

ECOLOGICALLY GUIDED BIOPROSPECTING IN PANAMA

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ABSTRACT

This ICBG program links the discovery of drugs and genes for agriculture with biodiversity conservation. We employ a combination of approaches including technology transfer to Panama, the development of Panama's scientific infrastructure for drug and gene discovery, the training of Panamanian scientists, the involvement of seven Panamanian laboratories in research, and the equitable sharing of revenues among a diversity of Panamanian institutions. In Panama, we will conduct assays to detect activity against cancer, HIV, malaria, Trypanosoma cruzi (the causative agent of Chagas' disease), leishmaniasis and agricultural pests. Monsanto Company will also test samples in their pharmaceutical and agricultural screens, provide NMR spectra, as well as provide training for Panamanian scientists. Any royalties or milestones received from commercialization will be shared among (i) a fund of the Panamanian government that supports the national park system, (ii) a Panamanian foundation funding conservation and sustainable development projects, and (iii) the three collaborating scientific institutions. In collaboration with Conservation International, we will help indigenous communities record their ethnobotanical traditions and provide educational and internship programs for indigenous participants. For collecting biological materials, we employ the use of novel ecological criteria to increase the probability of discovering active compounds. Our data suggest that the

application of ecological principles to the collecting of plants, insects and fungi will provide samples with greater activity in biological assays than samples collected at random. Using ecological insight for collection, promoting infrastructure development, and returning benefits to a broad range of institutions will have maximal impact on conservation both in the short- and long-term.

INTRODUCTION

Over 50% of the most common prescription drugs originate from plants, animals, fungi and bacteria (Grifo et al., 1997), and over 60% of anti-cancer and anti-infective agents developed between 1984–95 are of natural origin (Cragg et al., 1993, 1997). Hence, the drug discovery process continues to rely considerably on screening extracts or compounds from natural sources. Currently, commercial interest in finding novel genes for use in agriculture and in herbal medicinal products is also high. In addition, many, if not most, new drugs from natural products or new genes for agriculture are likely to come from countries that are high in biodiversity. Despite the commercial interest, the biodiversity-rich nations have had limited involvement in such research. In fact, the search for drugs and genes from natural sources holds the potential to enhance the research and commercial capacities of biodiversity-rich countries. Developing nations must obtain experience in the scientific, legal and commercial aspects of bioprospecting before they, along with industry, can effectively exploit biodiversity and thereby obtain benefit. Hence, we argue that involving developing countries directly in the scientific, commercial and legal aspects of natural products research and development will provide substantial

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benefits to all parties: novel medicines and genes for industry and scientific and commercial growth for developing nations.

The search for new drugs and genes also can play a role in conservation. The tropics contain the most diverse habitats in the world, yet are increasingly threatened with destruction. However, this trend might be slowed if the economic value of keeping a forest intact were greater than that of destructive uses, such as logging or agriculture. One promising mechanism for generating sustainable revenue from intact ecosystems is the commercialization of drugs, genes or herbal medicinal products derived from natural sources (Reid et al., 1993; Rosenthal, this volume). However, as was noted, bioprospecting in biodiversity-rich nations involves serious technical, legal, political and logistical difficulties. If bioprospecting is to be embraced by industry and biodiversity-rich nations as a tool for economic development and conservation, it is imperative to produce successful examples quickly (Inamdar et al., 1999).

Our recently-funded ICBG project has several innovative attributes that should help us meet the goals of linking effective drug and gene discovery with conservation and sustainable development in Panama. First, we use extensive ecological research on plant defenses to target collections towards species and tissues that have not been the focus of typical bioprospecting programs, thus improving the possibility of finding novel compounds. Second, considerable emphasis has been placed on training in and technology transfer to Panama. The project assists laboratories in Panama in the establishment of numerous biomedical and agricultural bioassays, and in enhancing their capabilities for the purification and identification of active compounds. Maximizing the degree of host country involvement also increases the number of professionals and students whose careers and studies are linked to the country's biological diversity. Third, the contractual arrangements that we have developed in the context of this project specify that any royalties and milestone payments will be shared among the Panamanian government, a local foundation that promotes conservation and sustainable development, and three Panama-based scientific institutions.

Our ICBG project is administered by the Smithsonian Tropical Research Institute (STRI) which is a branch of the Smithsonian Institution and is located in Panama. STRI has a 75-year tradition of conducting ecological research in Panama where it employs a staff of 34 scientists, and annually hosts over 500 scientists visiting from other countries. Agricultural and medi-

cal bioassays will be conducted in three different laboratories at the University of Panama, the major educational institution in the country. Purification of active compounds will be accomplished at the Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN) at the University of Panama, a laboratory that has conducted natural products research for the last 20 years. Bioassays for tropical diseases will be conducted at the Gorgas Memorial Institute for Health Research (Gorgas Memorial Institute), a branch of Panama's Ministry of Health with a long history of investigation in tropical diseases (Wright, 1970). Plant specimens obtained from bioprospecting efforts and specimens obtained in a beetle biodiversity inventory will be added to the curated collections at the University of Panama. The ethnobotanical component of the project is being managed in collaboration with Conservation International. Our industrial collaborator is Monsanto Company in St. Louis, MO, USA. The project participants are summarized in Table 1. Panama has numerous attributes that make it a logical site to implement a bioprospecting scheme. In particular, Panama is a "biodiversity hotspot", enjoys political and economical stability, has relatively accessible and intact forests, and has a long tradition of research on ecology and organismal biology.

SITE OF ICBG: THE REPUBLIC OF PANAMA

Panama is a biodiversity hotspot. In the most comprehensive analysis of the global distribution of vascular plant diversity, Barthlott et al. (1996) calculated regional diversity on the basis of the number of species per 10,000 square kilometers. In their analysis, the region from eastern Costa Rica, through Panama and including western Colombia, was ranked as having the highest plant biodiversity on the globe. This regional measure of diversity is the most useful spatial scale for evaluating diversity with respect to bioprospecting. Diversity measured at smaller scales, such as the number of species per hectare (local or alpha diversity) cannot be used to calculate diversity in a larger area. For example, Malaysian forests have extremely high local diversity (per hectare), but the same species occur throughout the country, resulting in a relatively low level of regional diversity (Condit et al., 1996). At the other spatial extreme, it is misleading to compare species lists for entire countries if the countries differ in size. The regional biodiversity measured by Barthlott et al. (1996) may prove to be the scale most relevant to

Table 1. Collaborators in the Panama ICBG project.

Associate Program 1: Collections, coordination and database management Associate Program Leaders: Drs. Phyllis D. Coley, Todd L. Capson and Thomas A. Kursar, Smithsonian Tropical Research Institute (STRI) Management and coordination of the Panama-based program Dr. Todd L. Capson ^{1,2} Collection of plants, preparation of extracts and culturing of endophytic fungi Drs. Phyllis D. Coley ^{1,2} , Thomas A. Kursar ^{1,2} and Mireya D. Correa ^{1,3} (STRI, ² U. of Utah and ³ U. of Panama)
Associate Program 2: Panama-based screening, isolation, and characterization of biologically active natural products Associate Program Leader: Dr. Mahabir P. Gupta, Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN), U. of Panama Anti-cancer screens and purification and identification of active compounds Drs. Mahabir P. Gupta and Pablo N. Solís (CIFLORPAN, U. of Panama) Screening of biological materials for activity against HIV Professor Basilio Gomez (Faculty of Medicine, Department of Microbiology, U. of Panama) Screening of biological materials for activity against agricultural pests Drs. Daniel A. Emmen, Cheslavo A. Korytkowski and Dora I. Quiros (Department of Zoology and Entomology Program, U. of Panama)
Associate Program 3: Panama-based screening for tropical diseases Associate Program Leader: Eduardo Ortega-Barría, M.D., Gorgas Memorial Institute for Health Research, Ministry of Health, Republic of Panama Screening of biological materials for activity against Chagas' disease Dr. Eduardo Ortega-Barría (Gorgas Memorial Institute, Panama) Screening of biological materials for activity against leishmaniasis Dr. Luz I. Romero (Gorgas Memorial Institute, Panama) Screening of biological materials for activity against malaria Dr. Phillip J. Rosenthal (U. of California at San Francisco)
Associate Program 4: Biodiversity inventories Associate Program Leader: Dr. Donald M. Windsor, Smithsonian Tropical Research Institute Inventory of herbivorous beetles Drs. Donald M. Windsor (STRI) and Hector Barrios (U. of Panama) Plant collections Professor Mireya D. Correa (STRI and U. of Panama)
Associate Program 5: Monsanto Company. Development of novel pharmaceutical agents and products for agricultural biotechnology Associate Program Leaders: Leslie A. Harrison (Agricultural Sector, Insect Control) and Margaret A. Wideman (Searle, Discovery Research)
Associate Program 6: Ethnobotany and Conservation Associate Program Leaders: Manuel Ramirez (Conservation International) and Dr. Todd L. Capson (STRI)

bioprospecting. The extraordinary regional diversity in Panama results from an unusual mosaic of habitat types and a blend of species from both Central and South America (D'Arcy, 1987; Condit et al., 1996). The high regional plant diversity in Panama greatly facilitates collections, as new species are encountered across short distances.

Panama's unique geographic position and high biodiversity make it a critical area for both regional and global conservation planning. These forests have served as important migratory corridors between Central and South America for the last 3.5 million years (Coates & Obando, 1996). The role of Panama as a biological corridor is particularly vulnerable because the isthmus is only 100 km wide. Thus, habitat destruction can easily disrupt this important link between the hemispheres. Under conditions of climate change, it will become important for species to migrate in response to adverse conditions. Hence, maintaining the Panamanian corridor will be vital for the long-term persistence of many tropical and temperate species. In recognition of the key role of this biological corridor, seven Central

American countries recently pledged to help preserve this forested connection, termed the Mesoamerican Biological Corridor (Illueca, 1997). Despite their importance, Panama's forests are under immediate and substantial threat (Gutiérrez, 1992).

COORDINATION OF THE ICBG THROUGH STRI

As described below, the vast majority of work associated with this ICBG is carried out in Panama, including preparation of extracts from biological materials, distribution and screening of extracts, purification of compounds with medicinal activities, an insect biodiversity inventory, training of students in all of these areas, and preservation of ethnobotanical traditions. The infrastructure and facilities at STRI have made an ICBG with such extensive Panamanian involvement possible. Efforts among collaborators in the US and Panama are closely coordinated from Panama. Due to the complexity of this project, we found it essential for a Ph.D.-level scientist to work full time in Panama on the design

of this program, the development of the accompanying legal arrangements, and the implementation of the ICBG program. Since June of 1997, Dr. Capson, an organic chemist, has worked at STRI facilities in Panama City and played this critical role.

ENHANCING THE POSSIBILITY OF FINDING NOVEL PHARMACEUTICAL AND AGRICULTURAL PRODUCTS

Using ecological insight to guide plant collections targets tissues not typically used in bioprospecting. Tropical rainforest plants have both higher concentrations and a greater diversity of chemical defenses than plants from any other biome (Coley & Barone, 1996) and are a potential source of new medicines (Balick et al., 1996). However, despite the historical importance of plants in drug discovery (Cragg & Boyd, 1996), there are relatively few recent successes. We suggest that "hit" rates may be low because samples are generally collected at random, and extracts are made from dried plant samples. In order to increase hit rates, we have invested considerable thought and experimentation in the design of an ecologically-guided collection scheme for plants, endophytic fungi and beetles. The collections are complemented by an extraction protocol in which we use exclusively fresh materials.

In this section we provide evidence for the rationale behind our collection scheme for plants. We have spent over two decades evaluating field damage on different plant species in tropical forests and more recently have run laboratory trials for toxicity against insects, fungi, bacteria and cancer cells. Here, we report results that are pertinent to bioprospecting. Specifically we show that a) young leaves contain greater concentrations of secondary metabolites than mature leaves, b) young leaves have greater activity in biological assays than mature leaves, c) many biologically active compounds are unique to young leaves, and d) biological activity is higher in extracts from fresh as compared to dried leaves.

Chemical defenses are higher in young leaves. In conventional bioprospecting programs, collectors frequently maximize the number of samples through random collections, using readily accessible mature leaves. However, mature tropical leaves are defended against pathogens and herbivores primarily by tannins and toughness (lignin and cellulose) (Coley, 1983; Coley & Aide, 1991; Coley & Barone, 1996), two ecologically effective defenses which hold little promise of

therapeutic or agricultural potential. In contrast, young leaves rely on an enormous diversity of chemical defenses (reviewed in Coley & Kursar, 1996). Young leaves are particularly vulnerable to herbivores because of an inevitable constraint of development: young leaves cannot toughen and require high levels of protein while they are growing. Thus, for young leaves in the tropics, secondary metabolites are the most effective option for defense. Concentrations of mono-, sesqui- and diterpenes are significantly higher in young as compared to mature leaves (Crankshaw & Langenheim, 1981; Langenheim et al., 1986). This pattern is in marked contrast to the temperate zone where young leaves are typically not as well defended chemically as mature leaves (Coley & Aide, 1991).

Our results for alkaloids also show higher concentrations in young leaves. The first five authors, in collaboration with Dr. Solís of the University of Panama, selectively extracted leaves for alkaloids from 18 species, and analyzed these using TLC and HPLC. TLC analyses of the alkaloidal extracts showed that alkaloids generally had higher concentrations in young leaves. HPLC analyses of the extracts showed that for compounds found in both young and mature leaves, peaks in young leaves were twice as high as the same peaks in mature leaves (paired *t*-test, $p < 0.01$).

Biological activity is higher in young leaves. To evaluate our prediction derived from ecological research that young leaves have more medicinally useful activities, we screened the extracts of a random collection of young leaves using an anticancer assay with a human colon tumor cell line (HCT-116). The National Cancer Institute considers crude extracts "active" or "extremely potent" when they inhibit cell growth at concentrations of 20 and 5 $\mu\text{g ml}^{-1}$, respectively (Cordell et al., 1993). By these criteria, 4.3% of the National Cancer Institute's samples are "active" (Suffness & Douros, 1982). Of the 109 species tested as young leaves, 41% were "active" and 8% were "extremely potent" (Fig. 1), suggesting that young tropical leaves may be an excellent source of biologically active compounds.

Very few studies, none in rainforest plants, have directly compared young and mature leaves to determine if they have different biological activities. To test this, we examined extracts from over 100 woody species from Panama for activity in two non-biomedical assays, *Bacillus subtilis* and *Artemia salina* (brine shrimp) (Solís et al., 1993). Activity against *B. subtilis* was measured as the width of the zone of growth inhibition around a disk containing crude

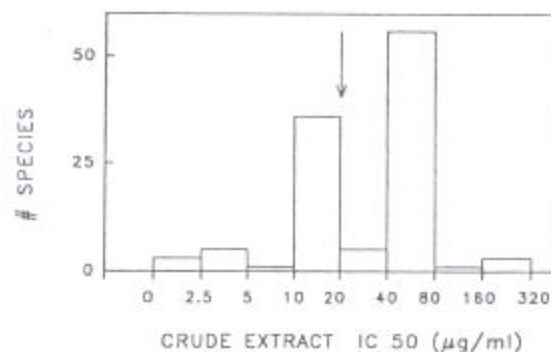


Fig. 1. Inhibition of the human tumor cell line HCT-116 by crude extracts of young leaves from 109 species of Panamanian rainforest plants. Leaves were freeze-dried and frozen. Extracts from 45 of the species showed significant activities at a concentration (IC_{50}) of less than $20 \mu\text{g ml}^{-1}$ (indicated by arrow).

extracts of fresh leaves. Inhibition zones averaged almost twice as large for young as compared to mature leaves (paired *t*-test, $p < 0.0001$, $n = 114$ species). In addition, 40% of the young leaves showed activity as compared to only 23% of the mature leaves. Results were similar for the amount of extract required to kill 50% of the *A. salina*; extracts from young fresh leaves were significantly more potent than those from mature leaves (paired *t*-test, $p < 0.0001$, $n = 108$ species).

Young leaves contain distinct secondary metabolites. In our alkaloid survey, we found that young leaves contained alkaloids not found in mature leaves of the same species. In addition to distinct alkaloids, young leaves also contain unique, abundant proteins (Kursar & Coley, 1992). We carried out a gel electrophoresis analysis of protein accumulation of five rainforest species, focusing on abundant proteins not likely to be associated with photosynthesis or primary metabolism. We found 12 such proteins that were abundant in young and absent from mature leaves (Table 2). Based upon analogy with secondary metabolites, these proteins may have defensive functions. Defensive secondary metabolites accumulate to high levels, differ in identity among species, and are developmentally regulated. Since the proteins in Table 2 manifest all of these properties, we hypothesize that they serve as plant defenses. In fact, plants are sources of many classes of defense proteins, such as trypsin inhibitors, ribosome-inhibiting proteins, etc. (Mehta & Boston 1998; Koiwa et al., 1997). Protein-based defense genes are of enormous interest in agricultural biotechnology (Xu et al., 1996; Jansens et al., 1997; Greenplate et al., 1995), and our preliminary data suggest that young leaves are more likely to produce leads for novel defense genes. Thus, a major goal of this ICBG project is to discover defense proteins that can be used in plant transformation.

In summary, we have found that many alkaloids and proteins are only present in the young leaves and we argue that this pattern may hold for most secondary metabolites. Therefore, because a higher concentration and diversity of secondary metabolites occur in young leaves, novel, bioactive compounds may be more abundant in young leaves. It should be pointed out that mature leaves do contain unique compounds, though not as many, so collection schemes might do well to include both age classes. However, by focusing collections only on mature leaves, we suggest that much of the chemical diversity of tropical forests may be missed.

Biological activity is higher in extracts from fresh as compared to dried leaves. A large body of evidence from traditional healers and scientists suggests that extraction of fresh samples yields greater activity, presumably because drying causes loss of active compounds (Schultes & Raffauf, 1990). To test the hypothesis that fresh samples have higher biological activity, we compared leaf samples that had been either extracted immediately or first subject to a mild drying treatment (4 days in the shade at ambient temperature). This comparison was made for both young and mature leaf samples from over 100 Panamanian species in assays with *A. salina* and *B. subtilis*. On average, extracts of fresh leaves had higher activities, with the difference being especially pronounced for young leaves (Fig. 2). Not only do these results suggest fresh extracts have more biological activity in some cases, but they also suggest that the active compounds in young leaves may be most sensitive to drying.

The comparison of particular interest is between our approach with fresh young leaves and more conventional approaches with dried mature leaves (Fig. 2). Our approach yielded significantly greater activity in the *A. salina* bioassay and 2.7-times greater activity in

Table 2. Proteins in young leaves. Proteins that are abundant only in young leaves, either early or late during leaf expansion. Both soluble and membrane proteins were extracted from leaves and analyzed by polyacrylamide gel electrophoresis in the presence of lauryl sulfate. Molecular weights (in kDa) are indicated for the proteins that were abundant in young leaves but not in mature leaves. Data from Kursar & Coley, 1992.

Species	Time of appearance during leaf expansion	
	Early	Late
<i>Ouratea lucens</i>	15.7, 17.0, 30.5, 31.5	—
Ochnaceae ((H.B.K.) Engler in Mart.)		
<i>Conarus panamensis</i>	—	14.6
Connaraceae (Griseb.)		
<i>Xylopa macrantha</i>	—	17.2
Annonaceae (Tr. & Planch)		
<i>Desmopsis panamensis</i>	10.5, 10.8, 15.0, 52.3	20.6
Annonaceae ((Rob.) Saff.)	—	—
<i>Annona spraguei</i> Annonaceae (Saff.)	39.0	—

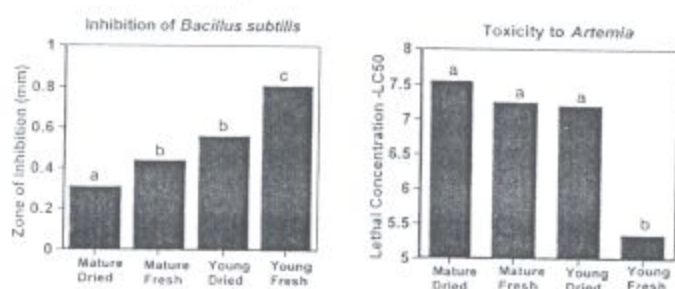


Fig. 2. Left panel: Inhibition of *Bacillus subtilis* by extracts from fresh and air-dried samples of young and mature leaves. Values with different letters are significantly different at $p < 0.01$ by both ANOVA and paired t -tests. Right panel: LC_{50} values for *Artemia salina* with extracts from fresh and air-dried samples of young and mature leaves. Values with different letters are significantly different at $p < 0.01$ by both ANOVA and paired t -tests.

the *B. subtilis* bioassay. Therefore, by combining our ecological insight with rapid extraction of fresh leaves, we are substantially increasing our ability to find active compounds.

There are two additional advantages to the use of fresh leaves. First, we can more effectively extract proteins from fresh, as compared to air-dried leaves. Second, collection of fresh leaves allows isolation of endophytic fungi from inside the leaves (see following section).

Beetles and endophytic fungi. We will also use ecological insight to guide collections of two other groups of organisms, herbivorous beetles and endophytic fungi. Both groups are extraordinarily diverse and both have complex, chemically mediated interactions with their hosts and predators. Herbivorous beetles are frequently well protected by chemicals against their predators (Pasteels et al., 1988; Windsor et al., 1992). Ancestral species tend to synthesize their own, and recently evolved species tend to sequester plant toxins. Knowledge of the anti-predator behavior and host-plant use of beetles can suggest both beetle and plant species that may contain useful compounds. Thus, decades of

ecological research on beetles that have chemically mediated interactions may indicate previously unexplored sources of biologically active chemicals.

The other group to be collected for screening in bioassays is endophytic fungi that live asymptotically inside of leaves (Carroll, 1988). Endophytes are orders of magnitude more abundant in the tropics than in the temperate zone, and tens of species can co-occur in a single leaf (Lodge et al., 1996; Bayman et al., 1997) and may in fact be hyperdiverse (Arnold et al., 2000). Endophytes synthesize biologically active compounds that protect the leaf from herbivores and pathogens and that may also regulate competition among endophytes within the leaf (Clay, 1990; 1992; Powell & Petrowski, 1992; Petrini et al., 1992; Breen, 1994). For example, taxol is a medically important compound that may be produced by a number of species of fungal endophytes (Stierle et al., 1993).

Tropical endophytes may be a particularly promising group to screen for biological activity because they are more diverse and less studied than temperate species. Even in the relatively species-poor temperate grasslands, endophytes have evolved an impressive

chemical arsenal that is effective against herbivores and perhaps against other fungal competitors (Clay, 1990). Given that species interactions are more intense in the tropics, one might predict greater selection for active secondary metabolites in tropical endophytes, a pattern similar to that seen for higher plant chemical defenses (Coley & Barone, 1996). In addition, since endophytic fungi are derived from a number of unrelated ancestral taxa (Verhoeff, 1974; Petrini, 1986), a diversity of chemical classes should be expressed within the group. Thus, we suggest that the enormous unexplored diversity of tropical endophytes may provide many novel leads. Cultures of endophytic fungi will be classified based upon morphology and color. Preliminary DNA-based analyses of the relationships among fungal isolates suggest that such classifications correspond well with species (G. Gilbert, personal communication).

MAXIMIZING OPPORTUNITIES FOR HOST COUNTRY INVOLVEMENT IN BIOPROSPECTING RESEARCH

Promoting conservation by providing short- and intermediate-term benefits. In the previous section, we suggested that the extraordinary biodiversity in Panama, as well as our innovative collecting and extraction program, would increase the probability of finding promising leads. Perhaps the most difficult challenge is effectively linking successful drug discovery with sustainable development and conservation. Increasingly, it is recognized that royalties alone will not provide sufficient incentive for preserving biodiversity because the probability of receiving royalties is too low (1 in 100,000 extracts for random screens) (Artuso, 1997; Miller & Brewer, 1992), and the time-scale is too long (approximately 10–20 years). Instead, significant efforts must be made to provide short- and intermediate-term benefits (King & Carlson, 1995; Iwu & Laird, 1998; Capson, 1998).

Arguably, immediate benefits to the host country can be provided most effectively through substantial technology transfer, training and capacity building. To that end, much of the screening of biological materials for biomedical and agricultural properties, as well the purification of active compounds, will occur in Panama. These steps can increase the value of the biological extract, as they add information important to the drug discovery process (Artuso, 1997). For example, a compound with demonstrated activity against clinically and economically important diseases will be worth

orders-of-magnitude more than a crude extract (Artuso, 1997).

In the following sections we outline various mechanisms that should contribute to a sustainable bioprospecting program in Panama. We emphasize the involvement of numerous institutions, training and technology transfer, inclusion of screens applicable to diseases and agricultural pests that are important in both temperate and tropical environments, preservation of ethnobotanical knowledge, and the need for frequent and transparent communication with the government and the public.

Collaborating with laboratories in Panama. A major goal of this ICBG is to maximize the benefits of bioprospecting research for the scientific community in Panama. Accordingly, we are working in a number of laboratories in two Panamanian institutions, as opposed to developing or supporting a single institute or facility. Benefits available to collaborators include technology transfer, training and internship opportunities, equipment purchases and improvement of research facilities. These efforts to assist infrastructure development in Panama are complemented by training opportunities for university-level students and post-doctoral scientists. Exposure to research in an active laboratory provides a valuable training experience. All of the scientific laboratories collaborating in this ICBG project are involved in training university students. We are collaborating with five research groups at the University of Panama (including the National Herbarium) and two laboratories at the Gorgas Memorial Institute (Table 1). In this ICBG program, 14 Panamanian scientists and 13 student assistants are involved. Meetings within the laboratory groups as well as regular meetings between all the laboratories will contribute to the learning opportunities.

The choice of biomedical and agricultural screens was based upon the research interests of the participating Panamanian scientists. All of the disease targets selected are of global importance and in need of innovative treatments. All of these assays are whole cell assays, which complement the highly specific receptor- and enzyme-based assays typically used by pharmaceutical companies. Additionally, our collaborators will screen for activity against agricultural pests that are important in Panama as well as worldwide.

The University of Panama. In the departments of Pharmacy and Microbiology, plant extracts are being screened for activity against cancer and HIV, respectively. The anticancer screens use three cancer cell lines, NCI-H460 (lung), SF-268 (central nervous sys-

tem) and MCF-7 (breast), that were selected in collaboration with the National Cancer Institute, and are established in the laboratories of Dr. Gupta. Extracts are assayed for activity against HIV in the Department of Microbiology at the School of Medicine in collaboration with Professor Gomez. The HIV assay was established in collaboration with the National Cancer Institute's AIDS Drug Screening and Development Laboratory, and employs a non-infective strain of the HIV virus, Dtat/RevMC99, which can be used in standard laboratory facilities (Kiser et al., 1996).

Gorgas Memorial Institute for Health Research. Scientists at the Gorgas Memorial Institute will screen extracts for activity against three tropical diseases. In Dr. Ortega-Barría's laboratory, extracts are tested for activity against *Trypanosoma cruzi*, the causative agent of Chagas' disease. In the laboratories of Dr. Romero, extracts are tested for activity against leishmaniasis.

Extracts also will be screened for activity against tuberculosis and malaria, two diseases for which laboratories in Panama do not currently run bioassays. Tuberculosis screens will be conducted in collaboration with Dr. Franzblau at the GWL Hansen Disease Center (Baton Rouge, USA) while testing for activity against malaria will be conducted at the Walter Reed Army Institute of Research. In the future, these latter two assays may be established in Panama.

Purification of compounds. Crude extracts that show activity in the biological assays mentioned above will be fractionated and the actives identified by Drs. Gupta and Solís in the CIFLORPAN laboratory at the University of Panama. Initially, extracts that show activity in the screens at Gorgas Memorial Institute also will be purified at the University of Panama, although Gorgas Memorial Institute intends to develop facilities to purify compounds active against tropical diseases. As Panama does not yet have an NMR or mass spectrometry facility, Ms. Wideman at Monsanto (Searle) has offered to provide spectra on a routine basis and to assist with dereplication and the identification of active compounds. In addition, Monsanto Company will annually host senior scientists from Panama, providing them an opportunity to use Monsanto's facilities and expertise in natural products chemistry.

Screening plants for activity against agricultural pests. In a collaborative effort between STRI, Monsanto Company, and the University of Panama, plant extracts will be screened for activity against the agricultural pest, whitefly (*Bemisia tabaci*). Agricultural pests cause the loss of about one third of all crops in the US and even more worldwide (Pimentel et al.,

1975; Oerke & Dehne, 1997). Recent experience demonstrates that chemically based anti-pest technologies are not adequate to lower crop loss rates and can cause serious human health problems (Pimentel & Greiner, 1997). To confront these issues, Monsanto and other companies are currently investing considerable resources to identify protein-based defenses that, following transformation into crop plants, will allow plants to protect themselves. Harrison of Monsanto Company will help establish a whitefly assay in Panama that will be run by Drs. Emmen, Quiros, and Korytkowski in the Entomology Program at the University of Panama.

Herbarium support. Professor Correa, a botanist, director of the University of Panama Herbarium and STRI staff scientist, has worked for 35 years on Panamanian flora. Professor Correa will confirm our identifications for the plants used in the bioprospecting and ethnobotany programs. In return, the ICBG program provides support for her collecting and curating program.

Insect biodiversity. Our ICBG includes an inventory of leaf-eating beetles, a better understanding of which will accomplish several goals that are consistent with this program. First, this survey will provide valuable information on the distribution of species of herbivorous beetles, including identification of habitats of endemism, high diversity and other priority areas for conservation. Second, information on host-plant associations and the structure of herbivorous insect assemblages is essential for basic ecological studies. Third, this program will create training and research opportunities for Panamanian systematists, as well as professional exposure. Fourth, as the survey will be repeated annually, trends associated with habitat modification can be monitored. Finally, because herbivorous beetles are often associated with toxic plants, this inventory will contribute both beetles and plants for use in screens.

Beetles are a logical choice for a biodiversity inventory program. The number of beetle species described world wide (>300,000) exceeds that of any other animal taxon, and the majority are leaf feeders (Mitter et al., 1991; Farrell et al., 1992; Futuyma & McCafferty, 1990). An inventory of leaf-feeding beetles (in the super-families Curculionioidea, Cerambycoidea and Chrysomeloidea) would thus present an important contribution to our understanding of Panamanian biodiversity (Windsor et al., 1992). This inventory will be carried out by Drs. Windsor (STRI) and Barrios (University of Panama) and student assistants. They will survey insects at 18 sites representing a diversity of habitats across Panama, from wet to dry, and from sea

level to 2000 m mountain tops. Collections will be housed at the University of Panama.

WORKING WITH PANAMA'S INDIGENOUS COMMUNITIES

According to the census of 1990, Panama, with a total of 2.4 million people, has an indigenous population of 195,000 inhabitants comprised of seven distinct groups (Ventocilla et al., 1995). Panama's indigenous cultures have a profound understanding of their physical world, reflected in their culture, knowledge and management of resources. Presently some indigenous groups live in extreme poverty within ecosystems that are still largely intact: the fragile ecosystems along the Caribbean coast, where the Naso, Ngöbe, and Kuna are found, serve as examples (World Bank, 1997). All three groups inhabit ecosystems of "high priority" for biodiversity conservation (World Bank, 1997), the fate of which is intimately linked to the well-being of the indigenous inhabitants (Conservation International & General Secretariat of the Organization of American States, 1994). Thus, any conservation strategy for this critical biological corridor must include participation by the indigenous communities that live there. The two basic components of our strategy for working with Panamanian indigenous communities are described below.

Recording ethnobotanical traditions. First, we work with communities to record ethnobotanical traditions. These traditions are still alive, but most of them have not been recorded. Unfortunately, in the communities in which we work most of the individuals that have an extensive knowledge of traditional medicine are elderly. The death of such individuals represents a great loss of specialized knowledge to those communities. By recording these traditions, local residents benefit by having a permanent record for present and future generations. This ICBG's ethnobotany program is directed by Manuel Ramirez of Conservation International and Dr. Capson, and involves the Ngöbe and Naso indigenous groups. Conservation International has worked with the Naso since 1992 on a number of initiatives, including ethnobotany to ecotourism. These programs with the Naso provide an ideal opportunity for the ICBG to contribute to ongoing projects and to initiate new projects. The ICBG program will pay for young men and women in these communities to study with their traditional healers such that this information will be passed on to the younger generations. A permanent written record of traditional plant-based medicines will

be produced along with collections of botanical vouchers. We will also provide the necessary equipment and logistical resources to support the project. The ethnobotany project will sponsor workshops and exchanges among the indigenous communities in Panama. For example, we will support visits by representatives of the Ngöbe indigenous group to the Naso so they can observe first-hand the program for assisting the Naso to record their ethnobotanical traditions. It is our hope that such indigenous exchange programs will ultimately promote cooperation and collaboration among Panamanian indigenous communities on a range of issues.

The ethnobotanical records that are produced in this work are not intended for use in bioprospecting, but will belong to the indigenous communities who will decide upon their use within and outside their communities. Should any of the indigenous groups we work with indicate at some future date a desire to enter into a bioprospecting collaboration with us, we will discuss the possibilities. Any such partnership would require appropriate legal agreements assuring recognition of intellectual property and equitable sharing of benefits.

While we hope to ensure that traditional medicine will continue to play an important role to Panama's indigenous cultures, modern medicine complements traditional health care systems. Accordingly, Dr. Ortega-Barría from the Gorgas Memorial Institute, who has an M.D. and training in pediatric infectious disease and parasitology, has agreed to visit the communities with which we are working, and to provide health care and supplies when appropriate.

Indigenous internship program. Another component of our work with indigenous communities aims to broaden the scope of involvement of Panama's indigenous groups in biodiversity conservation in Panama. The project will provide the opportunity for Ngöbe and Naso representatives to participate in internship programs at Conservation International in Washington, D.C. Conservation International's involvement in a large range of projects that promote biodiversity conservation and sustainable development worldwide will provide many opportunities for an indigenous intern to participate and learn; subjects of potential study include intellectual property, land-use planning, and the sustainable marketing of rainforest products.

BENEFIT SHARING AND CONSERVATION

As pointed out in previous sections, this ICBG places great emphasis on immediate capacity building in

Panama. These efforts, including training, technology transfer, and enhancing research in seven laboratories at two Panamanian institutions, are all aimed at establishing a self-sustaining bioprospecting program in Panama that will continue to generate benefits in the short- and long-term. Any milestones and royalties that result from commercialization of products from bioprospecting would provide additional incentives for biodiversity conservation (Reid et al., 1993). To that end, we have developed a series of comprehensive and equitable contractual arrangements. Dr. Capson has been the primary architect of the contractual arrangements for this project, and has worked closely with a number of individuals, in particular Dr. Kursar, and also with the attorneys, Ms. Marianne Guerin-McManus of Conservation International and Mr. Michael Gollin of the law firm Venable, Baetjer, Howard & Civiletti. Throughout the development of these contracts, we also consulted closely with the previous director of the Autoridad Nacional del Ambiente (ANAM), Ms. Mirei Endara, as well as colleagues from the Panamanian institutions with which we are collaborating. To adequately address the needs of each particular institution and to facilitate the drafting of the legal arrangements, we have developed a series of two-party contracts between STRI and each institution (ANAM, University of Panama, Gorgas Memorial Institute, Fundación Natura, Monsanto Company).

These contractual arrangements cover nearly every component of the work in Panama. They are explicitly designed for an equitable bioprospecting program that has a large component of host country involvement and explicit links to conservation. Contractual issues such as the obligations of the institutions, joint ownership of inventions, management of intellectual property, and division of royalties and milestone payments are carefully and clearly addressed, in addition to more standard contractual provisions. We have employed a "club" model for the division of royalties between STRI and its Panamanian collaborators in which each collaborating scientific institution and STRI will receive an equal fraction of royalties, irrespective of their relative contribution to the development of an invention. This arrangement, as opposed to one in which the fraction of royalties is proportional to inventorship or ownership, is intended to foster collaboration among institutions and laboratories. In the event that future bioprospecting activities include collaborations with indigenous communities, the community also would participate as a member of the "club". We are currently preparing for publication of redacted versions

of the contracts. These may be useful for other bioprospecting programs that have a large degree of participation of host country institutions, and that seek to promote biodiversity conservation.

An important provision in the contract with the government of Panama provides that an environmental trust fund will receive a share of all revenues generated from the bioprospecting activities. The trust fund was set up in collaboration with a Panamanian foundation, Fundación Natura, that promotes a broad range of conservation and sustainable development initiatives throughout Panama. Fundación Natura dispenses funds to non-governmental or community organizations through a competitive grants program. Proposals are reviewed by highly qualified local scientists and environmentalists. This encourages grass-roots participation, a diversity of approaches, and evaluation of projects based on merit. We anticipate that this will productively address a variety of conservation issues. Our collaboration with Fundación Natura is comparable to the approach we have used in working with academic collaborators in Panama: working with and strengthening local institutions that have demonstrable productivity but that can nonetheless benefit from receiving additional support.

Lastly, one portion of the revenue from royalties and milestones goes to the Fondo Nacional de la Vida Sylvestre, a branch of ANAM that is directly involved in protection of National Parks. Our collections will all be made in protected areas, so it is particularly fitting that revenues will help to protect these areas.

SUMMARY

While extensive areas of Panama are still forested, these forests are under immediate and substantial threat. This is therefore a particularly critical time when effective planning can still make a difference to conservation. A central goal of this ICBG project is to contribute to conservation and sustainable use of biodiversity in Panama, and to serve as a model for other biodiversity-rich nations. A cornerstone of our approach is the emphasis on capacity building through training and technology transfer. The majority of all royalties and milestones will go to Panama. These funds will be divided among a diversity of institutions, each with a different agenda, but all contributing to the development of a sustainable use of biodiversity.

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