The Effect of Altitude and Environmental Variables on Butterfly Activity in Puerto Rico

Thesis

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Thesis statement

None of the material offered has been previously submitted by me for a degree or other qualification to the Open University or any other university or institution. None of the material has been published yet.

Thesis abstract

The focus of this research project was at 735m and above 915m within the cloud forest on El Yunque Peak in the Luquillo Experimental Forest (LEF) in Puerto Rico. From 2001 to 2005, numbers of *Calisto nubila* encountered on El Yunque Peak decreased between 915m and 1000m, while numbers of *C. nubila* recorded along the Tradewinds trail between 735m and 765m increased. A climate anomaly was recorded on the peaks of the LEF by the climate data between May and September 2001.This was reflected in numbers of *C. nubila* encountered on El Yunque peak above 915m in the following field visit in 2002. Numbers of *C. nubila* on El Yunque Peak had not recovered by 2005.

Calculations using the general linear model on the Tradewinds trail 2003 data showed that all the climate variables had a close relationship with *C. nubila* numbers encountered between 735m and 765m. Because all the climate variables had a close relationship with each other as well as with *C. nubila* numbers, no single climate variable on its own had a close relationship with numbers encountered. The timing of the transect walks also had a close relationship with *C. nubila* numbers encountered on the Tradewinds trail. Annual precipitation in the preceding 365 days is shown to be a controlling mechanism on *C. nubila* population numbers on the Tradewinds trail by possibly acting on immature stages and foodplant condition at this site.

Numbers of *C. nubila* encountered on the 965m to 1000m El Yunque Peak transect had a close relationship with wind speed. *Calisto nubila* numbers also had a close relationship with the other climate variables combined.

The results show that *C. nubila* can be used as a biological indicator of climate change in forest ecosystems above 735m.

Chapter One

Introduction

1.1 Aims and objectives of research

The aim of this thesis has been to investigate butterfly activity in the Luquillo Experimental Forest (LEF) and to understand if this is determined by or related to climate factors. In order to fulfil this aim, the following objectives were set:

1. To find out if there were any variations in the distribution patterns of butterfly species in the LEF, particularly above 915m in the cloud forest zone.
2. To ascertain whether the climate variables measured by the portable climate station had any significant relationship with butterfly activity encountered per ten minutes on the transects.
3. To visit different ecological zones in Puerto Rico, set up transects and record species encountered.

It was anticipated that the end result would be a localised biological climate monitoring system using butterflies as the indicator species. The system then had the possibility of being set up in different altitudinal forest regions in Puerto Rico and could also be used as a template for research in other montane forest regions throughout the tropics.

1.2 Overview of chapters one to five

This thesis consists of five chapters. Chapter one is the main introduction to the butterfly species used for data calculations. Available background information is given on environmental and other factors affecting butterfly and other relevant invertebrate activity. Papers are cited in relation to physiological factors, environmental constraints on butterfly populations and life history information. Published papers on *Calisto* spp. are also discussed. A detailed description of the study site is provided in chapter two, this chapter outlines the materials and methods used for data collection and handling and gives a description and distribution for each of the species studied. Chapter three contains the results and is divided into three sections. It first concentrates on *Calisto nubila* Lathy (Satyridae) in the first part, *Antillea pleops pleops* Drury (Antillea) and *Wallengrenia drury* Latreille (Hesperiidae) in the second part with the last section focusing on results from other regions of Puerto Rico. The results are discussed in chapter four in the context of published papers which then puts forward the conclusions reached. The final chapter, chapter five comments on future opportunities for research related to the thesis findings and proposals on how this could be undertaken. The appendix section presents the butterfly data collected including daily *C. nubila* data from the Tradewinds trail transect one and two combined.

This thesis investigates the effect of environmental variables and altitude on three butterfly species, *C. nubila, A. pleops pleops and W. drury*. Most of the data for the thesis is derived from work on *C. nubila*. Previous studies on this and other butterfly species indicate that several environmental variables affect butterfly activity via their effects on factors such as thermoregulation, food availability for larva and reproduction.

1.3 Life history- reproduction

There are a number of published papers that relate to the life history of buttefly species and to *C. nubila* that are relevant to this thesis. This information is presented in the following sections.

Calisto nubila is known to attach the egg to the substrate with glue secreted by the female. The eggs are therefore flatter at the base (Sourakov, 1996). Eggs of some other *Calisto* species are not attached to the substrate, instead, they are dropped into the favoured position by the female. *Calisto pulchella* Lathy is known to drop the egg into the deep pocket between the stem and leaf of sugar cane. A. *Calisto tasajera* Gonzalez, Schwartz and Wetherbee female has been recorded sitting on a leaf and dropping the egg into the middle of an unidentified bunch grass. The grass is so tough and dry that the larvae is thought to feed at the very base of the grass stem. *Calisto zangis* Fabricus has been described in Jamaica dropping eggs while flying over grass in Jamaica (Brown and Heineman, 1972).

The mating system of *Celaenorrhinus approximatus*, Williams and Bell, *Astrapetes galesus cassicus*, Evans and *Mesosemia asa asa*, Hewitson have been studied in Costa Rica (J. Alcock, 1988). Territorial males of all three species patrolled the area around their perching sites regularly and responded to intruders with circling chases and ascending pursuit flights. Patrol flights in C. *approximatus* lasted from three to thirty two seconds. One copulation was seen in twenty hours of observation and was discovered after it had been initiated. The estimated mating time for *C. approximates* was one hour. Patrol flights of *A. g. cassius* lasted from four to twenty five seconds and one male was recorded perching at the same site for twenty five days. Three copulations were observed for *M. asa asa* and these were timed at fifty two minutes and between forty and seventy five minutes. Female pheromone evokes the male pursuing response and generates continued courtship, while male pheromones may cause a female to accept a male. The duration of copulation varies according to species, can be affected by temperature and lasts from thirty minutes to three hours (J. Scott, 1973).

In another study in Costa Rica, the dry season was found to influence the reproductive parameters in female butterflies (F. J. Odendal, 1990). Mature egg number was found to be larger in the early period than the late period. One explanation put forward for this was that the dry season in parts of Costa Rica caused decreased growth in the foodplant.

Research on the temperate satyrid species *Maniola jurtina* has shown that males and females show similar dispersal rates. Males were noted to show a characteristic exploratory flight to seek mates and to fly more often than females. Females in the study were predominantly flying to lay eggs and to feed and male and female *M. jurtina* were found to occupy different ecological niches (Brakefield, 1982a). *Maniola jurtina* was found to have a low rate of movement from areas of favourable habitat. Males were found to fly more often than females and, male behaviour when seeking mates was intermediate between that of patrolling species and of perching species of butterflies. Males were also found to alternate between periods of flight and longer periods of resting during which any butterfly that flew within sensory range was investigated .

The longevity of *M. jurtina* was found to vary between the two sampling years (Brakefield, 1982a). In the first year the mean known length of life for males was seven days and for females, five days. In the second year it was twelve days for males and nine for females. At a separate study site it was noted that adult survival of *M. jurtina* was lowest during the hot and dry summer of 1976 and that there was no obvious difference in survival between the two sample years. The peak period of egg production was found to be between two and five days after mating and that seventy five percent of eggs had been laid by ten days. Although *M. jurtina* is a temperate species, it belongs to the same family as *C. nubila* and behavioural wise, the two butterflies have a number of similarities with the larvae being grass feeders and feeding under the cover of darkness. The maximum lifespan of *M. jurtina* was recorded as twenty two days which was regarded as approaching that reached under favourable laboratory conditions.

Further research on temperate satyrids showed that following a population crash, numbers of *Aphantopus hyperantus* took several years to approach carrying capacity in the most favourable transects. Density–dependent dispersal would then only occur once the density had increased in the optimal transects (Sutcliffe et al 1996). Some temperate satyrid species are thought to be susceptible to desiccation of food-plants through drought. Associations with rainfall were more frequent using pooled three month data which suggested that the main effect was on the growth of plants (Pollard 1988).

In Southeastern Brazil, research was carried out to ascertain the selection of oviposition sites by a lepidopteran community (A.V.L. Frietas et al, 1999). It was found that out of 182 immatures, 90% were eggs or first instar larvae which were assumed to have low mobility within the leaves of the host plant. New leaves were preferred to mature leaves and a clear pattern was found for the occupation of newer leaves that were longer.

1.3.1 Feeding

Many of the *Calisto* spp. found on the Dominican Republic are known to use more than one species of grass as a foodplant (Sourakov, 1996). However many of the rare *Calisto* species on the island are hostplant specific and are thought to reflect their hostplants' present distribution patterns. Satyrinae and Hesperinae species are known to be the only grass feeding species in the Antilles (Scott, 1972).

In the study sites in Costa Rica, leaves of many plants wilted in the later period of the dry season and some species lost most of their leaves (F. J. Odendal, 1990). Further research on the antiherbivore defences of plants concluded that caterpillars feeding on plants which utilise quantitative protective compounds, whose efficiency increases with aging foliage, synchronise hatching with the appearance of fresh leaves (E. Kuehn et al (Eds), 2005). In sweep samples of tropical foliage insects carried out in Costa Rica there was a strong movement of insects into moist refugia during the dry season and a strong reduction in numbers of species and individuals in areas with a severe dry season. However in areas with a mild dry season, the numbers and species of insects appeared to rise (D. Janzen, 1973).

Maniola jurtina first instar larvae are particularly susceptible to low food quality and that a dry environment will decrease the normally low moisture and high fibre content of many grasses (Watson 1951). The longevity of *M. jurtina* has also been found to be higher in cool damp summers than when there was hot and dry conditions. Hot and dry conditions resulted in long periods of flight activity which was thought to have led to a faster rate of wing deterioration and a quicker use of energy reserves. Wetter habitats are thought to have buffered certain populations against hot and dry weather, as high adult density remained in these areas after a hot summer (Brakefield 1982).

Aphantopus hyperantus and *Pararge aegeria*, both Satyrids whose larvae prefer moist and semishaded habitats, show strong associations between decreased abundance and hot and or dry weather in the previous summer (Roy et al 2001). In the early part of previous years *A. hyperantus* and *P. aegeria* also had strong associations with high precipitation levels in the early part of the previous year (Pollard 1988). In a separate study, *A. hyperantus* was thought to have become absent from a location for several years due to the effects of drought on the vegetation (Sutcliffe et al 1996).

Calisto nubila has been successfully reared in captivity (Sourakov, 1999). Eggs were obtained from a single female *C. nubila* collected from El Yunque. No location is given within the forest area. Twelve of the twenty first instar larvae that accepted rye grass developed normally to the last instar, when most were preserved and a few allowed to pupate. Larvae were exposed to a range of grasses for a minimum of four days before any feeding commenced, thus indicating host plant specifity. No record was taken of the longevity of freshly pupated *C. nubila* specimens (A. Sourakov, personal communication).

White colouration has been noted in *Calistos* spp. (A. Sourakov, 1996). Two forms of *Calisto confusa confusa* Lathy which have white colouration, have been found on the island of Hispaniola. This species is the most widespread of the *Calisto* family on the island. *Calisto confusa debarriera* Clench lacks any white colouration on the underside whereas *C. confusa* has dark stripes bordered by white stripes of different width and intensity. The white stripes are noted as varying significantly from one population to another and are stronger in females.

1.3.2 Thermoregulatory behaviour

During a previous study on *C. nubila* at El Verde at 400m in the LEF (Ludwig and Shelly, 1985), it was found that flight activity was greatly reduced during cloudy conditions. Ambient temperature at the study site at 400m was found to be similar during cloudy and sunny conditions. It was noted that *C. nubila* perched almost exclusively on leaf surfaces and was never seen perching on the ground and that flight activity was greatly reduced during cloudy conditions. Ambient temperature at the study site was found to be similar during cloudy and sunny conditions. The reduction in flight activity was related to the importance of basking in elevating thoracic temperatures to levels where flight activity was possible. Males were observed occasionally to tilt their body to increase exposure to solar radiation. Tilting was observed in 21% of basking individuals. Tilted butterfly specimens heated more rapidly than upright ones. The selection of sunlit perches and high thoracic temperatures of basking butterflies suggested that *C. nubila* males, elevated thoracic temperatures through basking.

Kingsolver (1983, 1984) has done extensive studies on *Colias* butterflies in the central Colorado Rockies. These papers are of interest because *Colias* species use thermoregulatory behaviour and specifically lateral basking to elevate their body temperature prior to flight activity. The studies included different populations from low to high elevation sites. Research has additionally been done in America on the thermoregulation and flight activity of the Satyrid *Coenonympha inornata*, Edwards (Heinrich, 1986). Kingslover found that *C. inornata* has been found to be highly constrained by temperature in its activity and even on sunny days may spend a large proportion of its time grounded. It will stop flight in order to heat up by lateral basking and because of its small size cools more rapidly than larger species. The males patrol open grassy areas in search of receptive females by flying in a jerky manner along the grass tops. Butterflies flew readily for five minutes or more without stopping and when they stopped it was generally less than forty seconds. The results in the discussion section of this thesis indicate activity is thought to be affected by previous weather conditions, with flight activity starting later on in the day even with perfect conditions if the weather on the previous day was cooler. *C. inornata* close their wings dorsally and tilt over to one side until they are orientated at approximately right angles to incoming radiation.

Individuals perching at under 24.5 ^{0}C all assumed a lateral basking posture. Those perching at temperatures above 28 ^{0}C assumed a heat avoidance position where the head points away from the sun and wings are closed dorsally away from the sun. At intermediate temperatures, butterflies had ambiguous behaviour. Slight deviations from the normally observed lateral basking posture resulted in large changes in thoracic temperature. A shift in posture from basking to heat avoidance reduced the thoracic temperature by 10 ^{0}C in 90 seconds. *C. inornata* basks laterally after cooling convectively in flight. Flight metabolism is only sufficient to bring thoracic temperatures to 9 ^{0}C above ambient temperatures, so if a thoracic temperature of around 29 ^{0}C is required during flight, then butterflies must periodically bask to store heat. Basking in *C. inornata* functions both as a pre-flight warm-up mechanism and as a means of prolonging flight periods.

The four *Colias* spp. studied require body temperatures of between 30 ^{0}C to 40 ^{0}C for steady state flight. Like *C. nubila*, they bask laterally by holding the wings together over the dorsum and orientating the wings perpendicular to solar radiation. During heat avoidance behaviour, the wings are orientated parallel to incoming solar radiation. For *Colias* spp. only the basal wing is relevant to thermal regulation due to low thermal conductance of the whole wing itself. Basal wing colour is dependent upon the relative proportion of pteridine and melanin-pigmented scales which are under both genetic and physiological control. The thickness of fur which covers the ventral thorax also differs both in and between *Colias* spp. With elevation, the thickness of the fur between the pro and mesothoracic coxae increases among some populations. The thickest fur is found in a population at an altitude of 3,600m with the thinnest fur found at 1,700m. The thickness of fur was more variable between *Colias* populations at 2,700m and 2,915m. The same results were found with solar absorptivity of the ventral hind wing with *Colias* spp. High elevation populations had the highest absorptvitity and low elevation populations the lowest absorptivity. The thoracic diameter also increased with height.

Intermittent clouds cause flight activity of *Colias* spp. to cease throughout the day and direct solar radiation was essential for flight at mid to high elevations. Available flight time was less at mid elevation sites than at the 1,700m site. Analysis of the results indicated that wing absorptivity and total solar radiative flux density strongly influence the body temperature excess over ambient air temperature. Orientation of the body to incoming solar radiation produces similar results. It was also found that wind speed, thickness of fur, fur thermal conductivity and body size affect body temperature to a lesser degree.

Basking temperature of *Colias* spp. could be regulated by changing perch location and the density of the surrounding vegetation. Females at the 1,700m site, restricted flight activity most of the time to within the 35 ^{0}C to 38 ^{0}C range. General flight activity started when basking temperatures of male butterflies exceeded 30 ^{0}C. The air temperature at the high elevation site at 3,600m varied between 17 ^{0}C and 20 ^{0}C while the body temperature of male *Colias* spp. was generally 12 ^{0}C above these values. General flight activity therefore started when the air temperature exceeded 18 ^{0}C. For the mid elevation site at 2,700m, the body temperature was more volatile and generally ranged from 8 ^{0}C to 11 ^{0}C above air temperatures. At the low level site at 1,700m, body temperature was generally between 6 ^{0}C and 9 ^{0}C above the measured air temperature. The solar radiative flux perpendicular to the solar beam was found to remain relatively constant at 110 m/Wcm^2 throughout the day under clear skies at high elevation. Since *Colias* spp. rarely fly except under direct sunlight, it was noted by Kingsolver that this is the rough value for solar radiative flux under which there will be flight activity.

1.4 The effects of climate and elevation on Lepidoptera and anoline communities.

Moth assemblages have been found to increase with elevation on Mount Kinabalu in Borneo over a period of forty two years. The study found that the average altitudes of 102 montane moth species increased by a mean of 67m over the forty two year period. The authors suggest that large numbers of tropical insect species could be affected by climate warming and stated the need for additional sampling of tropical insect species in relation to historical papers (Chen et al, 2009).

Yellow sulphur butterflies, *Ashrissa satira,* have been recorded showing population changes to climatic events and are thought to lay their eggs on new leaves produced by vines only four or five days after the rains begin. Drier years resulted in more new leaves. The number of migratory butterflies was greatest in El Nino years, with one exception when there was an unusually wet El Nino year. Butterfly migrations were therefore linked to climate change and El Nino (Sygley, 2009).

There is evidence from Canada that butterfly diversity is responding to climate changes that have occurred over the last few decades. The Gorgone checkerspot (*Chlosyne gorgone*) and the Delaware skipper (*Anatrytone Iogan*) have recently established breeding populations well beyond the previous northern limits of their respective ranges (Kerr, 2001). A paper on the extinction risk from climate change, used projections of species distributions for future climate scenarios. When the average of the three methods and two dispersal scenarios is taken, the projections ranged from 18% to 35% for species extinction rates by 2050 (Thomas et al, 2004).

Two types of population structure were contrasted by Roy et al (2001). The paper studied patchy populations where individuals move freely among habitat patches, and metapopulations in which the majority of individuals stay within one habitat patch during their lifetimes. In a study of thirty one species of butterfly in Britain, the highest butterfly counts were not correlated with either temperature or sunshine. The conclusion was that peak counts and index values were strongly related to changes in population size.

Gian-Reto et al (2002) stated that it is generally agreed that climatic regimes influence species' distributions, often through species-specific physiological thresholds of temperature and precipitation tolerance. Species are expected to track the changing climate and shift their distribution poleward in latitude and upward in elevation. A further research project by Thomas et al (2004) predicted that on the basis of mid-range climate warming scenarios for 2050, that 15% to 37% of species would be 'committed to extinction.'

The Rapoport-rescue hypothesis (Stevens 1992) states that low elevation populations are rarely intolerant of environmental stochastity because short term climatic variability is positively correlated with elevation (Stevens, 1992). Many species at low elevations are thought to be approaching their upper elevational limits, while species that inhabit higher elevations have larger climatic tolerances and can therefore be found across a greater elevational range. It was hypothesised by Murphy and Weiss (1992) that because many butterfly species are restricted to specific larval host plants, 34% of the butterflies in the Toiyabe Range in the Great Basin, U.S.A. would be extinct in a 3 ^{0}C warming scenario. Dennis (1993) found that because cloud cover, precipitation and wind speed increase and ambient temperature decreases at higher elevations, time available for flight decreases as elevation increases. Time available for flight is also positively correlated with opportunities for egg laying (Kingsolver 1983).

Tropical lizard spp. have recently been shown to be sensitive to changes in local climate (Huey at al, 2008). Huey et al propose that the impacts of any warming will depend on the number of species at risk, physiological sensitivity to warming and options for behavioural and physiological compensation. The paper suggested that forest lizards are key components of tropical ecosystems and appear to be vulnerable to the physiological effects of climate warming.

Chapter Two

Study area, materials and methods

2.1 The Caribbean area, Puerto Rico and tropical montane forest areas

The Caribbean is regarded as a conservation hot spot where 11.3% of the region's original primary vegetation contains 2.3 and 2.9% of the worlds' endemic plants and vegetables, respectively, (Myers at al. 2000) and is one of the world's centres of biodiversity (Helmer et al, 2002). Tropical mountainous areas comprised 11 of the 25 biodiversity hotspots identified with seven of the hotspots including tropical island areas. It was concluded that most tropical islands fall into one or another hotspot due to high species endemism combining with extensive habitat loss (Myers at al, 2000). The altitude band of cloud formation on tropical mountains is limited and the tropical montane cloud forest (TMCF) occurs in fragmented strips and has been likened to island archipelagos. This isolation and uniqueness promotes explosive speciation, exceptionally high endemism and a great sensitivity to climate (Foster, 2001). TMCFs can be found on various island locations throughout the world such as New Guinea, Hawaii, Borneo, Micronesia and both the Greater and Lesser Antilles

TMCFs often occur in a relatively narrow altitudinal zone where the atmospheric environment is characterized by persistent, frequent or seasonal cloud cover at the vegetation level. The net precipitation in these ecosystems is significantly enhanced through direct canopy interception of cloud water. Solitary trees in this biological zone have been shown to collect more horizontal precipitation per surface area than forests of the same species due to the efficiency of the vegetation to collect and condense cloud moisture through exposure to wind (Vogelmann, 1973). TMCFs typically have a high proportion of biomass as epiphytes. Soils are wet, generally waterlogged and highly organic. Endemism is often high in these ecological zones (Hamilton et al, 1995) and the arboreal flora in cloud forests have an exceptionally climate-sensitive nature (Benzing 1998). Foster (2001) commented that nearly every aspect of the cloud forest is affected by regular cloud immersion from the hydrological cycle to the species of plants and animals within the forest. This isolation and uniqueness promotes explosive speciation, high endemism and a great sensitivity to climate.

These ecosystems are increasingly vulnerable to climate change because specific conditions are required to sustain their specialized and frequently endemic biota. Steep environmental gradients are associated with their boundaries and island biota are particularly at risk to invasion and displacement by non native plants and animals (Lloyd et al, 1998). These ecosystems are likely to be sensitive to cloud cover loss, changes to the cloud condensation level and to changes in relative humidity and rainfall. Increased variability of rainfall has been put forward as a likely characteristic of any global warming scenario. TMCFs are also an increasingly threatened ecosystem with many sites still outside protected areas. A study in 1997 by the World Conservation Monitoring Centre, showed that out of a total of 605 identified cloud forest regions, 264 had some form of protected status. Mexico had 64 identified sites, with 7 being protected. The highest concentration of identified cloud forest regions is Latin America, where 46% of global TMCF sites are found in twelve countries.

The cloud forests in the Caribbean and on the peaks of the Luquillo Experimental Forest (LEF) are known as elfin cloud forest and are characterised by short, contorted vegetation. This forest type is usually found on exposed slopes and ridges above 915m in the LEF and is frequently enveloped in dense fog . The canopy height is typically 2m to 6m and the branches are covered with mosses and epiphytes. Annual rainfall in the region increases from 2600mm to 5000mm with elevation (Garcia-Martino et al, 1996).

Regions of cloud forest in the Caribbean region are sensitive to disturbance from hurricanes, the incidence of which are expected to increase in any global warming scenario. For example in the LEF in Puerto Rico the following observations were recorded; regenertion of woody species by seeds was found to be more important in lowland forest than in cloud forest ecosystems and were less responsive to increased nutrient availability after disturbance. Tree growth diameter was also found to be 10-fold greater at lower elevations (Walker et al, 1991).

Puerto Rico is the smallest island within the Greater Antilles, lying east of Hispaniola. The Virgin Islands lie to the East of Puerto Rico which includes the U.S. Virgin Islands and British Virgin Islands with the most easterly island of Anegada. The Central mountain range, El Cordillera Central, runs from east to west across the island and the LEF is on the Eastern side of the island with the town of Luquillo around 20 km from El Yunque Peak, on the coastal plain. The highest peak on the island is 1338m in the Cordillera Central and the highest peaks in the forest are the Pico del este, referred to as East Peak in this thesis, at 1051m, El Yunque Peak at 1065m and El Toro, which is the peak associated with the Tradewinds trail, at 1074m. Annual precipitation levels in Puerto

Rico are variable, with the peaks of the LEF receiving around 5000mm. The Northern coastal plains where Cambalache Forest Reserve is situated have 1700mm of annual precipitation and the South West section of the island receives less than 1000mm (Garcia-Martuneo et al, 1996).

There is evidence that the climate of Puerto Rico has been changing in recent years. 1991, 1994 and 1997 were in the top six driest years of the last century (Larsen, 2000). Eight precipitation stations throughout Puerto Rico have been analysed for trend analysis (M. Molen, 2002). The precipitation records from these stations range from sixty four to ninety three years worth of historical precipitation data, thereby covering most of the last century. From the data, May to September recorded the highest levels of precipitation, December to April has the least and May is the month which historically receives the most rainfall. The Southern part of Puerto Rico is drier than the North due to orographic rains drying out the ocean air. All the stations showed a trend towards lower annual precipitation totals and most are either situated on the northern or southern coastal areas.

Deforestation occurred in Puerto Rico in the early twentieth century with parts of the Luquillo mountains and the El Yunque Forest region being used for shade coffee, timber harvesting, charcoal production and subsistence agriculture. Since the mid-1940s the forest area has been allowed to redevelop naturally, with the LEF being declared a reserve in the 1930's. The peaks themselves have remained largely untouched except for the access roads, masts and small buildings on East Peak and El Yunque Peak. The El Toro region remains untouched, but this peak is also the hardest to get to, normally requiring an overnight stop to traverse from the Tradewinds trail over El Toro peak and down to the access road on the other side. The LEF, where the field visits and transects were undertaken, is also known as the Caribbean National Forest and has been given international recognition by the United Nations, being designated as an International Biosphere Reserve.

The use of a protected region of cloud forest for the research project has the obvious benefit of ensuring that there is no logging or other forms of ecological disturbance to the transect areas except for natural events such as hurricanes, which have played a part in shaping the forest ecosystems on Puerto Rico throughout the historical period. Hurricanes are a common event in the Caribbean and strike Puerto Rico on average every twenty one years (Salvia, 1972). Hurricanes with a category 3 hurricane force or greater pass through the Luquillo Experimental Forest every 50 to 60 years causing landslides, widespread defoliation and the uprooting of trees (Schaefer et al, 1990).

There have been research projects on the effects of canopy gaps and logging activities in the tropics. Canopy gaps smaller than 0.02 ha were found to have little impact on the soil chemistry in Costa Rica, whereas cut forest clearings larger than 0.05 ha showed increases of nitrogen, calcium and magnesium for up to two years after the event. Potassium, ammonium and phosphate did not respond to disturbance (Parker, 1985). However, canopy gaps created by windblow or subsidence differ from logging in that they do not remove plant material from the site. Woody material often acts to reduce soil nutrient availability by microbial immobilisation (Zimmerman et al, 1995). The slow growth rate and water-logged anoxic soils of the regions of elfin cloud forest in the LEF dictate that the recovery period after disturbance takes longer in this ecological zone. There are areas of natural disturbance along sections of the Tradewinds trail transect, particularly where there has been subsidence. From observations, these events have resulted in more open grassy areas which provide a favourable habitat for butterfly activity and for foodplant growth.

In 1992, Puerto Rico had 364,000 hectares of closed forest which accounted for 41.6% of the land cover on the island, with around 5% of the forest area being under protection. Depending where they were found on the island, 45% to 68% of cloud forest areas were described as well protected, 36.7% of land use was pasture and grassland, 5.9% was under agricultural use, 2.4% under coffee production, 10.5% was classed as urban with remaining land covers comprising the remaining 1% (Helmer et al, 2002).

The trees within the cloud forest area (generally above 915m on the peaks of the LEF) receive a major percentage of incoming water through a process known as cloud stripping. This process describes the filtering of low clouds and wind driven mist by the vegetation types at high altitudes. The cloud forest ecosystem is particularly vulnerable to any lifting of the cloud base which would effectively dry out vegetation types adapted to the high atmospheric moisture levels and annual precipitation. The drying out scenario has already been reported as occurring on the lower slopes of the cloud forest in the Monteverde region of Costa Rica (Holmes, 2000).

Four main forest types can be found in the LEF (Wadsworth, 1951), tabonuco forest occurs below 600m and approximately seventy percent of the LEF is comprised of this forest type. Tabonuco forest consists of trees between 20m to 25m in height with an understorey of palms and is found on the Bisley trail and the Big Tree trail where transects were set up for the project. Annual precipitation is around 3537mm yr^{-1} (Garcia-Martino et al, 1996). *Dacryodes excelsia*, the tabonuco tree, is the main species present in this forest type.

Palo colorado forest generally occurs above the cloud condensation level at 600m, is described as mid-elevation forest and comprises around seventeen percent of the total forest area. Trees reach a maximum height of 15m and there is less of a variation between the canopy and understorey height. Annual rainfall in this forest region is around 4191mm yr^{-1} (Garcia-Martino et al, 1996). The main species is *Cyrilla racemilflora*, the palo-colorado tree. Species richness of bromeliads and invertebrates within the total forest area has been found to be the highest in this forest type. The forest found along the Tradewinds transects from 735m to 785m is classed as palo colorado forest. (Richardson et al, 2000)

On peaks and ridges above 750m is the dwarf cloud forest covering two percent of the total forest area in the LEF and palm forest occurs in around eleven percent of the total forest area. Palm forest is confined to steeper slopes where the soil is saturated, the drainage is poor and annual precipitation is in the region of 5000 mm yr^{-1} (Garcia-Martino et al, 1996). Palm height can be up to 15m and both Colorado and dwarf cloud forest can be found within this main forest type. *Prestoa montana*, the sierra palm is the dominant species.

Trees in tall cloud forest are typically around 6m in height whereas the canopy height in elfin cloud forest is 3m. Elfin cloud forest is found on ridges and exposed high level slopes and sixty percent of the total forest biomass consists of root systems (M. Molen, 2002). The most common species found in this ecosystem in Puerto Rico are *Pilea krugii*, a herb species, *Wallenia yunquensis*, a semi woody plant and *Calycogonium squamulosum*, which is a woody canopy plant (M. Molen, 2002). Seven tree species are found in elfin forest and of these three species, *Tabebuia rigida*, *Ocotea spathulata* and *Calyptranthes krugii* account for 80% of tree density (Howard, 1968).

The understorey is dominated by bryophytes and the soils are usually saturated and have slow permeability (Brown et al, 1983). Low soil oxygen saturated soils are thought to limit the function of roots in nutrient uptake (Walker et al, 1996). There are also additional environmental factors which affect tree growth at altitude, such as wind stress and low air temperatures. A study at East Peak in the LEF found annual precipitation to be 4,200mm of which 7% was intercepted mist, with annual temperatures ranging from 17^{o}C to 20 ^{o}C (Brown et al, 1983). The decline in animal abundance with elevation in the LEF mirrors the decline in nutrient inputs and decreasing decay rates along the forest elevational gradient (Richardson, 1999).

2.1.1 Sampling locations in Puerto Rico

The wealth of climate data available for the region was invaluable when doing the data calculations and along with the regions of cloud forest on the peaks, was the main reason for chosing to do the field visits and set up transects in this location. The other main reason was that the trail system was easily accessible through a well maintained road which went all the way to the peaks of both El Yunque and East Peak. An additional factor was that because there was a well maintained trail system, the path edges of the transects provided a good habitat for the foodplant to grow in and as a result, there was ample opportunity for *C. nubila* egg laying along good lengths of the trail system at altitude. *Calisto nubila* activity could be viewed away from the path areas where there had been recent landslides, tree windblow or where there were other grass areas. Access for data collection would have been difficult and only areas off the trail system on the Tradewinds trail between 735m and 765m could have been used, if needed.

The timing of the field visits was chosen because it was thought that this would provide the best opportunity throughout the year to record maximum butterfly activity. January to April is the traditional dry season in Puerto Rico and even during this period, it could rain all day on the peaks. During the field visits, rainfall events in the day were infrequent, although there were a number of days when transects had to be abandoned when it became clear that the precipitation events of that day were going to be prolonged on the peaks. Precipitation levels recorded by the East Peak climate station, the portable climate station during M. Molen's data collection and the portable climate station when it was set up on El Yunque Peak in July 2006, showed that outside the dry season, prolonged precipitation events could last for days and this would have made it difficult to collect data on the peaks. High levels of rainfall would also have made the trail system more difficult to navigate.

There were a number of river systems in the region which had been sampled for up to thirty years and the data was readily accessible through various web sites. The majority of these stations recorded streamflow and water height rather then precipitation, the Rio Icacos station records both precipitation and streamflow and is situated near the start of the Tradewinds trail first transect. The data from this station alone provided a wealth of data which could then be correlated with the rainfall data collected by the portable climate station set up near the start of the trailhead.

The East Peak static climate station also collected daily rainfall data. Daily minimum and maximum temperature data from various static climate stations in the El Yunque Peak region provided data which was useful in isolation but could also be correlated with measurements from the portable climate station. Additional temperature data was also available from the El Verde work station at around 400m which proved useful for measuring the climate anomaly experienced on the peaks was not recorded at this altitude.

Each of the field visits was around four weeks in total. The main focus of each field visit was to get as much sampling done within the El Yunque region and particularly the peaks. It was also decided early on in the project that consecutive daily data from sites above 735m would give a good data set to use with the climate measurements taken every ten minutes by the portable climate station. The first two years covered all the initial transects set up in 2001 in the LEF. In the third and fourth visit the transects above 735m were concentrated on, as higher elevation transects above 735m and the regions of cloud forest above 915m were the focus of the project.

While not always relating directly to the climate data from the portable climate station, the combined El Yunque Peak transects above 915m and the Tradewinds trail transects above 735m were able to give a good insight into *C. nubila* activity levels along much longer total transect lengths. It took approximately forty minutes to drive up the mountain to the Tradewinds trailhead and fifty minutes to get to the start of the combined El Yunque Peak transects at 915m. El Yunque is probably unique in this respect, service roads go right up to both El Yunque Peak and East Peak and on El Yunque Peak, the public can get to the Closed road car park where the trail winds its way up to the peak itself. With easy access, one other major factor in the forest region is that there has been no forestry operations on the higher slopes with the exception of the initial clearance routes for the roads. This has resulted in a largely untouched ecosystem which has been left alone for hundreds of years and in a lot of places has had no clearance at all.

Cambalache Forest Reserve, Guanica Biosphere Reserve and Carite Forest Reserve were also visited for data collection. Cambalache forest reserve is a lowland forest of 1050 acres in the limestone region of the North coast between the municipalities of Barcelonita and Arecibo. The vegetation grows on top of the limestone substrate and karstic formations are a regular feature.Forest vegetation gradually dries out towards the coast on Puerto Rico and semi-deciduous forest dominates karst substrates (Helmer et al, 2002). The site was first visited in 2002 to get a lowland comparison of *C. nubila* numbers encountered. Between 2002 and 2005, the forest was visited on four occasions to collect data. The transect used was the track from the car park to the picnic area as it was thought this gave a good general representation of vegetation present at the site, including tree cover, height and overall levels of sunlight and shade. In 2002 the transect length was longer on 3/3 and went further than the campsite on the right hand fork of the path. No exact finish location was noted, although there is photographic evidence of it. It took between one and a half and two hours to get to the forest from the El Yunque region, depending on how busy the roads were around the San Juan area.

Guanica was the second international biosphere reserve in Puerto Rico to be designated by the United Nations and is located on the southwestern coast of Puerto Rico between the towns of La Parguera and Guayanilla and is classed as a subtropical dry forest. Annual precipitation is around 915mm and it is locally referred to as the driest part of Puerto Rico with full sun temperatures being recorded around 40^{0}C. Growth in the forest is limited by water availability, salt deposition and wind speeds. It is dominated by succulents, thorny trees and shrubs with the canopy height typically being around 5m. There are endemic species in the reserve, such as the cactus species, *Leptocerus quadricostata* and *Guaiacum officinale. Calisto nubila* had been recorded previously in this region (D.S.Smith, personal communication). It was a two and a half hour drive each way from the El Yunque region to get to the reserve and the heat during the transect walks was intense. The parched nature of the vegetation can be seen from the photograph. In comparison, during the dry season in the El Yunque region there is just less rain and while it is predominantly dry along the coast, there are still frequent precipitation events on the peaks. Runs of dry days have also been recorded above 735m.

Carite Forest Reserve lies at the start of the Central mountain area near Caguas and contains 6,600 acres of forest areas. The forested area ranges from a height of 250m to 903m. Carite Forest Reserve was established in 1935 to protect river basins in the region from erosion that had developed as a consequence of logging and agricultural land use. It was a two hours drive west to this area from the El Yunque region.

Carite Forest Reserve and Guanica Biosphere Reserve were also visited after consultation with D. S. Smith. Records of *C. nubila* exist for both sites (D. S. Smith, personal communication). The El Radar trailhead and Doppler radar weather station were at similar heights to the start of the El Yunque trail at 915m and the El Seis trail also started at a comparable height to the higher level transects. There was no cloud forest found at Carite and the whole forest system is of recent origin and although the paths were muddy, indicating recent rainfall, the structure and vegetation of the forest is very different to El Yunque. There was no swirling mist or cloud cover and the grasses were noted as different both structurally and species wise from a visual perspective.

The Guanica and Carite visits were simplified data collection exercises in that there was no associated climate data and they were also single visits. The main reason for this was due to time constraints and that although the butterfly numbers recorded were relevant to the project, the sites were not considered as promising as Cambalache Forest Reserve for a second forest area to focus upon. The LEF data set was the primary focus of the field visits and the data from the transects above 735m was producing promising results, so when faced with the option of visiting other less fruitful sites as far as *C. nubila* was concerned, the decision was made to cover the above sites in one visit, Cambalache once or twice each field visit and to focus on the high level transects in the LEF.

Guanica Biosphere Reserve was visited on 13/3/03 and a short circular trail was chosen from the parking area. The transect started at 13:05 and finished at 14:15. Carite Forest Reserve was visited on 8/3/02 The Charco Azul trail was walked to the natural pool and back. The El Radar trailhead and Doppler radar weather station were visited and part of the El Seis trail was walked which starts at the recreation centre. Cambalache Forest Reserve was visited on 17/3/02, 9/3/03 and 16/3/03 and 3/4/05. The transect used was the main path leading to the campsite from the car park area. The transect finished where the path forked, with the left hand track leading to the campsite.

2.1.2 Butterfly study species in Puerto Rico

The genus *Calisto* contains all of the Satyridae found in the West Indies, most of which are found on the Greater Antilles. *Calisto* spp. share the swollen bases of the forewing subcostal and cubital viens found in other satyrids and are predominantly brown in colouration. Members of the genus are more closely related to each other than to any genus on the mainland. The larvae of species for which information is available, are mainly green in colouration, have forked tails and all feed on canes or grasses. The pupae is stout, green to brown and suspended form the substrate by the cremaster. *Calisto* spp. are considered to be weak fliers with a fragile wing structure (Miller et al, 1993). The forty described species so far are endemic, *C. nubila* is found on Puerto Rico, is the only satyrid found on the island and has also been recorded on the nearby island of Culebra (D. S. Smith, personal communication). *Calisto nubila* is widely distributed, from the mountain peaks, lowland woods and their grassy edges, to the dry forest in Guanica and flies throughout the year. The sexes are noted as similar, although this is now open to question with the findings in this thesis, with the female having a lower forewing measurement of 22mm and the male 21mm. *Calisto nubila* has a slow erratic flight pattern at higher altitudes, which speeds up at lower elevations and it unpredictabily darts into undergrowth when flying along forest tracks.

Wallengrenia drury and *A. pelops pelops* together with *C. nubila* were the other butterfly species encountered relatively frequently along the transects in 2001. At some point along the high level transects these species would be encountered and particularly when the sun broke through the clouds, there could be a flurry of activity in a more open grassy area.

Wallengrenia drury is recorded on a number of islands in the West Indies including the Southern Bahamas, Turks and Caicos islands and Hispaniola. A wide range of nectar sources are known to be used including *Bidens alba.* The larvae are noted as feeding on rice and sugar cane as well as grasses. This species has been observed to lay eggs on one of the grass species on the 965m transect on El Yunque Peak.

Antillea pleops pleops is found on the Greater Antilles as far as St. Kitts in the lesser Antilles. It is noted as occurring in small isolated colonies in both mountain and coastal localities. It flies throughout the year but is noted as being most frequent from June to August. The life history of *A. pleops pleops* is unknown. Larvae were found *on Justica martinsoniana* in June 1990 on El Yunque Peak, in areas severly damaged by a hurricane the previous year (Torres, 1992).

2.1.3 Butterfly transect data

Ten transect sites were selected covering three biogeographical regions of East Peak, El Yunque and the Tradewinds trail in the LEF in Puerto Rico. A walk and count method was used as it allows continuous assessment of all butterfly species present. The sampling method used in this field project is the line transect as described by Pollard (1977), recording all butterflies seen 2.5m either side of the transect route and up to 5m in front were recorded. Transect counting has become a standard method both in monitoring and undertaking ecological studies on butterflies (Pollard and Yates 1993). It has been concluded that seven to nine counts with regular time intervals will generate enough data to give a reasonably good sample of the local butterfly species pool. Numbers of observed butterfly species rise considerably in the first few additional counts in the sample.

The numbers of butterfly species encountered per ten minutes, are average values which were calculated from the mean number of species encountered per ten minutes for each transect walk section, and then calculating the mean of the value generated from the first calculation.

With a larger number of counts the accumulative curve of the observed species number started to level off and it was found that monitoring for a relatively short time period can give a clear picture of both local and regional butterfly biodiversity and species composition (Gutierrez and Menendez, 1998). The minimum transect sample for the higher level transects in a single year was six outward and six return transect walks on the Tradewinds trail transects one and two above 735m, and four outward and four return transect walks on the El Yunque Peak transects above 915m over a three to four week period during the field visits. One population turnover is the normal minimum duration for studying community stability (Gutierrez and Menendez, 1998) and sampling was done consecutively for the first three years of the project. There was no field visit in 2004 as it was thought that by having a years' break in the butterfly data collection, future climate fluctuations would be reflected in the *C. nubila* data set in the final visit in 2005. The data was analysed using correlation calculations, a proven method for doing statistical analysis of butterfly populations (Gutierrez and Menendez, 1998) and using the General Linear Model.

Pearson's correlation coefficient was used on the butterfly data recorded on the Tradewinds transects from transects above 735m to 765m and from 965m to 1000m on El Yunque Peak and climate data from the portable climate station and static climate stations. This is a parametric test for the strength of the relationship between pairs of variables which gives a value between plus 1 and minus 1. It is normally used as a measure of the strength of linear dependence between two variables. At plus one, all data points lie on a line for which Y increases as X increases. Minus one indicates that all data points lie on a line for which Y decreases as X increases. A value of 0 indicates there is no linear correlation between the variables.

The General Linear Model was used as the main statistical method of dealing with the *C. nubila* and climate data recorded by the portable climate station. This model can be seen as an extension of linear multiple regression for a single dependent variable. The reason for doing multiple regression calculations on the data set was to quantify the relationship between several independent or predictor variables. In this thesis the predictor variables were the the climate variables and the dependent variable was numbers of *C. nubila* encountered per ten minutes on the

Tradewinds transects one and two combined from 735m to 765m, and the third El Yunque Peak transect from 965m to 1000m.

The lengths of the transects were defined by selected marker points along the trail system. Six transect sites were on El Yunque along the Bisley trail at 250m, the Big Tree trail at 515m, the Closed road at 750m and on El Yunque Peak itself from 915m to 935m, 935m to 965m and 965m to 1000m. Two transect sites were on East Peak at 715m in colorado forest and 1000m on East Peak. The East Peak sites were only visited during sunny periods. If there was no sun when the 1000m transect was visited, no butterfly activity was recorded. This was the most problematic transect to do, as often at the start of the short windy road to the peak, it would be clear of cloud cover but by the time it was reached, it was overcast at the transect. The last transect site was on the Tradewinds trail between 735m and 765m. After the 2001 visit, this transect was split into two sections, the distance of the second section being much shorter. For the 2005 visit, an extra consecutive transect between 735m and 765m was added.

The Tradewinds trail and El Yunque sites were along existing forest tracks and paths, the East Peak sites covered grass verge and colorado forest at 715m, grass verge and cloud forest at 1000m and were not along any tracks or trail system. The El Yunque transects at 915m, 935m and 965m followed consecutive path systems. The Tradewinds transects ran from the start of the trail at 735m on the El Yunque side. The location of the transects and portable climate station, main static climate stations used, roads and two river systems mentioned in the text are shown in Figure 1 which is provided to give an initial insight into where all the various sites referred to in the thesis are illustrated. It is not intended to be an exact illustration.

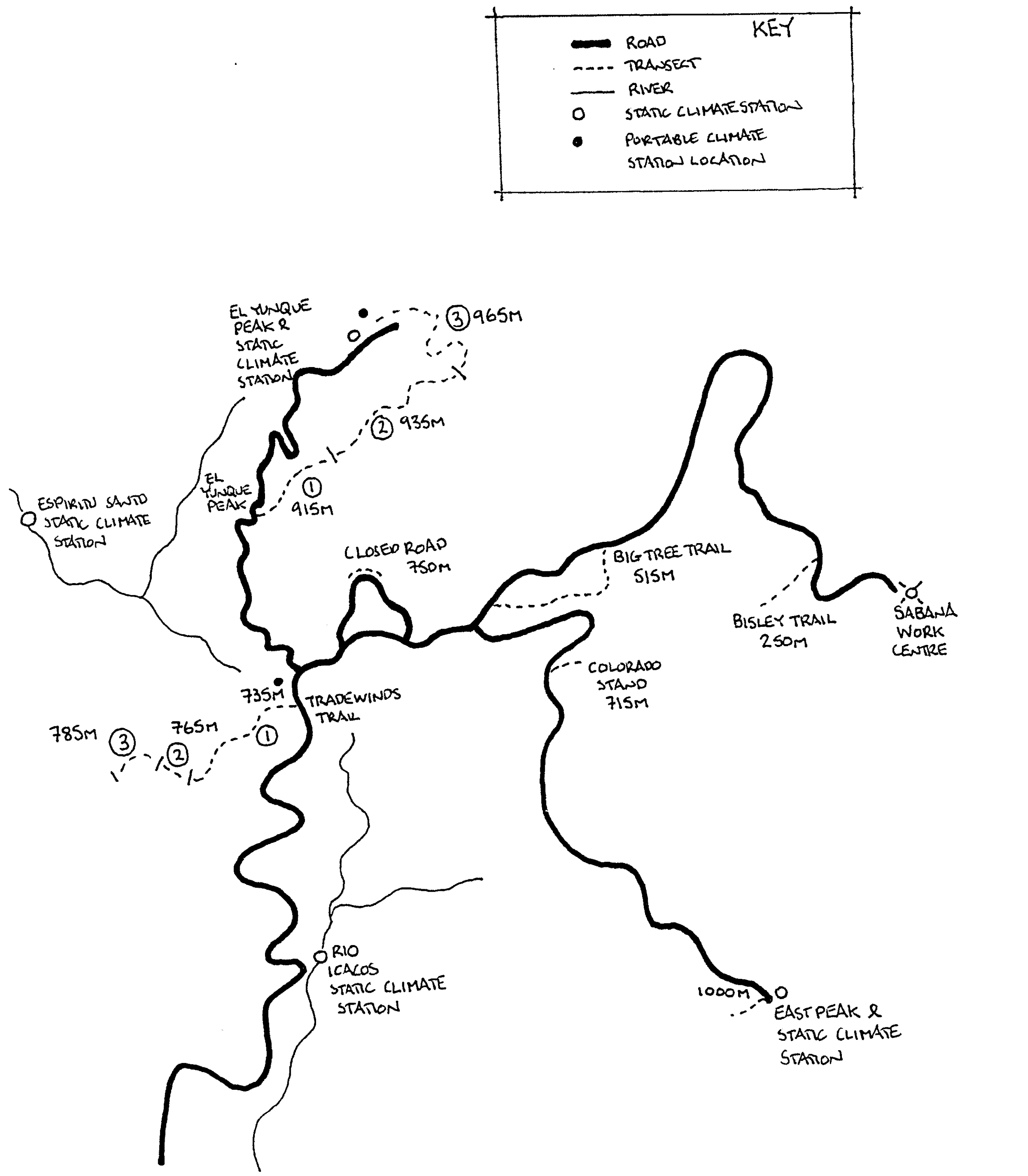
The data for this thesis was collected between:

1. 4th to 22nd of April in 2001.
2. 28th February to 21st March 2002.
3. 27th February to 19th March 2003.
4. 19th March to 6th April 2005.

The first field visit in 2001 was carried out during the dry season which normally lasts from January to the end of April as it was thought that butterfly activity and therefore numbers encountered would be high during this period, particularly on the peaks. Subsequent visits were done during the same period to ensure continuity of data collection and analysis.

The duration of the highest three consecutive El Yunque transects one way was approximately fifty minutes and the duration of the Tradewinds transects one and two combined was an average of thirty five minutes each way. Transects were walked in a range of weather conditions, including rain. Walks were carried out throughout the day, normally between 09:00 and 15:30 hours.

Figure 1. Map showing the transects used in the LEF, road systems, the main static climate stations used in the data calculations and the locations of the portable climate station. Illustration is not to scale.



In 2001 each transect was walked 6 times out and back during March 2002, most transects were walked 3 times, with the Tradewinds trail walked 9 times and the El Yunque Peak transects above 915m walked 7 times. The

Closed road transect was walked 4 times in 2002. In 2003 the Tradewinds trail and El Yunque Peak transects were focused upon, these being walked 14 and 4 times respectively. During 2005 the Tradewinds transects were walked 15 times and the El Yunque transects walked 4 times. An additional consecutive higher transect from 765m to 785m was also completed twice in 2003 and 15 times on the Tradewinds trail during the 2005 visit.

The portable climate station consisted of a 3.5m mast with temperature and humidity sensors (Campbell Scientific Ltd, HMP35AC) and cup anemometers (Vector instruments A100M/ A100ML) placed at 1.15m and 3.5m above the ground. A rain recording guage and a Q7 net radiometer were also included.

The portable climate station was situated at 1000m on El Yunque Peak from 27th February to 2nd May 2002 and near the start of the Tradewinds trail from 1st to 18th March 2003, 17th February to 6th April 2005 and 20th July to 2nd August 2006. Minimum and maximum temperature and humidity, wind direction and speed (2002- 2005) and total and standard total irradiation (2002 to 2003) were measured every ten minutes. Portable climate station data collected in 1997 and 1998 (M. Molen, 2002) has also been used.

Rainfall records are available for climate stations within the LEF, some stations have annual totals from 1920, with additional records dating back to 1896. The East Peak climate station is situated at 967m where monthly mean average maximum and minimum temperatures and precipitation are available covering a thirty year period to the present. This data is recorded by the S.E.R.C.C. (South East Regional Climate Centre) in the United States. The U.S. Geological Survey has undertaken continuous hydrologic studies in the region since 1969.

Chapter Three

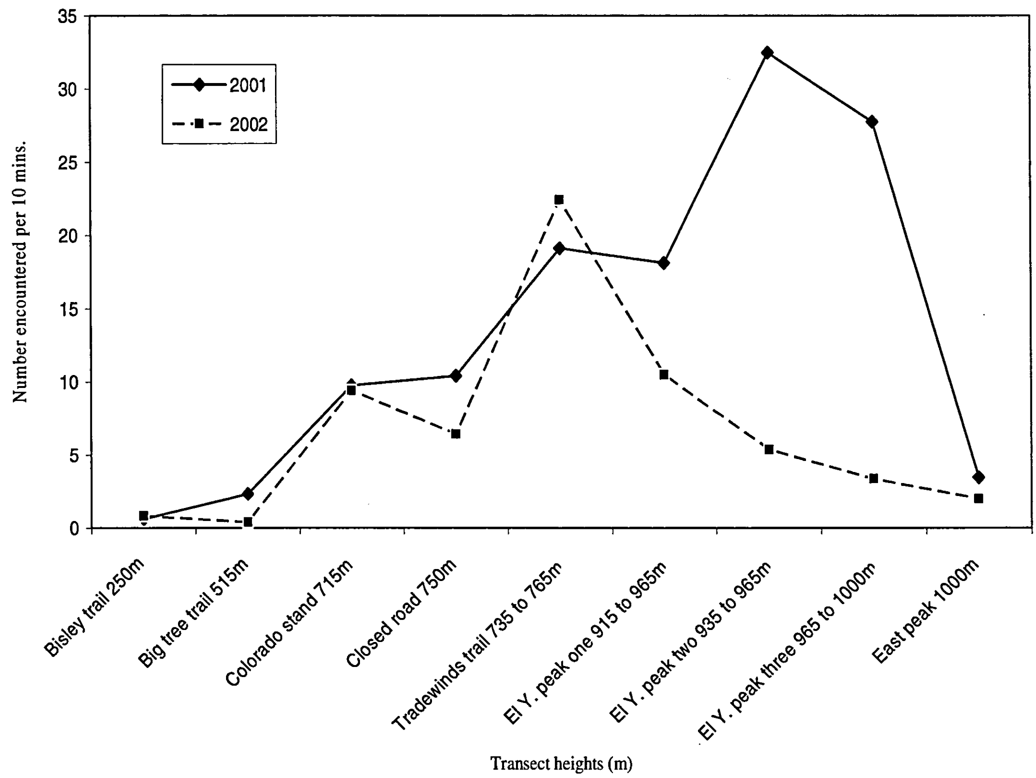
Results

This chapter will consider the altitudinal distribution patterns for *C. nubila,* numbers encountered per ten minutes and climate data from the Luquillo Experimental Forest (LEF). The main aim of the first section of this chapter is to ascertain whether there are any significant relationships between numbers of *C. nubila* encountered per ten minutes along the Tradewinds trail transects from 735m to 765m and El Yunque Peak transects above 915m and the climate parameters. The portable climate station was set up on El Yunque Peak at the end of the 965m to 1000m transect during the 2002 field visit and near the start of the Tradewinds trail transect at 735m during the 2003 and 2005 visits.

3.1 *Calisto nubila*

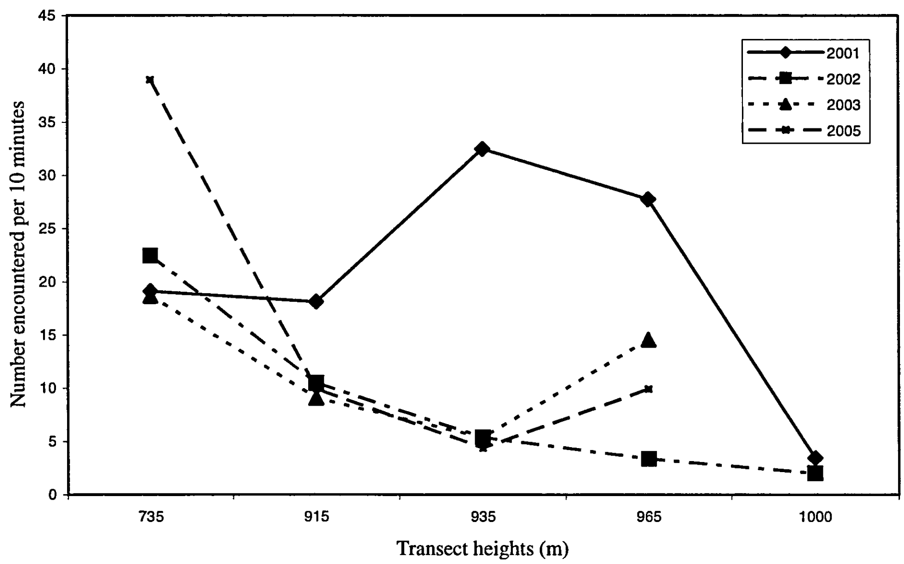
In 2001 *C. nubila* was found to have an altitudinal distribution pattern and was encountered in higher numbers in the LEF above the 735 to 765m Tradewinds trail transect with numbers increasing up to the El Yunque Peak third transect from 965 to 1000m (Figure 2). In 2002 there was an increase up to the 735 to 765m Tradewinds trail transect and then a decline.

Figure 2. Numbers of *C. nubila* encountered per 10 minutes in the LEF. In 2001 each transect was walked 6 times In 2002, the Closed road transect was walked 4 times, the Tradewinds trail transects walked 9 times, the El Yunque Peak transects 7 times, all other transects were walked 3 times. The transects have been inserted in the figure according to the highest point of the transect walk.



Numbers encountered along the three highest El Yunque transects and the Tradewinds trail transects were variable between the years sampled (Figure 3).

Figure 3. Numbers of *C. nubila* encountered per 10 minutes along the Tradewinds trail at 735m, El Yunque Peak transects from 915m to 965m and East Peak at 1000m in the LEF 2001, 2002, 2003 and 2005. Numbers encountered per 10 minutes for the Tradewinds one and two transects have been combined. In 2001 transects were walked 6 times and in 2002 the Tradewinds transects were walked 9 times, the El Yunque Peak transects walked 7 times and the East Peak transects walked 3 times. In 2003 the Tradewinds and El Yunque Peak transects were walked 14 and 4 times respectively. In 2005 the Tradewinds transects were walked 15 times and the El Yunque Peak transects walked 4 times.



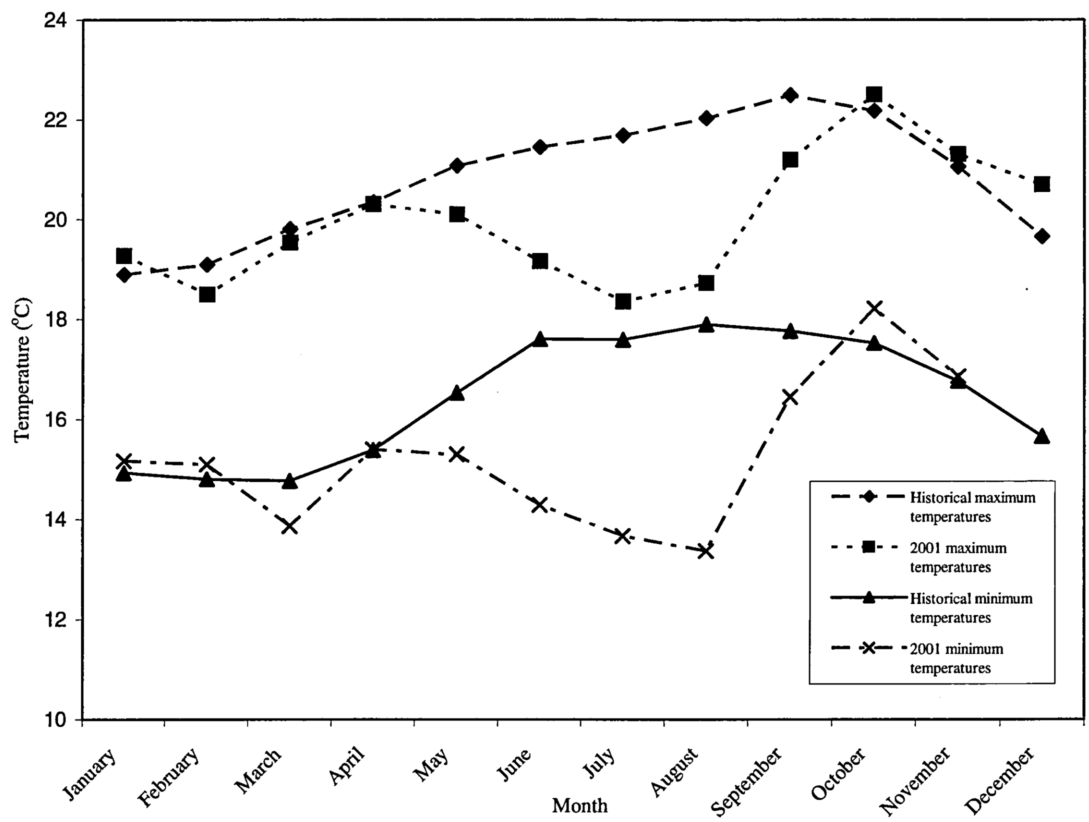
The difference in numbers of *C. nubila* encountered per ten minutes above 735m on the main field transects between 2001 and 2005 are illustrated in Figure three. In 2001 *C. nubila* numbers on the El Yunque peak transects between 915m and 965m were at higher levels than they were in subsequent years. There was a slight recovery in numbers along the 965m to 1000m El Yunque Peak transect. Numbers of *C. nubila* along the Tradewinds trail transect between 935m and 965m fluctuated between the years, while numbers of *C. nubila* encountered on the East Peak transect at 1000m remained low.

Data collected by M. Molen (2002) from 19th June to 28th July 1997 from tall cloud forest at 995m and from 29th April to 17th June 1997 and 3rd March to 14th May 1998 in short cloud forest at 1025m support the theory that the rise in *C. nubila* numbers along the 965m to 1000m El Yunque Peak transect only from 2002 to 2005 may be due to changes in the climate variables along this section because of the change in canopy height and more open vegetation structure. The data show that on clear days, relative humidity readings drop to lower levels and for longer periods in the short cloud forest in comparison with the taller cloud forest. Both data collection sites were on East Peak.

3.1.1 The 2001 climate anomaly recorded in the data

Monthly mean average maximum and minimum temperatures for 2001 for the East Peak climate station are shown in Figure 4. Of particular interest in both Figures are the data presented from May to September in 2001 when temperatures were lower than the historical mean.

Figure 4. Thirty year historical monthly mean maximum and minimum temperatures and 2001 monthly mean maximum and minimum temperatures from the East Peak climate station.



Reynolds sea surface temperature data from the NOAA-CIRES climate diagnostics centre, Colorado, U.S.A. and radiosonde observations performed by the National Weather Service indicate that Puerto Rico experienced average sea surface and air temperatures at ground level from May to September 2001. The El Verde climate station at 400m in the forest area also returned standard maximum and minimum temperatures for this time period. The East Peak climate station recorded that from the 30/5/01 to 11/9/01 there were 94 days out of the 105 day total during this period where the recorded daily maximum temperature was the lowest in the thirty one year period that the station had been collecting data. For the same time period there were 60 days where the lowest daily minimum temperature was recorded in the station's thirty one year data bank. From the climate information available to date only the peaks in the LEF experienced cooler conditions.

3.1.2 Climate station data and *C. nubila* numbers encountered above 735m

Table 1 illustrates the *r* values and levels of significance between minimum humidity and other climatic variables on the Tradewinds one transect in 2003 and 2005 during transect walks. The climate variable which has the closest relationship with minimum humidity in these calculations is maximum temperature. The calculations show that minimum temperature, humidity, maximum temperature, and wind speed all have a close relationship with each other at 735m in the LEF.

Table 1. *r* values and levels of significance for the total time period the portable climate station was set up on the Tradewinds trail in 2003 and 2005.

	Minimum humidity (%)	Level of significance	Year
Maximum temperature (°C)	-0.91	1%	2003
	-0.79	1%	2005
Minimum temperature (°C)	-0.89	1%	2003
	-0.77	1%	2005
Wind speed (Ms^{-1})	-0.44	1%	2003
	-0.24	1%	2005

Table 2 illustrates the r values and levels of significance between minimum humidity and other climatic variables on the El Yunque Peak 965m transect in 2002 during transect walks. The calculations show that minimum temperature, minimum humidity, maximum temperature and wind speed all have a close relationship with each other at 965m in the LEF.

Table 2. *r* values and levels of significance for the total time period the climate station was set up on El Yunque Peak in 2002.

	Minimum humidity (%)	Level of significance
Maximum temperature (°C)	-0.7	1%
Minimum temperature (°C)	-0.69	1%
Wind speed (Ms^{-1})	-0.06	1%

An additional calculation was done for daylight wind speeds and minimum humidity levels to see if there was a change in the *r* value. There was a higher *r* value, but the difference in values when compared to the twenty four hour calculation was minimal.

Figures 5 and 6 display the close relationship between minimum humidity and maximum temperature at 735m and 1000m in the LEF.

Figure 5. Relationship between minimum humidity and maximum temperature on the Tradewinds trail at 735m from 1/3/03 to 18/3/03 ($p<0.01$, $r = 0.9$, $n=2152$)

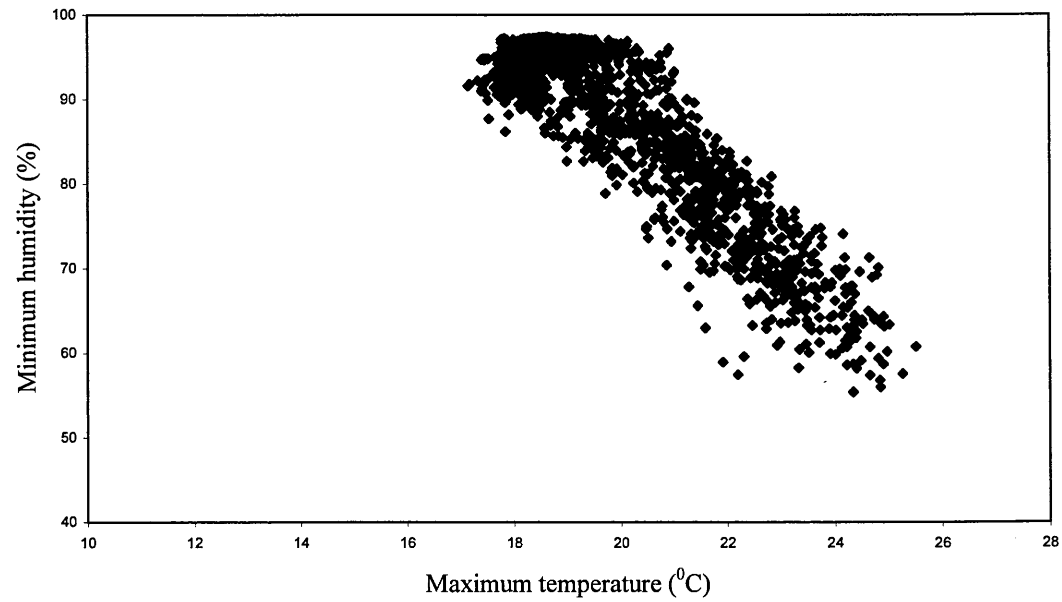
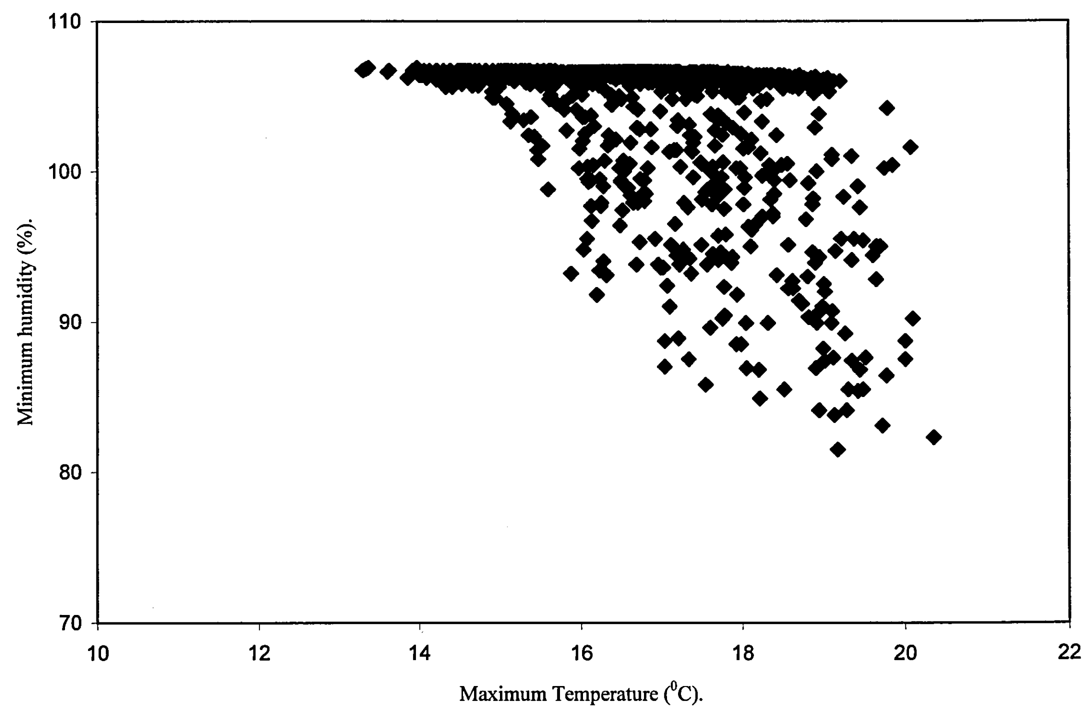


Figure 6. Relationship between minimum humidity and maximum temperature at 1000m on El Yunque Peak from 2/3/02- 13/3/02 ($p<0.01$, $r=0.41$, $n=1730$).



In further correlation calculations, minimum humidity was the climate parameter which had the closest relationship with *C. nubila* activity in 2003 and 2005 on the out transect (Table 3). Minimum and maximum temperature and humidity and wind speed per ten minutes during transect walks were used in these calculations.

Table 3. Correlation coefficient (*r*) and corresponding level of significance on the out transect for *C. nubila* numbers and minimum humidity on the Tradewinds trail one transect in 2003 and 2005. The portable climate station was not always on site for the duration of the complete set of transect walk data.

Tradewinds trail 2003, n=10			Tradewinds trail 2005, n=13		
r values on out transect for butterfly numbers vs:		Level of significance	r values on out transect for butterfly numbers vs:		Level of significance
Minimum Humidity (%)	- 0.872	1%	Minimum Humidity (%)	- 0.77	1%

Table 4. Correlation coefficient (r) and level of significance on the Tradewinds trail return transect for *C. nubila* numbers encountered per 10 minutes and minimum humidity.

Tradewinds trail 2003, n=10			Tradewinds trail 2005, n=13		
r values for return transect, butterfly numbers encountered per 10 minutes vs:		Level of significance	r values for return transect, butterfly numbers encountered per 10 minutes vs:		Level of significance
Minimum Humidity (%)	- 0.12	NS	Minimum Humidity (%)	- 0.509	5%

In correlation calculations for the return transect, minimum humidity was the only climate variable which displayed a close relationship with *C. nubila* activity (Table 4). When the 2005 return transect data is compared with the return transect data from 2003, this indicates that as the time of the walks gets later, activity levels could be less constrained by minimum humidity and may be independent of the other climate variables. Return transect times were done later in 2003 than in 2005. Minimum and maximum temperature and humidity and wind speed per ten minutes during transect walks were used in these calculations.

Table 5. Relationship between *C. nubila* numbers and minimum humidity during out and return transect walks combined on the third transect at 965m on El Yunque Peak in 2002 (n=14).

El Yunque Peak, third transect, 2002		
r values for butterfly numbers vs:		Level of significance
Minimum humidity (%)	- 0.723	1%

On El Yunque Peak, minimum humidity was also chosen as the climate variable used in calculations, because along with maximum humidity, it had the closest relationship with *C. nubila* numbers encountered (Table 5). Maximum and minimum humidity and temperature, wind speed and standard total irradiation and total irradiation were used in the calculation.

During the 2003 field visit the general linear model shows that there was a significant relationship between numbers of *C. nubila* encountered every ten minutes on the Tradewinds one transect between 735 and 765m and average values measured by the portable climate station during walks for the following climatic measurements; minimum humidity, maximum temperature, maximum humidity, minimum temperature and wind speed. For the calculations, average mean values of the climate variables during the transect walk times were used.

In the general linear regression model used for analysis, all climate variables are closely correlated with each other so only one climate variable can be used as an explanatory variable for *C. nubila* activity on the Tradewinds trail (Table 6). Minimum humidity has been chosen as the climate variable to be used because it has the highest *r* value in correlation calculations with butterfly numbers encountered per ten minutes on the out transects and was the only climate variable which had a close relationship with activity levels in the correlation calculations on the return transects on the Tradewinds trail.

The result of the overall general linear model for the Tradewinds trail in 2003 is 52.1% ($p<0.001$, $r^2 = 0.521$). This is the percentage of *C. nubila* numbers encountered that can be explained by the variation of minimum humidity and transect time. Minimum humidity and the time the transect is walked are therefore both significant predictors of *C. nubila* numbers encountered on the Tradewinds one transect in 2003 (Table 6). Mean *C. nubila* numbers with the standard deviation on the out and return transects are shown in Table 7.

Table 6. Significance of each predictor of *C. nubila* numbers encountered on the Tradewinds trail 2003 with the F value and degrees of freedom.

Climate/other variable	$F_{1,17}$	Level of significance (p)
Minimum humidity	8.52	0.0096
Timing of transect	16.27	0.0009

Table 7. Mean values and standard deviation for the out and return transects on the Tradewinds trail transect one in 2003 (n=14 each for out and return).

Transect	Mean *C. nubila* numbers	Standard deviation
Out	14.07	4.87
Return	20.49	4.21

There was a close relationship between numbers encountered per ten minutes along all three Tradewinds transects in 2005. Tradewinds one and two ($p<0.001$, $r=0.848$, $n=30$), Tradewinds transects two and three ($p<0.001$, $r=0.846$, $n=30$) and Tradewinds one and three ($p<0.01$, $r=0.850$, $n=30$). Daily numbers encountered per ten minutes along each of these transects indicate that there are mutual factors controlling activity levels.

The result of the overall general linear model for El Yunque Peak in 2002 is 65% ($p<0.001$, $r^2 = 0.65$). This is the percentage of *C. nubila* numbers encountered that can be explained by the variation of minimum humidity and wind speed. Minimum humidity and wind speed are therefore both significant predictors of *C. nubila* numbers encountered on the El Yunque Peak 965m transect (Table 8).

Table 8. Significance of each predictor of *C. nubila* numbers encountered on El Yunque Peak 2002 with the F value and degrees of freedom.

Climate variable	$F_{1,17}$	Level of significance (p)
Minimum humidity	23.22	0.00023
Wind speed	7.85	0.013

The 2002 survey period lasted 59 days and covered a complete *C. nubila* life cycle on El Yunque Peak, with transects above 915m walked from the end of February to the end of April. This 59 day period covered the estimated 60 day *C. nubila* life cycle under laboratory conditions (A. Sourakov, personal communication). Numbers encountered on the El Yunque Peak transects from the end of February through to the end of April confirmed that the *C. nubila* life cycle remained at low numbers when compared to the 2001 field visit and that the complete population cycle had been affected. One other point from the additional data collected in April 2002 was that two of the walks were completed under good to very good conditions for *C. nubila* activity, thus ensuring that maximum population activity would have been covered by these transects.

In 2002 the El Yunque transects were walked outside the field visit on 10^{th}, 13^{th} and 27^{th} April and show that periods of high rainfall are a limiting factor on population activity (Table 9). The extra data collected also indicates that numbers encountered around the Closed road transect area did not show the variations recorded above 915m between 2001 and 2002 and therefore the climatic anomaly only appears to have affected the *C. nubila* population above 915m.

Table 9. El Yunque transects above 915m walked in April 2002 by B. Bryan. Data also included for extra section walked along roadside up to start of Mount Britton trail on 10/4/02

Date	Transect and number of *C. nubila* encountered per 10 minutes			Weather conditions
	915m- 935m	935m- 965m	965m- 1000m	
10/04/02	4.7	0.57	0.67	Overcast rain
13/04/02	12	16	7.27	Sunny
27/04/02	17.4	9.38	13.5	Sunny periods
	Additional section walked. Number encountered per 10 minutes			
10/04/02	20			Overcast/drizzle with patches of sun

3.1.3 Comparison of the remaining climate and streamflow data

Maximum daily temperatures on El Yunque Peak are 3.7°C warmer in March and 3.39 °C warmer in April than on East Peak (Table 10). Minimum temperatures on El Yunque Peak are 2.13 °C and 3.3 °C cooler in March and April respectively than on East Peak. Overall average daily temperatures are similar for both months. This data illustrates that there are differences in the El Yunque Peak and East Peak maximum and minimum daily temperatures recorded, but the combined averages are similar. It is the East Peak static climate station at 1,000m that recorded the climate anomaly during the 2001 season which was reflected in the numbers of *C. nubila* encountered on The El Yunque Peak transects above 965m in the subsequent season. For data collection reasons the 14th, 15th, 16th and 17th of March were not included in the El Yunque Peak calculations.

Table 10. Maximum, minimum and average daily temperatures for El Yunque Peak and East Peak in March and April 2002.

	El Yunque Peak March 2002	East Peak March 2002	El Yunque Peak April 2002	East Peak April 2002
Maximum Temperature (^{0}C)	23.27	19.57	23.78	20.39
Minimum Temperature (^{0}C)	13.3	15.43	12.5	15.8
Average Temperature (^{0}C)	17.26	17.51	17.75	18.1

May to September 2001 was when monthly mean average maximum and minimum temperatures on East Peak were below the January to April means, with the result that the cooler temperatures associated with the dry season were extended through the summer months. Maximum temperatures from May to September 2001 on East Peak were the lowest ever recorded for that period. When compared to monthly mean average maximum temperatures from the historical records in the dry season from January to April however, they are not unusual.

Monthly mean average minimum temperatures for July and August 2001 on East Peak are amongst the lowest ever recorded. There are only seven other months in the recent historical record leading up to the 2001 season where monthly mean average minimum temperatures fell below 13.8 °C and these all occurred in the dry season when monthly precipitation totals are lower. Three of these months were February 1999, March 2000 and March 2001 where monthly precipitation totals were low, with 2001 having the second driest March on record after the 2005 season.

Historical monthly means and monthly precipitation totals are shown for February 1999, March 2000 and 2001 and July and August 2001 from East Peak (Table 11). These selected months from the historical record all had average minimum daily recorded temperatures under 13.8^{0}C, as did temperatures measured on East Peak during May to September 2001. The monthly precipitation totals were up to three times higher in July and August 2001 than during the above selected months. This indicates that it is the combination of high precipitation levels and low daily temperatures that affected the *C. nubila* population on El Yunque Peak.

Table 11. Monthly precipitation totals and mean values for selected months 1999- 2001 from the East Peak static climate station.

Month	Monthly precipitation	Annual monthly mean
February 1999	158 mm	261.6 mm
March 2000	111.5 mm	252 mm
March 2001	108.7 mm	252mm
July 2001	275.6 mm	330.2 mm
August 2001	512.8 mm	386.1 mm

Annual streamflows for two rivers in the Caribbean national forest are recorded in Table 12. The Rio Icacos static climate station is near the start of the Tradewinds transect at 735m, the Quebrada Espiritu Santo has its source below El Yunque Peak and the static climate station records the data at a reasonably early point. If the streamflow measurements are taken to be an indication of annual precipitation in the immediate surrounding areas, then the data shows that El Yunque Peak received above average precipitation levels between 2001 and 2004.

Table 12. Average monthly streamflows 2000 to 2004 and historical monthly means for The Rio Icacos and Quebrada Espiritu Santo.

	Rio Icacos ($m\ s^{-1}$)	Quebrada Espiritu Santo ($m\ s^{-1}$)
Historical Mean	4.77	2.31
2000	4.03	1.86
2001	4.13	2.51
2002	3.76	2.46
2003	5.08	2.89
2004	4.66	3.74

Annual precipitation totals from the East Peak and Rio Icacos climate stations returned similar values from 2000 to 2003. In 2004 there was a significant difference between the totals recorded (Table 13). East Peak is regarded from the historical data as the wettest point in the region, the Rio Icacos data however indicates that the Tradewinds trail at 735m can receive more rainfall that the peaks of the LEF.

Table 13. Annual precipitation totals for the East Peak and Rio Icacos climate stations 2000- 2004.

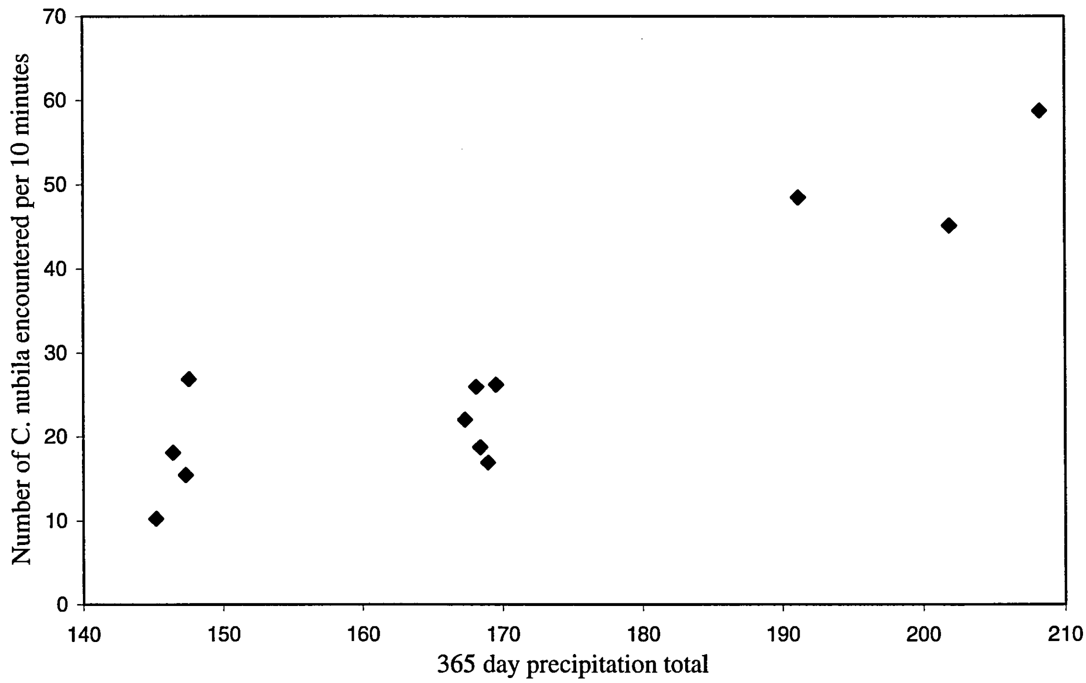
Year	East Peak (mm)	Rio Icacos (mm)
2000	3563	3601
2001	4132	4220
2002	3572	3689
2003	4726	4738
2004	3549	4995

There was a close relationship ($p < 0.01$, $r=0.9195$, $n=1948$) between daily streamflow and daily precipitation totals for the United States Geological Survey (USGS) Rio Icacos static climate station at 673m from 01/02/2000 to 01/05/2005. This showed that precipitation and streamflow data follow similar patterns and demonstrated why streamflow levels from the Quebrada Espiritu Santo, with its source point below El Yunque Peak, is considered to give a good indication of external hydrological inputs into a region where soils are constantly waterlogged. There was a similar relationship ($p<0.01$, $r=0.9568$, $n=46$) from 18/2/2005 to 4/4/2005 and from twelve days between 2/3/2003 and 17/3/03 ($p<0.01$, $r=0.8415$, $n=12$) between daily precipitation totals from the portable climate station, situated near the start of the Tradewinds trail and the Rio Icacos static climate station. Daily precipitation totals from the U.S.G.S station can therefore be used to calculate approximate historical daily precipitation totals on the Tradewinds trail.

3.1.4 The significance of 365 day precipitation totals and *C. nubila* activity

Figure 7 shows the relationship between 365 day precipitation totals from the Rio Icacos station and numbers of *C. nubila* encountered on the Tradewinds one and two transects on the return walk only, for 2001, 2002, 2003 and 2005. The daily rainfall totals from the Rio Icacos station and the portable climate station have previously been shown to have a close relationship. The first and second transects were combined for the calculation as the separate walks were treated as a continous transect in 2001. The return transect only data has been used as this has been calculated to be the data set where butterfly activity is more likely to be acting independently of climate factors. For each field visit, data from the first and last transects walked each season on the Tradewinds trail was used and also a walk from the middle of the data set. This ensured reasonable lengths of time between each data point from each visit. A significant relationship ($p< 0.01$, $r= 0.893$, $n= 12$) was found between precipitation levels during the previous year and *C. nubila* numbers encountered on the following field visit. The greater the precipitation totals for the previous year are, the more *C. nubila* individuals encountered per ten minutes on the Tradewinds one and two transects on the next field visit.

Figure 7. Relationship between 365 day precipitation totals before selected walks and *C. nubila* numbers encountered per 10 minutes 2001- 2005 on the Tradewinds trail. Numbers encountered on the first, middle and last transects walked each year were used.



3.1.5 Observational results from El Yunque Peak at 915m and Cambalache Forest Reserve

Mating events were rare along any of the transects in Puerto Rico. Only two matings were observed from start to finish. Information on the length of the matings is given in Table 14.

Table 14. Length of time of matings observed fron start to finish

Date of mating	Length of mating (minutes)
18/3/02	75
8/3/03	50

During the field visit in 2002, a number of observational data collection periods were set up along the roadside verge at the start of the 915m El Yunque transect. Flight times of male and female *C. nubila* are recorded in

Table 15. *C. nubila* flight times on three separate days is shown in Table 16 and flight times and distance covered is recorded in Table17. A ten metre section of roadside verge was also monitored for a period of time to record numbers of *C. nubila* entering, exiting and perching within the area (Table 18).

Table 15. Flight times of male and female *C. nubila* were recorded in overcast/ bright and full sun conditions on the roadside at the start of the El Yunque one transect on 11/3/02 between 13:35 and 14:25.

Female *C. nubila*. Flight time (seconds)				Male *C. nubila*. Flight time (seconds)			
Overcast/ bright		Full sun		Overcast/ bright		Full sun	
3		2		3		14	
4		2		4		12	
6		1		3		11	
3		20		15			
14							
Mean	6	Mean	6.25	Mean	6.25	Mean	12.33

Table 16. Observation periods were undertaken under clear skies. The start time on 11/3/02 was 13:35, the start time on the 14/3 was 10:45 and on the 20/3 13:00. The mean for all the observations is 16.53 seconds and for 11/3 and 20/3, which were undertaken during clear skies and at similar times, 18.95 seconds.

Flight time (seconds) 11/3/02		Flight time (seconds) 14/3/02		Flight time (seconds) 20/3/02	
22		14		7	
31		15		10	
16		7		8	
37		15		9	
30		7		9	
11		9		23	
17				27	
20				9	
				27	
				12	
				8	
				16	
				30	
				23	
				6	
Mean	23	Mean	11.7	Mean	14.9

Table 17. Measured flight times under clear skies and approximate distance covered on 11/3/02 and 14/3/02 on the roadside at the start of the El Yunque Peak transect at 915m.

Flight time (sec.)	Approximate length covered (m)
37	30
14	14
50	50

Table 18. For a total period of 22 minutes between 12:43 and 13:25 on 20/3/02 in full sun, a 10 metre section of roadside verge at the start of the El Yunque one transect at 915m was observed to record the number of *C. nubila* entering the area. Of the *C. nubila* entering the area, the number that perched within it was also recorded. 29.27% of the *C. nubila* that entered the area perched within it.

C. nubila entering area	*C. nubila* perching in area
82	24

A single *C. nubila* female was kept in a netted enclosure with a sugar solution for six days in vegetation at the end of the Tradewinds trail transect one. During this time the white streak on the forewing faded, as did the dark brown colouration of the wings. At the end of this period, the faded brown colouration of the female was still darker than a lot of butterflies seen on the transect walks indicating that females encountered on the transects were older.

During the 2002 field visit, females with a white streak were counted on the Tradewinds trail transects to ascertain if there was any relationship between numbers encountered and climate data from the portable climate station. No direct links were found. There were two dates in particular where numbers of fresh females on the transects were high and although climatic conditions were good on these days, the overall data collected was not markedly different to that on other days when lower numbers of fresh females had been recorded. Calculations were also done with temperature and humidity levels earlier on in the morning and numbers of fresh females encountered later on in the day when the transects were walked, but again, no relationship was found.

The life cycle of *C. nubila* in the field above 735m has been estimated at 73 days and under laboratory conditions at around 60 days (A. Sourakov, personal communication). The 73 day estimate comes from the 2003 climate data from the Portable climate station and the Rio Icacos daily precipitation data. Heavy rainfall was recorded in the Tradewinds region throughout most of December in 2002, precipitation levels however fell after a certain date indicating a possible increase in opportunities for *C. nubila* activity and mating. When recording *C. nubila* numbers per 10 minutes in March 2003, numbers of freshly emerged females suddenly increased on the 5th indicating possible good conditions for mating in the previous cycle. The first day where reasonable conditions for mating would have occurred was 73 days previously after the prolonged period of heavy rainfall.

3.1.6 Observational notes

Selected observational notes from 2002:

9/3/02- On the Closed road transect, a lizard was observed jumping at *C. nubila* as it flew past. This incident was noted, but the lizard activity recorded was not a rare event when walking the transects and was thought to be the main reason for *C. nubila* specimens seen with what appeared to be bite marks missing from their wings.

10/3/02- Two *C. nubila* were seen flying in light rain between 10:20 and 10:30 on the Tradewinds trail one transect. On the same transect, a female was seen flying just after a heavy precipitation event. This supports other observations that up to a height of 735m on El Yunque Peak, *C. nubila* will occasionally undertake flight activity during light precipitation events. This was never noted above 915m. A male *C. nubila* was noted nectaring on *Elephantophus mollis* for durations of 3,12,6 and 2 seconds. A female *C. nubila* was nectaring on *E. mollis* on the Tradewinds trail one transect for durations of 22,20,10,5,18,6,15,10,7,44,18,10 and 5 seconds in successive intervals. Recorded nectaring events were rare and it was proposed that the female specimen mentioned above may have just mated and was preparing to lay eggs.

11/3/02- On the El Yunque Peak third transect, *C. nubila* was observed flying in the mist during bright patches on El Yunque Peak at 12:30. This event shows that above 965m, on El Yunque Peak third transect, brief periods of sunshine are used by *C. nubila* to its full advantage.

12/3/02- On the Tradewinds trail one transect *C. nubila* flight velocity was noted as visibly increasing when the sun came out and on landing, a specimen was seen to adopt a heat avoidance position which would indicate overheating during flight activity.

15/3/02- On the Tradewinds trail one transect, during the middle of the day, there was less activity recorded in areas that received full sunlight for a period of time. In a grass glade area, beside a section of the Tradewinds trail one transect where observation periods were often undertaken, when there were periods of sun, *C. nubila* was seen to seek areas of shade and there was little activity.

On 16/3/02 on the outwards walk of the Tradewinds one transect at 11:33 a mating was recorded where, on the commencement of mating, the couple were pursued by another two male butterflies until they landed on vegetation. After 12 minutes the couple were blown off their position by the wind, took off and landed briefly four times and settled in the middle of the path where a further two male *C. nubila* attempted to dislodge the mating male unsuccessfully. The couple then flew up into the trees where visual contact was lost. At 12:22 on the same day, there was another mating observed at the end of the Tradewinds one transect. A passing male briefly tried to dislodge the mating male at 12:32 and the couple then flew into the shade on a tree leaf. At 12:41 the couple changed position and at 13:00 the male flew off. The female was noted to go immediately into basking condition in full sunlight. At 13:03 she flew higher up into the tree and then flew down a steep slope and visual contact was lost.

18/3/02- On the Tradewinds trail transect one, a male *C. nubila* was recorded as landing on a grass glade and putting its probiscus out. It appeared at the time that this was due to overheating in flight during full sunlight.

21/3/02- On the Bisley trail, a female *C. nubila* was observed adopting a heat avoidance position three times consecutively after flight activity. On the third occasion, on landing, the specimen adopted a heat avoidance position at the side of the path for 9 minutes before the next flight. On landing, it went into a heat avoidance position again. These notes record *C. nubila* overheating events after flight activity in full sunlight below 735m on El Yunque Peak. The activity noted on the Bisley trail indicates that at 200m on El Yunque Peak, temperature levels during flight at this altitude would appear to inhibit *C. nubila* activity.

Selected observational notes from 2003:

27/2/03- Weather conditions were more overcast on the return transects and this was recorded as being reflected in the numbers of *C. nubila* encountered which were lower.

28/2/03- The first *C. nubila* activity was noted on the Tradewinds one transect at 10:37. On the return transects, the vegetation was noted as drier and conditions sunnier which was reflected in *C. nubila* numbers recorded. This was a general observation, that *C. nubila* activity on the Tradewinds trail transects appeared to be weather dependent.

1/3/03- Thirteen fresh white females with a white streak were counted on the Tradewinds trail transects one and two and it was thought there may have been an emergence event as this was an unusually high number.

2/3/03- High numbers of *C. nubila* were recorded but the weather conditions were noted as being mostly overcast and changeable. It was proposed that the high activity in these conditions may be a result of the high numbers of fresh females recorded on the previous day, seven white females were recorded on 2/3/03.

3/3/03- Activity levels noted as being back to normal in overcast sunny conditions, six fresh white females were recorded on the Tradewinds trail transects one and two.

5/3/03- Twenty eight fresh females recorded. This was the highest number recorded throughout the field visits at any one location. Weather conditions were overcast on the out transect and overcast bright on the return transect.

7/3/03- First matings of the field visit so far were recorded with three in the same transect walk. Weather conditions were full sun for the duration of the recording period. This note indicated that above 735m full sunlight was required for successful mating opportunities.

Selected observational notes recorded on the Tradewinds trail transect in 2005:

21/3/05- *C. nubila* noted as flying during a precipitation event past the first shelter on the Tradewinds one transect at 12:00, it then stopped and basked in an intermittent bright spell.

22/3/05- The first *C. nubila* activity was noted on the Tradewinds one transect by the first shelter at 10:23.

26/3/05- *C. nubila* flight noted as much faster and erratic in sunny weather.

30/3/05- Lot of excrement being found in grasses which was presumed to be left by caterpillar activity overnight. The material was known to be excrement as it was visually the same substance seen to be excreted by *C. nubila* caterpillars when disturbed.

2/4/05- Length of Tradewinds one, two and three transects combined estimated to be 0.7 miles in length by a walking group using electronic equipment from the Tradewinds trailhead which marked the start of the Tradewinds one transect.

6/4/05- In intermittent weather, it was noted that as soon as it got brighter, there would be a flurry of *C. nubila* activity.

Plate 1. The end section of the El Yunque Peak transect at 1000m showing the canopy height of the cloud forest.

Plate 2. A further section of the 965m to 1000m El Yunque Peak transect to illustrate the path and vegetation structure.

Plate 3. The LEF. The communications masts on top of El Yunque Peak are just visible.

Plate 4. Looking over to El Yunque Peak from the Tradewinds trail. A communication mast can just be seen on the peak in the middle of the picture. This photo gives some idea of the visual distance between the two transects.

Plate 5. A section of the Tradewinds trail transect one at 750m showing the canopy height of the palo colorado forest.

Plate 6. The parched grassland of the Guanica transect during the dry season.

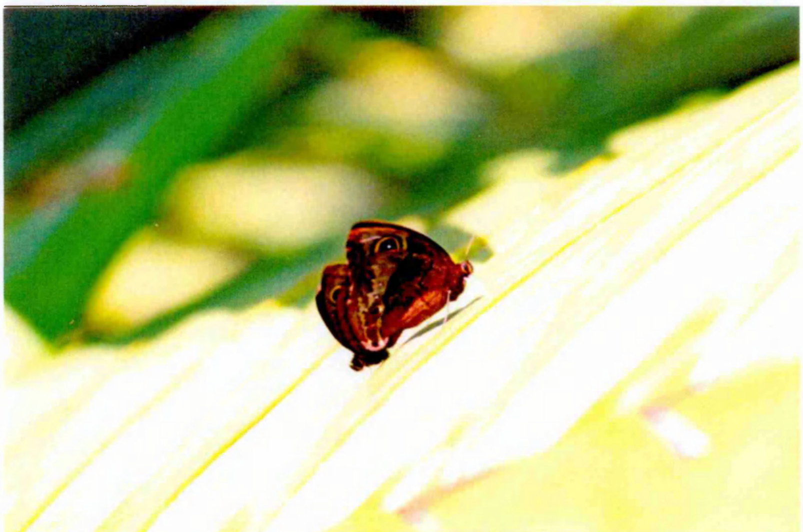
Plate 7. The *C. nubila* hostplant on the El Yunque Peak 965m transect. Even at this altitude with high annual precipitation, the lower grass blades can dry out.

Plate 8. A *C. nubila* first instar larvae showing the forked tail.

Plate 9. A grass tip being unfurled to show a *C. nubila* first instar larvae. The blade also shows signs of recent eating activity.

Plate 10. A typical larvae position inside the top grass blade and growing tip. When the sheath is pulled back, the caterpillar normally adopted a threatening posture with the head up, as in this picture.

Plate 11. *A C. nubila* mating on the Tradewinds one transect by the second shelter at 965m. The white streak on the female can clearly be seen in the photograph.



3.1.7 Overview of *C. nubila* results

The abundance of *C. nubila* is different for each biogeographical site. The main sites in the study within the LEF were the Tradewinds trail from 735m to 765m, El Yunque Peak from 915m to 1000m and East Peak at 1000m. Highest numbers in 2001 were encountered above 915m within the cloud forest on El Yunque Peak . Numbers encountered along transects above 915m in 2002, 2003 and 2005 were lower. Numbers of *C. nubila* encountered along the Tradewinds trail above 735m reflected a different trend, with numbers counted per 10 minutes increasing from 2001 to 2002, declining in 2003 and increasing again in 2005. Numbers recorded on the East Peak transect at 1000m remained similar in 2001 and 2002.

From the climate and activity data, minimum and maximum humidity and minimum and maximum temperature and wind speed are the climate factors which are significant predictors of *C. nubila* numbers on the Tradewinds trail in 2003. Because these climate factors have a close relationship with each other as well as with *C. nubila* numbers encountered, it was not possible to ascertain how each climate factor on its own affected butterfly numbers encountered using the general linear model. Minimum humidity was chosen as the explanatory variable in the general linear model as separate correlation calculations showed that minimum humidity had the closest relationship with *C. nubila* activity in 2003 and 2005 on the Tradewinds trail one and two transects combined.

The time of day when the Tradewinds transect one was walked was also a significant predictor of *C. nubila* numbers encountered, with increased numbers encountered on the return leg of the transect. Up to 15:30, the later in the day the return transect was walked, the greater the numbers encountered per ten minutes when compared to the out transect walk on the same day.

The preceeding 365 days precipitation had a close relationship to *C. nubila* numbers encountered in the following dry season on the Tradewinds trail one and two transects combined. The greater the precipition levels, the more *C. nubila* numbers encountered in the following dry season.

On the El Yunque Peak third transect above 965m in 2002, minimum humidity was chosen as the explanatory variable for the general linear modal as separate correlation calculations showed that minimum humidity had a close relationship with *C. nubila* numbers encountered in 2002 on the El Yunque Peak 965m transect. The outward and return transects were combined at this site, as the walks were done continuously, unlike on the Tradewinds trail. The difference at this altitude is that the general linear model showed wind speed is a significant predictor of *C. nubila* activity on its own, above 965m.

Section 3.2 *Wallengrenia drury* and *Antillea pleops pleops*

The main aims of this section were to investigate if there were any changes in numbers of *W. drury* and *A. pleops pleops* encountered as the transect height increased from 250m on the Bisley trail to 1000m on East Peak, if any relationships would be found between activity levels of *W. drury* and *A. pleops pleops* and the data recorded by the portable climate station and what numbers of *W. drury* and *A. pleops pleops* per ten minutes would be encountered along transects in other regions sampled in Puerto Rico.

3.2 Results

Numbers of *W. drury* recorded per ten minutes on the El Yunque transects between 2001 and 2005 are illustrated in Figure 8. Highest numbers were recorded along the 965m transect in 2005 after high annual precipitation in the region. In comparison to 2001, numbers encountered per ten minutes in 2002 were lower above 915m and similar along the Closed road transect at 715m. This indicates that *W. drury* population numbers were affected by the climate anomaly above 915m. *Wallengrenia drury* numbers recovered along the 965m transect only in 2005.

Figure 8 Numbers of *W. drury* encountered per 10 minutes on the El Yunque transects 2001- 2005. Transect walk details are the same as displayed for Figure 2 and Figure 3. The transects have ben inserted in the figure according to the highest point of the trnsect walk. Heights are in metres.

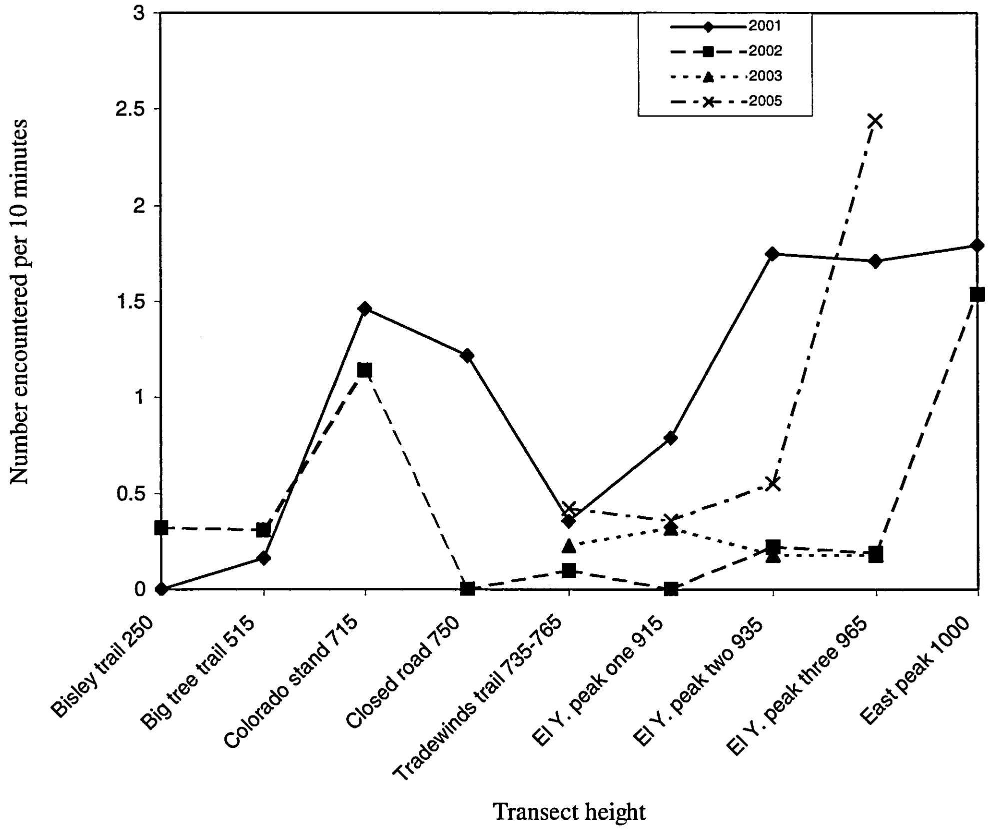


Table 19 indicates the *r* values for numbers of *W. drury* encountered per ten minutes on the Tradewinds trail transects one and two combined and minimum humidity values during transect walks in 2003 and 2005. As with *C. nubila*, humidity has been chosen as the indicator value for the climate variables as it has the closest relationship with numbers encountered. With *W. drury* however, it is maximum humidity which has the closest relationship.

Table 19. The *r* values and corresponding level of significance on the out transect for *W. drury* numbers and maximum humidity values during walks on the Tradewinds trail one and two transects combined in 2003 and 2005.

Tradewinds trail 2003			Tradewinds trail 2005		
r values on out transect for *W. drury* numbers vs:		Level of significance	*r* values on out transect for *W. drury* numbers vs:		Level of significance
Maximum humidity	-0.22	NS	Maximum humidity	-0.76	1%

Numbers of *A. pleops pleops* encountered per ten minutes on the El Yunque Peak transects from 2001 to 2005 are displayed in Figure 9. Apart from the 2001 field visit when this species was frequent above 915m on El Yunque Peak, it was rarely recorded in subsequent years. The climate anomaly of 2001 appears to have had a long lasting negative impact on numbers recorded in subsequent years.

Figure 9. Numbers of *A. pleops pleops* encountered per 10 minutes on the El Yunque transects 2001- 2005. Transect walk details are the same as Figures 2 and 3. The transects have been inserted in the figure according to the highest point of the transect walk. Heights are in metres.

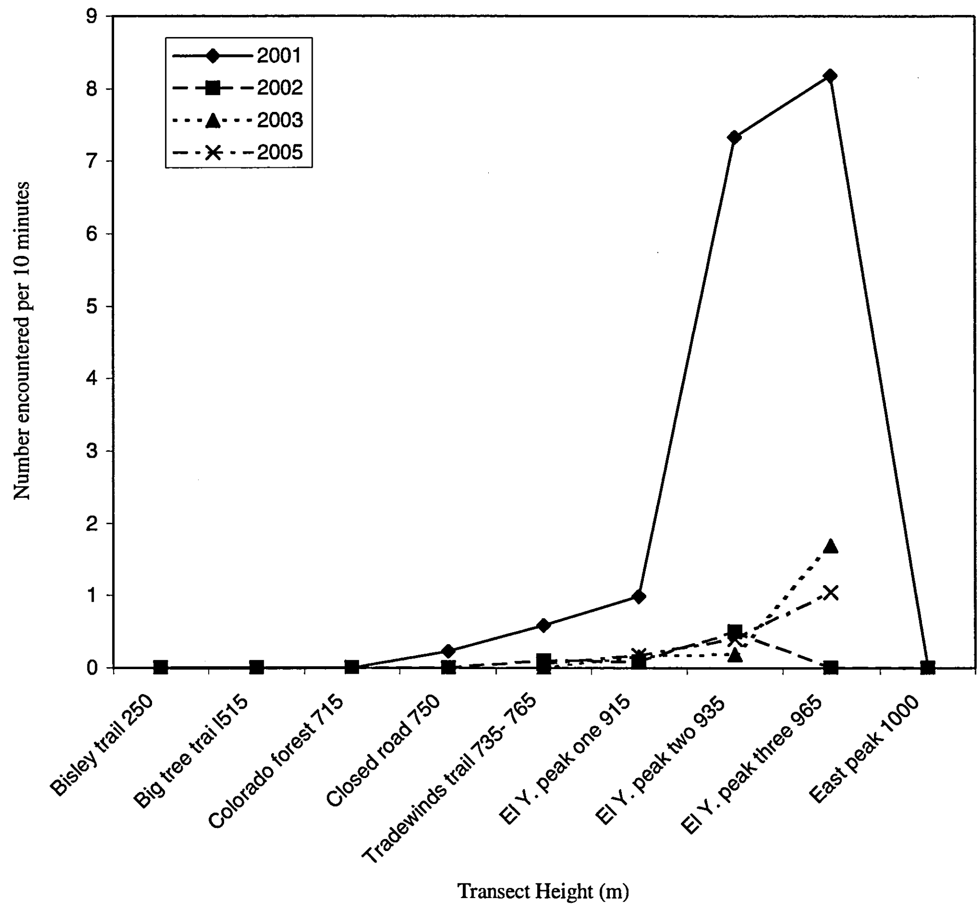
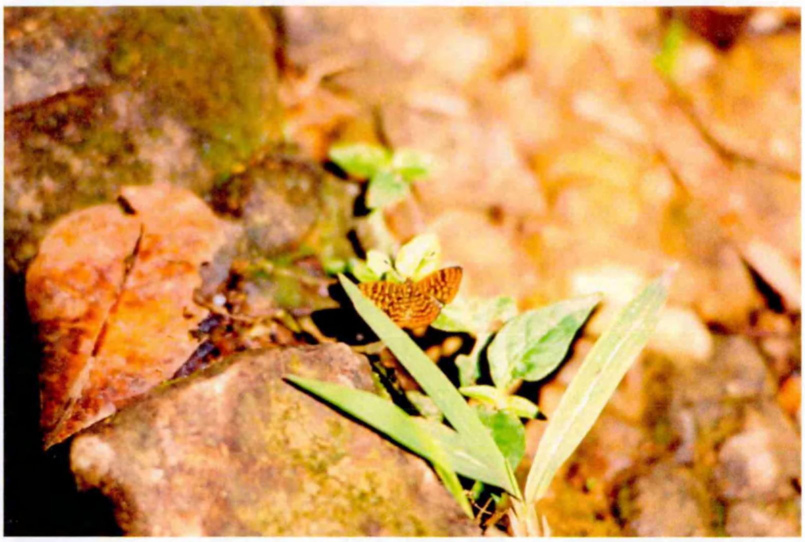


Plate 12. A *W. drury* specimen in the LEF.

Plate 13. Illustration of an *A. pleops pleops* specimen.



3.2.1 Additional observations on *W. drury* and *A. pleops pleops*

A *W. drury* mating was observed on the Closed road transect in 2002 at 14:30. The female was noted as flying on to a leaf with the male behind and fluttering her wings for about five seconds as the male approached, the two butterflies then flew off locked in a mating position together. In the observations recorded for 2003 and 2005, *W. drury* and *A. pleops pleops* activity was associated with drier, sunny conditions on the Tradewinds trail and El Yunque Peak transects above 915m. On the El Yunque Peak transect above 965m on 5/4/05, higher than normal *A. pleops pleops* numbers were recorded on the last section of the path after a footbridge. This section is where the canopy cover reduces in height and although conditions are generally windy the chances of periods of sunshine are higher in relation to the rest of the El Yunque Peak transects above 915m.

3.2.2 Overview of *W. drury* and *A. pleops pleops*

Numbers recorded for *W. drury* were tested by statistical methods with the climate data and although *W. drury* recorded close relationships with humidity and temperature levels in 2005, numbers encountered were considered to be too low to reach any conclusions. The *A. pleops pleops* data collected was not tested statistically due to the low numbers recorded throughout the transect walks when the portable climate station was set up between 2002 and 2005. Activity levels of both species however reflect both the climate anomaly and distribution patterns of *C. nubila* along the 915m to 965m El Yunque Peak transects.

Numbers of *W. drury* encountered along the transects did increase with altitude, but not in the same linear pattern as *C. nubila*. There was a peak in numbers at 750m at the Colorado stand transect with highest numbers recorded along the El Yunque Peak transects above 915m in 2001. In 2002, numbers of this species also reflected the climate anomaly on the peaks and as with *C. nubila*, numbers were low above 915m.

There was a recovery in *W. drury* numbers along the 965m El Yunque Peak transect only, where the highest numbers were recorded for this species along any transect in 2005. The reasons for this are proposed to be the same as for the increase of *C. nubila* numbers along the same transect, in that due to increased wind speeds and the more open habitat structure and low canopy height, humidity levels are lower which may lead to an increase in activity levels particularly when the sun breaks through the cloud cover. An interesting aspect of the *W. drury* data collected was that although numbers of this species reflected the climate anomaly in the 2002 season on El Yunque Peak, numbers encountered on the East Peak transect returned similar values to 2001. It is proposed that this population has different survival strategies to cope with what is considered to be harsh environmental conditions for any butterfly, especially a small skipper such as *W. drury*. These strategies appear to have buffered the population from the recorded climate anomaly.

Antillea pleops pleops was the third species recorded in reasonable numbers along the higher level transects in 2001. This species displayed an altitudinal distribution pattern similar to *C. nubila* on the transects above 915m. Numbers of this species never recovered on any of the transects above 915m after the 2001 climate anomaly. Numbers encountered were at low levels along all of the transects below 915m from 2001 to 2005. There was a small recovery recorded in numbers along the 965m transect from 2002 to 2005, but because numbers recorded were so low it was felt that no conclusions can be drawn from this.

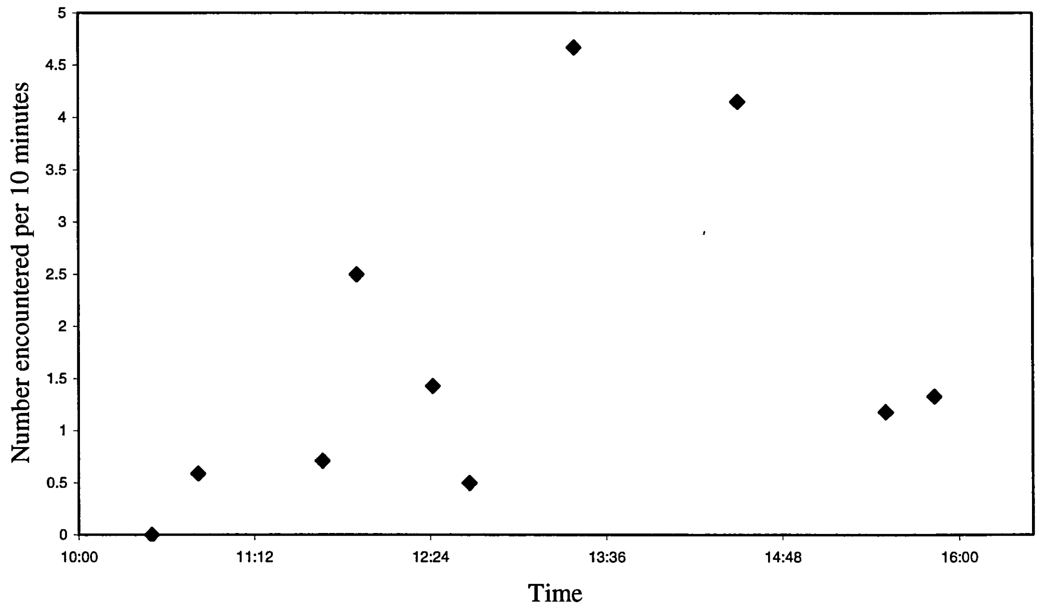
Section 3.3 Other regions of Puerto Rico

3.3 Results

The aim of this section is to find out numbers of *C. nubila* encountered at other locations in Puerto Rico and compare the findings with those from the LEF.

Calisto nubila was not recorded along the Guanica transect walked, but it was recorded at Carite Forest Reserve in low numbers. Precise numbers of the species found along the various transects walked at Carite cannot be presented as this data is not available. *Calisto nubila* activity was recorded during the Cambalache Forest Reserve visits and some of this data is presented here. In the 2003 season this site was visited in the morning and afternoon on two separate days under similar weather conditions to give an insight into daily activity in the forest area (Figure 10).

Figure 10. Cambalache Forest Reserve was visited on two separate days under similar weather conditions to record activity throughout the day. On the 9/3/03 transects were done from 10:30 to 12:52 (n=6) and on the 16/3/03 transects were done from 13:23 to 16:05 (n=4).



Numbers of *W. drury* encountered per 10 minutes in Cambalache Forest Reserve are presented in Table 20. This species was present in 2003 only.

Table 20.Numbers of *W. drury* encountered per 10 minutes on the Cambalache transects in 2002, 2003 and 2005 are shown. The total time spent each year walking the transects is included. The Cambalache transect was walked once in 2002, four times in 2005 and a section walked ten times in 2003.

	W. drury	Minutes
2002	0	61
2003	0.4	199
2005	0.04	230

Findings for *A. pleops pleops* from the Cambalache Forest Reserve visits are shown in Table 21. These findings indicate the overall low numbers of this species encounterd. As with *W. drury*, the total time spent walking the various transects is shown in the Tables to give an indication of how much time was spent recording in the various locations.

Table 21. Numbers of *A. pleops pleops* encountered per 10 minutes on the Cambalache transects in 2002, 2003 and 2005. The total time spent each year walking the transects is included. The Cambalache transect was walked once in 2002, four times in 2005 and a section walked ten times in 2003.

	A. pleops pleops	Transect minutes
2002	0	61
2003	0.45	199
2005	0	230

Observational notes from Cambalache Forest Reserve in 2003:

9/3/03- *Calisto nubila* resting periods on the path and vegetation were noted as very brief indicating that basking activity was absent at sea level. By 15:30, most of the path used as a transect was covered in shade and the larger butterflies were seen higher up in the tree canopy where there was still sunlight.

16/3/03- As the day gets later, *C.nubila* were noted as being less likely to stop on vegetation to be observed. This was additionally seen as indicating that weather conditions negated the need for basking behaviour at sea level.

3.3.1 Overview of section three

The main findings from this section were that *C. nubila* was encountered in higher numbers at Cambalache Forest Reserve at sea level than along any of the LEF transects below 715m. Results also indicated that *C. nubila* may well have a different life history to cope with the harsh conditions found at Guanica where the species has been recorded during the wet season, but was not recorded during the field visit there. *Wallengrenia drury* and *A. pleops pleops* were not recorded at all or encountered in low numbers at other locations in Puerto Rico.

Chapter Four

Discussion

The information in this chapter has the following aims:

1. To provide the results of *C. nubila* activity and an overview of published papers which are or relevance.

2. To discuss the main findings with sections on *C. nubila* numbers encountered and the climate variables measured by the portable climate station.

3. To look at the effects of the climate anomaly of 2001, streamflow and precipitation data, other sites sampled in Puerto Rico.

4. To give details on the observational notes made during the field visits in relation to published material.

Sections on *W. drury* numbers encountered and the climate data from the portable climate station and *A. pleops pleops* activity levels found on the transects then follow.

4.1 Thesis results in relation to published papers

No other similar research appears to be available relating to butterfly numbers, climate measurements and altitudinal transects in tropical forest regions. However the research done in Costa Rica (Ponds et al, 1999) showed that twenty anuran species and two anoline species had disappeared from Monteverde Cloud Forest and that it was runs of dry days in the dry season that appeared to be the main contributing factor. This was presented with the lifting cloud base hypothesis, whereby during dry periods the cloud cover would sit above the mountain, rather than enshrouding it in mist.

The drying out scenario would be described in *C. nubila* numbers encountered on the Tradewinds trail at 735m in the Luquillo Experimental Forest (LEF), as numbers are related to the preceeding 365 day precipitation total. The general cloud condensation level in the tropics is 600m, so this would be considered to be the lowest level where any changes in the cloud base would be discernable. From the data to date however, there is no indication that the cloud base is lifting in the LEF. From the precipitation and streamflow data during the project the trend was for increased hydrological inputs into the region during the study period.

The data shows however that changes in the climate variables above 735m are reflected by changes in *C. nubila* numbers encountered on the Tradewinds trail transects one and two combined from 735m to 765m and along the El Yunque Peak transect from 965m to 1000m. The correlation calculations show that humidity may be the main climate factor acting on activity levels, however the linear regression model calculated that all the climate variables were interlinked with each other as well as with butterfly activity, so no one overall climate variable can be directly linked to changes in numbers encountered. The exception to this was on the El Yunque Peak transect above 965m where wind speed had a close relationship to numbers of *C. nubila* encountered. *Calisto nubila* however is a species that can be used to monitor the effects of changes in the combined climate variables at altitude and particularly within the fragile environment of the cloud forest zone above 915m in the LEF. There appear to be no other published papers where butterfly activity has been directly linked to changes in climate variables above 735m in palo colorado forest and above 915m in the cloud forest zone. From the linear regression calculations on *C. nubila*, it is doubtful whether any invertebrate species above 735m can be used to monitor changes in any one climate variable in the LEF, instead numbers encountered will reflect changes in the overall local climate in the particular biogeographical area concerned.

The results also show that at 735m on the Tradwinds trail, *C. nubila* will also indicate changes in the levels of annual precipitation in this region of the forest and numbers can indicate whether this ecological zone is either drying out or experiencing increased annual hydrological inputs into the system in localised areas. The differences in precipitation recorded by the East Peak and Rio Icacos static climate stations indicate that precipitation levels can be variable in different areas of the LEF that are in reasonably close proximity to one another.

The temperate satyrids *A. hyperantus* and *P. aegeria*, where the larvae of both species prefer moist and semi shaded habitats displayed strong associations between decreased abundance and hot or dry weather in the previous summer (Sutcliffe et al 1996). This may well turn out to be the case on the Tradewinds transect above 735m, in that wetter years may favour *C. nubila* larvae in terms of foodplant condition. The dry season in Costa Rica had an impact on the quality and palatability of foodplants (F. J. Odendal, 1990) and in a further temperate satyrid, *M. jurtina*, first instar larvae were susceptible to low food quality and adult females had greater longevity in cool, damp summers which would increase the period available for egg laying opportunities. Hot and dry periods resulted in longer periods of flight activity, but also in quicker deterioration in wing condition and quicker use of energy reserves in *M. jurtina* (Brakefield, 1982a). In a further paper it is stated that the onset of premature ageing of host plants, often the result of heat and drought, can increase larval mortality and lead to reductions of population size (Weiss et al, 1988).

If the information from these papers is used in relation to the *C. nubila* results then a general picture starts to emerge which, with further research may give more information on the life history and ecology of *C. nubila.* The above papers certainly fit into the data collected and conclusions reached for *C. nubila.* It is a species where it is proposed that the larvae are susceptible to low foodplant quality and palatability. High precipitation levels are needed to sustain the quality of the foodplant and this can be clearly seen on the Tradewinds trail 735m transect in the dry season, when the foodplant has been recorded as drying out. This is further supported by the point that the first instar are found in the curled leaf tips only, where there is fresh growth and again, the plant is visibly greener towards the top of the plant on the high level transects. It would also explain why the species is encountered in high numbers at altitude only in the LEF.

From the thesis data, further insight can be gained into adult behaviour and numbers of *C. nubila* above 735m. Numbers encountered, particularly above 915m on El Yunque Peak have been noted as being linked to periods when the sun shone through the cloud cover on more overcast days. This is thought to be linked to humidity levels and average temperatures per ten minutes, but bursts of activity are more readily associated with this species at altitude, which ties into the behavioural patterns for *M. jurtina.* Wetter habitats were also thought to have buffered *M. jurtina* populations from dryer periods and this may well happen to *C. nubila* along sections of the Tradewinds trail during drier conditions such as those found in 2001during the survey period.

Studies on recovery time in copper butterflies after they have been submitted to low temperatures have shown them to be a sensitive index of climatic adaptation and acclimatisation. The recovery time was found to vary strongly across populations and groups being raised at different temperatures (E. Kuhn et al, 2005). If there is a sudden change in the lower temperature range that butterfly species are adapted to, such as the prolonged low temperatures on El Yunque Peak in 2001, then these conditions may be detrimental to the recovery time of *C. nubila*. In a study of *Colias* spp. it was found that populations have similar ranges of body temperature for basking, flight and heat avoidance (Watt, 1968) but that the same populations have different thermal optima for larval growth and feeding. Adult *Colias* thermal preferences were independent of those for the larvae (Sherman and Watt, 1973). Larvae development rates have been found to be temperature sensitive in some butterfly species (Scriber and Lederhouse, 1983, Ritland and Scriber 1985). These arguments are supported in that numbers of *C. nubila* recorded in March 2002, decreased with altitude above 915m, indicating that a humidity/temperature and or precipitation gradient may be the determining factor(s) relating to the population decline. If *C. nubila* larvae are adapted to temperature maxima and minima on El Yunque Peak, then the 2001 climatic anomaly would have had an adverse effect on the immature stages.

For two species of Colias butterflies (Kingslover, 1983,1984) it was found that due to the difference in wind speeds and solar absorptivity of the wings there was a 20% to 25% difference in the meteorological space in which they can fly. The presence of fur was also found to increase thoracic temperatures by up to 6 ^{0}C. The difference noted in the thickness of fur for the Colias spp. is an interesting point. There is a collection of *C. nubila* held in Oxford Natural History Museum from various elevations and it may be possible to ascertain whether, like Colias spp. there is any difference with elevation in the thickness of fur which covers the ventral thorax.

Calisto nubila appears to adopt behavioural aspects of patrolling and perching species. Males and females will perch for periods on vegetation and will seek out any other butterfly that flies past, usually spiralling upwards in the typical butterfly encounter behaviour. This is similar to the behaviour recorded for *M. jurtina* which was also found to be intermediate between that of patrolling and perching species.

Drier periods may have an impact on the vulnerability of *C. nubila* immature stages to parasites and pathogens, however this is thought to be less likely in the LEF. As mentioned in the introduction, *C. nubila* eggs have been reared in captivity from a female collected at 400m in the LEF. Although standard environmental conditions would have been created for the eggs, there appeared to be no specialist techniques or intricacies involved in rearing the instars. Twelve out of the twenty first instar developed normally to the last instar, at which point most were preserved. The main factor relating to mortality was whether the instars accepted any of the wide range of grasses they were exposed to. It took a minimum of four days before any of the instars began feeding and they were offered food continually, thus demonstrating host plant specifity (Sourakov, 1999).

During the last field visit in 2005, many hours were spent checking the hostplant grass for eggs and larvae in areas where *C. nubila* numbers were high, such as on the Tradewinds trail transects. *C. nubila* larvae were easily found within the growing tip of the hostplant grass, no larger larvae were found during this period and because of the size and larvae measurements given by Sourakov (1999), all those found were thought to be first instars. In

2005, on the first transect of the Tradewinds trail by the first shelter, it was estimated that around 25% of the grass tips were occupied by *C. nubila* larvae. The hostplant height was short around the shelter and therefore after emerging from thre growing tip, the larvae had less distance to travel to the relative security of the leaf litter. As in the Southeastern Brazil study (A.V.L. Frietas et al, 1999), it was concluded that the larvae had low mobility and stayed within the furled growing tip of the hostplant until they were large enough to make their way down the grass and into the leaf litter. Any larvae that were disturbed during observations made their way down into the growing tip as far as possible, leaving only the top of the head showing.

No *C. nubila* eggs were found on the hostplant above 735m on El Yunque Peak. All eggs found on the foodplant were marked and inspected on transect walks. Only one egg type was found infrequently and the larvae was not that of *C. nubila* and was suspected to be that of a moth because of the size of the pupae. The same egg was found on sedges at 765m on the Tradewinds two transect. It is therefore thought from these observations that *C. nubila* eggs are laid or dropped into the growing leaf tip by the female. If the egg is dropped into the growing tip then this is a different strategy used from the specimens reared in the laboratory where the egg was fixed to the grass (Sourakov, 1999).

The use of the tip of the grass as a foodplant for first instar larvae also agrees with the results from the Southeastern Brazil study in that a pattern was found for the occupation of newer leaves. By using the furled growing tip as a first instar refuge during daylight hours, *C. nubila* is ensuring that the larvae is near fresh growth and is well hidden. Fresh leaves may also be more palatable to fresh instars. No other grasses on the transects above 735m had the same growth pattern as the foodplant used by *C. nubila* in that no furled growing tip was available for use as a first instar larvae refuge. The implication for *C. nubila* eggs and larvae on the Tradewinds trail, is that during prolonged dry periods foodplant growth may be restricted and the condition of the leaves less palatable and more dry.

Due to the nature of the hostplant grass which dries out from the bottom leaves upwards, even during relatively wet periods, it is thought that predation may be a major factor involved in controlling population numbers. After the first instar, *C. nubila* larvae are not found on the hostplant in the day time and are known to be night feeders.

When the instars do feed, if the lower leaves are dried out, they have further to go up the plant to get fresh leaves and also are further away from the safety of the leaf litter, so this may be a contributing factor to mortality rates in drier periods above 735m in the LEF. A study of lowland tropical forest butterfly species in Costa Rica found that there was a decrease in the number of eggs layed by female butterflies in the later period of the dry season. One explanation for this was that in the later part of the dry season there was decreased plant growth as well as loss of leaves (Odendaal, 1990). Because of the apparent strategy used by *C. nubila* of laying eggs in the growing furled tip of the hostplant, the drying out of plant material should pose less of a problem until the instar has left the confines of the growing tip.

The hostplant specifity noted may well be one of the explanations why *C. nubila* numbers increase with altitude in the LEF, as although no research was done, the field notes indicated that the foodplant abundancy increased with height and appeared to be at a maximum above 735m. Humidity and precipitation levels may well therefore dictate hostplant abundancy as well as *C. nubila* numbers encountered on the transects.

The hostplant used by *C. nubila* on El Yunque above 735m was not found at Cambalache Forest Reserve or Guanica Biosphere Reserve and there are therefore future research opportunities in this line of study in the light of the hostplant specifity findings by Sourakov for *C. nubila* instars. Another question relating to this is that instars often have foodplant specifity because those species of plants lack certain chemicals or have a feeding stimulant (Janzen, 1988). If more than one hostplant is used by *C. nubila* then this would appear to indicate biological differences in separate *C. nubila* populations in geographical areas.

Voltinism was studied in some temperate butterfly species and was found to be linked to quantitative protective compounds and that the monovoltinism of some species is an environmentally imposed constraint. *Calisto nubila* is found throughout the year in Puerto Rico, however numbers may be constrained by plant compounds or the palatability of the foodplant in certain environmental conditions, as discussed above, numbers may be constrained by foodplant condition, particularly in dry periods above 735m in the LEF. At altitudes below 735m *C. nubila* was not recorded using lateral basking behaviour on any of the transect walks, flight periods were generally noted as being quicker and more sporadic with less distance covered. The extremes of behavior patterns can be seen on the El Yunque transects above 915m and the Cambalache Forest transect. *Calisto nubila* is rarely recorded out of the shady path edges at Cambalache Forest, specimens normally only stop very briefly and flight is much quicker and more erratic. On the El Yunque transects above 915m, *C. nubila* individuals stop frequently in full sun along the path edges, flight is slower and longer flight distances can be observed. It is also much easier to get close up to butterflies for recording purposes on the high altitude transects than when transects are being walked which are closer to sea level. *Calisto nubila* has however been recorded doing lateral basking under the forest canopy at 400m (Ludwig and Shelley, 1985) at El Verde in the LEF.

Noted *C. nubila* mating times fall into roughly the same time of day range as those of the Costa Rican species (Alcock, 1988) and are not often seen. After one *C. nubila* mating was observed on the Tradewinds trail, the female was noted to immediately go into lateral basking behaviour on the path edge. Whereas lateral basking normally consists of small wing angle changes in response to the direction and intensity of sunlight and the overall temperature of the thorax, in this case the female almost appeared to lie on her side to get the maximum intensity of sunlight on her wings. This was the only occasion such extreme behaviour was recorded. Normally after mating the female *C. nubila* flew out of sight.

The chosen vegetation on which the female lands can be near the path edge, higher up on a palm on in the middle of an area of high *C. nubila* activity. It is generally in a position of sunlight but *C. nubila* mating has also been recorded in the shade. From the observations, there appears to be no general rule, height or particular plant species that is chosen or adhered to for mating to commence.

The paper on *C. inornata* (Heinrich, 1986) is of relevance to the *C. nubila* research. 61 out of 289 observed stops of *C. inornata* were to feed on flowers and at all temperatures, butterflies stopped to feed once every 12 to 15 minutes. Observed frequency of potential matings was rare and was recorded only 3 times out of 79 chases, no actual matings were observed. There are similarities with *C. nubila* in that nectaring, potential matings and observed matings were only rarely recorded. As with *C. inornata*, basking in *C. nubila* above 735m appears to act as a pre-flight warm up mechanism and may also allow prolonged flight periods. It may be the case that *C. nubila*, particularly at altitudes above 735m, cools convectively in flight.

No mention was made in the papers cited, of other male butterflies of the same species trying to dislodge a mating male. This happened on a number of occasions during *C. nubila* matings. Although there were only two complete matings recorded from start to finish, there were other matings noted during the course of the transect walks, some of the more detailed observations are noted in the results section. Attempts to dislodge the mating male were not infrequent and could happen at any stage of the mating process whenever a passing male discovered the mating couple. There appears to be no chosen safe areas for mating to ensue, it just seems to be initially a random spot where the female, carrying the male, lands. Often however, after disturbances, the mating couple were noted to move onto higher vegetation where the chances of being disturbed were lower, as most *C. nubila* activity is amongst vegetation along the path edges.

Comparison between *C. nubila* flight times and those recorded for the Costa Rican species were also similar (Alcock, 1988). This probably reflects the case that general butterfly activity anywhere is dependent on environmental conditions and flight times will reflect these environmental changes. The difficulty in recording flight times and distances is that both the start and end of the flight need to be recorded. What often happens is that *C. nubila* will fly down onto the vegetation on the path edges or start the flight from a clear viewing position but the end of the flight is lost in the vegetation on the slopes. As with other observations made, the purpose of doing these recordings was to get some insight into the ecology of *C. nubila,* as to date, very little has been recorded. There was a difference recorded in male and female *C. nubila* activity in full sun and shade conditions. Female flight times were similar under both conditions whereas male flight times increased by a factor of two in full sun. The wing size of female *C. nubila* is larger than that of the male, indicating that the female may be less reliant on weather conditions for activity than the male, but the above observations probably reflect the need for males to seek out females and patrol or perch within the communal territories. Males are therefore more likely to use the advantages of full sun for activity in this role than the females. From the mating observations, full sun may be more beneficial for the female directly after mating. It is worth noting that the area used for the above recordings was at 915m at the start of the El Yunque one transect and where *C. nubila* is close to the environmental extremes of its activity range. This is a region where the amount of sunlight received by the peaks plays a major role in the life cycle of *C. nubila.* Periods of sunlight are used to their full potential and there are often flurries of *C. nubila* activity when the sun emerges from the clouds. The close relationship between *C. nubila* numbers encountered and wind speeds above 965m may indicate that wind distribution of the mist and cloud cover on the peaks could increase periods of sunshine. From a visual perspective, this appeared to happen when walking the El Yunque Peak transect above 965m.

The annual precipitation levels recorded by the static climate station on the Rio Icacos situated near the Tradewinds trail 735m transect were also interesting in that this region of the LEF, during the years of the study period from 2001 to 2005, had more annual precipitation that East Peak at 1000m and the 2004 season recorded the highest precipitation levels. East Peak is historically associated with the highest measured precipitation levels in the region because this weather station is situated on one of the highest peaks in the region over 1000m.

Although *C. nubila* numbers encountered are closely related to the climate variables, they may be related to changes in the overall population size (Pollard 1988). There was one particular transect walk on the Tradewinds trail in the 2003 field visit where numbers suddenly increased from the previous day, above what would normally be expected when conditions were good for activity levels, as they were on this particular day. The number of fresh females encountered was the highest of the survey period in 2003. Numbers encountered on this walk and on the subsequent day may have been higher than expected because the population size had increased. This transect was walked a couple of days later and although weather conditions were similar, numbers encountered were lower. Another relevant point made in this paper was that following a population crash, butterfly numbers can take time to increase to levels where density-dependent dispersal can take place. This may have happened on the El Yunque Peak transect between 935m and 965m with the result that *C. nubila* is no longer found along large sections of this transect.

The effects of the climate anomaly on the *C. nubila* population above 915m on El Yunque Peak reflects this population's vulnerability to climatic events and, agrees with the ecological theory which predicts less stable populations in physically harsh environments. Butterfly populations at high altitude sites were found to be more unstable in terms of abundance rankings and absolute abundances in the Picos de Europa in northern Spain (Gutierrez and Menendez, 1998). One explanation put forward for butterfly populations being less stable at higher altitudes was that a reduced flight period at high altitude sites may make adult butterflies more vulnerable to periods of bad weather (Thomas et al, 1994). For the *C. nubila* El Yunque Peak population a reduced flight period during the climate anomaly of 2001 will have had an effect on subsequent population numbers, however it is the unusually low minimum temperatures which are attributed to the ultimate decline in numbers encountered in 2002.

The findings in this thesis support the conclusions of the above papers in that monitoring for relatively short periods can give a good insight into abundances and species of butterfly present in an area (Gutierrez and Menendez, 1998). The climate anomaly recorded on the peaks of the LEF above 915m in 2001 also show that *C.*

nubila, *W. drury* and *A. pleops pleops* populations at this altitude in the LEF are less stable in what would be considered to be a harsh environment for butterfly species. In a study such as this however there needs to be at least one species which is found in numbers at altitude to get detailed insights into butterfly numbers in relation to climate parameters. When combining butterfly abundance with climate data, it is concluded from this present study that continuous daily transect walks over a minimum period of seven days can result in good data sets which will give detailed insights into daily butterfly numbers encountered in relation to the climate data collected.

4.1.1 *Calisto nubila* activity levels and the climate variables between 735m and 765m and above 915m in LEF.

During periods of low precipitation, moisture levels still remain high enough on the El Yunque Peak transects above 915m to ensure that good foodplant condition is provided for immature stages and it is this weather cycle on the peaks which appears to control population recruitment from 915m to 1000m in the LEF. There were a number of dry years with low annual precipitation in the period leading up to the 2001 season, the year when numbers of recorded *C. nubila* were at high levels above 915m. After the climate anomaly in 2001, numbers of *C. nubila* between 2002 and 2005 never recovered above 915m because annual precipitation levels, linked to measured streamflow levels in this region, remained high throughout this period when compared to the historical mean. There was only a partial *C. nubila* recovery recorded along the 965m transect between 2002 and 2005 which is proposed to be due to the more open vegetation structure and canopy height found at this altitude, which favours lower humidity levels and therefore could affect activity levels recorded along this transect particularly when the solar input increases with a break in the cloud cover. The close relationship between *C. nubila* numbers encountered and wind speeds above 965m on El Yunque Peak may support this argument.

Above 915m in the LEF, because the monthly mean average minimum temperatures under 13.8°C experienced during the climate anomaly of 2001 are rarely recorded on the peaks, it is proposed that they have an adverse effect on *C. nubila* numbers encountered, particularly when these temperatures are experienced on a daily basis for long time periods. It is thought butterflies were unable to raise thoracic temperatures high enough after being exposed to unusually low temperatures during the night, to fly in conditions where relative humidity must have remained close to 100% and ground conditions would have been wet on successive days. *Calisto nubila* flight activity has been recorded as being much lower during cloudy conditions at 400m at El Verde in LEF and that male behaviour is strongly dependant on high thoracic temperatures and sky conditions (Ludwig and Shelley, 1985). From numbers of *C. nubila* encountered on El Yunque Peak from 2001- 2005 and streamflow data from the Quebrada Espiritu Santo, population numbers are linked to low annual precipitation and longer periods of dry weather. Above 915m precipitation levels during dry periods are still high enough to ensure that the foodplant does not dry out. This is supported by visual observations and the Quebrada Espiritu Santo streamflow data. Population size above 915m is therefore dependent on levels of daily activity which has a close relationship to the climatic variables.

The Tradewinds populations between 735m and 765m are able to undertake normal levels of activity for long periods throughout days when the climate station was set up. In this region of the forest, population numbers and recruitment levels are linked to annual precipitation levels in the preceding year. The greater the annual precipitation, the higher the population numbers encountered the following year. The climate data and observations made during transect walks indicate that condition of the foodplant appears to be a limiting factor on population levels and that good foodplant condition requires high precipitation levels.

The precipitation levels recorded by the Rio Icacos station indicate that *C. nubila* would appear to have an area of maximum population growth and dispersal in the Tradewinds trail region. This is due to some of the highest annual precipitation levels in the region which keep the foodplant in good condition for larval growth, but the area also has the added benefit of higher temperatures than the transects above 915m. The results of this are that the larvae are catered for and adult activity can be maximised for both mating and egg laying activities during the dry season due to the higher temperatures at 735m.

The start and finish times of the transects on the Tradewinds trail in 2003 were generally later than in 2005. This factor is thought to explain why there is no close relationship in 2003 between any of the climatic parameters and activity levels on the Tradewinds one return transect in the correlation calculations. Return walks were being undertaken later on in the day when the climatic variables levels pose less of a constraint on activity. This argument is supported by the timing of walks having a close relationship relating to numbers of C. nubila encouintered in the general linear model on the Tradewinds trail between 735m and 765m.

The optimal time to start the Tradewinds transects is around 10:00 and the El Yunque Peak transects should be done around 10:30. This is the start of the main period of activity at both sites and also means that the return transect walks will encompass the main period of activity with maximum solar input. The three El Yunque Peak transects from 915m to 1000m should be walked on a lesser frequency. If numbers recover in this region of the forest to their 2001 numbers, then the portable climate station should be set up at the Tradewinds trail location and this should then be supplemented with a smaller portable device on El Yunque Peak at 1000m. On the El Yunque Peak transects above 915m, there is high annual precipitation, but humidity levels are shown to be greater throughout the day in the dry season and minimum and maximum daily temperatures are lower than at 735m on the Tradewinds trail. This means that opportunities for periods of sustained activity, such as those needed for mating and egg laying, are reduced. The Tradewinds trail from 735m to 765m is considered to be the optimal research transect in the LEF, as the climate anomaly of 2001 appeared to affect the peaks above 915m only and numbers of *C. nubila* encountered on this trail were always high.

Although lateral basking has been frequently noted on both the Tradewinds trail transect at 735m and the El Yunque Peak transects above 915m, the East Peak population was not noted as using this behaviour during the transect walks, either because the periods of sunshine were thought to be to brief or because there were no periods of sunshine at all. This backs up the proposal that this population has life history variations to cope with the conditions noted on East Peak during the dry season. These conditions could further deteriorate during the wet season.

4.1.2 The effects of the 2001 climatic anomaly on *C. nubila* populations

From the 2001 field data the climatic conditions of the months highlighted in the results section during 1999 and 2000 are thought not to have affected *C. nubila* numbers on El Yunque Peak because numbers encountered per ten minutes in the 2001 season were high. Similar monthly mean average minimum temperatures below 13.8°C in July and August 2001 alone are therefore not responsible for the population decline. The low monthly precipitation totals for the selected months from the historical record are thought to have ensured that because ground conditions may have been dry on most days, the climate variables were favourable enough to allow periods of normal population activity. Periods of high rainfall are a limiting factor on population activity above 915m both from recorded observations during transects and from the B. Bryan data collected at the end of the dry season. It is therefore proposed that it was the combined effects of the low temperatures with the precipitation levels that led to the reduction of *C. nubila* numbers encountered above 915m on El Yunque Peak in the 2002 season.

On the first occasion when extra transects were walked by B. Bryan in April 2002, an extra section was done along the roadside leading up to the start of the 915m transect. This walk has been included in the results section because this section is just above the Closed road transect and therefore gives an insight into *C. nubila* numbers encountered between the Closed road transect and the start of the El Yunque Peak transects at 915m. From the Closed road data at 750m it is unclear whether the climatic anomaly affected *C. nubila* populations at this site. The Tradewinds populations at 735m were stable between 2001 and 2002 and the *C. nubila* populations along the 715m Colorado stand transect on the East Peak road were also stable during this period. The Closed road transect *C. nubila* population declined from 2001 to 2002, but the decline in numbers was not as indicative as along the high level transect above 915m. On the Closed road, *C. nubila* could still be found in relative abundance during the 2002 field visit. The indications are that the climatic anomaly either did not affect *C. nubila* populations at 750m, or else the drop in numbers found in 2002 reflect the climatic anomaly, but at an altitude of 750m on El Yunque Peak, the effects were less severe and *C. nubila* could still be encountered frequently. The extra section of walk undertaken may therefore help to resolve this question. Numbers of *C. nubila* recorded along this extra section in April 2002 were high and were similar to those encountered along the 915m El Yunque Peak transect in 2001. It is therefore concluded that the climatic anomaly affected *C. nubila* populations above 915m only and appears to have been confined to high elevations above 915m.

The third biogeographical site of East Peak was walked to a much lesser degree. This was more of a satellite site to give a brief insight into numbers in another area of the LEF. The lack of any trails mean that this site will always remain a peripheral area, but the similarity in numbers encountered at this site in 2001 and 2002 appear to indicate that this *C. nubila* population was not affected by the climate anomaly of 2001 and that there may well be life history differences between this population and populations of *C. nubila* on the Tradewinds trail and El Yunque Peak transects which allow individuals to survive the harsh conditions found on East Peak and therefore allowed this population to deal with the continuous low minimum temperatures recorded during the climate anomaly more successfully.

4.1.3 Streamflow, precipitation and *C. nubila* numbers

When East Peak and the Tradewinds regions were experiencing dry years, El Yunque was having above average annual rainfall from 2001 onwards and in 2004 the Rio Quebrada Espiritu Santo had high streamflow levels indicating that, like the Tradewinds region, it also had high annual precipitation that year. In 2000 however, Quebrada Espiritu Santo streamflows indicate that El Yunque had a drier than average year, as did the other regions of the peaks.

The lack of *C. nubila* recovery form 2002 to 2005 can be attributed to climatic conditions on El Yunque Peak which did not favour high activity levels. Conditions may well have been ideal for immature stages and foodplant condition but not for population activity and mating. The increase in numbers on the third El Yunque transect above 965m appear to indicate that conditions for population activity were better than the lower two transects. Above 965m there is a noticeable change in canopy height and the vegetation is much more open. Considering the low numbers recorded along the second transect between 935m and 965m, *C. nubila* activity can be surprisingly high along the end of the third transect at 1000m just before the peak is reached. The low canopy height and vegetation structure are thought to favour changes in the climatic variables and thus higher population activity.

Numbers recorded on the Tradewinds transects increased between 2003 and 2005 along the first two transects combined. This increase is thought to be due to the high levels of precipitation in the region in 2003 and 2004. The climate data shows that even in the dry season from January to mid April, when daily maximum temperatures are at their lowest levels, there is *C. nubila* activity during precipitation events. This is thought to be due to maximum daily temperatures being higher at 735m than at 1000m on El Yunque Peak. The high rainfall has ensured vigorous growth and good foodplant condition for immature stages, while temperatures have been high enough throughout the year to allow good levels of population activity, mating and egg laying.

The level of rainfall recorded from the static Rio Icacos climate station for 2003 and 2004 is over the annual mean for East Peak, regarded as the wettest point in the region. The 2004 data is even more interesting when the distance between the two measuring stations is three kilometres and the Icacos station is almost 400m lower. Less rainfall would be expected to be recorded here as it is lower than the areas of cloud forest above 915m, which generally receive the maximum levels of precipitation (Brown et al., 1983). This data fits in with numbers of *C.*

nubila recorded on the Tradewinds trail transects between 2001 and 2005, relating to annual precipitation in the preceding years. After dry years, numbers recorded have been low, *C. nubila* populations have always increased after wetter years, with highest numbers recorded so far on any transect after the high annual precipitation in 2003 and 2004 in this region of the forest.

4.1.4 *Calisto nubila* activity at Cambalache Forest Reserve, Guanica Biosphere Reserve and Carite Forest Reserve

At Cambabache Forest Reserve, *C. nubila* numbers encountered were generally low and although no direct temperature data is available, it was always noted as being hot and dry during visits, contrasting with the conditions found above 735m in the LEF. There was not enough data collected for conclusions to be reached on *C. nubila* activity in this area of Puerto Rico, the transect data however gives a good insight into *C. nubila* activity levels in a different region of Puerto Rico in contrast to the LEF.

The data from 2003 was collected to give an idea of *C. nubila* activity throughout the day in the forest. The first activity was recorded late morning and continued throughout the day at low levels, reaching a peak in the early afternoon. *Calisto nubila* activity and behaviour at Cambalache was notably different from that on the peaks of El Yunque, flight was recorded as being quicker and much more erratic. Most of the activity was on the edge of the forest verges in the shade, with butterflies rarely straying beyond this region onto the sunlit path itself Perching periods were very brief and although flight times were not recorded, they were observed as being much shorter than they were at altitude.

Most recording notes were made during the 2005 visit, primarily because more life history details were known about *C. nubila* by this stage of the project. It is likely that a different foodplant is used by *C. nubila* at Cambalache forest. There may be different grasses found within the forest canopy, but given the dry nature of the vegetation at this site it is thought unlikely that the foodplant grass used on El Yunque Peak would be found here and it has been observed to show signs of desiccation in a region where rainfall levels are very high. Eggs were found on the predominant grass encountered at Cambalache, but it is not known whether these were *C. nubila* eggs or not. Caterpillars and instars were also found, but these were not that of *C. nubila*, unless different forms are found at low altitudes. The highest numbers of *C. nubila* at Cambalache were recorded during the 2005 visit when numbers were double those of the highest numbers found on previous visits. An important note was made in 2005 that it was cooler during that visit and this may have helped to improve activity levels. Lizards appear to remain a predatory force at Cambalache, as they are at altitude in the LEF. A number of *C. nubila* were noted with bites out of the wing when the Cambalache transects were walked. Fresh females with a noticeable white streak on the wing were recorded as well. One of the most interesting points to come out of the Cambalache visits was that numbers encountered were higher than would have been anticipated. Generally *C. nubila* activity levels were higher at Cambalache Forest Reserve than those found at 515m and below in the LEF.

At Carite Forest Reserve *C. nubila* was recorded on the El Seis trail, however numbers were noted as lower than any of the transects above 765m on El Yunque Peak, from the field notes *C. nubila* was also recorded in the grass beside a number of car park areas. Numbers were however noted as being lower than at the respective heights in the LEF.

Guanica was visited as the habitat and rainfall were very different to the El Yunque region. The LEF, which includes El Yunque Peak, is lush and green and has one of the highest global annual precipitation totals recorded, while Guanica in the dry season has parched dry vegetation, low annual rainfall, is at sea level and endures high temperatures. Given the climatic and vegetation conditions encountered at Guanica, it was surprising that there were records of *C. nubila* at the site. When shown illustrations of *C. nubila*, site staff indicated that it was present, but only during the wet season. This poses an interesting question, as it indicates that *C. nubila* either has a seasonal diapause to cope with the harsh environmental conditions, or else it is present, but in very low numbers, in areas that are not accessible to site staff.

4.1.5 Comments on additional *C. nubila* observations made during the field visits in relation to published papers

The noted host plant specifity (Sourakov, 1999) is interesting when the hostplant noted above 735m on El Yunque Peak was not found at Cambalache Forest and no green grass at all was found at Guanica Biosphere reserve. When Carite Forest Reserve was visited, the foodplant used on El Yunque was not known, so it may or may not be present at this site. It is also not recorded below 735m on El Yunque. Above 735m the grass used is readily available, although the relative abundance of it has not been recorded. It is probably most frequent along the Tradewinds trail which indicates again that it is fluctuations in the climatic variables and rainfall patterns which regulate *C. nubila* population numbers, as opposed to foodplant availability above 735m. This is due to the high numbers of *C. nubila* recorded along the El Yunque transects above 915m in 2001.

Female *Calisto* spp. on the Dominican Republic are noted as being rarely seen in flight, even when the species is abundant and are often associated with the hostplant, spending time on the plant itself (Sourakov, 1996). Older *C. nubila* females are not that infrequent along the transect walks above 735m, fresh specimens, as previously noted, however are. The hostplant used on the peaks of the LEF was not discovered until the last field visit in 2005. This was after many hours spent not only walking the transects, but also carrying out observational periods along the grass edges of the transects and also grassy glades where there was *C. nubila* activity. It may be the case that eggs are laid outside the main periods when the transects are walked, but this is thought to be unlikely since the transects are walked during the main period of *C. nubila* activity.

The observations of the larvae were enough to clarify that it was that of *C. nubila*. The main identification feature was the forked tail at the end of the caterpillar. This is a feature of Satyridae (Smith et al, 1994) and *Calisto* species (Sourakov, 1996). *Calisto nubila* is the only Satyrid found on Puerto Rico (Smith et al, 1994). There are indepth photographic records of a number of *Calisto* species from first instar to pupae which have been documented (Sourakov, 1996, 1999). *Calisto nubila* is one such species where there are photographs of each larval stage, with the previously noted forked tail. The colourations were not exactly the same as those found on the Tradewinds trail and El Yunque Peak, but this is to be expected when comparing single printed photographs in a paper and specimens in the field.

The larvae of *C. pulchella*, feeds in a deep pocket between the stem and leaf of sugar cane where it is completely hidden. It comes out to feed at night, in later instars only (Sourakov, 1999). The pattern for *C. nubila* feeding is thought to be similar. The first instar was only once found outside the growing tip of the grass during daylight hours and this first instar was noted making its way down the foodplant where it subsequently disappeared. Despite frequent searches on the foodplant, no later instars were found on it during daylight hours. It is presumed later instars hide away in the leaf debris, from which they emerge at night to feed. There were numerous photographs taken of the El Yunque Peak caterpillars which were not found on any other grass above 735m on El Yunque Peak and no other grass appears to provide the same shelter, in the sheath of the growing tip of the grass.

Once the larvae were located, they were found to be frequent on the hostplant along the footpath edges and were found right up to the end of the highest transect on El Yunque Peak at 1000m. Larvae were also found in numbers where there was good *C. nubila* activity. On certain days, particularly on the Tradewinds trail in 2003, fresh female *C. nubila* were recorded early on the first transect in unfavourable flying conditions along the path edges. It was initially thought that these were freshly emerged females waiting for daily activity to start. It may be the case that they were engaged in egg laying activity, which would explain the lack of male activity at the time.

Although there is no direct data, it was the general opinion of the recorder that when observed, females were more active along the footpath edges, which seemed to form an important part of the territory, particularly above 735m. Males appeared to be in greater numbers overall along all transects walked above 735m. This would fit in with the grass growth, which was mainly along the footpath edges, except where there had been a landslide or other activity which had opened up the forest floor. On the Tradewinds trail however, one grass glade just off the footpath on transect one was observed on several occasions in full sun and there was surprisingly little *C. nubila* activity seen within it. One reason for this is that the grass used for a foodplant at altitude grows along the footpath edges and does not seem to survive well anywhere else. The grassy glades studied along the transects above 735m all consisted of different species of grasses, which had a different growth structure and much thinner leaves.

The main predator of *C. nubila* appeared to be lizards, who could be observed infrequently jumping up at passing *C. nubila* specimens as they flew by. Lizards could often be seen perched on the edges of leaves of vegetation when walking the transects. *Calisto nubila* specimens were also noted throughout the field visit period with bite marks on their wings, presumably having survived a lizard jump. The only other predation found on adult *C. nubila* was one specimen that was found entangled in a spiders web, this was only observed on one occasion on the Tradewinds trail.

The remaining points from the observational notes are that the three matings recorded on the Tradewinds trail in 2003 demonstrate that full sun is needed for this activity and therefore ties into the previous conclusion that the low temperatures coupled to the high monthly precipitation on El Yunque Peak in 2001 meant that opportunities for mating and therefore for recruitment levels would have been low. The argument put forward that the Cambalache *C. nubila* population must differ from the El Yunque Peak populations is underlined with the observations of *C. nubila* overheating on the Tradewinds trail after flight activity and the successive heat avoidance activity recorded on the Bisley trail in full sun and after flight activity. No heat avoidance or other overheating behaviour was recorded at Cambalache.

These events would appear to show that *C. nubila* activity is restricted at lower altitudes due to temperature fluctuations and even at 735m after the heat of the day, overheating occurs after flight activity. The temperatures at Cambalache at sea level, although not measured, were recorded as feeling much higher than in the LEF. When compared to the LEF *C. nubila* data, *C. nubila* activity found at Cambalache would have been expected to be lower than numbers under 515m in the LEF. This is due to the higher temperatures encountered at Cambalache. On the Bisley trail at 250m, the majority of *C. nubila* activity was recorded along the footpath edges in the dappled shade. Numbers at Cambalache, as previously mentioned, were higher than any of the transects below 515m in the LEF.

Although there is no detailed recording on tilting behaviour on landing there is both photographic, movie and annotated evidence showing tilting immediately after landing. This behaviour would be attribuTable to most of *C. nubila* landing above 735m. The habitat at El Verde where a previous study was undertaken was noted to be in a forest tract that was characterised by a 15-20m canopy which had a deeply shaded but relatively open understorey (Shelley et al, 1985). At altitudes above 735m in the LEF, *C. nubila* activity occurs in open habitat along footpath edges, grassy glades in the forest and any other areas where there is full sunlight. Very little activity was recorded under the canopy or other shaded areas. At 250m on the Bisley trail *C. nubila* numbers were lower than those found at higher altitudes and activity was noted as more sporadic.

The numbers of *C. nubila* entering an area of roadside verge at 915m and then perching within it reflect the dual activity behaviour of the species. Perching within an area for males and seeking out any passing butterfly is an important aspect of normal daily activity, however most individuals flew through the recorded section. What individual *C. nubila* are doing in this role is seeking out any other flying butterflies in that section of the territory rather than just sitting on a perch and waiting for another butterfly to fly past. Perching is of course important for lateral basking and this activity certainly has more than one role for *C. nubila*. On landing, many butterflies start lateral basking, there will be a slight change in the angle of the wings noted after landing so that maximum value can be gained from the incoming sunlight (Ludwig and Shelley, 1985). *Calisto nubila* have also been noted to perch on the tarmac beside the roadside vegetation to bask at the start of the El Yunque one transect and also to bask on the boulders which make up the footpath on the El Yunque and Tradewinds transects.There may well be more or less perching within the transect during different periods of the day. When this exercise was undertaken, it was during the period of maximum solar radiation under clear sky conditions.

The average flight times recorded on three days at 915m on El Yunque Peak in 2002 at the same location, again reflect the fact that even under similar visual conditions, activity levels appear to reflect localised environmental conditions above 915m. The other roadside observations at 915m were done at similar times during the heat of the day. Direct humidity and temperature measurements would have been needed to ascertain whether there were any real changes in environmental conditions between the days that may have reflected the differences in flight time on the days used for recording.

Distances covered by *C. nubila* during flight indicate that there is an average speed during flight and therefore distances covered relative to flight times appear to be similar. The data for this part of the observations is minimal, but it does help to give an overall insight into the life history of the species. After watching *C. nubila* for many hours, the fifty metre flight distance was a surprise, even although the individual concerned did skip the ground very briefly twice. It was even more noTable given that it occurred at the 10:30 recording period at 915m, which generally is the start of daily *C. nubila* activity and commencement of the lifting of the cloud cover on an average day. This flight time was not included with the other flight times recorded on that day due to the point that the flight was not properly maintained for the full fifty metres

Fresh specimens of female *C. nubila* were observed to have a white streak on the forewing, which makes them particularly noticeable when walking transects. Specimens are known to be fresh because as *C. nubila* ages, the wing colouration fades from dark to a much lighter brown. The white streak becomes unnoticeable on older females, this probably being a reason why it has not previously been recorded. The white streak itself varies slightly from female to female and has also been recorded on females at Cambalache Forest Reserve. All mating females both in the LEF and Cambalache were all fresh females with a dark brown wing colouration and a white streak. No white colouration was noted in any male *C. nubila*.

There was no observed perches, if *C. nubila* is disturbed while perching it was not observed to return to it, instead flight activity was continued and individuals usually ended up perching at different sites within the territory. Encounters therefore indicate the importance of the grassy footpath edges for general perching behaviour rather than specific perching spots within the communal territory. *Calisto nubila* will also fly in a patrolling manner which generally ends up with the butterfly either having an encounter or perching again on vegetation. When patrolling, there appear to be no individual territories, rather a communal territory through which butterflies exit and return. Communities are then spread along more open sections of the path system. There is also a lot of activity in other open areas where there have been landslides, or along more open river valleys. When the trail goes under the forest canopy itself, particularly along the Tradewinds trail where the canopy height is higher, there is very little *C. nubila* activity and communities are split up from each other by the vegetation structure. There appears to be little movement between *C. nubila* populations as a whole. Individuals with a noticeable wing bite mark or similar feature were seen in the same location on successive transect walks. Larger populations on the Tradewinds trail are often found in distinct open areas with little activity found outside these areas until another favourable region is encountered. Only on more open sections, do *C. nubila* communities appear to have the opportunity for any degree of mixing.

Other *Calisto* species are known to live in harsh environmental conditions similar to those found at Guanica. *Calisto crypta*, Gali has emergence and breeding times which are correlated with the sporadic rains in the deserts of Hispaniola (Sourakov, 1996). This butterfly is closely associated with *Uniola virgata,* where most of the time is spent in the shade of these bushy grasses. Females are rarely recorded flying ouside the grass unless disturbed while males occasionally fly between bushes. Activity was recorded between 09:00 and 12:00 before the desert heat reached the frequently recorded temperature of 40^0C.

During the Guanica visit, a few places along the roadside were checked for any signs of general butterfly activity and none were found. On the trail walked, there was virtually no green vegetation and all the grasses were brittle and dried up. The vegetation here reflected what was generally noted on the reserve at the time of the visit. There was butterfly activity, but the species count and numbers recorded were low. Another reason Guanica was visited was that the vegetation structure here was similar to that found on Anegada where *Calisto anagadensis,* Smith, Miller and McKenzie is found. This is the furthest east any member of the genus is found. There is only one colony known and it is restricted to an area along the northwest coast. Again, the tussock grass *U. virgata* is thought to be used as a foodplant. It is not known at present whether *U. virgata* is present at Guanica, but given the similarity of the habitat between Guanica and Anegada, it would not be surprising if it was present. This would pose the question of the possibility of it being used by *C. nubila* as a foodplant in a harsh environment from where it radiates during the wet season or whether as mentioned previously, there is a diapause and other grasses are used when the growth conditions are right.

Calisto nubila is found in Puerto Rico from sea level to 1,000m and may well be found above this height in the Central Cordillera mountain range. It does not therefore appear to have any intolerance to elevational limits on the island. Because of its suspected use of different species of grasses for foodplants at different elevations as well as the large climatic tolerances experienced from sea level to the highest peaks, *C. nubila* would appear to be well placed to withstand any global warming scenarios. Overall numbers on Puerto Rico would however be expected to fluctuate, particularly at altitudes above 735m where the species is found in numbers, in response to precipitation and climate fluctuations.

4.1.6 *Wallengrenia drury* numbers encountered in the LEF, Cambalache Forest Reserve, Carite Forest Reserve and Guanica Biosphere Reserve

Wallengrenia drury showed a similar distribution pattern *to C. nubila,* with numbers increasing with altitude. The only exception was that higher numbers were encountered along the Closed road transect at 715m than at 750m on the Colorado stand transect and 735m along the Tradewinds trail. The Closed road transect was chosen for data collection as it provided a different habitat to the other transects above 700m.The vegetation structure and orientation therefore appear to have favoured *W. drury* habitat requirements, with the increases in numbers recorded at this site. Although numbers were variable on the Tradewinds transect and the El Yunque Peak transects above 915m between 2001 and 2005, numbers encountered are regarded as too low to draw any significant conclusions from the data

The purpose of doing the East Peak transects was to investigate out what butterfly species were present in these areas of the forest and the grassy verges along the roadside. By focusing on a small area only at East Peak, the numbers of *W. drury* encountered may be slightly higher than normally recorded, but the results are still seen as a valid indication of numbers at this site. In more open grassy areas which caught the sunlight *C. nubila* was generally encountered very occasionally flying within the area of cloud forest at this site, *W. drury* was only found in the open grassy area. Activity was also only recorded during short periods where the sun broke through the cloud cover. East Peak being the highest transect, is the first place where cloud cover gathers on a clear day and is also the last place where cloud disperses from and is often covered in cloud all day. It may be that *W. drury* has a diapause at this site during the wetter periods of the year. This would help to explain why numbers appear to have been unaffected here by the climate anomaly, while the populations on El Yunque Peak record the climatic anomaly in the 2002 data.

Wallengrenia drury was recorded at Cambalache Forest Reserve infrequently, but in 2003 higher numbers were encountered here than on any of the El Yunque transects above 735m. This is not regarded as a significant result, as overall numbers of *W. drury* were still low on all the transects walked in 2003. Numbers are considered to be too low for any clear conclusions to be made. At Guanica, *W. drury* was not recorded on the transect walked or seen anywhere else while walking around this area. At Carite Forest Reserve the notes on *W. drury*, indicate that no significant findings were made at the El Radar Trailhead, Doppler weather station or on the El Seis trail.

4.1.7 Portable climate station data and *W. drury* activity

Wallendrenia drury numbers on the Tradewinds trail were low throughout the sample period. What would often happen is that this species would not be recorded for long sections of the transect and then when there was a period of sunshine in a more open area, there would be some activity recorded. Again, the total numbers encountered are not regarded as high enough to reach any significant conclusions, but the overall activity patterns recorded are remarkably similar to that found for *C. nubila.*

As with *C. nubila*, it is humidity which has the closest relationship with *W. drury* activity levels in the climate correlation calculations, except it is maximum rather than minimum humidity values which have the greatest significance. Because there was no correlation found between any of the climate measurements in 2003 and *W. drury* numbers, it is felt that no strong conclusions can be drawn from the data. This is an interesting point, bearing in mind the contrasting size difference between the two butterflies. The data also indicates that the rise in numbers in 2005 on the El Yunque Peak transect above 965m appear to correspond to the increase in *C. nubila* numbers along the same transect. Numbers of *W. drury* were felt to be too low in 2002 on the El Yunque Peak transects above 915m to carry out statistical analysis of numbers encountered and data from the climate station.

The climate anomaly does appear to be recorded above 915m on the El Yunque Peak transects, as there is a decrease in *W. drury* numbers encountered from the 2001 to the 2002 season.

It could be argued that *W. drury* numbers encountered on their own are not high enough for serious data and climate analysis, but when combined with the *C. nubila* data, most of the general conclusions reached for both species are the same. Like *C. nubila*, *W. drury* numbers showed an increase on the Tradewinds three transect above 765m in 2005. Both species increase in abundance with height above 735m and both *C. nubila* and *W. drury* activity patterns have the closest relationship with humidity levels in the correlation calculations. The two species again recorded the climatic anomaly in the 2002 season and registered increases in numbers along the 965m El Yunque transect in 2005 after high annual precipitation on El Yunque Peak.

4.1.8 *Antillea pleops pleops* Activity Levels in the LEF, Cambalache Forest Reserve, Carite Forest Reserve and Guanica Biosphere Reserves

In the 2001 field visit, *A. pleops pleops* was another butterfly that could be encountered in numbers along high level transects. Overall activity encountered was less than *W. drury* and after the 2001 season it was recorded infrequently along all transects in the LEF. As a result no climate analysis has been done with the *A. pleops pleops* data, as it was felt that numbers were generally too low to make it worthwhile. Any comments from the data analysis would not have had sufficient numbers encountered per ten minutes recorded to make them conclusive.

The overall conclusions from the numbers of *A. pleops pleops* recorded are the same as that for *W. drury*. It was surprising to find this small butterfly at all at altitudes above 915m, and in 2001 numbers were high. 2001 was the only year where numbers of *A. pleops pleops* were relatively abundant above 915m on El Yunque Peak. Activity levels increased with height to the peak itself. This species was not recorded at all on the El Yunque Peak 250m, 515m or on the 750m and 1000m East Peak transects.

In 2002 *A. pleops pleops* was not recorded at all on the LEF transects above 915m, so this species seems to record the climate anomaly of 2001 as well. Numbers of A. pleops pleops however on the 965m El Yunque Peak transect did not increase in the 2003 and 2005 visits as *C. nubila* and *W. drury* did. There was an increase in activity levels recorded in the 2003 field visit above 915m, but overall numbers remained low. The climatic anomaly therefore appears to have had a long lasting and detrimental effect on numbers.

Like *W. drury*, when numbers of *A. pleops pleops* are found, it is usually in periods of sunshine. *Antillea pleops pleops* is however less associated with open grassy areas than *W. drury* and is often found perching with open wings within the forest canopy. This observation may explain why numbers remained low in subsequent years after the climatic anomaly, with above average annual rainfall at 915m indicated by the streamflow measurements.

Antillea pleops pleops numbers encountered did increase along the third Tradewinds transect above 965m in 2005, but again, numbers remained low. Given that this is a small butterfly whose activity levels increased in full sunshine on the peaks, it was surprising that it was not recorded at all on transects below 715m in the LEF. *Antillea. pleops pleops* was recorded on the Closed road transect at 715m in 2001. The altitude and open habitat of this transect was initially thought to have provided species such as *A. pleops pleops* good opportunities for activity. It may well be the case therefore that the distribution of *A. pleops pleops* follows that of its foodplant which is less abundant than the grasses used by *C. nubila* and *W. drury*. This finding may support the view that it is noted as occurring in small isolated colonies (Miller et al, 1994).

Because annual rainfall levels, as indicated by the streamflow data on El Yunque Peak, continued to increase from 2001 to 2005 and there was no recovery recorded for *A. pleops pleops* on the high level El Yunque transects above 915m, this species may well reflect dry years on the peak, so lower annual precipitation recorded in the streamflows from El Yunque Peak in the 2000 season gave this species an opportunity to increase in numbers at high altitudes. *Antillea pleops pleops* appears to be unable to increase recruitment levels in the more normal wetter conditions found in the regions of cloud forest above 915m on El Yunque Peak. While *C. nubila* and *W. drury* are good indicators of the general climatic conditions and annual precipitation levels found on the peaks in the LEF, *A. pleops pleops* would appear to be an indicator species of lower annual precipitation levels above 915m.

At Cambalache Forest reserve, *A. pleops pleops* was not recorded at all in 2002 or 2005 and was found in low numbers in 2003. Activity levels of this species found on the Cambalache transects came as a surprise. One of the reasons that Cambalache was visited in 2002 was due to the high numbers of *A. pleops pleops* found on the El Yunque peak transects above 915m, numbers of *A. pleops pleops* had previously been encountered in numbers at Cambalache as well (D.S. Smith, personal communication). It was therefore felt that Cambalache Forest Reserve would provide a good lowland comparison for this species. Cambalache was always visited in the dry season, so numbers of *A. pleops pleops* may increase at this site during the wet season in relation to foodplant abundance. It was not surprising that *A. pleops pleops* was not recorded from Guanica in the dry season, given the dry and brittle nature of the vegetation found along the transect walked at this site. It is unclear if this species was found on any of the Carite transects. From the notes there is no mention of the species and it was certainly not found in any numbers if it was recorded.

4.1.9 Overview of the initial objectives set

The project has been successful in meeting the main aim and objectives of this thesis. Three butterfly species were identified in the LEF which displayed altitudinal distribution patterns and *C. nubila* was found in numbers above 915m. The main species displaying an altitudinal distribution pattern was *C. nubila*, but *A. pleops pleops* and *W. drury* provided information which backed up the main *C. nubila* data set. *Calisto nubila* was recorded in numbers on the transects above 735m in the LEF and correlation calculations were used on numbers encountered per ten minutes along the Tradewinds trail transects one and two combined between 735m and 765m and the El Yunque Peak third transect between 965m and 1000m and data collected by the portable climate station. In addition, the general linear model was used on *C. nubila* numbers encountered on the Tradewinds transects in 2003. The result was a substantial data set from the high level transects above 735m for *C. nubila* numbers and the climate variables which in turn generated the results in chapter 3.

Chapter 3 established that *C. nubila* numbers encountered per ten minutes could be used as an indicator of local changes in climate on the Tradewinds trail transects above 735m and the El Yunque Peak transect above 965m as there was a close relationship in the correlation calculations with the climate variables. The 2002 El Yunque Peak general linear modal calculations showed that wind speed and the other climate variables combined were significant factors relating to *C. nubila* activity. The 2003 general linear model calculations concluded that all the climate variables and the time transects were walked were significant factors relating to *C. nubila* activity on the Tradewinds trail from 735m to 765m.

The thesis therefore met the anticipated end result of setting up a local climate monitoring system on the peaks of the LEF using butterflies as the indicator species. The recording of the climate anomaly of 2001 in the 2002 butterfly data set showed how the biological monitoring system works and the recovery, or lack of it, on the El Yunque Peak transects above 915m reflected how the *C. nubila* populations were affected by the local climatic conditions. The other transects set up in various forest regions in Puerto Rico provided an additional data base for the island as a whole and further insights were gained into the mostly unrecorded life history of *C. nubila*.

Chapter Five

Closing discussion and future research.

Section 5.1 Final comments on *C. nubila*, the transects used and the LEF

After the final field visit in 2005, it became apparent that *C. nubila* population numbers on the Tradewinds trail had increased to the highest levels so far recorded anywhere in the Luquillo Experimental Forest (LEF) and other sites in Puerto Rico. What was not clear at this point was why there had been such an increase. On the Tradewinds trail transects, there was a rise in population numbers from transect one to transect three which, when added together, constituted a continuous transect of around forty five minutes walking time which went from 735m to 785m. Because of the numbers of *C. nubila* involved and the daily continuity of the data collection, the data from this year in particular, is regarded as the best set from all the field visits and transects sampled. It was a combination of all the factors such as the length of the combined transects, the increase in altitude, the ecology of the colorado forest and the high proportion of foodplant found along the track edges. The addition of the third transect in 2005 was also an important addition to the overall data set.

Numbers of *C. nubila* recorded along the Tradewinds trail in 2005 were surprising. Particularly along sections of the third transect, they were literally everywhere and this is reflected in the data. There is brief movie footage of activity levels along this transect and it was particularly during sunny periods where there would be flurries of *C. nubila* activity along the grass edges of the path system. It also made the data collection a positive experience, as obviously when large population numbers are being recorded each day then the quality of the data set is going to be high.

One reason that the climate station was always situated on the Tradewinds trail after the 2002 visit was that although *C. nubila* numbers fluctuate along the transects in this region of the LEF, numbers were always reasonably high and data calculations were more relevant on a daily basis. The siting of the portable climate station on El Yunque Peak in 2002 however did produce a good data set which gave an insight into how the climate variables were interacting with *C. nubila* numbers above 965m.

The other reserves visited, although relatively brief, helped to give an insight into *C. nubila* activity levels throughout Puerto Rico. Both the northern and southern coasts were sampled, with El Yunque near the east and Carite Forest Reserve on the edge of the central mountain region, the only area not covered was the west coast. It is anticipated by the author that the peaks in the Cordillera Central may also have large *C. nubila* populations at altitude, as conditions in some areas are similar to the El Yunque region in that there are pockets of elfin cloud forest on the peaks. Carite Forest Reserve however showed that if the forest type is not relatively unmanaged colorado forest or elfin cloud forest, then *C. nubila* populations at altitude can be low.

The LEF is also probably unique on the island. There are static climate monitoring stations throughout Puerto Rico, but not in the density that is found in the LEF. The other point is that it is not just the density, but the actual river systems covered by that data set that are important. Again, the LEF is ideal as the Rio Icacos has a static station not far away from the Tradewinds trail which allowed precipitation comparisons with the data from the portable climate station. The East Peak static station was also of high significance to the project, as it showed the climate anomaly in the temperature data which, when compared to the El Verde work station temperature data, indicated that it affected the peaks only. The static station on the Quebrada Espiritu Santo with the source directly below El Yunque Peak also gave invaluable data on streamflow levels which was shown to correlate with precipitation levels on the peak itself. Without the wealth of local climate data therefore, some of the more important results and conclusions contained in this thesis could not have been made.

Now that there has been a base study undertaken which indicates how *C. nubila* interacts with the various climate parameters, it would be possible to take a location in somewhere like the Cordillera Central and with portable climate data collecting equipment, set up a comparative study and have another elfin cloud forest area where *C. nubila* was being used as a biological indicator of changes in local climate variables on the peaks of the island. It is suggested that this would be an ideal research project for a locally based student. It requires a reasonable amount of effort to collect data at altitude, but there are few areas where this can be done as easily as in Puerto Rico due to the road and trail systems. At around the 1000m mark, the mountain peaks are also relatively low compared to other cloud forest regions in the world.

With the present data set, it would be possible to do infrequent annual transect counts on the Tradewinds trail at 735m and on El Yunque Peak above 915m and record *C. nubila* numbers. When correlated with the regional and portable climate data, this would give an indication of changes in the local climate variables and previous annual precipitation on the peaks. This would then give a general overview of the localised climate in regions of cloud forest and mid elevation colorado forest which are tied into the ecological cycle in a region where precipitation levels are one of the main driving forces for the health of the local flora and fauna. Portable hand held data collectors which can be set up along trails for reduced time periods are widely available and most can withstand the rigours of the climate on the peaks of the LEF.

With further transect counts in any region of Puerto Rico, all butterfly species encountered would be expected to be recorded. Although it is thought to be unlikely that other species would be found in high numbers along high altitude transects, there may be other species encountered in reasonable numbers which can be used in calculations with any climate data collected. *Antillea pleops pleops* and *W. drury* are two such species, both of which can generate good data sets at altitude. The main issue with additional species however is that they should be regarded as such and any data collected is supplementary information. When numbers encountered are at a much lower level, it is difficult to reach conclusions with the same clarity as one can when dealing with large data sets.

In setting up a project such as this from a distance, it is obviously unclear how successful it is going to be in meeting the initial objectives of finding variations in butterfly distribution patterns and whether any changes were related to the climate variables. Collecting the butterfly data was never going to represent much of a problem and as has been previously discussed, there were many valid reasons for choosing the LEF to do the field visits in. What was surprising during the initial visit of 2001 was the activity levels and population numbers of *C. nubila* found on the peaks above 735m. In 2001 in particular, above 915m on El Yunque Peak, numbers of *C. nubila* were high. The activity encountered on the Tradewinds three transect in 2005 was even more so. To find high levels of butterfly activity in what would generally be regarded as a harsh climate was rewarding, as it means that you have large data sets to work with, which can be gone into in some detail when doing calculations with the climate data. In terms of finding a butterfly species that can be used as a biological indicator of changes in local climate in regions of cloud forest, this research project has been regarded as being successful. It is hoped that the data set and conclusions reached will provide a starting point for future research projects.

There are butterfly species encountered in cloud forest regions around the world, but to date, *C. nubila* may well be the only one that is consistently found in high numbers at altitude. It is however suspected that other *Calisto* species may be found in sufficient numbers at altitude in both the Dominican Republic and on Cuba and therefore that comparative research in these regions would be possible as well as fruitful.

5.1.1 Other possible research locations

The Dominican Republic is the main *Calisto* hot spot and has a variety of protected areas. Two thirds of all virgin forest on the island has been felled. The Amando Bermudez National Park however is 766 sq km and contains the highest peak in the Caribbean, the Pico Duarte at 3087m. Within the park area, annual precipitation ranges from 1000mm to 4000mm. There is no mention in literature of areas of TMCF occurring on the island and the annual rainfall levels are short of the typical 4800mm mark on the peaks of the LEF.

There are a number of other islands in the Greater Antilles where *Calisto* spp. are found, what is of interest to this research project though are the species found at altitude and it is this point that will narrow down the possible locations on other islands where comparative research can be undertaken. From the written material currently available, a number of mountainous regions in the Dominican Republic would be suitable and also areas of the Blue mountains in Jamaica where *C. zengis* has been recorded. The secondary factor involved would be whether the *Calisto* spp. concerned is found in numbers at altitude. Again, there is a high probability that numbers of separate *Calisto* spp. can be found in mountainous regions of the Dominican Republic, the density of *C. zengis* is however more uncertain. It is unlikely that the wealth of climate data available in the El Yunque region would be available at any of these locations. What is more likely is there would be some localised temperature and rainfall data that could be related to any data collected. Small electronic data collectors that can take frequent measurement of set climate parameters such as humidity and precipitation levels, which could be set up along the transects, could then be compared to the butterfly data collected.

Calisto delos would also make an ideal candidate for a further biological indicator species at altitude in Cuba. The main problem would be in finding a forested area, preferably including TMCF zones, large enough at altitude that was either in a protected area or was sufficiently isolated and inaccessible. To get to the high level transects used in Cuba required hours of walking with overnight stops or the use of a mule. A rough vehicle track was noted as ending just after La Esmajagua at 560m.

There are of course other butterfly species found at altitude in the Lesser and Greater Antilles and during the initial stages of the project Saba, St. Kitts, Nevis and Dominica also appeared to have pockets of cloud forest on mountainous peaks. None however had the accessibility of the El Yunque region and it was not always easy to ascertain whether there had been any forestry activity, land clearance or just how protected from any future disturbance certain areas were. The bonus of setting up transects in areas of cloud forest in the Caribbean was that the mountain peaks and areas of cloud forest are at lower elevations that on the mainland in Central or South America. This generally cuts down on travel time and often, as is the case in Puerto Rico with the El Yunque region and Central Cordillera mountain region, there are access or main roads which cut through cloud forest regions. Costa Rica has readily accessible regions of cloud forest, but butterfly species as well as overall numbers encountered can be low.

There are numerous high level trail systems in cloud forest areas in this area of the world which could have been used, but if transects had been set up on other trail systems around the peaks of the Caribbean, then it would probably have necessitated camping out and the data collection would have been done in a much more limited time period. On El Yunque around five hours each day were spent travelling to the sites and collecting the data and then around a further hour in the evening compiling notes. The ease of access meant that a large proportion of the time was spent recording notes and collecting data which is a major issue when only a limited timeframe is available to do each field visit. Sampling could additionally be done in July and August in the LEF. Additional climate data from the wet season (not included in this thesis) shows that there can be high levels of rainfall for several days on the peaks in the LEF. It also indicates that temperatures are higher during this period of the year when compared to the dry season, *Calisto nubila* activity would therefore be sporadic for consecutive days. This kind of data collection would be ideally suited to a local researcher who would be willing to travel up to the peaks each day for a defined period and collect *C. nubila* activity data from the transects.

5.1.2 Recommendations for future research

Future recommendations have been partially covered in previous sections, however the main recommendation would be for further research using the baseline data presented within this thesis. The simple option would be to continue using the El Yunque transects and set up some additional ones in the Central Cordillera region in Puerto Rico. There will already be scattered climate data available for that area and additional data collection with palm sized digital collecting stations along the chosen transects should not present any difficulties. Data collection and handling could be carried out by students from academic facilities on the island and the work incorporated into existing syllabuses. This option would provide a robust island climate monitoring scheme in regions of cloud forest on Puerto Rico with relatively small effort and resources.

The ideal scenario would be for a number of butterfly monitioring projects to be instigated in various regions of cloud forest in the Caribbean. This is possible, but there would be difficulties to be overcome. To make the scheme work there would need to be constant reliable climate data from each region concerned. On site historical data would be highly desirable. Each of the chosen regions would need to be coordinated and transects would ideally be walked on a regular basis. Probably the best opportunites would arise from island university or college ecology departments becoming involved and incorporating the climate monitoring scheme into existing courses as on site experience of field work and data handling.

The positive elements of this would be numerous and additional funding needed would be minimal if the transects were coordinated from within each island. The actual data collection would be straightforward to carry out and the data sets would be easily transferable into existing learning modules. Data calculations, although possibly initially daunting, are also uncomplicated and not confined to a single statistical method of evaluation. The cost of on site climate recording equipment is reasonably low, it usually just requires the data collecting from it at regular but infrequent intervals dependant on how many measurements are being taken in each 24 hour period. Travel to and from the transects would be more straightforward on some islands than others, but could easily be done in most locations in a day visit .

The end result would be a substantial climate monitoring scheme in one of the worlds most threatened ecological habitats that would highlight research in the Caribbean region, present a comprehensive data set and add to the ongoing global debate on climate change.

5.1.3 Acknowledgements

There are a number of people whose input was indespensible in relation to this thesis and the field visits. The International Institute of Tropical Forestry in Puerto Rico provided logistical support and staff were always helpful. Fred Scatena was a key Figure throughout the data collection period. From arriving for the first time in the LEF, everything was straightforward and simple, including access arrangements and setting up the portable climate station in new locations. It cannot be stressed enough that when you have spent months setting a project up from a distance and then arrive on location, it is of immense benefit to have a helpful person on site who is prepared to put themselves out to make sure it all goes smoothly along with having a very busy and demanding job to do themselves. Fred moved to mainland America after the first two visits, but remained a vital contact for arranging various things throughout the five years worth of field visits. Carlos Estrada was the other person on site at the Sabana work centre, who gave up a lot of time to ensure that the climate station was set up on location and that the data set was being collected properly. There were other members of staff who lent a hand from time to time in Puerto Rico, but Fred and Carlos were the main contacts and could not have been more helpful.

The late David Spencer Smith was another vital contact. He had been to Puerto Rico over thirty times and was an expert in the field of Lepidoptera in general and particularly in the Caribbean region. Each year, on at least one occasion, a meeting was set up to look at that years data collected and which butterfly species had been recorded where and there were countless small comments made, some of which are included in the thesis. Unfortunately he was not able to see the end results, but it was during the field visits that his input and shared knowledge were very much appreciated as external supervisor to this project.

Dr Mike Gillman as internal supervisor has calmly guided the thesis through the various stages and went to Puerto Rico during the first two field visits. Mike provided invaluable advice on many occasions, ensured that the whole research process went as smoothly as possible and had various external contacts who provided input to the ongoing research.

There were also numerous people who helped with computer questions in the early days of data collection and in particular Chris Park from Nene Park, U.K. came to the rescue on numerous occasions. There were also staff at the Southern Regional Climate Centre in the United States who answered data questions or supplied historical data sets quickly relating to the historical and real time climate data collected from the static stations in Puerto Rico.

Nick Greatorex-Davies helped with collating some of the temperate butterfly data from Monks Wood in Cambridgeshire. Everyone else who lent a hand from time to time will know who they are and their help was appreciated.

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Appendix one

Additional information on buterflyspecies encountered on the transect walks

Table 22.

Numbers of *C. nubila* encountered per 10 minutes on El Yunque Peak above 915m and the Tradewinds trail transects 1 and 2 combined from 735mto 765m and transect three from 765m to 785m.

	2001	2002	2003	2005
	Number encountered per 10 minutes			
El Yunque Peak				
915m- 935m	18.12	10.48	9.07	9.93
935m- 965m	32.47	5.38	5.37	4.38
965m- 1000m	27.75	3.37	14.56	9.93
Tradewinds trail				
735m- 765m	19.12	22.45	18.69	38.95
765m to 785m	/	/	/	91.12

Numbers of *W. drury* encountered per ten minutes on the Tradewinds trail transects one and two combined at 735m to 765m and on the El Yunque Peak transects above 915m are shown in Tables 23 and 24.

Table 23. *Wallengrenia. drury* numbers encountered per ten minutes were recorded on the Tradewinds trail transects one and two combined from 735m to 765m and transect three from 765m to 785m from 2001 to 2005.

Year	Species	Tradewinds transects 1 and 2 combined, 735m- 765m	Tradewinds transect 3, 765m- 785m
2001	*W. drury*	0.361	/
2002	*W. drury*	0.096	/
2003	*W. drury*	0.234	/
2005	*W. drury*	0.419	1.75

Table 24. Numbers of *W. drury* encountered per 10 minutes on the El Yunque Peak transect 1 from 915m to 935m, transect 2 from 735m to 765m and transect 3 from 965m to 1000m in 2001, 2002 ,2003 and 2005.

Year	Species	El Yunque 1 915m- 935m	El Yunque 2 935m- 965m	El Yunque 3 965m- 1000m
2001	*W. drury*	0.792	1.75	1.71
2002	*W. drury*	0	0.219	0.194
2003	*W. drury*	0.317	0.183	0.179
2005	*W. drury*	0.357	0.548	2.44

Tables 25 and 26 display the numbers of *W. drury* encountered on the other transects in the LEF. *C.alisto vitellius* numbers are also included as this species is visually similar to *W. drury* and is encountered in the same mid-altitude habitats.

Table 25. Other El Yunque and East Peak transects walked from 250m to 1,000m in 2001 and numbers of *W. drury*, *C. vitellius* and *A. pleops pleops* encountered per 10 minutes. Total transect walking time is indicated.

Transect name	*W. drury*	*C. vitellius*	Transect minutes
Bisley trail 250m	0	0	237
Big Tree trail 515m	0.16	0	378
Closed road 715m	1.46	0.23	130
Colorado stand 750m	1.22	1.22	49
East Peak 1000m	1.79	0	67

Table 26. Numbers of *W. drury* and *C. vitellius* encountered per 10 minutes on various transects in the El Yunque Experimental Forest in 2002. Total transect walking time is indicated.

Transect name	*W. drury*	*C. vitellius*	Transect minutes
Bisley trail 200m	0.32	0.1	96
Big Tree trail 515m	0.31	0	228
Closed road 715m	1.14	0.23	175
Colorado stand 750m	0	0	18
East Peak 1000m	1.54	0	26

The total time spent walking the various transects is also given for Tables 25 and 26 to give some indication of how long was spent walking the transects when *W. drury* was not recorded at all.

Numbers of *A. pleops pleops* encountered per ten minutes along the various transects in the LEF in 2001 and 2002 are shown in Tables 27 and 28. The results indicate that in the LEF, this is a scarce butterfly below 715m.

Table 27. Numbers of *A. pleops pleops* encountered per ten minutes on various transects in the El Yunque Experimental Forest in 2001. Total transect walking time is indicated.

Transect name	*A. pleops pleops*	Transect minutes
Bisley trail 250m	0	237
Big Tree trail 515m	0	378
Closed road 715m	0.23	130
Colorado stand 750m	0	49
East Peak 1000m	0	67

Table 28.Numbers of *A. pleops pleops* encountered per 10 minutes on various transects in the El Yunque Experimental Forest in 2002. Total transect walking time is indicated.

Transect name	*A. pleops pleops*	Transect minutes
Bisley trail 200m	0	96
Big Tree trail 515m	0	228
Closed road 715m	0	175
Colorado stand 750m	0	18
East Peak 1000m	0	26

Tables 29 and 30 show the numbers per ten minutes of *A. pleops pleops* encountered on the higher level transects above 735m in 2003 and 2005. This species was only encountered in reasonable numbers at higher altitude on the El Yunque Peak transects above 915m in 2001.

Table 29. Numbers of *A. pleops pleops* encountered per 10 minutes on the El Yunque trail transects 1,2 and 3 in 2001, 2002, 2003 and 2005.

Year	El Yunque 1	El Yunque 2	El Yunque 3
2001	0.99	7.34	8.18
2002	0.083	0.5	0
2003	0.159	0.183	1.7
2005	0.179	0.411	1.05

Table 30. Numbers of *A. pleops pleops* encounterd per 10 minutes on the Tradewinds trail transects 1 and 2 combined and transect 3 in 2001, 2002, 2003 and 2005.

Year	Tradewinds transects 1,2	Tradewinds transect 3
2001	0.593	/
2002	0.207	/
2003	0	/
2005	0.051	0.112

Table 31. Species and number encountered per ten minutes on the Cambalache Forest Reserve Transect in 2003. Total time spent walking the transect was 181 minutes.

Species	Number encountered per 10 minutes
C. nubila	2.82
H. charitonius	2.38
B. hysteria	1.49
M. petreus	0.99
A. g. arecosa.	0.28
Archaepropona	0.11
D. iulia	0.55
P. o. oileus	0.06
W. drury	0.44
A. p. pleops	0.44
H. amphichloe	0.06

Table 32. Species and number encountered per ten minutes on the Tradewinds transects one and two combined 2003. *Calisto nubila* data is not included. Total transect time was 1022 minutes for the two transects combined. *Adelpha gelania arecosa* was an additional species encountered on one occasion during the Tradewinds three transect walk in 2003.

Species	Number encountered per 10 minutes
Colobura dirce	0.04
W. drury	0.25
P. o. oileus	0.04
A. p. pleops	0.02

Table 33. Species and number encountered per ten minutes on the El Yunque Peak teansects one, two and three 2003. *Calisto nubila* data is not included. Total transect time for El Yunque transect one was 47 minutes, El Yunque transect two was 123 minutes and El Yunque transect four was 86 minutes.

Species	El Yunque one, 915m	El Yunque two, 935m	El Yunque three, 965m
A. p. pleops	0.85	0.24	1.98
W. drury	0.21	0.08	0.23

Table 34. Species and number encountered per ten minutes on the Cambalache Forest Reserve Transect in 2005. Total time spent walking the transect was 61 minutes. *Eunica tatila tatilista* was seen on the way back from one of the shelters outside the transect area.

Species	Number encountered per 10 minutes
C. nubila	4.26
H. charitonius	4.26
B. hysteria	5.08
A. g. arecosa	0.98
Archaepropona	0.66
D. iulia	0.66
P. o. oileus	0.33

Table 35. Species and number encountered per ten minutes on the Tradewinds transects one and two combined 2005. *C. nubila* data is not included. Total transect time was 978 minutes for the two transects combined. *Euides melphis melphis* was noted flying outside the transect area by a large overhanging rock on 6/4/05.

Species	Number encountered per 10 minutes
Colobura dirce	0.01
W. drury	0.43
P. o. oileus	0.04
A. p. pleops	0.05

Table 36. Species and number encountered per ten minutes on the El Yunque Peak transects one, two and three 2005. *Calisto nubila* data is not included. Total transect time for El Yunque transect one was 44 minutes, El Yunque transect two was 108 minutes and El Yunque transect four was 64 minutes.

Species	El Yunque one, 915m	El Yunque two, 935m	El Yunque three, 965m
A. p. pleops	1.36	0.37	1.41
W. drury	0.45	0.56	1.72

Table 37. Species and number encountered per ten minutes on the Tradewinds three transect 965- 985m, 2005. *Calisto nubila* data is not included. Total transect time was 177 minutes.

Species	Number encountered per 10 minutes
A. p. pleops	0.17
W. drury	1.64

Table 38. Average number of butterfly species encountered per 10 minutes on the Bisley trail, Big Tree trail, Closed road and El Yunque Peak transects above 915m in the LEF, 2001. *Antillea pleops pleops*, *C. nubila*, *C. vitellius* and *W. drury* are not included as they are discussed separately.

Butterfly species Encountered.	Bisley trail 250m.	Big Tree trail 515m.	Closed road 715m.	El Yunque one 915m.	El Yunque two 935m.	El Yunque three 965m.
Colobura dirce	0.04	0.46	0.24			
Danaus plexippus		0.03				
Dismorphia spio	0.12	1.54				
Dryas iulia iulia	0.16	0.23	1.56			
Euphyes singularis			0.06			
Pyrgus oileus oileus	0.08	0.03	6.59			
Perichares philetes			0.78			
Siproeta s. stelenes	0.04	1.01	6.77	0.5	0.04	

Table 39. Average number of butterfly species encountered per 10 minutes on the Tradewinds trail, Colorado stand, grass verge at 750m, East Peak and grass verge at 1000m in the LEF 2001. *Antillea pleops pleops*, *C. nubila*, *C. vitellius* and *W. drury* are not included as they are discussed separately.

Butterfly species Encountered.	Tradewinds trail one 735m.	Tradewinds trail two 765m.	Colorado stand 750m.	Grass verge 750m.	East Peak Cloud forest 1000m.	Grass verge 1000m.
Colobura dirce wolcotti	0.14	0.15				
Danaus plexippus						
Dismorphia spio						
Dryas iulia iulia				1.67		
Euphyes singularis						
Pyrgus oileus oileus						
Perichares philetes						
Siproeta s. stelenes						

Table 40. Average number of butterfly species encountered per 10 minutes on the Bisley trail, Big Tree trail, Closed road and El Yunque Peak transects above 915m in the Luquillo Experimental Forest 2002. *Antillea pleops pleops*, *C. nubila*, *C. vitellius* and *W. drury* are not included as they are discussed separately.

Butterfly species Encountered.	Bisley trail 250m.	Big Tree trail 515m.	Closed road 715m.	El Yunque one 915m.	El Yunque two 935m.	El Yunque three 965m.
Colobura dirce wolcotti	0.08	0.37	0.16			
Cycloargus t. woodruffi	0.08					
Danaus plexippus						
Dismorphia spio		1.06	0.06			
Dryas iulia iulia	0.08		0.06			
Euphyes singularis			0.16			
Eurema patens	0.08					
Pyrgus oileus oileus	0.7		0.11			
Perichares philetes			0.55			
Siproeta s. stelenes		0.11	0.06			

Table 41. Average number of butterfly species encountered per 10 minutes on the Tradewinds trail, Colorado stand, grass verge at 750m, East Peak and grass verge at 1000m in the Luquillo Experimental Forest 2002. *Antillea pleops pleops*, *C. nubila*, *C. vitellius* and *W. drury* are not included as they are discussed separately.

Butterfly species Encountered.	Tradewinds trail one 735m.	Tradewinds trail two 765m.	Colorado stand 750m.	Grass verge 750m.	East Peak Cloud forest 1000m.	Grass verge 1000m.
Colobura dirce wolcotti						
Danaus plexippus						
Dismorphia spio						
Dryas iulia iulia	0.002	0.01				
Euphyes singularis						
Pyrgus oileus oileus						
Perichares philetes						
Siproeta s. stelenes						

Table 42. 2001 *C. nubila* data for the Tradewinds trail transects one and two combined.

Date	Number encountered	Transect time minutes	Numbers per 10 minute
6/4/01	107 out	32	33.4
	94 return	35	26.9
8/4/01	32 out	27	11.9
	34 return	22	15.4
11/4/01	89 out	32	27.8
	58 return	32	18.1
13/4/01	56 out	34	16.4
	75 return	32	23.4
15/4/01	22 out	32	14.5
	38 return	38	10
17/4/01	45 out	35	12.9
	56 return	37	15.1

Table 43. 2002 *C. nubila* data for the Tradewinds trail transects one and two combined.

Date	Number encountered	Transect time minutes	Numbers per 10 minute
2/3/02	69 out	46	15
	99 return	45	22
4/3/02	96 out	45	21.3
	63 return	35	18
7/3/02	56 out	37	13.1
	60 return	39	15.4
10/3/02	70 out	40	17.5
	66 return	39	16.9
10/3/02	36 out	48	7.5
	75 return	42	17.9
13/3/02	212 out	47	25.7
	101 return	45	22.4
15/3/02	138 out	44	31.4
	138 return	31	44.5
16/3/02	160 out	43	37.2
	84 return	36	23.3
18/3/02	89 out	32	27.8
	60 return	32	18.8

Table 44. 2003 *C. nubila* data for the Tradewinds trail transects one and two combined. Data was collected on the 11/3, but the transect had to be abandoned due to weather conditions.

Date	Number encountered	Transect time minutes	Numbers per 10 minute
27/2/03	89 out	48	18.5
	45 return	44	10.2
28/3/03	41 out	40	10.3
	68 return	39	17.4
1/3/03	59 out	33	17.9
	80 return	37	21.6
2/3/03	56 out	45	12.4
	84 return	50	16.8
3/3/03	49 out	35	14
	59 return	37	15.9
4/3/03	63 out	37	17
	87 return	37	23.5
5/3/03	65 out	35	18.6
	63 return	32	19.7
6/3/03	55 out	34	16.2
	79 return	30	26.3
7/3/03	93 out	35	26.6
	61 return	27	22.6
8/3/03	62 out	32	19.4
	83 return	32	25.9
15/3/03	42 out	32	13.1
	58 return	25	23.2
17/3/03	46 out	33	13.9
	103 return	35	29.4
19/3/03	78 out	38	20.5
	97 return	37	26.2

Table 45. 2005 *C. nubila* data for the Tradewinds trail transects one and two combined.

Date	Number encountered	Transect time minutes	Numbers per 10 minute
19/3/05	175 out	33	53
	188 return	32	58.8
20/3/05	123 out	35	35.1
	122 return	33	37
21/3/05	97 out	36	26.9
	94 return	30	31.3
22/3/05	45 out	32	14
	95 return	32	29.7
23/3/05	132 out	33	40
	136 return	34	40
24/3/05	116 out	31	37.4
	180 return	32	56.3
25/3/05	119 out	32	37.2
	152 return	33	46.1
26/3/05	147 out	34	43.2
	148 return	34	43.5
27/3/05	165 out	33	50
	149 return	33	45.1
28/3/05	97 out	33	29.4
	155 return	32	48.4
30/3/05	96 out	29	33.2
	144 return	33	43.6
1/4/05	130 out	31	41.9
	171 return	30	57
2/4/05	73 out	32	22.8
	109 return	33	33

Table 46. 2005 *C. nubila* data for the Tradewinds trail transects one and two combined continued.

Date	Number encountered	Transect time minutes	Numbers per 10 minute
4/4/05	91 out	33	27.6
	133 return	35	38
6/4/05	52 out	32	16.25
	160 return	33	48.5

Table 47. 2001 monthly mean maximum amd monthly mean minimum temperatures from the East Peak static climate station and 30 year historical monthly mean maximum and momthly mean minimum temperatures from the climate records for the East Peak static climate station.

	Historical monthly mean maximum temperature (^{0}C)	2001 monthly mean maximum temperature (^{0}C)	Historical monthly mean minimum temperature (^{0}C)	2001 monthly mean minimum temperature (^{0}C)
January	18.9	19.28	14.93	15.17
February	19.1	18.5	14.81	15.1
March	19.81	18.5	14.81	15.1
April	20.35	20.31	15.39	15.4
May	21.08	20.1	16.53	15.3
June	21.45	19.17	17.61	14.29
July	21.69	18.36	17.6	13.67
August	22.03	18.73	17.9	13.37
September	22.49	21.2	17.77	16.45
October	22.18	22.5	17.53	18.22
November	21.06	21.31	16.77	16.86
December	19.66	20.7	15.66	15.71

Additional butterfly species encountered throughout Puerto Rico

Although the overall numbers are low, *A. pleops pleops* is encountered more frequently in 2003 on the El Yunque Peak third transect above 965m than on the lower two transects or on the Tradewinds trail transects. There are no conclusions drawn from this due to the low numbers recorded, but it is an interesting point which shows that conditions along this transect were better for this species during the previous season.

Wallengrenia drury shows an almost constant distribution along the Tradewinds trail transects one and two combined and along El Yunque Peak transects one and three in 2003. As with *A. pleops pleops*, overall numbers were low throughout the walks. It was rarely recorded on El Yunque Peak transect two where the canopy height is higher than transect three and the vegetation structure much denser. The frequency may be higher along El Yunque Peak transect one due to its proximity to the access road where canopy and vegetation conditions are much more open and numbers may reflect the occasional individual flying in from the surrounding road area.

The single specimen of *Adelpha gelania arecosa* Hewston recorded on the Tradewinds three transect in 2003 was noted as a surprise at the time. It was seen for around thirty seconds and was recorded as landing on the tops of trees at around 3m high to bask briefly on three occasions. This species appears to be very mobile and has a noticeably agile flight pattern, almost the oppostite to that of *C. nubila* which flutters in and out of the vegetation along the path system.

It is probably worth underlining the point that *C. nubila* was the most frequently recorded butterfly along most of the transects walked at the various sites in Puerto Rico. That it is still the most recorded butterfly at Cambalache Forest Reserve as well as on El Yunque Peak above 965m is noteworthy.

On the Tradewinds trail in 2005, *W. drury* and *A. pleops pleops* were rarely recorded. Numbers of both species increased along the third transect walked in 2005, but the increases recorded were small. The note made during overcast and intermittent rain shows that during such conditions *C. nubila* activity can be surprisingly high at altitudes between 735m and 765m, *A. pleops pleops* and *W. drury* are however not recorded during such conditions. This backs up the argument for the decline of both *A. pleops pleops* and *W. drury* on the El Yunque Peak transect three above 965m after 2001. In harsh environmental conditions when precipitation levels increase and conditions are overcast for long periods, activity levels of both these species would appear to very minimal.

Cambalache Forest Reserve had the highest species diversity recorded of any of the sites sampled. This is perhaps somewhat surprising in that mid elevation sites are often regarded as being the most species rich for flora and fauna. Palo colorado forests are thought to provide better habitats for species most vulnerable to desiccation. This intermediate ecological zone is put forward as being the the most favourable habitat for invertebrate survival as it has lower wind velocities than the elfin cloud forest and higher rainfall than the tabunuco forest (Richardson et al, 2000).

The number of species recorded at Cambalache varied throughout the years the site was visited. *Heliconius charitonia charitonia* Linneas is the species that is close to that of *C. nubila* in terms of numbers encountered per ten minutes and this species could be used for further research in lowland areas relating to climate variations. It would also be possible to use *C. nubila* at Cambalache for climate data comparison. Numbers encountered are not on the same scale as on El Yunque Peak above 735m, but it is still the most frequent butterfly recorded in this lowland habitat and its frequency is high enough to be used in calculations.

Probably the most noteworthy species recorded at Cambalache Forest Reserve from a visual perspective was *Archaeprepona*, a large butterfly and a powerful flier. At rest with wings closed, it resembles the tree trunks upon which it rests after flight, but in flight you can see the blue colour of the forewings as it flashes past, defending its territory from other butterflies.

During the 2002 Cambalache Forest Reserve field visit, the highest species count of any transect was recorded. Some of the species were encountered only in that visit and some of them, such as *Panoquina Panoquinoides* Skinner are normally found on coastal and beach habitats which would generally be drier than forest regions. Whether this therefore reflects a lower annual precipitation in the Cambalache region for the 2001 period is unclear.

Individual butterfly species encountered on the transects throughout Puerto Rico 2001- 2005

The main reference book used for the following species section was Butterflies of The West Indies and Southern Florida, Miller et al, 1994. This section introduces the additional species positively identified and found along all the transects walked throughout Puerto Rico. Unless the species was widespread, there are notes identifying which transects the species was encountered on and any extra findings of interest.

Colobura dirce Linnaeus- This is the single species in this genus which occurs in the Antilles. It is often seen alighting on the trunks of trees in the shade. This species was most frequently encountered on the Tradewinds trail transects one and two, where it would briskly fly through the tree understorey until alighting on a trunk with its wings closed. It is noted to readily visit traps baited with fruit (Miller et al, 1994), however it was never found in any of the fruit traps set up along the Closed road transect in 2001.

Hamadryas amphichloe Boisduval- Three species of this genus are found in the Antilles, only one however has been recorded on Puerto Rico. Greatest numbers are to be found on Hispanoila and there are no defined habitats noted. In Puerto Rico it is seen most frequently in the dry southwestern areas of the island and in Guanica Biosphere Reserve. This species was encountered in Cambalache Forest Reserve and was not recorded during the visit to Guanica.

Adelpha gelania arecosa Hewitson- This is a subspecies found in Puerto Rico and is classed as larger than the Hispaniolan species with a broader white spotband. It has been noted as frequent in Cambalache Forest Reserve, which is where it was recorded, but has also been seen above 1000m which would indicate that it is very mobile throughout Puerto Rico.

Biblis hyperia Cramer- Noted as occurring from Hispaniola, Puerto Rico, Culebra and some Lesser Antillean islands, it is thought the species originally colonised Puerto Rico by wind bourne specimens from Hispaniola. It was recorded in Cambalache Forest Reserve, where it has previously been noted. It is a very conspicuous species and was recorded with relative frequency when the forest transects were walked at Cambalache.

Siproeta stelenes stelenes Linnaeus- This is a subspecies found on Jamaica, Hispaniola, Puerto Rico, St Kitts and St Croix. Regarded as a typical butterfly of forest edges and shady paths, this butterfly has a slow and gentle flight pattern. Some seasonal variation has been recorded in Cuban species and on Hispaniola *S. stelenes* has been found from sea level to 1700m.

Dryas iulia iulia Fabricus- Recorded in a number of transect locations, this butterfly is easily recognisable by its mixture of brown and orange colouration and gliding flight. This is a Puerto Rican subspecies and is widespread and thought to generally avoid very dry regions. It was found throughout the El Yunque region and also in

Cambalache Forest Reserve, which would generally be classed as a dry habitat. The Puerto Rican subspecies may therefore be more drought tolerant than its close relatives on surrounding islands.

Heliconius charitonia charitonia Linnaeus- Found in a wide range of habitats, this butterfly was encountered in Cambalache Forest Reserve where the abundancy was similar to *C. nubila.* When nectaring it holds the wings outspread and it has a delicate gliding flight. Adult males are known to attend female pupae and this was observed at Cambalache on a freshly emerging specimen. This is a subspecies which has been noted on Mona island, Puerto Rico and some of the Leeward islands. Because of the frequency with which this species was always recorded at Cambalache, there would probably be enough numbers encountered to do some data analysis on, either on the general ecology of the species or with locally collected climate data.

Dismorphia spio Godart- Members of this genus mimic *heliconiinia* spp. and the flight pattern is almost an exact replica. This is another elegant flier, gently gliding through the forest edges, along roads and paths. It is found on both Hispaniola and Puerto Rico where it is classed as local and normally found flying in shade. The peak emergence of this species in Puerto Rico is noted as between January and March. There are three colour morphs and the yellow form is described as being particularly close to *H. charitonia.* It was never found in any number in the El Yunque region and was not recorded above 515m.

Pyrgus oileus oileus Linnaeus- The genus *Pyrgus* contains black and white chequered skippers which are generally found in open land, along roadsides or path edges. During transect walks it was most frequently recorded at the start of the walk, where the vegetation structure was more open and at Cambalache Forest Reserve it was observed on open sections of path edges where there were adjoining fields at the start of the transect or where paths forked off and there was a more open grassy area at the fork. It was mostly seen at rest on vegetation with unfolded wings in sunlight. This is a subspecies and has been recorded in Florida and the Antilles to Montserrat.

Perichares philetes Gmelin- This species is a large brown skipper which was only recorded on the Closed road transect at 715m. It was infrequently recorded, but was fairly conspicuous due to its size and darting flight pattern. The Closed road was chosen as a transect due the more scrubby nature of the vegetation there, with most woody species unable to get a foothold on the thin layer of soil over the tarmac surface. This different vegetation structure would appear therefore to be more favourable to this species. It is found on the islands of Cuba, Hispaniola, Jamaica, Mona island and Puerto Rico.

Danaus plexipus Hubner- A well known species which has been the focus of recent documentaries following its migratory pattern from Canada to California and the pine forests of Mexico. It is regarded as localized on Puerto Rico and has also been recorded on Culebra. It has a slow majectic flight and is an unmistakable butterly when encountered. A very mobile species which has occasionally seen in Europe, presumably blown off course by transatlantic weather systems. Recorded at the start of the Big Tree trail transect at 515m and three individuals were also observed by the roadside at around 200m on the homeward route in the El Yunque forest region where they were egg laying.

Euphyes singularis Herrich- Schaffer- Classed as widespread but uncommon on Puerto Rico, this butterfly was only encountered infrequently on the Closed road transect at 715m. It is found throughout the Greater Antilles. Reported as patrolling forest edges, this was the location it was recorded in, during this project.

Heraclides androgeus Cramer- Found from Mexico to Argentina, in the Caribbean, this species is found on Cuba, Hispaniola and Puerto Rico. Noted as uncommon and a butterfly found in mountainous areas in Cuba, it is widespread on both Hispaniola and Puerto Rico and is found in both mountain and lowland areas. Adults are noted as most frequent from June to August, but fly in numbers throughout the year and is especially found near citrus trees. It was recorded on the Cambalache Forst Reserve transect in 2002.

Panoquina, panoquinoides Skinner- The range of this butterfly is from California to Brazil on the mainland and occurs throughout most of the Greater and Lesser Antilles. Populations are described as small and localised. Normally a butterfly associated with beachside scrub and coastal land. Cambalache Forest Reserve, where this species was recorded in 2002, is near the coast but the terrain and ecology of the surrounding region vary from the coast. It is not known whether it has been recorded at Cambalache before but is certainly found on the Cayman Islands in differing habitats.

Panoquina sylvicola Herrich- Schaffer- The range of this species is from Texas to Argentina and is found throughout the Greater and Lesser Antilles though not on the Bahamas. It flies throughout the year and is found in beach and grassy areas and woodland habitats, but avoids deep shade. It was recorded on the Cambalache Forest Reserve transect in 2002.

Anaea troglodyte Fabricus- Noted as found throughout the Amaricas and on Hispaniola. This butterfly was positively identified at Cambalache Forest Reserve in 2002 and would therefore appear to be the first recording of it on the island. It is widespread and sometimes abundant on Hispaniola where it is found in lowland woods and countryside where there is tree cover. Also known as a fruit feeder, it has also been recorded as being attracted to mud.

Ephyriades arcas philemon Fabricus- This a subspecies which occurs throughout the Antilles with the exception of some of the northern Leeward islands. It is a butterfly of dry lowlands and occurs throughout the year. It was recorded on the Cambalache Forest Reserve transect in 2002.

Archaeoprepona Fruhstorfer- This is the only representative of the species found in the Caribbean region. It was always recorded during the various Cambalache Forest Reserve visits and is restricted to lowland woodland. Along with *D. plexipus*, this is probably the most visually stunning species recorded during the various field visits. It is a large butterfly with a fast powerful flight and would coming flashing past while walking the transect before seeming to disappear. This is a territorial species which flies from tree trunk to tree trunk, covering a reasonable distance as it does so. If the recorder has a keen eye and notes where it lands, it can readily be viewed while resting with closed wings.

Achlyodes mithridates sagra Evans- This is a subspecies which ranges from Hispaniola and Puerto Rico to the Virgin Islands. It is noted as selecting a wide range of habitats and has a low, fast flight. It was recorded on the Cambalache Forest Reserve transect in 2002.

Rhinthon Cubana Herrich-Schaffer- Noted as being infrequently collected throughout its range, this skipper is found in Cuba, Jamaica, Hispaniola and Puerto Rico as well as on mainland Central and South America. It has

only recently been added to the Puerto Rican butterfly list, having been encountered in the LEF and is generally regarded as solitary in Jamaica. This species was infrequently encountered on the Closed road transect at 715m and was also recorded for the first time (D.S. Smith personal comm.) at Cambalache Forest Reserve during the 2002 visit.

Cyclargus thomasi woodruffi W.P. Comstock & Huntington- This is a subspecies found on Mona Island, Culebra island, Puerto Rico, the Virgin Islands and St Kitts. Most common in dry localities and beach areas, this species was recorded along the Bisley trail at 400m in the LEF. This was the lowest of the transects walked in the area and would also be the driest.

Marpesia petreus Cramer- This species is thought to have dispersed into the Lesser Antillean chain via Trinidad and then on to Puerto Rico and Mona Island from which Hispaniola is 64 km away. The other Greater Antillean islands are inhabited by *Marpesia eleuchea*, Hubner. It is described as common and locally abundant and frequents partly sunlit paths. It had already been noted as occurring at Cambalache Forest Reserve and this was where it was recorded in the 2003 visit.

Eurema portoricensis Dewitz- Endemic to Puerto Rico, this butterfly has been observed in many localities throughout the island. It is found from sea level to the peaks at 1000m and was recorded on the Bisley trail at 400m and at Cambalache Forest Reserve in 2002. It has been recorded throughout the year.

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