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Secondary metabolites of vertebrate-dispersed fruits: evidence for adaptive functions

Metabolitos secundarios de frutos dispersados por aves: evidencia de funciones adaptativas

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ABSTRACT

In this paper, I discuss recent evidence concerning the adaptive significance of secondary metabolites in ripe fleshy fruits, and implications for seed dispersal by birds and other vertebrates. Specifically, I revisit a number of adaptive hypotheses originally presented and discussed by Cipollini & Levey in 1997, including the Attraction/Association, Seed Germination Effects, Attraction/Repulsion, Protein Assimilation, Gut Retention Time, Directed and General Toxicity, and Defense Tradeoffs hypotheses. I also present and discuss a new adaptive hypothesis, the Direct Nutritional Benefits hypothesis, posed to reflect recent discoveries concerning the positive dietary effects of some secondary metabolites. From this review, I conclude that focused studies are much needed to provide direct tests of these hypotheses. Evidence addressing many of the hypotheses is either observational or indirect, and gleaned from studies not directly designed to address these hypotheses. Despite this, most hypotheses find at least some level of support — even when the same metabolite is being considered (e.g., anthocyanins and carotenoids having functions as pigments as well as nutritional antioxidants). I conclude with a discussion of the nature of multiple molecular targets of plant secondary chemicals. In doing so, I reinforce the notion that synergistic interactions and multifunctionality of secondary metabolites may provide economical evolutionary solutions for plants facing the disparate and temporally variable selective pressures that impinge upon fruits and seeds. As such, it may not be surprising that specific secondary metabolites serve more than one adaptive function in ripe fleshy fruits; finding support for several non-mutually exclusive hypotheses is likely. Comparative studies designed to address these hypotheses should be undertaken with a careful consideration of the potential underlying effects of phylogeny and physiological constraints on such patterns.

Key words: frugivory, fruit pulp toxins, plant secondary metabolites, *Solanum* glycoalkaloids, vertebrate seed dispersal.

RESUMEN

En este articulo discuto evidencia reciente acerca del significado adaptativo de los metabolitos secundarios en la fruta madura y sus implicaciones para la dispersión de semillas por aves y otros vertebrados. Específicamente, reviso las hipótesis adaptativas originalmente presentadas y discutidas por Cipollini & Levey en 1997. Estas incluyen: atracción/ asociación, efectos sobre la germinación, toxicidad general y dirigida, y compromisos en defensa. Además, presento y discuto una nueva hipótesis adaptativa: efectos nutricionales directos. Esta hipótesis es postulada para reflejar los descubrimientos recientes sobre los efectos positivos resultantes del consumo de metabolitos secundarios. Concluyo que se requieren estudios con un foco estrecho para examinar estas hipótesis de un modo directo. La evidencia en favor o en contra de cada hipótesis es observacional o indirecta, y obtenida de estudios no diseñados específicamente para probar estas hipótesis. A pesar de esto, la mayor parte de las hipótesis encuentran cierto nivel de apoyo - en ocasiones, incluso cuando el mismo metabolito es considerado (e.g., antocianinas y carotenoides que funcionan como pigmentos además de antioxidantes nutricionales). Finalmente, discuto la naturaleza de los blancos moleculares de los compuestos secundarios encontrados en plantas. Al hacerlo, refuerzo las nociones que sugieren que la existencia de interacciones sinérgicas y que la acción multifuncional en estos compuestos pueden proveer de soluciones económicas a plantas que se enfrentan a selección temporalmente variable, y a veces multidireccional, sobre frutos y semillas. Puede no resultar sorprendente el que los compuestos secundarios en plantas cumplen funciones múltiples; siendo probable el apoyo a muchas hipótesis no mutuamente exclusivas. Estudios comparados que permitan examinar estas hipótesis deben ser diseñados considerando cuidadosamente los efectos potenciales de las relaciones filogenéticas y las restricciones fisiológicas pueden tener sobre estos patrones.

Palabras clave: frugivoría, toxinas de pulpa de frutos, metabolitos secundarios, *Solanum glycoalkaloids*, dispersión de semillas por vertebrados.

(Managed by F. Bozinovic)

INTRODUCTION

Secondary metabolites: mediators of fruit-frugivore interactions?

Some years ago, Daniel Janzen (1983a) stated the following, "...a given species of ripe fruit is undoubtedly designed by natural selection to be uninteresting or even distasteful to the majority of the frugivores in the habitat." Despite years of work since this and similar adaptive hypotheses, it has become evident that patterns thought to result from reciprocal selection pressures between fruiting plants and frugivores are, quite simply, not very tractable and/or explainable (Howe 1984, Herrera 1985b, Herrera 1986, Jordano 1987a, Willson & Whelan 1990, Witmer & Cheke 1991). At first glance, it seemed that differences in the quality of seed dispersal among frugivores (cf., Schupp 1993, Loiselle & Blake 1999) should result in patterns of fruit morphological and chemical make-up that "fit" nicely with the behavioral, physical, and physiological make-up of specific dispersers and/or disperser assemblages (Janson 1983, Janzen 1983c, Gautier-Hion et al. 1985, Johnson et al. 1985, Moermond & Denslow 1985, Wheelwright 1985, Debussche et al. 1987, Debussche & Isenmann 1989, Martinez del Rio et al. 1989, Willson et al. 1989, Gautier-Hion 1990, Stiles & Rosselli 1992). Yet, phylogenetic studies have failed to demonstrate that frugivores have strongly influenced morphological and nutritional traits of fleshy fruits (Herrera 1987, Jordano 1987b, Bates 1992, Bremer & Eriksson 1992, Herrera 1992). While it is acknowledged that fruit-frugivore interactions have influenced very broad-scale fruit traits (e.g., small round shapes, color contrast when ripe, nutrients ready for uptake) that can be interpreted as adaptations for dispersal, it is the finer scale patterns that remain rather unresolved. In short, we still have difficulty explaining who eats what and why.

In a previous review, Douglas Levey and I proposed that one reason for the lack of better understanding of fruit-frugivore interactions is that previous studies have generally overlooked the effects of fruit secondary metabolites (Cipollini & Levey 1997b). This is a bit surprising, since such compounds are likely important mediators of fruit-frugivore interactions (Janzen 1977, Herrera 1982, Sorensen 1983, Cipollini & Stiles 1992b). It was our hope that intensive study of such compounds could yield a new perspective on fruit-seed disperser interactions (see also Levey & Cipollini 1999). In this review, we presented a series of adaptive hypotheses to explain the functional significance of secondary metabolites in ripe fleshy fruits, and which might help explain the apparent paradox that some fruits seem to be toxic to the very organisms that disperse the seeds. A secondary goal of our review was to encourage focus on participants affecting fruit-frugivore interactions other than the vertebrate dispersers and the fruits they consumed. By considering other organisms involved in these interactions (e.g., microbes, insect pests, vertebrate seed predators or pulp thieves (cf., Cipollini & Stiles 1993a, 1993b, Cipollini & Levey 1997a), we hoped that greater insight might be gained into the nature of the interaction between the primary participants.

At the time of our review, the field was represented primarily by theoretically-oriented papers (Herrera 1982, Sorensen 1983, Herrera 1985b, Izhaki & Safriel 1990, Mack 1990, Sedinger 1990) and a few studies documenting patterns and/or effects of fruit secondary metabolites (e.g, Goldstein & Swain 1963, Dement & Mooney 1974, Janzen 1975, Janzen 1979, Janzen 1983b, Wrangham & Waterman 1983, Ballington et al. 1988, Izhaki & Safriel 1989, Gargiullo & Stiles 1991, 1993, Cipollini & Stiles 1991, Cipollini & Stiles 1993a, Ehrlen & Eriksson 1993, Murray et al. 1994, Bairlein 1996, Cipollini & Levey 1997a, 1997c). It is encouraging to see more ecological studies currently being undertaken with these goals, although there is still much to learn from the non-ecological literature. And, it is becoming more widely accepted that secondary metabolites of ripe fleshy fruits are probably the product of interactions among the various consumers of these fruits and the plants that bear those fruits (Harborne 1997).

The primary goal of the current paper is to provide an update of this earlier review. Following a general review of fruit secondary metabolites, I: 1) review some of the more recent findings that bear upon the proposed hypotheses, 2) introduce a new adaptive hypothesis (the Direct Nutritional Benefits hypothesis), 3) discuss the non-mutually exclusive nature of many of the adaptive hypotheses in light of the synergism and multifunctionality of phytochemicals, and 4) remind researchers to consider the potential effects of phylogeny when undertaking comparative studies in this area. For a full background to the hypotheses discussed in this paper, refer to the original paper (Cipollini & Levey 1997b) and to a follow-up exchange (Cipollini & Levey 1998, Eriksson & Ehrlen 1998).

Fruit secondary metabolites: why fruits aren't just like leaves

It is generally accepted that a primary function of secondary metabolites in immature fruits is defense from all consumers (i.e., pathogens and predispersal seed predators and pulp thieves). Consumption of immature fruits should always be detrimental, because the seeds are not yet viable. Thus, models that predict patterns of leaf chemistry (e.g., carbon/nutrient balance, optimal defense, and resource availability theories) should apply to immature fruits (Rhoades & Cates 1976, Bryant et al. 1983, Coley et al. 1985, Rhoades 1985, Coley 1986, Bazzaz et al. 1987, Cipollini & Levey 1991). Indeed, it is common for unripe fruits to possess potent biological activity (cf. Eigenbrode & Trumble 1994, Kreuger & Potter 1994, Tsujuno et al. 1995, Harborne 1997, Quidde et al. 1998, Sandrock & VanEtton 1998).

The selective pressures faced by ripe fruits differ, however, from those of leaves. Whereas leaf consumption should always negatively affect plant fitness (but see Paige & Whitham 1987), negative, neutral, or positive effects upon plant fitness are possible, depending upon how seeds are treated during and after fruit consumption. And, because fruits are not essential to plant growth (whereas leaves are), plants can more readily adjust fruit number in response to resource level variation (cf., Stephenson 1981, Sutherland 1986). Many studies have shown, for example, that fruit size and chemical composition within a species remains rather constant across great changes in environmental conditions, whereas crop size can vary enormously (cf., Papp et al. 1984, Cipollini & Whigham 1994). Thus, there is no reason to expect carbon/nutrient balance or resource level to affect fruit pulp secondary metabolite level in any predictable way (because resources available to each fruit may be relatively invariant). Also, within a fruit, resources can be allocated in nearly any way imaginable to seeds and pulp.

Changes with fruit ripening and the issue of physiological constraints

From a horticultural perspective, fruit ripening has been defined as a highly co-ordinated process of organ transformation which yields an attractive edible fruit with an optimum blend of color, taste, aroma, and texture (Prabha & Bhagylakshmi 1998). In the past, this process was considered to be the fortunate (for fruit consumers like us) result of organ senescence, little different from the physiological processes leading to leaf or whole plant senescence. In contrast, fruit ripening is presently considered to be tightly controlled genetically (compare Biale 1975 with Brady 1987 and Manning 1998). Pigments, cell wall structure, texture, nutrients, and secondary chemicals can all change independently during ripening, and the genetic regulatory mechanisms underlying these changes are being uncovered. For example, Manning (1998) has reported that at least 26 independent genes affect ripening of strawberry (*Fragaria ananassa* Duch.) fruits via changes in gene expression. Single genes and families of genes are involved in changes in key metabolic pathways related to color, texture, respiration, carbohydrate metabolism, and flavor. Changes with ripening do not necessarily result in complete loss of secondary chemical defenses, as many species retain significant levels of defense against pests and pathogens following ripening (cf., Cipollini & Stiles 1991, Cipollini & Stiles 1993a, Legal et al. 1994, Cipollini & Levey 1997a, Stiles & Oudemans 1999, Denoyes-Rothan et al. 1999). The extent to which either residual or novel secondary chemicals of ripe fruits also affect legitimate seed dispersers is an important question.

Various aspects of the fruit ripening process can be unlinked, resulting in fruits that show only some of the changes typically associated with full ripening. For example, Rick et al. (1994) reported that subpopulations of *Lycopersicon esculentum* var. *cerasiforme* (Wild Tomato) have very high glycoalkaloid (tomatine) levels (about 500-5000 mg/g dry mass) in ripe fruits. This is a simple recessive trait and the mature fruits are very bitter to the human palate. The ease with which secondary chemical production can be turned on and off via gene regulation in this species and others argues strongly against the concept of physiological constraints in the allocation of secondary metabolites in ripe fruits. Whether high tomatine level is adaptively significant in certain subpopulations or is the happenstance result of genetic variation among populations remains to be determined.

It is unlikely that many physiological mechanisms exist that preclude either the differential retention of particular compounds and/or the de novo synthesis of new compounds during ripening. In fact, a very common pattern for ripening fruits is a general decline of most secondary compounds (with some compounds decreasing less than others), as well as the de novo appearance of new compounds. For example, as flavanones (narirutin, naringin, and neohesperidin) decrease with ripening in grapefruits (*Citrus* spp.), the level of nootkatone increases dramatically (see below; Ortuno et al. 1995).

Secondary compounds are certainly present in ripe fruits of many wild and horticultural species (cf., Aoki et al. 1976, Herrera 1982, Moller 1983, Hikino et al. 1984, Perera et al. 1984, Bandyopadhyay et al. 1990, and many others); in fact, some compounds seem to be found exclusively in ripe fruits (cf., Barnea et al. 1993, Legal 1994, Ortuno et al. 1995, Farine et al. 1996). While none of the adaptive hypotheses proposed in this paper requires or predicts the de novo evolution of secondary metabolites found uniquely in ripe fruits (see Cipollini & Levey 1998 in response to Eriksson & Ehrlen 1998), the occurrence of such compounds does suggest adaptive roles.

Evidence that unique secondary chemical profiles exist in ripe fruits can also be seen indirectly in data showing the use of secondary chemicals by specialist insects as foraging cues. For example, both apple maggots (*Rhagoletis pomonella*) and blueberry maggots (*R..mendax*) are attracted to secondary chemical extracts of ripe fruits of their respective host species (*Malus pumila* and *Vaccinium corymbosum*) and lay higher numbers of eggs in response to these extracts (Bierbaum & Bush 1990). However, extracts from unripe fruits do not elicit such high responses. Other specialist insects that attack ripe fruit show similar responses to their host species (e.g., Oriental Fruit Fly, *Dacus dorsalis*; Melon Fly, *D. cucurbitae*; Queensland Fruit Fly, *D. tryoni*; Bierbaum & Bush 1990).

Finally, there is evidence that some plants retain potentially lethal levels of secondary compounds in ripe fruits (e.g., *Atropa belladonna* - tropane alkaloids, *Solanum* spp. - steroidal glycoalkaloids, *Sapindus* spp. and *Phytolacca* spp. - saponins; Heiser 1969, Myers 1983) despite low levels or virtual absence of such compounds in foliage of some species (Cipollini & Levey 1998). While I focus on the full spectrum of secondary metabolites, it is the presence of such high levels of vertebrate toxins in ripe fruits that is especially difficult to explain without careful consideration of all factors influencing fruit-frugivore mutualisms.

Adaptations for tolerance in birds

It has been postulated (Herrera 1985a) that avian frugivores may be particularly adapted for the dispersal of fruits containing secondary metabolites as evidenced by the fact that many such species are able to feed upon toxic insects. Adaptations may be seen in large livers with specific detoxifying enzymes in some species, in the lack of specific enzymes needed to induce toxic effects in post-ingestional toxins, in mechanisms that prevent intestinal uptake, and in systems that facilitate rapid elimination following uptake. Evidence that bird species, under certain situations, can evolve tolerance to broadly toxic substances can be seen in the example of seed predation by the endemic Hawaiian finch, Palila (*Loxioides bailleui*), which feeds almost exclusively during the breeding season on seeds of Mamane (*Sophora chrysophylla*; van Riper 1981).

Embryos at the stage consumed by Palila contain high levels of at least seven different quinolizidine alkaloids, including the alpha-pyridone cytisine which can exceed 2 % of seed dry mass (Murakoshi et al. 1984, P. Banko, M. Cipollini, G. Breton, & M. Wink, unpublished data). Since seeds contain levels of alkaloids known to cause severe neurological symptoms and death in humans, birds, and other animals, it is likely that Palila has evolved a tolerance to these alkaloids. While not an example of adaptations to toxins of fleshy fruits, the Palila-Mamane interaction illustrates that tolerance to specific secondary metabolites may be associated with restricted diets of avian species.

CASE STUDIES

Adaptive hypotheses for secondary metabolites in ripe fruit

In the following section, I focus on seven adaptive hypotheses posed previously to explain the presence of secondary metabolites in pulp of ripe vertebrate-dispersed fruits (Table 1). I briefly discuss each hypothesis and, where possible, summarize recent case studies that directly or indirectly address each hypothesis.

Attraction/association hypothesis

This hypothesis maintains that secondary metabolites provide foraging cues (e.g., colors, odors, and flavors) that can be readily recognized by frugivores and are thus associated with fruit rewards (e.g., sugars, lipids, proteins, vitamins, and minerals; van der Pjil 1969). This is the most obvious and widely accepted function of secondary metabolites in ripe fruits.

Examples of visual attractants include anthocyanins (water-soluble reds, blues, and blacks) of fruits such as *Vaccinium* spp. (Harborne 1979, Ballington et al. 1988) and carotenoids (lipid soluble yellows, oranges, reds and browns) of fruits such as *Capsicum* spp. (Britton & Hornero-Mendez 1997). Studies of flavor and aroma components of fruits have revealed exceedingly diverse and interactive mixtures of compounds

(Crouzet et al. 1997, Sanz et al. 1997). Even a "negative" taste perception such as astringency may be considered attractive to certain organisms under certain conditions, if associated in some way with rewards (Clifford 1997). Very rarely have phytochemists been able to attribute the characteristic color, flavor or aroma of fruits to single chemicals (Crouzet et al. 1997). In some cases, they can. For example, a sesquiterpine (nootkatone) increases considerably with ripening in grapefruits (*Citrus* spp.); this compound serves as the principal ripe fruit odor (Ortuno et al. 1995). The signature notes of ripe bananas and raspberries are other examples, attributable to isopentyl acetate and 4-(4'-hydroxyphenyl)-butan-2-one (raspberry ketone), respectively. These, of course, are the principal compounds detected and associated with these fruits by humans.

This brings out a major point about attractants and about fruit secondary metabolites in general. The characteristics of these metabolites must be determined with reference to the species that interact with the fruits. Knowledge of how fruit chemicals are perceived by and affect humans may reveal only part - or none - of the picture.

Seed germination effects hypothesis

This hypothesis posits that secondary metabolites are retained within ripe fruit pulp to either inhibit pre-dispersal seed germination or to enhance post-

TABLE 1

A summary of adaptive hypotheses for the role of secondary metabolites in ripe fleshy fruits

Resumen de las hipótesis adaptativas para el rol de los metabolitos secundarios en frutos maduros

Hypothesis	Biological Activity	Effect on Vertebrate Dispersers	Advantage to Plant
Attraction/association	Affects visual olfactory, tactile, or taste receptors.	Attractant: Food discovery and food intake rates are enhanced.	Rapid seed dispersal is promoted upon fruit ripening.
Seed germination effects	Inhibits germination of seeds within pulp or promotes post-dispersal seed viability.	None; Dispersers remove negative effects by consuming pulp.	Initiation of germination process is cued by pulp removal and/or seeds have greater viability.
Attraction/Repulsion	Deterrent to vertebrate seed dispersers.	Dispersers are repulsed soon after beginning to feed on fruit.	Seeds are dispersed away from parent plant in small groups.
Protein assimilation	Binds protein in diet of vertebrate seed dispersers.	Dispersers are induced to seek alternative protein sources.	Seeds are disperses away from parent plant in small groups.
Gut retention time	Alters gut passage rates of vertebrate seed dispersers.	Dispersers pass seeds through the gut either more quickly or more slowly.	Seeds either pass in intact (viable) condition or are carried further by dispersers.
Directed and general toxicity	May be deterrent to vertebrate seed dispersers.	Dispersers are unaffected when dispersal is predictable; diffuse interactions constrain selection for directed toxins.	May screen out seed predators and pulp thieves detrimental to seed dispersal.
Defense tradeoffs	Deterrent to microbial and invertebrate pests.	Secondary effects; dispersers may or may not be deterred.	Preserves fruits in absence of visitation by dispersers.
Direct nutritional benefits	Reward for fruit pulp consumption by vertebrate dispersers.	Greater fitness in dispersers due to improved health, longevity and performance.	Rapid dispersal is promoted upon ripening.

dispersal seed germination (this is a modification of the original hypothesis which referred only to inhibition). By removing fruit pulp during consumption, frugivores permit the germination process to be initiated. Or, via residual compounds distributed with seeds following dispersal, seed viability and/or seed germination is enhanced.

Seed germination inhibitors are most certainly found in the ripe pulp of some species. This type of prevention of premature seed germination has been termed "chemical dormancy" by Baskin & Baskin (1998). For example, ripe fruits of *Solanum* species often contain glycoalkaloids (hereafter, GAs). These compounds have been shown to delay or suppress seed germination (Campbell & Van Staden 1998), even after passing through the gut of birds (Wahaj et al. 1998). Likewise, in a study of the fruits of *Sorbus commixta*, Yagihashi et al. (1998) demonstrated rather unequivocally that seed germination inhibitors were present in the pulp of this species, and thus ingestion and removal of the pulp by birds was essential for seed germination. Preliminary evidence for the enhancement of seed germination comes in the case of the distribution of seeds by birds consuming *Capsicum* fruits containing capsaicin. Tewksbury & Nabhan (1999) reported that, in some experiments, seeds defecated after birds were fed capsaicin-laced diets germinated more often than those defecated after birds fed on control diets. Positive effects on seed viability, in this case, might have been due to defense of seeds from microbial and invertebrate pests and pathogens, rather than via a direct promotion of seed germination.

Experiments aimed at detecting chemical dormancy are very difficult to design, as effects upon seed germination may be indirect via effects on light, pathogens, pests, and other extrinsic factors (Cipollini & Levey 1997b). Laboratory experiments showing effects of chemical extracts (or lack thereof) on seed germination in Petri dishes are generally considered inadequate tests of this hypothesis. However, such tests may lead to insights if conducted at expected tissue concentrations in artificial fruit-pulp media.

Attraction/repulsion hypothesis

The Attraction/Repulsion hypothesis states that, while primary nutrients serve as rewards for attracting frugivores to the fruiting plant, secondary metabolites might induce frugivores to leave the plant early in a foraging bout (Sorensen 1983). Early departure from the plant might reduce the number of fruits consumed, but might enhance the likelihood of seed dispersal away from the parent plant, with consequent benefits.

In the last two years, I have seen no direct tests of this hypothesis. Tests of this and the following related hypothesis need, at the minimum, to demonstrate that a primary factor causing frugivores to diversify their diets is the presence of secondary compounds in fruits of certain species. For example, considering all other factors, do frugivores spend less time at plants or plant species known to have high levels of deterrent secondary metabolites in their ripe fruits? If so, what are the consequences for seed dispersal for the plant?

Protein assimilation hypothesis

As with the Attraction/Repulsion hypothesis, this hypothesis suggests that secondary metabolites induce frugivores to leave plants early in the course of foraging. In this case, however, the mechanism involves interference with protein metabolism, not direct chemical deterrence. By interfering with protein metabolism, frugivores would need to leave fruiting plants to find alternative sources of protein, such as invertebrates (Izhaki & Safriel 1989, 1990).

Since the original review, I have found no direct tests of this hypothesis, which was challenged by Mack (1990) and Sedinger (1990). Support for this hypothesis may come through better understanding of the protein and amino acid profiles of fruits and metabolic requirements of avian frugivores (cf., Izhaki 1998).

Gut retention time hypothesis

This hypothesis suggests that fruit secondary metabolites act to alter seed passage rates; either via laxative (Putz 1993, Murray et al. 1994) or constipative (Wahaj et al. 1998) effects.

Rapid gut passage can increase seed viability by reducing seed damage (Russi et al. 1992, Gardener et al. 1993); this in one fitness advantage for the plant. Also, frugivores may select fruits that have rapid gut transit times as a means of avoiding the limitation that gut passage rate has on food intake rate (Sorensen 1984, Levey & Grajal 1991). On the other hand, constipative effects may cause dispersers to carry seeds in the gut for longer periods of time, thus promoting seed dispersion (Murray 1988). In this regard, fruits dispersed by relatively immobile animals (e.g., small mammals) seem more likely to benefit from constipative effects than those dispersed by more mobile animals (e.g., passerine birds and bats). To date, very little is known about the laxative/constipative effects of secondary constituents of ripe fruits on the animals that disperse these fruits. Studies focusing on this issue should consider the potential effects of primary nutrients (e.g., sugar concentration) as well as secondary metabolites on gut passage rate (Witmer 1996).

Sorbitol is common in tissues of Rosaceous plants and may generally play a physiological role in tissues for dealing with water stress (Moing et al. 1997). But, it is also present at high levels in ripe fruits of many species, where it is associated with a laxative effect in humans. For example, the primary carbohydrates of ripe Saskatoon (*Amelanchier alnifolia* Nutt.), plum, and nectarine fruits are glucose, fructose and sorbitol (Rogiers & Knowles 1997). This suggests that the presence of sorbitol at high levels in ripe fruits of such species may be an adaptive pattern associated with the promotion of seed passage.

One recent test of this hypothesis is that of Wahaj et al. (1998). In this study, crude extracts of ripe *Solanum americanum* fruits accelerated seed passage rates for *Solanum* seeds consumed by Cedar Waxwings (*Bombycilla cedrorum*). *Solanum* GAs, which occurs at low concentrations in fruits such as *S. americanum* and at high concentrations in other fruits, did not accelerate gut passage. In fact, GAs slowed seed passage rates; so the positive effect of the crude *S. americanum* extract on seed passage rate must be attributed to other secondary chemicals.

GAs seem to be highest in mammal-dispersed Solanum fruits, being very low in the primarily bird-dispersed *S. americanum* (Cipollini & Levey 1997b, 1997c). If constipative effects are also seen in mammals, then greater retention times might result in broader seed dispersion. While we do not know how GAs actually affect seed transit times in mammals that disperse *Solanum* seeds, diarrhea is a principal symptom of acute toxicity in domestic mammals (Zitnak 1979). Thus, the activity of these compounds on mammals may differ from the effect seen on seed-dispersing birds. If seed transit times are actually accelerated in the guts of seed dispersing mammals, these compounds could have a role in decreasing seed damage during gut transit, which is likely to be much higher for mammals than it is for passerine birds (Cipollini & Levey 1997c).

Directed and general toxicity hypotheses

These hypotheses relate to the differential activity of secondary metabolites toward beneficial and detrimental vertebrates. The directed toxicity hypothesis posits that particular secondary chemicals are directed towards potentially damaging vertebrates (seed predators or pulp "thieves"), while remaining non-toxic to beneficial vertebrates (seed dispersers; Janzen 1979, Sorensen 1983).

There is certainly evidence for differential activity toward animals by particular fruit pulp secondary metabolites, as well as some support for the directed toxicity hypothesis. One of the best recent tests of this hypothesis is that of Struempf et al. (1999). These authors reported that amygdalin, a cyanogenic glycoside which is present in ripe fruit of species such as *Prunus* and *Sambucus* spp., does not deter fruit consumption by Cedar Waxwings. In just four hours, birds can consume up to five or six times the dose of amygdalin known to kill laboratory rats. Unlike other plant tissues, ripe fruits apparently lack the hydrolytic enzymes needed to release cyanide during and following ingestion by birds. The compound is apparently absorbed by the intestine, but is excreted intact in the urine (thus cyanide is not released). Because cyanide is normally released by b-glucosidase enzymes in invertebrate and mammalian guts, this compound seems to be directed toward those (presumably detrimental) consumers.

Another example is that of quinolizidine alkaloids in fruits of *Viscum cruciatum,* which is hemiparasitic on *Retama sphaerocarpa* (Martin-Cordero et al. 1997). Alkaloids within fruits of this mistletoe are acquired via the phloem of the host plant. But, of the host alkaloids, the most broadly and lethally toxic types (the alpha-pyridones: cytisine, anagyrine, N-methylcytisine) are not found in ripe fruits (only in leaves and other tissues). This suggests specific exclusion from the ripe fruits of the alkaloids which may be most toxic to vertebrate seed dispersers. The alkaloids that remain in the ripe fruits, the bipiperyl alkaloid ammodendrine and the quinolizidine alkaloids retamine and lupanine, while somewhat toxic, may be directed primarily toward other (damaging) organisms and do not seem to negatively affect avian frugivores of this species.

Even "primary" chemicals such as sugars and simple organic acids might act as defensive compounds in fruits and thus serve secondary roles (Cipollini & Stiles 1991). For example, high levels of sucrose in ripe fruits might explain why mammals tend to disperse *Vaccinium stamineum* (Deerberry) whereas passerine birds tend to disperse related *Vaccinium* species high in glucose and fructose (Darnell et al. 1994). In addition to accumulating high levels of sucrose, which many passerine birds cannot digest (Martínez del Río et al. 1989), *V. stamineum* fruits also tend to drop to the ground and to remain green when ripe, which fits other aspects of the classical mammal dispersal syndrome. We might ask if this and other "primary" compounds (e.g., citric acid) that occur at very high levels in some fruits are actually "secondary" defenses directed toward non-dispersing organisms. Or, more generally, (and to go out on a limb here) we might ask if all nutrients such as glucose and fructose that occur at high levels in ripe fruits ought to be considered "secondary metabolites" since their main roles at those concentrations in those tissues are not physiological, but ecological.

When unpredictable variation exists among fruit consumers in their effects upon seed dispersal, secondary metabolites should be general in toxicity. Under this scenario, seed-dispersing and non-seed-dispersing frugivores alike should be affected adversely by pulp secondary compounds. At the very least, patterns of toxicity should be unrelated to the potential fitness benefit of the consumer for the plant. This general toxicity hypothesis - an alternative to the directed toxicity hypothesis - suggests that diffuse interactions between frugivores and fruiting plants should constrain selection for directed toxicity (Cipollini & Levey 1997a, 1997b, Levey & Cipollini 1998). This could explain the presence of phytochemicals with broad-spectrum activities found in many ripe fruits.

Defense Tradeoff Hypotheses

Whereas the directed and general toxicity hypotheses as stated above refer only to vertebrates, the various defense tradeoff hypotheses are based upon the explicit assumption that toxins in ripe fruit are directed primarily at microbial pathogens and invertebrate pests that never act as dispersal agents. That is, these hypotheses assume a priori a particular level of directed toxicity. Because effects of microbes and invertebrates upon seed dispersal are presumed to be uniformly negative (cf., Buchholz & Levey 1990, Cipollini & Stiles 1993a, 1993b), the occurrence of secondary metabolites within ripe pulp should represent a tradeoff between defense from damaging agents and negative effects on dispersers (Herrera 1982). Fruiting plants that are at great risk of pest/pathogen attack should be under greater selection pressure for fruit defense than are plants with low risk of pest/pathogen attack.

For example, Kreuger & Potter (1994) conducted an experiment using fall armyworms (*Spodoptera frugiperda*) and Japanese beetles (*Popilla japonica* Newman) fed diets containing crude secondary extracts of *Ilex opaca* (American Holly). Holly fruits contained secondary chemical defenses against insects; saponins were highest in unripe fruits, whereas tannins were highest in ripe fruits. While fall armyworm grew more slowly on a diet containing dried, milled ripe fruit than on a control diet, only extracts from unripe fruit were repellant to Japanese beetles. The authors concluded that "Levels of secondary compounds in fruits of *I. opaca* may be a compromise [resulting from] the continued need to protect the seeds from pre-dispersal damaging agents without deterring potential vertebrate dispersers." This conclusion is consistent with the defense tradeoffs concept.

Plant fitness is primarily a product of the numbers of seeds brought to maturity, the number of seeds dispersed to safe sites, and seed germination rates. Selection for the retention of secondary metabolites within ripe fleshy fruits might reflect the relative effects of a particular chemical profile on pre-dispersal fruit survivorship, deterrence or attraction of effective dispersers, and effects on post-dispersal success. Any secondary chemical profile, which, on average, nets an overall gain in fitness, ought to be retained in the ripe fruit. As such, fruits that are highly deterrent to most frugivores (via general toxicity) may obtain high fitness by being extremely persistent (via chemical defense). Therein lies the defense-tradeoff. As long as seeds are dispersed before the next opportunity for successful seed germination arrives, it matters not how long the dispersal process takes. A prediction arising from this conclusion is that well-defended fruits ought to be especially common in highly seasonal environments just before the inimical season (e.g., the temperate winter). This seems to be the case for what has been termed "fall, low-quality" temperate fruits (Cipollini & Stiles 1992a, 1992b).

Removal rate and relative risk models

Based on Herrera's framework, Cipollini & Stiles (1992b, 1993a) developed a set of alternative models for fruit defense tradeoffs. The first hypothesis, the Removal Rate model, suggests that fruits, which are typically rapidly removed by frugivores upon ripening, should require relatively low levels of chemical defense. The second hypothesis, the relative risk model, suggests that fruits, whose intrinsic and/or extrinsic characteristics result in fast microbial growth and/or high levels of pest attack, should require relatively high levels of chemical defense. To avoid a "chicken and egg" problem, it is important to keep in mind that these hypotheses consider all factors that contribute to variation in dispersal rates or damage in absence of secondary chemicals (Cipollini & Stiles 1992b).

These two models lead to contrary predictions for the broad-spectrum defense of fruits that vary in nutrient content. The removal rate model predicts that nutrient-rich fruits, being rewarding for frugivores, should naturally be removed from plants quickly and thus should require very little chemical defense. The relative risk model, on the other hand, predicts that nutrient-rich fruits, being especially susceptible to pest and pathogen attack, ought to contain high levels of general toxins.

Most evidence seems in favor of the removal rate hypothesis. Species whose fruits are high in nutrient quality and which are removed very predictably and rapidly by consumers tend to show dramatic losses of broad-spectrum defenses with ripening (Cipollini & Levey 1997a, 1997c). For example, while the arils of Guarana (*Paullinia cupana*) fruits show a complete loss of caffeine during ripening, caffeine remains in the mature seeds at levels 3.5 times higher than in coffee bean (Baumann et al. 1995). The toucans (*Ramphastos* spp.) and guans (*Penelope* spp.), which disperse Guarana seeds are presumably very sensitive to caffeine (based on experiments with domestic birds), as are most vertebrates. However, after ingestion of Guarana seeds by birds, most of the caffeine remains in the seeds as the arils are stripped and digested during gut passage. Even incidental seed destruction (as by a seed predator) releases much caffeine. A similar pattern can be seen in the bright yellow arils of the Ackee Fruit (*Blighia sapida*), in which the complete conversion of generally toxic hypoglycin A to the presumably non-toxic hypoglycin B occurs during ripening (D'Mello 1997, Brown 1999). Because observations suggest that these fruits are typically removed very rapidly and predictably by frugivores, the data indirectly support the Removal Rate model.

Another example, which may support the removal rate hypothesis, is the fate of ebulin f, a ribosome-inactivating protein (RIP) that is found exclusively in immature fruits of *Sambucus ebulus* (Citores et al. 1998). RIPs are plant-derived N-glycosidases that impair protein synthesis in sensitive ribosomes from animals, fungi, plants, and bacteria. Many are antiviral compounds (e.g., anti-HIV) with no negative effects on animal cells (e.g., Non-toxic Type 1: pokeweed antiviral, gelonin, etc.; Non-toxic Type 2: nigrin B and ebulin 1). Other forms are also toxic to animal cells and thus are broad-spectrum (e.g., Toxic Type 2: ricin, abrin, etc.). Ebulin f, the "fruit form" of ebulin 1, is broadly toxic to animal cells and is lost almost entirely during ripening of *S. ebulus* fruits (Citores et al. 1998). Rapid removal of *S. ebulus* fruits by seed dispersing frugivores is necessary to prevent destruction of fruits and seeds by organisms that had been deterred by the RIP in unripe fruits. Again, this is consistent with the Removal Rate model. An interesting question is whether non-toxic type RIPs (listed above) are more likely to be found in ripe fruits than in other plant tissues. The Microbe/Pest Specificity model (see below) would predict this.

In contrast, extracts from ripe Chinaberry (*Melia azedarach*) fruits have been found to be toxic to larvae of the diamond back moth, *Plutella xylostella* (Chen et al. 1996). Fruit extracts affect survivorship, feeding rates, and growth of this insect pest and are probably due to two tetranortriterpenoid limonoids: 1-cinnamoyl-melianolone and 1-cinnamoyl-3,11-dihydroxy-meliacarpin. These compounds may show toxic effects on vertebrates, however, this has been little explored. We do know that the one-seeded drupes of chinaberries are considered to be very malodorous and obnoxious to humans. They are very persistent when ripe and tend to have low rates of removal by frugivores in areas where the species is naturalized (personal observation). This pattern is likewise consistent with the removal rate hypothesis.

Nutrient/toxin titration model

This defense-tradeoffs model (Cipollini & Levey 1997a, 1997b) is similar to the relative risk model in that it postulates a positive relationship between general toxin content and nutrient content. It suggests that nutrient-rich fruits should be profitable enough for frugivores to allow the retention of higher levels of general defenses. Toxin levels in fruits are, in a sense, titrated against fruit nutrient content by foraging animals. Two factors make the Nutrient/Toxin Titration model distinct from the Relative Risk model: 1) High nutritional quality must be able to compensate for high levels of toxins (a factor unique to the Nutrient/Toxin Titration model) and 2) Low nutrient fruits can contain high levels of toxins — but only if they are otherwise at great risk of microbial/pest damage (a factor unique to the Relative Risk model).

The only direct test of this hypothesis is that of Cipollini & Levey (1997a, 1997b) focusing on

GAs of *Solanum* fruits. Because nutrient variation within the diets of frugivores did not compensate for the deterrent effects of GAs at levels typical of "toxic" *Solanum* fruits, this hypothesis was not supported. In fact, both the relative risk and nutrient/toxin titration models have little support thus far. Based upon these studies, it is predicted that high levels of broad-spectrum toxins should be rarely found in highly nutritious fruits. Thus, if highly rewarding fruits are to remain attractive to frugivores, and yet require defense from pests and pathogens, they should possess only compounds with few or no negative effects on the frugivores (e.g, microbe/pest specific defenses).

Microbe/pest specificity model

This defense-tradeoffs model borrows heavily from the Directed Toxicity hypothesis (Cipollini & Stiles 1992b, 1993a). It proposes that fruits requiring high palatability and/or low toxicity for dispersal should contain toxins specific to microbes and invertebrate pests, with little or no effect upon vertebrate seed dispersers. In contrast, the Directed Toxicity hypothesis focuses exclusively on effects among vertebrates. This may not be an important distinction, and one may consider the Microbe/Pest Specificity model to be analogous to the Directed Toxicity hypothesis. Yet, microbe/pest specific toxins are more like to evolve than are toxins directed at particular vertebrate species or groups. This is because of the similarity of molecular targets possessed by vertebrate species in comparison with the relatively large differences between vertebrates and non-vertebrate pests. It is chemically more plausible for a compound to evolve that is directed against microbes or insect pests than it is for a compound to evolve that is active only against certain vertebrate species.

There are a number of recent papers suggesting that secondary metabolites of fruits may be directed primarily toward microbes and or other pests with little or no effects upon vertebrates. In fact, ripe fruits might be prime locations to search for such phytochemicals. For example, octanoic acid is a secondary metabolite found in ripe fruit only of *Morinda citrifolia* (Legal et al. 1994, Farine et al. 1996). This and related compounds defend *M. citrifolia* from generalist *Drosophila* species which consume only ripe fruit pulp. Only a single species of fruit fly, *D. sechellia* is known to tolerate the compound. In fact, this secondary compound may be involved in speciation of *Drosophila* (R'Kha et al. 1990, R'Kha et al. 1997), as *D. sechellia* is a specialist only on *M. citrifolia* and uses octanoic acid as a foraging cue. At the levels found in ripe fruits, octanoic acid is presumed to be relatively non-toxic to vertebrates, thus suggesting that this compound is pest-specific (with possible utility as a natural pesticide). In fact, fruit juice and dry extracts from *Morinda citrifolia* (Noni) are popular herbal health remedies commonly consumed by humans.

Another possible microbe/pest specific defense pattern is the allocation of persin in avocado (*Persea americana*) fruits (Rodriguez-Saona et al. 1997). Idioblast oil cells of ripe fruits contain persin ((12Z, 15Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene), which has strong anti-insect and anti-fungal properties. For example, persin has strong effects on larval growth, mortality and food preferences in *Spodoptera exigua* (Hubner) larvae, a generalist herbivore. But, idioblast cells are not toxic to vertebrates, because the tough walls are apparently not broken down by mammalian or avian digestion. This allocation pattern is apparently directed toward insect and microbial pests that break open the idioblast cells during consumption.

Physico-chemical defenses might also be directed primarily toward pests and pathogens. For example, fruit skins (exocarps) of ripe fruits are often laden with lignified schlerenchyma cells, calcium oxalate crystals, tannins, and other physical and chemical defenses. These apparently function primarily as defenses against microbes and insects, whereas the skins are often passed through the gut intact following consumption by frugivorous animals. Plant species can vary in the thickness and composition of the fruit skin. For example, *Ilex opaca* fruits have thicker, more continuous layers of lignified sclerenchyma cells than the congener *I. verticillata* (Gargiullo & Stiles 1993), and apple (*Malus* spp.) varieties vary in the amount and distribution of tannins and other phenolics within the skin (Lees et al. 1995). While animals may have to deal with excess indigestible ballast, which may entail some costs, fruits that have these layers may be better protected against pests and pathogens than those without.

Under some circumstances, compounds (e.g., capsaicin in *Capsicum* spp.) may be directed toward non-vertebrate pests and pathogens (e.g., fungi and insects) as well as toward non-dispersing vertebrates (e.g., seed predatory mammals), while remaining inactive toward dispersers (e.g., frugivorous birds). For example, Tewksbury & Nabhan (1999) studied over 150 hours of video tape of *Capsicum annuum* var. *aviculare* and discovered that ripe fruits were eaten only by birds, mostly by the curve-billed thrasher (*Toxostoma curvirostre*). Ripe fruits contain high levels of capsaicin, yet birds lack the molecular receptor for capsaicin and are not deterred by this compound (Mason & Clark 1995). In experiments with captive animals, packrats and mice strictly avoid both real fruits (Tewksbury et al. 1999) and artificial fruits containing capsaicin (Mason et al. 1991, Norman et al. 1992). While seeds passed through mammals failed to germinate in the Tewksbury et al. (1999) study, seeds passed through birds germinated at high frequencies, demonstrating that the birds were the only effective dispersers.

A NEW ADAPTIVE HYPOTHESIS: DIRECT NUTRITIONAL BENEFITS

An implicit assumption in all of the above is that secondary metabolites of ripe fleshy fruits have no direct nutritional benefits for consumers. Based in part upon review of recent work in the nutritional, agricultural, and medicinal literature (cf., Gehm et al. 1997, Madhavi et al. 1998, Sun et al. 1999), I propose a challenge to this assumption in the form of a new adaptive hypothesis: the Direct Nutritional Benefits hypothesis. This hypothesis posits that some secondary metabolites are retained in ripe fruits because they have direct dietary benefits; they are, in this sense, no different than commonly understood rewards such as water, carbohydrates, lipids, proteins, minerals, and vitamins. Cipollini & Stiles (1993a) and Bairlein (1996) hinted at this conclusion when reporting that consumption of artificial fruit pulp media by birds is occasionally stimulated directly by secondary chemical extracts.

Some fruit secondary components may even promote animal health and welfare via roles as natural medicines, which prevent or cure chronic diseases and disorders. For example, *Solanum* GAs within fruits of *S. lycocarpum* eaten by the Maned Wolf (*Chrysocyon brachyurus*) have been associated with prevention of endoparasitism by *Dioctophyma renale*, the Giant Kidney Worm (Motidome et al. 1970, Dietz 1984, Gorog 1999). Up to 65% of Maned Wolf diets consist of this fruiting species. Perhaps antifungal and other antimicrobial agents within ripe fruits might also serve similar functions in helping to prevent or cure disease in dispersers (cf., Zafriri et al. 1989, Howell et al. 1998), thus serving as rewards rather than repellants.

In another case, note that we usually assume that the function of dark or contrasting fruit pigments is to increase the probability of discovery by frugivores (Gross 1987). Hypotheses for the evolution of fruit pigments are generally formulated with respect to the light absorptive characteristics and hence color perception of these compounds (cf., Willson & Whelan 1990). Nevertheless, pigments are very often found throughout the interior of ripe fruits and may consist of a bewildering diversity of chemical forms that contribute little to overall color variation (Britton & Hornero-Mendez 1997, Brouillard et al. 1997). For example, while leaves contain as few as five types of primary carotenoids (which function if photosynthesis), about 60 different secondary carotenoids have been identified within ripe *Capsicum* fruits (Britton & Hornero-Mendez 1997). Interior pigments can hardly function as attractants, so why are they present, why are they so diverse, and what are their adaptive functions, in any? One possibility is that they are nutritionally beneficial.

Are some fruit pigments self-advertising nutrients? So far, the case for direct dietary benefits of particular fruit pigments is rather compelling. For example, ripe *Vaccinium* (Wild Blueberry) fruits are one of the richest sources of antioxidant phytonutrients (Prior et al. 1998). The antioxidant capacity of ripe fruits is associated with elevated anthocyanin (flavonoid) pigments. In some cases, antioxidant capacity can increase over 200% during ripening. Of what significance is this antioxidant capacity? It seems that diets high in fruit-derived anthocyanins and other antioxidants are associated with lower levels of cancer (Hertog et al. 1997, Giovannuchi & Clinton 1998, Nishino 1998, Pastori et al. 1998, Swanson 1998), heart disease (Leake 1997), and age-related physiological and performance declines in both animals and humans (German & Dillard 1998, Joseph et al. 1998, Joseph et al. 1999, Rao & Agarwal 1999). These effects can be seen even in very short-lived animals such as mice and rats (Joseph et al. 1998, Joseph et al. 1999). Possible direct nutritional benefits of fruit pigments bring to light a central issue concerning fruit secondary chemicals; the likelihood that many compounds may serve multiple roles within ripe fleshy fruits.

GENERAL DISCUSSION AND NEW DIRECTIONS

Synergism: The batter is more than flour, milk, and eggs

Because plant tissues typically contain hundreds of different chemicals, the effect of any given secondary metabolite may depend upon the particular chemical mixture in which it is found. Some examples include the interaction of plant polyphenols and polyphenoloxidase enzymes (Appel 1993) and the greater repellant/toxic effects of crude extracts over isolated components (Jakubas et al. 1992). In light of this, studies of secondary metabolites of fruits are perhaps best accomplished by focusing on the activity of the secondary chemical mixture or crude extract, rather than that of isolated compounds. Efforts to attribute biological activity to single compounds often fail (cf., the search for the "principal" antimicrobial compound in cranberries; Ofek et al. 1991). Questions might focus on the activity of the secondary chemical profile as a whole, or on the functions of particular compounds in a mixture. Either way, the chemical mixture and the likelihood of synergism ought to be considered (cf., Hay 1996, Pennings 1996). This holistic view is sometimes hard for traditional phytochemists to swallow because it seems to lack focus on specific structure-activity relationships of individual compounds. This isn't true, of course; the interactions that result from structural features of different molecules are simply more complex than that envisioned from focus on the bioactivity of individual compounds. And, animals do not interact with individual compounds.

Multifunctional metabolites: Molecular "jacks of all trades"?

The assumption that most secondary chemicals are highly specific in function and narrow in biological spectrum is probably incorrect. Consider the multiple activities of the GAs of *Solanum* species, some of which have already been discussed. Like other secondary metabolites, GAs have multiple effects which may reflect an economical solution to diverse selective pressures. 1) Human perception of GAs includes flavors ranging from "hot" to "spicy" to "bitter" to "attractive" depending upon concentration and pH (Zitnak 1979). 2) GAs can inhibit seed germination (Ghazi 1997), including that of seeds of the fruits from which they were derived (Wahaj et al. 1998, Campbell & Van Staden 1998). 3) GAs may have constipative effects on birds (Wahaj et al. 1998) and may cause laxative effects in mammals (Zitnak 1979). 4) At high levels, GAs seems to be generally toxic and deterrent to vertebrates (Zitnak 1979, Cipollini & Levey 1997c). Deterrent and toxic effects are partly due to anti-acetylcholinesterase activity of the steroid component of these molecules (Roddick 1989). 5) At high levels, GAs show broad-spectrum antifungal and anti-invertebrate activity, apparently via the disruption of cellular membranes via sterol binding (Tingey 1984, Van Gelder 1990, Keukens et al. 1992). 6) GAs show synergistic interactions with other GAs (Roddick et al. 1990) and with organic acids (via pH-dependent toxicity and taste perception; McKee 1959, Zitnak 1979, Roddick 1987). 7) GAs have potent anti-cancer properties (Cham et al. 1987, Cham & Meares 1987), via their direct effects on the regulation of apoptosis (programmed cell death; Chang et al. 1998). 8) While toxic to vertebrates at high concentrations, at low concentrations GAs may positively affect abnormal brain functioning via their anti-acetylcholinesterase activity (Perry 1997). 9) GAs may be active against parasites of vertebrates (Motidome et al. 1970, Dietz 1984, Chataing et al. 1996, Gorog 1999). My adaptationist conclusion from all of this is that the allocation of a particular level of a given GA in ripe fruits may be the net result of various beneficial functions outweighing the costs of toxic and deterrent effects toward dispersers.

Other examples of multifunctionality are likely to be found, once focus is properly placed. For example, refer to the seed predator deterrence of capsaicin in *Capsicum* fruits (Tewksbury & Nabhan 1999). Capsaicin may also have a role in the defense of fruits from fungal and invertebrate pests (Zitnak 1979). In addition to its defensive roles, Tewksbury & Nabhan (1999) also reported effects on seed germination and retention times of seeds in the guts of dispersers, strongly supporting the concept of multifunctionality for this compound. Finally, Surh et al. (1998) report potent anticancer/antimutagenic properties for this compound, which is a possible direct dietary benefit for frugivores.

Evidence from studies of *Solanum* GAs and other fruit chemicals argues strongly against the hypothesis that different compounds must mediate distinct functions. In fact, it is becoming more widely recognized that multiple functions exist for biological compounds as molecular techniques (especially) continue to identify multiple physiological roles for single compounds. It would be nice to be able to say, for example, that a particular secondary compound evolved via selection for a singular function and that this function alone has been maintained throughout the rest of evolutionary time. However, molecular structures and profiles are certainly modeled over time and such traits affect plant fitness regardless of how and for what purpose a given carbon skeleton originally evolved. The trait "secondary metabolites of ripe fleshy fruits" is thus determined not only by the genes allowing a particular chemical structure, but also by genes affecting the allocation patterns of that chemical and those that regulate patterns of potential chemical synergists or inhibitors (i.e., phenotypic effects may involve epistatic and pleiotropic gene interactions). While multifunctionality could be the accidental result of selection for a singular important function, I believe that it is more likely to be the product of evolutionary pressures favoring economical solutions to multiple selective pressures.

Metabolic pathways are usually elaborations of primitive pathways, as seen from the chemotaxonomic utility of secondary metabolites. It is parsimonious for novel functions to evolve via the regulation and modification of existing pathways in different tissues and at different times, and by slight chemical modification of existing carbon skeletons. In contrast the de novo evolution of highly distinct compounds in response to selection for novel functions should be relatively uncommon. Even slight modifications of carbon skeletons can vastly affect function, for example, in the case of capsaicin and related compounds (Mason et al. 1991, Jakubas et al. 1992, Mason & Clark 1995) and in variations in the glycosides of *Solanum* GAs (Chang et al. 1988).

In explaining the multiple molecular target sites of ergot and quinolizidine alkaloids, Michael Wink (1998) states: "If we accept the hypothesis that alkaloids were developed as chemical defense compounds through a process of 'evolutionary molecular modeling', the 'cross reactivity'... makes sense: any compound that can interfere with more than one target or with more than one group of adverse organisms is likely to be more toxic and thus has a better survival value in general than a more selective allelochemical". He concludes by saying that nature has obviously tried "to catch as many flies with one clap as possible" in the selection of alkaloids during evolution. These statements agree in general with the concept that, due to the inherent unpredictability of plant-animal interactions, compounds with multiple functions should be favored during evolution. Because fruit-frugivore interactions may be among the most diffuse of ecological mutualisms (Jordano 1987b), with both positive and negative effects resulting from fruit consumption, selection for multifunctionality ought to be especially common. From the adaptive perspective, it only remains to be determined which functions are paramount and which are happenstance.

CONCLUSIONS: WHERE DO WE GO FROM HERE?

I have re-examined seven hypotheses that attempt to explain the presence of secondary metabolites in ripe fleshy fruits, and have presented and discussed a new hypothesis called the Direct Nutritional Benefits hypothesis. Despite recent work, experimental studies focusing explicitly upon these hypotheses continue to be rare. With the exciting potential that this research promises, the area remains wide open for exploration, and I look forward to seeing more direct tests of these hypotheses. Researchers should keep in mind that it is likely that evolution of secondary metabolites in fleshy fruits may result in selection for multifunctionality and that synergism may be common. Because secondary metabolites may be expensive to produce, selective pressures may lead to the economical solution of retaining chemicals that have different functions under differing sets of circumstances. This means, of course, that many of the proposed adaptive hypotheses are not mutually exclusive.

I also wish to reinforce the notion that patterns of secondary chemistry in ripe fleshy fruits might also be the non-adaptive result of phylogenetic and/or physiological constraints (Ehrlen & Eriksson 1993, Eriksson & Ehrlen 1998), but see Cipollini & Levey (1998). This position stands as an alternative to the adaptive hypotheses presented in this paper. As such, comparative studies of patterns among plant species should attempt, where possible, to incorporate a phylogenetic framework into the study design. Regardless, continued study of the current functions of secondary metabolites of ripe fleshy fruits can lead to great insight into current fruit-frugivore interactions regardless of the original "raison d'etre" (sensu Fraenkel 1959) of such compounds.

On another note, rarely have fleshy fruits been examined for biologically active components of value to humans (cf., Cutler 1988). Because ripe fleshy fruits are designed to be consume by vertebrates, they should be an ideal place to look for compounds that are directed toward organisms other than vertebrates (including humans). For example, potential benefits for humans for the most common *Solanum* GAs include the prevention and elimination of cancers (Cham & Meares 1987), weed control (Campbell & Van Staden 1998), inhibition of endoparasites (Motidome et al. 1970, Dietz 1984; Chataing et al. 1996), alleviation of mental diseases (Evans & Somanabandhu 1977, Perry 1997), lowering of cholesterol levels (Sidhu & Oakenfull 1986), and control of molluscs (Medina 1984). Thus, a potential side-benefit of attempts to delineate the roles of secondary metabolites in mediating fruit-frugivore interactions lies in the discovery of such chemicals of importance to human health and welfare. Ripe fruits consumed by mammals and birds ought to be a primary focus of efforts to identify such beneficial compounds.

Returning to my principal questions, I conclude that secondary metabolites may serve a variety of functions, many relating to the mediation of fruit-frugivore interactions. Frugivores may eat the fruits they do, not only because of their nutritional or morphological characteristics, but also because of the particular profile of secondary metabolites they contain. In fact, secondary chemistry might even be a primary determinant of the patterns of frugivory that we see. Studies addressing these issues involve the integration of a wide variety of areas including phytochemistry, plant, animal, invertebrate and microbial physiology and ecology, comparative evolutionary biology, etc. In this regard, collaborations of ecologists with phytochemists certainly should be encouraged. Despite the inherent difficulty of conducting good experimental work in this area, there is much to be gained through such efforts. Like it or not, biologists have assumed a huge task in facing the rapid rates of habitat destruction and global change that are presently taking place. Understanding the nature of the interactions between vertebrates and the fruits upon which they depend may help efforts directed toward the conservation of these interacting species (cf., Tewksbury et al. 1999).

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