α2-globin mRNA. Before the development of this method of analysis, direct sequence analysis of cloned α1- and α2-globin genes or their cDNA's would have been required. The hybrid-selection approach should allow a more rapid analysis of other structural mutations and may result in additional examples of sequence conversion between the human α-globin genes or between duplicated loci in other gene clusters. Such information may further define the frequency of other DNA recombination events in the human genome.

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# Larval Development and Dispersal at Deep-Sea Hydrothermal Vents

Abstract. *Deep-sea hydrothermal vent communities exhibit an array of reproductive strategies. Although a few vent species undergo planktotrophic, high-dispersal modes of development, most exhibit relatively low dispersal, but probably free-swimming nonplanktotrophic development. This predominance of nonplanktotrophy may be largely a reflection of phylogenetic constraints on the vent colonizing taxa; intervent dispersal among these forms may be facilitated by reduced developmental rates in the cold abyssal waters away from the vents. It is proposed that for those vent species with nonplanktotrophic development, larval dispersal is a stepwise process with oceanic ridge axes serving as discrete dispersal corridors.*

The biological communities associated with deep-sea hydrothermal vents have been the subject of intense study since their discovery in May 1976 (*1*). Active vent systems accompanied by dense benthic assemblages are now known at a number of widely separated sites along midocean rift zones in the eastern Pacific, extending from 48°N along the Juan de Fuca Ridge to 22°S along the East Pacific Rise. One basic question concerning the ecology and evolution of the vent biota is how the species and associations are established and maintained in a habitat that is markedly patchy in time and space. Most of the species are sedentary, and it has been suggested (*2, 3*) that free-swimming larval stages are the principal agents of recruitment and gene flow among hydrothermal sites (*4*).

We surveyed the modes of larval development in 18 molluscan species collected from two vent sites, the Galápagos spreading center near the equator and the East Pacific Rise at 21°N. Mollusks are the only vent taxa that lend themselves to such a survey because their early ontogenetic history is recorded in the shell of juveniles and well-preserved adults (*5, 6*). Although a few vent species undergo planktotrophic, high-dispersal modes of development, most of these mollusks exhibit relatively low dispersal, but probably free-swimming nonplanktotrophic development. The sparse data accumulated to date on the reproduction of other vent taxa [for example, decapod crustaceans (*6*) and ampharetid polychaetes (*7, 8*)] suggest a similar diversity of larval development modes in deep-sea hydrothermal systems. Our results indicate that not all vent species require a high-dispersal larval stage to persist in these ephemeral environments and suggest that the reproductive strategies in the hydrothermal vent community are more complex than previously believed.

Hundreds of minute mollusks with at least portions of the larval shell intact were isolated from the washings of biological materials collected by the deep-sea research vessel *Alvin* from the Galápagos and 21°N sites (*9*). The specimens were immediately fixed in 10 percent buffered seawater formalin for 48 hours and subsequently preserved in 80 to 95 percent ethanol. Cleaned shells were mounted on copper tape, coated (under vacuum) with approximately 400 Å of gold-palladium or a combination of gold and carbon, and examined under an ETEC Autoscan or AMR 1000 scanning electron microscope.

The diversity of larval shell (protoconch) morphologies in the vent gastropods is illustrated by the representative species in Fig. 1. Modes of larval development can be inferred on the basis of protoconch size and form (*5, 10*); although there may be pitfalls to this technique, such a comparative approach has

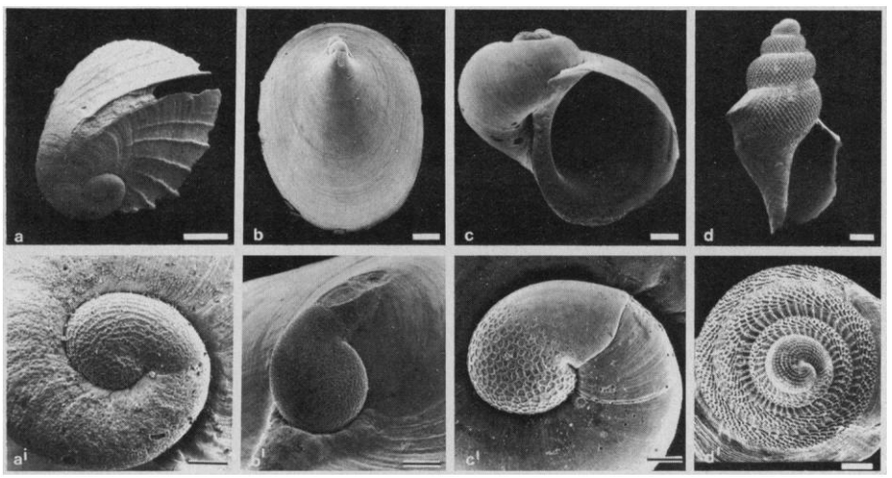

Fig. 1. Scanning electron micrographs of the shells of unnamed juvenile gastropods from the 21°N hydrothermal vent area. (a and b) Archaeogastropod limpets; (c) coiled trochoid archaeogastropod; and (d) turrid. Scale bars, 100 μm. (a′ through d′) Protoconch morphologies of (a) through (d), respectively. Scale bars, 50 μm.

proven effective in a wide variety of taxa and environments, including the deep sea (*11*). Of the 13 species of archaeogastropod limpets present at the two sites (*12*), ten have been collected with well-preserved protoconchs. All ten of these limpet species (Fig. 1, a and b) have a protoconch I with fewer than one-and-a-half whorls and lack a protoconch II; maximum protoconch I dimensions range from 175 to 325 μm. Comparison with the larval shell morphology of limpets for which development is known (*13*) suggests that most or all of the vent limpets undergo nonplanktotrophic development with a free-swimming, but nonfeeding, larval stage. A possible exception is a species with a protoconch of 325 μm, which indicates development from a large, yolky egg and perhaps direct development with the absence of any free-swimming stage.

Five of the six species of coiled trochoid gastropods present at the vents have well-preserved protoconchs. All have a typical trochacean archaeogastropod protoconch I consisting of one-and-a-half whorls or fewer and lack a protoconch II; maximum protoconch I dimensions range from 245 to 270 μm (Fig. 1, c and c′). Such dimensions suggest nonplanktotrophic larval development with a free-swimming stage (*5, 13, 14*).

Two neogastropod species, both turrids, are present at the vents, with one species at each site. In both species, the protoconch I consists of one-and-a-quarter whorls, exhibiting cross-hatched ornamentation, and the protoconch II is multiwhorled and elaborately sculptured (Fig. 1, d and d′). The morphology of each species is strikingly similar to that of shallow-water and deep-sea turrids for which planktotrophic development has been inferred (*15, 16*). Protoconch I dimensions fall within the range of egg sizes for turrids known to have planktotrophic development and outside the range for species known to exhibit nonplanktotrophic development (*17*).

Two species of bivalves occur at the two hydrothermal vent sites in significant numbers: *Calyptogena magnifica* (Vesicomyidae) (*18*) and an undescribed mytilid (*2*). No well-preserved juvenile specimens of *C. magnifica* have been collected, but the maximum diameter of 309 μm recently reported for the large yolky egg of this species (*18, 19*) suggests the existence of a nonplanktotrophic larval stage. In contrast, numerous well-preserved juvenile mytilids with intact larval shells (prodissoconchs) have been collected at the Galapágos site (*20*). The prodissoconch I of this vent mytilid is small [approximately 100 μm (*21*)] and is accompanied by a large prodissoconch II (more than 400 μm), indicating the presence of a planktotrophic larval stage with long-range dispersal capabilities (*2, 22*).

A few data are available for benthic crustaceans (*6*) and polychaetes (*7, 8*) from the vents. Modes of larval developments of four species of vent decapods have been inferred from analyses of egg size and fecundity, and a few zoeal stages have been isolated from vent plankton tows (*6*). As in the vent mollusks, there appears to be an array of reproductive adaptations among crustaceans; two (the galatheids *Munidopsis* cf. *subsquamosa* and *M. lentigo*) appear to have nonplanktotrophic development, whereas both the brachyuran *Bythograea thermydron* and the caridean shrimp *Alvinocaris lusca* apparently have widely dispersing planktotrophic larval stages. Desbruyères and Laubier (*7*) have suggested, by analogy with other ampharetid polychaetes, that the vent ampharetids have nonplanktotrophic benthic larvae and that the presence of *Paralvinella grasslei* at the Galápagos, 13°N, 21°N, and Guaymas Basin hydrothermal vent sites is maintained by the swimming of adults rather than by larval dispersal.

On the basis of these results for mollusks and available data on vent crustaceans (*6*) and polychaetes (*7*), we conclude that the hydrothermal vent fauna presents a heterogeneous assemblage of reproductive strategies (*23*). Larval shell morphologies indicate that only three of the molluscan species at the hydrothermal sites undergo a planktotrophic, high-dispersal mode of development; the remaining 15 species apparently have managed to persist in the ephemeral and patchy vent environments despite the possession of nonplanktotrophic, seemingly low-dispersal modes of development. In fact, all six molluscan species (*C. magnifica* and five limpets) shared by the two hydrothermal vent sites exhibit nonplanktotrophic modes of development. The two sites are 3300 km apart and yet the nonplanktotrophic larvae of shallow-water molluscan species having comparable larval shell morphologies remain in the plankton for only a few hours to a few days (*5, 13, 14*).

It is possible that the hydrothermal vent species have similarly brief free-swimming stages and disperse between vents in a stepwise manner, but large-scale surveys of midocean ridge systems studied to date (*24*) suggest that many of the active vent areas are not close enough for such dispersal to be feasible. Alternatively, because cold ambient bottom waters of environments away from the immediate vicinity of the vents would lower developmental rates, nonplanktotrophic larvae from the vent organisms may be capable of remaining in the plankton for far longer periods and thus be able to disperse far more widely than their shallow-water analogs (*25*). A similar temperature-related reduction in metabolic rate has been suggested as a means of prolonging the dispersive stage of the larvae of *Amphisamytha galapagenesis*, an ampharetid polychaete common at the Galápagos hydrothermal vent area (*8*).

One cost of planktotrophic development entails the transport of developing

larvae away from suitable habitats before the larvae are competent to settle and metamorphose (*26*). Planktotrophy may thus be disadvantageous for vent organisms, despite the broad dispersal capability it confers, because of the scale of the hydrothermal vent environment, with patches that are exceptionally small yet widely separated (*24, 27*). By analogy with the flexible development observed in many shallow-water opportunists (*28*), the nonplanktotrophic larvae of many of the vent organisms may either: (i) rapidly exploit local resources, since competency to metamorphose is attained very soon after release, or (ii) disperse relatively widely if metamorphosis is delayed in the presence of a deteriorating habitat and larvae enter cold, metabolism-slowing waters of the abyss. However, the predominance of nonplanktotrophic development in the vent assemblages need not indicate that this mode is optimal for this habitat. Just as the temperate rocky intertidal zone is populated by mussels (planktotrophic development) and limpets (nonplanktotrophic development), the hydrothermal vent communities contain a mixture of developmental types that probably reflects both demographic strategy and phylogenetic history (*29*). As members of the Archaeogastropoda, for example, the vent limpets and coiled trochoid gastropods are phylogenetically constrained to undergo a nonplanktotrophic mode of development. Free-swimming nonplanktotrophic larvae of these species may have a dispersal capability sufficient to maintain a chain of far-flung populations but insufficient to overcome isolation by chance founder events or extinction of intervening populations with local cessation of hydrothermal activity. Such allopatric processes may underlie the prolific speciation within the vent limpet families (*12, 30*).

More biogeographic data (and laboratory confirmation of the link between larval shell morphology and developmental pattern in these unusual taxa) are needed before we can fully understand the role of larval development in the origination and persistence of hydrothermal vent species. If larval dispersal is indeed a stepwise process for those vent taxa with nonplanktotrophic development, each spreading axis should be a discrete dispersal corridor. Allelic frequencies and taxonomic compositions should be more homogeneous among widely separated sites along a single spreading axis than among sites that are equally separated but belong to different spreading systems. In contrast, the more widely dispersing planktotrophic species should tend to be genetically and taxonomically homogeneous along mean vectors of bottom currents rather than having a genetic population structure or taxonomic distribution exclusively dependent on the configuration of ridge systems. The presence of active hydrothermal vent areas throughout the world's midoceanic ridge system (*24, 31*) affords an excellent opportunity to investigate mechanisms that maintain species identities among widely separated sessile populations as well as the evolutionary consequences of different developmental strategies.

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# Platelet-Activating Factor–Induced Aggregation of Human Platelets Specifically Inhibited by Triazolobenzodiazepines

Abstract. *Platelet-activating factor (PAF), a naturally occurring phospholipid, is a potent activator of various biological processes, including platelet aggregation. The mechanisms by which PAF acts are largely unknown, partly because of the lack of specific inhibitors for PAF-elicited responses. It was found that in washed human platelets the psychotropic triazolobenzodiazepine drugs alprazolam and triazolam potently inhibited PAF-induced changes in shape, aggregation, and secretion. The effects were specific for PAF activation, since the responses of human platelets to adenosine diphosphate, thrombin, epinephrine, collagen, arachidonate, and the calcium ionophore A23187 were not inhibited by the triazolobenzodiazepines. These psychotropic drugs should be useful in investigating the possibility that PAF or PAF-like phospholipids play a role in neuronal function and in elucidating biochemical mechanisms activated specifically by PAF in a variety of cells.*

Platelet-activating factor (PAF) is a naturally occurring phospholipid (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) that is released into the blood in vivo during immunoglobulin E–induced anaphylaxis (*1*). It is released from basophils in vitro on immunological challenge (*1*) and from platelets (*2*), neutrophils, and macrophages (*3*) in response to specific stimuli. This phospholipid is a potent mediator of inflammation (*1*), has antihypertensive activity (*4*), induces bronchoconstriction and contraction of smooth muscle (*1, 5*), and is one of the most powerful platelet activators known, inducing platelet shape change, aggregation, and secretion (*6*). The specific mechanisms underlying the responses of cells to PAF are unknown, and specific antagonists of PAF's action have not been identified.

Benzodiazepines are used clinically as anxiolytic and hypnotic agents. A derivative with a triazole ring (triazolobenzodiazepine), alprazolam, is particularly useful in the treatment of panic disorder with or without agoraphobia (*7*). A second triazole derivative, triazolam, is used as an effective, short-acting sleep medication (*8*). Benzodiazepine receptors are present primarily in the brain (*9*), but have also been found in platelets (*10*). We examined the effects of several benzodiazepines on human platelets activated by various agonists. We report that alprazolam and triazolam are potent and specific inhibitors of PAF-induced activation of human platelets.

The effects of alprazolam on PAF—induced aggregation of platelets and shape change (*11*) in human platelet-rich plasma are shown in Fig. 1A. At a concentration of 10 μ*M*, alprazolam inhibited the second wave of aggregation that is associated with granular release. At 40 μ*M* alprazolam completely inhibited PAF-induced shape change as well as the primary wave of aggregation (Fig. 1A). The concentration of alprazolam producing a 50 percent decrease in the initial velocity of PAF–induced aggregation in platelet-rich plasma was about 5 μ*M* (Fig. 1B). Triazolam was even more potent, showing a median inhibitory concentration ($IC_{50}$) of less than 2 μ*M* (Fig. 1B). Platelets from different individuals varied in their sensitivity to PAF. Accordingly, the $IC_{50}$ values for alprazolam and triazolam inhibition also varied, ranging from 2 to 12 μ*M* and 1 to 7 μ*M*, respectively. A benzodiazepine without the triazole ring, diazepam, did not inhibit PAF-induced platelet activation even at concentrations at which alprazolam and triazolam produced complete inhibition (Fig. 1B).

Subsequent experiments were performed to determine whether the effects

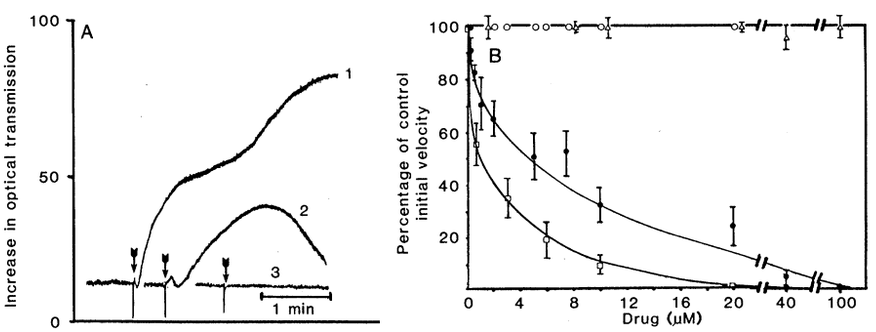


Fig. 1. (A) Inhibition of PAF-induced aggregation of human platelets in plasma by alprazolam. Blood was taken from normal individuals after obtaining their informed consent and the approval of the Institutional Human Experimentation Committee of the University of Vermont. The donors had fasted for at least 10 hours to prolong platelet responses to PAF (*12*) and claimed not to have taken any medication during the preceding 2 weeks. Blood was collected into 3.8 percent trisodium citrate (1:9), layered over a Ficoll Hypaque gradient, and centrifuged at 150*g* for 15 minutes at 22°C to obtain platelet-rich plasma. The platelet-rich plasma was removed and 0.45-ml portions ($2 \times 10^8$ to $4 \times 10^8$ platelets per milliliter) were incubated in a Chronolog-Lumi aggregometer for 1 minute at 37°C with stirring under the following conditions: (trace 1) no drugs added [addition of ethanol at 0.034 and 0.12 percent (final concentrations when added with alprazolam) had no effect on this activity]; (trace 2) 10 μ*M* alprazolam; and (trace 3) 40 μ*M* alprazolam (both concentrations were final). Alprazolam (8-chloro-1-methyl-6-phenyl-4H-*s*-triazolo[4,3a][1,4]benzodiazepine) (provided by R. Purpura, Upjohn) was dissolved as a 1 m*M* stock solution in 3 percent ethanol. Platelet aggregation was initiated by adding PAF at a final concentration of 51 n*M* (arrows). PAF (L-α-lecithin,β-acetyl,γ-*O*-alkyl; lot 386021) was obtained from Calbiochem-Behring as a pure lyophilized preparation, with the length of the alkyl chain consisting mainly of $C_{16}$ and $C_{18}$. A 10 m*M* stock solution dissolved in a solution of 0.35 percent bovine serum albumin (BSA) and 0.15*M* NaCl was stored frozen at −20°C. (B) Dose-response curves of inhibition of PAF-induced aggregation of platelets by alprazolam and triazolam. Platelet-rich plasma was stirred for 1 minute at 37°C with one of the following: (○) Tyrode's buffer or ethanol (controls); (●) alprazolam; (□) triazolam[8-chloro-6-(*o*-chlorophenyl)-1-methyl-4H-*s*-triazolo[4,3a][1,4]benzodiazepine]; or (△) diazepam. PAF (51 n*M*) was used to initiate platelet aggregation. Triazolam, prepared as a 1 m*M* stock solution in 30 percent ethanol, was provided by Upjohn. Diazepam, freshly prepared as a 1 m*M* stock solution in 3 percent ethanol, was provided by H. Sheppard and S. Spector (Hoffmann–La Roche). Each point is the mean ± standard deviation of the initial velocity of aggregation (*14*) for three separate experiments, each involving platelet-rich plasma from a different donor.