Frontiers in Ecology and the Environment

Ecological and evolutionary bioprospecting: using aposematic insects as guides to rainforest plants active against disease

Julie E Helson, Todd L Capson, Timothy Johns, Annette Aiello, and Donald M Windsor

Front Ecol Environ **2009; 7, doi:10.1890/070189**

Ecological and evolutionary bioprospecting: using aposematic insects as guides to rainforest plants active against disease

Julie E Helson[1,2], Todd L Capson[1,3*], Timothy Johns[1], Annette Aiello[3], and Donald M Windsor[3]

We examined Coleoptera and Lepidoptera assemblages feeding on two different groups of plants: one in which plants were active against cancer cell lines and/or protozoan parasites responsible for tropical parasitic diseases, and a second group that was inactive in the same bioassays. Aposematic species were found on nine of the ten active plant species, but on only four of the ten inactive plant species. Non-aposematic insects did not show a significant difference in their association with active versus inactive plants. Our results suggest that the presence of aposematic, herbivorous insects can be used to facilitate the identification of plants with compounds active against important human diseases.

Front Ecol Environ 2009; 7, doi:10.1890/070189

Natural products derived from plants, marine organisms, and microbes continue to play an important role in drug discovery for a broad range of diseases (Koehn and Carter 2005). In the case of plants, a variety of strategies have been employed to select candidates for drug discovery. A random screening of extracts from approximately 12 000 plant species by the US National Cancer Institute yielded a number of active chemotypes, two of which advanced to commercial products for the treatment of cancer (Cragg and Newman 2005). Ethnobotanically guided collections are responsible for nearly three-quarters of the 119 plant-derived drugs in current use, but the use of traditional knowledge to guide plant collections has proven highly controversial due to the difficulty of linking traditional knowledge with intellectual property ownership and benefit sharing (Cragg and Newman 2005; Rosendal 2006).

While the field is still in its infancy, ecological and evolutionary theories based on the chemical defense patterns of rainforest plants have been used to direct searches for active plant compounds by the Panama International Cooperative Biodiversity Groups Program (ICBG), a program involved in drug discovery for cancer and tropical disease, scientific capacity building, and biodiversity conservation (Coley *et al*. 2003; Kursar *et al*. 2006; Capson in press). Young leaves have been preferentially collected and tested in the drug discovery bioassays of the Panama ICBG program, because young leaves typically have higher concentrations and a greater diversity of chemical defense compounds than do mature leaves. This strategy has proven successful for discovering novel compounds with activity against tumor cell lines (Hussein *et al*. 2005), and protozoan agents of cutaneous leishmaniasis (*Leishmania mexicana* and *Leishmania panamensis*) and Chagas' disease (*Trypanosoma cruzi*; Jiménez-Romero *et al*. 2007). To broaden the collecting strategies based on ecological and evolutionary criteria used by the Panama ICBG program, and to provide additional insight into the nature of plant–insect chemical ecology, we examined associations between tropical insect herbivores and their host plants.

It has been proposed that the co-evolution or "arms race" between insect herbivores and plants has encouraged the development of defense mechanisms (eg plant secondary metabolites) and fostered the diversification of both groups (Ehrlich and Raven 1964; Kareiva 1999; Thompson 1999; Rausher 2001). Similar interactions between insects and their predators may also have led to the development of conspicuous aposematic ("warning") coloration in insects, to advertise their distastefulness or toxicity to visually oriented predators (Guilford 1988; Ruxton *et al*. 2007). Aposematic coloration typically consists of bright and contrasting colors, such as reds, oranges, yellows, blues, and purples, alternating with black, dark brown, or dark gray (Salazar and Whitman 2001). Moreover, aposematic insects often feed on toxic host plants that possess potent plant secondary compounds, which insects can use for defense against predators (Prieto *et al*. 2007). Some of these plant secondary compounds also have medicinal properties (Nishida 2002). However, because many edible insects mimic aposematically colored, distasteful species (Batesian mimicry; Brower *et al*. 1967), we cannot expect all aposematically colored, herbivorous species to be reliable indicators of plants with high levels of secondary compounds. As the presence of Batesian mimics in insect populations may obscure possibly useful linkages between aposematic herbivores and toxic host plants, our study investigates

[1]*Department of Plant Science, McGill University, Ste Anne de Bellevue, Quebec, Canada;* [2]*current affiliation: University of Toronto at Scarborough, Scarborough, Ontario, Canada;* [3]*Smithsonian Tropical Research Institute, Balboa, Ancón, Panama, Republic of Panama* [*]*(capsont@si.edu)*

whether there is a net positive benefit to using the presence of aposematic insects to guide the search for plants with biologically active secondary compounds.

We test the hypothesis that plants with compounds that have activity against cancer cell lines and/or tropical disease-causing parasites are more likely to have feeding associations with aposematic insects than are inactive plants. To test this hypothesis, we selected ten plant species that demonstrated activity in one or more bioassays against protozoan agents responsible for cutaneous leishmaniasis (*L mexicana* or *L panamensis*), Chagas' disease (*T cruzi*), and malaria (*Plasmodium falciparum*), or breast, lung, and central nervous system cancer cell lines, and ten plant species that showed no activity in the same bioassays. Each of the species was then surveyed for its associated coleopteran and lepidopteran herbivore assemblages in four Panamanian Protected Areas.

Methods

Criteria for the choice of plant study species

Plant species included in this study were chosen from among 1380 species tested by the Panama ICBG program in bioassays for activity against breast, lung, and central nervous system cancer cell lines, and the parasites *P falciparum*, *T cruzi*, and *L mexicana* or *L panamensis*, from January 1999 through January 2004. Plant species were chosen from six plant families (Asteraceae, Boraginaceae, Bignoniaceae, Convolvulaceae, Rubiaceae, and Solanaceae) on the basis of: (1) consistent activity or inactivity in the bioassays, (2) accessibility in the field, and (3) abundance in the field. Plants were deemed "active" if they displayed activity in one or more of the bioassays (Table 1). Plants categorized as "inactive" yielded extracts that showed no activity in the bioassays. Plant extracts were prepared using the protocol described by Montenegro *et al.* (2003).

Active and inactive plant species were paired by family whenever possible, in order to optimize the similarity of general characteristics within the plant pairs (Table 1). Phylogenetic relationships were used to pair those active and inactive plants in cases where there was more than one potential plant pair per plant family. Phylogenies for Asteraceae were obtained from Bremer (1994), Solanaceae from Hunziker (1979), and Rubiaceae from Pereira and Barbosa (2004) and Andreasen and Bremer (2000). Rafael Aizprúa of the Smithsonian Tropical Research Institute (STRI) collected and subsequently confirmed plant species identities. Voucher specimens of each plant species studied have been deposited in the herbaria of the University of Panama and STRI (WebTable 1).

Monitoring and collection of insects

Research was carried out in Panamanian Protected Areas, two of which are located in areas of lowland moist tropical forest (Soberania National Park [9°7' N, 79°42' W, 60 m above sea level (asl)] and Barro Colorado Nature Monument [9°9' N, 79°51' W, 80 m asl], and two of which are located in mountainous areas of humid tropical forest (Chagres National Park [9°13' N, 79°22' W, 950 m asl] and Altos de Campana National Park [8°13' N, 79°22' W, 800 m asl]).

Sampling time – the time actively spent searching a plant for herbivores – was equalized between active and inactive plants to the greatest practical degree. Individuals of active and inactive plant species were searched for approximately 210 and 190 minutes, respectively, during each census. Time was used as a measure of sampling effort, because the study plants were of diverse types (including herbs, lianas, shrubs, and trees) and because the abundance of the study species varied greatly, both within and among study sites. The minimum number of individuals of each species searched during each census is given in Table 1. Each plant included in the study was marked and observed a total of 16 times, at weekly or biweekly intervals, between May 2004 and November 2004, during the Panamanian rainy season (typically April through December), which is when insects are most abundant (Windsor *et al.* 1992).

Monitoring for insect herbivores consisted of thoroughly checking all leaf surfaces of the plant, paying particular attention to whether insects were actually feeding. When necessary, a hooked pole was used to carefully bring branches within reach. All adult and larval Coleoptera and larval Lepidoptera found on study plants were brought to the laboratory. Each insect was placed in a container with an uneaten leaf of the plant species upon which it was found, in order to test whether it had been eating the plant or had simply been resting on it. Only insect species that ate the plant sample in the laboratory were included in the analyses.

Larvae of Coleoptera and Lepidoptera found on study plants were reared to the adult stage, mounted, and identified. All adult Coleoptera found on the study plants were also mounted and identified. Voucher specimens of all collected insect species have been deposited in the Fairchild Museum of Invertebrates, University of Panama, and the STRI Synoptic Insect Collection.

Identification of insects

AA identified the Lepidoptera, and H Stockwell and DMW identified the Coleoptera. In a few cases, insect specimens were identified from photographs sent to experts in a particular field. Insects were considered aposematic if their integuments were colored, visibly to the human eye, wholly or in part, in red, orange, yellow, blue, or purple, with or without black, dark gray, or dark brown bands or other markings (Salazar and Whitman 2001). In contrast, insects were considered non-aposematic if they were green, black, brown, or gray, or used "imitation" for defense (eg

Table 1. Pairs of biologically active and biologically inactive plants and the minimum number of individuals of each plant species searched for insects during each census

Biologically active (type of bioactivity in the Panama International Cooperative Biodiversity Group screens)	*Approximate number of plants examined*	*Biologically inactive*	*Approximate number of plants examined*
Tithonia diversifolia (Asteraceae) (cancer, *P falciparum,T cruzi*)	20	*Tilesia baccata* (Asteraceae)	15
Neurolaena lobata (Asteraceae) (cancer, *T cruzi*)	15	*Wedelia calycina* (Asteraceae)	40
Baccharis trinervis (Asteraceae) (cancer, *P falciparum, T cruzi*)	50	*Alibertia edulis* (Rubiaceae)	20
Melampodium divaricatum (Asteraceae) (cancer, *T cruzi*)	50	*Brugmansia candida* (Solanaceae)	10
Phryganocydia corymobosa (Bignoniaceae) (cancer, *P falciparum, T cruzi*)	7	*Jacaranda copaia* (Bignoniaceae)	12
Cordia curassavica (Boraginaceae) (cancer)	15	*Tournefortia hirsutissima* (Boraginaceae)	7
Bonamia trichantha (Convolvulaceae) (cancer, *T cruzi*)	10	*Maripa panamensis* (Convolvulaceae)	10
Chomelia recordii (Rubiaceae) (malaria, *T cruzi* , *L mexicana*)	8	*Chiococca alba* (Rubiaceae)	10
Hamelia axillaries (Rubiaceae) (cancer, *P falciparum*)	10	*Isertia haekeana* (Rubiaceae)	10
Witheringia solanacea (Solanaceae) (cancer, *P falciparum, T cruzi*)	15	*Solanum jamaicense* (Solanaceae)	25

geometrid larvae mimicking "sticks" and *Oxytenis modestia* larvae mimicking either bird droppings or snakes, depending on the instar). Aposematic insects were scored according to the feeding stage observed in the field, which were larval Lepidoptera and adult Coleoptera, with the exception of the beetle *Physonota alutacea*, in which both the larval and adult stages were observed feeding.

Statistical analyses

Chi-squared goodness of fit tests were used to test distributions (numerical) observed in the field against expected distributions, determined by the null hypothesis (no difference). A Fisher's exact test was used in cases where the sample size was small (when an expected value was less than ten). A Wilcoxon matched-pairs signed rank test was used to compare the number of insects between plant pairs because of non-normal data distributions and the prevalence of zero values.

Results

Table 1 shows the biologically active and inactive plant pairs that we examined in this study, the bioassays in which the extracts of active plants tested positive against one or more of the tumor cell lines or tropical protozoan parasites used in this study, and the approximate number of individuals of each plant that we searched for insects. The aposematic and non-aposematic insects that we found to be associated with each species are shown in WebTable 2. Forty-six insect species were collected from plants that were active in tropical parasite or tumor cell line bioassays, whereas 25 insect species were collected from plants whose extracts were inactive in the same bioassays. Given that sampling efforts of active and inactive plants were similar, the total numbers of insect species collected from active plants was significantly greater than that from inactive plants (χ^2 [1] = 6.22, $P = 0.013$).

Aposematic species were found on nine of the ten active plant species, but on only four of the ten inactive plant species (one-tailed Fisher's exact test, $P = 0.03$). Two of the aposematic insects found on biologically active plants are shown in Figure 1. Aposematic species constituted 19 of the 46 insects collected from active plants, whereas aposematic insects constituted only five of the 25 insects collected from inactive plants (χ^2 [1] = 8.167, $P = 0.01$; Figure 2). On average, 1.9 aposematic insect species were found on each active plant species, compared to only 0.5 on each inactive plant species (Wilcoxon matched-pairs signed rank test, $P = 0.03$).

Non-aposematic insect species were found on eight of the ten active plants and on all ten of the inactive plants (one-tailed Fisher's exact test, $P = 0.24$). No significant difference was found between the number of non-aposematic insects on active plants (27 of 46) versus inactive plants (20 of 25; χ^2 [1] = 1.043, $P = 0.31$; Figure 2). On average, 2.7 non-aposematic insect species were found on each active plant species and 2.0

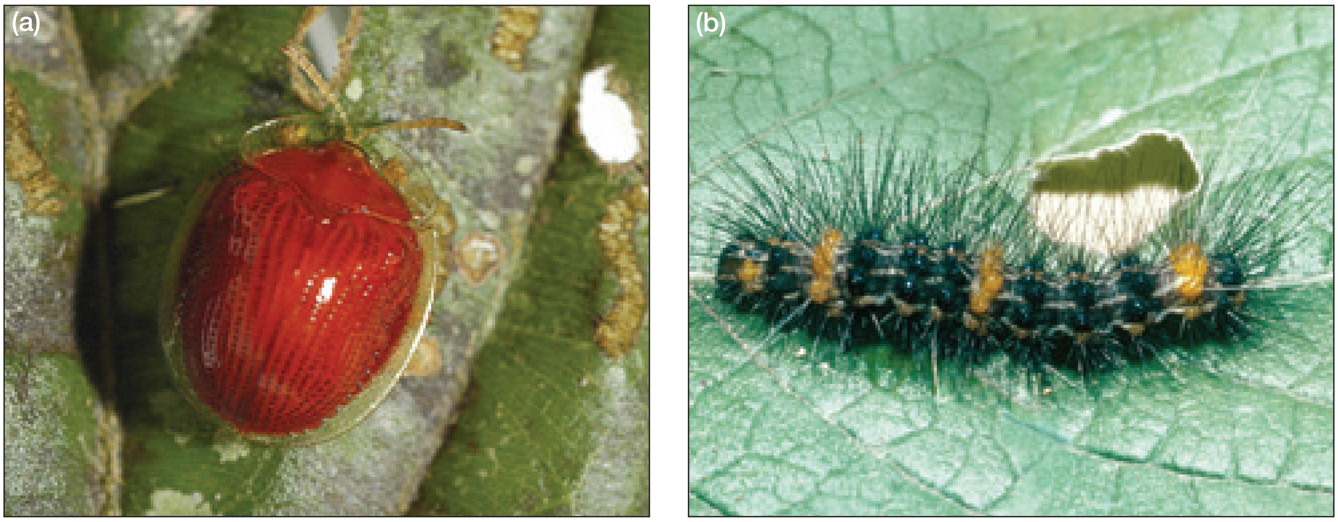

Figure 1. *Two of the aposematic insects found to feed on biologically active plants. (a) The adult stage of the coleopteran,* Charidotis coccinea*, and (b) the larval stage of the lepidopteran,* Dysschema jansonis.

on each inactive plant species (Wilcoxon matched pairs signed rank test, $P = 0.38$).

Discussion

The results of this study suggest that plants containing biologically active compounds are more likely to have associations with aposematic insects than are inactive plants. The presence of aposematic insects can therefore indicate that a particular tropical plant may contain biologically active compounds. As non-aposematic insects are more equally associated with active and inactive plants, using all the insects collected on plants may not be as informative as using only aposematic insects. These observations also suggest that the plant secondary metabolites that show activity in the bioassays used in this study may be among those compounds that are exploited by the aposematic insect herbivores as protection against predation. Insects have also been examined for their potential to yield biologically active compounds, independent of their host plants; however, an ICBG program based in Costa Rica reported difficulties in collecting sufficient quantities of insects for the bioassay-guided isolation and characterization of chemical compounds (Sittenfeld *et al.* 1999; Nielsen *et al.* 2004).

Although a significantly greater number of aposematic insects were found to eat plants containing biologically active compounds, they were also found to feed on inactive plants. One explanation is that these aposematic insects do not contain toxic compounds and are mimics of other toxic/distasteful aposematic insects, which do in fact eat plants containing biologically active compounds (Batesian mimicry). Another possibility is that the aposematic insects are actually sequestering biologically active defensive compounds, but that these compounds were not active in the bioassays used in this study. Although aposematic insects were found on inactive plants, they did not affect the net positive benefit of using aposematic insects as guides to plants containing biologically active compounds. In considering the ecological significance of these results, it should be recognized that many unpalatable insects are not aposematic, especially if visual predation is not of primary importance (Schaffner *et al.* 1994), that not all brightly colored members of mimicry complexes are necessarily toxic (Fordyce 2000), that some insects are able to synthesize defensive chemicals de novo, and that not all insect species feeding on a plant species with defensive compounds will sequester them (Nishida 2002).

Our results come from only ten plant species pairs. Additional studies of active and

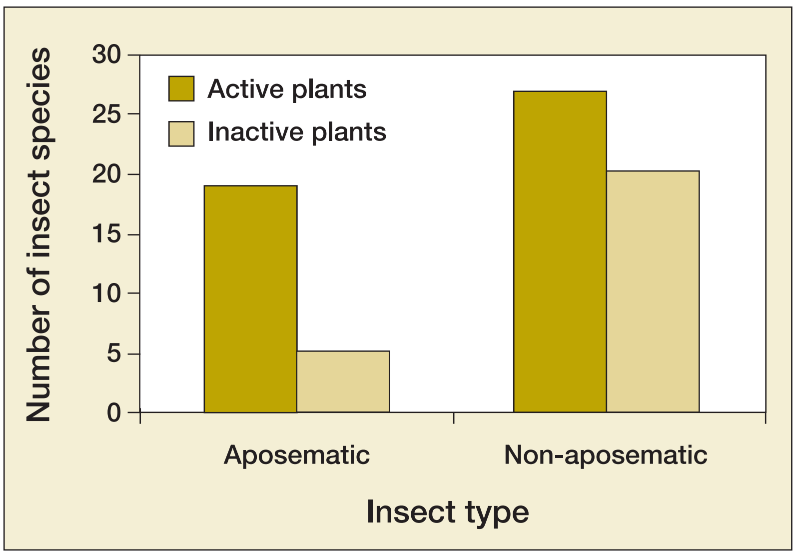

Figure 2. *Number of aposematic and non-aposematic insect species found to feed on the ten biologically active and ten biologically inactive tropical plants studied.*

inactive plant species would increase the generalizability of our findings. Nevertheless, these results suggest that aposematic insects can guide researchers to plants that contain biologically active compounds, a finding that provides additional evidence that insight from ecology and evolutionary theory can be used to a practical end, in this case, enhancing the likelihood of encountering plant species with secondary metabolites active against human diseases. Our results also provide a demonstration of the benefits to human health from the protection of tropical biodiversity, where the study of species from multiple trophic levels can facilitate the discovery of novel medicines.

Acknowledgements

The authors thank E Ortega, L Romero, and their colleagues for performing the bioassays reported in this study, R Aizprúa for confirming plant identifications, H Stockwell for helping with insect identifications, the Panamanian National Authority of the Environment for plant and insect collection permits (permit numbers for plants: SC/P-17-03, SC/P-22-03, SC/P-23-03, SC/P-24-03, SC/P-25-03, SC/P-26-03, SC/P-27-03, and SC/P-28-03; permit numbers for insects: SC/AP-1-05), ICBG participants P Coley, T Kursar, and M Heller for the use of laboratory facilities, and J Bede for input and support. We gratefully acknowledge financial support from the US National Institutes of Health, the US National Science Foundation, and the US Department of Agriculture through the International Cooperative Biodiversity Groups Program (grant numbers 1U01 TW01021-01 and 1U01 TW006634-01), an Engineering Research Council of Canada (NSERC) PGS-A Fellowship, and the Levinson Fellowship from the STRI–McGill NEO program.

References

Andreasen K and Bremer B. 2000. Combined phylogenetic analysis in the Rubiaceae–Ixoroideae: morphology, nuclear and chloroplast DNA data. *Am J Bot* **87**: 1731–48.

Bremer K. 1994. Asteraceae cladistics and classification. Portland, OR: Timber Press.

Brower LP, Cook LM, and Croze HJ. 1967. Predator responses to artificial Batesian mimics released in a neotropical environment. *Evolution* **21**: 11–23.

Capson TL. Biodiscovery research in Panama: linking science, technology, human health, and conservation in the host-country context. In: Carrizosa S and McGuire P (Eds). Legal and scientific implications of bioprospecting contracts: a handbook for policymakers. Bonn, Germany: IUCN Environmental Law Centre. In press.

Coley PD, Heller MV, Aizprua R, *et al.* 2003. Use of ecological criteria in designing plant collection strategies for drug discovery. *Front Ecol Environ* **1**: 421–28.

Cragg GM and Newman DJ. 2005. International collaboration in drug discovery and development from natural sources. *Pure Appl Chem* **77**: 1923–42.

Ehrlich PR and Raven PH. 1964. Butterflies and plants: a study of coevolution. *Evolution* **18**: 586–608.

Fordyce JA. 2000. A model without a mimic: aristolochic acids from the California pipevine swallowtail *Battus philenor hirsute*, and its host plant, *Aristolochia californica*. *J Chem Ecol* **26**: 2567–78.

Guilford T. 1988. The evolution of conspicuous colouration. *Am Nat* **131**: S7–S21.

Hunziker AT. 1979. South American Solanaceae: a synoptic survey. In: Hawes JG, Lester RN, and Skelding AD (Eds). The biology and taxonomy of the Solanaceae. New York, NY: Academic Press.

Hussein AA, Barberena I, Correa M, *et al.* 2005. Cytotoxic flavonol glycosides from *Triplaris cumingiana*. *J Nat Prod* **68**: 231–33.

Jiménez-Romero C, Torres-Mendoza D, González LDU, *et al.* 2007. Hydroxyalkenylresorcinols from *Stylogyne turbacensis*. *J Nat Prod* **70**: 1249–52.

Kareiva P. 1999. Coevolutionary arms races: is victory possible? *P Natl Acad Sci USA* **96**: 8–10.

Koehn FE and Carter GT. 2005. The evolving role of natural products in drug discovery. *Nat Rev* **4**: 206–21.

Kursar TA, Caballero-George CC, Capson TL, *et al.* 2006. Securing economic benefits and promoting conservation through bioprospecting. *BioScience* **56**: 1005–12.

Montenegro H, Gutierrez M, Romero LI, *et al.* 2003. Aporphine alkaloids from a *Guatteria* spp with leishmanicidal activity. *Planta Med* **69**: 677–79.

Nielsen V, Hurtado P, Janzen DH, *et al.* 2004. Recolecta de artrópodos para prospección de la biodiversidad en el Área de Conservación Guanacaste, Costa Rica. *Rev Biol Trop* **52**: 119–32.

Nishida R. 2002. Sequestration of defensive substances from plants by Lepidoptera. *Annu Rev Entomol* **47**: 57–92.

Pereira MS and Barbosa MR. 2004. A família Rubiaceae na Reserva Biológica Guaribas, Paraíba, Brasil. Subfamílias Antirheoideae, Cinchonoideae e Ixoroideae. *Acta Bot Bras* **18**: 305–18.

Prieto JM, Schaffner U, Barker A, *et al.* 2007. Sequestration of furostanol saponins by *Monophadnus* sawfly larvae. *J Chem Ecol* **33**: 513–24.

Rausher MD. 2001. Co-evolution and plant resistance to natural enemies. *Nature* **411**: 857–64.

Rosendal GK. 2006. Balancing access and benefit sharing and legal protection of innovations from bioprospecting impacts on conservation of biodiversity. *J Environ Dev* **15**: 428–47.

Ruxton GD, Speed MP, and Broom M. 2007. The importance of initial protection of conspicuous mutants for the coevolution of defense and aposematic signaling of the defense: a modeling study. *Evolution* **61**: 2165–74.

Salazar BA and Whitman DW. 2001. Defensive tactics of caterpillars against predators and parasitoids. In: Ananthakrishnan TN (Ed). Insects and plant defence dynamics. Plymouth, UK: Science Publishers.

Schaffner U, Boeve JL, Gfeller H, and Schlunegger U. 1994. Sequestration of *Veratrum* alkaloids by specialist *Rhadinoceraea nodicornis* Kono (Hymenoptera, Tenthredinidae) and its ecoethological implications. *J Chem Ecol* **20**: 3233–50.

Sittenfeld A, Tamayo G, Nielsen V, *et al.* 1999. Costa Rican International Cooperative Biodiversity Group: using insects and other arthropods in biodiversity prospecting. *Pharm Biol* **37**: 55–68.

Thompson JN. 1999. The evolution of species interactions. *Science* **284**: 2116–18.

Windsor DM, Riley EG, and Stockwell HP. 1992. An introduction to the biology and systematics of Panamanian tortoise beetles (Coleoptera: Chrysomelidae: Cassidinae). In: Quintero D and Aiello A (Eds). Insects of Panama and Mesoamerica: selected studies. Oxford, UK: University Press.

WebTable 1. Plant voucher numbers

Alibertia edulis (Rich) A Rich ex DC	Aizprúa 2901
Baccharis trinervis Pers	Aizprúa 2853
Bonamia trichantha Hallier F	Aizprúa 2845
Brugmansia candida Pers	Aizprúa 2843
Chiococca alba (L) Hitchc	Aizprúa 2850
Chomelia recordii Standl	Aizprúa 2844
Cordia curassavica (Jacq) Roem and Schult	Aizprúa 2842, 2851
Hamelia axillaries Sw	Aizprúa 2849
Isertia haekeana DC	Aizprúa 2905
Jacaranda copaia (Aubl) D Don	Aizprúa 2906
Maripa panamensis Hemsl	Aizprúa 2897
Melampodium divaricatum (Rich) DC	Aizprúa 2848
Neurolaena lobata (L) R Br ex Cass	Aizprúa 2902
Phryganocydia corymobosa (Vent) Buereau ex K Schum	Aizprúa 2947
Solanum jamaicense Mill	Aizprúa 2856
Tilesia baccata (L) Pruski	Aizprúa 2903
Tithonia diversifolia (Hemsl) A Gray	Aizprúa 2855
Tournefortia hirsutissima L	Aizprúa 2846
Wedelia calycina Rich	Aizprúa 2852
Witheringia solanacea L'Hér	Aizprúa 2854

WebTable 2. Aposematic and non-aposematic insects, identified to the lowest taxonomic level possible, found on each of the 20 study plants

Plant species	*Aposematic insect species*	*Non-aposematic insect species*
Baccharis trinervis	Alticinae sp (Coleoptera: Chrysomeloidea: Chrysomelidae) *Pachybrachis reticulata* Fabr (Coleoptera: Chrysomeloidea: Chrysomelidae: Cryptocephalinae) *Diabrotica* sp (Coleoptera: Chrysomeloidea: Chrysomelidae: Galerucinae)	*Myrmex vicinus* Ch (Coleoptera: Curculionidae) Geometridae sp (Lepidoptera: Geometroidea) *Semiothisa* sp (Lepidoptera: Geometroidea: Geometridae) Noctuidae sp (Lepidoptera: Noctuoidea)
Neurolaena lobata	*Platyphora ligata* Stal (Coleoptera: Chrysomeloidea: Chrysomelidae: Chrysomelinae)	*Calephelis* sp (laverna?) (Lepidoptera: Papilionoidae: Riodinidae) Noctuidae sp (Lepidoptera: Noctuoidea) Torticidae sp (Lepidoptera: Tortricoidea) Pyralidae sp (Lepidoptera: Pyraloidea)
Tithonia diversifolia	Melitaeinae sp (Lepidoptera: Papilionoidea: Nymphalidae) *Platyphora ligata* Stal (Coleoptera: Chrysomeloidea: Chrysomelidae: Chrysomelinae) *Dysschema magdala* Bois (Lepidoptera: Noctuoidea: Arctiidae: Pericopinae) *Chlosyne hippodrome* Gey (Lepidoptera: Papilionoidea: Nymphalidae)	Gelechiidae sp (Lepidoptera: Gelechioidea)
Melampodium divaricatum	*Systena s-littera* L (Coleoptera: Chrysomeloidea: Chrysomelidae: Alticinae)	*Rhabdopterus* sp (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae) Noctuidae sp (Lepidoptera: Noctuoidea)
Phryganocydia corymbosa	Unknown Lepidopteran sp	

WebTable 2. ***(Continued)***

Plant species	*Aposematic insect species*	*Non-aposematic insect species*
Cordia curassavica	*Physonota alutacea* Boh (larvae) (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)	*Polydacrys depressifrons* Boh (Coleoptera: Curculionidae)
	Lebia sp (Coleoptera: Carabidae: Harpalinae)	*Polychalma multicava* Latr (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)
		Physonota alutacea Boh (adult) (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)
		Galerucinae sp (Coleoptera: Chrysomeloidea: Chrysomelidae)
		Noctuidae sp? or Pyralidae sp? (Lepidoptera)
		Alticinae sp (Coleoptera: Chrysomeloidea: Chrysomelidae)
Bonamia trichantha	*Chersinellina heteropunctata* Boh (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)	
Chomelia recordii	*Charidotis coccinea* Boh (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)	*Coelocephalapion nodicorne* Shp (Coleoptera: Curculionidae)
	Charidotis erythrostigma Champ (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)	*Omiodes* sp (Lepidoptera: Pyraloidea: Pyralidae: Pyraustinae)
	Phanaeta ruficollis Lef (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae)	Geometridae sp (Lepidoptera: Geometroidea)
	Unknown Lepidopteran sp	Unknown Lepidopteran sp Unknown Lepidopteran sp Unknown Lepidopteran sp Unknown Lepidopteran sp
Witheringia solanacea	*Lema bitaeniata* (Coleoptera: Chrysomeloidea: Chrysomelidae: Criocerinae)	*Ithomia iphianassa panamensis* Bates (Lepidoptera: Papilionoidea: Nymphalidae: Ithomiinae)
	Noctuidae sp (Lepidoptera: Noctuoidea)	*Plagiometriona gibbifera* Champ (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)
		Geometridae sp (Lepidoptera: Geometroidea)
Wedelia calycina	*Colaspis* sp (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae)	*Brachypnoea* sp (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae)

WebTable 2. *(Continued)*

Plant species	*Aposematic insect species*	*Non-aposematic insect species*
Brugmansia candida	Percopinae sp (Lepidoptera: Noctuoidea: Arctiidae)	Torticidae sp (Lepidoptera: Tortricoidea) Geometridae sp (Lepidoptera: Geometroidea) Rutelinae sp (Coleoptera: Scarabaeidae) Unknown Lepidopteran sp
Tournefortia hirsutissima	*Ischnocodia annulus* Fabr (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae) *Dysschema jansonis* Butl (Lepidoptera: Noctuoidea: Arctiidae: Pericopinae)	*Charidotis vitreata* Perty (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)
Tilesia baccata	*Microctenochira flavonotata* Boh (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae)	*Brachypnoea* sp (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae)
Alibertia edulis		*Oxytenis modestia* Cram (Lepidoptera: Bombycoidea: Saturniidae: Oxyteninae) Pyralidae sp (Lepidoptera: Pyraloidea)
Jacaranda copaia		Geometridae sp (Lepidoptera: Geometroidea)
Maripa panamensis		*Microctenochira cruxflava* Champ (Coleoptera: Chrysomeloidea: Chrysomelidae: Cassidinae) Unknown Lepidopteran sp
Chiococca alba		Geometridae sp (Lepidoptera: Geometroidea) Noctuidae sp (Lepidoptera: Noctuoidea)
Isertia haekeana		*Perigonia lusca* Fabr (Lepidoptera: Sphingoidea: Sphingidae) Alticinae sp (Coleoptera: Chrysomeloidea: Chrysomelidae) Eumolpinae sp (Coleoptera: Chrysomeloidea: Chrysomelidae)
Solanum jamaicense		*Colaspis sanjoseana* Bechyné (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae) *Colaspis nr flavipes* (Coleoptera: Chrysomeloidea: Chrysomelidae: Eumolpinae) Unknown Lepidopteran sp
Hamelia axillaries		Pyralidae sp (Lepidoptera: Pyraloidea)