

ORIGINAL PROJECT PROPOSAL

ANT DIVERSITY ALONG AN AMAZON-ANDES ALTITUDINAL GRADIENT

A key question that has interested ecologists for decades is how species and communities respond to their environment. Understanding this can help ecologists to determine how fluctuations in climate, the presence of invasive organisms or other disturbances may influence natural systems. Recently there has been a resurgence in montane research in an attempt to answer some of these questions (e.g. Lomolino, 2001; Rickart 2001). Mountains have become one of the last refugia for a large, albeit undetermined number of species, and it is essential and timely that ecologists once again take up the challenge of describing and understanding patterns of biological diversity of montane ecosystems (Lomolino, 2001).

Altitudinal gradients provide an ideal set-up to explore how factors such as energy and water availability, and habitat type and heterogeneity affect diversity (Rahbek 1995, Sanders 2002, Botes et al. 2006, Sanders et al. 2007). This project thus aims to investigate changes in the composition of ant assemblages along an altitude gradient in Peru from the lowland Amazon rainforest, through to the highlands, and link ant diversity to various biotic and abiotic habitat measures (including vegetation cover, litter load, productivity, temperature, rainfall).

The altitudinal gradient in the study spans 3431m, ranging from 194m asl in the lowland

Amazon rainforest, up to 3625m asl in the montane grassland. This is most extensive altitudinal gradient ever sampled for ants. Sampling will take place at all 12 sites along the altitudinal gradient in conjunction with an existing carbon-dynamics project run by Prof. Malhi. Identification of samples may be limited to fewer sites at a later stage due to time constraints.

Ants will be sampled using a range of techniques including leaf litter sampling (passive winkler sampling), and pitfall trapping. At each altitude, sampling will take place in four sites spaced at least 300m apart. At each site 10 pitfalls will be set in a grid (2 x 5) with 10m spacing between traps (total of 40 traps per altitudinal band).

Traps will be set for a 48 hour period. In addition, at each site leaf litter will be sampled from ten 1m² quadrats. Litter will be placed in mesh bags within the winker sacks, and suspended for 48 hours. During this time, any live insects work themselves out of the litter and fall into cups containing preservative at the bottom of the bags.

Samples will be returned to the UK where all ant specimens will be identified to species level. Taxonomic assistance will be provided by Dr Kate Parr. A range of analytical techniques will be employed (see also Botes *et al.* 2006).

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Ant diversity along an Amazon-Andes altitudinal gradient

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ABSTRACT

Ants play a significant role in almost all tropical ecosystems due to their near ubiquity and ability to effect

substantial changes to their environment. The distribution of species richness along altitudinal gradients is an enigmatic aspect of the discipline of biogeography with ongoing debate occurring, born of conflicting study results. This study aims to investigate the relationship between altitude and ant species richness along a 2800m elevational gradient situated in Peru, running from lowland Amazon to Andes cloud forest. The results exemplify the mid-domain effect i.e. species richness peaking at a mid-range elevation, in this case Tono at 948m above sea level. This effect is attributed (in this study) to the coincidence of the peak in species richness with a peak in vascular plant species richness recorded by Grytnes and Beaman (2006) along a similar transect. Peak plant species richness and primary productivity frequently occur mid way along elevational gradients due to the preferential conditions particularly the increased amount of rainfall associated with the mid-range.

INTRODUCTION

The mechanisms underlying the distribution and richness of species within and between ecosystems are a

focus of intense scientific interest. Despite the implementation of innumerable projects investigating biodiversity/habitat interactions at sites worldwide; the field necessitates the consideration of such large numbers of influential variables that it is in its relative infancy with regards to accumulated knowledge set against the gulf of discoveries yet to be made.

Aside from scientific curiosity, advancement in our understanding of the patterns dictating species distribution is of great significance in a number of related fields, such as the control of disease in both reservoir and vector, management of invasive species and in ascertaining the probable effects of climate change on biodiversity (Botes *et al* 2006) so that appropriate reactive strategies may be tailored to its preservation.

Of particular interest is the relationship between the latitudinal gradient of the Earth and species diversity. Such an encompassing study area, it would be pertinent to assume, could not be easily assigned

explanatory premises but there exist several hypotheses that have only received sporadic opposition, that attempt to reduce the enormity of the

data collected by their studies to simple hypotheses to

explain the patterns of global species distribution. The most relevant of these to this study is Rapoport's Rule which states, simply that; latitudinal ranges of plants and animals are generally narrower at low than at high latitudes.

This is consistent with the observed increase in biodiversity towards the tropics, away from the poles (Colwell & Lees 2000). In 1991, George Stevens published a paper which drew strong parallels between the effects observed for latitude with those attributed to altitude with the argument that Rapoport's rule could be applied in the vertical plane as well as latitudinal, with the extrapolation that increasing altitude may be considered proportional to increasing latitude; species ranges reduced in low compared to high altitude. Many of the changes in biotic and abiotic factors associated with latitude are also applicable to altitude, for example: temperature, vegetation (diversity, height, canopy cover, species etc), primary productivity, humidity, rainfall, soil characteristics (texture, pH, organic content, geological content etc), diluvian dynamics and light levels, with a subsequent list of subtler variables also

playing a key role in shaping the ecosystems. While atmospheric oxygen partial pressure and cloud density exemplify attributes exclusive to altitudinal change.

This adaptation of the theory was notably applied in the study of ants by Nathan Sanders in 2002 who examined "patterns in ant species richness along elevational gradients in three states in the western US: Colorado, Nevada, and Utah".

Ant species are also the focus of this study, but the site under observation is located in the Peruvian Andes.

The formation of the Andes began during the Jurassic period 200 – 145 million years ago, becoming recognisable as contemporary by the mid Cretaceous period, approximately 105 million years ago, around this time also; phylogenetic analysis suggests that the ant species evolved from a lineage within the vespoid wasps.

From an evolutionary biological point of view, it is interesting to consider the relationship between the continuous variation in habitat represented by the Andes and the multitude of ant species evolved to live specifically in every available ecological niche; as a result, there has been considerable study devoted to species diversity along

environmental gradients but this study represents the largest thus far conducted into the effect of altitude on ant species in South America, altitude being a relatively neglected environmental gradient (Stevens 1992).

ANALYSIS OF LITERATURE

In order to predict the possible outcome of this study and also place the results in context, we must review the contemporary and preceding published literature regarding the effect of altitude upon ant species distribution.

Immediately apparent from first inspection of the literature, it is virtually impossible to find a paper that does not open with the phrase: "Much attention has been paid recently to faunal changes along environmental gradients, in terms of number of species and their relative abundances" (Turner & Broadhead 1974); as is thus universally attested to, this field is large but relatively well studied, despite this however, there is no clear consensus as to the general trends in the distribution of species over altitudinal gradients.

The literature contains a wide range of contradictory examples; several papers have attempted to reconcile the opposing views such as the study published by Rahbek in 1995, in which he compares 97 papers citing 163

examples focusing upon a large number of different study organisms. The breadth of the Rahbek paper gives a useful overview to the idea of species distribution; the consensuses of the paper are as follows:

"The decline in species richness with increasing elevation is widely accepted as a general pattern i.e. because of reduced temperature and consequent decrease in productivity" (Rahbek 1995).

"The analysis of [the] empirical data support the view that species richness declines with elevation, but not the view that this decline is necessarily monotonic" (Rahbek 1995).

This last point is somewhat of an understatement in the face of the diversity of the conclusions reached by the different studies; the following pie chart (Figure 1) depicts the relative proportions of outcomes of 20 relevant studies concerned with insect species richness in response to altitude.

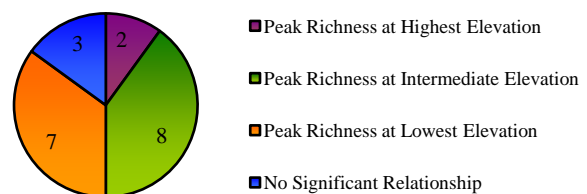


Figure 1 Pie chart showing the relative proportions of different conclusions of a range of published papers

The proceeding passages will describe in detail a representative study from each of the opposing categories that yielded a significant relationship; with the addition of other reference material around this framework.

PEAK RICHNESS AT HIGHEST ELEVATION

The discovery of the largest number of species (and individuals in this case) towards the higher study sites is an outcome that seems contrary to logical expectations, i.e. that species richness is highest at the lowest elevation site as concluded by Rahbek; 10% of the studies examined showed this trend. The study by Turner and Broadhead was of greatest relevance to this project; the experimental organism was Psocoptera, often called the booklouse (or barklouse), described by Turner and Broadhead as “predominantly small arboreal herbivores grazing on the micro-epiphytes growing on leaf and bark surface” with the 1067m study transect situated along the southern slopes of the Blue Mountains in Jamaica.

At each of the 8 study sites, a representative branch was beaten rapidly 25 times over a 1m² collecting tray; at each of the sites also; temperature and relative humidity were recorded over a period of 19 months.

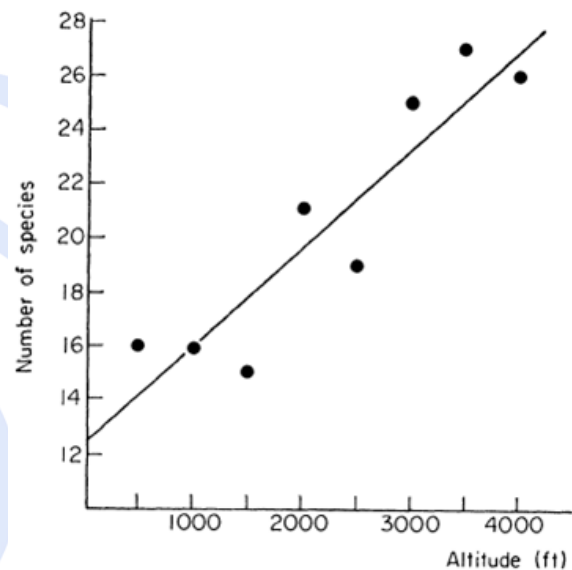


Figure 2 Graph from Turner and Broadhead (1974) of arboreal invertebrate herbivore species richness over altitude from a 4000ft transect in Jamaica

“Over the altitudinal range 500 - 4000 ft there is a linear increase in the number of psocid species with increasing altitude at a mean rate of 3.3 species per 1000 ft (1 species / 100m)” (Turner & Broadhead 1974) (Figure 2).

In their analysis of vegetation, Turner and Broadhead found that “the variety and quantity of micro-epiphytes (algae,

lichens and fungi) on mango leaf and bark surfaces increase with increasing altitude. The diversity of the psocids and the diversity of the microepiphytes on which they feed, as measured by Simpson's diversity index, are significantly correlated." Clearly, epiphyte species distribution is the driving factor behind the generation of this somewhat unexpected relationship with the higher diversity of epiphytes at the upper altitudes providing a greater range of ecological niches for a greater diversity of psocids. Turner and Broadhead attribute the distribution of epiphytes to prolonged human influence: "Since the English occupation of Jamaica in 1665 the original mesophytic forest of the lower slopes of the St Andrew Hills has been largely replaced by horticulturally and agriculturally desirable trees and there has been a progressive encroachment on the montane forests for timber and crop land". It may be argued that this study is not valid due to the disturbed nature of the site but this argument represents the habitual tendency to regard mankind as abstract from the rest of the world; lowland agricultural practices have occurred for thousands of years over immeasurable areas of land across the globe; it is therefore unsurprising that

this has had an impact upon the distribution of species with montane habitats acting as bastions of faunal diversity due to their agricultural inadequacy in a number of examples. This having been said, our study focuses upon the 'natural' distribution of species in relatively undisturbed areas; alongside this focus, however, we must be mindful of the far reaching, often subtle and extending influences of man upon the natural environment.

PEAK RICHNESS AT INTERMEDIATE ELEVATION

A mid elevation peak in species richness was the most commonly observed result of the literature survey; occurring in 8 out of the 20 papers.

In 1973, Janzen conducted a survey investigating the effects of elevation (along with seasonality, vegetation types, time of day and insularity) upon tropical leaf litter insect assemblages in Costa Rica. The following graph (Figure 3) depicts Janzen's elevational findings, these values are for the secondary dry season; this being the most relevant measurement to our study which was conducted during the month of June; the start of the Peruvian dry season.

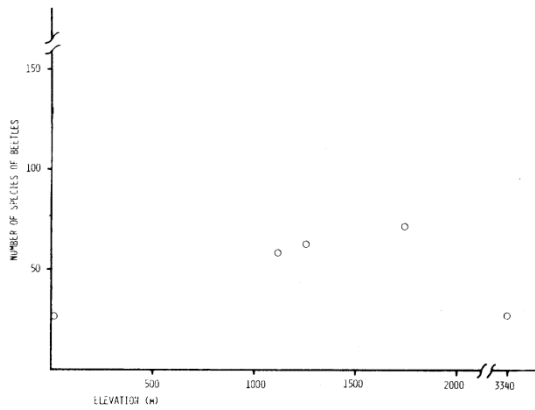


Figure 3 Graph from Janzen (1973) of tropical leaf litter insect species richness over altitude from a 3340m transect in Costa Rica

Species richness peaks at approximately 1750m (48% of the way up the gradient). This phenomenon is known as the mid-domain effect (MDE); a term first coined by Cowell and Lees in 2000 following their work on geographic species distribution, in which a mid range peak is also evident.

Janzen summarises the graph with the following paragraph: “The numbers of insects and species above intermediate elevations show a general decrease, and intermediate elevations appear to have the highest insect density. This is believed due to a higher harvestable productivity from the plant community at intermediate elevations owing to lowered plant maintenance costs on cool nights” (Janzen 1973).

Explanations for this phenomenon generally fall into a simple dichotomy;

referred to as “the ends are bad” and the complementary “the middle is good”. These colloquial terms pervade the literature with the subsequent explanation for the latter included in McCoy:

“Photosynthetic rates and respiratory rates of plants are assumed to be high at low elevations and low at high elevations; and, as a result, the net accumulation of photosynthate is highest at mid-elevations. The “extra” photosynthate provides a larger resource base for herbivorous insects, which allows more species of both herbivorous insects and their dependent carnivores to pack in” (McCoy 1990).

‘The ends are bad’ argument was summarised succinctly by Gagne in 1979, who proposes that higher elevations are limited by climatic severity, as are lower elevations but that the lower limits are subject to the pressure of predation to a greater degree. The reduction in species richness at high elevations, as inferred by Rapoport’s rule, is more easily understood than the reduction at low elevations; the next section (Peak Richness at Lowest Elevation) deals with the hardships associated with higher altitude in more detail.

Human influence is a potential explanation for much of the loss in diversity at lower altitudes, as discussed in the previous section, but this argument is unsatisfactory to explain most of the low diversity observed at low altitudes as most of the studies are conducted at sites deliberately free from anthropogenic influences.

If the relationship between productivity and species diversity is examined exclusively, as it was by Rosenzweig and Abramsky (1993); an interesting relationship emerges; as productivity increases (e.g. with decreasing altitude), species diversity initially increases then declines giving a hump shaped curve (Rosenzweig & Abramsky 1993) (see discussion).

PEAK RICHNESS AT LOWEST ELEVATION

Upon general acceptance of the application of Rapoport's rule to altitudinal gradients, it is pertinent to assume that species richness is inversely proportional to altitude, with peak species richness occurring at the lowest elevation due to protracted species ranges. This outcome was shared by 7 out of the 20 papers reviewed, of these; the 1987 study by Wolda will be explored in detail. Wolda

used light trapping to collect a wide variety of insects; his study is of particular relevance due to the fact that the study site was situated in The Republic of Panama, a country separated from Peru only by Colombia. Wolda's findings are represented in the following graph (Figure 4):

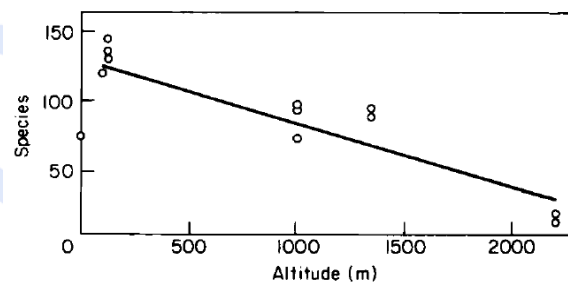


Figure 4 Graph from Wolda (1987) of light-trapped insect species richness over altitude from a 2000m transect in Panama

"In all insect groups tested, the present data show a decrease in species richness with increasing altitude. With the exception of the earwigs and, possibly, the chrysomelid beetles, where the decrease only started after 1000m, the decrease was gradual from the lowlands to 2200m" (Wolda 1987).

There are numerous factors postulated to be influential in the generation of this relationship; the most significant of these

being: reduced habitat area at high altitudes (Hebert 1980), reduced resource diversity (Gilbert 1984), increasingly harsh, variable and unpredictable environments at higher altitudes (Thiery 1982) and reduced primary productivity at high altitudes (Connell & Orias 1964) (Lawton *et al* 1987).

Despite the application of Rapoport's rule to altitudinal gradients combined with the arguments outlined above; the majority of papers reviewed contested the validity of this premise with 8 showing a mid-domain peak and 2 depicting the antithesis (a high elevation peak); with a further 3 showing no significant relationship. The prevalence of this contradictory finding in the preceding research is addressed directly by Wolda who attributes it to flaws in experimental design, inferring that minor inconsistencies in the experimental execution between similar studies are the cause of their different conclusions: "There is no sign of an intermediate level maximum such as is indicated in the literature. The collecting technique as well as the habitats sampled may have something to do with this" (Wolda 1987). Wolda criticises the study by Janzen (used in this report to exemplify a mid-domain peak) along with two other

studies claiming peak species richness at an intermediate elevation: "The data from Costa Rica (Janzen 1973) are confounded by sweep samples having been taken in different seasons. Morón & Terrón (1984) found only one extra species of scarab beetle at 1120m than at 650m. For the light-trapped moths in Papua New Guinea (Hebert, 1980), no data from low altitudes are presented, so it is not clear at what altitude the decrease of species abundance started." But can all of the many examples of mid-domain peaks to be found in the literature really be a result of poor experimental design; as Wolda seems to suggest?

This is unlikely; despite the fact that the study conducted by Wolda which involved extensive experimentation showed an inverse proportionality between altitude and species richness, "mid-elevation peaks are probably the rule rather than the exception" (Sanders 2002) due to the variety and quantity of studies that have yielded this result.

In attempting to summarise the literature, it is clear there exist a range of conflicting views, both in terms of the relationship between altitude and species distribution and in terms of the explanations offered for the same relationships.

One of the major points that the studies do agree upon is the notion that further study is needed into the enigmatic discipline of biogeography.

AIMS OF THE PROJECT

The broad aim of this study is to determine how leaf litter ant assemblages vary along an elevational gradient in the eastern Andes from lowland Amazon to upland cloud forest. The samples were packaged and returned to Oxford (with permission from the relevant Peruvian authorities) where they were identified to species level where possible. The data were then manipulated using the statistical tools outlined in the subsequent section entitled 'Statistical Analyses' and used in the corroboration or contradiction of the following hypothesis:

HYPOTHESIS

Consistent with the majority of the preceding studies; species richness will peak at an intermediate elevation

Increasing range size with increasing altitude is also to be expected in accordance with Stevens' application of Rapoport's rule, but the effects of this phenomenon are unlikely to be detected

in this study due to the inadequate number of sites.

METHODS

Leaf litter ants were collected using Winkler sampling. This passive technique involves the collection of all of the leaf litter to a humic layer depth easily dislodged by hand over a one metre square quadrat (Majer *et al* 1997). The litter is placed into a sieve bag and the smaller elements, including ants, are filtered through by shaking. The siftate is then emptied into a holding bag and is then transported from the field to a suitably dry place that is unlikely to be disturbed or exposed to wind or excessive heat etc. The siftate is decanted into a mesh bag 42 × 24 cm with holes 4 × 3 mm, the mesh bag is suspended within a larger bag which is completely sealed and a vessel containing 50ml of 80% ethanol (King & Porter 2004) (and a label with the sample's number and site) is placed at the bottom, underneath the mesh bag to collect invertebrates that fall out. This arrangement is left for 48 hours, during which time the ants migrate out of the particulate litter and fall into the alcohol. At the end of the period, the alcohol containing the preserved ants (along with other insects and abiotic matter) is

transferred to a 50ml NASCO Whirl-Pak® bag which is sealed for transit. At each site sampling was conducted along six replicate transects spaced 300m apart (where possible), with five 1m² quadrat samples collected every 3m along each transect.

With the aim of reducing proceeding ambiguity, the 5 altitudinal sites will be referred to simply as 'sites', the replicate transects at each site are called 'replicates', while individual quadrat samples will be called 'samples'.

For each of the sites, a description of the main vegetation was recorded; GPS coordinates were taken (which also provided an estimation of altitude) and a crude estimation of gradient was performed.

The sites were chosen because they form part of an altitudinal transect that is the focus of study for a number of research projects as part of a collaboration between Oxford University, The University of Edinburgh, The University of St Andrews, Universidad San Antonio Abad (Cusco, Peru) and Wake Forest University (North Carolina, USA), this means that historical data are available regarding rainfall; a measure impossible to estimate reliably in the time available for this study. Data regarding oxygen partial pressure will

also be provided from an external source (West Respiratory Physiology 2000) as this is also a challenging variable to measure. A number of other sites along the transect were potentially available but time constraints necessitated the utilisation of the minimum number of sites that would allow the data to be represented graphically; the five sites were chosen for their (as far as possible) even spread along the transect combined with the strength of their historical data.

Alongside this historical data; for each transect, the following contemporary environmental variables were recorded as part of this study:

- Percentage Canopy Cover (estimated by eye by viewing the canopy from the forest floor through a 3.5cm diameter tube) – the canopy cover determines the amount of light that reaches the understory which determines the nature of the vegetation (Grundel *et al* 1998).
- pH (universal indicator soil test) – despite the publicised irrelevance of soil pH on ant species exemplified by the 1995 study by Lavelle; this simple measure has far reaching implications with regard to soil chemistry.
- Humidity – ant species are strongly influenced by humidity, tending to favour higher relative humidity: "brood tending

workers of all species tested showed a marked preference (negative hydrokinesis) for saturated atmospheres (near 100 % RH). The responses are presumably due to the broods' higher susceptibility to desiccation (Potts *et al* 1984)".

- Ambient and Soil Temperature – being thermophilic organisms; temperature is potentially the most influential variable in the distribution of ant species, "The combined effects of temperature and rainfall explained 80% of the variation in ant species richness" (Sanders *et al* 2003).
- Rainfall – an important predictor of species distribution due to the wide range of factors it influences, such as vegetation, productivity, humidity and diluvian dynamics.
- Soil Texture and Humic Layer Depth – little is known about the interrelationship between soil mechanics, chemistry and biology and ant species distribution (Andersen & Sparling 2008) but the reliance of many leaf litter ant species upon the soil for food, shelter etc means that this variable is unlikely to be negligible in their distribution.
- Oxygen partial pressure – the decline in oxygen with increasing elevation must be considered in the distribution of species above approximately 1000m as

the subsequent decline may strongly select against certain species (see discussion section for more detail on this point).

For every site, the following biotic and abiotic factors were recorded in terms of presence / absence:

- Moss – bryophytes, along with other epiphytes form a critical part of many ecosystems, particularly with regard to nutrient dynamics (Nadkarni 1984).
- Fern – like mosses, Pteridophyta are present in such quantities all the way along the transect that their potential impact upon the ecosystem cannot be underestimated (Poulsen & Nielsen 1995).
- Large Tree (over 10 cm Diameter at Breast Height (DBH)) – trees represent an important habitat for ant species, providing a transition between canopy and understory (Wilson 1987), it is therefore important to take the proximity of a tree to the sample site.
- Rock – rocks provide a habitat for ants, predators and prey; influencing the distribution of proximal species.
- Dead Wood – Several species of ant (e.g. certain *Chrematogaster* species) live in dead wood as do many prey organisms.
- Other Ground Vegetation – this measure gives an impression of the

three dimensional complexity of the sample site.

- Bare Soil – also gives an impression of complexity, relevant when the ant species under observation inhabit the leaf litter.
- Bamboo – bamboo was found to exhibit a non-homogenous distribution along the transect, being more prolific at the higher elevations. Bamboo can have a strong impact upon ant species assemblages; particularly the species that form symbiotic or commensal relationships. Bamboo is prevalent in areas previously disturbed e.g. by tree fall or the actions of man; relevant when we consider the potential influence of the latter upon the results.

THE STUDY ORGANISM

Since their divergence, the spread and proliferation of ant species, facilitated by the evolution of flowering plants, has been profound, with the differentiation of over 15,000 species, more than 12,000 of which have been described; a figure postulated to represent only a third of total species (Hölldobler & Wilson 1990). The known species are divided into 283 genera, belonging to 21 subfamilies within the family Formicidae (antweb.org 2008). The importance and ubiquity ants to the rainforest ecosystem as a whole cannot

be underestimated, as expounded by E. O. Wilson: “There are more species of ants in a square kilometre of Brazilian forest than all the species of primates in the world”. Ants are highly influential in a wide range of biological processes such as soil perturbation and predation of other insect fauna (Folgarait 1998). The experimental technique utilised in this study (Winkler sampling) is designed to collect ants (and other invertebrates) found in the leaf litter only (see section: ‘Strengths and weaknesses of the experimental and analytical methods used’). All species of ground ants can be classified as allogenic ecosystem engineers – organisms that create or modify their habitat (Jones *et al* 1994). This modification is exemplified by the extent to which leaf-cutter species (e.g. *Atta colombica*) reduce the annual production of tropical forests, up to 17% in some cases (Cherret 1989). Many organisms live in association with ant species; these organisms, known as myrmecophiles, may be symbiotic, commensal or parasitic (Ito & Takaku 1994). “Anthills may create patches of mycorrhizal enrichment (Friese & Allen 1993) which can help the establishment of plants (Allen & Friese 1989) due to the dispersion of fungal spores (McIlveen & Cole 1976)” (Folgarait 1998).

The presence of ant nests and other turbation within the soil changes its physical and chemical properties, potentially resulting in increased drainage, aeration and the incorporation of nutrients by food storage combined with the accumulation of faeces and corpses (Brian 1978).

The physical movement of soil by ants in the construction of their mounds can have a significant impact upon an ecosystem; Paton *et al* recorded one tropical species to have mounded 10 metric tonnes of soil in a single hectare within the space of a single year

In the South American Tropics, the leaf-cutter ants are extremely influential species, described by Alvarado *et al* (1981) as the most important agents of soil modification.

A species of leaf-cutter (*Atta sexdens*) in Brazil excavated 40 tonnes of soil that covered an area of rainforest 100m² (Autori 1947) whilst a chamber, excavated by another species (*Atta capiguara*), was discovered that measured 1.5m in width and 5m in height and contained over 500kg of organic matter (Amante 1964). Leaf-cutter species are also important

as they bypass several of the usual stages involved in the decomposition of

leaves with their active farming of the Lepiotaceae fungus (Speight *et al* 1999) (Lugo *et al* 1973). Various studies have demonstrated an increase in organic phosphorous, nitrogen and potassium (Salem & Hole 1968) also sodium, calcium and magnesium in ant mounds relative to the surrounding soil (Petal *et al* 1992), facilitating the growth of certain species of plant.

Decomposition processes carried out by fungi and amonifying bacteria, are very active and more represented in ant mounds in comparison to adjacent soils (Folgarait 1998). Nests of *Atta colombica* in Panama have been shown to increase by 38 times the fluxes of 13 chemical elements in comparison to surrounding areas of forest due to the greater root activity close to the ant nests (Haines 1978).



Figure 5 Bullet ant *Paraponera clavata* in Tambopata, the bullet ant is the largest ant species in the world (2.5cm) with a sting so painful it has

been likened to being shot. (See photo reference)

As a belle-view for species diversity in general, ants have certain strengths and weaknesses: the ubiquity of leaf litter ants means that passive techniques may be employed in their collection, many samples may be collected and catalogued in a single study due to the compact nature of the species; the importance of ants as a major component of baseline conservation inventories is a strong attractive factor in their study, also; the relatively unexplored nature of the ant species is alluring from the point of view of pure scientific curiosity. This last point is

conversely a major detriment to the use of ants to make inferences about species distribution in general, with a postulated two thirds of species yet to be classified; taxonomic identification is challenging but often easier than working with many other study species, e.g. beetles. Despite the fact that invertebrates comprise the bulk of terrestrial diversity and are critical to the function of many ecosystems, there are further objections to the use of ants as indicator species in general: they are protected by [more charismatic] umbrella species, are too numerous to survey and lack appropriate methods for rapid assessment (Fisher 1999).

THE STUDY SITE

We must consider the global distribution of ant species when deciding on the location of the study site, ants are absent only from Iceland, Greenland and Antarctica (although they are not native to a number of islands) (Brian 1978), the logical choice of site for this study should contain a high number of ants to give a strong representation of their distribution along the altitudinal gradient.

The second highest concentration of ant species occupies the Neotropic ecozone which runs from Florida to Cape Horn including all of South and parts of Central America; it contains over 2162 (known) species, the African continent being home to the greatest number of species at ~2500 (Hölldobler & Wilson 1990). When considering the investigation of the effect of altitude on ant species, there are few places (outside of Africa) more perfect for the study site than the Peruvian Andes, home to a large number of individual ants distributed over steep altitudinal gradients. The study site was situated over a 250km area in the Madre de Dios and Cusco Regions of Peru, the study sites were: Tambopata, Tono, San Pedro, Trocha Union

8 and Wayquecha; representing an altitudinal gradient spanning 2.8 vertical kilometres and starting 200m above sea level at Tambopata.

One of the major strengths of the project is the choice of study site for the altitudinal gradient, the richness of ant species at Tambopata is discussed directly in E. O. Wilson's seminal work *The Diversity of Life*: "At the Tambopata Reserve, Terry Erwin used a bug bomb to collect all the insects from a single leguminous tree in the rain forest. I identified the ants in his sample and found 43 species in 26 genera, approximately equal to the entire ant fauna of the British Isles" (Wilson 1992). Table 1 summarises the attributes of each site

	Tambopata	Tono	San Pedro	Trocha Union 8	Wayquecha
Height Above Sea Level	200m	948m	1490m	1800m	2997m
GPS Coordinates	UTM8581566 19L0470611	UTM8565972 19L0221473	UTM8555996 19L0224992	UTM8553910 19L0222585	UTM8540336 19L0219573
Gradient	0°	0° (slight incline)	5-45° shallow to medium	45°, medium to very steep	0° (slight incline)
Soil	brown colour, leaf litter abundant	low organic matter, black humic layer, yellow / beige clay, clay often on surface	dark colour, lighter soil beneath	more continuous layering, very moist, rocks and roots plentiful, dark soil	dark brown soil, discrete layering
Humic Layer	1-2cm	10cm	2cm	10cm	10-12cm

<p>Vegetation</p>	<p>tall canopy (>50m in places), lianas present, largest single trees, closest resemblance to stereotypical Amazonian forest, discrete canopy arrangement</p> 	<p>tree ferns abundant, fewer than higher elevations, similar arboreal fern coverage to San Pedro, very few bromeliads, structured canopy, largest trees, large bamboo prolific, less moss than higher altitudes, arboreal moss cover similar to San Pedro, less moss on ground dead wood, palms common, high diversity, lianas present.</p> 	<p>structured canopy, taller canopy than at higher altitudes, higher diversity than at higher altitudes, no Waymania, tree ferns prolific, ferns present on ground and trees but not as greater number as higher up, less bamboo, Cecropia common, bigger trees, bromeliads, less moss than Trocha Union 8, thinner understory than Trocha Union 8.</p>	<p>Waymania present tree ferns predominant also, Waymania, Clusia and Alzatea comprise the</p>  <p>Wayquecha but less uniform, bamboo prolific, ferns present upon forest floor and trees, dead bamboo covering forest floor, small number of plans, greater diversity, open canopy with no evident disturbance, moss very prolific,</p>	<p>average humic layer depth 10-12cm, dark brown soil, discrete layering, Vegetation: Waymania predominant tree genus over 60% of plot, Clusia second most predominant genus, Clethra comprises top storey, bromeliads present over 4-5m above the ground, moss ubiquitous over tree trunks, range of other epiphytes, bamboo middle storey, ferns on ground and</p>
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				bottom storey very diverse	trees, leaf litter abundant, ground cover sparse.	
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Table 1 Summary Box of site specific attributes

STATISTICAL ANALYSES

Species accumulation curves were constructed for each site (using Microsoft® Excel 2007); site species richness over altitude graphs were also plotted, along with the number of individual ants found at each site. Linear regression analyses were performed to identify any significant relationships between species richness and each of the relevant environmental variables. A graphical representation was constructed of the statistical significance of: altitude, canopy cover, humidity, ambient temperature, soil temperature,

humic layer depth, rainfall (historical), oxygen (historical), moss, fern, large tree, rock, dead wood, ground plant, bare soil and bamboo.

RESULTS

The following graph (Figure 6) depicts the observed relationship between altitude and species richness, no species were recorded at the highest elevation (Wayquecha 2997m) using Winkler sampling but two individuals of different species were collected manually (obviously these are not included in the results). Consistent with the majority of

the literature, a mid-domain effect is observed with species richness peaking at 948m (Tono). The relationship between altitude and species richness was found to be statistically significant $p=0.03769$.

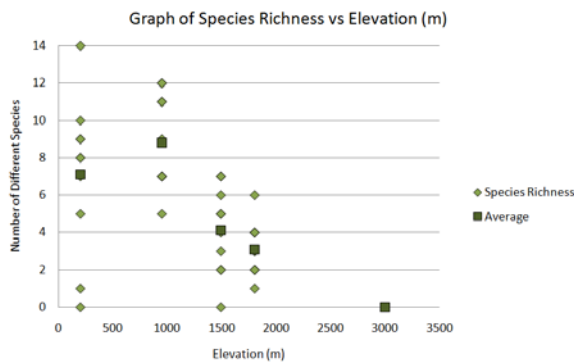


Figure 6 Graph of elevation (x) vs. species richness (y) showing the much publicised mid-domain effect

The relationship between altitude and the number of individual ants collected at each site was also significant ($p=0.03900$) but the number of individuals peaked at the lowest elevation (Tambopata); this is represented graphically in Figure 7.

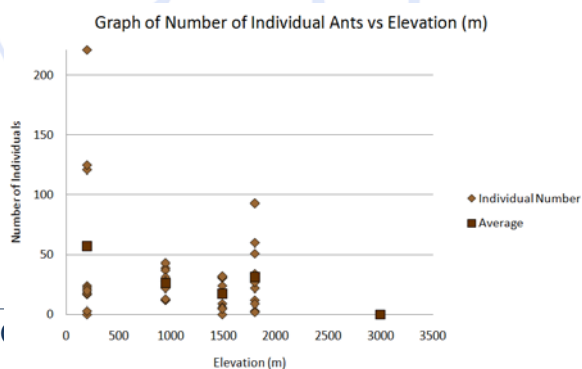


Figure 7 Graph of elevation vs. number of individuals showing the inversely proportional relationship between the number of individuals with increasing elevation

The data for recorded and historical biotic and abiotic factors on species richness and the number of individuals are represented in Figure 8. The F values were calculated using regression analysis performed by Microsoft® Excel 2007. The red line indicates the threshold of statistical significance; only datapoints in excess of this threshold are significant i.e. altitude, canopy cover for species richness, soil temperature for species richness and oxygen partial pressure.

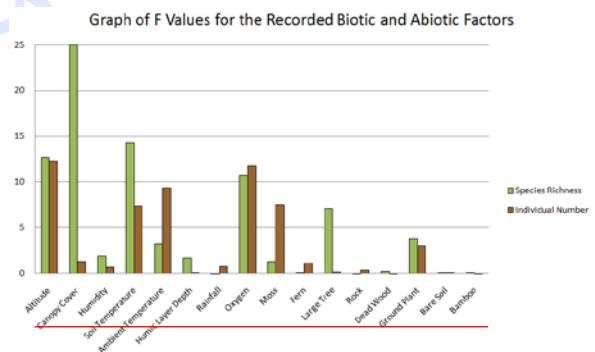


Figure 8 Graph of F values for each biotic and abiotic factor

Microsoft® Excel was also used to construct species accumulation curves for each of the sites, represented in Figure 9.

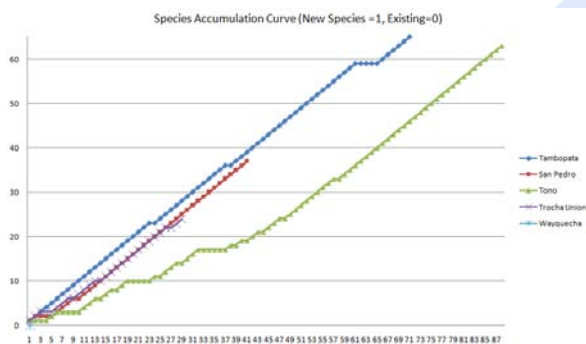


Figure 9 Species accumulation curve

Each of these graphs is represented full-page as appendices 2 to 5 inclusive.

DISCUSSION

After the collection and analysis of a suitably large dataset, the conclusions of this report corroborate the hypothesis. Species richness peaks at Tono, 948m above sea level with 24% more species than Tambopata (lowest elevation 200m) the site with the next highest diversity. There is a rough linear decrease in species richness at sites above Tono (San Pedro, Trocha Union 8 and Wayquecha respectively). The largest number of individual ants was found at the lowest elevation with 182% more species than Trocha Union (1800m), apart from the result for Trocha Union 8 there is a rough exponential decrease in the number of ants as altitude increases. The reasons for the MDE (mid-domain effect) given in the literature review include climatic severity at the extremes of altitude (Gagne 1979), higher predation rates at lower elevations (McCoy 1990), human disturbance at lower elevations (Turner & Broadhead 1974), primary productivity peaking at

the mid altitudinal range (Rosenzweig & Abramsky 1993) (Janzen 1973) and also, as claimed by Wolda (1987), inadequate sampling efforts. The only statistically significant climatic variables are soil temperature (on species richness) and oxygen partial pressure (on species richness and individual number). Soil temperature exhibits a linear decline with altitude from 21.6°C in Tambopata to 10.6°C in Wayquecha, as previously mentioned, temperature is an important predictor of species distribution, it is also little surprise that soil temperature is more significant than ambient temperature in the distribution of leaf-litter species. It is probable that the decline in temperature is largely responsible for the decline in species richness at elevations above 948m, or possibly higher, as a more intensive sampling effort may reveal. This does not account for the reduction in richness at Tambopata relative to Tono with Tambopata experiencing an average temperature 12% higher than Tono during the experimental period; clearly another factor is affecting the distribution. The explanation posed by Rosenzweig & Abramsky (1993) of a mid elevation peak in productivity carries little credence in the face of the data generated by *this* study showing

Tambopata (the lowest elevation) to facilitate the subsistence of the greatest number of individual leaf litter ants, a measure proportional to primary productivity in accordance with elementary energetic ecosystem dynamics. By this same logic, predation cannot be held culpable for reduced richness at Tambopata, as suggested by McCoy (1990) as pressures from predation would not reduce the number of species independent from the number of individuals. While predation may well reduce the number of individuals in an area, the selective evolutionary pressure it exerts upon a population may cause a response whereby the species differentiate to negate the impact of the predation so increased predation at Tambopata may act as a driver of increased richness rather than a constrictor.

Anthropogenic disturbance is also unlikely as an explanation for the mid domain effect, as postulated by Turner & Broadhead (1974) in the explanation of their upper elevation peak, Tambopata has been a protected nature reserve since January 26th 1990. While even very low impact human presence may have an effect upon the presence of larger fauna; an extensive change in

land use, such as the conversion of rainforest to farmland, would be required to significantly reduce the number of ants in an area. Wolda's argument of a mid range peak in species richness being attributable to an inadequate sampling effort is also unlikely as the MDE is the most frequent outcome of experiments of this type conducted in undisturbed areas and is regarded by the majority of the scientific community as the rule rather than the exception; a view corroborated by this study. Why then should the lowest elevation accommodate a higher number of individual ants of fewer species than the next lowest elevation in this study? In attempting to answer this question, it is important to bear in mind the last point made; that scientific studies are ubiquitously subject to inconsistencies of perspective. The sampling methodology of this study is not flawless (see subsequent section) and may have selected for or against certain species, for example; from observation, there seemed to be a greater number of larger species of ant at Tambopata than higher elevations, Winkler sampling may be slightly more inclined towards the collection of smaller species and thus may have underrepresented the number of species at Tambopata. However,

sampling inconsistency alone cannot account for the substantial discrepancy in species richness at Tambopata compared to Tono particularly when the mid-domain effect is corroborated by the majority of the other studies.

As previously alluded to, primary productivity exhibits a strong intermediate peak (Rosenzweig & Abramsky 1993), although this seems inconsistent with the results of this study (peak individual number at the lowest elevation); it is important to consider the effect of plant species richness distribution with altitude; potentially the most influential driving factor behind the distribution of all invertebrate species richness. Since the ecosystem flora forms a large part of the scenery upon, around and within which the tableau of invertebrate subsistence unfolds, a strong correlation between ant species richness and floral species richness is, therefore, to be expected. A greater variety of plants would naturally result in the evolution of a greater variety of ants to fill the whole range of ecological niches e.g. the 24 different species of the *Acromyrmex*, leaf-cutter, genus evolved to harvest different species of plant. But where then does floral species richness peak? The answer comes from a paper published by Grytnes and

Beaman (2006) investigating the effect of elevation on vascular plant species richness distribution at Mount Kinabalu, Borneo, a tropical forest gradient running from 300 to 3900m; they claim: "Total species richness has a humped relationship with elevation, and a maximum species richness in the interval between 900 and 1200m." This conclusion fits precisely with the results of this study i.e. that species richness peaks at 948m (Tono); a pertinent statement considering the similarity of the two study sites i.e. tropical, roughly equatorial virgin rainforests of similar altitude. However, Grytnes and Beaman are unsure of the theory behind their trend: "Relating this pattern directly to explanatory factors is difficult". O'Brien (1993) describes the relationship between climate and photosynthesis as first order and also that "climate accounts for 77.8% of the variation" of woody plant species richness over latitude and altitude. O'Brien claims that greatest species richness is found "where the amount and duration of energy is maximised, and decreases as the amount or duration of energy moves above or below optimal conditions or as moisture decreases". These extensive quotes from O'Brien are included in this report to preserve the wording used as

there are some important points raised. Since the energy required for photosynthesis comes from sunlight, maximum useable energy is likely to come further up the valley (depending upon its gradient), at higher elevations still the increased light levels cannot facilitate photosynthesis further due to the constrictive nature of a number of factors such as reduced temperature and even reduced CO₂ partial pressure. As well as a decline in energy; O'Brien claims that levels above the optimum will result in reduced productivity and, therefore, reduced species richness. This may be the case at Tambopata; that the plant species must close their stomata during the heat of the day to preserve water while the cooler; and often better illuminated valley sides can continue photosynthesising throughout the day. The last point O'Brien makes is to postulate that a decline in moisture acts as a constrictor of species richness, this idea also fits with the results generated by this study as Tono not only experiences a far greater amount of rainfall than Tambopata (7950mm y⁻¹ compared to 2400mm y⁻¹) but it was also more humid on average during the study period (98% compared to 94%). This discrepancy is likely to be due to the increased amounts of precipitation

experienced by mountainous regions compared to those nearer sea level. As air masses are forced over rising terrain, the clouds cool adiabatically, since cooler air cannot carry as much moisture as warm air, resulting in precipitation, this effect is called orographic lift (Whiteman 2000).

Furthermore, the mid-elevation peak in primary productivity observed by Rosenzweig and Abramsky is also consistent with the above postulations.

It is evident from the F value graph that the other parameter statistically significant in the determination of ant species richness distribution (apart from altitude itself) is oxygen partial pressure. The data used to investigate the relationship came from 'Respiratory Physiology' (West 2000); a general source could be used in this case as the geographic oxygen partial pressure changes in air can be confidently regarded as negligible compared to the variation with altitude. Ants do not possess lungs; they respire by passive gas exchange with the air via simple openings in the exoskeleton called spiracles which lead to tracheae then tracheoles. As a result of this morphology; ant size is governed strongly by the surface to volume area ratio; placing a theoretical limit upon the

maximum size of an ant and also, more subtly, the surface area to volume ratio dictates the altitudinal ranges of species based on their tolerance of hypoxic conditions since oxygen partial pressure decreases exponentially with elevation. The two species manually found at Wayquecha (highest elevation, 2997m) were small in size; although this cannot form the basis of a strong conclusion, it does support the postulation of oxygen as a limiting factor in the distribution of ant species richness.

The remaining significant factor that influences the distribution of species richness is canopy cover, the reason for the strength of the significance of canopy cover ($p=0.015$) becomes apparent once plotted alongside species richness (see Appendix 1). This relationship has been documented several times prior to this study, following their research into leaf litter ant species on coffee plantations in Mexico; Armbrrecht and Perfecto (2003) concluded that "Canopy cover was the most important habitat variable causing differences in ant species richness". Species richness is directly proportional to canopy cover which is in turn roughly proportional to primary productivity. It is pertinent to assume that canopy cover is also proportional to the amount of leaf litter.

The fact that altitude is statistically significant in the determination of the distribution of species richness and number is unsurprising as it represents the cumulative effect of all the contributory factors, as summarised by Lomolino (2001): “elevational gradients in species diversity result from a combination of ecological processes, rather than the presumed independent effects of one overriding force.”

The data were also used to construct species accumulation curves (Figure 9) for each of the sites in order to gain an estimation of the sampling effort relative to the extrapolated total number of ant species in the ecosystem. Unsurprisingly the curves reveal the predicted presence of a great deal more species than those collected and identified; the curves for all the sites have an average gradient of 0.86, even with the thorough sampling effort exercised in this study, a much greater effort indeed would be required to gain even a rudimentary idea of the overall ant species richness of the Amazon.

STRENGTHS AND WEAKNESSES OF THE EXPERIMENTAL AND ANALYTICAL METHODS USED

THE EXPERIMENTAL METHOD

Due to logistical and time constraints, there was only time for a single collection method; Winkler sampling (see methods section). As with any scientific procedure, there are relative inconsistencies and points where it may be improved, this technique is no exception. There are a number of weaknesses associated with Winkler sampling, each of which places caveats upon the conclusions drawn, for example, we cannot claim to draw any inferences regarding ant diversity in general as we have only sampled leaf litter species. Independent from this conscious selection i.e. the choosing of Winkler sampling in the knowledge that it only collects leaf litter species are a very large number of subtle mechanisms by which Winkler sampling does not result in the collection of all ant species in the proportions in which they exist in their habitat. The technique is selective for smaller species due to the gauge of the mesh inner bag; despite the potential of all but the largest few species to physically fit through the mesh, they are less probable to exert the effort involved, a higher proportion of smaller species will therefore be expected; this is corroborated by a 2001 study designed

to test Winkler sampling against pitfall trapping: "Winkler sampling was found to catch greater numbers of smaller ants than pitfall trapping, whereas pitfall trapping caught more larger ants" (Parr & Chown 2001)(this selectivity is unlikely to affect the results significantly as most litter dwelling ants are small (Parr personal communication)). Winkler sampling may also be selective for species better adapted for movement through soil as these are also probabilistically more likely to emerge from the inner mesh bag within the 48 hour period; exaggerating the proportion of soil-dwelling species relative to those less adapted to a hypogean existence. More sedentary species may also be selected for; as the disturbance caused by the collection of leaf litter may result in the radiation of more mobile or more perceptive species away from the study site. Highly gregarious ant species, similar to highly evident species, are selected against due to the fact that the experimenter is unlikely to collect leaf litter obviously crawling with ants (e.g. *Eciton burchelli*; one of the New World army ants; common in Tambopata). Another weakness of Winkler sampling is the intra-bag predation that may occur between ant species or predation of ants by other invertebrates inadvertently

collected during the 48 hour period, however, this weakness is unlikely to pose any significant detriment to the validity of the results.

The use of Winkler sampling alone represents a major flaw in the experimental method; as highlighted by the study conducted in the Amazon by Majer and Delabie: "A comparison of the litter and soil ant fauna have shown that a combination of pitfalls, litter sifting [(Winkler)], baiting and hand sorting increase the efficiency of species captures in comparison to any single method by itself" (Majer & Delabie, 1994). This flaw was unavoidable due both logistical and time constraints; the carrying of the litter traps and spade into the jungle along with the Winkler sampling equipment combined with the tight timescale for sampling at each site proved impractical.

A further weakness of the experimental design lies with the potential influence over the results held by environmental factors affecting each site in the short period of time spent sampling; for example: it rained at both Trocha Union 8 and San Pedro and Tono experienced a significant drop in temperature from the average 22.1°C to 18°C during sampling; due to a meteorological phenomenon known locally as a friajé.

Despite the many apparent flaws of the technique; “the use of Winkler [sampling] is highly effective for sampling leaf litter arthropods, particularly ants” (Fernández 2002). The weaknesses of Winkler sampling are offset by the comparability it affords with other studies that have used the same technique. The weakness of using Winkler sampling alone may not affect the strength of the conclusions to a degree of ‘unpublishability’; many studies have been published where Winkler sampling represented the sole collection method; a study conducted by Fisher in the tropical forests of Madagascar found Winkler sampling to be more efficacious than pitfall trapping: “The efficiencies of pitfall and Winkler sifting methods to collect ants foraging or nesting in the leaf litter were not equal. It was more cost effective to use Winklers alone in the rain forest of eastern Madagascar. Pitfall traps were redundant; they collected fewer individuals of the same species sampled.” (Fisher 1999).

The pioneering nature of the study is also a great strength; despite the aforementioned richness combined with the changes in elevation, there has never been a study of this magnitude conducted into the effect of altitude upon

ant species on the continent of South America.

The number of sites is optimal; being the minimum number of data points for the creation of a graph and also the maximum number possible within the time available.

THE ANALYTICAL METHOD

The statistical analyses were robust and appropriate with the novel F value graph acting as a useful comparison of the significance of each of the explanatory variables. The only potential criticism of the statistics is the scope of the analyses; if more time was available then, for example; Sørensen’s similarity index could have been employed to gain a measure of beta diversity. Limited time was available for the analysis of the results due to unforeseen setbacks in the returning of the samples to the UK meaning that identification could not commence until late into the period allocated for analysis, the next section addresses the activities hypothetical extra time and greater resources would be used to facilitate.

IF GREATER RESOURCES WERE AVAILABLE

If more time were available then sample sizes would have been increased to

reduce the influence of anomalous outliers on the conclusions drawn. The number of sites along the transect would also be increased to give a more accurate representation of species turnover in relation to altitude; indeed a frequent enough sampling protocol may even reveal the constriction in range sizes associated with Rapoport's rule expected to occur with decreasing altitude. These extra replicates would be concentrated around Tono with the aim of ascertaining both the true peak in species richness (it is unlikely that the peak coincided with Tono study site). Extra replicates would also be used to confirm the altitude at which the methodology yields no specimens, to fill the 1200m gap between Trocha Union 8 and Wayquecha. If the project were to be repeated with greater time available then sampling effort may be included as a major consideration to increase the comparability of the results with other studies.

FUTURE PERSPECTIVE

An interesting aspect that may be investigated in the future is the idea of the relationship between the evolutionary lineages of the different species and their position along the elevational

gradient with the aim of inferring evolutionary direction along the gradient.

CONCLUSIONS

Strong parallels exist between the long-studied latitudinal distribution of species richness and the distribution over altitudinal gradients. One of the explanatory premises of latitudinal distribution, Rapoport's rule (range size constriction with increasing latitude), has been demonstrated to be applicable to altitude in some instances (Turner and Broadhead 1974). The vast range of contributory factors make the relationship between species richness and altitude challenging to study comprehensively; as a result, many of the relevant studies have yielded contradictory results. A trichotomy exists with the existence of trends showing a linear increase in species richness with altitude, a linear decline and, most commonly, a mid-altitudinal peak (mid-domain effect) along with a number of studies showing no significant relationship. Despite the general acceptance of the mid-domain effect as the rule rather than the exception, sources conflict again in their postulated explanations of the phenomenon. Theories include: differential predation rates, anthropogenic influence, climatic

extremes and primary productivity distribution.

This study relied on the use of Winkler sampling in the collection of leaf litter species at 5 replicate 1m² sites spaced 3m apart repeated at sites along a 2800m altitudinal transect running from Amazon basin habitat to the Peruvian Andes. Limited amounts of historical data regarding rainfall were available from previous and ongoing research into this transect.

The collection protocol yielded 1323 individual ants of 172 distinct species. The results show a clear mid-domain effect with ant species richness peaking at Tono (948m). The highest number of individual ants was found at the lowest elevation (Tambopata). Regression analyses revealed a significant relationship for both individual number and species richness for altitude (and oxygen partial pressure); this indicates that the range of biotic and abiotic factors, the cumulative effect of which is represented by the parameter of altitude itself.

The strong positive correlation between canopy cover and species richness observed in this study is believed to be due to the increased amounts of leaf litter generated by the thicker canopy cover.

Species accumulation curves projected a steep increase in the accumulation of species beyond the range of this study; this was expected due to the acknowledged biodiversity of Amazonia.

Several of the explanations of previously observed mid-domain effects are inadequate in the understanding of the reasons behind the trend in this study e.g. human disturbance of the lower elevation. It would be pertinent to assume, before observation of the previous literature, that the lowest elevation would yield the highest species richness (due to the occurrence of the highest temperatures etc). The observed peak in species richness was found to be consistent with the findings of Grytnes and Beaman (2006) whose study showed a peak in vascular plant species richness between 900 and 1200m on a suitably similar transect. The relationship between floral and faunal species richness is strong enough to largely explain the mid-domain effect trend.

The methodology and analyses can be confidently regarded as effective despite a number of minor inconsistencies and constrictions.

SUMMARY

This ambitious study has been successful, complying with and corroborating the previously published literature. Upon its eventual publication, it is likely that this study will serve as a useful resource for similar research into this enigmatic and fascinating field.

FURTHER INFORMATION

Should an electronic copy of this report or any of the data tables or graphs be

required, they may be downloaded from: www.darwin.nexo.com/ants

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PHOTO REFERENCE

Figure 5 Bullet ant

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All other photos taken by myself

COMPARISON OF ORIGINAL PROJECT TIME PLAN COMPARED TO ACTUAL
TIME PLAN

Date Planned Event

4th June Flight from Heathrow to Lima

5th Flight from Lima to Cusco

6th Flight from Cusco to Puerto Maldonado, Sample Tambopata - Transects 1-3
and Record Environmental Data

7th Leave Winkler Bags to Hang

- 8th Sample Tambopata - Transects 4-5 and Record Environmental Data
- 9th Leave Winkler Bags to Hang, Flight from Puerto Maldonado to Cusco, Get provisions
- 10th Drive from Cusco to Wayquecha, Sample Wayquecha - Transects 1-3 and Record Environmental Data
- 11th Leave Winkler Bags to Hang
- 12th Sample Wayquecha - Transects 4-6 and Record Environmental Data
- 13th Leave Winkler Bags to Hang
- 14th Contingency
- 15th Drive from Wayquecha to Trocha, Sample Trocha Union 8 - Transects 1-3, Record Environmental Data
- 16th Leave Winkler Bags to Hang
- 17th Sample Trocha Union 8 - Transects 4-6 and Record Environmental Data
- 18th Leave Winkler Bags to Hang
- 19th Drive from Trocha Union 8 to San Pedro
- 20th Sample San Pedro - Transects 1-3 and Record Environmental Data
- 21st Leave Winkler Bags to Hang
- 22nd Sample San Pedro - Transects 4-6 and Record Environmental Data
- 23rd Leave Winkler Bags to Hang
- 24th Drive from San Pedro to Patria, Stay Overnight in Patria
- 25th Drive from Patria to Tono and Hike to Site, Sample Tono - Transects 1-3 and Record Environmental Data
- 26th Leave Winkler Bags to Hang
- 27th Sample Tono - Transects 4-6 and Record Environmental Data
- 28th Leave Winkler Bags to Hang
- 29th Drive from Tono to Patria
- 30th Drive from Patria to Cusco
- 1st July Contingency
- 2nd Contingency
- 3rd Flight from Cusco to Lima, Flight from Lima to Heathrow
- 4th Arrive back in the UK
- 5th Sort and identify all samples until July 26th
- 27th Compile data and complete analyses of results until July 31st

1st August Write report until deadline

13th Dissertation deadline



Dr

KATE PARR

Date Actual Event

This Colour Highlights Deviation from Plan

4th June Flight from Heathrow to Lima

5th Flight from Lima to Cusco

6th Flight from Cusco to Puerto Maldonado, Sample Tambopata - Transects 1-3 and Record Environmental Data

7th Leave Winkler Bags to Hang

8th Sample Tambopata - Transects 4-5 and Record Environmental Data

9th Leave Winkler Bags to Hang, Flight from Puerto Maldonado to Cusco, Get provisions

10th Drive from Cusco to Wayquecha, Sample Wayquecha - Transects 1-3 and Record Environmental Data

11th Leave Winkler Bags to Hang

12th Sample Wayquecha - Transects 4-6 and Record Environmental Data

13th Leave Winkler Bags to Hang

14th Contingency

15th *Sampling Impossible Due to Rain*

16th *Sampling Impossible Due to Rain*

17th Sample Trocha Union 8 - Transects 4-6 and Record Environmental Data

18th Leave Winkler Bags to Hang

19th Drive from Trocha Union 8 to San Pedro

20th Sample San Pedro - Transects 1-3 and Record Environmental Data

21st Leave Winkler Bags to Hang

22nd *Sampling Impossible Due to Rain*

23rd *Sampling Impossible Due to Rain*

24th Drive from San Pedro to Patria, Stay Overnight in Patria

- 25th Drive from Patria to Tono and Hike to Site, Sample Tono - Transects 1-3 and Record Environmental Data
- 26th Leave Winkler Bags to Hang
- 27th Sample Tono - Transects 4-6 and Record Environmental Data then Drive from Tono to Cusco
- 28th Hang Winkler Bags in Cusco
- 29th Contingency
- 30th Contingency
- 1st July Contingency
- 2nd Contingency
- 3rd Flight from Cusco to Lima, Flight from Lima to Heathrow
- 4th Arrive back in the UK
- 5th Waited for ants to be returned by third party due to the absence of the export permit at time of return trip
- 25th Received samples from all sites apart from Tambopata, began identification
- 15th Received Tambopata samples from fourth party returning from Peru, ID completed August 19th
- 20th Analysis and write up
- 27th Deadline, extended due to extenuating circumstances regarding the absence of the export permit being the fault of a third party, new deadline Sunday August 31st but Exam Schools closed over the weekend



Dr
KATE PARR

Considering the logistical complexity of the project, there are many areas that may be regarded as successful. However, heavy rain prevented the collection of replicate samples from Tono and San Pedro as any attempt at collection in these conditions would have yielded highly spurious results because of the extensive degree to which precipitation affects invertebrate species i.e. many individuals would seek shelter, thereby biasing the results against their species. The main problems came with the news that we would be unable to bring the samples back to the UK ourselves as the export permits were not ready, despite our application for them months in advance

upon the advice of our Peruvian contact. A two week deadline extension was immediately sort upon my return and granted. But the setback meant that identification was not completed until 24 days after the date originally planned leaving approximately one week for analysis and write up of the project, for which 17 days were originally allocated even with the extension of time.

THE JOURNAL OF BIOLOGICAL CHEMISTRY INSTRUCTIONS FOR AUTHORS

The following is a compiled list of the requirements of a journal article submitted for publication in The Journal of Biological Chemistry, a format chosen because it has been used successfully in a previous study by myself and does not differ vastly from the format of journals that specialise in ecology and biogeography. These guidelines were adhered to in the creation of this report. Instructions that contravene specific course instructions (length, referencing style etc) are omitted.

- ☑ All submitted manuscripts should contain original research not previously published and not under consideration for publication elsewhere
- ☑ Papers must be written in English
- ☑ Prepare the text in Microsoft Word
- ☑ **In preparing the Word text document create a format that produces a manuscript that has an appearance similar to that of a published paper**
- ☑ All of the text should be single spaced with one-inch margins on the left and right sides
- ☑ Once the text of the manuscript is completed in Word, convert the Summary through Discussion sections from a single column format to double column format
- ☑ Title sections should remain in single column format
- ☑ Prepare figures at publication quality resolution, using only applications capable of generating high resolution TIFF or EPS files
- ☑ Number each figure
- ☑ Formatted for 8.5 x 11 inch paper
- ☑ Single spacing throughout
- ☑ Title – as short and informative as possible, should not contain non-standard acronyms or abbreviations and should not exceed two printed lines

