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Monitoring of butterflies in semi-natural grasslands: diurnal variation and weather effects

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Running title: *Monitoring of butterflies in semi-natural grasslands*

Abstract The butterfly fauna was monitored in six semi-natural grasslands in southeastern Sweden. The aim was to evaluate monitoring criterias for wind, sunshine, temperature and time of day for butterfly species richness and abundances when using the line transect method. A total of 30111 butterflies belonging to 46 species were recorded. Data from this study suggests somewhat stricter criteria for temperature and sunshine than stated in the widely used “Pollards walk”. A sharp decline in butterfly numbers were detected at temperatures below 19°C if the proportion of sunshine of the transect walk was below 80–85 %. No effect of wind speed, up to five on the Beaufort scale, on observed numbers of species or individuals were found. Several butterfly species showed well-defined diurnal rythms of flight activity, and the results indicated that transect walks can be performed between -4.5 and +4 h from the time when the sun reached its highest point. The results of this study can be used to adjust general criteria in national monitoring and also for detailed regional and local monitoring where it may be important to adjust for diurnal rhythm and weather related bias.

Keywords Lepidoptera · Line transect method · Species richness · Weather conditions

Introduction

During the 20th century, butterfly species have declined rapidly throughout much of Europe and the decline has been especially pronounced over the last 50 years (Warren 1992, Pullin 1995, New 1997a, Maes & van Dyck 2001). The major cause of this decline is thought to be the loss and fragmentation of suitable habitats due to the intensification of agriculture and changes in land use. Butterflies are often associated with low-productive, unfertilized,

semi-natural grasslands (van Swaay et al. 2006). These habitats have faced one of the greatest declines in Europe during the last century and are expected to become increasingly marginalized as agriculture intensifies (Pullin 1995, van Swaay & Warren 1999). Therefore there is an increasing need of monitoring biodiversity in these habitats. Many butterfly species are sensitive to changes in habitat quality and they react faster to environmental changes than other organisms, for example plants (Erhardt & Thomas 1991). Butterflies are also relatively easy to monitor and can therefore be used as indicators or umbrella species when looking at local habitat status, environment conditions, biodiversity and climate change (New 1997b, Oostermeijer & van Swaay 1998, Blair 1999, van Swaay et al. 2006).

Accurate, repeatable and cost-effective methods of monitoring are important components of successful policies to conserve and enhance butterfly biodiversity (Pywell et al. 2003). In a number of European countries, butterfly monitoring schemes have been in use for many years, especially the UK, the Netherlands and Belgium. All of those countries use the line transect method (Pollard 1977). The method states criteria which should be followed in order to provide a degree of uniformity. However, many of these criteria have not been thoroughly tested, and rarely outside the UK. Sweden has recently (2006) started a nation-wide monitoring scheme (Glimskär et al. 2006) and there is a need to evaluate the criterias under Swedish conditions.

The aim of this study was to evaluate monitoring criterias for wind, sunshine, temperature and time of day for butterfly species richness and abundances using the line transect method. Further, the number of sampling visits are also discussed.

Material and methods

Study area

The study was conducted during 2006 in six semi-natural pastures, all within a maximum of 15 km from each other, in the county of Östergötland in south-eastern Sweden (58°20'N, 15°42'E). Pastures were identified from regional inventory records of meadows and pastures in the Nature Conservation Programme by the Municipality of Linköping. The pastures used in this study were all classified as being of national interest, regularly grazed, and had an area of 5.9–8.8 hectares.

The Swedish monitoring guidelines

The criterias for monitoring in the newly started (2006) nation-wide monitoring scheme (NILS; Glimskär et al. 2006) differs somewhat from the criterias in Pollard (1977). The Swedish guidelines are: (i) temperature >17°C and sun or temperature >25°C irrespective of sun; (ii) wind below 8 m s⁻¹; (iii) between 9.00 and 16.30 h. The number of visits per site during the season is limited to three.

Butterfly recordings

Butterflies (*Rhopalocera*) and burnet moths (*Zygaenidae*) were recorded using the line transect method (Pollard 1977, Pollard & Yates 1993). The transects were walked at a speed of approximately 50 metre per minute and each butterfly individual found within five meters on each side and in front of the recorder was noted. The transects were located in straight lines 25 meters apart, covering the whole of each site. In those cases where

individuals had to be caught in order to be identified the inventory was temporarily paused and later resumed from the same spot. One pair of species was difficult to identify in the field, *Leptidea sinapsis*/ *L. reali*, and were therefore treated together. Nomenclature follows Eliasson et al. (2005) and, for convenience, all species are referred to as “butterflies” in the following text. The field work was carried out from the 22nd of May until the 16th of August between 09:00 and 16:30 Swedish summer time (Greenwich Mean Time + 2) under all different weather conditions except for rain. Every site was visited at least 15 times during the study period. In order to eliminate the possible effect of diurnal fluctuation in the number of observed butterflies, a rotating scheme was used so that the six sites were visited during different times of the day.

In order to evaluate the percentage of total species richness recorded at a site at different numbers of visits, 20 combinations of butterfly counts from different time periods (Table 2) were randomly selected and an average calculated for each pasture. The line equations of each pasture were then used to calculate an average curve of all six pastures.

Diurnal variation

The variation in butterfly abundance over the day was studied in one of the pastures (Ringetorp) during an intense, shorter period of 16 days at the end of June. In this site, between three and seven (71 in total) counts were conducted each day between 08:00 and 19:00. During this period, the other sites were also visited, but with a slightly longer time-span in between. For the analyses of diurnal variations, only those days in the intense study period with a minimum of five counts were used. This gave a total of 10 days and 56 counts. As we were interested in the variation within days, we eliminated the between-day

Table 1 Butterfly species and their abundance, number of sites where they were found and group belonging at six pastures in Östergötland, SE Sweden.

Species	Abundance	No. of sites
<i>Aphantopus hyperantus</i>	7554	6
<i>Maniola jurtina</i>	5102	6
<i>Coenonympha arcania</i>	4450	6
<i>Argynnis aglaja</i>	2346	6
<i>Brenthis ino</i>	2174	6
<i>Boloria selene</i>	1543	6
<i>Coenonympha pamphilus</i>	816	6
<i>Polyommatus amandus</i>	674	5
<i>Polyommatus icarus</i>	622	6
<i>Pieris napi</i>	609	6
<i>Melitaea athalia</i>	568	6
<i>Ochlodes sylvanus</i>	444	6
<i>Lopinga achine</i>	390	1
<i>Thymelicus lineola</i>	381	6
<i>Lasiommata maera</i>	329	4
<i>Polyommatus semiargus</i>	287	6
<i>Pieris rapae</i>	217	6
<i>Argynnis paphia</i>	216	6
<i>Lycaena virgaureae</i>	191	6
<i>Leptidea sinapis</i> / <i>L. reali</i>	174	6
<i>Boloria euphrosyne</i>	119	6
<i>Gonepteryx rhamni</i>	115	6
<i>Aglais urticae</i>	114	6
<i>Hesperia comma</i>	98	5
<i>Zygaena filipendulae</i>	96	3
<i>Inachis io</i>	74	6
<i>Pieris brassicae</i>	72	6
<i>Lycaena phlaeas</i>	69	6
<i>Lyceena hippothoe</i>	66	6
<i>Antochardis cardamines</i>	40	6
<i>Aricia artaxerxes</i>	39	4
<i>Argynnis adippe</i>	38	6
<i>Zygaena lonicerae</i>	29	3
<i>Zygaena viciae</i>	15	2
<i>Nymphalis antiopa</i>	13	4
<i>Issoria lathonia</i>	6	3
<i>Favonius quercus</i>	4	2
<i>Erebia ligea</i>	4	2

<i>Pararge aegeria</i>	3	1
<i>Cynthia cardui</i>	3	2
<i>Pyrgus malvae</i>	2	1
<i>Callophrys rubi</i>	1	1
<i>Adscita statices</i>	1	1
<i>Hipparchia semele</i>	1	1
<i>Polygonia c-album</i>	1	1
<i>Lasiommata megera</i>	1	1

Table 2 Time periods from which 20 combinations of butterfly counts were randomly selected for each pasture.

No. of visits	Time periods
1	May 22 nd – August 16 th
2	May 22 nd – July 4 th , July 5 th – August 16 th
3	May 22 nd – June 19 th , June 20 th – July 18 th , July 19 th – August 16 th May 22 nd – June 12 th , June 13 th – July 4 th , July 5 th – 26 th , July 27 th –
4	August 16 th
5	May 22 nd – June 8 th , June 9 th – 26 th , June 27 th – July 13 th , July 14 th – 30 th , July 31 st – August 16 th
6	May 22 nd – June 21 st , June 22 nd – July 20 th , July 21 st – August 16 th

variation by normalising the data on the number of observed individuals and species on a per day basis. I.e., daily means were calculated, and the deviation from these means were used. The time for each count was calculated as the middle between start and stop time.

Weather conditions

At the end of each transect walk, temperature, wind speed, percentage of sky covered by clouds and percentage sunshine (percentage of the transect walk carried out during sunshine) were recorded. The temperature was measured about 70 cm above ground. The wind speed was estimated by observing the swaying vegetation using the Beaufort scale.

The percentage of sky covered by clouds was classified into four categories: 0–25%, 26–50%, 51–75% and 76–100%. The percentage of the transect walk carried out during sunshine was estimated to the nearest 10%.

As an initial step to evaluate the importance of the different weather variables on the recorded butterfly assemblages, a Redundancy Analysis (RDA) was conducted on data from the intense study period using CANOCO 4.5 software (ter Braak and Smilauer 2002). RDA was used due to low beta diversity of the data from the intense period. P-values were established for the RDA in Monte Carlo tests with 9999 permutations. In order to diminish the influence of species with high abundance, species data were log-transformed before analysis.

As the intensive period coincided with stable weather conditions, only wind exhibited sufficient variation for a meaningful analysis of its impact on species richness and number of individuals. Only those days with a minimum of five counts were used in the calculations and values were normalized within each day to minimize possible between-day differences in butterfly numbers.

For evaluation of variation due to temperature, overall cloudiness of the sky and the proportion of time during transect walked in sunshine, data from the entire season were used. A minimum of three butterfly counts made during a period of less than 14 days (the butterfly fauna was assumed not to change in any major aspects during such a short time period) were standardized for each pasture, which gave a total of 79 counts. An inspection of the data on variation in number of butterfly species and individuals with temperature and sunshine revealed that it had linear properties, and therefore a multiple regression was carried out using STATISTICA software version 7.0 (StatSoft Inc. 2004). The dependent

variable was number of butterfly species or individuals recorded (both standardized) and the independent (i) temperature, (ii) the proportion of time during transect walked in sunshine.

Results

A total of 30111 butterfly individuals belonging to 46 species (Appendix 1) were observed in the six pastures. Of those, 23558 butterfly individuals of 35 species were observed during the 16-days intensive study period conducted in Ringetorp. The increase in the percentage of species richness found with number of visits approximated a characteristic logarithmic distribution for all six pastures (Fig. 1). The increment was rather sharp between one, two and three visits, and started to reach a plateau at five visits. The average percentages of species richness found at 1, 2, 3, 4, 5 and 6 visits were 31, 49, 73, 78, 85 and 89, respectively. The average curve based on the line equations from all sites was calculated as follows:

$$y_{average} = 38.06 + 67.95 * \log_{10}(x) \quad (1)$$

SDs for the two estimates of the constants were 4.397 and 11.124, respectively.

Diurnal variation

Between 08:00 and 17:00 Swedish summer time (the sun reached its highest point at 13:01 in Linköping during the intensive study period), most counts of species were above or close to the normalized mean value. Later in the afternoon all counts were made below the mean value (Fig. 2). The number of individuals had a sharp increase in the morning, with all

Fig. 1 Percentage of species richness found at different numbers of visits in six pastures. The symbols represent the average value of 20 random selections of counts from different time periods (Table 2).

counts above or close to the mean value from 08:00 to 17:00. After 17:00 all counts made were far below the mean value (Fig. 2).

The number of individuals of *Brenthis ino* and *Aphantopus hyperantus* (33.2 % of total butterfly observations) reached a peak between 09:00 and 10:00, after which they decreased, and from 12:00 to 16:00 most counts were below the mean value (Fig. 2). After 16:00, the number of individuals of *Brenthis ino* and *Aphantopus hyperantus* once again

Fig. 2 Number of observed butterfly species (a) and individuals (b) in Ringetorp at different times of the day (Swedish summer time). Number of observed individuals of *Brenthis ino* and *Aphantopus hyperantus*, and *Zygaena* species (c) at different times of the day. Trend line set to lowess with a stiffness of 0.3. Arrow indicates when the sun reached its highest point (13:01).

increased. *Zygaena* species (*Zygaena filipendulae*, *Zygaena viciae*, *Zygaena lonicerae* and *Adscita statice*) showed a different pattern of diurnal activity (Fig. 2), with counts below the mean value up until 12:00, after which they increased slightly, and later in the afternoon around 16:00 they decreased.

Impact of weather

Results from the RDA (Fig. 3) on data from the intense study period showed the relationship between weather variable and the butterfly assemblages. The RDA was highly significant ($F=12.032$, $P=0.0001$), and the weather variables explained 42% of the variance (sum of all canonical eigenvalues). The first axis (eigenvalue 0.25) mainly coincided with temperature, while the second axis (0.16) did so with wind speed (Fig. 3a). The butterfly species were clearly associated with high temperatures and many also with high amount of sunshine and low wind. However, a number of species seemed unaffected by wind speed (Fig 3b).

Wind speed below five on the Beaufort scale did not seem to affect the number of observed butterfly species or individuals (Fig. 4). Both the number of observed butterfly species (Fig. 5) and individuals (data not shown) followed the same basic pattern concerning temperature and proportion of time during transect walked in sunshine. In temperatures of 10–19°C there had to be at least 80–85 % sunshine to obtain values above the standardized mean value (i.e. >0). A threshold in proportion of sunshine could be seen at around 20°C. Above 20°C the proportion of sunshine that produced values below the standardized mean value decreased rapidly. At 22°C, counts made in around 40 % sunshine were over the mean value, and in temperatures above 29°C counts were made over the

Fig. 3 Redundancy Analysis of the butterfly data collected during two weeks at one site, using four explanatory variables describing weather. The solution explained 42% of the variation in butterfly assemblages.

mean value without any sunshine at all. With results from the multiple regression (there were no significant correlation between sunshine and temperature, $R^2=0.048$), the standardized number of butterfly species ($F_{(2,76)}=6.28$, $P=0.003$, $R^2=0.14$) and individuals

Fig. 4 Numbers of observed butterfly species (a) and individuals (b) per count in different wind speeds in Ringetorp according to the Beaufort scale. One is $0.3\text{--}1.5\text{ ms}^{-1}$, and five is $8.0\text{--}10.7\text{ ms}^{-1}$ on the Beaufort scale.

($F_{(2,76)}=8.55$, $P=0.004$, $R^2=0.18$) at different temperatures and percentage of sunshine can

be estimated as follows:

$$\text{Species} = -0.96 + 0.022 * \text{temperature}(\text{°C}) + 0.0072 * \text{sunshine}(\%) \quad (2)$$

$$Individuals = -1.21 + 0.032 * temperature(^{\circ}C) + 0.0072 * sunshine(\%) \quad (3)$$

Both the number of observed butterfly species (Fig. 5) and individuals (data not shown) followed the same basic pattern concerning temperature and the overall cloudiness of the sky. If 76–100 % of the sky was covered by clouds, it had to be at least 28°C for counts to be above the standardized mean value. At 51–75 % coverage, it had to be approximately 22°C, at 26–50 % 13°C, and at 0–25 % coverage around 12°C.

Discussion

This study provides detailed data on weather impact and diurnal pattern of butterfly numbers. The results are important in two ways. First, to elaborate the general criteria for monitoring on a nationwide scale; second, for detailed monitoring in the local scale where it may be important to adjust for weather related bias. Even though variation in large scale monitoring is expected to be averaged out over the large number of transects conducted per year, it is of interest to minimise the irrelevant variation in data as much as possible as that increases the power (i.e. the chance of detecting real changes over time; van Strien et al. 1997). The criteria should be strict enough to minimise variation but on the other hand, not so restricted that monitoring only can be done during weather conditions so rare that it in practise is impossible to cover many sites in a season.

Monitoring of species richness is also of general interest (Balmford et al. 2005). The increase in the percentage of species richness found with different number of visits approximated a characteristic logarithmic distribution for all pastures, which is in agreement with Dennis et al. (1999). Vessby et al. (2002) found that the number of

Fig. 5 a) Standardized numbers of observed butterfly species at different temperatures and percentage sunshine (proportion of time during transect walked in sunshine) in six pastures. b) Standardized numbers of observed butterfly species at different temperatures and cloud coverage in six pastures. The percentage of sky covered by clouds was classified into four categories: 1=0–25 %, 2=26–50 %, 3=51–75 % and 4=76–100 %.

observed butterfly species reached an asymptote between 5 and 10 visits, which corroborates the results from the present study. The increase in the proportion of total species found was especially pronounced when comparing one, two and three visits. Obviously, only making a few visits leaves many species undetected. One or two visits to the same site during a season can clearly not be regarded as enough if the goal is to investigate the species richness, and will result in low power to detect changes. Increasing sampling intensity from three to five visits in the Swedish monitoring system would increase the species richness found by approximately 12 percent units on average. However, the gains of increasing sampling effort need to be weighted against the cost, since funding is often scarce in conservation work. Roy et al. (2007) showed that three sampling visits were enough to detect a 25% decline for 20 widespread butterflies with an effort of an average of 430 monitoring sites. The visits is then restricted to July and August and one-generation early summer species will in that case be ignored.

Impact of weather

The development of reliable methods for monitoring is crucial in order to evaluate conservation efforts and setting conservation priorities. Pollard & Yates (1993) have stressed the importance that butterfly counts reflect "real" changes in abundance rather than variations caused by varying conditions during sampling. If the influence of factors such as weather conditions is known, efforts can be made to minimize their impact on the data by adjusting the guidelines for monitoring. A somewhat surprising finding in our study, was that more than 40% of the variation in a species assemblage at a site during two weeks could be accounted for by weather variables. Hence, it seems very plausible that

supplementary data on important weather variables collected during monitoring, may be important if used directly in the analyses to "adjust" for such uninteresting variation (e.g. Rothery & Roy 2001, Benes et al. 2006).

In this study, no effect of wind speed, up to five on the Beaufort scale, on observed numbers of species or individual was found. This is in agreement with other studies (Pivnick & McNiel 1987, Swengel & Swengel 2000) but in conflict with Dover et al. (1997), who reported that counts were particularly low above wind speeds of 8 knots (three on the Beaufort scale). Butterflies are known to spatially redistribute as wind speeds increase, and activity in the components of the landscape offering shelter have been shown to increase as wind speed increased (Dover et al. 1997). Adults of *Thymelicus lineola* change their activity pattern under strong winds by moving close to the ground and never attempt flight (Pivnick & McNiel 1987), which makes counts during such conditions unreliable. It is possible that the influence of wind speed on butterfly counts is greater during periods with lower temperatures than the temperatures during this study. Counts in the early spring have been shown to be unproductive as the species present tend to fly mainly in areas sheltered from the wind (Pollard 1977). Effects of wind speed can also vary greatly between different biotopes (Pollard & Yates 1993), and recommendations concerning wind conditions are therefore needed to be interpreted somewhat differently depending on the current weather conditions, time of season and the exposure of the study site. Wind speeds above five on the Beaufort scale were not encountered during the intensive study period, but experience from a few days with wind speeds of six and seven during the rest of the season suggests that this might indeed be the upper limit for conducting meaningful butterfly census. Despite the lack of detectable wind effects on

species richness or number of individuals, the butterfly assemblages were clearly affected by wind speed. I.e., species must respond differently to wind.

The activity of butterflies is to a large extent limited by temperature since most butterflies require a high and restricted range of body temperature in order to fly. They often achieve these temperatures through behavioural thermoregulation such as basking in the sun (Kingsolver 1985, Pivnick & McNiel 1987, Dennis et al. 2006). Cloudiness has been shown to affect counts of *Danaus plexippus* (Meitner et al. 2004, Davis et al. 2002), and to inhibit flight activity of Hesperiidæ species even if temperature conditions remained favorable (Pivnick & McNiel 1987). However, for most species, flight will not require sunshine if the temperature in the shade is sufficiently high (Pollard & Yates 1993). The criteria in Pollard (1977) are that counts may be made if the temperature is over 17°C, irrespective of sunshine, and below 17°C if at least 60 % of the walk is made in sunshine. Data from this study suggest that somewhat stricter criteria would be preferable in Sweden (which, incidentally is in line with the current Swedish guidelines). A sharp decline in butterfly numbers were detected at temperatures below 19°C if the proportion of sunshine of the transect walk was below 80–85 %. At 20°C the butterflies were active at a lower proportion of sunshine (70 %), and at 22°C around 40 % sunshine. Above 29°C, the amount of sunshine seemed to be of minor importance. Consequently, it seems that the UK Pollards methods developed in the British Isles should not be uncritically transferred to other areas.

Our data also suggest that the overall cloudiness of the sky can be used as a criteria when considering the overall weather situation, and act as help in decision-making concerning the suitability of a particular day for recordings. The activity of the butterflies

showed a threshold when cloudiness exceeded 50 %. At this cloudiness, the number of species showed a drastic fall unless the temperature was at least 22°C.

Diurnal variation

The British recommendation is that transect walks should be undertaken between 10:45 and 15:45 British Summer Time to counter some of the influence of differing activity profiles of the butterflies (Pollard 1977). However, the data that these recommendations are based upon seem to be rather scarce (Moore 1975). Moore (1975) presents data from two days and concludes that the maximum density of butterflies for the day was found to occur at any time from about three hours before noon to about four hours after noon. Pollard and Yates (1993) have tried to assess variation in butterfly counts due to time of day, and found that it had a small, but significant effect on the counts of *Aphantopus hyperantus* compared with the variation due to week and year of recording. However, the data used in their analysis were spread out over the whole season during two years (57 counts) and not only directed to estimate diurnal variation. In our study, 56 counts, spread out from -5 to +6 h from the time when the sun reached its highest point, collected during two weeks were used to assess the diurnal variation in number of observed butterfly species and individuals during the peak abundance of the season at a species-rich site. Our results indicate that transect walks could be performed between -4.5 and +4 h, i.e. 2.5 hours longer than recommended by Pollard (1977). The Swedish criteria for time (-4 and +3.5) do seem to be sound. Before and after these time periods, it is likely that unfavourable air temperatures and light conditions may reduce butterfly activity (Rawlins 1980, Shreeve 1984, Meyer & Sisk 2001). The number of observed butterfly individuals peaked in the morning, and started to

decrease earlier in the afternoon than the number of species. After +2 h, most counts were below the normalized mean value. The reason for this decline was however largely due to the activity pattern of two of the most abundant species: *Brenthis ino* and *Aphantopus hyperantus*. These species had much fewer active individuals, but still many compared to other species, in the early afternoon.

It is clear that single counts taken at one time of the day might be misleading since many butterflies show well-defined diurnal rhythms of flight activity (New 1997a). Results from our study also show that the number of observed individuals of a species might fluctuate greatly over the course of the day, and that there can be large differences in diurnal rhythms of flight activity between species. For example, on the 3rd of July, 145 individuals of *Aphantopus hyperantus* were recorded on a count carried out between -4 and -3 h, while only 60 individuals were recorded between 0 and +1 h, although the weather conditions stayed practically the same. The opposite activity pattern was shown by *Zygaena* species (*Zygaena filipendulae*, *Zygaena viciae*, *Zygaena lonicerae* and *Adscita statice*) with lower abundance in the morning, and a peak in the afternoon. Yamamoto (1975) and Frazer (1973) have described variations in counts of butterflies at different times of day. Frazer (1973) showed that populations of *Aglais urticae* are maximal within an hour or two of noon, and emphasized that a serious source of sampling error might lie in which time of day the counts are carried out. The diurnal behavioural pattern of *Lopinga achine* showed that low number of individuals were captured before 10 am and after 3 pm (Konvicka et al. in press) These differences in diurnal activity pattern of butterfly species are important to consider since it might bias the results from especially in a local monitoring scheme. If counts at a site are carried out in the morning one year and in the afternoon the following

year, the abundance of some species might be either over- or underestimated. If the diurnal activity pattern of each butterfly species is known, this could be used to adjust for time of the day of the count. When the aim of a monitoring scheme is to study changes in abundance of some individual species, the counts should preferably be performed at the same time of day every year to minimize the effect of diurnal activity patterns.

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