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Shallow gene pools in the high intertidal: extreme loss of genetic diversity in viviparous sea stars (*Parvulastra*)

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We document an extreme example of reproductive trait evolution that affects population genetic structure in sister species of *Parvulastra* cushion stars from Australia. Self-fertilization by hermaphroditic adults and brood protection of benthic larvae causes strong inbreeding and range-wide genetic poverty. Most samples were fixed for a single allele at nearly all nuclear loci; heterozygotes were extremely rare (0.18%); mitochondrial DNA sequences were more variable, but few populations shared haplotypes in common. Isolation-with-migration models suggest that these patterns are caused by population bottlenecks (relative to ancestral population size) and low gene flow. Loss of genetic diversity and low potential for dispersal between high-intertidal habitats may have dire consequences for extinction risk and potential for future adaptive evolution in response to climate and other selective agents.

1. Introduction

An important discovery of empirical population genetics is the strong correlation between geographical genetic variation and a few key life-history variables [1]. Small demes or pools of mates (the effective population size, N_e) and limited dispersal of propagules among demes (the migration rate, m) can reduce within-population genetic diversity and increase among-population genetic differences. Evolutionary biologists have long recognized [2–4] the emergence of these effects on long time scales in comparative studies of species-specific differences in mating-system traits (especially the evolution of self-fertilization) and dispersal traits (especially the dispersive properties of gametes, pollen, seeds or larvae). Conservation biologists working on shorter time scales have more recently recognized [5] similar effects of small population size (e.g. from over-harvesting) on increased local inbreeding among fewer mates (and smaller N_e) or of habitat fragmentation on demographic connectivity (and lower m). On both time scales, these correlations have important implications for population dynamics, local adaptive evolution and extinction [6,7].

Life-history traits [8,9] that can shape population genetic patterns [10,11] have repeatedly evolved among closely related and ecologically similar marine invertebrate species [12,13]. These make useful case studies, because population genetic patterns among such species can reasonably be ascribed

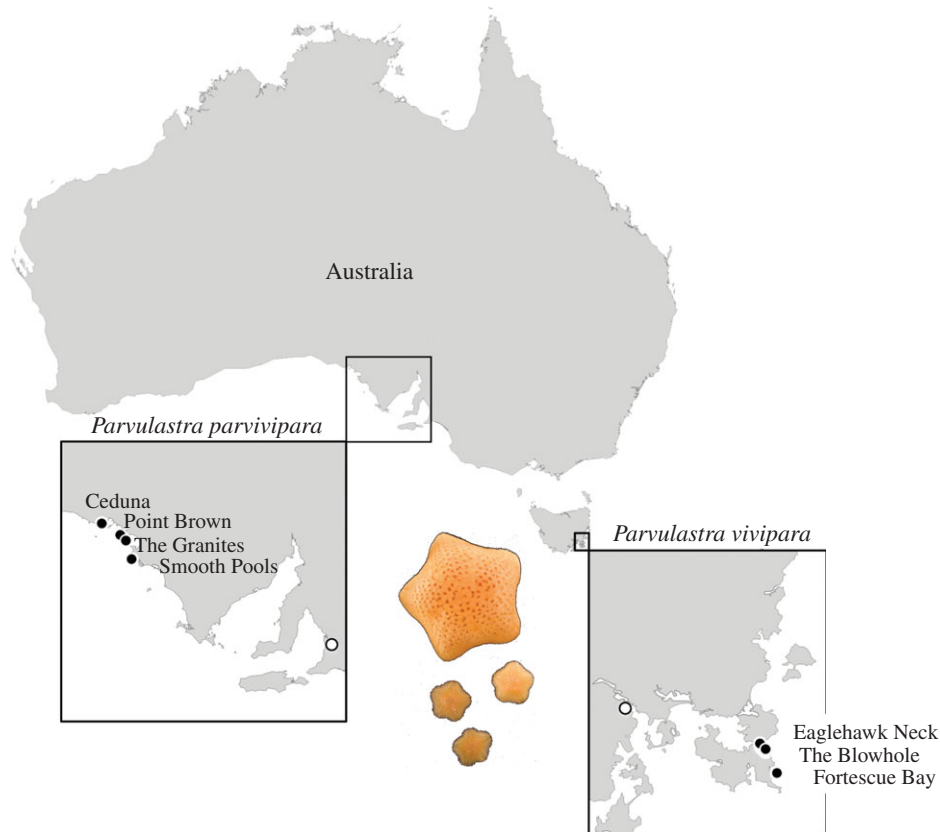


Figure 1. Geographical distribution of seven *Parvulastra* populations (filled circles) in South Australia (*P. parvivipara*) and Tasmania (*P. vivipara*); two open circles show state capitals (Adelaide and Hobart). Inset: a hermaphroditic *P. vivipara* parent and its brood of three live-borne offspring. (Online version in colour.)

to their specific life-history traits. In the sea star family Asterinidae (cushion stars), four lineages in three genera have evolved self-fertilization, internal brood protection of larvae and live birth of benthic juveniles (figure 1) [14]. We showed that extreme inbreeding and loss of genetic diversity in a live-bearing self-fertile hermaphrodite (*Cryptasterina hystera*) has evolved on a time scale of a few thousand years (since its divergence from *Cryptasterina pentagona*), similar to the temporal scale associated with ecological variables in conservation genetics studies [15]. Here, we extend this comparison [16] to two additional species of self-fertile live-bearing asterinids: the sister species *Parvulastra parvivipara* and *Parvulastra vivipara*. Both species are restricted to high-intertidal pools on the western Eyre Peninsula (*P. parvivipara*) and eastern Tasmania (*P. vivipara*, which is listed as ‘vulnerable’ [17]; figure 1). Adults (less than or equal to 1 cm diameter) and clutches (less than 100 offspring) are small, and larvae metamorphose into juvenile sea stars in the gonad followed by birth of non-dispersing benthic offspring [14]. This life history probably arose in the common ancestor of these two species in the Pleistocene [18] after their divergence from *Parvulastra exigua*, in which adults have external fertilization, a mix of selfing and outcrossing, and no brood care [10,19,20]. We found nearly complete loss of genetic diversity in all populations of both live-bearing *Parvulastra* species (similar to *C. hystera*), with small population sizes and zero gene flow. Both species face a high risk of extinction and have limited potential for local adaptive evolution (owing to lack of genetic diversity) or population replacement (after local extirpation) in response to climate change and other threats to coastal habitats.

2. Material and methods

We sampled tube feet of 241 adults from seven populations (19–51 per population) that span most of the range of *P. parvivipara* (approx. 120 km) and part of the range of *P. vivipara* (approx. 25 km) (table 1). We characterized allele-size variation at seven microsatellite loci [19,21], and nucleotide sequence variation in a nuclear intron (glucose-6-phosphate isomerase or GPI) [15,21] (GenBank accession no. KC866341-42) and two parts of the mitochondrial genome concatenated together as a single locus (several transfer RNA genes plus the control region) [21,22] (KC876801-859). All nine loci are highly variable in other asterinids, including *P. exigua* [10,19,20]. We used some of the same microsatellite markers to genotype offspring and brooding females from four populations (The Granites, Smooth Pools, Eaglehawk Neck, The Blowhole; table 1) to look for evidence of sexual reproduction and outcrossing.

We used standard frequency-based population genetic methods to characterize diversity within populations and differentiation between pairs of populations for each species [15,21]. We fitted isolation-with-migration models to data from polymorphic loci for pairs of populations in each species (table 1) using IMA [23] (see the electronic supplementary material).

3. Results

Genetic diversity within all populations was extremely low. For seven population samples, each genotyped at seven microsatellite loci, 34 of 49 cases were fixed for a single microsatellite allele (table 1); in 10 cases (locus c114 in *P. parvivipara*; loci c104 and c113 in *P. vivipara*), no alleles were observed (these populations appear to be fixed for

Table 1. Counts of alleles and haplotypes for nine loci sampled from seven *Parvulastra* populations. Microsatellite alleles are labelled by size (bp); GPI and mtDNA haplotypes are labelled by letter (A–B, A–N). Blank entries indicate probable microsatellite null alleles. Italicized values indicate data used in isolation-with-migration analyses for two population pairs.

| | <i>P. parvivipara</i> | | | | <i>P. vivipara</i> | | | |
|-------|-----------------------|-------------|--------------|--------------|--------------------|--------------|---------------|--|
| | Ceduna | Point Brown | The Granites | Smooth Pools | Eaglehawk Neck | The Blowhole | Fortescue Bay | |
| | S32°12.3' | S32°32.0' | S32°52.8' | S32°55.3' | S43°0.5' | S43°1.8' | S43°8.3' | |
| | E133°44.3' | E133°51.8' | E134°5.0' | E134°4.6' | E147°55.9' | E147°56' | E147°58.1' | |
| a110 | 214 | 0 | 0 | 0 | 38 | 56 | 74 | |
| | 228 | 40 | 102 | 100 | 0 | 0 | 0 | |
| | 232 | 0 | 0 | 0 | 0 | 0 | 0 | |
| b202 | 198 | 40 | 102 | 100 | 38 | 56 | 74 | |
| c104 | 226 | 40 | 102 | 100 | 72 | | | |
| c113 | 248 | 40 | 102 | 100 | 72 | | | |
| c114 | 263 | | | | 38 | 56 | 74 | |
| c212 | 265 | 40 | 96 | 88 | 0 | 0 | 0 | |
| | 267 | 0 | 0 | 0 | 38 | 56 | 72 | |
| | 268 | 0 | 6 | 12 | 72 | 0 | 0 | |
| c219 | 178 | 40 | 102 | 100 | 72 | 0 | 0 | |
| | 203 | 0 | 0 | 0 | 0 | 34 | 56 | |
| | 206 | 0 | 0 | 0 | 0 | 4 | 16 | |
| GPI | A | 40 | 82 | 96 | 70 | 0 | 0 | |
| | B | 0 | 0 | 0 | 0 | 30 | 26 | |
| mtDNA | A | 0 | 0 | 0 | 0 | 0 | 20 | |
| | B | 0 | 0 | 0 | 0 | 0 | 2 | |
| | C | 0 | 0 | 0 | 0 | 15 | 0 | |
| | D | 0 | 0 | 0 | 0 | 0 | 17 | |
| | E | 0 | 0 | 0 | 0 | 0 | 1 | |
| | F | 21 | 17 | 0 | 7 | 0 | 0 | |
| | G | 0 | 0 | 0 | 9 | 0 | 0 | |
| | H | 0 | 0 | 0 | 1 | 0 | 0 | |
| | I | 0 | 0 | 3 | 0 | 0 | 0 | |
| | J | 0 | 0 | 0 | 1 | 0 | 0 | |
| | K | 0 | 0 | 0 | 1 | 0 | 0 | |
| | L | 0 | 0 | 16 | 0 | 0 | 0 | |
| | M | 0 | 0 | 1 | 0 | 0 | 0 | |
| | N | 0 | 0 | 1 | 0 | 0 | 0 | |

null alleles) [19]; and in only five cases were polymorphisms segregating within a population. Alleles differed in size by a maximum of only one microsatellite repeat (for the trinucleotide loci c212 and c219) or two repeats (for the dinucleotide locus a110) within either species. We found one heterozygous genotype (a110^{228/232}) in three individuals from Smooth Pools. This was also the only population with a fixation index less than 1.0 ($f = 0.83$). Standardized allelic richness and observed heterozygosity averaged across loci were correspondingly very low for all seven populations ($R_s \leq 1.20$, $H_o \leq 0.01$). We found no intraspecific polymorphism in our large sample of GPI intron sequences from either species, which were each fixed for a single (different) haplotype.

We found some evidence of outcrossing in one species. Among 20 *P. vivipara* parents brooding 74 offspring, we found only homozygous parental genotypes at three loci (b202, c212 and c219; table 1) and only maternal alleles among all offspring. However, among 42 *P. parvivipara* parents brooding 181 offspring genotyped at two loci (a110 and c212), we found two parents that were a110^{232/232} homozygotes brooding both a110^{232/232} homozygous offspring as well as one or two offspring that were a110^{228/232} heterozygotes. We conclude that these three offspring were sired by a male with the more common a110²²⁸ allele (table 1). The result is also surprising evidence for sperm transfer between individual hermaphroditic sea stars that lack obvious

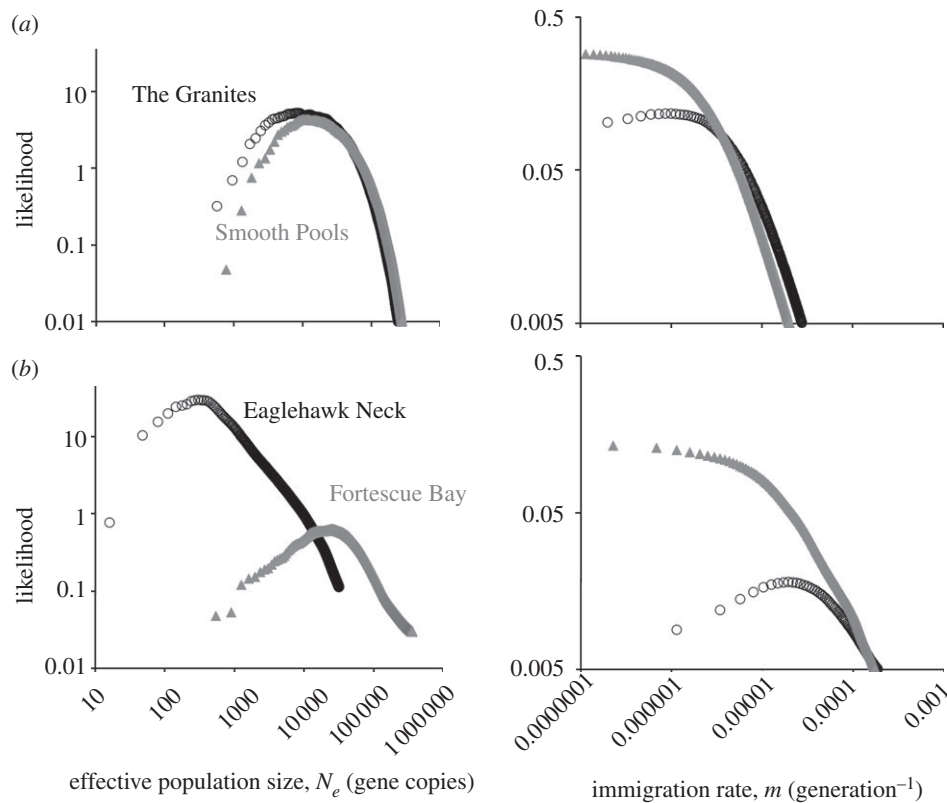


Figure 2. Posterior probability distributions for population demographic parameters (N_e , m) from isolation-with-migration model fits to genetic data (a) for two *P. parvivipara* populations and (b) for two *Parvulastra vivipara* populations.

morphological or behavioural adaptations for copulation and sperm storage.

We observed seven mtDNA haplotypes from *P. parvivipara* populations and five from *P. vivipara* (table 1); within both species, haplotypes differed by 4 or fewer substitutions or insertion–deletions. Four of seven populations were fixed for a single haplotype, and only at Smooth Pools did we find more than one relatively common haplotype (F, G).

Population differentiation varied across population pairs and loci (some fixed for the same allele; others fixed for different alleles). For example, microsatellite pairwise F_{ST} was less than or equal to 0.06 and not significantly different from zero among the three northernmost populations from Ceduna, Point Brown and The Granites, and all three of these were strongly differentiated from Smooth Pools ($F_{ST} \geq 0.79$). By contrast, analyses based on mtDNA showed that the Smooth Pools population was not differentiated from Point Brown ($F_{ST} = 0$), with strong differentiation among some northern population pairs (e.g. $F_{ST} = 0.73$ for the more highly variable control region sequences from Point Brown and The Granites).

Isolation-with-migration model fits are shown in figure 2 for the least depauperate population pair from each species. In both cases, the two historical population parameters (ancestral N_e ; divergence time) were estimated with low probabilities and poorly defined posterior distributions (see the electronic supplementary material). For *P. parvivipara*, the two population sizes were similar to each other ($N_e \sim 10\,000$). Although the poorly defined posterior probability distribution for the ancestral population size limits our ability to compare it quantitatively with the two population size estimates, in both cases, the point estimate of ancestral population size ($N_{eA} > 1\,000\,000$) and its lower confidence limit were much greater than the upper confidence limit on each of the population size estimates. For *P. vivipara*, population size for Eaglehawk Neck was very small

($N_e \sim 300$), but much larger for Fortescue Bay ($N_e \sim 30\,000$, and similar to the maximum-likelihood estimate of ancestral population size). These results are provisional, and do not capture the possible effects of older metapopulation dynamics on the coalescent pattern [24], but both analyses tend to suggest an historical population size reduction that has been especially acute in some populations such as Eaglehawk Neck.

Gene flow into both *P. vivipara* populations was $m = 2.0\text{--}14.8 \times 10^{-6}$ generation $^{-1}$, and the likelihood ratio test (LRT) of nested population models in IMA suggested that both rates were not significantly different from zero ($p = 0.204$ with 2 d.f.). Immigration into *P. parvivipara* populations was also low, and the LRT suggested that neither of those single rates significantly differed from zero ($p = 0.083\text{--}0.917$, d.f. = 1); however, a third test rejected the hypothesis that both are zero ($p \ll 0.001$, d.f. = 2). The shapes of the posterior distributions (figure 2) suggest that nonzero gene flow might be more likely into The Granites, but the magnitude of that gene flow must be low ($m \sim 0.9 \times 10^{-6}$).

4. Discussion

This study documents unusually extreme inbreeding across the geographical ranges of two species of self-fertile, non-dispersing sea stars. Although our analysis of offspring genotypes shows that outcrossing does occur in at least some broods of one species, selfing is likely to occur as well and both selfing and loss of gene flow probably contribute to strong genetic drift within populations and the fixation of different alleles between populations at many loci. The additional contributions of selection and hitchhiking [3] to this process are not known in *Parvulastra* but could be significant. Comparable effects of selfing are well known among plants, in which mixed

mating systems are more common [3] and variation in selfing rates has been attributed to selection on components of fitness such as reproductive assurance [4].

Hermaphroditism and benthic brood protection have evolved in parallel as a suite of life-history traits among multiple asterinid lineages [14], in physiologically extreme high-intertidal habitats and in association with low genetic diversity and strong population differentiation [15]. The apparent ease with which many asterinid lineages can evolve benthic brood protection and self-fertility [20] raises the question of why such lineages have not replaced asterinids with gonochoric outcrossing adults and planktonic larvae? One hypothesis involves the associated high risk of local extirpation and extinction. All known asterinids with internal or external brood protection have small geographical ranges (less than 300 km) and have recently diverged from close relatives with other life histories [14,15,18,25].

Are viviparous sea stars doomed to extinction in the high-intertidal zone [26]? Our studies of *Parvulastra* and *Cryptasterina*

suggest that live-bearing asterinid populations are unlikely to harbour the additive genetic variation necessary for rapid adaptive evolution in response to strong selection on physiological tolerance or other traits associated with climate change [27] or to expand their geographical ranges via dispersal into new habitats in response to changing climate patterns [28]. The fate of such specialized, isolated and genetically depauperate populations seems at best uncertain, but if past is prologue [6], then such species seem to be marked for extinction in the shallow end of the high-intertidal gene pool.

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