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THE EFFECTS OF CONTRASTING MODES OF FERTILIZATION ON LEVELS OF INBREEDING IN THE MARINE INVERTEBRATE GENUS CORELLA

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Abstract.—A simple difference in the body design of two species of marine urochordates in the ascidian genus Corella suggested that these species may differ in their mating systems. The two coexisting species share common life-history traits and morphology with the exception of a difference in body design that affects site of fertilization and embryonic development. Corella inflata has internal fertilization and embryonic development, while C. willmeriana has external fertilization and embryonic development, while C. willmeriana has external fertilization and embryonic development. The natural mating system of these two species of solitary ascidians was inferred by comparing the relative survival of selfed and outcrossed fertilizations in the laboratory. Corella inflata, the internal fertilizer, showed no difference in survival between selfed and outcrossed fertilizations at any developmental stage through metamorphosis and early juvenile development. In contrast, self-fertilized crosses of C. willmeriana has results suggest that C. inflata may inbreed frequently in nature, while viable C. willmeriana offspring are primarily a result of outcrossing. The internally-fertilizing species, C. inflata, showed approximately 10% male sterility in laboratory crosses despite apparent morphological hermaphroditism. The externally-fertilizing, commonly outcrossing species, C. willmeriana, showed no difference in fertility between genders.

Key words.—Ascidian, Corella, gender, inbreeding, incompatibility, marine, mating system.

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Inbreeding, whether through self-fertilization or breeding with relatives, has traditionally been considered to produce offspring of reduced fitness compared to the progeny produced by outcrossing and thus should be rare (e.g., Darwin 1876; but see review in Baker 1983). Studies of natural populations reveal however that inbreeding commonly occurs and need not lead to the production of offspring with reduced fitness (reviews in Shields 1982; Jarne and Charlesworth 1993; Thornhill 1993; Waser 1993; Husband and Schemske 1996). Recent theory offers an explanation why highly selfing populations may be evolutionarily stable: rates of inbreeding and inbreeding depression may coevolve as deleterious recessive alleles are exposed to selection and purged (e.g., Lande and Schemske 1985; Charlesworth and Charlesworth 1990; Uyenoyama et al. 1993; Lande et al. 1994). From this perspective, and all else being equal, populations or species that persistently inbreed should exhibit lower levels of inbreeding depression upon close inbreeding than populations or species that regularly outcross.

Because all else is rarely equal, one approach to testing these predictions involves a comparison of closely related species that differ only with respect to a reproductive character that should lead to a difference in levels of inbreeding. Some botanical studies adopting such an approach confirm these predictions whereas others do not (e.g., reviews in Barrett and Eckert 1990; Waser 1993). In many plants, however, floral morphology, phenology, and pollinator behavior interact in a complex way to govern the breeding system (e.g. review in Waser 1983). At first sight, the mating systems of many sessile marine invertebrates appear similar to those of angiosperms. However, invertebrate mating systems are not complicated by the interaction of flowers with pollinators (reviews in Strathmann 1990; Grosberg 1991; Knowlton and Jackson 1993), potentially simplifying the relationship between reproductive attributes and mating systems, and therefore, expectations of inbreeding depression based on such traits.

Mating systems and the extent of self-fertilization and inbreeding depression in marine invertebrate populations are largely uninvestigated (reviews by Jarne and Charlesworth 1993; Knowlton and Jackson 1993). Marine organisms display a wide variety of life-history and reproductive habits that suggest that mating systems may be quite variable among taxa (e.g., Bell 1982) and inbreeding may be common (Jackson 1986; Strathmann 1986; Knowlton and Jackson 1993). Numerous representatives from many marine taxa should be inbred based on inferences from larval and adult traits thought to reflect limited dispersal and genetically subdivided populations (Strathmann 1986; Knowlton and Jackson 1993). In a very few cases, inbreeding has been documented in field populations (colonial ascidian, Botryllus schlosseri, Grosberg 1991; acorn barnacle, Balanus improvisus, Furman 1990; solitary ascidian, Corella inflata, Cohen 1992; anemone, Epiactis prolifera, Bucklin et al. 1984; Edmands 1994, seagrass, Zostera marina, Ruckelshaus 1994). However, conclusive results based on genetic tests and rearing of naturally fertilized field progeny to confirm inbreeding are rare (Bucklin et al. 1984; Grosberg 1991; Cohen 1992; Edmands 1994).

A recent review of philopatric marine invertebrate species provided evidence for self-fertilization in 17% and suggested that many more species may be biparentally inbred (Knowlton and Jackson 1993). Additionally, nonphilopatric species (not included in the review) ought to inbreed by self-fertilizing when mates are rare (e.g., Baker's Law 1955), a prediction supported in a field study of acorn barnacles (Furman 1990). There is a long list of marine taxa that do self-fertilize in the lab including ascidians (e.g., Morgan 1945; Anderson

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Habitat and abundance	Corella inflata		Corella willmeriana	
	docks, nets rocky subtidal muddy subtidal	high medium-low low	rocky subtidal muddy subtidal	low low
Reproductive morphology	atrium		no atrium	
Site of fertilization	internal		external	
Dispersing stages and duration	larvae	seconds to days	larvae embryos eggs	~ 18 hours to days ~ 24 hours viable for hours
	sperm (some)	minutes to hours	sperm	minutes to hours

TABLE 1. Life history attributes of Corella sp. in the San Juan Archipelago, Washington.

et al. 1976), barnacles (e.g., Barnes and Crisp 1956; El-Komi and Kajihara 1991), a bryozoan (Hughes 1989; but see Hunter and Hughes 1993), spirorbid polychaetes (Gee and Williams 1965; Beckwitt 1982), ctenophores (Pianka 1974), asteroids (Strathmann et al. 1984), chaetognaths (Reeve and Cosper 1975), scleractinian corals (Heyward and Babcock 1986), and bivalves (Sastry 1979). This widespread capacity for selfing in the laboratory suggests that mixed selfing and outcrossing may be common in nature.

In this study, I compare the mating systems of sympatric congeneric sessile solitary ascidians in the genus *Corella* that share habitats, life-history attributes, and morphology, but that differ in a key morphological attribute leading to internal fertilization and embryonic development in one species and



FIG. 1. Adult morphology of *Corella willmeriana* and *C. inflata*. Arrows show direction of water flow, in the branchial siphon and out the atrial siphon. Eggs are shown being released from the atrial siphon of *C. willmeriana*. Embryos and larvae are shown within the atrial brood chamber of *C. inflata*. Adults are portrayed in an orientation commonly seen in nature on rock walls.

external fertilization and development in the other (Table 1; G. Lambert et al. 1981; Young 1988; Cohen 1992). I expected both species likely to be inbred based on previous reports of near simultaneous release of self-compatible gametes (G. Lambert et al. 1981), limited dispersal due to the combination of a sessile adult phase and a potentially extremely shortlived larval planktonic phase, and a colonizing habit (Lambert 1968), which could enforce self-fertilization in isolated individuals. Although both species release gametes in response to light cues (G. Lambert et al. 1981; C. Lambert et al. 1995), one species, Corella inflata, possesses a swollen atrium, which functions as a brood chamber; the other species, C. willmeriana lacks a brood chamber (Fig. 1). Thus the presence of a brood chamber (atrium) in C. inflata, extends the period of proximity of eggs and sperm from the same individual and the potential for self-fertilization. Additionally, because the internally fertilizing species releases hatched larvae that are capable of immediate settlement and metamorphosis (C. Lambert et al. 1995), there is a strong potential for biparental inbreeding due to limited dispersal of offspring.

This study first compares the mating systems between the two species and then examines in further detail the internally fertilizing species, *C. inflata.* I investigated two components of the mating system: (1) relative survival of selfed and outcrossed offspring; and (2) relative success of male and female fertility in these simultaneous hermaphrodites. Both comparisons are important for inferring the natural mating system: they provide information on the relative probability that individuals in a population will produce viable offspring through selfing versus those produced by outcrossing. Experimental designs used in this study to test the viability of self-fertilized offspring also provide information on relative gender success in test crosses. I used different types of cross designs to compare the relative success of manipulated matings and addressed the following questions:

(1) Is self-fertilization likely in natural populations of either species? I evaluate this possibility by comparing the success of controlled self and outcross matings in the laboratory. Relative viability of selfed and outcrossed laboratory offspring gives an indication of the potential viability of the two types of offspring in nature (e.g., Sabbadin 1971; Charlesworth and Charlesworth 1987) in the face of possible self-incompatibility or inbreeding depression.

(2) If there is a difference between the two species in

viability of selfed offspring, at what stage can differential viability between types of matings be detected? Timing may distinguish the type of selection producing viability differences in different sorts of crosses (e.g., Seavey and Bawa 1986; Waser 1993; Sage et al. 1994; Husband and Schemske 1996), although practically it may be very difficult to distinguish between phenomena such as self-incompatibility systems and inbreeding depression, or between partial incompatibility and inbreeding depression (Waser 1993; Sage et al. 1994).

(3) Is there intraspecific variation within populations in the viability of self-fertilized offspring? And, in particular, are the individuals that are the most successful at outcrossing also the most successful at self-fertilization? If so, then some individuals are simply producing higher quality gametes regardless of the occurence of selfing.

(4) Is there intraspecific variation between individuals in relative reproductive success as a male and as a female? That is, do individuals vary in crossing success depending on whether they contribute male or female gametes to a cross? Although an individual may be a morphological hermaphrodite, lack of success in reproducing with one type of gamete may functionally define the individual as a single sex (Lloyd 1980; see Kearns and Inouye 1993 for clarification of terminology). Functional gender specialization may have a similar effect to actual differences in relative gender allocation in terms of increasing the effective individual and population outcrossing rates (Ross 1990).

MATERIALS AND METHODS

Study Species

Both C. inflata and C. willmeriana are annual solitary ascidians in the northeast Pacific (Ascidiacea, Phlebobranchia) that colonize subtidal rock walls, floating docks, and other hard substrate, where their sexually produced larvae settle and metamorphose into permanently attached juveniles (Lambert 1968; G. Lambert et al. 1981; Young 1988; C. Lambert et al. 1995). The two species coexist on rock walls in the San Juan Archipelago (Cohen 1992), and C. inflata is also commonly on floating docks (Lambert 1968; G. Lambert et al. 1981; Young 1988; Cohen 1990, 1992). Adults reproduce sexually throughout their lifetime and during all times of year (Lambert 1968; Cohen 1992). Neither species reproduces asexually. Corella willmeriana spawns eggs and sperm, usually within minutes of each other, into the plankton where fertilization and development occur (G. Lambert et al. 1981). Embryos may disperse away from the parent during the approximately 24 h of development prior to hatching as larvae. Corella inflata spawns both male and female gametes into a maternal atrium. Eggs in the atrium may be surrounded by a cloud of sperm (G. Lambert et al. 1981). Although the mechanism of entry is unknown, heterologous sperm may be present in the atrium as necessarily occurs in self-incompatible brooding ascidians (Bishop and Ryland 1993; Ryland and Bishop 1993). Embryos are held in the atrium through fertilization and embryonic development (Child 1927) until well beyond hatching in the laboratory (Lambert and Lambert 1978) and in the field (C. Lambert et al. 1995). When larvae leave the mother, they may settle and metamorphose immediately in the laboratory into sessile juveniles (C. Lambert et al. 1995) although, like *C. willmeriana* tadpoles, they may disperse for longer as well. Both species were reported to self-fertilize to at least some extent in the laboratory (G. Lambert et al. 1981).

Corella inflata were collected by scuba and from floating docks from locations in the San Juan Islands in 1990 and 1991. Corella willmeriana were all obtained by scuba from one location (Pt. George in San Juan Channel) on December 2 and 6, 1990; they are not abundant in the San Juan Archipelago so sample sizes for this study were limited. Only healthy animals of each species were used.

Crossing Technique

To obtain unfertilized gametes, I placed individual ascidians in separate darkened containers overnight or for a minimum of 6 h to induce spawning upon exposure to light. Unfertilized gametes were obtained by dissection in the following manner: the adult body was removed from the surrounding test, both gonoducts and the adjacent intestine were tied off with a tourniquet of human hair. The animal was then rinsed at least twice to remove any contaminating gametes. To collect gametes, gonoducts were separately nicked with forceps. Eggs were collected first with a pipette following their release from the nicked oviduct. After egg collection, the sperm duct was nicked, and sperm were collected with a pipette and temporarily stored in a separate dish.

Eggs were aliquoted with a pasteur pipette to different treatments and to a control dish of eggs without added sperm as fertilization controls. Aliquots were 25–160 eggs per dish $(35 \times 10 \text{ mm} \text{ plastic petri})$ depending on availability of eggs. Tests showed that > 25-40 eggs gave consistent results in replicated dishes. All eggs were aliquoted to dishes before any sperm were added to any dish. Care was taken during egg aliquoting to ensure that each dish received a similar subsample of the total clutch produced. This was done by serially pipetting small batches of eggs into each test dish so that each dish received several separate aliquots of eggs. In some cases, where size of the experiment permitted and number of available eggs allowed, 2–3 replicated dishes were prepared to test the repeatability of the crosses.

Sperm were diluted with fresh seawater in the holding dish just prior to fertilization and mixed with a glass pasteur pipette. An equal number of sperm drops was added to each fertilization dish. Sperm were diluted only enough to allow equal division of drops between dishes. This technique was thought to most closely approximate the natural situation in C. inflata where sperm released from the sperm duct are concentrated in a dense cloud in the atrium and eggs are released into that cloud. For lack of natural spawning information on the free-spawning species, and to allow comparison between the two species, the same technique was used for C. willmeriana (following the methods of G. Lambert et al. 1981). Additionally, preliminary tests using different sperm concentrations on aliquots of eggs from the same individual showed no difference in fertilization timing or success between treatments, with the exception that cultures kept with sperm concentrations much higher than any used in these experiments became contaminated with bacterial growth and died (S. Cohen, unpubl. obs.).

I kept dishes near ambient sea temperatures in a running seawater table through development. In three cases, the control (no sperm added) dish showed low fertilization (2%, 4%, and 6%, rather than the expected 0%), indicating that self sperm were not completely rinsed away from the releasing adult. For those cases, I used a corrected experimental fertilization success by subtracting the rate of fertilization found in the control dish from the value for the experimental cross dish.

Staging

Developmental success was scored using a dissecting microscope. After initial trials, I picked stages that could be rapidly assessed at lower powers of magnification to allow the greatest possible number of (1) embryos per replicate; (2) replicates; and (3) treatments to be scored in all dishes before the next developmental stage occured. I scored crosses at two major developmental stages: during embryonic development (ES) and after metamorphosis (MJS). Success through the embryonic development stage (ES) encompasses fertilization and early embryonic cleavages, gastrulation, and development of the unhatched tadpole larval form. Success at metamorphosis (MJS) includes settlement of the larva, metamorphosis, and 5-7 d of subsequent juvenile development. For a given stage, all individuals were counted and scored as normally developed or undeveloped. Success was estimated as the number reaching the ES stage divided by the total number of individuals. MJS success was estimated as the number reaching that stage divided by the number that had successfully passed through the ES stage. Metamorphosis may occur as soon as approximately 42 h after fertilization (C. Lambert et al. 1995). To score MJS for a cross set, I counted successful and unsuccessful individuals on a given day between 6-8 d after fertilization.

Cross-Design

For C. inflata, I used a series of small completely reciprocal diallel crosses $(3 \times 3 \text{ and } 4 \times 4)$ (Fig. 2). This design minimized any delay between gamete collection and fertilization and, it also allowed a more powerful test of hypotheses concerning gender-specific and cross-specific success (e.g., Lyons et al. 1989; Molau 1991).

For *C. willmeriana*, a paired design consisting of a single self-fertilization and outcrossed fertilization for each individual was used. The paired design allowed more individuals to be tested at one time than would have been possible with a diallel design. This avoids the need to test for a date effect since sample sizes were limited by the local rarity of the species. On a single day (December 8, 1990), batches of unfertilized eggs from each individual were separated and either selfed or outcrossed with another randomly picked individual resulting in a paired self and outcross for each individual.

Age of gametes may affect selfing rates, because the barrier to selfing is reported to break down with aging of gametes in other ascidian species (Kawamura et al. 1987). Thus, gametes held for longer periods of time before fertilization may



FIG. 2. Crossing design and analysis table for a sample 3×3 diallel set. Individuals A, B, and C each partipate in every possible cross (including self-fertilization) as both a male and a female parent (after Molau 1991).

show a greater tendency to self-fertilize successfully. *Corella willmeriana* gametes in the paired self/outcross experiment were held approximately 10 h after dissection before fertilizations were completed. Most *C. inflata* fertilizations were carried out within 2 h of initial dissection of adults; none exceeded 10 h. Thus, the experimental bias is conservative and would lead to an enhanced levels of self-fertilization in *C. willmeriana* relative to *C. inflata*. Additionally, in *C. inflata* there was no trend of differential self-fertility with aging of gametes (S. Cohen unpubl. obs.).

Analysis

I replicated *C. inflata* diallels with a second set of dishes for each cross as time and gamete availability allowed. To allow a combined analysis of replicated and unreplicated diallels, a single set of data was used from the replicated cross sets. In a few cases, one of the replicates had an unusually low rate of development (e.g., replicates of 18% and 96%, and 37% and 88%) most likely caused by contaminants in the dish. In these cases, for analysis I chose the highest value for any given pair under the assumption that extremely low outlying values were an artifact of dish contamination affecting gamete viability. This treatment follows the methods and results of Morgan (1938, 1945) by interpreting the high-



FIG. 3. Percent survival of *Corella* embryos to two developmental stages according to species and type of cross: (a) embryonic survival (ES); (b) survival through metamorphosis and early juvenile development. Error bars represent one standard error. Numbers above bars are sample sizes.

est value as the potential viability of a given cross. This is the most biologically reasonable explanation given aliquoting techniques described previously, which would reduce differences due to any natural variance within a batch of eggs. Additionally, in preliminary experiments in the lab, and in field observations of natural clutches, clutches were highly synchronized except for occasional spawning of batches of eggs containing obviously immature, smaller oocytes. These batches were discarded. Preliminary analyses using both replicates did not change the results. Inspection of deviates showed no pattern of variation between replicates or anomalously low values correlated with type of cross (selfed or outcrossed), site, or date.

Corella willmeriana crosses in the paired self/outcross experiment were not replicated to save time in doing crosses, because so many individuals had to be dissected. A replicated C. willmeriana 2×3 diallel showed no difference between replicates of each cross.

Frequency data were arc-sine transformed for analysis. Means and standard errors reported in the text are back-transformed. Paired t-tests and different ANOVA models were

TABLE 2. Three-way analysis of variance model for effects of site, date, and type of cross on embryonic survival in *Corella inflata*.

Source	Mean square	df	F	Р
Cross	0.113	1	2.38	0.262
Site	0.060	2	0.18	0.842
Date (site)	0.319	6	18.35	< 0.01
Site * cross	0.048	2	2.32	0.154
Cross * date (site)	0.017	6	0.17	0.985

used to determine the effects of type of cross and species on success at different developmental stages. In all ANOVA models, type of cross was always treated as a fixed factor and site as a random factor. I used SAS GLM (version 6.03) to analyze two- and three-way ANOVAs. Systat (vers. 5.0, Wilkinson 1989) was used for other analyses.

RESULTS

Survival in Embryonic Development (ES), Comparison of Selfed and Outcrossed Matings

Success for each *C. willmeriana* individual in paired selfed and outcrossed matings was scored at an embryonic (unhatched) tadpole stage (referred to as ES). Self and outcross values for ES differ significantly (paired *t*-test, t = 4.668, P < 0.001, df = 10). Mean selfing success was $18\% \pm 1.6\%$, (n = 11, range 0%-91%), whereas mean outcross success in the paired samples was $76\% \pm 0.6\%$, (n = 11, range 35%-97%) (Fig. 3A).

Survival to ES for all *C. inflata* individuals from all crosses in all diallels are summarized in Fig. 3A. Mean self success was $85\% \pm 0.4\%$, (n = 29), while mean outcross success was $89\% \pm 0.2\%$, (n = 29) (Fig. 3A). I tested for a difference in ES with type of cross across sites using a mixed model two-way ANOVA with site treated as a random effect, and type of cross treated as a fixed effect with date nested within site (SAS, GLM). Since date nested within site was the only significant effect (P < 0.005) (Table 2), I then tested each site separately (Table 3).

Within each site, I used two-way ANOVA to test embry-

TABLE 3. Two-way analysis of variance for effects of date and type of cross on embryonic survival in each site separately in *Corella inflata*.

Source	Mean square	df	F	Р
Site 1				
Cross Date Cross * date	0.001 0.449 0.003	1 3 3	0.01 3.23 0.02	$0.933 < 0.050 \\ 0.996$
Site 2				
Cross Date Cross * date	0.062 0.047 0.096	1 1 1	1.01 0.76 1.56	0.333 0.399 0.232
Site 3				
Cross Date Cross * date	0.116 0.260 0.000	1 2 2	0.12 3.19 0.00	0.243 0.058 1.000

1901

onic success using type of cross (fixed) and date (random) as main effects. There was no effect of type of cross at any site. A date effect was found for site 1 (P < 0.05), but there was no interaction between date and cross. The date effect is probably a result of a split between two pairs of dates in overall fertilization success rates independent of type of cross: date 1 (mean self success = 75%, mean outcross success = 71%) and date 2 (68%, 84%) versus date 3 (98%, 96%) and 4 (98%, 98%). To increase the power of the ANO-VA, I pooled dates within site 2 and within site 3 separately (since there was no date effect at either site), and I still found no significant effect of type of cross at either site. Variances between selfing and outcrossing success were homogenous with all sites combined, for site 2 separately, and for site 3 separately. Variances from site 1 were heterogeneous at the 0.05 level, but not the 0.01 level (F-max test; Sokal and Rohlf 1981). Therefore, in C. inflata there is no significant difference in viability of selfed versus outcrossed offspring in any analysis.

Comparison of Survival of Selfed and Outcrossed Offspring at Different Stages of Development

For both *C. inflata* and *C. willmeriana* sequential measures of embryonic survival (ES) and metamorphic and juvenile survival (MJS) were scored in the same cross sets. Figure 3B shows MJS in both species for those individuals that developed through metamorphosis. For *C. willmeriana* survival through metamorphosis and early juvenile development (scored at 5 d postmetamorphosis) does not significantly differ for selfed (mean = $71\% \pm 1.7\%$, n = 8, range 38%– 100%) versus outcrossed (mean = $47\% \pm 0.3\%$, n = 11, range 14%–71%) progeny (independent samples *t*-test with pooled variances, t = 1.915, df = 17, P > 0.07).

For C. inflata three diallels from the larger (ES) set were continued and resurveyed 10–19 d after fertilization for survivorship through metamorphosis and early juvenile development. A two-way mixed model ANOVA with cross (fixed) and date (random) showed no significant effects (type of cross, df = 1, F = 0.87, P > 0.3; date, df = 2, F = 0.90, P > 0.4; type of cross * date, df = 2, F = 0.07, P > 0.9). I therefore pooled samples across dates, and the resulting one-way ANOVA with cross only showed no significant difference in survivorship between selfed and outcrossed treatments (df = 1, F = 0.529, $P \ge 0.4$). Mean C. inflata MJS self success was $46\% \pm 1.4\%$ (n = 10) and mean MJS outcross success was $55\% \pm 0.5\%$ (n = 21).

For C. inflata, I compared survival at the earliest scorable stage following fertilization with survival through gastrulation. A single cross set (October 17, 1990, 4×4 diallel) was scored at two early developmental stages to determine whether there was an initial difference in developmental rate between selfed and outcrossed embryos. Developmental stages scored were two-cell (stage 1) and gastrula (stage 2). Separate one-way ANOVAs of stage 1 and stage 2 showed no significant effect of type of cross in either cross set (stage 1: df = 1, F = 0.178, P > 0.6; stage 2: df = 1, F = 0.023, P >0.8). Therefore, no differential timing effect was detected in self-fertilization compared with outcrossing at the earliest scorable postfertilization stage (two-cell stage), 1.5–2 h post-



FIG. 4. Frequency of *Corella inflata* embryos from the same diallel set reaching two-cell and gastrula stages. Solid bars represent self-fertilized crosses and striped bars represent outcrosses. Error bars represent one standard error. Numbers in parentheses are sample sizes.

fertilization. Figure 4 shows selfing and outcrossing survival for the two stages separately. The lower values for both selfing and outcrossing at the two-cell stage compared to the gastrula stage indicates that scoring was done as two-cell division was still occuring. Scoring during the cleavage divisions gives the greatest chance of detecting a delay in early development (either in fertilization or in development itself).

Individual Variation in Survival of Selfed Compared with Outcrossed Progeny

Using all *C. inflata* individuals, I compared survival of selfed versus outcrossed progeny with the ES dataset (Fig. 5A). For individuals, success at selfing is highly correlated with mean success at outcrossing (Spearman correlation coefficient = 0.571, n = 29, Bartlett chi-square statistic = 10.437, df = 1, P < 0.001).

For *C. willmeriana*, Figure 5B shows a similar plot comparing single selfed and outcrossed values. There is no significant correlation between survival of selfed and outcrossed progeny (Spearman correlation coefficient = 0.276, n = 11, Bartlett chi-square statistic = 0.670, df = 1, P > 0.4).

Individual Gender Variation: Are Male and Female Success Correlated in Individuals?

For *C. inflata* I used Schlichting and Devlin's (1989) model for a diallel with all possible crosses including self-fertilization to analyze the ES data set. Relative performance values are individual means adjusted to their cross set means (Schlichting and Devlin 1989). The relative performance of the *i*th male and *j*th female were calculated (see Fig. 2) according to the formulas (following Molau 1991):



% SURVIVAL IN OUTCROSS

FIG. 5. Scatter diagrams showing the correlation of individual survival at self-fertilizing and outcrossing: (a) Corella inflata individuals from ES data set; (b) C. willmeriana individuals from paired test.

 $A_{i} = \sum a_{i} / N_{i} - \sum a_{i} / N_{i}$

and

$$G_{j} = \Sigma g_{j.} / N_{j.} - \Sigma g_{..} / N_{..}$$
 (2)

(1)

where A = male performance and G = female performance for a given individual in its entire cross set, a = male ESand g = female ES in a particular cross, and N = number of crosses.

Figure 6A shows male performance plotted against female performance (using untransformed % ES in the performance calculation) for each individual. A Spearman correlation test $(r_s = 0.244, Bartlett chi-square = 1.629, df = 1, n = 29, P$ > 0.2) shows no correlation between male and female performance within an individual. Male values show a greater range than female values and are negatively skewed. These exceptionally low male values occur across sites and dates.

For C. willmeriana, figure 6B shows male versus female embryonic success plotted by individual (outcrosses only).

FIG. 6. (A) Scatter diagram showing the correlation of individual Corella inflata performance as a male and as a female in the ES data set. (B) Scatter diagram showing the correlation of individual C. willmeriana survival to the tadpole stage in self and outcrossed fertilizations.

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% EMBRYONIC SURVIVAL AS A FEMALE

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There was no correlation between male and female success (Spearman correlation coefficient = 0.204, n = 10, Bartlett chi-square statistic = 0.317, df = 1, P > 0.5). Sample size is limited (n = 10).

Individual Gender Variation: Is Gender-Specific Success **Related to Selfing Success?**

For C. inflata using each individual in diallels 1-9, I compared self to female or male outcrossing mean ES. There is no correlation of female mean outcrossed ES (arc-sine transformed values) with selfed ES (Pearson correlation coefficient = 0.100, Bartlett chi-square = 0.264, df = 1, P > 0.6); however, male mean outcrossing ES is significantly correlated with self ES (Pearson = 0.707, Bartlett chi-square =18.377, df = 1, P < 0.001).



FIG. 7. Embryonic survival of four *Corella inflata* individuals in a complete 4×4 diallel cross set. Each individual is outcrossed with three other individuals as well as self-fertilized.

Population Level Gender Variation: Is There an Overall Male or Female Effect on Fertilization Success or, an Interaction of Male and Female?

For C. inflata, I used the ES dataset in a three-way ANOVA with date, male, and female as full factors. All effects are fixed so that the relationship of the factors male and female can be investigated without assuming independence. This follows the model of Schlichting and Devlin (1989) for diallels with all possible crosses including self-fertilizations. Male and female variances are heterogenous at the 0.05 level (Fmax, Sokal and Rohlf 1981) due to the males with low success in all crosses that were discussed earlier. Date and date * male are highly significant (P < 0.001, date: df = 8, F = 6.86; date * male: df = 17, F = 6.93). No other effects are significant. Although the three males with the lowest success are from diallel sets from three different days, there is still a date effect because a single individual affects many cells in the diallel. Figure 7, a single full diallel, shows success arranged by male parent to illustrate the effect of a single individual with low male success on many crosses within a diallel. These individuals with low male success show no effect of type of cross. The three-way ANOVA analyzing all cross sets does not reveal an effect of the full factor male (male: df = 3, F = 1.08, P > 0.3) because there were so few individuals with low male success in total (3-4 of 29). There is no interaction effect of male*female associated with specific combinations of genotypes.

DISCUSSION

The two species of *Corella* show a striking contrast in the survival of self-fertilized offspring in the laboratory. Self-fertilization of the externally fertilizing *C. willmeriana* was significantly less successful than outcrossed fertilization when scored for normal development through the unhatched tadpole larval stage (ES). In contrast, selfed and outcrossed fertilizations of the internally fertilizing *C. inflata* were equally successful at all stages. Selfing is the strongest form of inbreeding and produces intense selection against deleterious recessive alleles. If viability differences are not found in self-

crosses, such as the case for the internally fertilizing C. inflata, it is likely that high levels of inbreeding may have occured in the populations these individuals came from, which purged the deleterious recessive alleles (Shields 1982; Lande and Schemske 1985; Charlesworth and Charlesworth 1987).

Controlled lab matings are one means of obtaining information on a species' mating system. Other factors such as the availability of self and outcrossing gametes and demographic population structure affect the types of matings that occur in nature. Lab matings may be taken to reflect the relative probability of success of different types of matings if they do occur in nature. It seems likely, given the reproductive morphology of the internally fertilizing C. inflata, that opportunities for selfing are frequent in the field. This study shows that selfed and outcrossed matings in the lab have equal viability when measured at key early developmental stages in the life history from fertilization through embryonic and larval development, settlement, and metamorphosis. Since morphology suggests that encounters of self-gametes are very likely and lab crosses show that selfed matings are viable through important developmental stages, it seems likely that C. inflata is inbreeding by selfing in nature. Using the same criteria, it seems much less likely that C. willmeriana is inbreeding by selfing in nature, since selfgametes are not held in the same place for any period of time and selfed matings show low viability in the lab. These two sympatric congeners appear to have a different potential for inbreeding by selfing in the field.

Because no survivorship difference was found for C. inflata embryos, I also looked for a developmental rate difference between self and outcrossed matings. In these experiments, an apparent rate difference might have appeared due to a delay in fertilization of selfed relative to outcrossed matings. This sort of delay has been found in many plant species where selfing is common in nature, but the genetic load remains high (e.g., Eckert and Barrett 1994). A delay could also be an indication of a partial genetic incompatibility system (Waser 1993; Sage et al. 1994) and might be expected in a species such as C. inflata where self-sperm appear to have access to self-eggs before outcross sperm reach the eggs. I found no difference in developmental rate between selfed and outcrossed matings scored after the first cleavage division or at gastrulation. So, a timing difference between selfed and outcrossed offspring was not detected at 2 h postfertilization when the first easily scorable cleavage event occurs. This is a shorter period of time than the 3 to 4 hours for breakdown of the block to selfing suggested to occur in some other ascidians (Scofield et al. 1982). Thus, again I found no evidence of either inbreeding depression or an incompatibility system in C. inflata.

The lack of any detectable inbreeding depression in *C. inflata* in this study should not be taken as evidence that there is no inbreeding depression at all in this species. Inbreeding depression may occur at a variety of life stages (Sabbadin 1971; Charlesworth and Charlesworth 1987; Barrett and Eckert 1990) including much later stages (reviewed in Husband and Schemske 1996). In fact, there is theoretical and empirical evidence that high levels of self-fertilization select for later expression of inbreeding depression (Husband and

Schemske 1996). Inbreeding effects in herbaceous plants (relative to longer-lived woody plants) may not be detectable until much later in the life cycle (Barrett and Kohn 1991). The protogynous colonial ascidian *Botryllus schlosseri* occurs in inbred (Grosberg 1987, 1991) but virtually nonselfing (Sabbadin 1971) populations and shows expression of later acting inbreeding depression when colonies are forced to selffertilize (Sabbadin 1971). Grosberg (1987) also found differential survivorship in *B. schlosseri* at later developmental stages, although in his study, the differences are due to outbreeding, rather than inbreeding, depression.

An additional caveat on extrapolating from laboratory crosses to natural mating systems is that measures of inbreeding depression are known to be environmentally dependent (reviews in Husband and Schemske 1996; Eckert and Barrett 1994; Pray et al. 1994). Although I did not detect inbreeding depression in selfed matings of the internallyfertilizing *C. inflata*, it may still occur in natural populations. Sabbadin (1971) found environmental variation in the expression of inbreeding depression in the colonial ascidian *B. schlosseri*. In that study, inbreeding depression was greater in colonies in the lab, but the relative viability of outcrossed and selfed offspring remained the same across habitats.

An earlier analysis of the mating system of the internally fertilizing species, *C. inflata*, found adult genotype frequencies in three dock populations to be in agreement with Hardy-Weinberg equilibrium frequencies predicted under the condition of random mating (Cohen 1990). Thus, the present laboratory cross results seem at odds with the previous study, which suggests this species outcrosses routinely. However, subsequent sampling of a greater number of *C. inflata* populations showed interpopulation variation in levels of outcrossing calculated from adult genotype frequencies (Cohen 1992). Additionally, progeny analysis of naturally fertilized *C. inflata* broods from a single population showed high levels of inbreeding consistent with the laboratory cross results of this study (Cohen 1992).

An alternative explanation for the difference in apparent levels of inbreeding depression in the two species is that the apparently selfing species (C. inflata) may have experienced a population bottleneck that led to elevated levels of inbreeding (by selfing or biparental inbreeding), which allowed purging of the genetic load in this species, while the other species did not encounter a bottleneck and go through this sort of selection. If this scenario were true, then the apparent difference in natural levels of inbreeding would be due to an environmental difference and perhaps only spuriously associated with the difference in site of fertilization and embryonic development. However, the purging effects of bottlenecks require a substantial amount of time at a very low population size (Nei et al. 1975; Chakraborty and Nei 1977). Demographic data on field populations of both species indicates that a bottleneck explanation for the difference in laboratory selfing ability between the two species is unlikely. First, local populations are too transitory for the genetic effects of a long-term bottleneck to occur. The populations are not at equilibrium and may appear and go locally extinct in a matter of months in these annual species (Cohen 1992). And second, the two species coexist on rock walls where processes leading to local extinction affect the species similarly (Cohen 1992).

At the individual level, *C. inflata* show a strong correlation of success at selfing and at outcrossing. Apparently, some individuals produce higher quality gametes than others, and their success is not influenced by the degree of inbreeding in a particular mating. Thus, I found no evidence of individual variation in inbreeding depression in this species. In contrast, *C. willmeriana* individuals showed no correlation of success at selfing and outcrossing. Some individuals are more successful at self-fertilization and others at outcrossing. This could indicate variation in inbreeding depression due to different individual genetic loads (e.g., Pray and Goodnight 1995) or differences in self-compatibility.

Whether the lower success of C. willmeriana from selfed matings represents an incompatibility system rather than postfertilization inbreeding depression remains uncertain, although some evidence suggests inbreeding depression as the more likely explanation. Success of selfed matings varies widely among individuals. In 8 of 11 cases, I found a selfsuccess rate of 0% to 91%. And, I found approximately equal success with selfing and outcrossing in 2 of 11 paired matings. In natural spawns of temporarily isolated individuals in the laboratory, G. Lambert et al. (1981) report self-sterility in 3 of 17 C. willmeriana. Resolving the issue of whether an incompatibility system exists in this species would require extensive cellular and genetic analysis, and it would perhaps still remain uncertain (Sage et al. 1994). A next step would be to look for evidence of sperm entry and fertilization by staining eggs in self-crosses and looking at the nuclei to determine if inviability was pre- or postzygotic (Seavey and Bawa 1986; Sage et al. 1994). Prezygotic inviability would suggest a partial incompatibility system. However, postzygotic inviability could result from either inbreeding depression or late-acting self-compatibility or even a combination of the two processes. Considerable research in plants has left the issue still unresolved in many cases (Waser 1993; Sage et al. 1994).

I found variation in relative gender success in both species; there was no correlation of success as a female and success as a male for individuals of either species. *Corella inflata* showed greater variation in male than female success. Female success was tightly clustered relative to male values, which included a few males with dramatically low success (lower minimum values) for both selfing and outcrossing and a larger number with relatively higher values than most of the female values. The few extremely low male values may indicate a low (about 10%) frequency of male sterility in the population.

A 10% frequency of male individuals in a population of self-fertile hermaphrodites may have strong effects on population genetic structure by increasing the outcrossing rate (Charlesworth and Charlesworth 1978). The low male viability detected in a few *C. inflata* in crosses may reflect non-heritable, unselected variation between individuals. Or, it may have a genetic basis as found in a number of selfing plant species (reviews in Gouyon and Couvet 1987; Ross 1990; Mazer 1992). Further investigation of this topic would require analysis of the heritability of the male-sterile condition in *C. inflata* using controlled crosses over several generations, or by population-level analysis of male fertility

components (Brown 1990). Mixed populations of hermaphrodites and females have been found in several other marine invertebrates as well (Fell 1974; Dunn 1975; Bucklin et al. 1984; Chornesky and Peters 1987; Edmands 1995).

The free-spawning species C. willmeriana shows no evidence of gender specialization such as partial male sterility. The comparison of the two species is interesting. Although, the sample size for the externally fertilizing, locally rare, C. willmeriana limits the power of the gender comparison in this study. Nonetheless, if the occurence of low male values in C. inflata is environmentally based, then given the phylogenetic and ecological similarity of these two species, one might expect a similar condition in C. willmeriana. However, if gender variation in C. inflata is genetically based, other mechanisms such as a partial incompatibility system or external fertilization may so effectively prevent or lower the incidence of selfing in C. willmeriana that there is little selection in this species for limiting hermaphroditism (e.g., Uyenoyama 1993). The botryllid family of colonial ascidians provides an example of closely related species that show a diversity of mechanisms that have the effect of promoting outcrossing. Some of the described systems include morphological separation of gametes in a simulataneous hermaphrodite, differential timing of gamete release, and perhaps, an incompatibility system as well (Oka 1970; Sabbadin 1971; Mukai 1977; Scofield et al. 1982; R. K. Grosberg, unpubl. obs.).

This study shows that a difference in site of fertilization and embryonic development, based on a simple morphological difference, may have profound consequences for the mating system. As predicted, the internally fertilizing species, C. inflata, shows no evidence of inbreeding depression in artificially self-fertilized matings. This suggests that in nature, inbreeding by self-fertilization is common for this species in some populations. Combined with other data on adult and progeny genotype frequencies in a number of C. inflata populations (Cohen 1990, 1992), this further implies a mixed mating system in field populations of C. inflata. In contrast, the externally fertilizing species, C. willmeriana, shows significantly lower viability in selfed relative to outcrossed matings, suggesting a low rate of inbreeding in nature likely due to external fertilization and perhaps enforced by inbreeding depression or a partial gametic incompatibility system. Comparison of these two sympatric congeners shows that site of fertilization may predict the extent of inbreeding and inbreeding depression in sessile marine invertebrates.

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