Jean Carlos Santos
Geraldo Wilson Fernandes *Editors*

# Measuring Arthropod Biodiversity

## A Handbook of Sampling Methods

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# Chapter 11
# Measuring Orthoptera Diversity


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## 11.1 Introduction

Orthoptera are insects with enlarged hind legs for jumping, well developed compound eyes, a large prothorax with a shield-like pronotum curving downward laterally, and mandibulate mouthparts for biting and chewing. This order occurs worldwide, in grasslands and forest areas, from the Arctic to tropical and desert regions. They are an ancient insect order, dating from the Paleozoic age, 300 million years ago (Grimaldi et al. 2005), and have 28,500 described species (Bidau 2014; Cigliano et al. 2020). The order has two suborders: (1) Ensifera, which comprehend true crickets (Gryllidae, Fig. 11.1a), mole crickets (Gryllotalpidae, Fig. 11.1b), camel crickets (Raphidophoridae), bush crickets or katydids (Tettigoniidae, Fig. 11.1c), grigs (Prophalangopsidae), wetas (Anastostomatidae, Fig. 11.1d), and Cooloola monsters (Coolooidae) and are characterized by long antennae (usually longer than the body), swordlike or needlelike ovipositors, and auditory organs, the tympani, on the front tibiae, and (2) Caelifera, which comprehend grasshoppers (Acrididae, Fig. 11.1e, f), ground-hoppers (Tetrigidae, Fig. 11.1g), and pygmy mole crickets (Tridactylidea), characterized by a shorter antennae, ovipositor with only two valvular pairs, and abdominal tympana.

Except for the subfamily Oecanthinae, all Orthoptera have a hypognathous head, with mouthparts directed downward and the head vertical in relation to the body axis (Johnson and Triplehorn 2005). The jaws of Orthoptera are large and very distinct between the groups, reflecting their varied eating habits. The presence of wings is prevalent. The first pair of wings comprehends the tegmina, which, in addition to protecting the second pair of wings, is related to sound production (stridulation) in Ensifera and some Caelifera. The second pair of wings is membranous, used for flight. The presence of developed anterior and posterior wings is not a rule in Orthoptera, since there are brachypterous, micropterous, and apterous species (Bidau 2014). Orthoptera presents paurometabolous development, i.e., from each egg hatches an immature, called nymph, that looks like an adult, but smaller, without wings and with fewer antennal articles (Sperber et al. 2012). The first and the second pair of legs are usually cursor-like, but in predatory species there are modifications with strong and curved spines (Sperber et al. 2012). In mole crickets and some species of grasshoppers (Tridactylidae), the first pair of legs is fossorial, allowing the excavation of burrows and galleries (Johnson and Triplehorn 2005; Bidau 2014).

In this chapter, we will present the diversity of habitats and behaviors of Orthoptera and the manner in which this diversity affects their sampling, presenting

**Fig. 11.1** (continued) P.G.B.S. Dias. (**e**) Grasshopper *Chromacris speciosa* (Thunberg, 1824) (Orthoptera: Caelifera: Romaleidae) from Belterra, Pará State, Brazil. Photo: L.D. Campos and P.G.B.S. Dias. (**f**) Unidentified grasshopper (Orthoptera: Caelifera: Acrididae) from Itatiaia, Rio de Janeiro State, Brazil; (**g**) Ground-hopper (Orthoptera: Caelifera: Tetrigidae) from Belterra, Pará State, Brazil. Photo: L.D. Campos and P.G.B.S. Dias. (**h**) Pterochrozinae (Orthoptera: Ensifera: Tettigoniidae) from Belterra, Pará State, Brazil. Photo: L.D. Campos and P.G.B.S. Dias

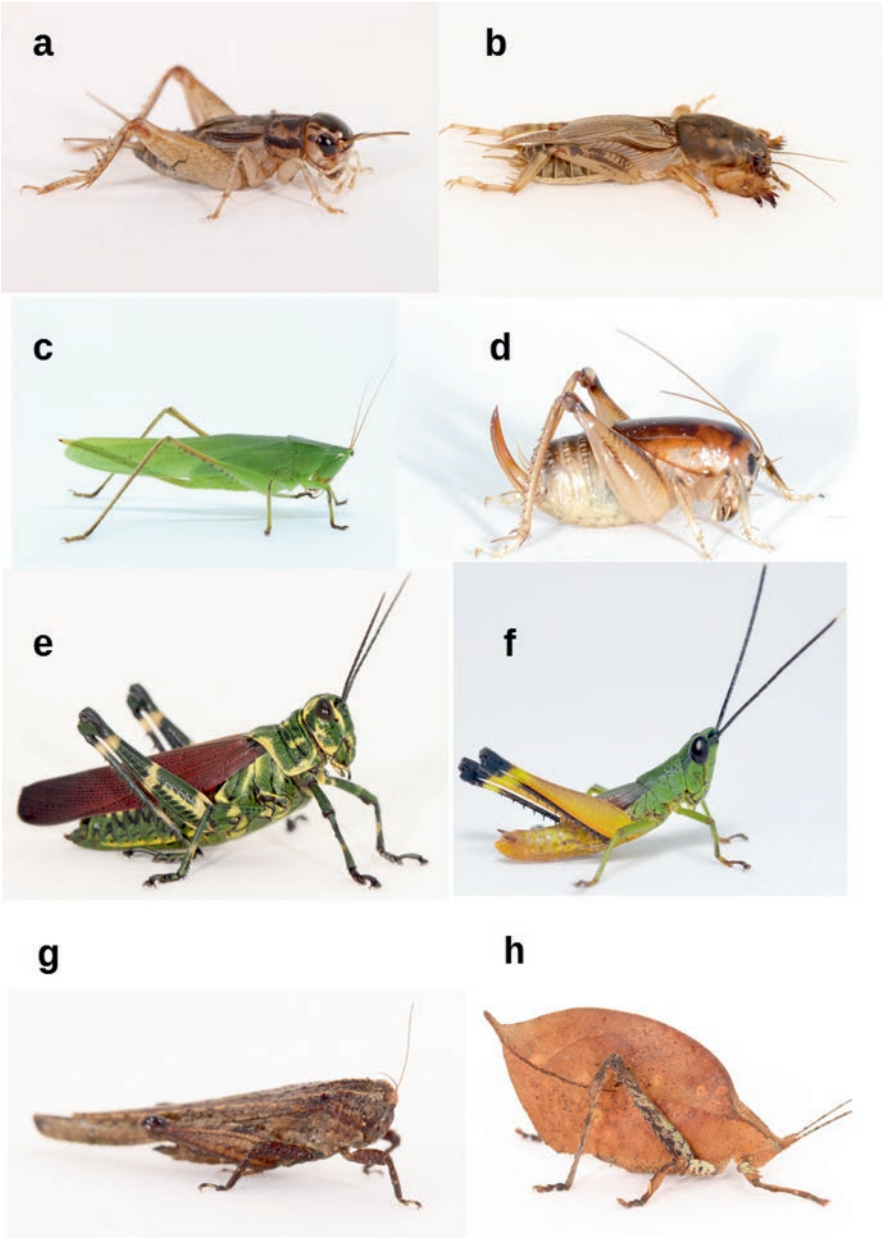


**Fig. 11.1** (**a**) Cricket *Miogryllus* sp. (Orthoptera: Ensifera: Gryllidae), from Belterra, Pará State, Brazil. Photo: L.D. Campos and P.G.B.S. Dias. (**b**) Mole cricket (Orthoptera: Ensifera: Gryllotalpidae) from Belterra, Pará State, Brazil. Photo: L.D. Campos and P.G.B.S. Dias. (**c**) Katydid *Neoconocephalus* sp. (Orthoptera: Ensifera: Tetigoniidae) from Boracéia, São Paulo State, Brazil. Photo: L.D. Campos and P.G.B.S. Dias. (**d**) Weta *Apotetamenus* sp. (Orthoptera: Ensifera: Anostostomatidae) from Veredas, Minas Gerais State, Brazil. Photo: L.D. Campos and

specific methods for Orthoptera sampling according to habitat, taxon, and study aims. We will dedicate a special section to studies on orthopteran behavior and breeding, bioacoustics, chromosomes, and DNA, as well as recommendations for specimen preservation for scientific collections. We will also discuss sampling design and cautions for data analysis in ecological studies on orthopteran populations and communities.

## 11.2 Habitats and Behaviors of Orthopterans

In general, Ensifera (crickets, katydids, and alike) are active at night, while Caelifera (grasshoppers and alike) are active during the day. Crickets are commonly seen walking and jumping on soil and litter but can also occur on tree trunks and shrub foliage, under fallen branches and trunks, in natural crevices, and on rocks. Several crickets use surfaces such as tree trunks or shrub foliage to stridulate, mostly males calling females for reproduction. Crickets are also an important component of natural cave faunas. Most species of katydids present arboreal habits, occupying from brushes of the understory to canopy; however, there are katydids that live under fallen wood trunks, litter, and soil or associated with macrophytes of continental aquatic ecosystems (Gwynne 2001; Rentz 2010). Most katydids are active at night (Rentz 2010), but there are species that are active during the day, as some species of *Conocephalus* (Tettigoniidae: Conocephalinae) (Chamorro-Rengifo et al. 2018). Grasshoppers are more often found in grasslands and at forest edges and ecotone habitats. There are semiaquatic orthopterans, such as semiaquatic grasshoppers, that feed on aquatic macrophytes.

The feeding habit of Orthoptera is highly diversified and comprises species that go from exclusively predatory to exclusively herbivorous. Omnivory, however, is the most common feeding habit in orthopterans (Gangwere 1961), except Caelifera, which are predominantly herbivorous. Popularly, the omnivorous eating habit was grounded in the laboratory, since insects housed in lab are fed with dog food, cat food, vegetables, fruits, and other food sources (Huber et al. 1989; Bidau 2014). Analysis of the proventricular and fecal content concluded that there are few oligophagous (that eat few species) or monophagous (that eat only a single species) orthopterans; even exclusively herbivorous species generally consume a wide variety of plant families (Gangwere 1961).

Grasshoppers are herbivores, with some species feeding on at least 14 plant species, from 11 different families (Sperber 1996). Herbivore grasshoppers may damage various crops, such as soybeans, corn, pastures, and grass. In North America and Africa, there are records of large grasshopper swarms, which consume about 60% of crops (Nerney 1960; Gallo et al. 2002; Simon 2020). Mole crickets are predominantly herbivores and may cause great economic impact on golf and football fields (Bello 2020); they dig tunnels and feed on roots of grass, killing the aboveground plant tissue (Hertl et al. 2001); they may also present predation habits, especially on sessile or little moving prey, such as eggs, pupae, aphids, mealybugs, and

larvae of insects and earthworms (Huber et al. 1989). In katydids, feeding habit is diverse, with subfamilies that feed on pollen or nectar, called "winged flower lovers," as katydids of the subfamily Zaprochilinae. There are katydids that feed on flowers and leaves (Phaneropterinae, Mecopodinae, and Pseudophyllinae) and on arthropods (Saginae, Lipotactinae, Tympanophorinae, and Listroscelidinae), but only in the subfamily Listroscelidinae all species are exclusively predatory (Gwynne 2001). Most species of katydids are omnivorous, with a tendency to being herbivorous; this trend is corroborated by the morphology of their mouthpieces, with adaptations that allow grinding of leafs (Gwynne 2001). In forest environments, crickets are abundant, especially on the litter. They coexist in this high productivity environment without evident segregation of trophic niches (Jesus 2015). Individuals of the same genus present a greater overlap of food resources than individuals of different genera, and as the number of coexisting species increases, there seems to be a narrowing of the trophic niche; in addition, there is great plasticity in the diet, since the same species can explore different resources in different areas (Jesus 2015).

Camouflage is a widespread characteristic in katydids, their appearance resembling the substrate where they rest (Gwynne 2001). Several Phaneropterinae and Conocephalinae are green. The wings of Phaneropterinae may resemble dicotyledon leaves (Magnoliopsida), whereas Conocephalinae can be polymorphous in color (as in some *Neoconocephalus*, with greenish and brownish individuals in the same population) with wings resembling monocotyledon leaves (Liliopsida, see Fianco 2019). Phaneropterines are generally associated with dicotyledons of forest environments, while conocephalines are generally associated with meadows and grasslands. Pseudophyllinae and Pterochrozinae of the Neotropics are generally brownish or with greenish stains, resembling tree trunks and associated biota (as mosses and lichen) or resembling dry leaves (Fig. 11.1h), and their habitat is the substratum they resemble.

## 11.3 Sampling Methodologies According to Habitat and Behavior

### *11.3.1 Sampling Ground Dwelling Orthopterans*

Orthoptera that move on the ground or litter can be sampled with pitfall traps (Fig. 11.2a–d). These traps may capture these organisms passively when there is no bait associated to the trap (Fig. 11.2b, d) or with baits, such as cotton soaked with sugary solution (Fig. 11.2c). The pitfall traps might be installed with covering, to reduce entrance of water during heavy rains, thus avoiding dilution of the killing solution, but at the same time covering might reduce capture of orthopterans.

There are several cricket species that spend most of their time moving through or above forest litter, as is the case of flightless crickets as the Nemobiinae and Phalangopsidae. The efficiency of the pitfall trap depends on its ability to capture


**Fig. 11.2** (**a**) Installing pitfall traps. Photo: L.D. Campos and P.G.B.S. Dias; (**b**) unbaited pitfall trap without covering. Photo: N. Szinwelski; (**c**) baited pitfall trap with covering. Photo: M.G. Lhano; (**d**) unbaited pitfall trap with covering. Photo: M.G. Lhano. The covering reduces entrance of water during heavy rains, avoiding dilution of the killing solution, but at the same time covering might reduce the capture of orthopterans

organisms that fall into the trap, and once the organism has fallen into the trap, the trap preventing the organism from escaping. Sperber et al. (2003) showed that using a solution containing alcohol increases the capture efficiency of orthopterans in pitfall traps, because the alcohol accelerates the cricket sinking and killing (Szinwelski et al. 2013), thus avoiding its escape. In sites where ethanol fuel is available, it might be used as a killing solution, preserving cricket's DNA (Szinwelski et al. 2012a, b). Sugarcane juice, which can be substituted by sugar or sugarcane syrup diluted in water, sprinkled on the vegetation or used in soaked cotton attached to the pitfall trap (Fig. 11.2c) attracts orthopterans and can be used as bait, to increase pitfall capture efficiency. Sugarcane syrup functions as an increase in local resource availability, promoting cricket aggregation and increasing local diversity (Szinwelski et al. 2015).

Sperber et al. (2007) showed that litter vibration through walking alters the number of captured orthopterans, probably because the substrate vibration provokes them to jump. The number of captures in pitfalls presents a nonlinear response to disturbance frequency, increasing in low to middle disturbance frequencies but saturating and eventually decreasing with high disturbance frequencies (Sperber et al. 2007). For studies that aim to estimate naturally occurring densities and diversities, we recommend avoiding the use of baits and minimizing the effects of litter

vibration. The number of orthopteran captures per trap is normally low (Ribas et al. 2005), so placing at least ten sets of pitfall traps parallel to each other at 15 m intervals, with each set consisting of a line of five traps 1 m apart (Szinwelski et al. 2012a, b) for each site, is recommend. More recently, we were using a larger sampling effort, of 150 sets of 5 traps each (Vargas 2013, 2018) per site, being each set at least 30 m apart. The original idea was to consider each set of five traps as a replicate, based on the assumption that 30 m distance would be sufficient to achieve independence. However, we realized that the drivers of orthopteran abundance, diversity, and occurrence might predominate at still larger spatial scales, requiring replicates to be further away. Thus, when studies focus on large-scale drivers, replicates have to be at least 10 km away to be considered real replicates, if landscape or biome drivers are being evaluated (Sperber et al. 2013). This does not apply to geographically restricted areas, such as mountain tops.

### *11.3.2 Sampling Shrub Dwelling Orthopterans*

For the quantitative sampling of shrub dwelling orthopterans, a commonly used methodology is sweeping with an entomological net, hitting the vegetation, and capturing the insects that are located on leaves and twigs directly with the net. This method has its shortcomings, however. Captured insects might be damaged and relevant specimens might escape. Thus, such methodology is of little use for inventories. Sweeping with the entomological net might be useful for homogenizing sampling effort, when the number of beatings per time and distance walked by the collector is standardized (Janzen and Schoener 1968). In this case, the collector has also to be standardized, to enable estimates of abundance or species richness comparisons among sites, habitats, or experimental treatments. For inventories, the most common sampling methodology is active searching, in which the researcher localizes the specimen and then makes a directed effort to collect it. This assumes that the researcher is focused on the taxon of interest.

### *11.3.3 Sampling Cave Orthopterans*

Cave crickets, along with other invertebrates (beetles, spiders, and harvestman) and vertebrates (fish and bats), are frequent in cave fauna (Gnaspini and Trajano 1994). Cave crickets are mainly represented by two families, with different geographical distributions: Rhaphidophoridae, mainly tropical (Asian and Australian), although there are few species of rainforest in Europe and North America, and Phalangopsidae, which occur along almost all Neotropical, African, and some Asian and Australian regions (Rampini et al. 1983).

Studies of cave fauna are of extreme importance, because in addition to the intrinsic fragility presented by the subterranean environment, these environments

have a key role in environmental licensing. Below are the most used collection methods for collecting cricket in underground environments:

**Direct Intuitive Searching (DIS)** Direct intuitive searching is targeting a microhabitat or environmental zone to address a research question (Souza-Dias et al. 2014; Bolfarini and Bichuette 2015). These microhabitats include flood detritus, penetrating tree roots hanging from ceilings or walls, guano deposits, edges of drip pools and ponds, muddy banks, and animal or insect carcasses. These microhabitats are likely to support a high diversity or present specific functional groups (e.g., guanophiles) (Wynne et al. 2019). This methodology involves searching for the greatest possible diversity of environments found inside the cavities, giving priority to sites where the specimens can be captured manually, with the aid of tweezers and brush. In this way, it is possible to quantify the number of individuals collected and thus estimate the impacts that the removal of their habitats may have.

**Quadrat Method** Quadrat sampling involves the visual inspection of delimited sample areas, optimizing collection of subterranean fauna. This method allows estimating sampling effort and enabling comparisons among subterranean systems (Krebs 1999; Bichuette et al. 2015). Sampling by the quadrat method has been used for a long time in ecological studies, mainly in plant ecology (Weaver 1918; Gleason 1920). In quadrat sampling, it is necessary to inform the sampling time and the number of captured individuals. Sampling locations can be qualitative, targeted to specific microhabitats (e.g., guano, carcasses).

**Leaf Litter Traps** This collecting technique was developed for cave ecology studies (Humphreys 1991; Weinstein 1994) and involves placing a permeable sheet on the floor to collect the leaf litter produced along a period of time and transporting it to the laboratory, where the litter can be weighted and screened for organisms. Leaf litter traps are constructed with rectangular plastic sheet containers by firstly screwing a wooden support into each corner of the container lid and next suspending a mosquito net between these four supports. A leaf litter trap of standard volume can be thus placed into the container base and transported into the cave as a sealed unit. These traps can be exposed for up to 7 days to accumulate material; a shortcomming of this method is that it often captures very young individuals who are difficult to identify, and might require its rearing to adulthood.

**Pitfall Traps and Vulcan Traps** Pitfall traps are also usually employed in caves (with or without bait, Fig. 11.2), but this method should be used with caution since it can impact the whole terrestrial fauna, oversampling some taxonomic groups, such as collembolans, orthopterans, and cockroaches (Sharratt et al. 2000). The traps are made of low-rimmed dishes or buried bottles (on unconsolidated terrestrial substrates), installed in places with and without guano on the substrate of the cave, with or without the aid of bait, in places where there are no natural pools, or these occur only on a seasonal basis. The traps are distributed in several locations in the caves. For consolidated substrates, the solution for fall traps are "vulcan traps," which are pitfalls with access ramps for fauna, so they do not need to be buried.

The traps must not exceed 48 h of exposure, because with time the traps accumulate dead animals and attract saprophages.

### *11.3.4 Sampling Semiaquatic Orthopterans*

Worldwide, about 80 Orthoptera species are considered semiaquatic and another 110 are water-dependent, from which about 50 species occur in the Neotropics, mainly represented by Acridomorpha, Tetrigoidea, Gryllidae, and Tettigoniidae. This semiaquatic habit seems to have evolved independently in the history of Orthoptera as a result of adaptive radiations, especially in Latin America, where there are important large river systems with considerable variations in water levels that generate immense surfaces of freshwater habitats (Amédégnato and Devriese 2008; Cover and Bogan 2015; Nunes-Gutjahr and Braga 2018). Semiaquatic orthopterans generally present a strict relationship with aquatic host plants (especially for host-specific oviposition, feeding habits, and nymph development). Despite its ability to swim and stay long periods under water, semiaquatic grasshoppers maintain the ability to leave water and perform similar ecological functions to their terrestrial relatives.

Semiaquatic grasshoppers are mainly associated to riparian habitats, living in marshes, floodplains, swamps, bogs, inundated grasslands, margins of rivers, lakes, flatwoods, ditch banks, inundation zones, and shores of running water, where they can complete their life cycles in macrophytes and herbaceous or graminaceous plants (Amédégnato and Devriese 2008; Nunes-Gutjahr and Braga 2018).

Like terrestrial Caelifera, semiaquatic grasshoppers are mostly diurnal, and some species are active both during day and at night. Semiaquatic orthopterans have physiological, ethological, and morphological adaptations that allow them to live permanently in the aquatic habitats on floating or rooted plants. Aquatic orthopterans might be able to swim, using paddle-shaped hind tibiae, and they may use a plastron respiration (a special adaptation to store oxygen, Thorpe 1950) when submerged, as well as morphological and chromatic mimicry, modified tibial spurs bearing hydrophobic bristles, modified ovipositor valves for egg insertion into or on the surface of aquatic plants (endophytic or epiphytic oviposition) (Braker 1989), and possess diving behavior in order to feed on submerged plants and to escape predators (Nunes-Gutjahr and Braga 2018).

Generally, semiaquatic Orthoptera can swim, walk, or skate over the water film surface. When attacked or in danger, they fly away or swim rapidly, even against currents greater than 1 m per second, hiding among roots of aquatic plants or deep-diving as long as 10 m and staying motionless under the water for more than 30 min until the danger has gone. Within Tetrigoidea, individuals of Scelimini are fully aquatic and can swim effectively (Rentz and Su 2003), feeding on aquatic weeds, aquatic algae, lichens, mosses, and debris (Kočárek et al. 2008). Besides, although Tridactylidae and Ripipterygidae are excellent jumpers, they are commonly found in galleries in moist soil, close to rivers, lakes, and beaches.

Given the relative scarcity of sampling of this semiaquatic fauna, it is very likely that this fauna is underestimated. The difficulty in collecting these grasshoppers is mainly due to access to wet habitats (often due to the vegetation associated with those habitats), the escape behavior of these insects (as they can be in both terrestrial and aquatic environments, changing habitats according to the situation), and not being suited for being captured in traps.

Semiaquatic orthopterans may be sampled through active collecting with sweep netting, transect counts, and hand capture. Passive methods for sampling are rarely used and don't provide reliable data for population or diversity estimates. On the other hand, sweep netting performance varies according to the surveyors or environmental conditions (O'Neill et al. 2003).

For a successful sampling, the essential is to answer these good and concise questions: what do I want to sample? What is my hypothesis? Where do I want to sample? With those question answered, it is a natural process to select the proper method to assess the semiaquatic fauna. For example, sweep netting is a good methodology in general, but for some Leptysminae as *Cornops aquaticum* (Bruner 1906), which swim swiftly, it is not the best choice. For semiaquatic Caelifera in general, the most common technique for sampling is sweep netting, which provides quick, low-cost, and accurate estimates of the grasshopper community composition. The entomological sweeping net is made of a sturdy structure, with a strong handle, and a strong net bag that does not snag on sharp vegetation (Samways et al. 2010). Standardized methods use an entomological net measuring 70 cm in diameter and a collecting bag of 100 cm in length, with easy handling and robust enough for the sampled vegetation. Typical methods include sweeping some predetermined number of times (Onsager and Henry 1977), along a predetermined time, and made by a predetermined number of collectors. According to O'Neill et al. (2003), the specific technique used in sweep sampling can vary among samplers and even among different samples taken by the same person. For them, including the net speed could affect the ability to capture grasshoppers since they have rapid escape responses. Differences among species composition among sets of samples could result from species-specific difference in escape behavior. O'Neill et al. (2003) recommend that the sampling method is described more accurately in the published studies, including the procedures taken for standardization.

For sweep netting standardization, the researcher may establish the distance from the vegetation, sweeping speed, intensity, height, angle of sampling, and standards the number of sweep beatings per meter, when the vegetation is homogeneous. In addition, transects can be standardized for ecological studies. A transect can consist in a number of sweeps taken on a number of consecutive strides, sweeping generally the net once with each step, keeping a consistent sweeping speed. It's highly recommended having a large sampling effort and reduced variability among samples, which might be achieved by standardizing the number and identity of the collectors, as well as the time of the day that the sampling is undertaken. Simultaneous collectors should sample simultaneously along parallel transects and keep a regular distance from each other (the optimal distance depends on the habitat where the sampling is made) (Onsager 1977; Onsager and Henry 1977).

Grasshoppers are generally difficult to sample due to their jumping and flying behavior (Browde et al. 1992). Adults jump only at higher temperatures; thus, sampling should be done in the early morning, when temperatures are not so high but sufficient for grasshoppers to begin their activity, or in the late evening, when temperatures are not too low and orthopterans haven't started to look for shelter to spend the night. In many wetlands, a boat should be used to access the vegetation, to help sampling and displacement in the study area.

To sample semiaquatic grasshoppers which live on macrophytes, Vieira and Adis (1992) developed an aluminum cage to increase sampling effectiveness. It is a 1 $m^3$ cage, with two openings (at the top and at the bottom), where grasshoppers can be trapped and removed manually afterwards. After a cautious approach with the boat, the previously assembled cage must be thrown (with the bottom open) by two collectors, which will capture macrophytes and grasshoppers inside the cage. After capture, the cage must be closed quickly and individuals can be collected through the opening at the top. This methodology was used in studies made in the Amazon and Pantanal and was proven to be efficient (Lhano 2002). The sampling effort for this methodology can include standardized techniques as quantity the number of samples, sampling area, etc.

Other focused methodologies can be used for specific groups, like for Tridactylidae and Ripipterygidae, which are insects easily attracted by light. Some grasshoppers, as the Leptysminae *Guetaresia lankesteri* Rehn, 1929, have arboreal habits in tropical wet forests and can be collected using canopy fogging.

After sampling, the recommended method to kill specimens is to place them in a freezer for at least 8 h and avoid using chemical jars. Besides, it is highly recommended to keep one hind leg in a labeled tube containing 95–99% ethanol for future genetic studies.

## 11.4 Sampling Methodologies According to Taxon

### *11.4.1 Sampling Katydids*

To collect katydids, the two most often sampling methods used are active searching and light traps (Rentz 2010). Active searching is done both during the day (4 h before sunset) and during the night (4 h after sunset), walking through trails inside or at the border of the vegetation, both searching visually and hearing attentively (Montealegre-Z 1997). Through visual searching, we detect individuals on the vegetation, while hearing enables us to first locate the region where the singing individual is active, following the produced acoustic sounds. During this localization, the space of observation is reduced, and this restriction of searching space enables visual detection of the singing katydid. Thus, hearing-oriented search leads generally to capturing males, since the males are the ones that produce more conspicuous sounds. During active searching we use a robust entomological net that must be

more resistant than the ones used to capture butterflies. This net is composed of a rim of ca. 40 cm of diameter attached to a cable of about 1.5 m; a tapered bag is fixed on the rim (the fabric must be resistant) with a tulle bottom and with a length of a little less than the length of the collector's arm, so that the collector can reach those individuals that are there deposited, after collecting them with the net. When the individual is located, it must be captured with the help of the entomological net, passing it rapidly and in an abrupt movement from under the vegetation upward, where the organism is located, as far as a common escape strategy of these organisms is to fall down to the floor. The net movement has to be swift, because several katydids fly away when perceiving the vibration of the vegetation. This method of collection is the most recommended for ecological purposes, since it's cheap and can be applied in areas which are inaccessible for other types of collection. We recommend collecting both during day and night, to better access the complete katydid fauna of the environment. For standardization, we suggest establishing a transect to be traveled in a predetermined time, and to standardize both time and distance traveled in each sampling unit, if possible replicating the sampling units, so as to have homogeneous sampling effort among sampling units.

A passive sampling method is using light traps, composed of a light source positioned in front of a white cloth that must be perpendicular to the soil (Fig. 11.3). This kind of trap must be mounted in a clearing or forest gap, surrounded by vegetation, so that the light can dissipate into a considerable distance and in this way attract a higher number of katydids (Rentz 2010). The light must be turned on just before sunset and stays on for about 6 h. The best dates to use this kind of sampling are during the darkest nights, with new moon, because katydids will be more active then. It's recommended to use sodium or mercury vapor lamps and/or black light. To standardize this sampling method we recommend homogenizing the time of sampling, carrying out at least 2 days of collection effort (sampling units) on each site.

Another sampling method for katydids are bat detectors, which are less frequently used because of their high cost. This equipment works complementing searching through hearing, as it transforms the acoustic signals that humans are not able to hear (above 20 kHz) to signals situated in a frequency band that humans can hear (Rentz 2010). The detector is directed toward the vegetation, capturing the high-frequency sounds and transforming them, thus enabling auditory orientation of the researcher toward the acoustic source, where the collector proceeds the manual capture of the stridulating individuals. To standardize this sampling method, we recommend doing it at the same time of the day, with the same sampling effort among sampling units.

A further device for sampling katydids are Malaise traps, rarely used for orthopterans; they have the advantage to enable capturing rare species of katydids (see Fianco et al. 2019). At ground level, it is apparently not effective, but the suspended mounting tends to collect rare species, especially species with sustained flight or flightless species that inhabit the understorey or canopy that make contact with the trap.

**Fig. 11.3** Active light collecting at white sheet. Photo: L.D. Campos and P.G.B.S. Dias

### *11.4.2 Sampling Crickets*

For practical reasons, we will refer to crickets *sensu lato*, which includes the monophyletic superfamilies Gryllotalpoidea (Gryllotalpidae and Myrmecophilidae) and Grylloidea. Crickets *sensu stricto* (or true crickets) are grouped into one superfamily, Grylloidea, one of the main lineages of Orthoptera, with more than 5,600 described species (Chintauan-Marquier et al. 2015; Cigliano et al. 2020). Grylloidea is subdivided into four families: Gryllidae, Mogoplistidae, Phalangopsidae, and Trigonidiidae. The relationships among cricket families and subfamilies are discussed in Chintauan-Marquier et al. (2015).

Crickets occupied almost all available habitats in terrestrial ecosystems, from grasslands to dense forests, from underground to forest canopies. Several lineages are adapted to inhabit different habitats in their environments: ground and leaf litter (Gryllidae, Phalangopsidae, Gryllotalpidae, Trigonidiidae), cavities under and

aboveground level (holes, caves, crevices) (Gryllotalpidae, Gryllidae, Phalangopsidae, Myrmecophilidae), living and dead tree trunks (Gryllidae, Mogoplistidae, Phalangopsidae), shrubs (Gryllidae, Mogoplistidae, Trigonidiidae), branches (Gryllidae, Trigonidiidae), and canopy (Gryllidae, Phalangopsidae, Trigonidiidae).

Crickets *sensu lato* are generally omnivorous, although there are some predatory and herbivorous species in Gryllotalpidae. Most crickets are nocturnal, spending daytime hidden in their shelters. The wide variety of habitats and feeding habits makes sampling crickets a challenge, with no specific trap for them. Thus, one must use different sampling methods to sample the main families of crickets in a given area. The main methods and techniques for collecting crickets are presented below.

#### 11.4.2.1 Active Searching for Crickets

The most effective method for collecting crickets, and the only one that can be specific to a clade, family, or species, is active searching (Fig. 11.4). This kind of sampling can focus on a large area, with a broad microhabitat range, such as litter, cavities, bushes, tree trunks, and foliage, or with a more narrow and restricted focus, such as specific sites and microhabitats, like cavities or caves. It is also possible to focus on adults instead of juveniles (nymphs), since juveniles are not useful for taxonomic purposes and are of difficult identification. Yet, if the objective is sampling tissue or raising species for behavioral or other laboratory studies, nymphs are welcome. The best period for sampling crickets is nighttime, where almost all crickets are active. However, daytime searches are important to find the shelters of nocturnal crickets and to collect diurnal species, such as trigs and nemobiines (Trigonidiinae), crickets of the genera *Lerneca* (Phalangopsidae, Luzarinae), and many eneopterines (Gryllidae, Eneopterinae).

There are some entomological equipment and techniques that can be used in active sampling of crickets:

**Entomological Net** This is the most common equipment for active sampling of insects and highly effective to collect grasshoppers (Caelifera). Considering the nocturnal habits of crickets, low density of populations, and their habitats (small cavities, tree trunks, leaf litter), this equipment is not recommended. Small nets used in fishkeeping are a good solution for collecting species inside cavities, trunks, or any other situation in which you are close to the cricket.

**Plastic Tubes and Pots** A highly effective way of catching a cricket is using plastic tubes and pots (transparent are preferable) and searching actively for these organisms, on the ground, cavities, bushes, etc.

**Entomological Umbrella (Fig. 11.5)** This is recommended for collecting crickets from shrubs and branches, as Mogoplistidae, Trigonidiidae (Trigonidiinae), and Gryllidae (Oecanthinae, Tafaliscinae, Eneopterinae, Podoscirtinae). This equipment

**Fig. 11.4** Active searching for crickets with plastic tube and jar. Photo: L.D. Campos and P.G.B.S. Dias

**Fig. 11.5** An alternative design for entomological umbrella. Photo: L.D. Campos and P.G.B.S. Dias

can be used in the traditional way, placing it above a branch, beating the branch with a stick to drop the specimens into the umbrella, and then capturing them.

**Light Sampling (Fig. 11.3)** Active sampling at strong light sources, that attract nocturnal insects, is effective for crickets, such as Gryllotalpidae, Trigonidiidae (Trigonidiinae), and Gryllidae (Eneopterinae, Oecanthinae, Podoscirtinae, Tafaliscinae, Gryllinae), besides other insect orders. The most commonly used technique is placing a light source near a white sheet.

**Baits** Using baits, laid along forest trails, is effective to attract some groups of crickets such as Gryllidae, Phalangopsidae, and Nemobiinae (Trigonidiidae). The baits can be oat or biscuit bran, small pieces of fruits, or even a molasses solution sprayed on the vegetation or on the ground. After some time (30 min or more), some individuals can be seen feeding on the baits. Using oat as bait on the ground has an additional advantage for night sampling as it helps focusing the collectors' eye.

#### 11.4.2.2 Passive Sampling Crickets

For Grylloidea and Stenopelmatoidea, it is recommended to use bird, banana, or watermelon baits, placed on the soil, at night, for direct manual collection or with the aid of wide mouth flasks. During the day, these organisms may be found under

rocks, fallen trunks, or tree bark, where they remain hidden until night. The most frequently used passive sampling method for crickets are pitfall traps. In this case, special care should be taken to keep the region maintained without human access, as far as vibration caused in the substrate alters the frequency of capture (Sperber et al. 2007).

Further devices and techniques that can be used for the passive sampling of crickets are:

**Malaise** Very frequently used in entomological surveys for sampling flying insects such as Diptera and several families of Hymenoptera and Coleoptera, Malaise traps are little effective for crickets. The main reason is that crickets are not good flyers. Thus, few cricket specimens are caught in Malaise surveys. Nevertheless, Malaise can collect specimens of Gryllidae (Eneopterinae, Oecanthinae, Podoscirtinae, Tafaliscinae) and Trigonidiidae (Trigonidiinae).

**Moericke (Fig. 11.6)** Yellow pan traps (Moericke traps) are not much used in crickets' surveys. However, in recent expeditions, this method has been efficient for capturing ground crickets such as Nemobiinae (Trigonidiidae) and Luzarinae (Phalangopsidae) and small flying crickets such as Trigonidiinae (Trigonidiidae).

**Pitfall Traps (Fig. 11.2)** Pitfall traps are the most common method of sampling ground crickets. It is widely used in crickets' surveys and taxonomical and ecological studies. Those traps are cheap and easy to construct and operate. Usually, each pitfall trap is filled with a liquid, which must kill and preserve the specimens. The chosen liquid also depends on the time that the traps will remain in the field and the resources available. It can be water + detergent (detergent is important for reducing waters surface tension) for short periods (~24 h), water + detergent + salt (salt is an alternative way of preserving DNA for short periods), and ethanol in different concentrations (for longer periods). The best killing solution is ethanol or ethanol fuel because it kills the specimens quickly, avoiding their escaping the trap, and preserves DNA (Szinwelski et al. 2012a, b, 2013). One can also use attractive liquids such as cane molasses diluted in water as a way to increase sampling efficiency.

### *11.4.3 Sampling Grasshoppers*

For terrestrial grasshoppers, sweep net sampling is the most common sampling technique used to determine species composition present in a specific area. The captured grasshoppers are manually placed in labeled plastic bags containing local vegetation and transported in thermoplastic boxes in order to avoid overheating and damage to the material. The sweeping net's fabric must be resistant to the type of vegetation present in the area where the study will be performed, and spare nets must always be carried to the fieldwork.

To avoid varying sampling effort and biasing ecological analyses, standardized methods of sweep sampling must include predetermined number of times of

**Fig. 11.6** Moericke traps. Photo: L.D. Campos and P.G.B.S. Dias

sweeping, duration of the sampling, number of collectors, distance among the collectors, and who will perform the sampling—it must be the same person for all sampling along the study. Typical methods used to standardize are two consecutive periods of 60 min with 10-min break (for density population studies, a minimum of 50 individuals is frequently used independently of time), two collectors, and at least 10 m distance from each other during the samplings. It is vital to put the collected grasshoppers of each sweeping period (or whatever the sampling unit established) in separate and adequattely labeled plastic bags, so as to be able to estimate density and diversity per sampling unit. For taxonomic and inventory studies, it is not necessary to control those variables, and just walking around the area and using the entomological net to capture the specimen after visual contact are efficient enough. However, absence of sampling unit control eliminates information on within area variation, and thus prevents comparisons among areas and any extrapolation of the results.

Different kinds of entomological nets (sweep net, aerial net, combination of aerial and sweep net, aquatic net, etc.) can be used according to habitat and taxa which will be sampled. For example, Carvalho (2010) designed a very efficient net to use in grasslands, 1.5 m long and a 1.3 m high, hold by two persons, and with the net almost touching the ground, walking a previously predetermined distance (5, 10 m, etc.).

We reccomend that, as soon as the fieldwork is completed, the sampled specimens are killed in a freezer at −20 °C. Before killing, we recommend keeping one hind leg in a labeled tube containing 90% alcohol for future genetic studies. After killed, each specimen must be prepared for conservation in a dry environment. For taxonomic studies, it is recommended to carry out an evisceration of the specimen, removing the internal organs with a longitudinal cut in the region of the cervical membrane. After evisceration, specimens must be carefully filled with antiseptic talc (neutral and unscented) and borax (1:1 proportion). Lastly, the grasshoppers must be pinned and mounted and then taken to the drying oven for 12 h at 40 °C or at room temperature. Using high temperature might damage the grasshoppers coloration. An alternative, quicker, method is to maintain the grasshopper specimens in the freezer for 8–12 days, mounting the specimens when they are still soft and flexible and maintaining them to dry at room temperature for 1 month. The advantage of this is avoiding the procedure of taking the guts out, which is an arduous work that takes a whole evening for each specimen.

## 11.5 Sampling Methodologies According to Study Aims

### *11.5.1 Sampling for Breeding and Behavioral Studies*

Behavioral studies depend directly on massive breeding of these insects to carry out the experiments. After collecting the individuals, it is recommended that the orthopterans be taken to the laboratory for maintenance at a temperature of around 23 °C ± 1, in a terrarium containing moist substrate, hiding places, a source of moisture and food ad libitum. We use sifted earth or sand, egg cartons, damp cotton to maintain humidity around 60%, and fish food to feed the insects. The moist substrate or cotton serves as a substrate for oviposition. Air humidifier might be necessary to maintain humidity.

For the experiments, it is ideal that the individuals be isolated before the last molt, thus guaranteeing the individuals' virginity. This separation should be done when males and females are already showing visible wing buds.

### *11.5.2 Sampling for Bioacoustic Studies*

Bioacoustic diversity might be studied using a set of sound recorders (Riede 1994; Chesmore 2004). Orthopterans have been a source of wonderment for their songs since Paleolithic times (Grimaldi et al. 2005). Orthopteran organs for the production of acoustic signals and hearing appeared independently in the two lineages that make up the group, Caelifera and Ensifera. Grasshoppers stridulate by scraping a row of denticles present in the inner part of the posterior leg femur on the lateral

region of the anterior wings or rubbing the anterior wing on denticles present on the posterior wing. Crickets and katydids produce sound by scratching the pallet, on the side of one of the anterior wings, against a row of denticles on the opposite wing.

The repertoire of orthopteran songs is diverse, but most species produce sounds of calling, courtship, and aggressiveness, the first being the most studied for its use in species taxonomy and for issues related to sexual selection. The acoustic signals emitted by orthopterans are classified as pure tones due to their emission in a species-specific frequency band. In crickets, the frequency values vary between 3 and 9 kHz, while in katydids, much higher frequencies are found, even reaching ultrasound ones. The values shown in the frequencies depend on the rigidity of the exoskeleton, body size, and the flexibility of the emitting organ, the tegmina.

The recording of the acoustic signals can be done in the field or in the laboratory, preferably with high-quality digital recorders and directional microphones. With the advancement of technology, simple equipment, even cell phones, enable sound recordings with enough quality for scientific analyses.

Here we suggest a protocol that should be followed before, during, and after sound recording, so as to meet minimum scientific requirements:

1. Locate the insect in the field and approach it without causing disturbance to the environment—care must be taken to avoid touching the vegetation.
2. When visualizing the insect, position the microphone and start recording, avoiding sudden movements, so that the stridulation is not interrupted.
3. After about 3 min of sound recording (the recording time may vary according to the situation), press the “pause” button on the recorder.
4. Photograph and collect the individual for later recognition of the species.
5. Immediately after collecting the individual, measure air temperature in the location as close as possible to the stridulation site, since temperature influences several parameters of the orthopteran sound.

After following the steps described above, some information should be verbalized following the recording of the sound produced by the insect; thus, release the “pause” key and pronounce the following information:

1. Individual code—it will be necessary to create a code for each registered individual; the same code must be kept with the collected specimen.
2. Location—country, state, city, etc.: if possible, insert geographic coordinates.
3. Description of the exact location of the stridulation.
4. Date.
5. Recording time.
6. Temperature.
7. Brand of the equipment used for recording.
8. Other information may be inserted as it deems pertinent, such as the position or behavior of the insect during stridulation; the type of the stridulation site, such as a leaf, trunk, branch, or crevice; if the insect uses the curvature of the leaf to amplify the sound; etc.

Software for analyzing and editing sounds can be found on the Internet, such as Avisoft, CoolEdit, Adobe Audition, Raven, Audacity, etc. Some of these are freeware or open source. With the advancement of technology and the emergence of portable digital recorders coupled with the creation of indexes used to reflect acoustic activities from audio files, passive fauna monitoring studies have become increasingly accessible and reliable. Passive acoustic monitoring is a technique used to monitor animals indirectly, using the individual acoustic communication to record data about the community, being minimally invasive to fauna and flora.

Among the devices currently used, two passive acoustic recorders, SongMeter (Wildlife Acoustics, Inc.) and AudioMoth, stand out, both enabling the recording of acoustic signals from the environment, obtaining good acoustic information quality. Both devices can be configured with recording protocols allowing total control of the sampling time, which can be adjusted according to the researcher's aim. The frequency bands covered by these recorders capture signals from 28 Hz to 48 kHz, enough to register crickets and grasshoppers. Depending on the species of katydid, special equipment that captures ultrasound is necessary, usually equipment developed for the bioacoustic studies of bats.

Orthopterans emit two types of sounds, trills (continuous sequence of pulses) and chirps (groups of pulses) (Fig. 11.7). Pulse is a chain of sound cycles produced during inward movement of the forewings. Here are some parameters for the description of the sound of a species:

(a) **Trill** (1) dominant frequency, highest intensity spectral component of the song; (2) pulse rate, number of pulses per second; (3) pulse period, elapsed time from the pulse's first sound wave, up to the beginning of the subsequent pulse; (4) pulse duration, elapsed time from the first to the last sound wave of a pulse; (5) pulse interval, elapsed time from the pulse's last sound wave, up to the beginning of the subsequent pulse; and (6) number of sound waves per pulse.
(b) **Chirps** (1) dominant frequency; (2) chirp rate, number of chirps per minute; (3) chirp period, elapsed time from the chirp's first pulse to the beginning of the subsequent chirp; (4) chirp duration, elapsed time from the first to the last pulse of a chirp; (5) chirp's interval, elapsed time from the chirp's last sound wave, up to the beginning of the subsequent chirp; (6) number of pulses per chirp; (7) pulse period, elapsed time from the pulse's first sound wave, up to the beginning of the subsequent pulse; (8) pulse duration, elapsed time from the first to the last sound wave of a pulse; (9) pulse interval, elapsed time from the pulse's last sound wave, up to the beginning of the subsequent pulse; and (10) number of sound waves per pulse.

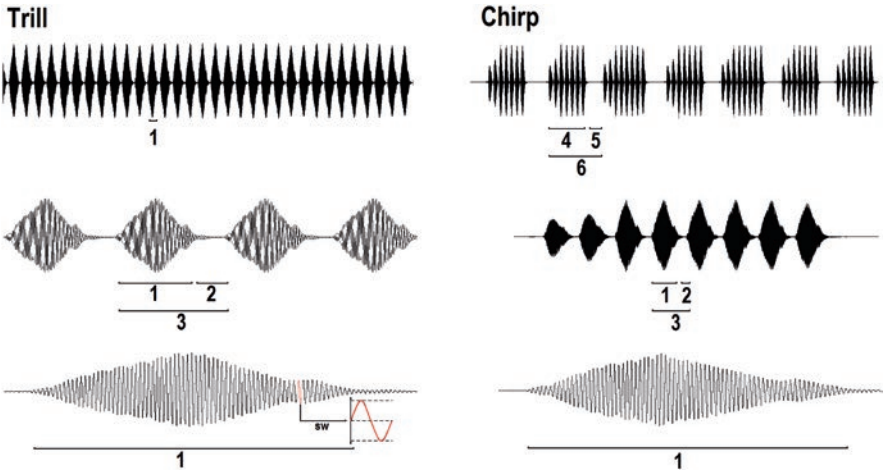


**Fig. 11.7** Calling song of the cricket *Miogryllus itaquiensis* Orsini et al. 2017 (Orthoptera: Ensifera: Gryllidae), composed of trill and chirp sequences. 1, pulse; 2, pulse interval; 3, pulse period; 4, chirp duration (chirp with seven pulses); 5, chirp interval; 6, chirp period; SW, sound waves (pulse with around 72 sound waves, each one produced by a tooth stroke)

### *11.5.3 Sampling for Chromosomal Studies*

Studies of chromosomes in Orthoptera have been widespread in recent centuries, since their karyotypes have a relatively small number of chromosomes, in general with a large size, facilitating their observation with optic microscope. For this reason, many important discoveries about mitotic and meiotic processes were made through the study of the chromosomes of these insects. The methodology for obtaining chromosomes from orthopterans (and also from other organisms) demands that individuals be collected and kept alive until the dissection process is carried out to obtain and fix the tissues that will be used for cytogenetic techniques.

The most practical way to keep individuals alive for a few hours until they are taken to the laboratory is to pack them in plastic bags with leaves and dry branches so that they remain sheltered in the bags so as to not collapse. It is not recommended to put green leaves in the plastic bags, as the leaves' perspiration may accumulate water droplets inside the plastic bag, which can be harmful to individuals. Plastic or glass tubes, about 13 cm long and 2.5 cm in diameter, are also good options for transporting individuals; the tube must be closed with a dry cotton plug, compacted firmly in the tube's opening.

Katydids can be collected on grasses and shrubs, following the same methodology described for grasshoppers. Night collections can be carried out passively, with light traps, or actively, searching the bushes and lower branches of the trees by means of manual picking, or with the aid of an entomological umbrella. Katydids can be put into plastic bags, as described for grasshoppers; however, large

individuals should be packed individually in glass tubes, as they usually have well-developed jaws, and it is not uncommon for them to damage other individuals if kept together.

The method of collecting and storing crickets is similar to the procedure described for katydids. However, unlike katydids, crickets are very abundant on soil litter, and in this environment, collections should be made with pitfall traps, which can be made with a 1–2 L pet bottle, with the tip cut and inverted forming a funnel. Considering the objective of getting the individuals alive, it is recommended to install pitfall traps in the late afternoon and remove them in the early morning of the following day, since coleopterans, ants, centipedes (Scolopendridae), spiders, and scorpions may fall into the trap preying or damaging the crickets. It is recommended to use oat flakes to attract them, as the oat flakes are dry, preventing the crickets from becoming trapped and dying, as occurs when using moist baits such as cane molasses, banana, or other fruits.

When orthopterans are collected for chromosomal analysis, preference is given to adult males, as the group's taxonomic features are mainly based on male terminalia. In addition, males have well-developed testicles, composed of follicles where intense mitotic and meiotic activity occurs. Thus, even with conventional chromosomal analysis, it is possible to determine the diploid number, the morphology of the bivalent chromosomes in mitotic metaphase (spermatogonial metaphase), as well as the sex determination mechanism, since most species have X0♂-XX ♀ sex system, the X chromosome being univalent and easily observed in prophase and metaphase I. On the other hand, collecting living adult females is important, as they are usually fertilized and can be raised in the laboratory until they lay eggs. The offspring can supply a large amount of material for obtaining chromosomes, with the advantage that some individuals can be kept until adulthood so that males are used for species determination. In addition, nymphs offer good material for obtaining mitotic chromosomes, which are generally more distended.

The technique for obtaining chromosomes from grasshoppers, katydids, and crickets is relatively simple. For mitotic chromosomes, a 0.05% solution of colchicine must be injected into the individual (male, female, or nymph). The individual must remain alive and after 3–5 h be dissected to remove the cecum and midgut. The tissues must be submitted to the hypotonic solution of KCl 0.075 M (filtered or distilled water can be used instead) for 5–10 min and transferred to an Eppendorf tube with the Carnoy I fixative solution (3 ethylic alcohol:1 glacial acetic acid). The gonads of male and female nymphs also provide good mitotic chromosomes. To obtain meiotic chromosomes, testicles from adult or preadult males are used. In this case, individuals do not need to be injected with colchicine, and the procedures for hypotonization and fixation are as described above. The Eppendorf tubes with the fixed material should be kept in the refrigerator, preferably at 4 °C. From these procedures, the tissues can be used for any type of cytogenetic technique, whether with conventional or differential staining.

### *11.5.4 Sampling for DNA Studies*

DNA sampling within Orthoptera follows basic protocols from molecular methods. First, DNA quality is an important factor for future analyses and will depend on collection and appropriate preservation of samples. Sampling methods like pitfall traps must use preservation liquid like fuel ethanol (Szinwelski et al. 2012a, b) or >96% ethanol as a killing solution. Using detergent spoils all DNA. For active searching, specimens or samples must be immediately stored in preservation liquid. For conservation during long periods, DNA samples must be conserved in >96% ethanol, frozen, and stored at −20 °C. Research dealing with stored DNA must avoid freeze/thaw cycles of samples, since this will affect the stability of the genomic DNA, leading to degradation (Shao et al. 2012). Generally, we use entire hind femora of smaller specimens or fragments of femora of larger specimens for DNA samples of Orthoptera. If the specimen is too small, we must use the entire individual to obtain a sufficient amount of DNA for further analysis. After sample preparation, DNA must be extracted from the nucleus of the cells. This can be done by either ready-to-use DNA extraction kits or conventional protocols; the steps are the same.

**DNA Extraction** First, cells must be separated from each other by a physical means or vortexing and put into a solution containing salt. A detergent is then added to break down the lipids in the cell membrane and nuclei for DNA release. DNA is then separated from proteins and other cellular debris using a protease and cleaning reagents like phenol chloroform. Finally, we use ice-cold alcohol (either ethanol or isopropanol) that, together with salt, precipitates the DNA. After cleaning, the precipitate is resuspended in a slightly alkaline and ready-to-use buffer.

**DNA Amplification and Sequencing** After extraction, fragments of DNA are amplified and sequenced. Polymerase chain reaction (PCR) is a DNA amplification technique that uses interleaved cycles of specific temperature for strand denaturation and primer binding and extension by Taq polymerase. These fragments can now be sequenced by either Sanger or next-generation sequencing (NGS) for molecular studies of taxonomy, diversity, and evolution in Orthoptera.

### *11.5.5 Preservation of Specimens for Scientific Collections*

Preservation methods depend on the group to which they belong. Caelifera and Tettigoniidae are preserved dry, remembering that the faster the drying, the better the color preservation of the integument. Katydids must the dissected, in order to remove internal organs; for this, it's necessary to make a cut using scissors in the pleura of the abdomen (of ca. 5 mm); the internal organs must be removed with tweezers (if possible the male genitalia must be kept in vials with glycerin). After being removed, it's necessary to stuff the internal part of the katydid's body with cotton and then fill the body with borax or cotton. After this, we mount the

individuals: the forelegs should be positioned forward and the rest back; male cerci must be mounted opened, for a better visualization. At least one individual of each morphospecies must be mounted with the left tegmen opened. After being mounted, the individuals must be dried in an oven, at 40 °C, for 48 h. After being dried, they must be conserved in boxes with mothball or camphor, in places with relatively low temperatures (less than 20 °C). Grylloidea and Stenopelmatoidea are preferably preserved in humid way, inside tubes with 70% alcohol or in ethanol fuel: however, hydration is needed to manipulate these organisms. When collected in pitfall traps, it is recommended to use a more concentrated alcoholic solution, due to the soaking and consequent softening of the specimens, when it rains in the field. For molecular purposes, we recommend that part of individual tissues (e.g., foreleg) be transferred to a tube with >96% alcohol and be stored in a fridge. For grasshoppers, killing and preserving in freezer (−20 °C) is the most efficient method to preserve the coloration. If it is necessary to kill in the field, use insect killing jars, fueled with ethyl acetate or cyanide, avoiding direct contact of the killing agent with the specimen. For drying, avoid using laboratory oven: let them dry at room temperature, in a dry room. After killing, remove the grasshopper's guts through a small incision along the specimen's neck, under the pronotum, using a small forceps. Introduce antiseptic talc (neutral and unscented) and borax (1:1 proportion), and, if necessary, stuff the specimen with cotton. An alternative, quicker method is to maintain the grasshopper specimens in the freezer for 8–12 days, mounting the specimens when they are still soft and flexible and maintaining them to dry at room temperature for 1 month. The advantage of this last technique is that it avoids taking the guts out, which is an arduous work that takes a whole evening for each specimen.

## 11.6 Sampling Design

If you have a strict hypothetical-deductive approach, you have to control sampling design so as to guarantee a homogenous sampling effort among sampling units, as well as to guarantee independence of sampling replicates, and you have to relate your data to the corresponding effort and environmental variables relevant to your tested hypotheses. If, on the other hand, you aim at an inventory, your focus will be on the broadness of the sampled fauna, aiming completeness and you may disregard control of sampling effort or of environmental variables. Of course, you may have a mixture of both approaches. The behavior of your focus organisms affects sampling efficiency and bias. Thus, you have to plan carefully your sampling according to microhabitat and behavior of your focus organisms, so as not to over-extrapolate your conclusions.

Most commonly sampling aiming at a hypothetical-deductive approach involves passive capture of organisms, such as pitfall traps for litter-dwelling crickets. Such sampling has shortcomings, since their efficiency depends on the organisms' mobility, and as such it has low efficiency for broad-scale inventories. The addition of attractive baits may enhance capture efficiency, but it generates bias in the spectrum

of captured organisms, which may invalidate the test of ecological hypotheses. For inventories, it is common to combine several simultaneous sampling techniques, including active day and night sampling.

## 11.7 Cautions for Data Analysis

When the studied orthopterans do not occur in swarms, they can be analyzed as unitary organisms; therefore, when analyzed as response variables (Y), being it the number or density of individuals or the number of species, the appropriate distribution of the data is the Poisson distribution, eventually corrected for overdispersion or substituted by negative binomial distribution (Crawley 2013; Zuur et al. 2009), instead of the classical normal distribution. Thus, it is necessary to adjust generalized linear models (GLMs), which can be done using free software such as R (R Core Team 2019). When the data contain many zero values, which may occur, e.g., with cricket individuals captured in pitfall traps, it might be necessary to use zero-inflated Poisson (ZIP) models (Zuur et al. 2012), or, alternatively, work with presence-absence data, using binomial distribution. Frequently, however, data with many zeros indicate insufficient sampling effort, maybe associated with incorrect spatial scale. Species occurrence analyses should privilege large spatial scales, with appropriate geographical data analyses.

To deal with nested data, great care must be taken. For example, you may have a single information on the explanatory variables of your model, e.g., if you are interested in the effects of forest remnant regeneration time on cricket species richness (Szinwelski et al. 2012a, b), you might pool several sampling units within each forest remnant, so as to avoid pseudoreplication (sensu Hurlbert 1984), or you might use mixed effects models to include the nested structure of your data into the model, which is essential if you have different spatial scales for local and regional explanatory variables. Most frequently mixed effects models consider random intercepts (Zuur et al. 2012), which means that you admit random variation in the intercept of the effect of your explanatory variable, e.g., the effect of the canopy cover on cricket species richness, where some remnants have higher richness than others, given a fixed canopy cover, but the slope of crickets' species richness driven by the canopy cover value is always the same (Farias-Martins et al. 2017). More complex mixed effects assume random intercept and random slope (Gelman and Hill 2006), which means that you admit random variation both in the intercept and in the slope of the effect of your explanatory variable, e.g., the effect of the canopy cover on cricket species richness. In this more complex, and probably overcomplex model, the effect of your explanatory variable on your response variable, e.g., of the canopy cover unto cricket species richness, varies randomly, among forest remnants, both in the intercept and in the slope, meaning that in some forest remnants, cricket species richness increases more steeply with canopy cover than in other remnants.

An important issue to consider in field studies is spatial autocorrelation, because this process leads to greater similarity between the closest sites due to factors that

are spatially correlated, such as environmental conditions (soil type, temperature, slope of our altitude) (Fleishman and Mac Nally 2006) and biological attributes (dispersion, home-range size, and biotic interactions) (Wilkinson and Edds 2001). To minimize this problem, it is possible to use statistical analysis dealing with spatial autocorrelation, and for univariate-dependent variables (e.g., species richness, density, abundance), generalized least squares (GLS) models are used to identify and control spatial autocorrelation (available in "nmle" R package, Pinheiro et al. 2020), while for multivariate-dependent variables (e.g., species, phylogenetic and functional composition), restricted ordinations (partial redundancy analysis, partial RDA; partial canonical correlation analysis, partial CCA) are used (available in "vegan" R package; Oksanen et al. 2019). However, modeling the spatial autocorrelation can be complex, so we suggest opting for sample designs that lead to greater sample independence, so as to avoid pseudoreplication. When possible, we suggest *a priori* sampling to identify the spatial connectivity of the variables in the study regions in order to elaborate a sample design with the smallest possible spatial autocorrelation.

To evaluate biodiversity metrics (e.g., richness, species diversity, abundance, density) at broad geographic scales, it is necessary to know the availability of these metrics in the studied space (e.g., continent, biome, biogeographical province) and the gradient of the predictors of biodiversity (e.g., climate, average annual temperature; landscape, forest cover; topography, slope and elevation). Thus, samples should be proportionally arranged on the geographical and environmental variation of the predictors, in order to sample the most representative sites both in geographical and environmental space. After this step, the spatial modeling of biodiversity metrics can be performed in two different ways: (1) direct modeling of biodiversity metrics, based on the correlation between diversity metrics, estimated at known sites, and environmental variables (macroecological modeling (MEM)) (see Gotelli et al. 2009), and (2) species distribution modeling in isolation, with subsequent overlap, to predict species richness index (stacked species distribution modeling (SSDM)) (see Guisan and Rahbek 2011). MEM usually uses regression models that depend on the metric used: count data, e.g., richness and abundance use (Generalized Linear Models (GLM) or Generalized Additive Models (GAM) with Poisson distribution or machine learning classification models; random forest (RF) and support vector machine (SVM)), and continuous data, e.g., diversity and density (GLM/GAM with Gaussian distribution, SVM, RF). SSDMs can be based on different statistical algorithms depending on the characteristic of the biodiversity metric used: presence data (Bioclim, Gower, Domain, Mahalanobis) and presence and absence data (GLM/GAM with binomial distribution, RF, SVM). Note that to make the spatial prediction of metrics, it is necessary to have raster layer maps (maps with matrix structure, composed of pixels which have values of spatial variable ending, e.g., temperature, altitude) with the values of the predictor variables for the entire region of interest, and the resolution of this raster depends on the size of the study region and the processes and factors to be studied, so that the spatial pattern of biodiversity metrics is demonstrated on a map of the study region.

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