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Spawning, copulation and inbreeding coefficients in marine invertebrates

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Patterns of population genetic variation have frequently been understood as consequences of life history covariates such as dispersal ability and breeding systems (e.g. selfing). For example, marine invertebrates show enormous variation in life history traits that are correlated with the extent of gene flow between populations and the magnitude of differentiation among populations at neutral genetic markers (F_{ST}). Here we document an unexpected correlation between marine invertebrate life histories and deviation from Hardy–Weinberg equilibrium (non-zero values of F_{IS}, the inbreeding coefficient). F_{IS} values were significantly higher in studies of species with free-spawned planktonic sperm than in studies of species that copulate or have some form of direct sperm transfer to females or benthic egg masses. This result was robust to several different analytical approaches. We note several mechanisms that might contribute to this pattern, and appeal for more studies and ideas that might help to explain our observations.

Keywords: F_{IS}; Wahlund effect; fertilization; population genetics

1. INTRODUCTION

Large positive F_{IS} values from marine invertebrate population genetics studies are commonly observed but less easily explained in relation to life history variation compared to among-population differentiation (high F_{ST}; e.g. Bohonak 1999). F_{IS} values reflect deviation from Hardy–Weinberg equilibrium (HWE) genotype frequencies and (indirectly) reflect relative population heterozygosity. High positive F_{IS} values (and heterozygosities significantly less than those predicted at HWE) may be caused by laboratory artefacts, unobserved null alleles, natural selection acting on the genetic markers, mating among relatives, or unrecognized spatial or temporal structure within samples known as the Wahlund effect (Zouros & Foltz 1984; David *et al.* 1997; Raymond *et al.* 1997; Whitaker 2003). Here we report the results of a literature survey that reveals a taxonomically broad association between one life history trait (spawned planktonic sperm versus copulation) and F_{IS} variation.

2. LITERATURE SURVEY AND ANALYSIS

We surveyed the literature (especially the journals *Marine Biology*, *Molecular Ecology* and *Evolution*) for studies that reported F_{IS} values from broad geographic sampling of multiple nuclear loci with large average sample sizes per population (see electronic supplementary material). For each case (a species within a study, $N=124$) we used the mean estimate of F_{IS} for all populations averaged across all polymorphic loci. We scored each species for three reproductive traits of primary interest: mode of larval dispersal (with or without prolonged planktonic larval dispersal), male spawning mode (planktonic sperm versus copulation or other direct sperm transfer) and female spawning mode (planktonic eggs versus internal or external benthic eggs). We compared F_{IS} values for bivariate traits using two-sample *t*-tests. We used the sequential Bonferroni correction for multiple *t*-tests (Sokal & Rohlf 1997) to identify individual test results (indicated by an asterisk) that were significant at the study-wide $\alpha=0.05$.

We examined the phylogenetic correlation between reproductive traits and F_{IS} values using independent contrasts. We used a phylogeny (figure 1) derived from recent syntheses (Ruppert *et al.* 2004) and a few molecular phylogenies (Romano & Cairns 2000; van Oppen *et al.* 2001; Le Goff-Vitry *et al.* 2004). Where we happened to survey two or more studies of a single species, we included all F_{IS} estimates. We resolved some parts of the tree by favouring monophyletic species and genera, and we arbitrarily resolved polytomies that did not include informative differences in any of the reproductive traits. We assumed all branch lengths to be equal. We used the Brunch method in CAIC (Purvis & Rambaut 1995) to estimate correlations between standardized contrasts in reproductive traits and standardized contrasts in F_{IS} values transformed as $\exp(F_{IS})$.

In other analyses (not shown here) we also scored each species for two aspects of the breeding system: asexuality (with or without a significant asexual stage) and hermaphroditism (with or without a prominent simultaneous hermaphrodite adult stage). Neither of these traits was significantly associated with F_{IS} variation in *t*-tests, but our survey was a weak test of these effects because the realized extent of either asexual propagation or selfing is poorly known for most of the species that we surveyed.

3. RESULTS

Most studies reported positive F_{IS} values (mean $F_{IS}=0.172$). Among the 23 highest F_{IS} values (more than 0.3), 22 came from anthozoans ($n=14$), bivalves ($n=4$), gastropods ($n=2$), polychaetes ($n=1$) and tunicates ($n=1$) with free-spawning males. Mean (variance) F_{IS} was 0.205 (0.054) for 89 studies of species with planktonic sperm (figure 2*a*). In contrast, mean F_{IS} was 0.081 (0.007) among 35 studies of sea stars, gastropods, cephalopods and crustaceans with direct sperm transfer or copulation ($^*p=0.000\,09$). We found a comparable difference when we analysed only allozyme results ($^*p=0.0003$, but not for the much smaller number of nDNA marker studies, $N=17$, $p=0.091$), without the four highest F_{IS}

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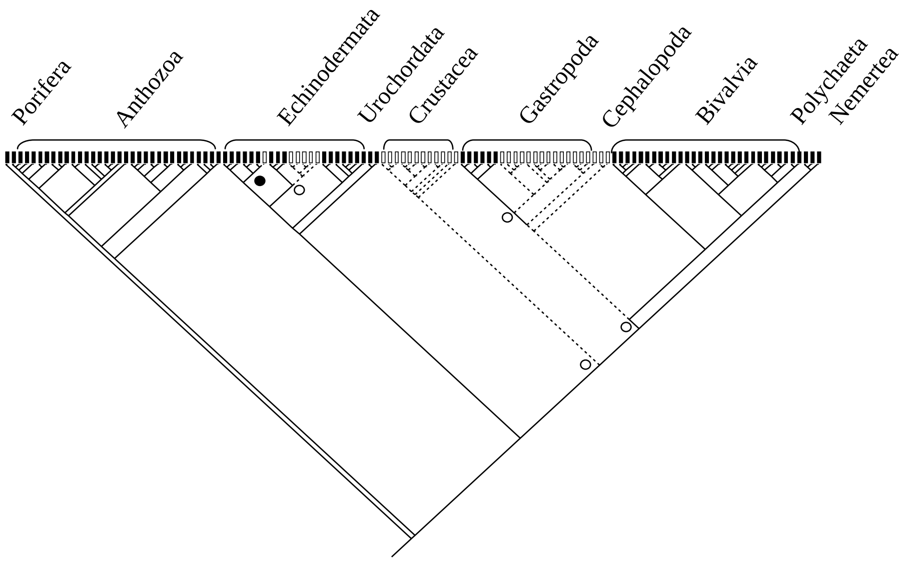

Figure 1. Phylogeny used in the independent contrasts analysis. Higher taxon names are shown above the branching pattern (species names are omitted for clarity). Black branches and boxes indicate lineages or species with planktonic sperm; broken branches and white boxes indicate copulation or direct sperm transfer in (left to right) an asterinid sea star, *Leptasterias* sea stars, crustaceans, caenogastropods (whelks, periwinkles, slippershells), opisthobranch gastropods (nudibranchs, sea slugs) and cephalopods (squids, octopuses). Open circles mark nodes in which copulating descendants had lower F_{IS} than the sister group with planktonic sperm; the black circle marks one contrast (asterinids) in which the copulator had a higher F_{IS} value.

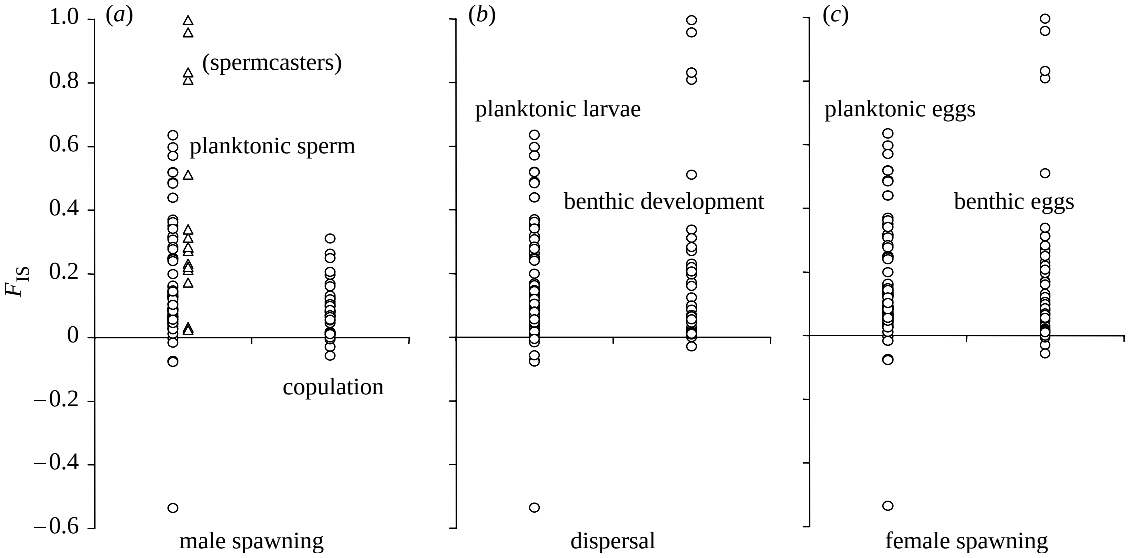

Figure 2. Marine invertebrate F_{IS} values. (*a*) Studies of species with planktonic sperm (left) versus species with copulation or other direct sperm transfer to eggs (right). Some species with planktonic sperm fertilize internal eggs or benthic egg masses (spermcasting, triangles). (*b*) Dispersing planktonic larvae (left) versus benthic development without larval dispersal (right). (*c*) Planktonic eggs (left) versus benthic fertilization of internal eggs or external egg masses (right).

values >0.8 (from *Epiactis* sea anemones and a cup coral; $^*p=0.0008$), without all sea anemones (in which cloning and selfing are suspected to cause deviations from HWE; $^*p=0.003$), or without all bivalves (in which selection on allozyme variation is suspected to cause deviations from HWE; $^*p=0.0002$). We found a comparable difference when we eliminated multiple studies for single species and used only the lowest ($^*p=0.0002$) or the highest ($^*p=0.0001$) observed value for each species.

Many of the highest F_{IS} values (mean $=0.353$) were found among 17 studies of spermcasting corals, sea anemones, sponges and tunicates with planktonic sperm and internal fertilization (triangles in figure 2*a*; Pemberton *et al.* 2003). F_{IS} values for spermcasters were significantly greater than for copulators ($^*p=0.002$) but not significantly greater than in species with planktonic fertilization after correction for multiple tests ($p=0.022$).

The phylogenetic analysis identified five independent contrasts between lineages with and without free spawning of sperm (figure 1). In four cases, the evolution of copulation or other direct sperm transfer (in *Leptasterias* sea stars; snails; other molluscs; and crustaceans) resulted in lower F_{IS} values ($r=0.787$). This result was marginally not significant ($p=0.063$), but an important assumption of the Brunch regression method was violated: after standardization there was still a significant association between contrasts in F_{IS} values and their standard deviations ($p=0.047$; see Purvis & Rambaut 1995). The source of this violation was the fifth contrast (between two species of asterinid sea stars) in which the lineage with direct sperm transfer had a higher F_{IS} value than its free-spawning sister group. When we dropped asterinid species from the Brunch analysis, the four remaining contrasts in F_{IS} values were no longer significantly correlated with their standard deviations and the correlation between copulation and lower F_{IS} values was highly significant ($r=0.972$, $p=0.006$).

The patterns above were not confounded by variation in other reproductive traits. Mean F_{IS} was 0.149 (0.030) among 89 studies of species with planktonic larval dispersal versus 0.235 (0.076) among 34 studies of animals with non-dispersing larval development ($p=0.096$; figure 2*b*). This difference is in the expected direction if some high F_{IS} values reflect mating among relatives in species without planktonic larval dispersal. Mean F_{IS} was 0.166 (0.035) among 70 studies of females with planktonic eggs and 0.184 (0.056) among 52 studies of internally fertilized females ($p=0.644$; figure 2*c*). The phylogenetic analyses identified a larger number of contrasts for these two traits ($N=14$–17), but both were weakly correlated with contrasts in F_{IS} values ($r<0.4$) and the associations were not significant ($p>0.1$).

4. DISCUSSION

The evolution of copulation (the derived character state; Parker *et al.* 1972; Nielsen 1998) is significantly correlated with the evolution of lower F_{IS} values in both our survey and phylogenetic analyses. This surprising pattern does not imply that marine invertebrates with planktonic sperm are typically inbred (via matings among relatives). Rather, it suggests that some aspect of the evolutionary genetics, demography, or mating system of these animals that is associated with the production of planktonic sperm for fertilization also tends to cause greater departures from HWE in the form of heterozygote deficits.

Additional planned comparisons (e.g. Cohen 1996) might help to reveal the underlying mechanisms that cause this pattern. Our survey does not point to a single general explanation that would plausibly connect planktonic sperm with greater departures from HWE, and we do not know what mechanism(s) caused this pattern. We suggest below three possibilities that could combine to influence deviations from HWE and that seem worthy of further exploration, but we hope that readers will critically evaluate these suggestions and propose better alternatives.

One likely mechanism is extensive within-population genetic structure (the Wahlund effect) caused by unrecognized spatial or temporal variation (David *et al.* 1997; Whitaker 2003) that results in large deviations from HWE. However, we are uncertain why the strength of this effect should be correlated with male spawning mode but not dispersal mode (which seems more likely to influence the magnitude of within-population genetic structure).

A second mechanism is based on higher rates of molecular evolution associated with some life history traits (e.g. Foltz 2003). Higher mutation rates in free-spawners may be caused by larger numbers of cell cycles leading to high sperm production (Beckenbach 1994). Under this mechanism, higher F_{IS} values in species with planktonic sperm would be consistent with more frequent null alleles that cause greater departures from HWE in comparison to copulating species with lower allocation to sperm production (Launey & Hedgecock 2001). Null alleles may be common at allozyme (Foltz 1986) and microsatellite (Launey & Hedgecock 2001) loci in free-spawning mollusks and echinoderms (McCartney *et al.* 2004), in comparison to copulating decapod crustaceans in which nulls may be rare (Jensen & Bentzen 2004).

A third mechanism is based on high variance in reproductive success (Purser 1966), which can cause deviations from HWE due to randomly induced differences in allele frequencies between sperm and eggs (Pudovkin *et al.* 1996; Luikart & Cornuet 1999). Under this mechanism, among-male variance in fertilization success (which promotes heterozygote excess relative to HWE) might combine with other factors (such as selection and null alleles that promote heterozygote deficits; David *et al.* 1997) to produce smaller overall departures from HWE in copulators (in which variance in reproductive success might be higher) compared to large departures from HWE in species with planktonic sperm (in which this variance might be lower). In terrestrial vertebrates and arthropods, copulation is associated with high among-male variance in paternity (Birkhead & Møller 1998) and intense sexual selection (Simmons 2001). In a free-spawning sea urchin, this among-male variance was surprisingly low (Levitan 2004), perhaps due to mixing of sperm in the water and the inability of males to limit access to eggs by the sperm of other individuals. However, the models predict heterozygote excess and high negative (rather than less positive) F_{IS} values, and the strength of this effect is expected to be of the order of $1/2N_e$ (that is, very weak in most marine invertebrates with very large N_e). This mechanism also does not predict the strikingly high F_{IS} values among spermcasters that share some life history features in common with free-spawners and with copulators (Bishop 1998).

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