Mate Selection and the Evolution of Highly Polymorphic Self/Nonself Recognition Genes

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Multicellular organisms use the products of highly polymorphic genes to distinguish self from conspecific nonself cells or tissues. These allorecognition polymorphisms may regulate somatic interactions between hosts and pathogens or between competitors (to avoid various forms of parasitism), as well as reproductive interactions between mates or between gametes (to avoid inbreeding). In both cases, rare alleles may be advantageous, but it remains unclear which mechanism maintains the genetic polymorphism for specificity in self/nonself recognition. Contrary to earlier reports, we show that mate selection cannot be a strong force maintaining allorecognition polymorphism in two colonial marine invertebrates. Instead, the regulation of intraspecific competitive interactions appears to promote the evolution of polymorphisms in these species.

By reducing the risk of inter- and intraspecific parasitism, the ability to distinguish self from nonself and close kin from distant relatives stabilized two major transitions in the history of life: the evolution of tissue differentiation and cooperative social behaviors (*1*, *2*). One of the hallmarks of these self/nonself recognition systems, such as the vertebrate major histocompatibility complex (MHC) (*3*), is their high specificity, conferred by loci with as many as 100 alleles. Natural selection may favor heterozygotes or carriers of rare alleles, leading to the evolution of extreme allelic diversity (*4*–*6*), through two mechanisms. Rare or heterozygous genotypes may be favored because of their enhanced somatic resistance to parasites or pathogens or their overall vigor (*5*, *6*), though specific associations between pathogens and MHC genotypes are elusive (*5*). Alternatively, rare genotypes may have higher reproductive success: Female mice (*7*) and humans (*8*, *9*) prefer mates carrying different MHC alleles, either to avoid inbreeding or to enhance the resistance of their offspring to pathogens and parasites. Selection mediated by such mating preferences may indirectly promote MHC and other allorecognition polymorphisms (*3*, *10*–*12*), though other attempts to find MHC-dependent mating preferences have been less successful (*3*, *13*, *14*).

Burnet (*10*) and others (*11*) proposed that the evolutionary antecedents to the mammalian immune system might be found in the allorecognition systems governing tissue fusion in colonial marine invertebrates (tunicates, cnidarians, bryozoans, and sponges) (*15*). The sessile life-styles, encrusting growth forms, and limited dispersal of many of these animals promote tissue contacts between allogeneic conspecifics and intense intraspecific competition for space (*15*, *16*). Spatial competition is mediated by complex somatic recognition behaviors, ranging from intergenotypic fusion to active cytotoxic rejection and aggression. For example, in the hermaphroditic colonial tunicate *Botryllus*, a single mendelian locus (*A*) with perhaps hundreds of codominant alleles (*1*, *2*, *3*, . . . *n*) controls allorecognition specificity (*11*, *16*–*18*). Because most alleles are rare, allele sharing is mainly restricted to close relatives. Colonies sharing one or both alleles at this locus will fuse (Fig. 1). Colonies lacking a shared allele reject each other and retain their individuality.

These same life cycle traits also increase the likelihood that close relatives can mate. Oka (*19*) first claimed that the somatic allorecognition locus in *Botryllus primigenus* could also reduce the potential for inbreeding. If sperm sharing an allorecognition allele with an egg-parent cannot penetrate the layer of diploid parental cells surrounding its eggs, then selection should favor carriers of rare alleles (*20*). Burnet (*10*) subsequently argued that the mate selection hypothesis could account for the high levels of polymorphism that are apparent in both invertebrate allorecognition systems and the vertebrate immune system. Scofield and colleagues (*11*) claimed to confirm these results for *B. schlosseri* and concluded, "Polymorphism at the *Botryllus* [allorecognition locus] may be maintained through linkage of histocompatibility genes with elements which enforce the heterozygous condition through control of fertilization or development."

Both somatic and mate recognition are plausible mechanisms for the maintenance of extraordinary allorecognition polymorphism. Decisive experiments that could discriminate between the effects of these two mechanisms are difficult to perform in vertebrates because of the complex and functionally interconnected behaviors regulated by the MHC and the difficulties in separating environmental from genetic sources of phenotypic variation.

Here we show that mate selection is not essential for the maintenance of high levels of polymorphism in somatic recognition systems—an intensely debated question among vertebrate immunologists. We used colonial marine invertebrates (a tunicate and a hydroid) to test three predictions of the mate selection hypothesis. In both species, individuals freely spawn sperm into the surrounding seawater. In the dioecious hydroid, females also spawn eggs. In the hermaphroditic tunicate, eggs are retained within parental colonies and are fertilized by sperm entrained in their feeding currents. The short-lived swimming larvae are released several days after fertilization. If, as predicted by others (*3*, *10*–*12*, *19*), the allotypes of colonies pleiotropically affect sperm-egg interactions, then for a one-locus allorecognition system (see Fig. 1) such as that of *Botryllus*, the following predictions could be made: (1) Matings between fusible colonies sharing both allorecognition alleles (that is, syngeneic) should yield no offspring (if allotypic similarity prevents fertilizations) or fewer offspring as compared to matings between colonies not sharing allorecognition alleles (if allotypic similarity reduces but does not eliminate sperm-egg compatibility). (2) In matings between fusible colonies sharing one allorecognition allele (that is, semiallogeneic), two allotypic classes, one of them homozygous, should be missing or underrepresented in their offspring (Fig. 1, C and D). (3) The segregation patterns in reciprocal crosses between hermaphroditic semiallogeneic parents should be reversed according to the identity of the sperm parent (Fig. 1D).

We tested prediction 1 in sperm competition experiments with *B. schlosseri*. We used the breeding design in Fig. 1 to generate replicate full-sibling families of F_1 offspring (*21*). We compared the in vitro fertilization success of full-sibling pairs of sperm parents (P_2): one sharing both allorecognition alleles with a sibling egg parent (syngeneic) and the other sharing neither allele (allogeneic) (*22*). In all comparisons, both sperm parents contributed roughly equally to the paternity of the F_2 offspring. There were no significant deviations from the 1:1 siring ratio expected if there were no fertilization bias due to the allorecognition locus (Table 1).

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We tested prediction 2 in crosses between semiallogeneic full siblings of *B. schlosseri*. In such crosses, a single sperm-donor colony (P_2) heterozygous at its allorecognition locus should produce equal numbers of sperm carrying each allorecognition allele. We conducted reciprocal matings between pairs of colonies collected from populations at Woods Hole, Massachusetts, and similar matings between colonies from Berkeley, California. In every trial, all four F_2 genotypes were produced in large numbers and at frequencies not significantly different from the expected 1:1:1:1 ratio under random fertilization (Fig. 1C and Table 2), including a homozygous allotype (Fig. 1C) that should be absent or rare if sperm are unable or less likely to fertilize eggs that are surrounded by an envelope of tissue bearing the sperm allorecognition allele. This outcome did not depend on which hermaphrodite parent provided sperm and which provided eggs: All four F_2 genotypes were produced in 1:1:1:1 ratio when either parent acted as a sperm donor. This falsifies the prediction of reversed segregation patterns under the model of fertilization bias (prediction 3). These results substantially expand on but are concordant with earlier preliminary analyses (*23*) and indicate that mate selection acting through the *B. schlosseri* fusion-rejection locus cannot promote the evolution of genetic polymorphism at that locus.

We also tested prediction 2 in the dioecious hydroid *Hydractinia symbiolongicarpus*. These colonies show heritable fusion-rejection behavior (*24*, *25*) and allorecognition polymorphism (*26*) comparable to that of *Botryllus*. Because the formal genetics of allorecognition specificity in these hydroids appear to be more complex than in *Botryllus*, we could not directly determine the allotypes of offspring from these matings (as we did in the *Botryllus* experiments). Instead, we compared the frequency of fusion-rejection responses among the F_2 offspring in the same mating design shown in Fig. 1 (*27*). Whatever the formal genetics of allorecognition, offspring from

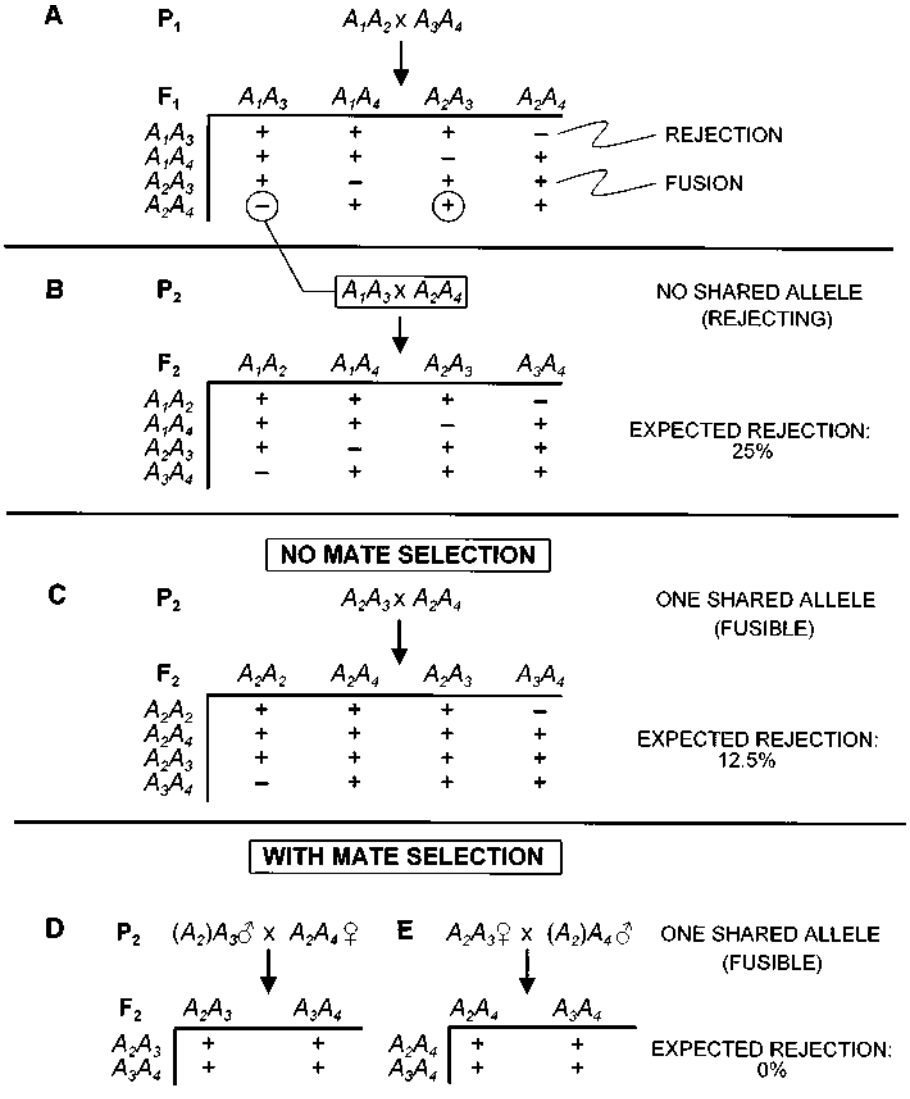

Fig. 1. Schematic of a mating design to identify the effects of a somatic compatibility system on fertilization patterns in a one-locus allorecognition system. In the case of *Botryllus*, a single mendelian locus (*A*) with many codominant alleles (*1*, *2*, *3*, . . . *n*) controls allorecognition specificity (*11*, *16*–*18*). Colonies sharing one or both alleles at this locus will fuse. The genetics of allorecognition in *H. symbiolongicarpus* are somewhat more complex, with compatibility probably being controlled by three or four independent loci with five to seven alleles per locus (*26*). Because these loci have not been cloned and characterized, the precise allelic matching rules for fusion remain difficult to specify. (**A**) Unrelated heterozygous parents (P_1) with four allorecognition alleles (A_1 to A_4) produce four different allotypic classes of F_1 offspring. We chose fusible and rejecting pairs of these offspring as parents (P_2's) to generate F_2's [example pairs are circled in (A)]. (**B** through **D**) The frequencies of F_2 allotypes, and thus frequencies of fusion and rejection, should vary according to the allotypes of the P_2's and to whether mate selection operates. These fusion and rejection frequencies are useful measures of the diversity of allotypes among offspring in the case of the hydroids in which allotypes cannot be determined directly. The predicted rejection frequencies shown for each panel are only quantitatively accurate for the *Botryllus* one-locus system. Quantitative predictions would be different for the multilocus *Hydractinia* system, but the qualitative prediction (B > C > D) still holds. (B) Regardless of the fertilization model, matings (P_2) between rejecting (allogeneic) brother-sister pairs ($A_1A_3 \times A_2A_4$) produce four allotypes among their F_2 offspring, of which 25% reject each other (dashes). (C) Without mate selection based on the somatic allotype, semiallogeneic parents that share one allele in common produce four classes of F_2 allotypes, including one homozygous allotype (A_2A_2) that rejects some siblings (A_3A_4). These four allotypes are expected in 1:1:1:1 ratios with 12.5% rejections. (D) In semiallogeneic crosses with gametic incompatibility mediated by the allorecognition locus, one class of sperm (A_2 in parentheses) is incompatible with eggs surrounded by A_2A_4 maternal tissue and sires no offspring (missing allotypes are shown in parentheses). Only two allotypic classes should be present among the F_2's, and these are a subset of allotypes expected without gametic incompatibility, as in (C). (**E**) If the sex roles of the hermaphroditic colonies are reversed in reciprocal crosses, a different allele (A_4) will be compatible with eggs (surrounded by A_2A_3 maternal tissue), and a different subset of allotypes is expected among the F_2's. In either case, no rejections should be observed because all progeny share at least one allorecognition allele.

incompatible parents should reject each other more often than offspring from fusible parents (Fig. 1, B and C). Moreover, if allorecognition alleles shared by fusible F_1 parents prevent some fertilizations between genetically similar gametes, this difference in F_2 rejection frequencies should be larger than in the absence of such mate selection (Fig. 1, B and D). Log-linear analysis shows that rejection frequencies among F_2 offspring from fusible and rejecting sibling parents were slightly different in the predicted direction (higher for offspring of rejecting F_1's), but this difference was not statistically significant at the $P = 0.05$ level (Table 3). This result implies that the difference in fusion-rejection behavior between F_2 offspring from fusible versus rejecting parents must be very small, and the potential for this difference to be affected by mate selection must be even smaller. As with the colonial ascidian *B. schlosseri*, the allotypes of hydroid colonies had no detectable effect on interactions between their gametes. Although other genetic polymorphisms regulating mate selection may exist in these species, these polymorphisms are not the same as (or linked to) the loci regulating somatic tissue interactions. Consequently, mate selection is not an essential mechanism for the persistence and maintenance of extraordinary genetic polymorphisms in either of these somatic recognition systems.

Outside of the context of pregnancy, the regulation of behaviors associated with tissue interactions is unlikely to represent a important selective force currently promoting the evolution of allorecognition polymorphism in mobile solitary organisms such as vertebrates (*28*). For many sessile colonial animals, the situation differs. It appears more likely that the maintenance of allotypic diversity in *B. schlosseri* and *H. symbiolongicarpus*, and perhaps in many other colonial organisms, arises from selection to ameliorate the risks of altruistic behavior and to reduce the costs of competing for growing space (*1*, *15*, *29–33*) through selective cooperation with close kin (*29*, *30*). Some of these risks are now well documented (*1*, *15*, *31–34*).

Nevertheless, the disparity between our findings and those of Oka (*19*) and Scofield *et al*. (*11*) suggests that the link between allorecognition and mating patterns may be evolutionarily labile. Multiple selective mechanisms must in some cases maintain apparently similar allorecognition polymorphisms, perhaps even in closely related species. Moreover, allorecognition systems mediating somatic interactions or resistance to pathogens appear to have been coopted repeatedly to regulate mating patterns and other complex social behaviors such as kin recognition in other species (*3*, *12*, *35–39*). It remains to be determined how the nature of selection on allorecognition and MHC alleles varies among populations and species, and consequently the general roles played by direct selection on MHC and allorecognition phenotypes versus indirect selection via mating preferences in the evolution of these extraordinary polymorphisms.

Table 1. Siring frequencies in *B. schlosseri* sperm competition experiments between sperm donors that are syngeneic and allogeneic to the egg donors. We inferred paternity on the basis of the presence of diagnostic paternal allozyme markers at the phosphoglucose isomerase (*PGI*) locus in the offspring from each cross. Syngeneic sperm parents shared both allorecognition alleles with the egg parent; allogeneic parents shared neither allorecognition allele with the egg parent. Initial log-likelihood tests showed that siring frequencies were homogeneous among trials within each of the three matings (all $G_{(2)} < 2.5$, all $P > 0.28$). *G* values (1 df) test the outcomes of the goodness of fit of the pooled data in each mating to an expected siring ratio frequency of 1:1 under the hypothesis that there is no association between parental allotype and fertilization success. All values of $G_{(1)}$ have $P > 0.12$.

Mating	Trial	Inferred sire of offspring		$G_{(1)}$
		Syngeneic	Allogeneic	
1	a	57	43	
	b	59	41	
	c	53	47	
	Total	169	131	2.42
2	a	46	54	
	b	53	47	
	c	42	58	
	Total	141	159	0.54
3	a	47	53	
	b	48	52	
	c	41	59	
	Total	136	164	1.31

Table 3. Rejection frequencies among offspring from matings between somatically fusible versus rejecting *H. symbiolongicarpus* colonies. *N* is the number of interactions between pairs of offspring colonies. Only rejections are shown (fusions are *N* minus rejections). Log-linear *G* tests show that neither the identities of each of the 16 families ($G_{(14)} = 15.29$, $P > 0.3$) nor the fusion-rejection interactions between the F_1 parents ($G_{(1)} = 3.17$, $P > 0.07$) significantly affected the frequency of rejections among F_2's.

		N	Number of rejecting pairs (%)
		Fusible parents	
Mating	1	209	84 (40)
	2	196	90 (46)
	3	208	98 (47)
	4	399	167 (42)
	5	33	11 (33)
	6	123	49 (40)
	7	196	76 (39)
	8	219	88 (40)
Total		1583	663 (42)
		Rejecting parents	
Mating	9	198	85 (43)
	10	216	106 (49)
	11	198	75 (38)
	12	122	48 (39)
	13	217	104 (48)
	14	217	103 (48)
	15	80	36 (45)
	16	40	20 (50)
Total		1288	577 (45)

Table 2. Segregation frequencies of allotypes among offspring from crosses between semiallogeneic sibling *B. schlosseri*. Reciprocal matings between colonies (P_2) from Woods Hole and Berkeley used the same genotypes functioning as male and female (1 through 6) and as female and male (1a through 6a). A_iA_j denotes the allotype of parents and offspring. For each mating, F_1 siblings were genotyped by somatic compatibility tests against colonies of known allotype, then a pair of sperm and egg parents (P_2) was selected so that the members of each pair shared one allorecognition allele (A_j) in common. Because this locus includes dozens or hundreds of alleles, we assumed that the shared allele (A_j) differed among pairs (for example, A_2 in the first mating, A_5 in the second, etc.). *N* is number of F_2 offspring genotyped (also by somatic compatibility testing against stock lines of known allotype). Based on log-likelihood ratio goodness-of fit tests, all four possible allotypes in each of the 12 F_2 families appeared in frequencies not significantly different from 1:1:1:1, which is the ratio expected with random fertilization between semiallogeneic parents (Fig. 1C). All values of $G_{(3)}$ have a $P > 0.50$.

		Parental (P_2) allotypes		*N*	F_2 allotypic frequencies				$G_{(3)}$
		Sperm P_2	Egg P_2						
		A_iA_j	A_jA_k		A_iA_j	A_iA_k	A_jA_j	A_jA_k	
				Woods Hole					
Mating	1	A_1A_2	A_2A_3	89	23	26	19	21	0.58
	2	A_4A_5	A_5A_6	50	12	10	15	13	0.53
	3	A_7A_8	A_8A_9	82	20	24	19	19	0.40
	1a	A_2A_3	A_1A_2	102	24	29	23	26	0.40
	2a	A_5A_6	A_4A_5	60	13	16	19	12	0.98
	3a	A_7A_8	A_7A_8	77	17	19	17	24	0.80
				Berkeley					
Mating	4	$A_{10}A_{11}$	$A_{11}A_{12}$	63	14	14	17	18	0.40
	5	$A_{13}A_{14}$	$A_{14}A_{15}$	55	10	16	13	16	0.96
	6	$A_{16}A_{17}$	$A_{17}A_{18}$	76	19	19	23	15	0.85
	4a	$A_{11}A_{12}$	$A_{10}A_{11}$	56	13	15	13	15	0.14
	5a	$A_{14}A_{15}$	$A_{13}A_{14}$	85	20	17	24	24	0.85
	6a	$A_{17}A_{18}$	$A_{16}A_{17}$	70	14	17	18	21	0.71

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20. This situation would be similar to gametophytic self-incompatibility (SI) systems of flowering plants. If both male alleles are expressed on the surface of sperm, this would be similar to plant sporophytic SI. The quantitative predictions of these two SI systems would differ depending, for example, on whether the number of shared alleles (one or two) in a system resembling sporophytic SI quantitatively influences gametic compatibility. Nevertheless, the qualitative prediction (more fertilizations for males sharing no alleles with females) would still hold [see D. Charlesworth, *BioEssays* **17**, 31 (1995)].
21. We reared *B. schlosseri* colonies in 500-liter recirculating seawater aquaria at 18°C using previously described protocols (*23*). We determined the allotypes of prospective parents and their offspring by performing serial pairwise compatibility tests on subclones (about five zooids each) isolated from these colonies against sibling or offspring colonies known to be homozygous for alternative allorecognition alleles (*23*). Fusing colonies displayed a characteristic confluence of their blood vascular systems, indicating that they shared at least one allorecognition allele with their fusion partner. The blood vascular systems of rejecting colonies never fused, and after 12 to 24 hours, we often found a distinct necrotic zone demarcating the two subclones.
22. We established three families of full siblings from controlled laboratory matings between colonies collected from the undersides of floating docks in the small-craft harbor at Berkeley, CA. We bred these families from parental colonies that had been previously determined [by allozyme electrophoresis (*40*)] to be heterozygous for two common marker alleles (designated *PGI-1* and *PGI-2*) at the phosphoglucose isomerase locus, a gene unlinked to the allorecognition locus in *B. schlosseri*. We identified offspring from each of these three matings that were either *PGI-1/1* or *PGI-2/2* homozygotes, and then determined [using fusion assays (*21*)] their genotypes at their allorecognition locus. From each family, we chose three sibling colonies for the sperm competition experiments. One of the sibling colonies, which supplied ripe eggs for fertilizations, was either a *PGI-1/1* or *PGI-2/2* homozygote. We fertilized these eggs in vitro (*40*) using sperm from two siblings, one a *PGI-1/1* (or *PGI-2/2*) homozygote syngeneic to the colony supplying the eggs, the other a *PGI-2/2* (or *PGI-1/1*) homozygote allogeneic to the colony supplying the eggs. In each of three replicate trials for each of the mate competition experiments, we added equal volumes (2.5 ml) of a standardized dilution (5×10^7 sperm per milliliter) of sperm from each sperm parent to a dish containing 5 ml of 0.22 μm–filtered seawater and ≈150 ripe ova surgically removed from the egg parent. Ninety minutes later, we transferred 120 to 140 zygotes into clean dishes containing 50 ml of filtered seawater. We held the dishes at 18°C, daily transferring the developing embryos to clean seawater. When the tadpole larvae hatched on day 6 or 7, we froze them individually at −80°C, pending electrophoresis. We then used cellulose acetate gel electrophoresis to assay 100 fully developed larvae from each mating, and assigned paternity to either the sygeneic or allogeneic sperm parent based on the presence of one or the other diagnostic *PGI* marker.
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27. We initiated lab pedigrees from field-collected parental colonies as described (*26*). Compatibility tests between F_1 colonies were conducted as for *Botryllus* with subcloned explants (three to five polyps each) of these progeny. Fusible or rejecting brother-sister pairs were then grown to maturity and mated, and pairs of F_2 offspring were settled in close proximity on glass slides (*26*). We scored the outcomes of interactions between the growing stolons of 33 to 399 pairs from each of 16 matings (8 between fusible sibs; 8 between unfusible sibs) as fusion (anastomosis of the growing stolons of the two colonies) or rejection (production of hyperplastic stolons, followed by tissue necrosis).
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How Snapping Shrimp Snap: Through Cavitating Bubbles

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The snapping shrimp (*Alpheus heterochaelis*) produces a loud snapping sound by an extremely rapid closure of its snapper claw. One of the effects of the snapping is to stun or kill prey animals. During the rapid snapper claw closure, a high-velocity water jet is emitted from the claw with a speed exceeding cavitation conditions. Hydrophone measurements in conjunction with time-controlled high-speed imaging of the claw closure demonstrate that the sound is emitted at the cavitation bubble collapse and not on claw closure. A model for the bubble dynamics based on a Rayleigh-Plesset–type equation quantitatively accounts for the time dependence of the bubble radius and for the emitted sound.

The oceans may be deep, but they are not at all quiet (*1*). Sounds in the oceans include those of waves; rain, hail, and snow; and the biological sounds of fish, dolphins, whales, and snapping shrimp. The latter, in particular, produce the dominant level of ambient noise in (sub)tropical shallow waters throughout the world (*2*). These shrimp usually occur in such large numbers that there is a permanent crackling background noise, similar to the sound of burning dry twigs (*3*). The snapping sound can be heard day and night (*4*), with source levels as high as 190 (*5*) to 210 dB (*6*) (peak to peak) referenced to 1 μPa at a distance of 1 m. This severely limits the use of underwater acoustics for active and passive sonar, both in scientific and naval applications. The frequency spectrum of a snap is broad, ranging from tens of hertz to >200 kHz (*5*). The noise of snapping shrimp is therefore also used as a source for creating pictorial images of objects in the ocean through ensonification (*7*).

A snapping shrimp of the species *Alpheus heterochaelis* (~5.5 cm in size) is shown in Fig. 1A. The shrimp produces the snapping

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