extreme sexual dimorphism (Baltzer 1931, Dawydoff 1959). Females have a cylindrical trunk that can be up to 8 cm long, and a prostomium in the form of a bifid proboscis that cannot be retracted into the trunk but which can be extended to over 1.5 m (Jaccarini and Schembri 1977a). In contrast, males are dwarf planarian like organisms that are only some 1–3 mm long and which behave like testicular parasites: they are initially fixed to the proboscis or trunk (Agius 1979) of the female but later enter and permanently inhabit the androecium, which is the anterior part of the modified nephridium of females that serves as a receptacle for the dwarf males (Jaccarini et al. 1983). The males reside in the androecium until required to fertilise the eggs (Baltzer 1931, Dawydoff 1959). In the laboratory, female *Bonellia* collected from Malta spawned from June to January each year (Schembri 1977). Individuals did not seem to spawn more than once during this period, so on average each female probably only spawns once a year, producing an egg string with some 1000 eggs at each spawning (Schembri 1977). The egg string is kept in the female's

burrow until the eggs hatch into trochophore larvae. Hatching is more or less simultaneous and takes place within 48 h from spawning (at 20°C). From hatching to day 7, the larvae are initially planktonic (although remaining close to the bottom) but from day 7 onwards they start settling on the bottom in loose aggregates, which are apparently not a result of an active swarming behaviour, but rather of their limited dispersal distances. Female *Bonellia* from Malta take at least two years to reach maturity. There are no data available on annual male and female mortality; in the laboratory, females were kept for a continuous period of three years without noticing any significant mortality (P. J. Schembri, unpubl.).

Bonellia viridis has also attracted considerable attention for its mechanism of sex determination (Charnov and Bull 1977). In a small fraction of larvae, sex determination is genetic (i.e. the larvae are syngamic); these larvae are predetermined to become either male or female (Jaccarini et al. 1983). In the remaining larvae, sex is determined environmentally (i.e. the larvae are metagamic); the environmental factor that triggers sex determination is the presence or absence of a masculinising stimulus normally due to a substance produced by females. Larvae exposed to females or their secretions thus metamorphose into males, while those that are not metamorphose into females. However, some larvae become intersexes (individuals showing a mosaic of male and female characteristics) and others stay permanently undifferentiated; both these types cannot reproduce and soon die (Jaccarini et al. 1983). Spontaneous male-to-female sex ratio of *Bonellia* larvae developing in isolation is rather low (Jaccarini et al. 1983). On the other hand, adult sex ratio is more or less male biased under natural conditions. Up to 85 males have been found to settle on the proboscis of a single female (Vollrath 1998), although on average only up to four of these males have been found to occur in the androecium (Jaccarini et al. 1983).

In the Mediterranean, *B. viridis* live on rocky bottoms where they seem to prefer rock bordering sandy substrata, or rock with small pockets of sediment or covered by fine layers of silt. The worms occupy spaces within piles of boulders or take over abandoned burrows in the rock excavated by other animals (Schembri and Jaccarini 1978). They do not burrow in the sediment itself but extend their proboscis over the sediment to feed on it, the females being deposit feeders (Jaccarini and Schembri 1977b). *Bonellia* therefore occur on heterogeneous bottoms and their distribution is highly clumped since they only occur on the rocky component of such bottoms. Also, female *Bonellia* are quite mobile and may change their burrows frequently. Extrapolating from laboratory observations of female *B. viridis* kept in artificial burrows, females will change from one burrow to another as often as every few days (Schembri 1977).

On the other hand, Wilczynski (1960) observed that once individuals of *B. viridis* kept in an aquarium were provided with hollowed rocks, they entered these cavities and did not change positions over a period of three months. In nature, the worms were never observed to venture in the open. In any case, once *Bonellia* females settle in a patch of rock, they most probably remain in the same patch for the rest of their lives and do not cross from one patch of rock to another if these patches are separated by a sandy substratum. The overall displacement over the lifetime of a female is therefore estimated to be no more than a few metres.

The model

Space

We model the littoral habitat as a lattice of 50×50 square sites. We allow each site to contain at most one female *Bonellia*, representing approximately an area that covers a single burrow and within which larvae react to the presence of females. Sites are classified as either inhabitable or not. Observed densities range between 1 and 12 females per m^2 (P. J. Schembri, unpubl.). Therefore, we assume the carrying capacity of the environment to be 12 females m^{-2} , with sites that do not overlap and fill up an area of 1 m^2 . This implies the average area of one site to be $1/12 = 0.0833 \approx 0.09 \text{ m}^2$, which gives the side length of one square site about 0.3 m and the habitat is thus about $15 \times 15 \text{ m}$ large.

To generate a clumped distribution of inhabitable sites, with r_p being the fraction of them on the lattice, we follow the procedure suggested by Wiegand et al. (1999). This consists of superimposing n_c two-dimensional Gaussian functions by placing them at random locations over the lattice, and then of placing a horizontal plane at an elevation along the elevational gradient within the three-dimensional map, producing two elevational zones in the landscape (Wiegand et al. 1999). High elevations are then associated with the inhabitable sites, while low elevations with the uninhabitable sites. As a null scenario, we also consider the environment in which inhabitable sites are distributed randomly. We fix $n_c = 5$ and $r_p = 0.2$ throughout the paper (Table 2 for all model parameters).

Time

Time runs in discrete steps (one step = one year). Within each time step, reproduction and larval dispersal are assumed to precede male and female mortality. Unless stated otherwise, simulations are started by a fraction $r_0 = 0.05$ of inhabitable sites made occupied. These sites are randomly chosen and made occupied by fertilised adult females (two-years-old females containing four males each).

Reproduction and genotypic sex determination

Each adult female that is fertilised (i.e. that contains at least one male and is at least two years old) produces a batch of n_c eggs at spawning. This number is reduced by the fraction r_i of intersexes plus permanently undifferentiated larvae (called reproductively invalid larvae further on) as these do not contribute to future generations. In a small fraction of larvae, sex determination is genotypic. Fractions of syngamic males and females are denoted r_m and r_f , respectively. The remaining fraction $1 - r_m - r_f - r_i$ of the larvae are initially sexually undifferentiated and will metamorphose into males or females depending on environmental conditions.

To find out the proportion of syngamic females, one would need to use the strongest masculinising signal possible (which is the pigmented mucus from the trunk, Agius 1978, 1979, while in nature it is the female itself). Analogously for syngamic males, one would need to use the strongest feminising signal possible (which of course is the complete absence of any female or its secretions). Experiments carried out by one of us (PJS) and collaborators showed that r_m varies between 0.04 and 0.1, r_f stays more or less consistent at about 0.03, and r_i varies between 0.02 and 0.14 (Schembri 1977, Agius 1978, 1979, Jaccarini et al. 1983, P. J. Schembri, unpubl.). In the model simulations, we vary the parameters r_m , r_f , and r_i between 0 and 0.15.

Larval dispersal

Larvae are assumed to disperse in a continuous plot superimposed on the lattice; we assume periodic boundaries that join left and right and top and bottom edges of the environment and thus mimic larger habitats. Individual larvae die during dispersal with probability d_1 and are assumed to make only one aggregate step, following random direction and distance given by a negative exponential probability distribution $1 - \exp(-x/\tau)$, where x measures the distance from the point attained by day 7 and τ is the mean settling distance since that day.

On average, 78% larvae survive the dispersal stage (Jaccarini et al. 1983) and we thus fix $d_1 = 0.2$ throughout the paper. Observations also suggest that larvae start to settle by day 7, some 50% of them settle by day 14, and all settle by day 31 (Schembri 1977). Moreover, larvae appear to disperse in the range of meters rather than tens of metres in the absence of bottom currents. We assume that dispersal is unidirectional, more time spent swimming means larger distances achieved, and the dependence is linear, with day 31 corresponding to $9 \text{ m} = 30$ sites. Day 7 at which larvae start to settle then corresponds to approximately 2 m. Fitting a negative exponential probability distribution to the observed settling frequencies gives mean settling distance since day 7 as $\tau \approx 1.6 \text{ m}$. Therefore, the overall dispersal

distance is the sum of 2 m (dispersal up to day 7) and the randomly generated settling distance (dispersal since day 7).

Larval settlement, competition for burrows, and environmental sex determination

Larvae die if they end up in an uninhabitable site. Other rules are summarised in Table 1.

The model assumes that one lattice site encompasses at most one burrow, so that at most one female can occupy a site. Table 1 prescribes that all additional syngamic females attempting to settle there will die. In nature, some of them will probably sooner or later move away to nearby burrows. The current rule thus represents the most pessimistic scenario. Our simulations showed that females quickly saturate at an equilibrium (Results) and females saved by changing the model rules would thus not qualitatively affect population and evolutionary dynamics. For the same reason, interactions between larvae that may lead to feminisation of some of them prior to settlement (Jaccarini et al. 1983) are likewise not assumed (Table 1).

Mortality

Females die with annual probability d_f , with all males within dying as well. Males settled in living females die with annual probability d_m , with no effect on female mortality. There are no data available that would help us in estimating d_m and d_f . To obtain at least some plausible values, we run the model for various values of d_m and d_f (0.1, 0.2, . . . , 0.6) and calculate the mean and maximum of the numbers of males within individual females at the end of simulation run (100 years) over 20 replicates (Fig. 1). We choose two "extreme" combinations consistent with the fact that at most 85 males have been found settling in one female (Vollrath 1998) for further

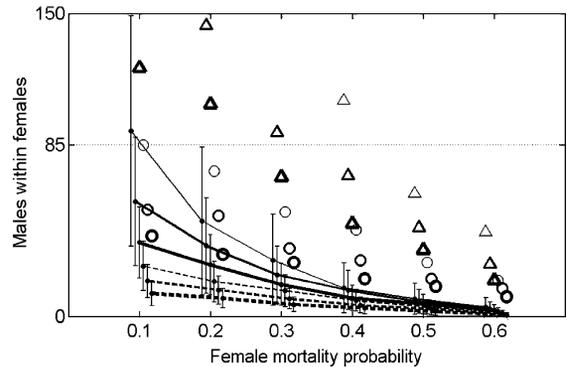


Fig. 1. Average means (dots), average standard deviations (vertical bars), and maxima (triangles and circles) of the numbers of males within one female for various combinations of male and female mortality probabilities d_m and d_f , calculated after 100 years and averaged over 20 replicates. Other parameters as in Table 2 and with $r_m=0.05$, $r_f=0.05$, $r_i=0.05$. Lines: solid thin- $d_m=0.1$, solid medium- $d_m=0.2$, solid thick- $d_m=0.3$, dashed thin- $d_m=0.4$, dashed medium- $d_m=0.5$, dashed thick- $d_m=0.6$. Triangles (circles) correspond to solid (dashed) lines of the same thickness. For clarity, different series are shifted slightly along the x-axis.

calculations: (i) $d_m=0.4$ and $d_f=0.1$, and (ii) $d_m=0.1$ and $d_f=0.5$.

Female dispersal

We do not consider that settled females could change burrows as they cannot cross borders between spatially separated patches of inhabitable sites and we consider temporal intervals of one year. This corresponds to the assumption that "moving" females do not modify the spatial pattern of lattice occupancy and hence the overall distribution of settling opportunities.

Evolution

One of the most interesting questions concerning *B. viridis* is the selective advantage of environmental

Table 1. Sex determination rules.

| Larvae ending up in a vacant inhabitable site | | | | |
|--|----------------|--|--|---|
| Condition | Only SM | Only SF | SF+SM and/or SU | SM+SU (with at least one SU) |
| Result | All larvae die | One unfertilised female settles, remaining females die | One fertilised female settles, SM+SU or SM+SU-1 males settle in it, depending on whether the female comes from SF or SU group; remaining females in SF group die | One fertilised female settles, SM+SU-1 males settle in it |
| Larvae ending up in a female-occupied (inhabitable) site | | | | |
| Condition | Only SF | SM and/or SU | SF+SM and/or SU | |
| Result | All larvae die | All larvae become males and settle in the female | All SF die, SM+SU males settle in the female | |

Abbreviations: SM – syngamic male larvae, SF – syngamic female larvae, SU – sexually undifferentiated larvae, excluding intersexes and permanently undifferentiated larvae.

sex determination (ESD) over genotypic sex determination (GSD). Why is ESD maintained in this species or why has it not been invaded by the much more widespread GSD? To gain an insight into this issue, we assume a mutant that produces no sexually undifferentiated larvae, i.e. the fraction of syngamic male larvae is effectively the primary sex ratio as we know it from GSD species, and all other larvae are females. We run simulations in which a small number of GSD individuals invade a resident ESD population at equilibrium (and vice versa), and examine outcomes of such invasion scenarios. We also explore evolution within pure ESD populations by performing invasion experiments with two ESD phenotypes differing in the fraction of syngamic males, syngamic females and reproductively invalid larvae, and within pure GSD populations where various phenotypes are assumed to differ in the primary sex ratio μ . Within the adopted notation, GSD populations correspond to $r_m = \mu$, $r_f = 1 - \mu$, and $r_i = 0$. Resident and invading populations are initialised with $r_o = 0.95$ and 0.05 , respectively.

Study of the evolution of reproductive strategies in *Bonellia viridis* is unfortunately hampered by two unresolved questions. First, it is not known which of the males present in the androecium fertilises the female, and whether more than one male is involved in mating or not. Secondly, no data are available on the pattern of reproductive strategy inheritance. We thus consider three hypothetical scenarios. The simplest possibility is to assume that female offspring inherit the mother's reproductive strategy (the maternal inheritance scenario). Another possibility that we explore is the classical inheritance scenario, in which a randomly chosen male from those that reside in a female fertilises it. If both the female and the chosen male are of the same phenotype, all larvae inherit that phenotype. If, on the other hand, male and female reproductive strategies differ, we let half of the larvae inherit the father's strategy and the other half the mother's strategy. The third possibility we consider is the following. Imagine that all males present in a female release their sperm into a sperm-storage organ of the female. Offspring arise from a union of an egg and a randomly chosen sperm cell, and we thus assume that half of the larvae inherit the mother's phenotype, while the proportion of phenotypes in the remaining larvae equals the proportion of phenotypes taken across all males within the female. We refer to this scenario as the proportional inheritance scenario.

Our model incorporates two major selective mechanisms: (i) competition for empty burrows among settling larvae and (ii) competition among males residing in a female to fertilise it. In order to assess their relative importance in the evolution and maintenance of ESD over GSD, we perform four more sets of experiments. In each of them, one of the two mechanisms has the ESD-

like rule switched off in ESD populations or switched on in GSD populations (Fig. 6). That is, if competition for empty burrows/mating is switched off in ESD populations, ESD larvae within an empty/female-occupied site behave nonadaptively as GSD ones. Likewise, if competition for empty burrows/mating is switched on in GSD populations, GSD larvae within an empty/female-occupied site behave adaptively, that is, like ESD ones.

Results

Population dynamics

Simulations show that starting even from low population sizes, female *Bonellia* soon establish at an equilibrium density. Vacant inhabitable sites are maintained through nonzero female mortality and existence of empty, difficult-to-reach patches that are located far from the occupied ones. Fig. 2 shows average dynamics of a few sample populations. They quickly saturate at an equilibrium and fluctuate around it due to the stochastic character of the model. ESD populations saturate more quickly; slight delay in saturation in GSD populations appears to increase with increasing primary sex ratio (Fig. 2). At high female mortality rates, moreover, ESD populations generally achieve higher equilibrium values; the equilibrium for GSD populations tends to decrease with increasing primary sex ratio (lower set of lines in Fig. 2).

Settling success of females, i.e. the ratio of successfully settled female larvae to all female larvae ending up in inhabitable sites, appears to be lower under GSD than under ESD for most parameter values (Fig. 3). It is maximised at 1 under ESD and no syngamic females; it moreover decreases with r_m and r_f (ESD), does not vary with r_i (ESD), and increases with the primary sex ratio

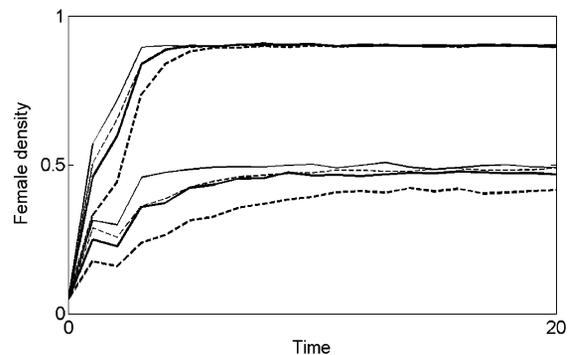


Fig. 2. Temporal dynamics of a few sample populations averaged over 20 replicates. Upper four lines: $d_f = 0.1$, $d_m = 0.4$, solid thin: $r_m = 0.05$, $r_f = 0.05$, $r_i = 0.05$ (ESD), solid thick: $\mu = 0.5$ (GSD), dashed thin: $\mu = 0.3$ (GSD), dashed thick: $\mu = 0.7$ (GSD). Lower four lines with the same parameters except $d_f = 0.5$, $d_m = 0.1$. Other parameters as in Table 2.

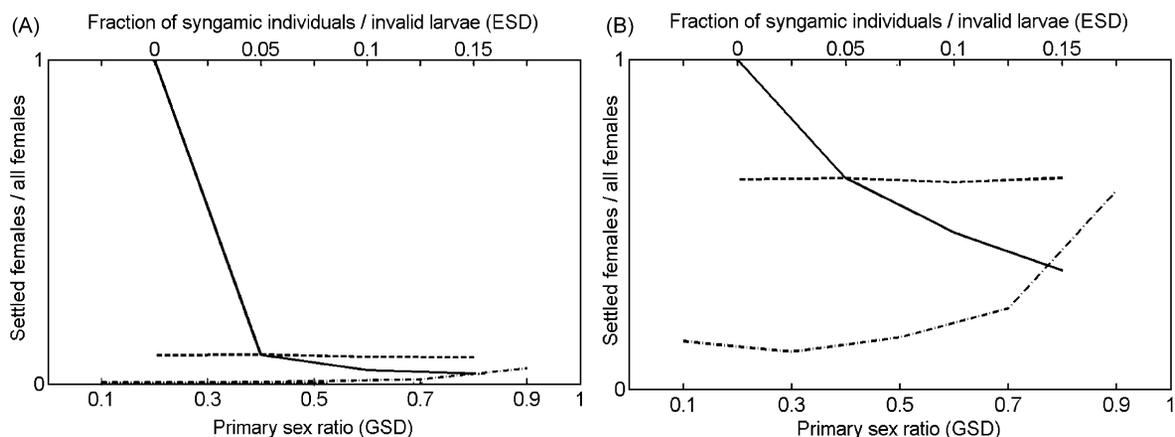


Fig. 3. Ratio of successfully settled female larvae to all female larvae ending up in inhabitable sites under ESD (solid line: $r_m = r_f$ varies, $r_i = 0.05$; dashed line: $r_m = r_f = 0.05$, r_i varies) and GSD (dash-dot line). A. $d_f = 0.1$, $d_m = 0.4$, B. $d_f = 0.5$, $d_m = 0.1$. Each value is calculated over the last 50 time steps in a single simulation run consisting of 100 years, and averaged over 20 simulation. Other parameters as in Table 2.

(GSD) due to increased, constant, and reduced competition for burrows among female larvae, respectively. Only for large values of μ and r_f (and r_m), ESD females have lower settling probability than GSD ones but as we show below, it is rather unlikely that evolution ever takes *Bonellia* populations to these values. Since populations of both reproductive types achieve similar equilibrium values (Fig. 2), absolute numbers of successfully settled females are comparable under ESD and GSD.

All else being equal, temporal dynamics in female density do not differ from those plotted in Fig. 2 when inhabitable sites are randomly distributed over the lattice (analogous to a high value of the number n_c of clumps consisting of inhabitable sites). Maximum observed number of males within females is lower compared to the spatially clumped case (not shown). This is due to the relatively short larval dispersal: if any two patches of inhabitable sites are small and separated by uninhabitable bottoms, more larvae are lost.

Adverse changes in the environment may lead to a decline in reproductive output of adults and/or survival

of both free living larvae and settled individuals, and possibly result in population extinction. We first examine the effects of annual mortality of females (Fig. 4A, C). Equilibrium density of females in persisting populations decreases linearly with d_f . At the same time, the fraction of extinct populations starts to abruptly increase beyond a critical value. Second, we examine the effects of egg batch size (Fig. 4B, D). Initially, equilibrium density of females in persisting populations shows no significant decrease with decreasing n_e , but it takes increasingly longer times for the population to equilibrate (not shown). Below a critical value, the fraction of extinct populations abruptly increases (Fig. 4B, D). Moreover, we observed that the lower is the initial number of females and the higher is the habitat fragmentation in terms of the number of patches of inhabitable sites, the higher is the fraction of extinct populations under otherwise fixed conditions (not shown). Populations thus demonstrate the Allee effect (Boukal and Berec 2002), presumably due to the lack of mating opportunities at low population sizes. Overall, Fig. 4 demon-

Table 2. Parameters used in the model.

| Description | Symbol | Value |
|--|--------|---|
| Number of patches of inhabitable sites | n_c | 5 |
| Fraction of inhabitable sites on the lattice | r_p | 0.2 |
| Fraction of inhabitable sites initially occupied by fertilised females | r_o | population-dynamic studies: 0.05 (=25 females); evolutionary studies: resident -0.95, invader -0.05 |
| Number of eggs per spawning female | n_e | 1000 |
| Mortality during dispersal | d_l | 0.2 |
| Mean settling distance | τ | 1.6 |
| Fraction of syngamic males | r_m | variable: 0–0.15 |
| Fraction of syngamic females | r_f | variable: 0–0.15 |
| Fraction of intersexes and permanently undifferentiated larvae | r_i | variable: 0–0.15 |
| Primary sex ratio in GSD populations | μ | variable: 0.05–0.9 |
| Annual mortality of females | d_f | 0.1 or 0.5 |
| Annual mortality of males | d_m | 0.4 or 0.1 |

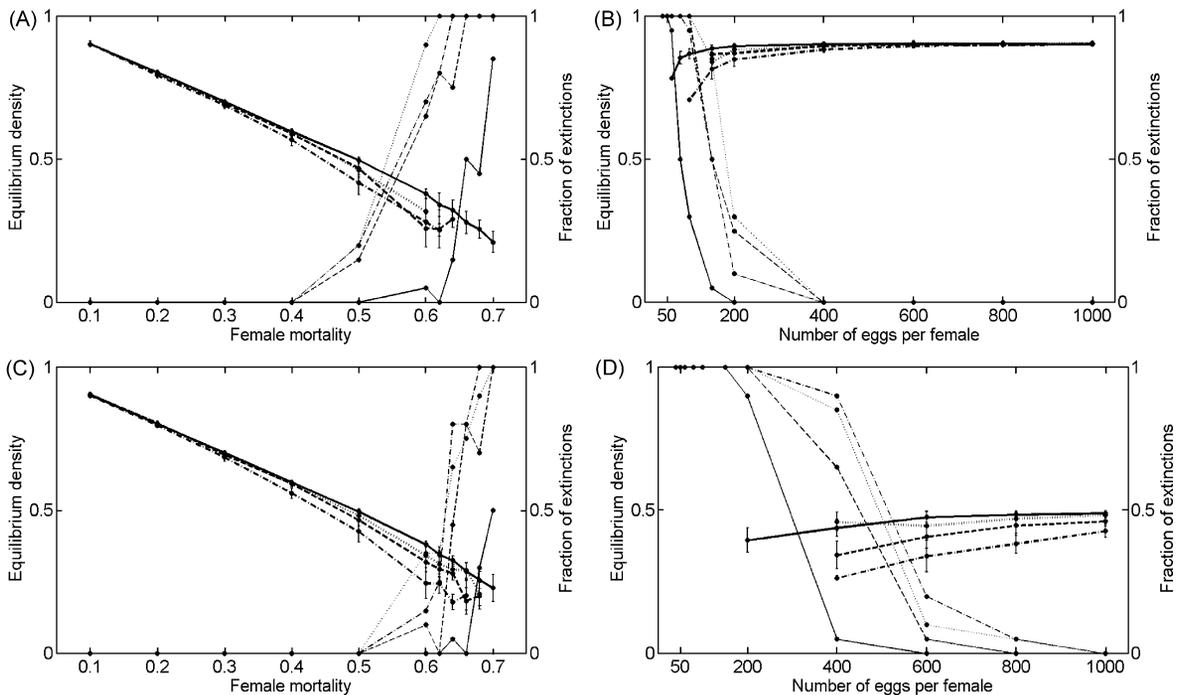


Fig. 4. Female equilibrium in persisting populations and fraction of extinct populations as a function of female mortality (A, C) and number of eggs per batch (B, D). Results based on 20 simulation replicates for each fixed parameter set. A. $n_e = 1000, d_m = 0.4$, B. $d_f = 0.1, d_m = 0.4$, C. $n_e = 1000, d_m = 0.1$, D. $d_f = 0.5, d_m = 0.1$. Other parameters as in Table 2. Lines – solid: $r_m = 0.05, r_f = 0.05$ (ESD), dashed: $\mu = 0.5$ (GSD), dotted: $\mu = 0.3$ (GSD), dash-dot: $\mu = 0.7$ (GSD).

strates that the fraction of populations going extinct under adverse conditions is much lower for ESD populations than for GSD ones.

Altogether, population dynamics indicate an advantage of the ESD reproductive strategy over the GSD one. However, only the following invasion experiments can properly examine conditions under which ESD populations may resist GSD invasions and invade GSD residents.

Evolution

Maternal inheritance

With maternal inheritance, ESD populations are relatively robust against GSD invaders. Fig. 5 shows invasibility plots in which invasion results are summarised. Apart from a few scenarios with low μ and high $r_m = r_f$ or r_i , GSD populations can neither invade nor resist ESD ones. Successful GSD invasions are due to an extraordinarily large number of female larvae produced by GSD females and a lower number of metagametic larvae, that are not reproductively invalid, produced by ESD females. Only in such “extreme” situations do GSD females outcompete ESD ones in the struggle for empty burrows that is decisive under the maternal inheritance scenario (below).

Within pure ESD populations, individuals with lower values of r_m, r_f and r_i , whether residents or invaders, fix in the population and evolution thus tends to produce populations in which there are no syngamic and reproductively invalid larvae. Within pure GSD populations, starting at sufficiently high primary sex ratios of both phenotypes, populations evolve towards increasingly female-biased primary sex ratios. The decrease in μ is limited by a threshold μ_t below which GSD populations cannot persist even when starting from all inhabitable sites initially occupied, as the low number of present males cannot fertilise sufficiently many females and populations gradually decline to zero. What happens if a chance mutation produces an invader phenotype with μ below μ_t ? Extensive simulations gave rise to three possible outcomes: extinction of both competing phenotypes, extinction of the phenotype with lower μ and persistence of the other, and coexistence of both phenotypes. However, we could not exclude the possibility that the observed cases of coexistence of both phenotypes are artefacts of relatively short simulation times. We explain these results by the following processes: the phenotype with $\mu < \mu_t$ is a superior competitor for empty burrows over that with $\mu > \mu_t$, but cannot sustain itself. It is the phenotype with $\mu > \mu_t$ that supplies that with $\mu < \mu_t$ with males needed to reproduce and spread (recall the rules of the maternal inheritance

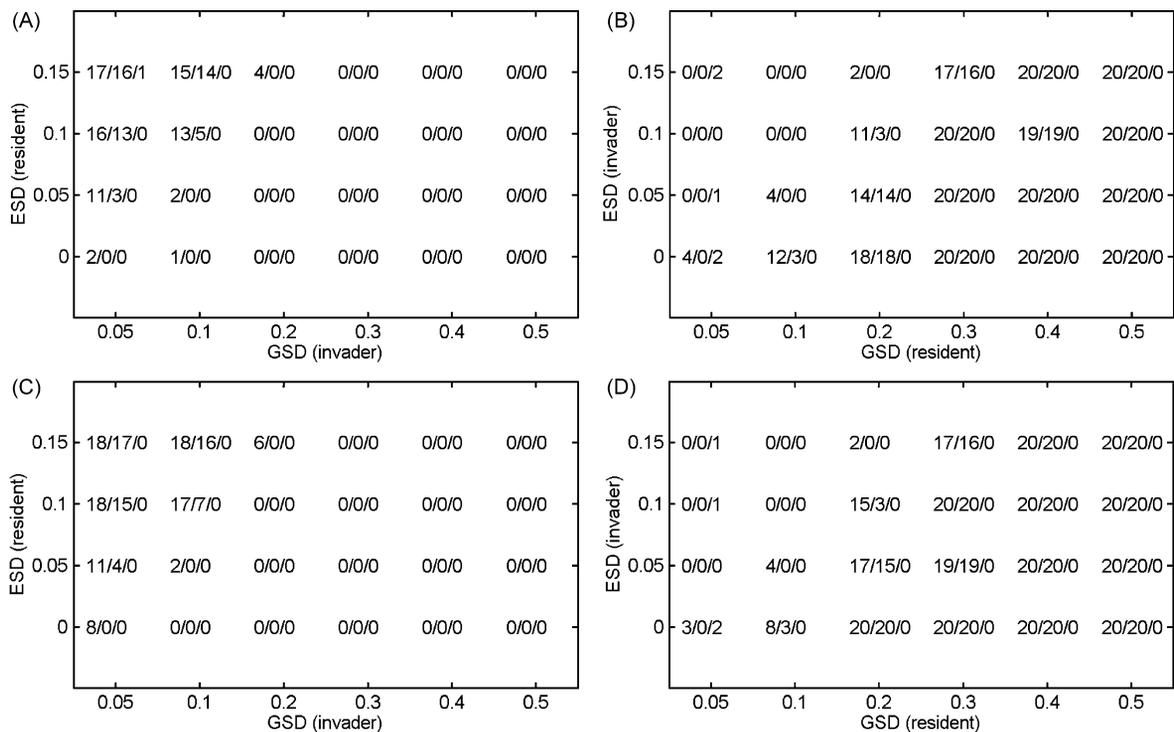


Fig. 5. Invasibility plots for the maternal inheritance scenario. For each parameter combination, 20 simulation replicates were run for 1000 years each. Legend: first number = number of successful invasions, second number = number of successful invasions that lead to complete replacement of the resident population, third number = number of cases in which the overall population went extinct. (A) Resident ESD and invading GSD, $r_m = r_f$ varies, $r_i = 0.05$; (B) Resident GSD and invading ESD, $r_m = r_f$ varies, $r_i = 0.05$; (C) Resident ESD and invading GSD, $r_m = r_f = 0.05$, r_i varies; (D) Resident GSD and invading ESD, $r_m = r_f = 0.05$, r_i varies. Other parameters as in Table 2, $d_r = 0.1$, $d_m = 0.4$. Results for $d_r = 0.5$, $d_m = 0.1$ were qualitatively identical, with a slightly larger fraction of extinct populations at low primary sex ratios under GSD.

scenario). Observed cases of extinction of both phenotypes are then caused by an overly increase of the invader population that virtually replaces the resident population and, not able to persist by itself, subsequently dies out.

Classical and proportional inheritance

These two scenarios are similar in nature and give identical results. Not surprisingly, GSD populations evolve towards the 1:1 primary sex ratio ($\mu = 0.5$), and ESD populations evolve towards no syngamic and reproductively invalid larvae, i.e. $r_m = r_f = r_i = 0$ (not shown). When ESD and GSD populations compete with each other, ESD is always fixed in the population regardless of being the resident or the invader strategy (not shown). GSD invader as well as resident populations are eliminated in less than 150 years, sometimes even more quickly.

Other life history parameters such as mortality values play negligible if any role in the outcome of our evolution experiments. We argue that they cannot affect the results unless they differ in ESD and GSD populations or give them different competitive ability in locating/occupying empty burrows and fertilising fe-

males, the two driving evolutionary forces present in our simulations.

Finally, to assess relative importance of competition for empty burrows and for female fertilisation, we run simulations with ESD-like traits switched on/off (described in detail in Fig. 6 and Methods). Under the classical and proportional inheritance scenarios, if ESD and GSD phenotypes differ only in their reaction to

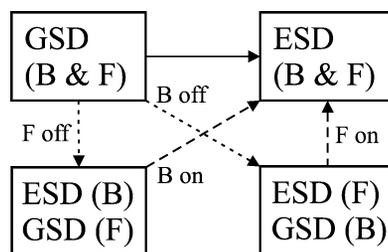


Fig. 6. Explored combinations within the ESD and GSD phenotypic space. Legend: B off – ESD populations behave like GSD ones with respect to competition for burrows, B on – GSD populations behave like ESD ones with respect to competition for burrows, F off – ESD populations behave like GSD ones with respect to competition for mating within females, F on – GSD populations behave like ESD ones with respect to competition for mating within females.

masculinising signals (presence of a female; “B off” and “B on” in Fig. 6), the resulting invasibility plots are identical to those under the full ESD and GSD competition (ESD is always fixed in the population regardless of being the resident or the invader strategy). On the other hand, if they differ only in their reaction to feminising signals (presence of an empty burrow; “F off” and “F on” in Fig. 6), we observed that the ability of ESD populations to resist invasions by/invoke low primary sex ratio GSD populations is weakened, and both phenotypes can even coexist in the population in some cases (not shown). Results for the maternal inheritance scenario are just opposite. The invasibility plots stay identical to those in Fig. 5 under the different reaction to feminising signals. If ESD and GSD phenotypes differ only in their reaction to masculinising signals, GSD populations are able to invade, but not replace, ESD ones for all primary sex ratios, with decreasing success as μ increases. On the other hand, ESD populations are able to invade, but not replace, GSD ones only in a few cases (results not shown). We conclude that (i) ESD strategy is more advantageous as a reaction to masculinising rather than to feminising signals under the classical and proportional inheritance scenarios, but the opposite is true under maternal inheritance, and (ii) for most explored parameter combinations, strategies with incomplete ESD-like traits are replaced by full ESD strategy.

Discussion

Population dynamics of *Bonellia viridis*

Marine invertebrates are probably one of the most difficult animal groups in which to study population dynamics empirically, and such studies may not be feasible at all for many species. Models predicting long term dynamics of such populations are therefore valuable tools for assessing their viability. We combine small pieces of evidence, mostly resulting from short term laboratory studies, in a model that tracks the fate of any single individual in a heterogeneous environment.

The echiuran worm *Bonellia viridis*, with its bizarre form of sex determination (the vast majority of sexually undifferentiated larvae settling on a female become males, while larvae that settle away from females become females in most cases), is a fascinating species to study from a population-dynamic as well as an evolutionary viewpoint. Are its population dynamics as peculiar as its sex determination?

Population dynamics of *B. viridis* appear rather simple and not too sensitive to parameter changes around the measured values, or to changes in distribution and sizes of patches of inhabitable sites. Starting even from low population sizes, female *Bonellia* soon attain their equilibrium density. Vacant burrows are maintained

through female mortality and existence of empty patches of inhabitable sites that are difficult to reach; under the adopted dispersal rule, only 1% of larvae disperse beyond 8.4m and only 0.1% of them reach 12.4 m or more. On the other hand, such patches are quickly colonised once reached. This high colonisation ability seems to be in agreement with the observation that *B. viridis* is a common species wherever it occurs.

Being a potent coloniser, *B. viridis* is nevertheless susceptible to habitat deterioration. One of us [PJS] observed a declining population at Marsaxlokk Bay, Malta. With industrialisation of the bay over the past 20 years, the levels of suspended matter in the water increased with time and the *Bonellia* population decreased until a few years ago, when no individuals could be found in a place where they used to be abundant. Our simulations gave rise to frequent extinctions under low female fecundities as well as high female mortalities, which are likely consequences of habitat deterioration. We also observed that under such unfavourable conditions, higher habitat fragmentation may cause extinction of populations that would be able to persist in a more contiguous habitat of the same size.

Evolution and maintenance of ESD in *Bonellia viridis* populations

One of the most interesting questions concerning *B. viridis* is why the ESD reproductive strategy is maintained in this species or what selective advantage ESD has over the more common GSD strategy. First, simulated population dynamics already suggested an advantage of the ESD strategy over the GSD one in terms of population growth rate, female settling success, and susceptibility to extinction.

Second, we considered three hypothetical scenarios for reproductive strategy inheritance (maternal, classical, and proportional), coupled with a direct, lottery like competition among female larvae for empty burrows. The GSD strategy was almost always inferior to ESD as ESD populations easily invaded and permanently displaced GSD ones. Only under the maternal inheritance scenario, high female production (low primary sex ratios) in GSD populations and low proportion of viable metagametic larvae in ESD populations reversed the outcome and lead to the fixation of GSD.

Taking into account that ESD is the present form of reproductive strategy in *B. viridis* populations and assuming that evolution advances in relatively small steps (although we will hardly ever know how sex determination strategies are linked to actual genes and mutations in their composition), it follows that to get a GSD mutant, sequential mutations must first push the fraction of metagametic larvae ($1-r_m-r_f$) down to zero. However, we showed that ESD populations should

evolve towards full metagamy. Consequently, if evolution really proceeds in this way, appearance of GSD mutants in current ESD populations by small mutational steps in the fraction of syngamic larvae is highly unlikely. Only if some GSD mutants appear through a one-step mutation, may they invade and eventually replace ESD residents. Nevertheless, this can happen only in a relatively small portion of parameter combinations under the maternal inheritance scenario. We therefore conclude that all examined reproductive strategy inheritance scenarios may explain the maintenance of ESD in *B. viridis*.

Although up to 85 males may settle on the proboscis of a single female (Vollrath 1998), on average only up to four of these males have been found to occur in the androecium (Jaccarini et al. 1983). One could therefore imagine another inheritance scenario in which only four randomly chosen males of those present in a female would father the offspring in a proportional manner. However, such an inheritance scenario is intermediate between the classical and the proportional one, and gives identical evolutionary results.

What are the major factors that maintain ESD in *B. viridis* populations? No previous study has explicitly investigated the evolutionary significance of ESD in this species, beyond a short verbal argument provided in Charnov and Bull's (1977) influential paper. They were probably the first to address the question of selective advantage of ESD over GSD by proposing that "labile sex determination (not fixed at conception) is favoured by natural selection when an individual's fitness (as a male or female) is strongly influenced by environmental conditions and where the individual has little control over which environment it will experience". As regards *Bonellia viridis*, they argued that "the resource of strongest sex-dependent value is the female, because of the ability of the male to remain small, live upon her and fertilise her eggs" (Charnov and Bull 1977).

Our study shows that this statement should be refined. We have identified two selective processes that affect the evolution of ESD vs GSD in *Bonellia*: (i) competition for empty burrows among settling larvae and (ii) mating competition among males residing in a female. In a lottery like model such as our, more common phenotypes will have a higher chance to win in a contest for an empty burrow or mating. Unlike GSD larvae, ESD larvae that are metagamic can adjust their sex to current environmental conditions. If the same amount of ESD and GSD larvae end up in an empty burrow, ESD larvae will contain more potential females unless the primary sex ratio under GSD is highly female biased. Likewise, if the same amount of ESD and GSD larvae end up in a female occupied site, ESD larvae will contain more potential males unless the primary sex ratio under GSD is highly male biased. Available burrows are an external

resource that cannot be actively created by the females. In Malta and also in other parts of the Mediterranean (for example Banyuls sur Mer and Naples in Wilczynski 1960), *B. viridis* occurs only in burrows excavated in rock by other organisms (e.g. thalassinid shrimps) or in stable accumulations of close-packed stones, both having multiple openings and allowing for a unidirectional respiratory stream of water to be pumped through by peristalsis (Schembri and Jaccarini 1977, 1978). Few organisms are able to excavate rock, not any kind of burrow will do for *Bonellia*, and apposite accumulations of stones may be just as isolated and patchily distributed in the environment as rock with suitable galleries. Thus, empty burrows are a resource that is patchily distributed and unpredictable for females (as females are for males) since *Bonellia* cannot dig burrows in soft sediment, cannot excavate their own burrows in rock, and cannot survive for long outside burrows (Schembri 1977, Schembri and Jaccarini 1978).

Settling larvae might face a number of decisions. Larvae that settle at/near a female have to decide whether they become males and compete for fertilisation opportunity or become females and compete for limited numbers of suitable burrows. Although we have no knowledge of the real reproductive behaviour within females and of the exact genetic mechanisms of reproductive strategy inheritance, it is likely that the more males there are within a female, the lower the likelihood each of them has to fertilise it. Larvae that settle in empty burrows usually face the same dilemma, since dispersing larvae end up in loose aggregates and the first coloniser is very likely to be followed by a number of other larvae. Anyway, larvae that metamorphose into females may initially occupy some small burrows and later on move to a larger burrow that becomes empty, or die if there is none.

To separate effects of ESD vs GSD in competition for these resources, we run simulations in artificial situations where adaptive sex determination processes are switched off in ESD populations or switched on in GSD populations (Methods). We showed that the strategy in which ESD affects only masculinisation appears more advantageous than if it affects only feminisation under the classical and proportional inheritance scenarios, and vice versa under maternal inheritance. For most explored parameter combinations, however, the joint functioning of both processes is fixed by evolution and leads to fully ESD populations. Therefore, both females, as Charnov and Bull (1977) hypothesised, and empty burrows are "resources of strong sex-dependent value", ensuring maintenance of the ESD reproductive strategy in *B. viridis* populations.

Apart from *B. viridis*, very few ecological and behavioural observations are available for any species within the Bonelliidae. Rietsch (1886) stated that *B. minor* Marion lives in the abandoned burrows of

boring bivalves, Dartnall (1976) reported *B. sabulosa* Dartnall from coral gravel, Singhal and Datta Gupta (1980) found *Achaetobonellia maculata* Fisher and *Acanthobonellia vulgaris* Fisher living in crevices and cavities in coral rock, and Edmonds (1987) reported *Metabonellia haswelli* (Johnston & Tieg) to live in crevices between rocks and under rocks (especially those abutting flats with seagrass beds), and *Pseudobonellia biuterina* Johnston & Tieg to live in coral and limestone reefs and under isolated stones resting on sand. Although this information is scanty, it does suggest that our results may also apply to other species of echiurans that also live in habitats that are relatively rare and patchily distributed, have dwarf males living within the androecium of the female (although the male of many species is unknown – Stephen and Edmonds 1972), and presumably show ESD. Surprisingly, ESD has not been demonstrated for any species other than *B. viridis*, offering a tantalising question for future field studies.

Our model simulations showed that ESD populations evolve towards no production of syngamic and reproductively invalid larvae. The observed fraction of these types is indeed rather low, with minimum values around 0.03 for each type (Schembri 1977, Agius 1978, 1979, Jaccarini et al. 1983, P. J. Schembri, unpubl.), but variability exists between various experiments that aim to estimate it. What makes this difference? Our simulations also showed that the closer the fraction of syngamic and reproductively invalid larvae is to zero (for both the resident and the invader phenotype), the longer it takes the invader to replace the resident (the latter having larger fractions of such individuals; results not shown). It might be, therefore, that selection and mutation may operate at the same time scale and their balance may thus keep these fractions away from zero. Another plausible explanation for a nonzero fraction of syngamic and reproductively invalid larvae may lie in the genetic basis of ESD in *Bonellia viridis*. Although currently unknown, it is possible that larvae are subject to a polygenic sex determination in which, depending on the ratio of female-determining to male-determining genes in a larva, “pure females” contain only female genes, “pure males” contain only male genes, and larvae with a mixture of female-determining and male-determining genes can be feminised or masculinised to different degrees depending on the strength of the environmental signal. In such a setting, the presence of reproductively invalid larvae can be explained by a weak masculinising/feminising signal that would not allow some almost pure females/almost pure males to completely overcome their genetic predisposition and finish their metamorphosis into the other sex. Anyway, more work is needed to resolve this issue.

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