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(Crustacea: Ostracoda)**

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Complete List of Authors:	Rossi, Valeria; University of Parma, Life Sciences Martorella, Alessio; University of Parma Scudieri, Dora; University of Parma Menozzi, Paolo; University of Parma
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Seasonal niche partitioning and coexistence of amphimictic and parthenogenetic lineages of
Heterocypris barbara (Crustacea: Ostracoda)

Rossi V., Martorella A., Scudieri D., Menozzi P.
Department of Life Sciences, University of Parma, Viale G.P. Usberti 11/A, I-43124 Parma (Italy)

valeria.rossi@unipr.it
alessiomartorella@libero.it
scudieridora@yahoo.it
paolo.menozzi@unipr.it

Author to whom correspondence should be addressed: Valeria Rossi
Department of Life Sciences, University of Parma, Viale G.P. Usberti 11/A, I-43124 Parma (Italy)
Tel.: +39 0521 905612;
e-mail: valeria.rossi@unipr.it

Abstract

Sympatry of amphimictic and parthenogenetic lineages in species with mixed reproductive systems is rarely observed in nature. On Lampedusa Island (Pelagic Islands, Italy) amphimictic and parthenogenetic lineages of *Heterocypris Barbara* (Gauthier & Brehm 1928) co-occur in a temporary pond. Their sympatric persistence calls for an ecological differentiation. We investigated the role of seasonal variation of temperature and photoperiod conditions by two different approaches: microcosms set up by inundation of dry sediments from the temporary pond and life table experiments. Microcosms recreate conditions similar to the field and in their sediments random samples of resting eggs of both amphimictic and parthenogenetic females are stored. Life table experiments supplied individual based estimates of survivorship, adult life span, fecundity and sex ratio in the progeny. We carried out the experiments at 24 °C 12:12 L:D (simulating fall conditions) and at 16 °C 10:14 L:D (simulating winter conditions). Males and amphimictic females were the most numerous forms at 24 °C 12:12 L:D; parthenogenetic females were dominant at 16°C 10:14 L:D. Life table experiments showed that amphimictic forms do not complete development at 16 °C 10:14 L:D. Our results suggest that sympatry of amphimictic and parthenogenetic females in the field depends on seasonal niche partitioning and the storage effect of resting eggs that allows survival through adverse-season conditions.

Keywords: storage effect; recruitment from egg bank; hydroperiod; seasonal succession; microcosms, *Heterocypris barbara*, ostracods.

1 Introduction

2 Sexual reproduction is far more common than parthenogenetic reproduction in spite of the fact
3 that, all else being equal, in the short run, sexual lineages will be outnumbered due to the cost of
4 producing males that gives a reproductive advantage to asexual reproduction (Maynard Smith 1978;
5 Jennions and Kokko 2010; Rankin et al. 2011). Lively (1996) estimated that a population of 1
6 million sexual individuals would be replaced in less than 50 generations by a parthenogenetic
7 lineage. Not surprisingly parthenogenetic forms are considered better colonizers because one
8 individual is enough to reproduce, and this gives advantage in low population densities (Bell 1982;
9 Horandl 2009). Although sexual (amphimictic) reproduction is a complex issue (Meirmans et al.
10 2012), the prevalence of sexual reproduction indicates that in the long run the benefit of sex
11 overcomes the cost of producing males. A classical argument is that sex generates new genetic
12 combinations, a pre-requisite for the action of natural selection (Agrawal 2006).

13 In several species both sexual and asexual reproductive systems have been reported but, with the
14 exception of cases where the coexistence is necessary (e.g. hermaphrodites, organisms with
15 environmental sex determination, cyclical parthenogens, gynogenetic and hybridogenetic sperm
16 dependent asexual forms) (Bell 1982). Sympatry of the two forms has seldom been reported (Lynch
17 1984; Case 1990; Agh et al. 2007). Geographical parthenogens, in which parthenogenetic lineages
18 and their amphimictic relatives have, by definition, disjointed spatial distribution, can be seen as an
19 example of spatial niche partitioning (Bell 1982; Lynch 1984; Peck et al. 1998; Schwander and
20 Crespi 2009). Ecological differentiation that allows niche partitioning is a classical explanation for
21 species coexistence (Tokeshi 1999). A strong ecological differentiation in the same habitat by
22 biologically similar organisms can easily be extended to the finest taxonomic units (Débarre and
23 Lenormand 2011) and seems to require special circumstances: for instance, niches of the different
24 forms that create temporarily competition-free spaces (Lehto and Haag 2010).

25 A few cases of coexistence in the same habitat of amphimictic and parthenogenetic forms have
26 been reported, although evidence of ecological differentiation is usually based on correlative

evidence, with scarce experimental support (Levine and HilleRisLambers 2009; Lehto and Haag 2010). A well studied case is the freshwater snail *Potamopyrgus antipodarum* (J. E. Gray, 1853) in New Zealand where amphimictic and parthenogenetic females are spatially structured by depth and vegetation zone, as result of niche partitioning among lineages (Fox et al 1996; Jokela et al. 1997; Negovetic and Jokela 2001; Jokela et al. 2009). Two examples are from freshwater ostracods, one of the largest groups of Crustacea. In the Australasian species *Candonocypris novaezelandiae* (Baird, 1843) from Lake Purrumbete (Southern Victoria, Australia) (Chaplin 1992) and in the cosmopolitan *Eucypris virens* (Jurine, 1820) from Southern Europe (Sicily and Central Spain) (Schön et al. 2000; Rossi et al. 2008; Adolfsson et al. 2010) amphimictic and parthenogenetic forms were shown to co-occur by genetic evidence of different breeding systems. In *C. novaezelandiae* the coexistence seems transient (Chaplin 1993). In *E. virens* it seems mediated by spatial heterogeneity in habitat stability (Schmit et al. 2013). Ostracods, are an important component of temporary pool communities (Meisch 2000). In non-marine species, transition from sexual to asexual reproduction is a common phenomenon (Chaplin et al. 1994; Schön et al. 2000; Rossi et al. 2008). *Heterocypris barbara* (Gauthier and Brem 1928) is widely distributed in the circum-Mediterranean area; males are commonly reported but they are absent from some populations occurring in saline inland waters (Baltanás et al. 1990; Meisch 2000). On Pelagie Islands (Sicily channel) we have observed several bisexual populations of *H. barbara* made up by males and amphimictic females. On Lampedusa Island, over three different years, we observed the coexistence of both amphimictic and parthenogenetic females in Vallone della Forbice C temporary freshwater pond (Bellavere et al. 2002, Rossi et al. 2003; 2004; 2007 and unpublished data).

The use of nuclear markers and mitochondrial DNA sequences supported a recent origin of parthenogenetic *H. barbara* lineages from sympatric amphimictic relatives and linkage disequilibrium due to heterozygote excess was found in the parthenogenetic females (Rossi et al. 2007). This is evidence of apomixis, a form of parthenogenesis experimentally demonstrated in closely related species (Rossi and Menozzi 1990; Rossi et al. 2008). Field and genetic data support

the correlation between reproductive mode and female morphotype (Bellavere et al. 2002; Rossi et al. 2007). A lamella hyalina on the posterior margin of the left valve of amphimictic females, distinguished them from parthenogenetic ones and we have no evidence of polyploidy (Rossi et al. 2003). Hatching phenology, recruitment events from egg bank and voltinism vary by thermal regime, pH, oxygen concentration and conductivity (Rossi et al. 2004; Rossi et al. 2013). Hypoxia and high conductivity prevent and delay, respectively, the hatching of resting eggs that may also remain dormant in hydration. In amphimictic lineages, a prolonged recruitment phase is observed at 24 °C and 12:12 L:D photoperiod and low conductivity, a mix of conditions that may be considered a proxy of rainy season and long hydroperiod (Rossi et al. 2013). Moreover, the mean generation time increased from 13 to 71 days with decreasing temperature and water conductivity probably because, in ephemeral ponds, temperature and conductivity covary as the pond refill or dry (Rossi et al. 2013). The presence and persistence of *H. barbara* in temporary ponds depend on life cycle completion (from resting egg hatching to resting egg production through 9 larval stages). A hydroperiod shorter than the species life cycle might eventually drive the population to local extinction due to egg bank depletion, while hydroperiods longer than one life cycle might allow overlapping generations and egg bank enrichment. Resting forms are the “storage” that allows survival through dry season and adverse-temperature.

Here, using microcosms and life table experiments, we investigated the role of temperature and photoperiod as factors for seasonal niche partitioning of amphimictic and parthenogenetic lineages of *H. barbara* in the Vallone della Forbice C temporary pond. Complementary information on individual based life history traits was then obtained by life table experiments. We hypothesized that niche partitioning allows the coexistence of amphimictic and parthenogenetic lineages.

Materials and methods

Study site

The ephemeral pond Vallone della Forbice C (hereafter FC) is found in a cleft located halfway up the rocky side of a deep valley, carved up by ancient fluvial erosion (Bellavere et al. 2002). Small (about 2 m across) and shallow (maximum depth 50 cm), the pond is fed only by rain water. The Mediterranean area has a predictable dry summer: the combination of rainfall and temperature is compatible with water presence (hydroperiod) only from October to March (Fig. 1). Rainfall pattern and, hence, the onset and duration of the wet winter season is highly variable from year to year.

Microcosm experiment

Life cycle and population dynamics of amphimictic and parthenogenetic lineages of *H. barbara* were evaluated in laboratory microcosms that recreate conditions similar to the field. Each microcosm was set up by hydrating dry sediments from FC temporary pond where a random sample of resting eggs, produced in the field by both amphimictic and parthenogenetic females, was naturally stored (Rossi et al. 2004; Rossi et al. 2013). Sediment inundation in different experimental conditions (temperature and photoperiod) allowed us to evaluate the egg hatching and the dynamics of sexual and asexual forms in experimental conditions that simulate seasonal succession. Eight microcosms were set up by hydrating 40 g of well-mixed, dry sediments from FC temporary pond with 500 ml distilled water in transparent cylindrical open top PET containers, 8 cm diameter, 18 cm height (Rossi et al. 2013). Microcosms were maintained in thermal chambers at two sets of controlled conditions chosen to reflect seasonal temperature and photoperiod (fall 24°C 12:12 L:D and winter 16 °C 10:14 L:D) for 3 or 6 months, depending on the temperature. Previous study showed that, the mean generation time of *H. barbara* increased from 13 to 71 days when decreasing temperature from 24 to 16 °C (Rossi et al. 2013). In all microcosms, water level was maintained constant by adding distilled water each day.

For each microcosm, once a week, water was siphoned out with a small pipe and all organisms were censused by hand-sorting under a stereo microscope. The number of juveniles, adult males, adult females and ovigerous females were recorded. Males and ovigerous females were

distinguishable by the long sperm ducts or the eggs in the ovary, visible through the transparent carapace. Amphimictic and parthenogenetic females were distinguished by the lamella hyalina that appears in the pre-adult stage on the posterior margin of the left valve of amphimictic females (Bellavere et al. 2002; Rossi et al. 2007). Each individual was measured and then returned to its original microcosm; water was reset to 500 ml. By considering the size frequency distribution of measured organisms and the time of appearance of ovigerous females, the number of generations per each microcosm was estimated (Rossi et al. 2013).

Conductivity, pH and oxygen concentration were measured every seven days. Each microcosm experiment lasted at least three months and was carried out from November to April 2010-11 (6 month long inundation) and from March to May 2011 (3 month long inundation) (Fig. 1). We used counts of individuals over the whole experiment, a number that, given the duration of the experiment, represents a sum over more than one generation.

We used a generalized linear model (GLM, computed with R) (McCullagh and Nelder 1989) to test whether the proportion of amphimictic and parthenogenetic females were different between the microcosms at 24 °C 12:12 L:D and 16 °C 10:14 L:D. We used quasi-binomial distribution error to account for the over-dispersion of the data (Crawley 2007). Observed sex ratios in the microcosms at different experimental conditions were tested against a null model of 1:1 expected sex ratio with a log-likelihood test ratio (LRT). The log-likelihood ratio is computed between the maximum likelihood model obtained with the observed proportion of sexes and the likelihood of observing these same proportions under the hypothesis of equal frequencies of the sexes. The log-likelihood ratio is distributed as a Chi-square with one degree of freedom under the null hypothesis. The effect of experimental conditions on number of amphimictic and parthenogenetic females was also tested using a repeated measures generalized linear mixed model (GLMM, with a binomial distribution error). The logit of the number of amphimictic and parthenogenetic ovigerous females was the response variable, the experimental conditions and time (in weeks) since inundation were fixed

explanatory variables and microcosm was used as a random explanatory factor. We used the `glmer` function from the LME4 library, in R.

Life table experiment

To obtain individual based life history traits of amphimictic and parthenogenetic females at different experimental conditions, we set up a life table experiment. Ninety-two early juveniles (first three instars) were sampled from four additional microcosms not used for the microcosm experiment (set up as the ones used in the experiment and kept at 24 °C 12:12 L:D (two microcosms) and at 16 °C 10:14 L:D (two microcosms). Individual organisms were maintained in 24-well plates, each in 2 ml mineral water, fed *Tolypothrix tenuis* (Kützting ex Bornet & Flahault 1886; Cyanobacteria), at the same conditions at which they were hatched (55 juveniles at 24 °C and 37 juveniles at 16 °C). They were checked daily for survival, gender and, in females, for reproductive mode (presence or absence of the lamella hyalina). At the age of about 20 days, amphimictic females were crossed with males from the same microcosm according to Rossi and Menozzi (2012). For each fecund parthenogenetic and amphimictic female, size, age at deposition of first egg, age at death, age at hatching of first egg and offspring number were recorded. We consider such traits as fitness related traits. For each fecund female, adult life span was computed as difference between age at death and age at deposition of first egg and F1 offspring was checked to adulthood to record sex ratio (as ratio between number of males and number of females) and female morphotype (with or without lamella hyalina).

ANOVA was used to test for differences in life history traits of amphimictic (AM) and parthenogenetic (P) females; the Scheffé test or the Dunnett test was used for pair-wise comparison according to the variance homogeneity.

Results

Microcosm experiment

During the experimental period, environmental conditions varied among microcosms. Mean oxygen concentration varied from 1 to 4 mg L⁻¹, mean pH value varied between 8 and 9 and mean conductivity varied from 0.9 to 2.2 mS cm⁻¹ (Table 1). In all microcosms hatchlings were observed and adults were found for the duration of the experiment (6 months for all microcosms but FC3 and FC4 which were observed for 3 months) (Table 1). Adults we counted, generally, belonged to more than one putative generation (Table 1).

We observed a clear difference in the proportion of amphimictic and parthenogenetic forms in different experimental conditions. At 24 °C 12:12 L:D amphimictic females were dominant: the only forms present in three out of four microcosms (Table 1). In the FC2 microcosms, 88% of females were amphimictic and their number decline with time (Table 1, Fig. 2). At 16 °C 10:14 L:D amphimictic females were not observed in two out of four microcosms and they represented the 86% and 13% females in the FC5 and FC6 microcosms, respectively (Table 1, Fig. 2). Considering the cumulated individual counts at the end of the experiment in all 8 microcosms, the numbers of amphimictic females and parthenogenetic females were significantly different between the two experimental conditions (GLM: $F_{6,7} = 18.06$, $p < 0.001$). At 24 °C 12:12 L:D, a total of 372 adult amphimictic females with lamella and 115 adult males were counted. This overall result was significantly different from a sex ratio of 1:1 (LRT: $\chi^2 = 135.62$, $df = 1$, $p < 0.001$) and in all the microcosms the sex ratio was significantly female biased except in microcosm FC1 (Table 1). At 16 °C 10:14 L:D, a total of 73 adult females with lamella and 64 adult males were counted: the overall result was not significantly different from a sex ratio of 1:1 (LTR: $\chi^2 = 0.59$, $df = 1$, $p = 0.44$). The numbers of amphimictic and parthenogenetic females were significantly different between the two experimental conditions along time since inundation (GLMM: LTR: condition $\chi^2 = 11.507$, $df = 1$, $p < 0.001$; time LTR: $\chi^2 = 42.248$, $df = 1$, $p < 0.001$). Interaction between experimental condition and time was not statistically significant.

Considering microcosms where both amphimictic and parthenogenetic forms were observed (FC2, FC5 and FC6) amphimictic females and males appeared earlier than parthenogenetic females at both set of experimental conditions (Table 1, Fig. 2).

Life table experiment

Results from life table experiment carried out at 24 °C 12:12 L:D photoperiod and at 16 °C 10:14 L:D photoperiod confirmed the preference of different thermal - light conditions by parthenogenetic and amphimictic forms. At 16 °C 10:14 L:D photoperiod, out of total of 37 isolated juveniles, only 25 parthenogenetic females (NAF = 25) reached adulthood (Table 2). At 24 °C 12:12 L:D photoperiod, out of total of 55 isolate juveniles, both amphimictic and parthenogenetic females reached adulthood and the number of adult amphimictic females (NAF = 20) was double with respect to the number of adult parthenogenetic ones (AF = 10) (Table 2). As a consequence, life span of adults and per capita number of offspring could only be compared for the 24 °C 12:12 L:D condition (Table 2). Mean life span of adults and mean number of offspring did not vary between amphimictic and parthenogenetic females at 24 °C. Adult parthenogenetic females were larger at 24 °C 12:12 L:D than at 16 °C ($F_{2,52} = 4.171, p = 0.021$).

At 24 °C 12:12 L:D, amphimictic females produced a total of 86 amphimictic females with lamella and 62 males. This overall result is different from a sex ratio of 1:1 ($\chi^2 = 3.89, df = 1, p = 0.048$).

Parthenogenetic females produced only females without lamella. However, males were also found in the progeny of three parthenogenetic females. In the progeny of one female born and grown at 24 °C 12:12 L:D, 28 females and 9 males were observed. In the progeny of two females born and kept at 16 °C 10:14 L:D we found 18 females and 5 males and 13 females and 4 males, respectively. Sex ratio, that is male proportion, was significantly higher in the progeny of amphimictic females than in the progeny of parthenogenetic females (Table 2).

Discussion

We showed how differences in the ecological niche of amphimictic and parthenogenetic lineages of *H. barbara* could allow their coexistence in a temporary pond through a highly differentiated seasonal cycle. Experimental conditions mimicked environmental conditions found in the field at the beginning of the rainy season (end of September-October) and at its peak (mid-winter). At conditions found at the beginning of the rainy season we observed mostly amphimictic forms. Parthenogenetic females were in similarly dominant proportions at conditions found at the peak of the rainy season. Warmer temperatures and longer day light of the beginning of the rainy season are associated with higher variability in rainfall and thus water presence is more uncertain than in the peak of the wet season. A similar seasonal dynamics of *H. barbara* amphimictic females was also observed in another population on Lampedusa, Aria Rossa, where only males and amphimictic females were found (Rossi et al. 2013). Although delayed hatching of both resting and non-resting eggs are likely to complicate distinction between true generations (Rossi et al. 2012; 2013; 2015), we consider that both amphimictic and parthenogenetic lineages have more than one recruitment cohort in our microcosms. The association of sexual reproductive systems with environmental heterogeneity is one of the predictions of ecological hypotheses on the maintenance of sex (Bell 1982). One possible reason for the difference in proportions of amphimictic and parthenogenetic forms in different seasonal conditions is suggested by our life table experiment: at 16 °C 10:14 L:D amphimictic females do not complete their life cycle. In microcosm experiment at the same conditions, we observed adult amphimictic females, although in limited numbers and only in 2 out of four replicates. An obvious explanation is the greater environmental heterogeneity in the microcosms. Free of the possible effect of temperature, in the three microcosms in which we found adults of both forms, resting eggs that will develop into amphimictic females hatch earlier than those that will develop into parthenogenetic females. A shorter time to hatch and to complete life cycle would be an advantage for amphimictic forms over parthenogenetic females when hydroperiod is more variable and uncertain (i.e. at the beginning of the rainy season).

The coexistence of amphimictic and parthenogenetic forms is allowed by resting eggs that hatch at different environmental conditions (Chesson 2000; Hairston and Kearns 2002). The storage effect (Chesson 1994) is a mechanism proposed to explain the coexistence of different species in temporally varying environments that could be extended to a finer distinguishable taxonomic level like lineages within species. Its three components are: temporal differences between taxa in their per capita competitive abilities, persistence through unfavorable periods, and positive temporal covariation of periods of high growth potential and low competition (Abrams et al. 2013). Our experiments were not designed as competition experiments and offer correlation based support for temporal niche partitioning. We think they supply evidence of presence and absence of the two forms in different moments of the seasonal cycle that could be considered a temporarily competition-free space, an example of the special circumstances required for the coexistence of biologically similar organisms in the same habitat (Lehto and Haag 2010). In simpler terms we can say that, if a form is not present and not adapted to a particular set of environmental conditions it obviously is an inferior competitor.

Our results could support a reasonable reconstruction of the evolutionary events involved in the coexistence in the same pond of amphimictic and parthenogenetic lineages. The Frozen Niche Variation (FNV) hypothesis is often used to illustrate the mechanism that leads to clonal differentiation and coexistence in parthenogenetic lineages (clones) arising within an amphimictic population (Vrijenhoek 1979; Vrijenhoek and Parker 2009). According to the FNV model, independently derived clones "freeze" parts of the variation present in the sexual progenitors and therefore can have different ecological characteristics. Selection is expected to favour the clones which have non-overlapping niches with other clones or the sexual progenitors.

Clones of *H. barbara* that arise within amphimictic population might "freeze" the ecological tolerance for the conditions found in the pond in the peak of the wet season while the amphimictic lineages maintain the ability to cope with a more variable environment. Both forms can use the storage represented by resting eggs to survive less favorable conditions and resting eggs may rest in

hydration and delay hatching. Population dynamic models consistent with this hypothesis and the role of selective asymmetries in mediating the spatial and temporal coexistence of parthenogenetic taxa and their close sexual relatives have been investigated in several theoretical studies (Weeks 1993; Rispe et al. 1998; Doncaster et al. 2003). The outcome of a clonal invasion in an ecological timescale under the frozen niche variation hypothesis depends on the inter-specific interactions between amphimictic and parthenogenetic competitors (Doncaster et al. 2000; Pound et al. 2002). Several studies have explored the threshold in niche overlap that permits coexistence (Case and Taper 1986; Pound et al. 2004). However, sympatric amphimictic and parthenogenetic morphs are rarely observed in nature. Few experimental studies have tested if ecologically relevant genotypic variation can be frozen in different clones originating from sexual ancestors in *Artemia*, *C. novaezelandiae*, *E. virens* and *P. antipodarum* (Barata et al. 1986; Chaplin 1992; Negovetic and Jokela 2001; Agh et al. 2007; Rossi et al. 2008; Schmit et al. 2013). Ecological theory predicts that genetic variation produced by sexual reproduction results in niche diversification and provides a competitive advantage both to facilitate invasion into genetically uniform parthenogenetic populations and to withstand invasion by parthenogenetic competitors (Tagg et al. 2005). In previous work, we have showed that clonal lineages of *H. barbara* from Vallone della Forbice C, arose within bisexual populations of Lampedusa and include at least 5 different multilocus genotypes (Rossi et al. 2003; 2007). In parthenogenetic lineages, clonal variability may balance the longer-term benefits of recombination and our preliminary data are suggestive of niche partitioning even among different specialized clones (Rossi et al. 2004). Actually, we previously described the coexistence and the seasonal succession of ecologically different clones of *H. incongruens* (Ramdohr, 1808) in Northern Italy rice-fields (Rossi and Menozzi 1993; Rossi et al. 1996).

Here, we have not carried out an actual competitive experiment but directly compared life history traits of parthenogenetic females and their sympatric closest sexual relatives. We did not find significant difference in fitness related traits between amphimictic and parthenogenetic females. Present results are not in accordance with other data on *E. virens* and *H. incongruens*. In *E. virens*

from western and central Spain, Martins et al. (2008) found that parthenogenetic lineages have higher potential growth than amphimictic ones due to earlier hatching response of resting eggs. In *E. virens* and *H. incongruens* Rossi and Menozzi (2012) found that parthenogenetic females have higher mean rate of reproduction over the adult life span than non-sympatric and not relative amphimictic ones. In the framework of the sex paradox, this discrepancy calls for the need of comparing parthenogenetic females to their closest sexual progenitors eliminating the confounding effects of sexuality, ploidy and local adaptation (Kobayashi et al. 2013).

In our life table experiment, sons are recorded in the progeny of 10% of fecund apomictic females. Due to the multi-X systems of sex determination, in the apomictic females of the genus *Heterocypris*, the occasional non-disjunction of the X-chromosome during oogenesis could generate XO eggs that develop into males (Schön and Martens 1998). The occasional production of sons by parthenogenetic females is documented in other taxa: it may be linked to the recent sexual ancestry of parthenogenetic females and could reduce their demographic advantage on amphimictic ones (Innes et al. 2000; Neiman et al. 2012). In our microcosms the sex ratio was generally female biased, as well as in the field (Bellavere et al. 2002). A similar phenomenon was described in amphimictic lineages of *E. virens* and *H. incongruens* (Vandekerckhove et al. 2007; Rossi and Menozzi 2012). Female-biased sex ratios are common among freshwater ostracods and it may be related to reduced longevity of males and/or to the sex determination systems (Cohen and Morin 1990; Chaplin et al. 1994; Schön and Martens 1998). Small bisexual populations from temporary ponds may suffer sperm limitation that might have favoured the spread of parthenogenetic lineages (Chaplin et al. 1994). Protandry and delayed recruitment of *H. barbara* parthenogenetic females with respect to amphimictic ones may prevent hybridization and sperm competition by parthenogenetic females (Smith 1984). Our preliminary observations suggest that male rather than female behaviour determine copulation frequencies but we have no data about the possible effect of female size on male mating preferences (Jennions and Kokko 2010; Nelson and Neiman 2011). Due to posterodorsal copulation, an intriguing hypothesis is that lamella hyalina, typical of amphimictic

females, represents an honest signal for mate recognition or resistance avoidance to male harassment (Cohen and Morin 1990; Rankin et al. 2011). We have laboratory evidence that male aggressiveness may cause female's death and lead to cannibalism of eggs and juveniles in *Heterocypris* (unpublished data). In the case, male mate preferences might act as a reinforcement mechanism (Servedio and Noor 2003).

In the ephemeral pool, organisms face important selective constraints resulting from a trade-off between hatching success of resting eggs and the risk of depleting egg bank hatching during inundations too short for completing life cycle. The niche partitioning between amphimictic and parthenogenetic lineages, and the effect of "seasonal" variation of temperature and photoperiod on hatching phenology of different *H. barbara* lineages may enable their coexistence that actually is limited in time. Achieving reproductive success in this context depends largely on hatching synchronization with water availability in the "right seasonal slot". However, hydroperiod variability and disturbance events, i.e. unpredictable drying up, may eventually reduce, revert or interrupt the sequence of competition (Brock et al. 2003; Park et al. 2014). Coexistence may, in theory, be extended indefinitely at appropriate rate of disturbance and environmental heterogeneity has been postulated as a factor enabling it (Chesson 2000).

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Table 1. Results from experimental microcosms set up with inundated sediment from Vallone della Forbice at two different experimental conditions 24 °C and 16 °C temperature (°C), and 12:12 L:D and 10:14 L:D photoperiod (L:D). Mean values of conductivity and standard error of the mean (based on 12 or 24 samples according to inundation of 3 or 6 months) is shown. Cumulative number of recorded and measured juveniles (J), adult males (M), ovigerous amphimictic females (AMF) and ovigerous parthenogenetic females (PF) over the experimental period are reported. In parentheses, the estimated number of generations are reported. Minimum recruitment time in days from sediment inundation to the appearance of first adult males (M), ovigerous amphimictic females (AMF) and ovigerous parthenogenetic females (PF) are reported. In the last column sex ratio (SR) was calculated as ratio between number of males and number of amphimictic females observed over the experimental period (** indicate significance of departure from 1:1 with $p < 0.001$).

Microcosm	°C	L:D	mS cm ⁻¹	J	Cumulative number			Recruitment time (days)			
					M	AMF	PF	M	AMF	PF	SR
FC1	24	12:12	0.9 ± 0.11	11	44	47 (3)	0	23	23	-	0.9
FC2	24	12:12	1.6 ± 0.31	143	40	102 (3)	14 (2)	13	13	27	0.4**
FC3	24	12:12	1.9 ± 0.49	15	24	121 (3)	0	21	21	-	0.2**
FC4	24	12:12	2.2 ± 0.58	42	11	102 (3)	0	21	21	-	0.1**
FC5	16	10:14	0.9 ± 0.15	53	36	32 (1)	5 (1)	71	38	198	1.1
FC6	16	10:14	1.3 ± 0.35	226	28	41 (1)	277 (3)	21	27	48	0.7
FC7	16	10:14	1.6 ± 0.21	375	0	0	163 (2)	-	-	41	-
FC8	16	10:14	1.6 ± 0.28	414	0	0	110 (2)	-	-	35	-

Table 2. Results from life table experiment carried out at two different experimental conditions (EC): 24 °C 12:12 L:D and 16 °C 10:14 L:D. The starting number of juveniles for each condition were: 55 newborns at 24° 12:12 L:D; 37 newborns at 16°C 10:14 L:D (see Material and methods). For each experimental condition (EC) and reproductive mode (RM) the number of amphimictic females (AMF) and parthenogenetic females (PF) that reached adulthood (NAF), the number of fecund females (NFF), mean size at adulthood (Size), mean adult females life span (ALS), mean offspring number (ON) and mean sex ratio (SR) in the progeny of fecund females are reported. Standard deviation is reported in parentheses. Different letters (a, b) indicate significant difference among female types at different experimental conditions. Scheffé test was used for size and adult life span, Dunnet test was used for offspring number and sex ratio.

EC	RM	NAF	NFF	Size (mm)	ALS (days)	ON	SR
24 12:12	AMF	20	15	1.51 (0.11) ab	23.00 (13.03) a	9.87 (7.83) a	0.69 (0.36) b
16 10:14	AMF	0	0	no data	no data	no data	no data
24 12:12	PF	10	8	1.58 (0.08) a	17.13 (13.47) a	10.75 (12.48) a	0.04 (0.11) a
16 10:14	PF	25	21	1.45 (0.14) b	19.57 (13.84) a	5.05 (6.10) a	0.03 (0.09) a

Captions

Figure 1. Mean values of minimum and maximum monthly temperature and precipitation on Lampedusa Island (50 yearly records collected in the period 1926-2007). Standard error of the mean is shown for precipitation and is not reported for temperature: it varies between 0.18 in July and 0.37 in February for minimum and between 0.32 in February and 0.77 in June for maximum.

Figure 2. Results from microcosm experiment. Percentage of amphimictic (AM) ovigerous females (of total ovigerous females) from Vallone della Forbice microcosms maintained at two environmental conditions. Top: 24 °C 12:12 L:D photoperiod. Bottom: 16 °C 10:14 L:D photoperiod. Number of days are from sediment inundation.

1

Draft

Figure 1