of thick samples, such as fracture surfaces, directly.

We anticipate that applications of this technique will comprise the characterization of phase-separated and -oriented polymeric alloys and their interfaces, composites, diamond-like carbon films, other carbon-containing advanced materials, as well as coal and coke. Absorption edges of elements other than carbon, particularly those of nitrogen and oxygen, could also be explored.

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14. We are grateful to the many people who have made this work possible. In particular, we thank J. Kirz and C. Jacobsen and their co-workers at the State University of New York (SUNY), Stony Brook, and the NSLS, who built and maintain the STXM, Sue Wirick for building a rotating sample mount, S. Subramoney for providing the samples, and A. Smith for helping with the data acquisition. J. Kirz also read the first draft of the manuscript and made helpful suggestions. Part of this work was performed while H.A. was at SUNY, Stony Brook, and was partly supported by the National Science Foundation (DIR 9005893). The work was performed at NSLS, which is supported by the Department of Energy, Office of Basic Energy Sciences.

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# Refuge Theory and Biological Control

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**An important question in ecology is the extent to which populations and communities are governed by general rules. Recent developments in population dynamics theory have shown that hosts' refuges from their insect parasitoids predict parasitoid community richness patterns. Here, the refuge theory is extended to biological control, in which parasitoids are imported for the control of insect pests. Theory predicts, and data confirm, that the success of biological control is inversely related to the proportion of insects protected from parasitoid attack. Refuges therefore provide a general mechanism for interpreting ecological patterns at both the community level (their species diversity) and population level (their dynamics).**

The practice of introducing parasitoids, those insects that parasitize and kill their arthropod hosts, for the biological control of insect pests has contributed much to ecological theory. Unfortunately, the converse is not true (*1*), because analytical parasitoid-host models that have been applied to biological control (*2*) have not identified simply measured, unambiguous parameters that actually improve the chances of successful pest control. Consequently, the practice of biological control through the introduction of natural enemies remains largely empirical and based on trial and error, in spite of the need to improve its scientific basis (*3*, *4*).

We propose that theory recently developed to account for variability in the species richness of parasitoid communities offers a simple methodology to evaluate the extent to which a parasitoid introduction will control a pest population. The model formalizing the theory (*5*, *6*) identifies proportional refuges from parasitism [defined as a fixed proportion of the host population immune to attack (*7*)] as a key constraint on the number of parasitoid species that can persist on a host (*5*). As a result of its population-dynamic structure, the model further quantifies the extent that parasitoids will depress host populations below the densities hosts would achieve in the absence of parasitoids (*6*).

It is this second property of the model that is relevant to biological control, the goal of which is to reduce and maintain pest populations below some critical density defined as their economic threshold. Basically, refuge theory predicts that hosts that occupy small refuges (that is, a low proportion of their population is in the refuges) will be highly exploitable by parasitoids, and as a result the host populations will be

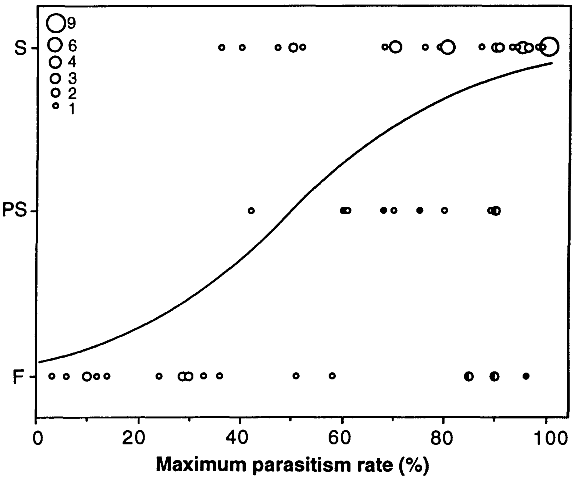

**Fig. 1.** Relation between maximum percentage parasitism (in two cases including host mortality from host feeding by the parasitoid) and the outcome of parasitoid introductions for classical biological control. Multiple cases are denoted by larger circles. Blackened symbols represent failures and partial successes attributed to climatically related factors. The regression line illustrated is based on a logistic regression when partial successes have been excluded (logit$y = -2.880 + 0.057x$, $\chi^2 = 28.48$, $n = 64$, $P < 0.0000001$). Regressions based on an economic evaluation of success (less than full control considered a failure) and a dynamic evaluation of success (full and partial successes taken as equal evidence of the ability of parasitoids to reduce pest densities) produced similar statistics (logit$y = -2.957 + 0.048x$, $\chi^2 = 23.76$, $n = 74$, $P = 0.0000011$ and logit$y = -2.669 + 0.058x$, $\chi^2 = 29.67$, $P < 0.0000001$, respectively).

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**Table 1.** List of parasitoid releases for biological control ranked by the maximum parasitism rate (Max.) recorded after parasitoid release and establishment. The outcomes of the introductions are based on information provided in the sources (Ref., reference number). Target species may appear more than once where control attempts have been made at different locations or with more than one parasitoid species. F, failure; PS, partial success; and S, success.

| Target species | Parasitoid species | Max. (%) | Outcome | Ref. |
|---|---|---|---|---|
| *Leucoptera coffeella* | *Mirax insularis* | 3 | F | (*11*) |
| *Hypera postica* | *Bathyplectes curculionis* + *Tetrastichus incertus* | 6 | F | (*19*) |
| *Aonidiella aurantii* | *Prospaltella perniciosi* | 10 | F | (*20*) |
| *Mayetiola destructor* | *Pediobius metallicus* | 10 | F | (*11*) |
| *Forficula auricularia* | *Bigonicheta setipennis* | 12 | F | (*21*) |
| *Rhabdoscelus obscurus* | *Lixophaga sphenophori* | 14 | F | (*11*) |
| *Brontispa longissima* | *Tetrastichus brontispae* | 24 | F | (*22*) |
| *Diatraea saccharalis* | *Paratheresia claripalpus* | 29 | F | (*11*) |
| *Neodiprion sertifer* | 14 native and introduced spp. | 29 | F | (*11*, *23*) |
| *Oryctes rhinocerus* | *Scolia ruficornis* | 30 | F | (*11*) |
| *Cydnia pomonella* | *Ascogaster quadridentata* | 30 | F | (*24*) |
| *Eoreuma loftini* | *Allorhogas pyralophagus* | 33 | F | (*17*) |
| *Brontispa longissima* | *Tetrastichus brontispae* | 36 | F | (*25*) |
| *Nipaecoccus viridis* | *Anagyrus indicus* + *A. kamali* | 36 | S | (*26*) |
| *Operophtera brumata* | *Agrypon flaveolatum* | 40 | S | (*11*) |
| *Acyrthosiphon pisum* | *Aphidius eadyi* | 42 | PS | (*27*) |
| *Cephus pygmaeus* | *Collyria calcitrator* | 47 | S | (*11*) |
| *Aleurocanthus woglumi* | *Prospaltella opulenta* | 50 | S | (*28*) |
| *Plutella xylostella* | *Apanteles plutellae* | 50 | S | (*29*) |
| *Phthorimaea operculella* | *Apanteles subandinus* | 51 | F | (*30*) |
| *Tryporyza nivella* | *Isotima javensis* | 52 | S | (*31*) |
| *Triathaba complexa* | 3 parasitoid spp. | 58 | F | (*11*) |
| *Coleophora laricella* | *Chrysocharis laricinellae* | 60 | PS | (*21*) |
| *Popillia japonica* | *Tiphia vernalis* | 61 | PS | (*11*) |
| *Pristiphora erichsonii* | *Mesoleius tenthredinis* | 68 | S | (*32*) |
| *Eriosoma lanigerum* | *Aphelinus mali* | 68 | PS | (*33*) |
| *Lamprosema octasema* | *Chelonus stratigenas* | 70 | PS | (*29*) |
| *Sirex noctilio* | 3 parasitoid spp. | 70 | S | (*24*) |
| *Phenacoccus manihoti* | *Epdinocarsis lopezi* | 70 | S | (*34*) |
| *Lepidosaphes beckii* | *Aphytis lepidosaphes* | 70 | S | (*11*) |
| *Proceras sacchariphagus* | *Apanteles flavipes* | 70 | S | (*35*) |
| *Leptinotarsus decemlineata* | *Doryphorophaga doryphorae* | 75 | PS | (*36*) |
| *Laspeyresia nigricana* | *Ascogaster quadridentata* | 76 | S | (*11*) |
| *Brontispa longissima* | *Tetrastichus brontispae* | 79 | S | (*25*) |
| *Phytomyza ilicis* | 4 parasitoid spp. | 80 | PS | (*11*) |
| *Operophtera brumata* | *Cyzenis albicans* | 80 | S | (*11*) |

severely reduced. Conversely, for hosts that occupy sufficiently large refuges, parasitoids will be unable to exploit the host population sufficiently to appreciably depress its density (*8*). The precise effect of the refuge capacity on host abundance depends on other parameters in the model (notably, the maximum population growth rates of the host and parasitoid), but if the conceptual basis of the theory is correct, measurement of the proportion of the host's population subject to attack should be an important parameter describing the amount of host population depression and thus should provide a reliable estimation of whether a parasitoid will be able to successfully control its host.

We tested this prediction by searching the classical biological control literature for reports of parasitoid introductions that provided field estimates of parasitism and an evaluation of the outcome. Because refuges from parasitism can arise from many sources [including intrinsic, physiologically based host defenses and extrinsic defenses arising from plant structures inhabited by hosts (*9*)], we recorded the maximum parasitism level achieved in any host population as the best estimate of the proportion of hosts not in the refuge (*10*). We identified 74 cases for which the required information was provided (Table 1).

Evaluations of biological control are typically based on economic criteria; a parasitoid introduction either fails to influence the status of the pest or, alternatively, the pest is partially or completely controlled. Because most evaluations of outcomes are nonquantitative, we analyzed biological control three ways, each by logistic regression. (i) We contrasted clear outcomes, those that resulted in complete failures or complete successes. In this test we excluded partial success, cases in which parasitoids reduced pest populations to some extent, but full economic benefits were not realized. (ii) We used a conservative economic criterion, in which partial successes were included and classified as failures. (iii) The final analysis was based on a more optimistic, dynamic criterion for which all successes, whether partial or complete, were pooled and tested against failures. This distinguishes cases in which parasitoids were able to exert at least some control on host densities from cases in which parasitoids were unable to significantly reduce pest densities.

As predicted by theory, there is a strong association between maximum parasitism rates and biological control outcomes (Fig. 1). Moreover, the relation is robust, even when the more conservative, economic criterion for success is applied. Thus, the maximum susceptibility of a host to parasitoid attack (estimated by maximum parasitism rates) provides a highly significant estimate of the probability that the parasitoid introduction will reduce host densities.

Consistent with the population dynamics models on which refuge theory is based (*2*, *5*, *6*), our results do not identify host

**Table 1.** (continued).

| Target species | Parasitoid species | Max. (%) | Out-come | Ref. |
|---|---|---|---|---|
| *Phenacoccus aceris* | *Allotropa utilis* | 80 | S | (*11*) |
| *Rhyacionia frustrana* | *Campoplex frustranae* | 80 | S | (*11*) |
| *Unaspis yanonensis* | *Aphytis yanonensis* | 80 | S | (*37*) |
| *Anastrepha* spp. | *Aceratoneuromyia indica* | 80 | S | (*38*) |
| *Phyllonorycter messaniella* | *Apanteles circumscriptus* + *Achrysocharoides splendens* | 80 | S | (*24*) |
| *Agonoxena argaula* | *Brachymeria agonoxenae* | 85 | F | (*11*) |
| *Anthonomus grandis* | *Bracon kirkpatricki* | 85 | F | (*11*) |
| *Antonina graminis* | *Neodusmetia sangwani* | 87 | S | (*39*) |
| *Brontispa mariana* | *Tetrastichus brontispae* | 89 | PS | (*11*) |
| *Carulaspis minima* | *Encarsia lounsburyi* | 90 | F | (*40*) |
| *Caliroa cerasi* | *Lathrolestes luteolator* | 90 | F | (*24*) |
| *Parabemisia myricae* | *Eretmocerus debachi* | 90 | S | (*41*) |
| *Leucoma salicis* | *Apanteles solitarius* | 90 | PS | (*21*) |
| *Parlatoria oleae* | *Aphytis maculicornis* | 90 | PS | (*42*) |
| *Rhabdoscelus obscurus* | *Lixophaga spenophori* | 90 | S | (*31*) |
| *Rastrococcus invadens* | *Gyranosoidea tebygi* | 90 | S | (*43*) |
| *Aleurocanthus woglumi* | *Eretmocerus serius* | 91 | S | (*11*) |
| *Coleophora laricella* | *Agathis pumila* | 91 | S | (*11*) |
| *Aleurocanthus woglumi* | *Prospaltella opulenta* | 93 | S | (*11*) |
| *Pristiphora geniculata* | *Olesicampe geniculatae* | 94 | S | (*19*) |
| *Oulema melanopus* | *Tetrastichus julis* | 95 | S | (*19*) |
| *Diatraea saccharalis* | *Lixophaga diatraea* | 95 | S | (*44*) |
| *Diatraea saccharalis* | *Apanteles flavipes* | 95 | S | (*44*) |
| *Mythimna separata* | *Apanteles ruficrus* | 95 | S | (*45*) |
| *Dasyneura mali* | *Prosactogaster demades* | 96 | F | (*14*) |
| *Plutella xylostella* | *Diadegma eucerephaga* | 96 | S | (*29*) |
| *Agromyza frontella* | *Dacnusa dryas* | 96 | S | (*46*) |
| *Siphoninus phillyreae* | *Encarsia partenopea* | 98 | S | (*47*) |
| *Homona coffearia* | *Macrocentrus homonae* | 99 | S | (*31*) |
| *Chromaphis juglandicola* | *Trioxys pallidus* | 100 | S | (*11*) |
| *Lepidosaphes ficus* | *Aphytis mytilaspidis* | 100 | S | (*11*) |
| *Levuana irridescens* | *Bessa remota* | 100 | S | (*11*) |
| *Nezara viridula* | *Trissolcus basalis* | 100 | S | (*29*) |
| *Plutella xylostella* | *Tetrastichus sokolowskii* | 100 | S | (*29*) |
| *Promecotheca coeruleipennis* | *Pediobius parvulus* | 100 | S | (*11*) |
| *Pseudococcus citriculus* | *Clausenia purpurea* | 100 | S | (*11*) |
| *Maconellicoccus hirsutus* | *Anagyrus kamali* | 100 | S | (*11*) |
| *Ceroplastes rubens* | *Anicetus annulatus* | 100 | S | (*48*) |

susceptibility as the only factor affecting host population depression. Parasitoid introductions sometimes fail even when maximum parasitism rates are high (Fig. 1). Complete or partial failure can occur for several reasons, including climatic mismatch (*11*), high incidence of hyperparasitism (*12*), extremely low economic thresholds of the pest (*13*), or poor synchrony with the host (*14*). The particular importance of climate to successful biological control is apparent in our data set; 4 of the 10 partial successes and 3 of the 5 clear exceptions to the outcome predicted by the theory involve climatic factors that reduce parasitoid reproduction, survivorship, or host synchrony (Fig. 1). Obviously, these cases are beyond the scope of refuge theory, which assumes that parasitoids are climatically adapted. Exceptions arising from interactions between parasitoids and climate are consistent with the widely recognized basic requirement for good climatic matching (*15*). On the other hand, even including these cases in our analysis does not alter our main conclusion that host susceptibility provides a good estimator of the outcome of classical biological control.

These results are important in at least four ways. (i) They represent a test of theory proposing that refuges from parasitism represent a major constraint on parasitoid-host interactions, thus influencing not only parasitoid community species richness (*5*, *6*, *16*) but also host population dynamics. (ii) They provide biocontrol workers with an unambiguous parameter that is relatively easy to measure in the field [either during cage studies (*17*) or field trials before general release of the agent, or during post-release evaluations] that may assist them in gauging the potential success of an introduction (*18*). (iii) They suggest that despite the inherent complexities in parasitoid-host systems, relatively simple theory and models can capture the main features of the dynamics. (iv) They suggest that refuges from parasitism provide a mechanistic foundation for the scientific basis of biological control.

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# Map-Based Cloning of a Protein Kinase Gene Conferring Disease Resistance in Tomato

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**The *Pto* gene in tomato confers resistance to races of *Pseudomonas syringae* pv. *tomato* that carry the avirulence gene *avrPto*. A yeast artificial chromosome clone that spans the *Pto* region was identified and used to probe a leaf complementary DNA (cDNA) library. A cDNA clone was isolated that represents a gene family, at least six members of which genetically cosegregate with *Pto*. When susceptible tomato plants were transformed with a cDNA from this family, they were resistant to the pathogen. Analysis of the amino acid sequence revealed similarity to serine-threonine protein kinases, suggesting a role for *Pto* in a signal transduction pathway.**

Gene-for-gene interactions, in which plant disease resistance involves a single resistance (*R*) gene in the plant that responds specifically to a single avirulence gene in the pathogen, have been described for numerous plant-pathogen pairs (*1*). Disease susceptibility results if either the plant *R* gene or the pathogen avirulence gene is absent from the interacting organisms. Avirulence genes corresponding to specific *R* genes have been cloned from bacterial and fungal pathogens (*2*). However, a lack of knowledge about the products of *R* genes has made their isolation difficult and has hindered efforts to examine their role in the recognition and defense against specific pathogens.

Tomato, *Lycopersicon esculentum*, offers many advantages for the cloning of *R* genes solely on the basis of their position on a genetic linkage map. Because tomato has been the subject of more than 50 years of plant breeding, over 27 loci have been identified that confer resistance to many agriculturally important fungi, nematodes, viruses, and bacteria (*3*). Many of these loci have been located to small intervals on genetic linkage maps (*4*). A high-density, restriction fragment length polymorphism (RFLP) map (*5*) and a yeast artificial chromosome (YAC) library have been developed for tomato (*6*). These resources, combined with the relatively small genome size of tomato (950 megabases) and low level of repeated sequences, have expedited map-

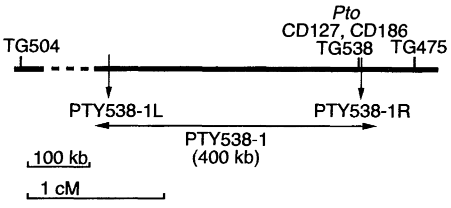

**Fig. 1.** Genetic map of the *Pto* region on chromosome 5 (bold line). The locations are indicated of tomato genomic RFLP markers (TG), the YAC end clones PTY538-1L and PTY538-1R, cDNA clones CD127 and CD186, and *Pto*. The YAC, PTY538-1, that spans this region is shown below. Genetic distance, in centimorgans (cM), is based on linkage analysis of 251 $F_2$ plants (*10*). The size of the YAC in kilobases was determined by pulsed-field gel electrophoresis with yeast strain AB972 as a standard (*31*).

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