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Studies with butterfly bait traps: an overview

Estudios empleando trampas de cebo para mariposas: una revisión

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Abstract: In the last decades, there has been a considerable increase in literature concerning ecological studies employing bait traps to capture butterflies. The growing interest in this kind of studies has given rise to a demanding group of young students and researchers looking for information and standardized protocols. Due to such growing interest in bait trap studies, this review aims to discuss (i) the basic aspects of the main technique of collection and sampling methods, and (ii) alternative solutions of different bait trap surveys in the Neotropics. Common mistakes that could undermine the quality and comparability of obtained data are also discussed.

Key words: Fruit-feeding butterflies. Butterfly sampling. Inventories. Nymphalidae. Neotropic.

Resumen: Existe, en las últimas décadas, un aumento sustancial de literatura sobre estudios ecológicos que emplean trampas con cebo para capturar mariposas. El interés creciente por los estudios con trampas motivó un grupo exigente de estudiantes jóvenes e investigadores a la búsqueda de información y protocolos estandarizados. Con base en lo anterior, esta revisión tiene como objetivo discutir (i) los aspectos básicos de los principales métodos, y (ii) soluciones alternas de los diferentes estudios con trampas en el Neotrópico. Además se discuten los errores comunes que pueden disminuir la calidad y comparación de los resultados.

Palabras clave: Mariposas frugívoras. Muestreos de mariposas. Inventarios. Nymphalidae. Neotropico.

Introduction

In the last decades, there has been a considerable increase in literature concerning ecological studies employing bait traps to capture butterflies, from simple local butterfly surveys and field guides (Uehara-Prado *et al.* 2004; Silva *et al.* 2010; Pedrotti *et al.* 2011; Santos *et al.* 2011; 2014a,b,c,d, Bellaver *et al.* 2012; Silva *et al.* 2013), detailed comparative studies of sites with different degrees of disturbance (DeVries *et al.* 1997; Shahabuddin and Terborgh 1999; Ramos 2000; Fermon *et al.* 2000; Shahabuddin and Ponte 2005; Bobo *et al.* 2006; Barlow *et al.* 2007; Uehara-Prado *et al.* 2007, 2009; Pardini *et al.* 2009; Ribeiro and Freitas 2012), to studies on population ecology (DeVries *et al.* 1999b; Uehara-Prado *et al.* 2005; Grøtan *et al.* 2012; Tufto *et al.* 2012), edge effects (Bossart and Opuni-Frimpong 2009), temporal (DeVries *et al.* 1999c; DeVries and Walla 2001; Pozo *et al.* 2005, 2008; Ribeiro *et al.* 2010; Ribeiro and Freitas 2011; Nobre *et al.* 2012) and spatial (DeVries 1988; Schulze *et al.* 2001; Fermon *et al.* 2003; Ribeiro *et al.* 2008; Marini-Filho and Martins 2010; Luk *et al.* 2011; Ribeiro *et al.* 2012; Santos 2013; Checa *et al*. 2014) patterns of butterfly distribution, countryside ecology (Horner-Devine *et al*. 2003; Pozo *et al.* 2005, 2008; Dolia *et al*. 2008), rapid assessment of local butterfly diversity (Daily and Ehrlich 1995), restoration ecology (Sant`anna *et al.* 2014), ecological modeling (Jost *et al.* 2010), behavior of selected species (Alexander and DeVries 2012), and broad applied monitoring programs (Pozo *et al.* 2008; Costa-Pereira *et al*. 2013).

Basically, bait traps capture several different organisms, specially the so-called fruit-feeding butterflies, which encompass a non-monophyletic group of butterflies whose adults primarily feed on rotten fruits and other decaying materials, and can be easily attracted with baited traps (this issue will be further discussed later in this paper). Thus, there is a series of practical aspects that favor the use of baited traps in comparative studies, being the most important the comparability of independent samples, which allow simultaneous sampling with standardized efforts at different

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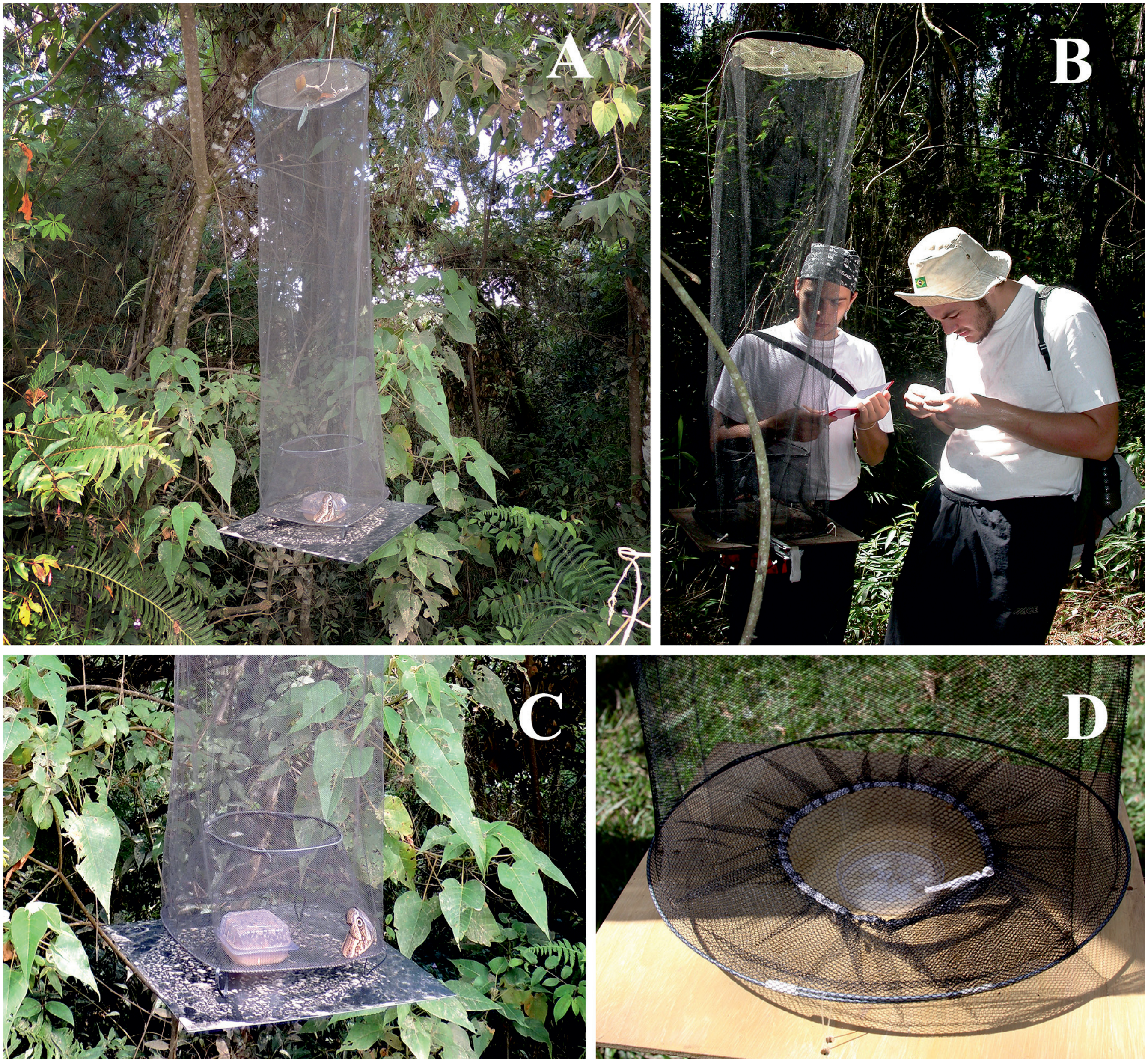

Figure 1. Details of bait traps. **A.** General view of a bait trap; **B.** Two researchers collecting and taking notes on captured butterflies; **C.** Detail of the trap base showing the internal inverted cone (with a brassoline – *Caligo arisbe* – trapped between the cone and trap netting) and the plastic pot containing the bait; **D.** Detail of a trap with a narrowing in the trap base (an alternative to the inverted cone showed before).

sites. Another practical advantage is the possibility of easily sampling butterflies along a vertical gradient in tall forests, including canopy or sub-canopy sampling.

Most fruit-feeding butterflies can be identified to the specific level in field trips, i.e., the majority of the captured individuals can be marked and released unharmed, so that recaptures can be evaluated with minimum handling. Moreover, the attraction of butterflies to a food resource reduces the fortuitous capture that might be present in other methods (DeVries and Walla 2001; Freitas *et al.* 2003; Uehara-Prado *et al.* 2005). Finally, experienced researchers do not influence sampling success in detecting and capturing butterflies with bait traps (capture is passive), a bias commonly present in other methods (Ebert 1969; Brown 1972; Pollard 1977; Caldas and Robbins 2003; Pozo *et al.* 2005; Nowicki *et al.* 2008; Iserhard *et al.* 2013).

The growing interest in bait trap studies (and alleged promises of publishing papers in good journals) has given rise to a demanding group of young students and researchers looking for information and standardized protocols. Due to such growing interest in this kind of study, this article aims to discuss (i) the basic aspects of the main methods, and (ii) alternative solutions of different bait trap studies in the Neotropics. Common mistakes that could undermine the quality and comparability of obtained data may be avoided by following basic and correct guidelines of the current methodology.

Butterfly bait traps: a historical approach. This section mostly follows Sevastopulo (1954), Rydon (1964), DeVries (1987, 1988), Sourakov and Emmel (1995), Austin and Riley (1995), Shuey (1997), Hughes *et al.* (1998), Freitas *et al.*

(2003, 2006), Uehara-Prado *et al.* (2005, 2007), as well as the authors' personal experience. There are many other studies discussing bait trap designs in the literature, and it is strongly suggested that anyone interested in starting a bait trap study become familiar with all alternative solutions to build a bait trap (See Pozo *et al.* 2008 and references therein).

A good historical review of butterfly traps can be found in Rydon (1964). Bait traps appeared in East Africa few years after the Second World War, and were originally designed to capture *Charaxes* butterflies (Charaxinae). Two kinds of traps appeared almost simultaneously, each of them with some advantages and disadvantages. The most used bait trap, also known as Van Someren-Rydon trap, (hereafter VSR traps) consisted of a cylinder made with netting and wire, closed at the top and open at the bottom, and attached to a base (usually a sheet of plywood or plastic) on which the bait is placed (Figs. 1A, B). There are no rules about the size of a bait trap, and dimensions vary amongst authors. However, a bait trap should be tall enough to prevent butterflies to escape after being trapped, ideally 100-130 cm tall, and its diameter should also be wide enough to allow the capture of the larger owl butterflies (Satyrinae: Brassolini), that is, not less than 25 cm wide. An internal inverted cone (about 20 cm wide at the opening) may be used to prevent butterflies from escaping (Fig. 1C) despite the lack of information in literature about cone efficiency, which has not been tested yet (an interesting variation of the internal cone is a narrowing in the trap base, as shown in Fig. 1D). Finally, whatever the shape, the base should be slightly wider than the netting cylinder, allowing butterflies to easily land at the base before entering the trap.

Although the effect of trap netting color in butterfly catchability has never been tested, dark colors such as green, black and grey have been successfully used, whereas conspicuous white netting has apparently kept away some butterflies (while attracting others, such as the big white species of *Morpho* in Southern Brazil). Light colors are also more conspicuous to people and its use may be problematic at inhabited areas.

Baits could be placed directly over the base, but ideally, they should be placed in plastic pots, thus facilitating cleaning and bait replacement. The plastic pot containing the bait can be covered with a perforated plastic cover, thus preventing butterflies from drowning in the liquid, avoiding feeding by other insects and reducing evaporation, while still allowing the odor to spread and attract butterflies. In addition, butterflies should land on top of the plastic pot, about 5 cm above the trap base, to access the bait. This places butterflies inside the trap and away from escape routes, also restricting their view fields and minimizing the light width they see between the trap base and the trap netting. We also suggest that researchers use resistant transparent plastic cover at the trap top; it does not only protect the bait and captured butterflies from heavy rains, but also prevents butterflies from escaping – once they tend to fly upward towards light passing through the transparent plastic.

Although this is the basic and most used design of a bait trap, this is not the only kind of trap used; several variations can be found, many of them practical and functional. An interesting variation is the horizontal bait trap, used by Accacio (2002), especially designed to be placed on the forest floor. This kind of bait trap has been conceived to enhance the capture of low flying satyrines, especially those belonging to the Neotropical tribe Haeterini (*Cithaerias*, *Dulcedo*, *Haetera*, *Pierella* and *Pseudohaetera*), which can be the most abundant fruit-feeding butterfly group in some sites in Amazonia. Most species of Haeterini usually fly very close to the forest floor, being rarely captured in bait traps placed higher than 30 cm above ground, as easily observed by reading species lists of most published bait trap studies in the Neotropics (see also Alexander and DeVries 2012). Besides Haeterini, horizontal traps proved to be very efficient in capturing several Morphini, Brassolini and small satyrines, consistently capturing them in higher numbers than traditional VSR vertical traps in every occasion when both traps were placed together. This type of trap can also be used in grasslands, where no trees are available for trap hanging.

Regardless of the trap type, it is extremely important that previous field tests are conducted so that all possible setbacks with the chosen design are solved before studies begin.

Fruit-feeding butterflies. Although baited VSR traps can attract and capture several different insect groups, the fruit-feeding (sometimes called 'frugivorous') butterflies are the main focus of the many published studies. Therefore, an important issue is to define them exactly.

Even when considering the complete diversity of all food sources used by adult butterflies, these can be roughly separated into two main feeding guilds (DeVries 1987): 1) the nectar-feeding, and 2) the fruit-feeding (See Hernández *et al.* 2008 for other classification, and Luis and Llorente 1990, Vargas *et al.* 1991, 1994, Luis *et al.* 1991 for studies in guilds).

Nectar-feeding butterflies gain most of their nutritional requirements from flower nectar, and most species are almost exclusively flower visitors, being the majority in practically all butterfly assemblages worldwide, and the totality in most temperate habitats and in some high mountain sites in the tropics. Conversely, fruit-feeding butterflies are typical of tropical and subtropical habitats, comprising, for instance, 50–75% of all Neotropical Nymphalidae (data obtained from the lists presented in Brown 2005; for other numbers see Vargas *et al.* 1994, 1999 and Pozo *et al.* 2008). They obtain their nutritional requirements from rotting fruits, plant sap and decaying material, as mammal excrement and carrion, and are infrequently observed visiting flowers (DeVries 1987, 1988).

In Neotropical habitats, the fruit-feeding butterflies are represented exclusively by the Nymphalidae subfamilies Satyrinae, Biblidinae, Charaxinae and also some Nymphalinae (a non-monophyletic group of genera previously treated as tribe Coeini) (following Freitas and Brown 2004 and Wahlberg *et al.* 2009) (Fig. 2). In the Neotropics, four tribes of Satyrinae are more commonly reported to be attracted to bait traps: Satyrini, Morphini, Brassolini and Haeterini (the latter easily captured with horizontal bait traps, as discussed before). Strictly, in any Neotropical site, only species in the groups aforementioned should be considered if fruit-feeding butterflies are studied. However, even in these groups there are some species that should not be included in trap studies, either because they are nectar feeders or are rarely attracted to traps (therefore, their abundances in sampling will not reflect the actual abundances in the field, characterizing a "methodological rarity" (Vargas *et al.* 1994, 1999). Within the Morphini, for example, species of high flying *Morpho* (the "*hecuba* group" of DeVries *et al.* 2010) are rarely captured in bait traps, even when they are locally abundant. In the Biblidinae, species of *Dynamine* are also nectar feeders

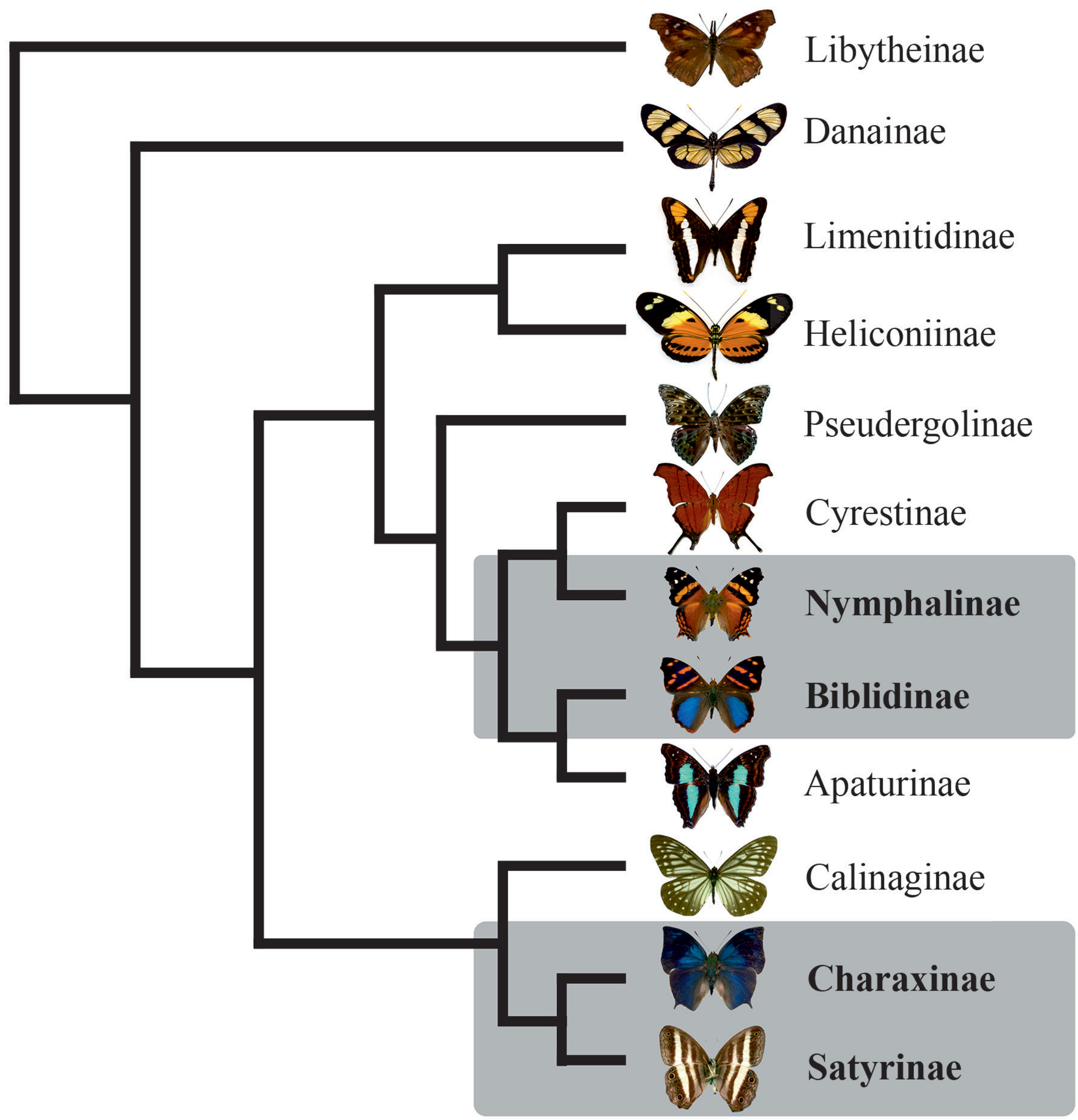

Figure 2. The phylogeny of Nymphalidae butterflies (based on Freitas & Brown 2004 and Wahlberg *et al*. 2009), highlighting the subfamilies of fruit-feeding butterflies present in the Neotropics (the grey rectangles).

and are seldom captured in traps; nevertheless, occasional captures should not be considered in bait trap studies (see below).

Several nectar-feeding butterflies (e.g. some Pieridae, Riodinidae, Hesperiidae, Nymphalidae subfamilies such as Limenitidinae, Cyrestinae and Apaturinae, and the tribe Ithomiini) are frequently captured on baits traps (although occurring in very low frequencies). Several published studies take account of such groups. However, as these butterflies feed primarily on flowers and could be influenced with uncontrolled variables (e.g. blooming), they should be considered as by-catches and ignored in bait trap studies. Additionally, although capture frequencies of these particular butterflies seem to be higher in drier environments, nectar-feeding species are usually represented by few individuals in trap studies; their inclusion, thus, "inflate" the number of rare species (singletons and doubletons) in the sample and strongly changes community parameters in alpha and beta diversity analysis, such as individual-based rarefaction, accumulation curves, estimation of species richness, and similarity indexes.

In conclusion, it is essential that only strictly fruit-feeding species be taken into account in bait trap studies. The inclusion of any additional species may strongly influence results biasing the analysis and influencing the robustness and accuracy of any diversity measurement in the study (as mentioned above).

The different types of baits. This section is mostly based on the authors' personal observations, as very little information about bait attractiveness is available in the literature (but see Molleman *et al.* 2005; Holloway *et* *al.* 2013; and Fucilini 2014). Moreover, negative results obtained with alternative baits are usually not reported, making this information unavailable for those interested in starting a bait trap study.

Several kinds of baits have been employed in bait trap studies, including many types of fruits, rotting fish and shrimp, liver and feces. These alternative baits are variable in attractiveness and can be used in specific occasions. They are discussed below:

Fruits. Virtually, any fermented fruit will attract fruit-feeding butterflies and can be used in bait trap studies. However, juicy fruits are much more effective in attracting fruit-feeding butterflies, particularly mango, pineapple, jackfruit and bananas (bananas will be discussed in details in the next topic). One or more of these fruits are regularly found in tropical places, and have been regularly used as baits. Avocado, melon, watermelon and citric fruits (such as grapefruit, orange and tangerine) have limited attractiveness. Some kinds of bananas (such as some large plantains) could be very dry and present little attractiveness, but they become excellent baits if soaked in liquid (see below). Fruits can be used alone or soaked in liquid to avoid fast drying in field conditions and enhance attractiveness. Sugar cane juice has been widely used in Brazil and in other tropical countries in Central and South America, but other fruit juices, beer, and even pure water have been used; the latter has proved to be very useful to moisten the bait in dry days or in dry locations (Vargas *et al.* 1994, 1999; Daily and Ehrlich, 1995). Finally, when commercial fruits are not available, native fruits can be collected, enriched with liquid, and used as baits, especially if the objective is to carry on a survey (when bait standardization is not needed).

The "standard bait" - banana and sugar cane juice. The traditional, widely used and successfully employed bait in trap studies in the Neotropics is a mixture of mature banana (processed, hand mashed or in pieces) with sugar cane juice, fermented to become attractive to fruit-feeding butterflies, hereafter known as "standard bait". Usually, a period of 48 hours is enough to provide very attractive baits. This kind of mixture has several practical advantages, including attractiveness to all fruit-feeding butterflies, low attractiveness to nectar-feeding species, usual ease of preparation, storing period over five/six days, and the possibility of standardization in sampling protocols (by controlling ingredients and fermentation time). However, although bananas can be easily found anywhere, sugar cane juice can be hard to find in some places. Recent attempts suggest brown sugar diluted in water as a good substitute for sugar cane juice, being that a very handy replacement in places or year periods when sugar-cane may be difficult to find (accordingly, diluted molasses can be also used in the same way). Some studies have suggested the use of alcoholic beverages, such as beer or rum, to turn the bait more attractive (e.g. Daily and Ehrlich 1995). However, this is not necessary, and can sometimes modify the mixture completely, making it non-attractive at all (by producing acetic acid instead of alcohol).

Rotting fish. Some studies have used rotting fish in addition to fermented fruits, or even as an alternative to it (Montero *et al.* 2009). Rotting fish is a very good bait, attracting not only fruit-feeding butterflies, but also several other butterfly groups, especially the genus *Adelpha* (Nymphalidae: Limenitidinae) and several Riodinidae. Although this bait is especially good to be used in short-term and maximized inventories, it is not recommended in comparative studies due to the difficulty to find the same kind of fish for bait standardization and unpredictability throughout rotting processes for each fish species. Finally, the strong and unpleasant smell of decaying fish should not be overlooked when using this type of bait, as it may commonly be emetic to people.

Feces. Feces of different types are frequently visited by many butterfly species, including some fruit-feeding groups. Although it seems an obvious bait choice, this is not true at all by three main reasons: 1) several species of fruit-feeding butterflies are not particularly attracted to feces, mainly Satyrini; 2) feces attract several nectar-feeding species as well as many other insects, e.g. flies and beetles (and, because feces are a preferred bait for other insects such as beetles, the concomitant use of butterfly and beetle traps can be conflicting); and 3) it is virtually impossible to standardize this kind of bait. However, besides the practical limitations that restrict the use of feces in comparative studies, these can be successfully used in general inventories, attracting many Charaxinae (*Prepona*, *Archaeoprepona*, *Fountainea* and *Memphis* are especially attracted to feces) and several Biblidinae (especially *Eunica*, *Nessaea* and some Callicorini). In addition, dog feces have been successfully used to attract the rarely collected *Narope* and *Aponarope* (Brassolini). As mentioned before for rotting fish, working with this kind of bait may be extremely unpleasant and demotivating for many people.

Other attractants. Besides the above cited examples, many other substances and substrates are known to attract fruit-feeding butterflies, and could be potentially used as baits. These include prawns, carrion, bird droppings and urine (Chermock 1952; Reinthal 1966; Payne and King 1969). However, most of the butterfly surveys on these alternative baits have been carried out in temperate countries, and detailed quantitative studies in tropical forests still lack. Again, the difficulty in standardization is the main limitation of using these alternative baits (but see Holloway *et al.* 2013 and Checa *et al*. 2014).

Bait attractiveness. As a final recommendation, it is important to highlight that bait exposure in field clearly decreases its attractiveness due to dryness, dilution by rain or decomposing. Bait replacement (between 24 to 48 hours at the utmost) is suggested to keep equivalent attractiveness throughout sampling days, regardless the kind of bait used (DeVries and Walla 2001; Uehara-Prado *et al.* 2007). If bait replacement is not possible, adding any spirits or beer to the bait is a good solution (this gave excellent results in studies from Mexico, J. Llorente, *pers. com.*).

Useful by-catches. Besides fruit-feeding butterflies, traps baited with the "standard bait" can attract and capture a plethora of other insects, including nectar-feeding butterflies, moths, beetles, many different dipterous families (especially fruit-flies), bees, and wasps. Even if bait traps were conceived to collect fruit-feeding butterflies, and consequently, are not ideal to collect most of the above groups, in some special cases these groups can be informative and useful, particularly in comparative studies (see below).

In addition to the fruit-feeding butterflies, Erebidae moths are a second group of Lepidoptera satisfying all premises to be used in bait trap studies (Süssenbach and Fiedler 1999; Freitas *et al.* 2003). These moths are primarily fruit-feeding, which means that their abundance in traps probably reflects the actual abundance of the species in situ (but this still should be tested). Moreover, many Erebidae are large in size, and some species can be easily identified in nature. Furthermore, there are virtually no studies with Erebidae moths employing bait traps (but see Süssenbach and Fiedler 1999, Ribeiro and Freitas 2010). Therefore, some groups of Erebidae moths are good candidates to be included as focal organisms in bait trap studies.

Ithomiine butterflies (Nymphalidae: Danainae: Ithomiini) are nectar feeders that can occasionally be captured in bait traps. However, capture success is variable in different sites, turning this group unpredictable to be regularly used in bait trap studies; whilst in some regions they are regularly attracted, in other places they can be completely absent (pers. obs.). However, there are at least two comprehensive comparative studies using Ithomiine specimens captured with bait traps. The first focused on fine scale Müllerian co-mimicry associations in a tropical forest (DeVries *et al.* 1999a), whereas the second evidenced that forest fragmentation affect mimetic and taxonomic composition (Uehara-Prado and Freitas 2009).

Many beetle species from different families are usually captured by bait traps, such as Cerambycidae, Sylphidae, Lucanidae, Scarabaeidae and Staphylinidae, and some of them are sufficiently abundant to generate useful data. A good example includes two Oedemeridae species in the study of Uehara-Prado *et al.* (2009), thus alleging that these beetles are more abundant in preserved areas than in disturbed ones. Bait traps usually attract several other arthropods, and it is strongly suggested that future researchers pay attention to them in order to get more data with the same trap effort. It is very important that each group be analyzed separately, so patterns become more consistent and comparable amongst different studies.

Species identification. The taxonomy of fruit-feeding butterflies is a common problem that inexperient researchers customarily face. Although some species are relatively easy to identify with the help of field guides and a certain period of training, this is not true for most Charaxinae (especially *Memphis*), several Biblidinae (the genera *Eunica* and *Callicore* can be problematic), and a large portion of the Satyrinae. The subtribe Euptychiina (Satyrini) is a special challenge, even to trained taxonomists, as many species within this group are very similar (the so called “small brown butterflies”) and remarkably variable, making it difficult to tell species apart (Marin *et al.* 2011).

Several field guides that include fruit-feeding butterflies have become available for neotropical localities in the last decades (D’Abrera 1987a, b, 1988; DeVries 1987; Brown 1992; Neild 1996, 2008, Luis *et al*. 2003; Uehara-Prado *et al.* 2004; Santos *et al.* 2011; 2014a, b, c, d; Warren *et al*. “Butterflies of America”: http://butterfliesofamerica.com/). Although most of the available guides are precise and complete for some specific localities, inexperienced people may either easily misunderstand the important traits that distinguish different species or place several species together based upon superficial wing patterns.

The following mistakes have been identified in recent literature and/or student projects:

1) Separation of species based upon general wing pattern. The two most common examples are: i) Separating Satyrinae (especially the Euptychiina) by the number of eyespots on the wings, resulting in a completely unnatural assembly of morphotypes, with no relation to real taxonomic entities, and ii) Separating species of the Anaeini tribe (Charaxinae) by considering either the amount of blue dye in dorsal wings, or the general ventral wing pattern.

2) Inclusion of species that do not occur in a given region either by using an inappropriate field guide (one from another geographical region, for instance) or by searching species in the field guide that resemble the one researchers actually have in hand.

3) Inclusion of species that do not occur in a given region by not identifying misplaced names in the guides, or by looking at the wrong figure caption.

4) Combination of two (or more) species together – one not figured in a field guide with another, which is figured – due to lack of taxonomic knowledge about the group.

All the above mistakes can be easily avoided with a minimum planning and consulting with experienced taxonomists. Thus, it is highly recommended to anyone interested in starting the study of butterflies to devote some time prior to field work to be familiar with the local fauna, which in turn might avoid common problems related to species identification.

In those cases when previous training is not possible, an alternative is to collect every single trapped individual and bring the collection for ulterior identification. Individuals should be killed and stored individually in glassine envelopes, frozen or kept in tightly sealed boxes with mold deterrent (to preserve general morphology and DNA) to prevent specimens from misidentification. Although almost all problems of species identification might be solved with such procedures, they may be destructive and prevent the obtainment of information on individual movement, longevity, and many other population data that could be relevant and interesting (e.g. Uehara-Prado *et al.* 2005, Marini-Filho and Martins 2010; Tufto *et al.* 2012).

Another approach based upon low identification capacity is to identify butterflies to a higher taxonomic level, namely genera, tribes, or subfamilies (Santos *et al.* 2014a, b, c, d). This approach is especially useful in places where data gathers are not specialists, and butterfly collecting is not a viable alternative (Costa-Pereira *et al.* 2013). Moreover, it has already been shown that fruit-feeding butterflies respond to environmental variation at high taxonomic levels (e.g. Brown and Freitas 2002; Uehara-Prado *et al.* 2007).

Sampling. Although the present article does not have the purpose of discussing a protocol for sampling with bait traps, some practical aspects should be focused to help students and researchers to plan a correct and well-developed sampling protocol in field studies.

The main goal in any bait trap study is the achievement of a comprehensive sampling that adequately reflects the local fruit-feeding butterfly community. Two conditions should be satisfied to attain such goal: 1) sampling unities should be defined objectively, and 2) sampling efforts should be adequate (See Pozo *et al*. 2008 for this topic).

Choosing specific placement sites may or may not be a concern in a butterfly trapping study, but inspecting the surroundings is desirable and some places must be avoided, as in some habitats traps are hardly able to compete with some local food sources, such as fruit or sap producing trees, in a short distance radius (10 meters or less).

There is no inferior or superior limit to the size of a sampling unit, but they usually consist of a group of three to five bait traps placed as to represent one habitat, or a portion of it (see for example DeVries *et al.* 1999c; Horner-Devine *et al*. 2003; Uehara-Prado *et al.* 2007; Marini-Filho and Martins 2010; Bellaver *et al.* 2012; Ribeiro and Freitas 2012; for transects, see recommendations in Pozo *et al*. 2008). Usually, a single bait trap should not be used as a sampling unit as local conditions could affect it hardly, which in turn strongly affects catchability; therefore, it might diverge forcefully from the local pattern. However, this is obviously related to the question to be answered, and some studies have successfully used single bait traps as sampling unities (Barlow *et al*. 2007; Ribeiro and Freitas 2012). Not uncommonly, bait trap studies have shown that some traps capture a large number of individuals, whilst a nearby trap can virtually attract no butterfly at all. By combining three or more bait traps, sampling unities become more homogeneous and equivalent, and more likely to represent the actual local fruit-feeding butterfly assemblage, instead of local idiosyncratic patterns.

It is also important that sampling unities are sufficiently distant to be spatially independent from one another. For example, Ribeiro *et al*. (2012) showed that the landscape within the nearest 200 m radius strongly affects sampling unities in the Atlantic Forest; it means that sampling unities should be distant at least 200 m from each other to be minimally independent in that biome.

As mentioned above, sampling effort is an important detail to be considered and planned before initiating any bait trap study. The total effort needs to be only barely sufficient to adequately represent the local assemblage, so that descriptions and comparisons can be reliable. If sampling is incomplete in space or time, or efforts are insufficient, general patterns can be masked and contradictory results are likely to be observed (see Hill and Hammer 2004). We also recommend that traps overnight in the field, or at least remain open from sunrise to sunset, since several fruit-feeding butterfly species fly preferentially at dusk (most Brassolini and some Satyrini and Morphini).

The ideal number of days to sample varies in localities and seasons. Ideally, one should test for sampling sufficiency before establishing the effort to be placed in each sampling period. Sampling success is variable in various habitats; i.e., a single trap can capture dozens of individuals in a single day at the end of the dry season in some semi-deciduous forests of Eastern Brazil, although results can be as low as 0.5 individual by trap per day in central Amazonia (Ribeiro and Freitas 2012). That means that the number of trap/days in the latter should be much higher than that in the former.

Sampling success is also related to season and weather conditions, such as rain and temperature. Ribeiro and Freitas (2010) found a positive relation between mean temperature and both richness and abundance of captured butterflies. Grotan *et al.* (2012) also found a positive relation of abundance with temperature and rainfall, but a negative relation between diversity and rainfall. However, despite the fact that warmer months would correspond to those with higher capture rates due to results obtained, such assumption is not always true. Recent studies have demonstrated that capture rates are higher during the dry season when in comparison with the wet season (unpublished results). These data do not mean that season is more important than temperature, but that both factors are interacting to produce the observed patterns of catchability tracked in most year round studies. Detailed population studies are to be further investigated in order to know to what extent catchability results reflect the real abundances of butterflies in the field.

A final detail to be considered in trap studies is the seasonal variation of local butterfly fauna. Ideally, sampling periods should be chosen to represent the majority of the local community. This is especially important in places where some abundant species are univoltine (adults with a single flight season), or at least concentrated in few months. For example, in Southeastern Brazil, summer months (December to February) are mandatory in a bait trap study as several abundant species of Morphini and Brassolini only fly in such period. In addition, the best months in this region are March to May (end of the rainy season) (Ebert 1969; Brown 1972, 1992). Consequently, researchers should extend the sampling period from December to April if for some reason a study had to be concentrated in five months. Any other five-month combination would be less than ideal, either because of the univoltine species absence or the inclusion of months with low butterfly densities.

All recommendations presented before are summed up as follows: 1) It is very important to define the number and size of sampling unities, as well as the total sampling effort and sampling months before initiating a bait trap study; 2) A good taxonomic training and a set of field guides with the local fauna are also desirable; 3) By following such procedures, the most usual problems can be avoided and reliable and useful results are to be achieved.

Concluding remarks. The first insights on bait trap techniques to sample fruit-feeding butterflies aimed to supply students and researchers interested in starting a study with this group with general guidelines. Given that information concerning bait trap studies is spread out in many publications, which are sometimes hard to be found, this revision is an attempt to combine most of the relevant extant knowledge into a single publication.

It is known that publishing representative species lists at any given site demands hard work and lots of time. Some researchers, however, seem to think antithetically, publishing unrepresentative lists, with sloppy sampling methods and low sampling effort, usually with insufficient or misleading information and several misidentifications. So, several research groups in Central and South America are collaboratively discussing the development of a general sampling protocol to be used in studies with fruit-feeding butterflies in different habitats and with distinct approaches.

The main objective of this paper is to discuss the implication of the use of bait traps, indicating the importance of sampling only the “true” fruit-feeding species, with special attention to their correct taxonomic identification. By following these steps correctly, both statistical designs and data obtained are likely to offer robust and reliable results about the diversity patterns of fruit-feeding butterflies in any study site.

Finally, this overview is scarcely a comprehensive revision on bait trap studies, although it is a first step to notify investigators and students about important points, and to provide information about the use of fruit-feeding butterflies in ecological studies. Likewise, all researchers interested in increasing knowledge on fruit-feeding butterflies in the Neotropics are invited to the discussion that is taking place in specialized media.

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