Assessment of biodiversity in Africa, Kakamega Forest N.R.: an evaluation of methods for recording Lepidoptera in relation to the gathering of statistically valuable data

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Summary
Quantitative and qualitative descriptions of lepidopteran biodiversity based on several collection methods are described. Each method produces data, which were typically used for comparisons of different biotops. Statistical evaluation of data requires an inherent data quality. Data have to be objective and, so far as possible, connected. In Kakamega forest N. R. in Western Kenya five methods were tested under tropical rain forest conditions. Their suitability to produce statistically valuable data was critically evaluated. Only one of five methods permits statistical use of the resulting data.

Introduction
Nature conservation and landscape planning require biological data. Area protection currently focuses on rare species, biotopes or the diversity of species. A nature conservation strategy which aims to suppress any human influence within an area, is not viable in most regions of the world. Successful protection of greater and smaller areas requires nowadays a defined development objective and professional management of this process. The goal of current landscape management is to accommodate biodiversity within moderate human activity.

Landscape planning is a helpful instrument to control land use and to preserve the genetic resources of a selected area. The planning process includes an evaluation of biotic and abiotic factors which require their own criteria. Those currently employed are very diverse: naturalness, rarity, endangeredess, diversity, aesthetics, scientific importance, etc. (Usher & Erz 1994). For practical purposes everyone needs to begin with an objective classification of the different values. Procedures for establishing criterion-classification of biological data also have to be objective (Plachter 1991, Jessel 1999).
Insects are often used as indicators for several parameters. More than a million species have been described and they are mainly distributed in the tropical forest zone. The species are adapted to different biotic and abiotic conditions. They are often highly specialized and occupy many ecological niches. The composition of several parameters such as climate, soil state and composition of the vegetation require the inclusion of phytophagous insect species like the Lepidoptera, with more than 146,000 described species (Heppner 1993). Lepidoptera are an important and well-known group, which allows their use as criteria for the evaluation of planning processes.

Within the BIOTA-Project - as a part of a three-year pilot study in Africa - communities of selected family groups of day-active and night-active Lepidoptera will be investigated in natural, seminatural and disturbed biotopes in the Kakamega Forest N.R. in Western Kenya. The initial project aims are the description of alpha- and beta-diversity, their comparison with each other and characterization of the taxocoenose. The results of this characterization are to facilitate an effective evaluation of biotopes in this region based on the structure of the Lepidoptera community and the determination of influence through different land usage on the insect communities, especially Lepidoptera.

At the starting phase of the BIOTA-project different methods were checked for their suitability in effectively recording Lepidoptera in tropical forests. Next, every method was critically evaluated to see whether it can bring out data from several sampling places and patterns which could be used in a serious statistical comparison of different biotops. Improvement of the standard methodologies was the main emphasis of the first stage of fieldwork. Crucial methods and results are represented here.

**Necessity of objective data**

It is typical to describe the diversity criteria of an investigated area or selected biotope based only on an inventory list of species. For this purpose several methods will usually be applied. Inventory is a very important aspect, but on the other hand it is generally impossible to extract from such list which biotopes of an area were investigated or on which methods the list is based on. Comparison of α-diversity of selected biotopes or β-diversity of an area and/or an arrangement of different land use can be useful, provided that indicators are sampled using a method which brings out objective data.

This means that the sampling must be repeatable and checkable by other scientists too. Only objective data allow serious statistical analyses, e.g. discussions of evenness, dominance or abundance. Individual impressions and manipulation or selection of data must be excluded. Furthermore, the manner of reiteration during a period of sampling has to remain equal. Data which are truly objective can only be produced using homogeneous procedures, which ideally exclude any subjective influence within the sampling; i.e. the human observer as an unquantifiable impact on data. Related data must also be recorded under the same sampling conditions. Sampling of biological data in particular can, in different places, frequently succumb to the influence of weather conditions, if they cannot be carried out at the same time in different places. Procedures which excluded these impacts on the data can be call standardized methods.
Many well-known methods for investigating Lepidoptera exist. Most of them were developed for effective general recording or for specific species and are suitable for an inventory. Only a couple of these techniques are appropriate for producing objective and/or ideally-combined data. However, for the goal of biodiversity research employed methods must produce comparable, objective and connected data (MÜHLENBERG 1993). In the case of Lepidoptera continuous field work over an entire year or over a period of several years would be ideal and the best groundwork for a statistical analysis, but this is difficult in practice.

The first step for the generation of objective data within the project was to select suitable standard methods for measurement and for a monitoring system which can be employed in different habitats under different conditions in the whole of Africa and in other parts of the world too at any time.

**Tested methods and results**

a) **Recording Butterflies on a transect line**

Butterflies have been studied in detail for their ecology and distribution and are mostly used as indicators for conservation. The first quantitative analyses of butterflies come from the nineteen-forties (Kovács 1958). The monitoring method was probably first used in Great Britain in the nineteen-seventies (Pollard & Yates 1993). Currently the walk-and-count-transect method is a standard method in Europe for ecological research. It has previously been applied in the tropics as well (Cheverton & Thomas 1982, Hill 1992, Wood & Gillmann 1998).

This method involves a periodical recording during a defined daylight time on a selected route within selected plot(s). All Butterflies seen within the bounds of the route and within an estimated distance of 5 m ahead of the recorder are counted. As far as is known, this method has never been used in Africa before. The selected route in Kakamega Forest N.R., with a length of 4 km, passed through several biotopes. Every biotope forms a segment. This transect was checked up to 10 a.m. at a slow speed (< 1.5 km/h).

Butterflies move slowly in the morning. Later in the sunshine it was difficult to record all species and specimens because they are many of them, often flying very fast or escaping from the route into the treetops or shrubs. Furthermore, it is impossible to identify all African butterflies via this action, even for well trained individuals. Currently 491 species of butterflies are known for the Kakamega Forest N.R. (KÜHNE et al. in press). However, Wood & Gillman (1998) recorded during their investigation in the Neotropical region a maximum of only 47 species within this sampling. In general, it seems likely that this method can successfully be used in regions where the total number of butterfly-species is less than 100 and all species can be easily identified in the field by the recorder. Therefore it is difficult to use this method as a standard measurement for diversity in a region outside the Holarctic which would have a rich butterfly fauna and difficult taxonomic groups. One could confine the taxonomic groups to families which are easy to observe and identify in the field. But in this case the important groups Lycaenidae and Hesperidae for a fine tuned assessment would have to be ignored.
Another frequently ignored fact is that the path for sampling in the forest is an artefact and simulates a borderline or gap. It is not known which species prefer these structures and what impact a path has on the general recorded butterfly fauna. It seems impossible to measure this impact. Furthermore, the weather changed very often during recording on the route. These circumstances result in different data because the important climate conditions are not the same for all segments during the recording time. For these reasons this method could not be used as a standardized method because individual and climatic conditions are not constant. However, the butterflies are good indicators in the tropics because they have been studied in detail. It is only possible to use semi-quantitative analysis of species in the sample plot.

b) Fruit (bait) traps

Slightly different constructions of fruit (bait) traps are known (Austin & Riley 1995). They are all a variant of a gauze-material cylinder. The traps were hung along the transect route in every sample plot in an attractive position and were filled with natural fruits. Smashed fruits such as bananas, pineapple, etc. and fish were used. The bait traps are mainly attractive for fruit-piercing butterflies of the family Nymphalidae, especially for the strong flyers Charaxes. For success it is necessary to install the fruit traps on the same place for a long period (see Wood & Gillmann 1998, Fermon & Dall’Asta 1994, Larsen & Dall’Asta 1994). Both diurnal and nocturnal insects could be recorded.

In contrast to Schultze (1995) in Borneo, Osborn et al. (1999) in Venezuela did not find statistical differences between traps in the understorey and in the canopy. Sourakov & Emmel (1995) found the best results with traps hanging 2 m high. The number of recorded species is normally rare. Differences between recorded species on the transect and in the traps were not observed in the Kakamega forest, but in other areas they were slightly different.

In Kakamega Forest N.R. problems were produced by monkeys. They often fed on the fruits in the traps. It is unclear when this happened. Sometimes a trap was destroyed during this action and data were lost. Furthermore, it has to be noted that specimens go in and out of the trap during the holding time. This was also reported by Sourakov & Emmel (1995) and Austin & Riley (1995). This very important fact is often ignored when using these data for statistical analysis. Tests in Kakamega Forest N.R. have revealed that in extreme cases all of six specimens escaped from inside the trap after one hour. These facts justify this method as useful for an inventory in this area, but not for a standard method which has to produce statistical data.

c) Recording nocturnal Lepidoptera by automatic light traps

Since the nineteen-fifties several constructions of light traps were used everywhere, especially in institutes for agriculture and pest control. An excellent overview of many aspects and investigations for this method are given by Muirhead-Thomson (1991). Views about this method remain divergent. For instance Malicky (1965) thought that was impossible to use this method in coenological investigations. On the other hand Rézbányai (1974) suggested that sampling with this method is absolute necessary for establishing a fauna and that the method has to used up 10 to 20 years for long term observations of population dynamics (Varga & Uherkovich 1974, Rézbányai 1974) and phenology (Jermy 1974). A critical review of this subject was given by Vojnitz & Mészáros (1974).
The method is well known but many aspects remain under discussion. However, the method was widely used in studies for instance in Europe: Britain (Hosny 1959), Austria (Wieser 1991), Hercinia (Meinecke 1984), Northern Germany (Kolligs 2000) and Africa: Kenya (Taylor & Brown 1972) and Tanzania (Robertson 1977).

Several names of different light trap constructions exist. They are calling Rothamsted traps (Williams 1948), Robinson traps (Robinson & Robinson 1950), modified Robinson traps (Williams et al. 1955) or Muguga traps (Brown 1965). It is known that the results are influenced by their construction (Taylor & Brown 1972, Jermy 1974, Worth 1979), sampling locality (Malicky 1974, Jermy 1974), altitude (Frost 1958, Taylor & Brown 1972), weather (Hosny 1959), light emission and spectrum (Hosny 1959, Mikkola 1972, Kolligs 2000), moonlight (Nemec 1971, Persson 1971, Bowden & Church 1973) and background illumination (Bowden & Morris 1975, Bowden 1984). Novák (1974) discovered that the sex ratio in a trap differs from natural conditions.

Contrary to popular opinion, a light trap has an unknown lure effect, already Cleve (1962) pointed out that the light can be calculated mathematically. The catchment or effected area of a trap with a 15 W light-tube is, for insects, 20-200 m (Bowden & Church 1973, Bowden 1982), for beetles 188 inches (ca. 4.8 m) (Onsager & Day 1973), for noctuid species 120 m (Stewart et al. 1969) or for moths 30 m (Kolligs 2000). The effectiveness of a trap is around 10-50 % (Hartstack et al. 1968) or 30-40 % (Kolligs 2000). The small drainage area of trap with a 15 W light (< 30 m) within a rain forest is the guarantee for a representative sample of the investigated biotope. Hartstack et al. (1968) and Hollingsworth & Hartstack (1972) found that the distances for a difference between background illumination and the light of the trap are 193 m for UV radiation and 225 m for all radiation without moonlight. This can be accepted as the maximum range for a trap in an open area. This guarantees that only the specimens within this biotope are attracted.

Our traps were constructed for tropical conditions, especially for strong rainfall. This has a bigger diameter for the roof than for the funnel and bucket (>12cm) which protect the trap against rain and retain dry conditions inside. The traps operated with one 15 Watt tube emanating superactinic light the whole night. The Lepidoptera fell across a plastic-funnel into a bucket where they were killed by chloroform. Field experience showed that species move inside the trap if the volume of chloroform is too low. Our traps worked well with 8-10 cl of chloroform per night. The chloroform was placed into a small glass which was covered by a metal-gauze.

The traps were situated for sampling in a small, open, representative place within the inspected biotopes. They ran the whole night. All plots had to be sampled on the same night. Different attitudes were tested as well.

The advantage of this method is that subjective influences on the results are impossible. Furthermore, all weather conditions homogeneously affect the results of each trap if they used on the same night. This allows a direct arrangement of the raised objective data. This method exhibits a very useful standard.

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d) Recording nocturnal Lepidoptera by manual observing at light

An historical overview of this famous method is given by Lödl (1989a). The importance for entomology is quite clear (see Lödl 1987). A lot of light sources and variations of the constructions of reflecting surface are known. Influence radii vary between 35 m at full moon to 519 m at new moon for a 125 W MV light source (Bowden & Morris 1975). These results show that the method can be used for investigation of a single biotope if the illumination is restricted. It can be carried out inside the biotope like, for instance, a forest. Kovacs (1958) discovered that different sampling places within a biotope bring out the same results. If it is impossible to find a place for recording without illumination of other biotopes, then reduction of the radiation using a cloth is possible (Kovacs 1958).

In Kakamega Forest N.R. we tested a light tower equipped with a 125 Watt vapour lamp, superactinic light (2x 15 Watt) and tubes of neon light (blue and white). Collecting started in the evening and finished 3 hours after dusk. All species of the selected families were recorded quantitatively.

The results of this method are very diverse. They depend mainly on the weather. Wind, temperature, rainfall and moonlight are important and affect the result. Especially in the rain forest temperature in the evening depends on the starting time and duration of rainfall in the afternoon and the possibility of water evaporation afterwards which is supported by the sunshine. It is not known why, but it is a fact that the time of flying activity differs from species to species and night to night. All these factors are very important and the observations with the light differ from night to night. A further variable aspect is the individual: how is the observer trained, is the person fast enough if a lot of species arrive over a short period, can the person note the brief visitors too. These factors have an important influence on the results.

The spectrum of recorded species differ from other methods, but it is the most successfully method for collecting at night with the greatest output of species (Lödl 1989b). The method is more attractive for the strong flying species and for species who inhabiting treetops. It is an important method to record a major part of the whole species spectrum for an inventory and therefore for the reference system.

A disadvantage is that this method can be applied only in one sample plot per night. For that reason it is impossible to compare the results from sample to sample directly, but they can be compared with the light trap of the same sample plot. Initial results showed that the species spectrum differs from those of the corresponding light trap.

e) attractive substances

Many methods are known which rely on attractive substances (Muirhead-Thomson 1991). These can include sugar, wine, weak alcohol or a mixture of substances. Different chemicals can also be used successfully (Priessner 1979, BoppRE 1995, 1999, Landolt et al. 2001). The bait can be placed onto bark or other surfaces or on hanging fruit discs. These spots are very attractive for nocturnal Lepidoptera.

A quantitative analyses seems to be possible (Kovacs 1958) and it can be standardized as well. But the lure depend on selected substances. They are predominantly restricted for a few species or groups. They are preferable for autecological questions or investigations on special groups, but unsuitable for synecological questions.
Discussion

Ecological investigations contain many aspects. For some questions it is necessary to analyse data statistically. The data have to meet exceptional recording demands (Mühlenberg 1993, Finck et al. 1995, Jessel 1999, Trautner 1992). First of all statistical analyses need objective data. Investigations which compare biotopes have to be based on comparable or better connected data. Comparison of the diversity of different places based on statistical analysis of a species group cannot be carried out seriously if it is not based on methods which produce linked or dependant data. Not all of the methods which are analysed here are able to meet these demands or they are restricted to some cases only. For instance the walk-and-count-transect seems to be useful outside Africa.

Two main aspects should be considered if a method is to bring out hard data for statistical analysis. First the investigator as an important variable has to be excluded, or their influence has to be strongly reduced. Second, the technique should make it possible to produce connected data, i.e. carried out at the same time in different sampling places. These demands are best met by light traps. They offer the possibility that this unique technique can be used simultaneously in several plots and produce data which are connected with each other. Other techniques always involve a different sampling time for the several sampling plots. This time factor implies different conditions during sampling. Most important are the weather and the attention or fitness of the observer.

Through a critical review of all these facts, only the light trap is able to meet all these demands. Lödl's (1989b) conclusion was the same. His opinion was that a methodical standard can only be achieved using light traps and this method exhibits a very useful standard which allows comparative analysis. Ecological research in future which includes statistical analyses should take into consideration this critical view.

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