particular groups, such as fishes, arthropods, and squid, is presumably a reflection of their composition. No mineralization occurred in the polychaete *Nereis* (1.48% phosphate by weight) under the same experimental conditions.

Phosphatization of Santana Formation fishes has been estimated to occur in life (*7*) or, on the basis of a comparison with decay rates of modern fish tissues, within 5 hours of death (*29*). In our experiments, however, the process was initiated within 2 weeks, but the degree of mineralization of muscle tissue increased up to 4 weeks or even longer. The rate of decay, and presumably mineralization, would have been further slowed at lower temperatures.

This study, together with the evidence of the fossil record, emphasizes that precise conditions must prevail in order for mineralization of soft tissue to take place. But, once the morphology is stabilized by initial mineralization, the potential for preservation of the soft tissue through subsequent diagenesis is enormously enhanced, although the material remains vulnerable to compaction unless subsequently enclosed in a concretion. There is a critical balance between decay to release phosphate and precipitation to preserve the soft tissue before much morphological detail is lost (*1*, *7*). This balance occurs in closed conditions where an initial drop in pH triggers phosphatization. The role of microbial films in fossilization may extend beyond the protection of carcasses and inhibition of decomposition (*30*) to the establishment of the conditions necessary for rapid mineralization (*4*).

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Population Structure and the Evolution of Virulence in Nematode Parasites of Fig Wasps

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It is often assumed that parasitic and disease-producing organisms tend to evolve benign relationships with their hosts over time. In contrast, theoretical arguments suggest that increased opportunities for parasite transmission will promote the evolution of increased virulence. The natural history of species-specific nematodes that parasitize fig-pollinating wasps permits the testing of these predictions in natural populations. For 11 species of Panamanian fig wasps, those species characterized by population structures that result in increased opportunities for parasite transmission harbor more virulent species of nematodes. In addition, differences in population structure are also associated with differences in other intra- and interspecific phenomena, including sex ratios among the fig wasp species, the degree of tension in the wasp-fig mutualism, and lethal combat among the males of parasitic wasps.

Parasitic and disease-producing organisms influence nearly all aspects of biological organization. These organisms have been implicated either directly or indirectly in (i) limiting the geographical range of host organisms (community composition); (ii) regulating host population densities and dynamics; (iii) mediating the outcome of competition or predation between their hosts and other organisms; (iv) maintaining polymorphisms in blood proteins, and possibly in maintaining general genetic polymorphisms; (v) underlying sexual selection; and (vi) serving as a selective force leading to the predominance of sexual reproduction (*1*–*6*). The proposed mechanisms by which

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parasites might affect all of these ecological and evolutionary phenomena depend on the parasite's virulence (reduction in the lifetime reproductive success of the host owing to the parasite), begging the question of what factors ultimately influence the evolution of virulence itself.

It is frequently assumed that, with time, parasites tend to evolve benign relationships with their hosts. However, the usefulness and general validity of this view have been questioned (*7*). Moreover, recent analyses have suggested instead that parasite virulence should depend on transmission, so that greater opportunities for transmission would permit or even promote the evolution of greater virulence (*7–13*). Conversely, systems characterized by restricted opportunities for transmission [for example, systems in which most or all parasite transmission is vertical (from parent to offspring), as opposed to horizontal (from among unrelated individuals)] are thought to promote neutral or even mutualistic interactions among would-be parasites and their hosts (*14*). Whereas evidence supports some aspects of these models (*10, 14*), many of these proposed relationships have been supported on theoretical grounds only. Unfortunately, limitations imposed by the natural history of the study organisms (for example, difficulties in measuring lifetime reproductive success of individual hosts as a function of parasitism and difficulties in measuring opportunities for parasite transmission) often hinder direct testing of the predictions made by these models in natural populations.

The natural history of fig-pollinating wasps and the species-specific nematodes that parasitize them (*15–18*) permits a direct measurement of these crucial parameters. In the fig wasps, a number of gravid, pollen-bearing foundress wasps enter a receptive syconium (the enclosed inflorescence that eventually ripens to become the fig fruit) nearly simultaneously, pollinate the flowers, lay eggs, and die. The bodies of the foundress wasps remain within the fig fruit and may be counted to determine the number of wasps potentially pollinating and laying eggs in any given fig. As the fruit ripens and seeds develop, the wasp offspring mature (each feeding on the contents of one seed), eclose, and mate inside the fig. The winged females gather pollen, leave the syconium, and disperse to begin the cycle anew. When only one foundress wasp enters a fig fruit, a count of the offspring gives a direct measure of the wasp's lifetime reproductive success (*15*).

Just as distinct species of fig-pollinating wasps are generally associated with distinct species of host figs, morphological work on the Panamanian species has established that distinct species of nematodes are associated with distinct species of host fig wasps (*15–18*). The nematodes' life cycle is thus intimately connected with that of the fig wasps. In nematode-infested figs, immature, dispersal-phase nematodes crawl onto newly emerged female fig wasps and are carried by them to the next fig. At some point, the nematodes enter the body cavity of the wasp and begin to consume it. Later, about six or seven adult nematodes emerge from the body of the dead wasp (personal counts, 16 to 18), mate, and lay eggs within the same fig in which the host wasp has laid her eggs. The nematodes' eggs hatch synchronously with the emergence of the next generation of fig wasps and begin their cycle anew. In almost ripe fig fruits that have been pollinated by only one foundress wasp, the presence of immature nematodes can be used to determine whether or not that individual wasp was infected. Therefore, the number of wasp offspring associated with nematode-infected single foundress wasps can be compared to the number of offspring associated with uninfected single foundresses in order to estimate the nematodes' effects on the lifetime reproductive success of their host fig wasps (that is, in order to measure the virulence of the parasite).

Studies initiated over 10 years ago (about 120 to 140 fig wasp and nematode generations) in the vicinity of the Panama Canal show that the 11 different species of host wasp considered here present a continuum of population structures (in this case, distributions of numbers of foundresses per fig fruit) (*19, 20*) (Table 1). The opportunities for nematode transmission among the individuals of any given wasp species depend, in turn, on the population structure of the host fig wasp. For example, if all the wasp broods in all of the figs in a population are founded by only a single foundress wasp, then all parasite transmission is vertical, from parent wasp to offspring. The offspring of the host wasp that the nematodes are directly parasitizing present the only opportunity for propagation of the nematodes' offspring. A nematode's fitness is therefore completely coupled to the reproductive success of the host wasp it is directly parasitizing. In such a system, any reduction in the wasp's reproductive success as a result of the nematode will ultimately result in the elimination of the nematode from the wasp population, in a manner analogous to the elimination of a deleterious dominant allele.

However, if some broods are founded by more than one foundress wasp, then the

Table 1. Species of fig, wasp, and nematode studied. The number of fruit crops sampled in order to estimate *Prop*, the proportion of single foundress broods in each species, is indicated by *n*; the number of crops sampled per species to estimate the virulence, *V* (the lifetime reproductive success of nematode-infected wasps relative to uninfected wasps), is given by *N*; *I* gives the total number of broods sampled from individual infected wasps; and *U* gives the total number of broods sampled from individual uninfected wasps. More virulent nematode species are associated with greater opportunities for transmission.

Fig species (*Ficus*)	Pollinator wasp species (*Pegoscapus* or *Tetrapus*)	Nematode species (*Parasitodiplogaster*)	Single foundress broods		Virulence estimates			
			n	*Prop.*	*N*	*I*	*U*	*V*
columbrinae	*P. orozcoi*	*colubrinema*	21	0.99	3	26	74	1.01
perforata	*P. standleyi*	*perfornema*	22	0.99	6	60	80	0.97
paraensis	*P. sp.*	*paranema*	39	0.92	4	56	44	1.00
pertusa	*P. silvestrii*	*pertanema*	16	0.90	2	33	9	0.99
obtusifolia	*P. hoffmeyer*	*obtusinema*	48	0.83	7	170	89	0.94
bullenei	*P. sp.*	*bullenema*	24	0.82	2	12	33	0.99
citrifolia	*P. aesutus*	*citrinema*	65	0.78	6	92	129	0.93
yoponensis	*T. ecuadoranus*	*yoponema*	15	0.58	1	4	14	0.92
nymphaefolia	*P. amabilis*	*nymphanema*	33	0.55	8	183	47	0.90
nr. trigonata	*P. sp.*	*trinema*	36	0.31	5	45	103	0.84
popenoei	*P. sp.*	*popenema*	61	0.24	8	61	80	0.89

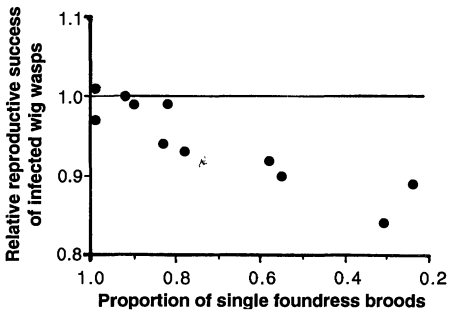

Fig. 1. Relation between virulence, measured as the lifetime reproductive success of nematode-infected relative to uninfected female fig wasps, and the proportion of multifoundress broods encountered in 11 Panamanian species. Increased incidence of multifoundress broods provides increased opportunities for nematode transmission. Those species of wasp characterized by increased opportunities for transmission of their species-specific parasitic nematodes contain more virulent species of nematodes. All statistical tests indicate a significant relation [$P < 0.001$ (*22*)].

opportunity exists for a nematode's offspring to propagate by parasitizing the offspring of wasps other than its direct host. As multiple foundress broods increase in frequency, a nematode's fitness, on average, becomes progressively decoupled from the reproductive success of the host wasp it is directly parasitizing because parasite transmission increasingly becomes horizontal, from parent wasp to the offspring of another wasp. In such a system, nematodes could afford to reduce the reproductive success of their direct hosts, particularly if reduced nematode propagation through their host's offspring were balanced by an equal or greater amount of horizontal transmission (*21*).

Therefore, within fig wasp species, the lifetime reproductive success of individual female wasps can be related to the presence or absence of nematode infections, thereby permitting the estimation of nematode virulence. Across species, the characteristically different population structures exhibited by the host wasp species present different opportunities for the transmission of the nematode species that parasitize them. Consequently, the estimates of virulence for the different wasp-nematode species associations can be compared in the context of the different opportunities for nematode transmission observed among the different host fig wasp species.

For each fig-wasp-nematode species association, I sampled almost ripe fruit from different crops and inspected the fruit for the bodies of the foundresses. In those broods with only one foundress, I checked for the presence of dispersal-phase nematodes and allowed the brood to complete development. The wasp offspring were then counted. For each fruit crop sampled, the mean number of offspring per infected foundress was divided by the mean number of offspring per uninfected foundress in order to provide an estimate of the relative reproductive success of infected versus uninfected single foundresses (virulence). Within each species, the weighted mean of such estimates provides the final estimate of nematode virulence (Table 1). I then compared the rankings of virulence estimates for each wasp-nematode species association with the rankings of the incidence of single foundress broods (those broods in which only vertical transmission is possible).

The nematode species with the greatest estimated virulence are associated with host wasp species that are characterized by population structures providing the most frequent opportunities for horizontal transmission of their parasites (Table 1 and Fig. 1) (*22*). This result indicates that host population structure and the resulting opportunities for parasite transmission can influence the ecological and evolutionary outcome of species interactions in natural populations. Moreover, even though these pairs of species all share the same basic natural history, estimates of the nematodes' influence on the lifetime reproductive success of the wasps define a continuum of relationships ranging from commensal to parasitic. Furthermore, the result supports the proposition that an increase in host density or crowding will be linked to increased opportunities for parasite transmission, which in turn will promote the evolution of increased virulence of the parasitic or disease-producing organisms infecting those host populations (*7–14*).

The preceding analysis addresses ongoing relationships in contemporary time. In addition, several lines of evidence suggest a longer term evolutionary context for these findings. The Panamanian nematode species are all members of the genus *Parasitodiplogaster*, established by Poinar from nematodes collected from the African fig wasp (*Elisabethiella stuckenbergi*) (*16*) (Table 1). The parasitic life cycle of this genus of nematodes represents a significant evolutionary shift in an otherwise phoretic group (*Diplogasteridae*) (*16–18*). Taken together, the species-specificity, biogeography, and phylogenetic affinities of this group of nematodes (*16–18*) and of their host wasps and host figs, as well as the DNA sequence divergence (*22*) and fossil record among both the fig and the wasp species (*18, 23–25*), indicate an ancient association between the nematodes, the wasps, and the figs that began as a benign, commensal relationship (*18*). This strongly suggests that, rather than evolving to become more benign, the nematodes have, if anything, evolved to become more virulent, and that the eventual outcome of any particular species interaction is largely dependent on the population structure characteristic of the host wasp species.

Independent of nematode virulence, differences in wasp population structure also affect other important aspects of wasp and fig biology. For example, because of the effects of local mate competition and inbreeding, wasp species characterized by lower numbers of foundresses produce, on average, more female-biased broods (*19, 20*). Because only female wasps perform pollination services for the host fig, brood sex ratios are an indicator of the degree of tension (conflict of interest) within the mutualistic association between the wasp and the fig, such that decreasingly female-biased sex ratios represent a shift away from the fig's reproductive interest (*15*). Furthermore, parasitic species of fig wasps are characterized by much less subdivided population structures than any of the pollinating wasp species (*15, 26*). These parasitic species are often characterized by lethal combat among the males along with the concomitant morphological adaptations. Therefore, species characterized by the most subdivided population structures have the greatest female biases in sex ratio, the least tension in their mutualistic interactions, the least tendency for fighting among males, and the most benign parasites. This observation points to the diversity of ways in which population structure can influence the outcome of evolution.

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Structure-Based Discovery of Inhibitors of Thymidylate Synthase

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A molecular docking computer program (DOCK) was used to screen the Fine Chemical Directory, a database of commercially available compounds, for molecules that are complementary to thymidylate synthase (TS), a chemotherapeutic target. Besides retrieving the substrate and several known inhibitors, DOCK proposed putative inhibitors previously unknown to bind to the enzyme. Three of these compounds inhibited *Lactobacillus casei* TS at submillimolar concentrations. One of these inhibitors, sulisobenzone, crystallized with TS in two configurations that differed from the DOCK-favored geometry: a counterion was bound in the substrate site, which resulted in a 6 to 9 angstrom displacement of the inhibitor. The structure of the complexes suggested another binding region in the active site that could be exploited. This region was probed with molecules sterically similar to sulisobenzone, which led to the identification of a family of phenolphthalein analogs that inhibit TS in the 1 to 30 micromolar range. These inhibitors do not resemble the substrates of the enzyme. A crystal structure of phenolphthalein with TS shows that it binds in the target site in a configuration that resembles the one suggested by DOCK.

Thymidylate synthase (TS) is a target for drugs against proliferative diseases and cancer because it is required for de novo synthesis of deoxythymidine monophosphate (dTMP) and, hence, for DNA production. Efforts have focused on the design of inhibitors similar to the substrate, deoxyuridine monophosphate (dUMP), or the cofactor, 5,10-methylenetetrahydrofolate (CH_2-H_4 folate). Administered as the premetabolite 5-Fluorouracil, 5-Fluorouridylate is a mechanism-based inhibitor of TS (*1*) that is used in chemotherapy, whereas 10-propargyl-5,8 dideazafolate (CB3717) is a cofactor mimic (*2*). Although CB3717 has an inhibition constant, K_i, of 40 nM (*3*) for TS, it shows liver and kidney toxicity (*4*), which emphasizes the need for more efficacious agents. The x-ray structure of TS (*5, 6*) has been used in iterative cycles of crystallographic analysis, synthesis, and inhibition assays to design high-affinity heterocyclic inhibitors that resemble the cofactor (*7*). Anti-TS drugs dissimilar to the substrate and cofactor remain attractive because they are less likely to have the side effects that are produced by the nucleotide and folate mimics.

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We sought novel inhibitors of TS with a computational screen targeted at the structure of *L. casei* TS determined at 2.3 Å resolution (*8*). We used the program DOCK (*9–11*) to explore the active site of TS with molecules from the Fine Chemical Directory (FCD) (*12*). For each FCD molecule, DOCK examined an average of 10^4 orientations for steric fit to TS and scored each on the basis of the energy of the electrostatic interaction with the enzyme. Of the 55,313 molecules searched, the 600 with the highest scores were saved. We applied a correction for ligand solvation, which was based on a modified Born equation (*13*), to sort the selected molecules.

The TS substrate, dUMP, and several known nucleotide and non-nucleotide inhibitors of the enzyme received good scores (Table 1). The program retrieved some molecules previously unknown to bind to TS. On the basis of DOCK score and lack of similarity to dUMP, we selected 25 of these molecules as putative inhibitors. Three of the compounds, including sulisobenzone (SB), inhibited TS with values of the inhibition constant IC_{50} (50% reduction) in the high micromolar range (Table 1).

The TS-SB complex was crystallized from both phosphate and tris buffers, and the structure of each was determined to 2.5 Å resolution (*14*) (Fig. 1). In neither structure was SB found in the binding mode favored by DOCK. We anticipated that the sulfonate group of SB would bind in the TS subsite that is occupied by a phosphate moiety in all of the other structures that we have solved (*15*). Instead, both of these structures showed a buffer-derived anion, either phosphate or sulfate from the $(NH_4)_2SO_4$ precipitant, bound at this site (Fig. 1B). In both structures, SB is displaced away from the nucleotide binding region to different but overlapping regions of the active site. In the structure crystallized from the phosphate buffer, the sulfonate moiety of SB forms hydrogen bonds with the sulfhydryl of the conserved active site nucleophile, C198, and with the backbone NH of D221, a conserved residue that is involved in cofactor binding (*6, 16*). The methyl of SB makes van der Waals contacts with L224, which also contributes to the cofactor binding site. In the structure that crystallized from the tris buffer, the SB is rotated 153° and translated 5.1 Å with respect to the configuration that crystallized from the phosphate buffer. The sulfonate is accessible to solvent in the vicinity of the TS carboxyl terminus (Fig. 1B). The substituted ring of SB is in van der Waals contact with W85 at the mouth of the active site. Taken together, the two SB configurations partially overlap the substrate and cofactor subsites of TS, as deter-