Sex in an uncertain world: environmental stochasticity helps restore competitive balance between sexually and asexually reproducing populations

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Abstract

Like many organisms, individuals of the freshwater ostracod species *Eucypris virens* exhibit either obligate sexual or asexual reproductive modes. Both types of individual routinely co-occur, including in the same temporary freshwater pond (their natural habitat in which they undergo seasonal diapause). Given the well-known two-fold cost of sex, this begs the question of how sexually reproducing individuals are able to coexist with their asexual counterparts in spite of such overwhelming costs. Environmental stochasticity in the form of 'false dawn' inundations (where the first hydration is ephemeral and causes loss of early hatching individuals) may provide an advantage to the sexual subpopulation, which shows greater variation in hatching times following inundation. We explore the potential role of environmental stochasticity in this system using life-history data analysis, climate data, and matrix projection models. In the absence of environmental stochasticity, the population growth rate is significantly lower in sexual subpopulations. Climate data reveal that 'false dawn' inundations are common. Using matrix projection modelling with and without environmental stochasticity, we demonstrate that this phenomenon can restore appreciable balance to the system, in terms of population growth rates. This provides support for the role of environmental stochasticity in helping to explain the maintenance of sex and the occurrence of geographical parthenogenesis.

Introduction

The ubiquity of sexual reproduction has stimulated a large body of research aimed at explaining the maintenance of this reproductive mode against the backdrop of competing asexual reproduction and the associated cost of sex (Maynard Smith, 1978). Among the hypotheses considered, there is a bias towards biotic interactions (particularly parasites) and genetic processes (especially the purging of mildly deleterious mutations). Because the ensuing theories come with caveats, such as the requirement for high transmissibility and

virulence of parasites (Howard & Lively, 1994) or atypical genome-wide mutation rates and epistasis (Kouyos *et al.*, 2007), attention has turned to pluralistic hypotheses (West *et al.*, 1999). Although demographic stochasticity is often explored in the context of finite population sizes and genetic drift (Howard & Lively, 1994; Park *et al.*, 2010), pluralism has generally not embraced the notion of environmental stochasticity [but see (Getz, 2001) for a resource competition model of environmental stochasticity and the maintenance of sex].

The freshwater crustacean *Eucypris virens* is an ostracod species with a wide geographical range and the maintenance of two distinct, obligate reproductive modes (purely sexual and purely asexual) (Horne *et al.*, 1998). The asexual reproductive mode has the larger

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geographical range, whereas the sexual reproductive mode is restricted to Mediterranean Europe (McKenzie, 1971; Horne *et al.*, 1998; Bode *et al.*, 2010; Schmit *et al.*, 2013a). Part of the life cycle involves the production by both reproductive modes of diapausing eggs that survive the seasonal drying of the ponds, which they inhabit (Martens *et al.*, 2008). Hatching of diapausing eggs is in response to environmental cues, including temperature and photoperiod (Martins *et al.*, 2008). Although these cues often coincide with the onset of the extended hydrated winter period, occasionally climate conditions cause ponds to dry up shortly after hatching has occurred, causing death of hatched individuals but not unhatched eggs. We term these initial rains that induce hatching followed by dry, inhospitable conditions a 'false dawn' event.

Published life-history data (Martins *et al.*, 2008) showed that eggs from asexual populations hatched earlier on average and more synchronously than eggs from sexual populations. These differences cannot be conclusively ascribed to differences in reproductive modes, as they could be due to geographical variation. They nevertheless suggest a mechanism through which asexually reproducing individuals could be potentially more negatively impacted by environmental stochasticity than their sexual counterparts. To what extent such environmental stochasticity can restore balance in relation to the two-fold cost of sex is an open question, and one that can greatly impact our understanding of the role of pluralism in the maintenance of sex (West *et al.*, 1999). By combining the life-history data and climate data in a population model, we illustrate that environmental stochasticity can impact population growth rates, restoring some of the competitive imbalance caused by the two-fold cost of sex and potentially contribute to the macroscale pattern of geographical parthenogenesis (Horne & Martens, 1998).

Materials and methods

Here, we outline the original experimental data on ostracod hatching and explain how we use it to derive estimates of key life-history traits. Analyses are novel, although no additional experiments were performed. Additionally, we introduce the climate data along with details of how we extract key information on rainfall. Finally, we describe how the data are incorporated into population models, which provide predictions on the fate of reproductive modes under different climatic regimes.

Source of life-history data

In a previously published experiment (Martins *et al.*, 2008), sexual and asexual *E. virens* were sampled at various sites in Europe. In this study, we focused only on those individuals sampled in Spain (latitudinal range 39°17′–39°54′N – see Appendix for details) as this was the country sampled in which individuals of both reproductive modes were often observed. Sampling was performed in three distinct regions (Cáceres, Ciudad Real and Valencia) varying primarily in their longitudinal coordinates (Appendix, Table A1). Within a region, individual sites (temporary freshwater ponds) containing exclusively female individuals were designated as 'asexual' and those containing males and females were designated as 'sexual'. In at least one of the eight sites, it is possible that sexual and asexual reproductive modes coexisted as a mating experiment suggested that only around 40% of females sampled from the same pond were sexual (Schmit *et al.*, 2013b). However, because in general sexual and asexual individuals did not coexist in these sampled sites, the potential differences in life-history traits are tentatively ascribed to their differences in reproductive mode. Under these assumptions, a 'pure' asexual population would exhibit even greater differences in hatching times than sexual populations. Sampled animals were transferred to the laboratory. By simulating the photoperiod and temperature associated with the onset of the summer desiccation of temporary ponds in Spain, it was possible to induce the animals to produce primarily diapausing eggs. Although various temperature treatments were used in the original experiment, we restricted our analysis to those diapausing eggs that were stored at room temperature (~ 20 °C) with subsequent hatching and development performed at 17 °C and 12 h light per day (conditions typical of the onset of winter in the region).

Life-history analysis

In the original experiments, life-history data were obtained by immersing eggs in mineral water and tracking the fate of ostracods (grown individually) from this time until their death by daily observation (Martins *et al.*, 2008). Individual development was conducted using a previously established nutrient regimen (Schmit *et al.*, 2007). For the subset of the original data, we conducted a survivorship analysis and calculated the probability of hatching as well as transition probabilities to each of the subsequent stages including the final, reproductively active adult stage. We also analysed the time of hatching relative to rehydration along with the time spent in each stage by fitting distributions for count data (comparing standard parametric distributions using likelihoods from generalized linear model fits). These data were used to construct life tables, coded as population projection matrices. In cases where time to an event (stage development) was best described by a negative binomial distribution, appropriate pseudo stages were added to the matrix (Caswell, 2000).

Climate data analysis

Using the Spanish national weather data repository (Datos climatológicos - Agencia Estatal de Meteorología - AEMET), we identified the weather stations closest to the sampling sites. Distances between sites and their nearest weather stations were approximately 30 km (see Appendix). From the data for these stations, we extracted rainfall patterns that reflect false dawn events. We restricted these patterns to x days of rain in the first week following the summer dry period, followed by x days of no rain (for $x = 3, 4, 5, 6$ & 7). We acknowledge that this is arbitrary, and precisely defining conditions for pond drying include unmeasured factors such as water volume and surface area, wind speed, temperature and humidity, among others. Our goal here is to illustrate the potential for environmental stochasticity to interact differentially with sexual and asexual populations. The scenario aims to capture sufficient conditions for egg hatching and initial development, and a sufficient number of dry days to render the environment unviable for subsequent development. Although more elaborate patterns can be searched for, exploratory analyses show that there is a rarefaction signature so that the addition of more patterns has a diminishing return on the detection of false dawn events in the data. The raw data allowed us to calculate the probability of a false dawn event occurring in a season, to test for differences between regions and to test for evidence of temporal autocorrelation between years (i.e. whether a false dawn year is more likely to be followed by another). Additionally, the length of the wet and dry seasons in each region was estimated using profiles for the daily probabilities of rain (Fig. 2). Each region's rainfall data (covering between 41–73 years) was aggregated by ordinal date, and the probability of rain on a given day was calculated as the proportion of times that ordinal date exhibited rainfall. The relative length of the wet season was taken as the proportion of days in which the probability of rainfall exceeded 10%. We acknowledge once again that this definition is arbitrary; our goal here is to illustrate that environmental stochasticity may contribute in decreasing the cost of sex. Exact inference of local parameters is beyond our scope because important information is lacking. However, our analyses do extend to consider other measures of wet season length (see Appendix).

Population modelling

From the projection matrices for sexual and asexual reproductive modes, we calculated population growth over 50 years. Projection forward in time was done under both constant environmental conditions and with the inclusion of stochastic false dawn events characterized in accordance with the climate data. Due to the stochastic nature of these events, projection was replicated (100 times per treatment). Comparison of sexual and asexual populations was made under identical, stochastically generated environments. Because the life-history data do not include direct competition between the asexual and sexual populations, estimated parameters on survivorship and hatching responses were included in separate population growth models.

To implement the projection model, both types of population (sexual and asexual) were assumed to begin with 1000 eggs. This number is large enough to avoid the undesirable effect of demographic stochasticity operating before differences in reproductive modes manifest due to environmental stochasticity. The eggs remained in diapause until a hydration cue. Hatching followed according to the parameterized negative binomial distributions for hatching frequency over time. Populations were projected forward within a season using a daily time step. Development rates and survivorship probabilities were applied in accordance with the data. The reproductive output of asexual females was assumed to be twice that of sexual females (100 vs. 50) in accordance with the two-fold cost of sex (Maynard Smith, 1978). The brood size of sexual females was in line with experimental evidence (Vandekerkhove *et al.*, 2007). In later analyses, the cost of sex was relaxed from strictly two-fold to ascertain the differences in reproductive output required for sexual and asexual populations to have equivalent growth rates and therefore be deemed equally competitive. Strict two-fold costs of sex relate to an assumption of a 1 : 1 ratio of males and females. Whereas field estimates of sex ratios in *E. virens* and related species often find a slight female bias (Chaplin *et al.*, 1994), hatchling experiments recovered an equal sex ratio (Vandekerkhove *et al.*, 2007). Additional reasons for an ameliorated cost of sex include the ability of sexual populations to better resist virulent parasites (Maynard Smith, 1978).

In the event that a female reached the final reproductive stage within the hydration period, she produced subitaneous eggs (immediately hatching), with brood sizes given by the fecundity term in the matrix model. At the end of the hydration period, all females of reproductive age produced a brood of diapausing eggs (with asexual females producing twice as many diapausing eggs as sexual females – except in cases where a reduced cost of sex was examined). These eggs alone provided the initial population for the following season (long-term egg bank storage was not considered).

Within each replicate, each of the 50 years over which a population was tracked was designated as a regular year or a false dawn year. In cases without temporal autocorrelation, this was implemented as a sequence of 50 binomial trials with probability, *P*, of a false dawn year estimated from the climate data analysis. In cases with temporal autocorrelation, 50 trials were performed iteratively. The first year was assigned a false dawn year with probability *P*. For subsequent

years, if the preceding year was a false dawn year, then the binomial trial for the current year was performed with probability of a false dawn year given by $P' + \delta$ (similarly, $P' - \delta$ if the preceding year was not a false dawn year). Values of P' and δ were chosen following empirical numerical investigation such that the average probability was P and the correlation coefficient was in agreement with the climate data analysis (see Appendix). If a false dawn year was selected to occur, a drought-onset day was chosen as a uniform random deviate in the range 8–14 days after initial hydration (i.e. in accordance with the definition of a false dawn event). Until the drought-onset day, population dynamics developed according to the hatching, development, survivorship and reproduction parameters for the nondrought years. At the drought-onset day, all individuals in stages other than the diapausing egg stage were removed. A rehydration day was selected according to the distribution of such days from the climate data (see Appendix). Between the drought-onset day and the rehydration day, no hatched eggs survived. Once the rehydration day was reached, population dynamics again reverted to those governed by the regular life-history parameters. Simulations in which censused adult population sizes dropped below one were discarded, and probability of extinction as a function of region and reproductive mode was recorded.

Results

Life-history analysis

Data for successfully hatched eggs from the original life-history experiment (Martins *et al.*, 2008) (n_{sexual} = 32, $n_{asexual}$ = 33) were used to establish development rates and survivorship. Survivorship analysis revealed no significant difference between the two reproductive modes (log-rank test: $P = 0.31$, Appendix, Table A5) and consequently were pooled together to estimate survivorship by stage. All eggs from both treatment groups hatched following hydration, and there was a significant difference in the hatching times (Kolmogorov–Smirnov test: $P = 0.002$). Parametrically, hatching times for both groups were best described by a negative binomial distribution (Fig. 1, Asex: $k = 23$, $\gamma = 0.6$; Sex: $k = 23$, $\gamma = 0.48$ – where mean and variance of the distribution are given by k/γ and $k (1 - \gamma)/\gamma^2$, respectively). Note, the dispersion parameter, k, is constrained to integer values as it is interpreted as the number of pseudostages in the projection matrix (Caswell, 2000). To fit these parameters, first the unconstrained maximum-likelihood estimate of k is obtained, then it is rounded to the nearest integer and the second parameter, γ, is re-estimated. For all stage durations after hatching, there were no significant differences
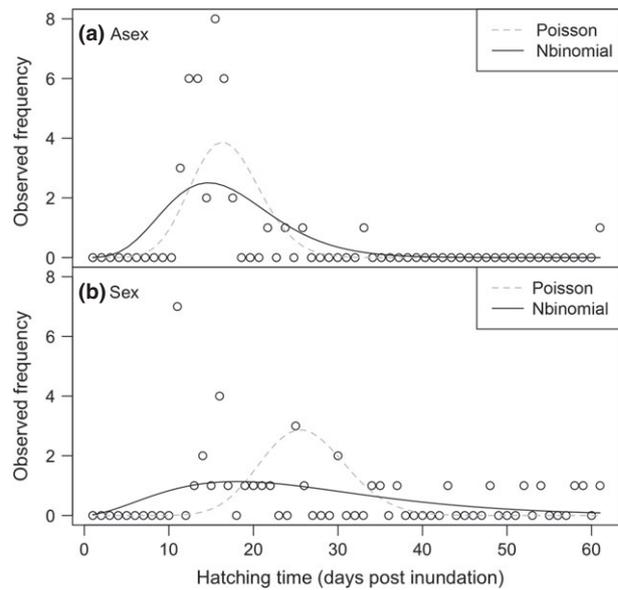

Fig. 1 Hatching times for resting eggs produced by a: asexually and b: sexually reproducing females. Open circles are raw count data to which maximum-likelihood fits of Poisson (grey dashed line) and negative binomial (black solid line) distributions are overlaid.

between sexual and asexual populations. Consequently, these data were pooled and parametric model fitting consistently showed that stage distributions were again best described by negative binomial distributions (see Appendix). Pseudostages were used in the matrix model to simulate duration within stages as a negative binomial distribution. For the subadult and adult stages, the number of pseudostages was constrained to be ≤ 4. This approach broadly captures the duration of stages and avoids over inflating the development time to adult (see Appendix).

Climate data analysis

The three regions (Cáceres, Ciudad Real, Valencia) had similar probabilities of experiencing a false dawn event ($P = 0.31$, $P = 0.25$, $P = 0.19$, respectively). There was no significant difference between these probabilities (chi-square test: $P = 0.44$). The mean probability of a false dawn event was 0.25. There was no 1-year lag autocorrelation in false dawn events in Cáceres and Ciudad Real (Spearman correlation test: $P = 0.70$ and 0.56, respectively). However, there was evidence of a 1-year lag autocorrelation in Valencia (Spearman correlation test: $P = 0.01$). The length of the wet season (liberally defined as the number of days in which the probability of rain ≥ 0.1) in Cáceres, Ciudad Real and Valencia was estimated as 296, 310 and 341 days, respectively (Fig. 2).
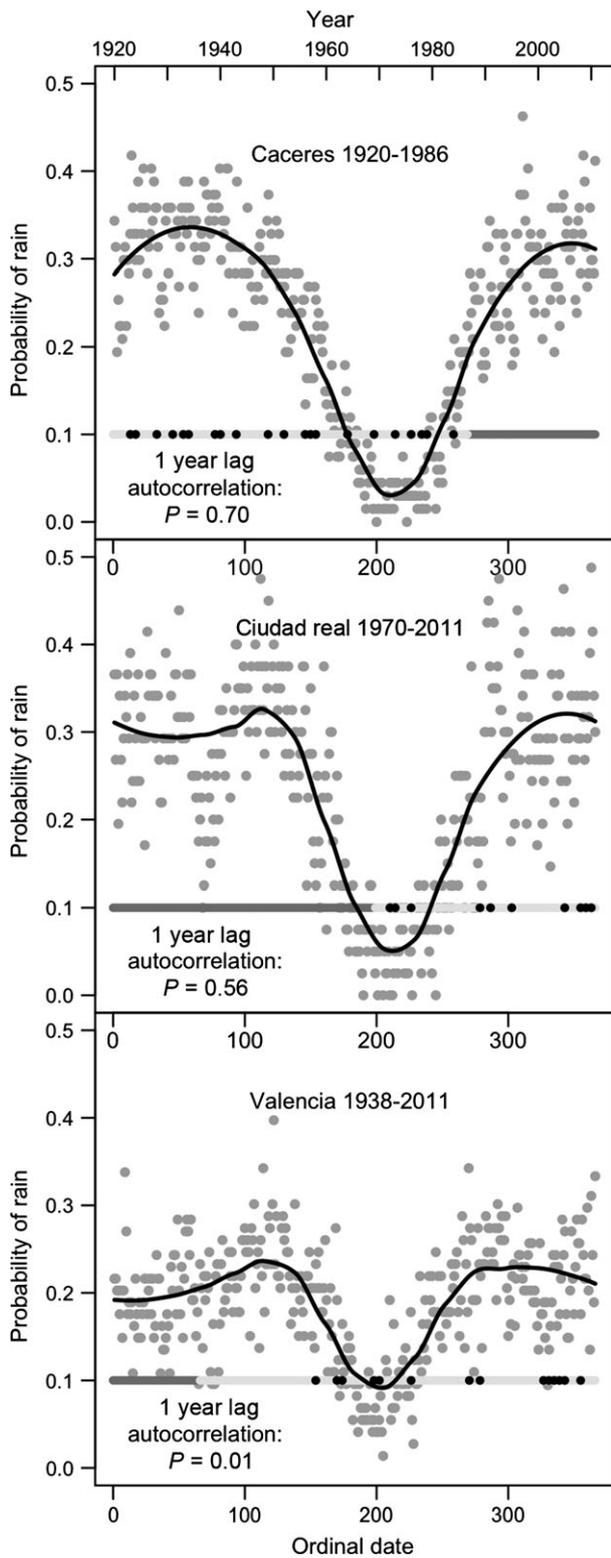
Fig. 2 Rainfall patterns in the three sampling regions. Multiple years of data were aggregated by ordinal date (lower x axis), discriminating days with a zero and nonzero rainfall to estimate the probability of rain on a given day (grey circles). The function of probability of rain by day was then estimated by a loess smoothing function (span = 0.5, solid black lines). The length of the wet season was then estimated as the large, contiguous proportion of the year in which the probability of daily rainfall ≥ 10%. This 10% threshold line also carries information about false dawn years (calibrated on upper x axis): dark grey segment = no data, black circles = false dawn years, light grey segments = regular years.

Population modelling

Population projections 50 years forward in predictable environments from an initial egg bank of size 1000 show that asexual reproduction results in a significant growth rate advantage (Fig. 3 – thick black lines below lower
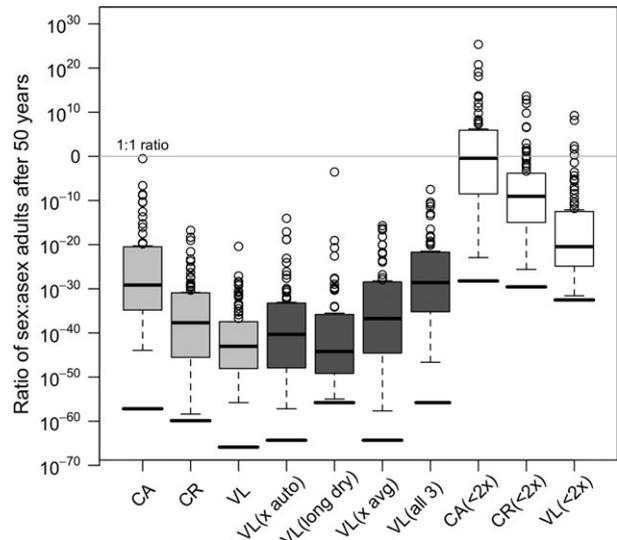

Fig. 3 Model predictions of the ratio of sexual to asexual population sizes after 50 years. Box-and-whisker plots correspond to simulations with environmental stochasticity, whereas solid black lines below the lower whisker correspond to equivalent conditions in the absence of environmental stochasticity. Light grey filled plots refer to predictions under a two-fold cost of sex assumption, with regional assumptions of probability of false dawn events, their temporal autocorrelation and length of wet season in accordance with data. Dark grey filled plots focus on the site (Valencia) where the sexual population is predicted to have the lowest relative growth rate and investigate the roles of probability of false dawn events, their temporal autocorrelation and the length of the wet season in suppressing population growth by modifying assumptions in turn ('x auto': removing temporal autocorrelation; 'long dry': substituting Valencia's short dry season for Cáceres' long dry season; 'x avg': substituting the pooled mean probability of a false dawn event for Cáceres' individual, higher value) and together ('all 3'). The unfilled plots are similar to the original light grey plots except that the cost of sex is assumed to be < two-fold (Asex: Sex brood sizes of 100 : 80).

whiskers). In the absence of false dawn events, asexual populations are predicted to outgrow sexual populations by a factor of 10^{58}–10^{66} over this time, with the variation due to the difference in the length of the wet period in the three regions. However, the inclusion of false dawn events greatly reduces this advantage to asexual populations, with typical (median) population size ratios in the range 10^{31}–10^{51} (Fig. 3 – light grey box-and-whisker plots). Sexual populations in the Valencia-type environment suffer the most. This site is characterized by the longest wet season and is the only site with temporal autocorrelation between false dawn years. Additionally, this site has the lowest probability of a false dawn year (although not statistically different from the other sites). Altering any of these characteristics to more resemble the westernmost Cáceres region, in isolation, does not have a major effect on the performance of the sexual population, whereas altering all three in combination does (Fig. 3 – dark grey box-and-whisker plots). In reality, populations would reach carrying capacity before such large population sizes were achieved. In the absence of life-history data stratified by strength of density-dependent competition, we opt for a simple model to estimate relative growth rates (relative population sizes after a fixed time interval and starting from an equal size).

Although environmental stochasticity in terms of false dawn events diminishes the two-fold cost of sex in this system, it does not fully compensate for it. None of the environmental conditions typical of the three regions interact with the life-history strategies to lead to equal long-term growth rates of the sexual and asexual populations. However, if the ratio of offspring from asexual/sexual broods is adjusted from 100 : 50 to 100 : 80 (a plausible cost of sex offset by advantages such as purging deleterious alleles, resisting parasite infection and female-biased sex ratios), then 1 : 1 growth rates are achieved in all regions and are the expected outcome in one of them, namely Cáceres (Fig. 3 – unfilled box-and-whisker plots). By including false dawn events, asexual populations are at a consistently higher risk of population extinction, measured as simulations in which the censused adult population dropped below one (40% vs. 30% in Cáceres, 28 vs. 22% in Ciudad Real, 22 vs. 20% in Valencia).

Discussion

Pluralistic hypotheses for the maintenance of sex are gaining credibility as they allow strict assumptions associated with any one mechanistic explanation to be relaxed to the point that they are better in line with observational evidence and are closer to a general explanation (Howard & Lively, 1994; West *et al.*, 1999; Park *et al.*, 2010). The role of environmental stochasticity is largely absent from the pluralist debate, in spite of the fact that diapause is often associated with a multiplicity of

reproductive modes (Kleiven *et al.*, 1992; Schierwater & Hauenschild, 1990; Horne & Martens, 1998; Simon *et al.*, 2002; Nambu *et al.*, 2004; Tojo *et al.*, 2006; Zierold *et al.*, 2007). Diapause is strongly influenced by environmental stochasticity because cues to produce diapausing eggs and cues for eggs to hatch are environmentally driven (Donaldson-Matasci *et al.*, 2013). Whereas it is always possible to conceive of life-history traits associated with reproductive modes interacting with environmental stochasticity to influence the fate of sexually reproducing populations, it is challenging to bring together relevant data to demonstrate that this occurs in nature, although the frequency of sexually reproducing individuals has been related to a decrease in the permanence of sites they occupy (Schmit *et al.*, 2013a,c). Here, we have combined life-history data for the freshwater crustacean *E. virens* and environmental data in terms of false dawn events to illustrate how environmental stochasticity can appreciably offset the cost of sex in these organisms. Although the sampling in the three regions was not sufficiently extensive to statistically test for differences in the occurrence of reproductive modes by location, the findings were at least consistent with model predictions. In all four sites around westernmost Cáceres, males were recovered. Males were also found at one of the two sites around the central region, Ciudad Real. No males were found at either of the sites around easternmost Valencia in the data associated with this study, and other studies find it to be rare in that region (Schmit *et al.*, 2013a). Life cycles involving diapause require resisting many unpredictable, often stressful environments. Interestingly, a separate set of experiments on *E. virens* aimed at testing for differences between reproductive modes in contending with stresses such as UV radiation and hypoxia found that sexual populations tended to have slightly earlier responses to hatching cues indicating the importance of considering high-dimensional niches in future studies of environmental stochasticity and sexual/asexual coexistence. The underlying mechanism for the difference between sexual and asexual individuals in their responses to cues was not directly assessed in this study, but could include genetically determined hatching responses that will be more variable in a population with recombination and the higher probability of such diverse populations to include individuals with plastic responses.

We acknowledge that our definitions of false dawns and wet/dry seasons are arbitrary. However, our goal here is illustrative rather than demonstrative. The model predictions suggest that due to the potentially early, synchronous hatching of asexual individuals (Martins *et al.*, 2008), they suffer disproportionately when faced with stochastic false dawn events. Although this works in the favour of the sexual population, on its own it is not predicted to be a sufficient force to fully compensate for the two-fold cost of sex, as it does not lead to equal population growth rates of the two reproductive modes (Fig. 3). Generally, sexual

populations will stand the greatest chance of success in regions where the probability of false dawn events is high, such as North Africa (Schmit *et al.*, 2013a). Additionally, regions with a relatively short winter period accentuate the advantage to sexual populations as the intrinsically higher population growth rate of asexuals has less time to generate large discrepancies between sexual and asexual population sizes. The temporal autocorrelation between false dawn years favours the asexual population in simulations in which neither population goes extinct. If there are a fixed number of false dawn years in a period of time, then by experiencing some of them in succession, naturally there are long sequences when early, synchronous egg hatching is not costly and the asexual population can grow much more quickly than the sexual population. Moreover, sexual populations are less likely to go extinct (both absolutely and relatively) in regions with temporal autocorrelation in false dawn years.

By relaxing the two-fold cost of sex so that sexuals produce 80% the number of daughters that asexuals produce, then long-term population growth rates of the two reproductive modes equalize (Fig. 3). Given that organisms are likely contending with parasites and the accumulation of mildly deleterious mutations and that asexual individuals are expected to pay a higher associated cost in fitness than their sexual counterparts (Howard & Lively, 1994; Hurst & Peck, 1996; West *et al.*, 1999), the role of environmental stochasticity can play a significant role in a pluralistic explanation for the maintenance of sex. Here, we restrict ourselves to treating sexual and asexual population growth separately (compared with a direct competition model). This is in keeping with the life-history data, which did not include direct competition experiments. However, with appropriate data, modelling efforts could be extended to consider the outcome of direct competition. By estimating population growth rates and extinction probabilities, the work presented here goes some way to explaining observed patterns of geographical parthenogenesis in *E. virens* complementing existing hypotheses (Horne & Martens, 1998; Schon *et al.*, 2000; Adolfsson *et al.*, 2010). Other extensions, such as long-term egg bank storage (Evans & Dennehy, 2005) could also be incorporated into the matrix modelling approach straightforwardly. However, the propensity for long-term viable eggs in this organism is not known, and all eggs exposed to hatching cues in the original experiment did all hatch (Martins *et al.*, 2008).

Acknowledgments

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Stage durations by reproductive mode

Fitting stage duration distributions

Eucypris virens individuals begin as eggs and have eight subadult instar stages before developing to the final adult stage. Only the egg stage (time between inundation and hatching) showed a significant difference in distribution of stage duration as a function of reproductive mode (Table A3). In this case, Poisson and negative binomial distributions were fitted to the separate asexual and sexual count data (observations of individuals transitioning to next developmental stage each day). Otherwise Poisson and negative binomial distributions were fitted to the pooled stage data. In all cases, the negative binomial distribution was a better fit to the data, shown by larger log-likelihood scores (Table A3).

Appendix

Sampling sites and weather stations

Life-history data involved ostracods sampled from three regions in Spain with approximately the same latitude but different longitude. Within each region, between two and four sites were sampled (Table A1).

For each region, the nearest weather stations were identified from the Spanish government weather station list (http://www.aemet.es/es/servicioclimaticos/datosclimatologicos) as detailed in Table A2.

Table A1 Sampling sites from where animals used in life-history experiments were obtained.

Region	Site ID	Reproductive mode designation	Specific location
Cáceres	CA1	Sexual	39°54′2″N, 06°03′37″W
Cáceres	CA2	Sexual	39°54′4″N, 06°03′36″W
Cáceres	CA3	Sexual	39°54′21″N, 06°03′36″W
Cáceres	CA4	Sexual	39°54′35″N, 06°04′10″W
Ciudad Real	CR1	Sexual	38°49′02″N, 04°03′52″W
Ciudad Real	CR2	Asexual	38°49′28″N, 04°03′52″W
Valencia	VL1	Asexual	39°20′30″N, 00°18′43″W
Valencia	VL2	Asexual	39°17′43″N, 00°20′42″W

Table A2 Closest weather stations to sampling sites, used for climate data.

Region	Closest weather station (ID)	Specific location	Mean distance from regional sites
Cáceres	Cáceres, ciudad (3469)	39°28′34″N, 6°22′21″W	54 km
Ciudad Real	Ciudad Real (4121)	38°59′19″N, 03°55′25″W	22 km
Valencia	Valencia (8416)	39°28′18″N, 00°22′43″W	18 km

Simulating temporal autocorrelation in false dawn years

Valencia, the most eastern region, demonstrated significant evidence of temporal autocorrelation (1 year lag) between false dawn years as defined in the main text. The pooled mean for the probability of a false dawn year was $P = 0.25$, and the correlation coefficient for the Valencia data was ~ 0.3. In order to stochastically generate a sequence of 50 years, the first year was assigned a false dawn year with probability P. For subsequent years, if the preceding year was a false dawn year, then the binomial trial for the current year was performed with probability of a false dawn year given by $P'+\delta$ (similarly, $P'-\delta$ if the preceding year was not a false dawn year). Following numerical investigation, values of $P' = 0.32$ and $\delta = 0.15$ were selected. A typical 50-year sequence of zeros (regular year) and ones (false dawn year) simulated under these conditions 1000 times has the properties demonstrated in the histograms of Fig. A1. In a separate analysis (main text – Fig. 3, dark grey boxplot), the unpooled estimate for the probability of a false dawn year in Cáceres ($P = 0.31$) was used for the Valencia population (as part of a study to isolate contributions of regional differences in explaining population patterns). Generating suitable sequences of 50 years was achieved with values of $P' = 0.37$ and $\delta = 0.16$. The sequence of years simulated under these conditions has the properties demonstrated in Fig. A2.

Rehydration following a false dawn event

Second hydrations (taken as the true onset of the winter hydration period) were determined by searching for the original inundation pattern (a week dominated by rains) after a false dawn event. The time interval between first and second inundations in the climate data is shown in Fig. A3.
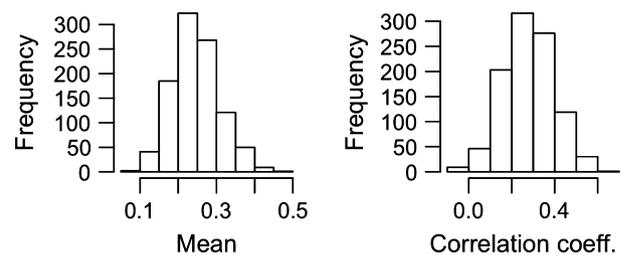

Fig. A1 Histograms of mean number (left) and temporal autocorrelation at 1-year lag (right) of false dawn years generated from 1000 simulations where probability of a false dawn year is $P' \pm \delta$. Probabilities are explicitly calculated using $P' = 0.32$ and $\delta = 0.15$, and $P'+\delta$ is used when the previous year was a false dawn year, otherwise $P'-\delta$ is used. Resulting mean probability is centred on 0.25, in accordance with the pooled mean for all three study regions, and correlation coefficient is centred on 0.3, in accordance with the Valencia data.
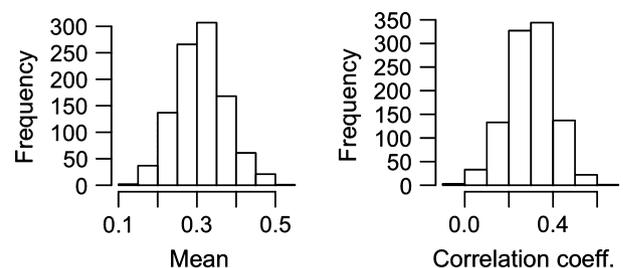

Fig. A2 Histograms of mean number (left) and temporal autocorrelation at 1-year lag (right) of false dawn years generated from 1000 simulations where probability of a false dawn year is $P' \pm \delta$. Probabilities are explicitly calculated using $P' = 0.37$ and $\delta = 0.16$, and $P'+\delta$ is used when the previous year was a false dawn year, otherwise $P'-\delta$ is used. Resulting mean probability is centred on 0.31, and correlation coefficient is centred on 0.3, both in accordance with the data.

Pairwise Kolmogorov–Smirnov tests show that these patterns do not vary significantly by region (CA-CR: $P = 0.44$; CA-VL: $P = 0.23$; CR-VL: $P = 0.73$). Consequently, they were pooled, and Poisson and negative binomial fits were performed on the pooled data so that time of second hydration could be calculated in simulations. A negative binomial fit (dispersion parameter 4.07, mean = 0.076) was the better fit (log likelihood = -50 vs. -85).

Selection of number of pseudo stages

In population projections using matrix models, it is often necessary to describe the time T spent in a stage by a negative binomial distribution with mean M and variance V. This is approximated by defining σ as the probability of an individual surviving in the stage and γ as the probability of developing from this stage to the next given current stage survival, then by constructing
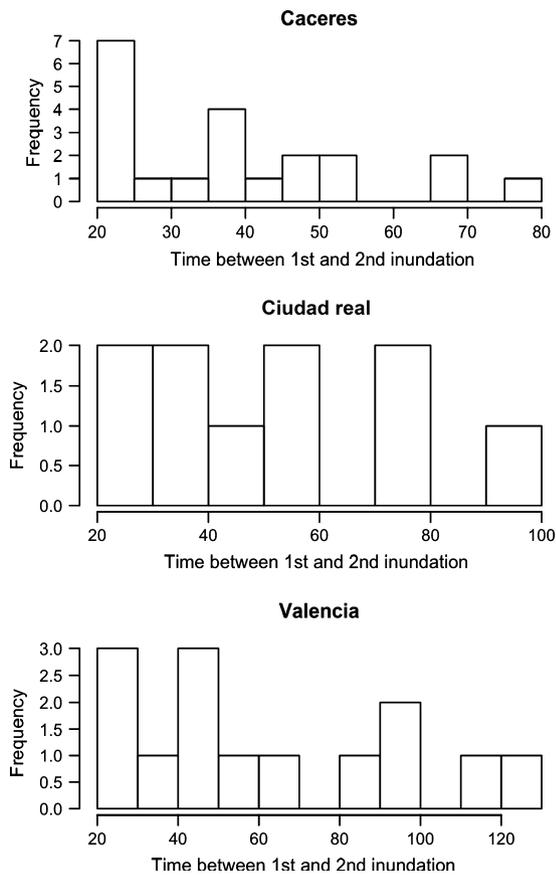
Table A3 Duration in each of nine post-hatching ontogenetic stages, for each reproductive mode. These analyses are performed on data originally collected by Martins *et al.* (2008), Oikos (cited in main text).

Stage	Average duration (days) for asexual individuals	Number of individuals (asexual)	Average duration (days) for sexual individuals	Number of individuals (sexual)
1	1.2	33	2.0	32
2	2.5	32	3.0	27
3	3.9	27	3.9	20
4	4.7	23	5.3	14
5	5.2	23	4.9	13
6	4.5	21	5.5	10
7	5.2	18	7.6	8
8	8.3	18	8.3	8
9	25.4	18	21.1	8

Fig. A3 Histograms of time intervals (days) between 1st and 2nd hydration events in the three regions.

k pseudostages (with $M = k/\gamma$ and $V = k\ (1-\gamma)/\gamma^2$) (Caswell, 2000). The low parametric dimensionality of the negative binomial can create a conflict in capturing the mean and dispersion in data. In this study, the distribution of hatching times following inundation follows a negative binomial for both reproductive modes. The two reproductive modes have significantly different parametric forms for this distribution and they are essential in describing the key aspect of their differential success in stochastic environments. Consequently, we use the 23 pseudostages that are estimated to accurately characterize the dispersion in the data reflecting time spent as eggs following inundation, at the cost of a slight overestimate of the mean time spent in this stage. For other stages, there is in fact no significant difference between the time spent in a given class for the two reproductive modes. Given that there are many such instar and adult stages, it is undesirable to use the high number of pseudostages suggested by routine analysis as this overestimates time spent in a class and this is propagated forward between classes so that the development time from egg to adult is unrealistically large. As a compromise, we allow the number of pseudostages for a stage be > 1 (i.e. we capture dispersion in the data), but we limit the number of stages to 4. This allows for the negative binomial character of the data for time spent in classes to be approximated, without an unrealistic overall development time (Fig. A4).

The restriction that all stages after the egg stage can have no more than four pseudostages does not compromise the model description of the number of days an individual will spend in a class (Fig. A5)
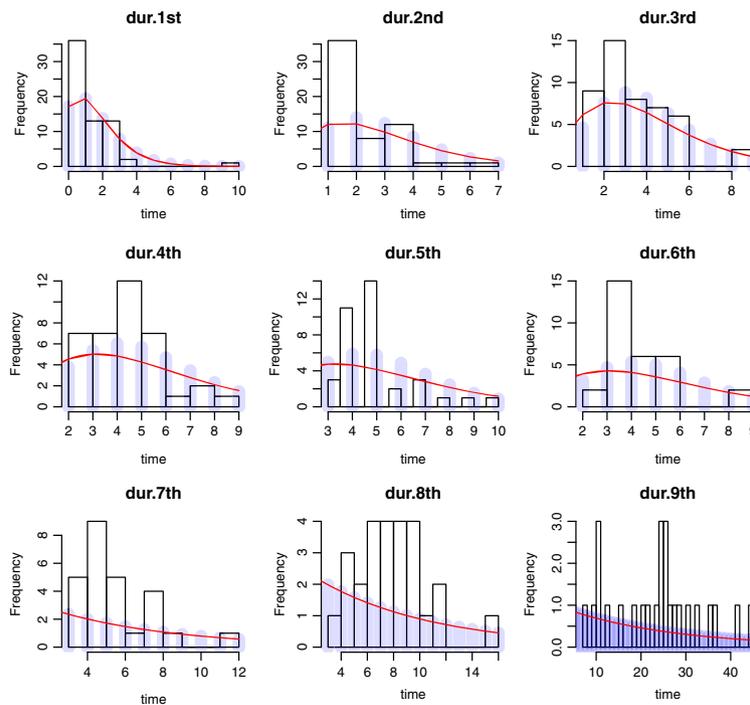

Fig. A4 Effect of the number of pseudostages (k, represented by circle radius) on agreement between model-generated and empirical mean life-stage durations (colour-coded). Egg stages are consistently modelled with the maximum-likelihood number of pseudostages given by negative binomial fitting. This number is 23 for eggs produced by both sexually and asexually reproducing females. Here, emphasis is on capturing the empirical themes of earlier and more synchronous hatching of asexual eggs and later broadly distributed hatching times of sexually produced eggs. For other life stages, there is no statistically significant difference in stage durations for sexual and asexual individuals. Using maximum-likelihood estimates for pseudostages (panel A) causes large discrepancies between model-generated and empirical mean subadult stage durations to arise, with the unrealistic effect of delayed progression to reproducing adult stage. Constraining subadult pseudostages to ≤ 4 (panel B) provides a closer agreement between model and data in terms of overall time spent as subadults, while still providing flexibility in the number of pseudostages to capture exponential-like stage duration distributions (one pseudostage) and more hump-shaped stage duration distributions (e.g. four pseudostages).

Fig. A5 Duration of subadult stages (1–8) and adult stage (9) pooled across reproductive modes. Unfilled bars are empirical data. Overlaid purple bars are negative binomial fits without constraining the dispersion parameter $k < 4$. The red line shows the negative binomial fit when constraining $k < 4$.

Survivorship by reproductive mode

A survival analysis was conducted on the ostracods (Fig. A6). Although there was a tendency for asexual individuals to have a higher survival probability, it was not statistically significant (log-rank test: P-value = 0.313). For each line style (solid black and grey dashed), the central line is the estimate of proportion surviving and the extreme lines are the 95% confidence interval.

Between-stage survival probabilities are given in Table A4. When pseudostages are used to generate negative binomial stage durations in the matrix population projection, these are adjusted as outlined in Caswell (2000), Chapter 6 (reference in main text).

Sensitivity of length of wet/dry season

In the main text, the length of the dry season is defined as the extended, contiguous portion of the year for which the daily probability of rainfall ≤ 10%. The maximum daily rainfall probability is < 35% and the communal maximum is ~ 20%. Varying the threshold condition between 10 and 20% (Fig. A7) does not change the overall pattern used in the main text: that Valencia has the longest wet season with the other two regions having a very similar wet season length (approximately 20–30 days shorter than Valencia). Consequently, the specific threshold choice is not expected to qualitatively change the outcome of model predictions.
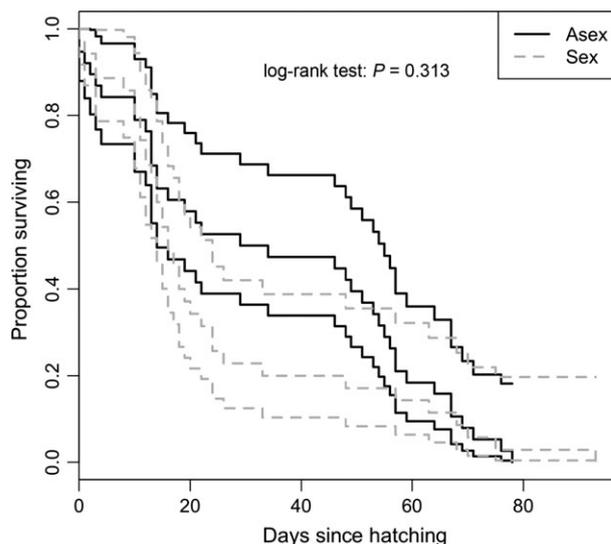

Fig. A6 Survival analysis by reproductive mode (asexual population: solid black lines; sexual population: grey dashed lines). Extreme lines (upper and lower) are 95% confidence intervals.

Table A4 Tests of difference of stage durations by reproductive mode (column 2 – Kolmogorov–Smirnov *P*-values) and fitting results of Poisson (P) and negative binomial (nb) distributions to asexual (Asex), sexual (Sex) and pooled (merged columns) populations (columns 3–6 –log-likelihood values).

Stage	K–S *P*-value	Asex: LogLik(P)	Sex: LogLik(P)	Asex: LogLik(nb)	Sex: LogLik(nb)
Egg	0.002	−75.10	−68.43	−49.19	−62.97
1	0.99	−24.26		−21.81	
2	0.89	−57.44		−23.05	
3	0.99	−36.63		−26.03	
4	0.97	−32.50		−24.12	
5	0.97	−40.27		−25.20	
6	0.80	−37.50		−21.68	
7	0.87	−31.91		−24.60	
8	0.92	−30.77		−28.76	
9 (Adult)	0.90	−46.02		−45.89	

Table A5 Between stage survival probabilities.

Transition	Probability
Egg-> 1	0.88
1-> 2	0.91
2-> 3	0.79
3-> 4	0.78
4-> 5	0.96
5-> 6	0.84
6-> 7	0.83
7-> 8	1
8-> 9	1

Projection matrix models implementation

Within a season, the basic projection model calculates life-stage population sizes at day $t + 1$ based on sizes at day t using data on hatching, development, survival and reproduction. The life stages are eggs (E), eight subadult stages (J1-8) and an adult stage (A).
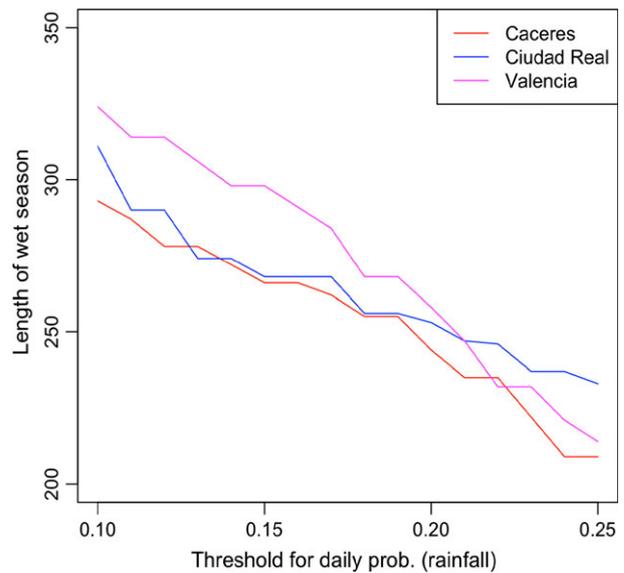

Fig. A7 Predicted length of wet season in the three regions as a function of the threshold for daily probability of rainfall selected as the criterion for demarcating wet and dry seasons.

$$\begin{bmatrix} E \\ J1 \\ \vdots \\ J8 \\ A \end{bmatrix}_{t+1} = \begin{bmatrix} \sigma_E\gamma_E & 0 & 0 & 0 & F \\ \sigma_E(1-\gamma_E) & \sigma_1\gamma_1 & 0 & 0 & 0 \\ 0 & \ddots & \ddots & 0 & 0 \\ 0 & 0 & \sigma_7(1-\gamma_7) & \sigma_8\gamma_8 & 0 \\ 0 & 0 & 0 & \sigma_8(1-\gamma_8) & \sigma_A\gamma_A \end{bmatrix} \begin{bmatrix} E \\ J1 \\ \vdots \\ J8 \\ A \end{bmatrix}_t$$

In implementing the full model, several life stages are divided into pseudostages, as explained earlier. At the end of a season, surviving adults produce diapausing eggs which begin hatching at a date in the following season according to climate (rainfall) data.