Economies of scale, social immunity, and host-parasite interactions in a social spider system

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the degree of Doctor of Philosophy

in Zoology

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Abstract
This thesis work addresses Anderson and May’s four main postulates of parasitism: (i) parasites are nutritionally dependent on the host, (ii) parasites cause the harm to their host, (iii) the host can evade parasites through immunity, and (iv) parasites must transmit (i.e., disperse) between hosts. To a parasite or pathogen, a host provides a suitable “habitat” through which they can move to complete various life stages, mirroring dispersal in metacommunities. Typically, host-parasite theory considers a single host-single parasite system. In reality, a host can potentially house multiple species, and parasites may be able to use multiple species of host, resulting in a metacommunity that occurs at multiple scales. My research aims to expand Anderson and May’s postulates in the context of a social host using modern metacommunity concepts. Using a community of kleptoparasitic invaders found in colonies of social and subsocial spiders that steal prey resources from their host, my PhD research is at the intersection of metacommunity ecology and host-parasite theory. First, we quantified energetic surplus in social spider colonies as a function of colony size, which may impose nutritional limits on parasitism. Next, we tested whether hosts engage in social immunity to limit kleptoparasite burden along an elevational gradient. Finally, we used DNA sequencing and laboratory experiments to quantify dispersal ability of kleptoparasites and harm to their host. We found that intermediately sized colonies have the greatest energetic surplus, but that kleptoparasite density is greatest in small colonies and in environments with greater productivity. Additionally, using genetic markers, we found that kleptoparasites can freely move between host colonies, while hosts are more limited in their dispersal abilities. Finally, we found that kleptoparasitism negatively affects host body condition, such that immature individuals may grow more slowly in parasitized colonies. This thesis has combined classic host-parasite theory with a modern metacommunity framework to demonstrate
Anderson and May’s four postulates of parasitism in action across multiple levels of organization within a social system.
Lay Summary
This thesis explores the interactions between social spider hosts and their kleptoparasites, other spiders that reside within the host colony and steal resources. First, we found that social spider colonies experience an energetically-driven optimal colony size. Next, we found that parasite burdens are strongly tied to elevation, where colonies at lower elevations experience a greater burden. Consequently, we likewise observed that social spiders use social immunity behaviours to deter parasitism, and that colonies in locales with higher parasite burden exhibit more anti-parasite behaviours. Using genetic markers, we found that kleptoparasites are able to move freely between colonies, suggesting their own fitness is decoupled from that of their host. Finally, we found that kleptoparasite presence has a negative effect on the growth rate of immature individuals, which may have downstream effects on a colony’s population trajectories.
Preface
A version of Chapter 2 has been published as:
Straus, S., González, A. L., Matthews, P., & Avilés, L. (2022). Economies of scale shape energetics of solitary and group-living spiders and their webs. Journal of Animal Ecology, 91, 255–265. I (SS), ALG and LA conceived the study. SS and ALG collected the data, with assistance from PM and undergraduate student Emily Dorey. SS and ALG analyzed the data. SS wrote the first draft, and all authors contributed to subsequent drafts.

A version of Chapter 3 has been published as:
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Chapter 4
I conceived of this study under the guidance of Leticia Avilés. I collected the specimens and data, with the help of undergraduate student Madeleine Ankenman, and I extracted DNA and prepared sequencing libraries. Sequencing was carried out by Génome Québec. I conducted the bioinformatics analysis and wrote the manuscript under the supervision of Leticia Avilés.

Chapter 5
I conceived of this study under the guidance of Leticia Avilés. I set up the laboratory experiment and collected the data, with the help of undergraduate student Madeleine Ankenman. I analyzed the data and wrote the manuscript under the supervision of Leticia Avilés.
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List of Symbols

Δ - denotes change over time
List of Abbreviations

3D - three-dimensional

A.s.l. - above sea level

BCI - body condition index

CI - confidence interval

mm - millimeter(s)
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Dedication

I dedicate this thesis to my parents, who are my best friends and biggest cheerleaders.
Chapter 1: Introduction

1.1 General Background

Anderson and May proposed four main factors influencing disease behaviour in host-parasite systems (1979). (i) The parasite is nutritionally dependent on the host, such that the host serves as the parasite’s habitat; (ii) the amount of harm to the host caused by the parasite; (iii) the host’s ability to evade parasitism via an immune response, and (iv) the parasite’s need to transmit between hosts. The harm caused to the host (i.e., “virulence”) tends to evolve towards some intermediate level (Lively, 2006). If a parasite is too virulent, it may fail to transmit by killing the host before an opportunity for transmission has occurred. Similarly, parasites that exhibit too little virulence would be outcompeted within the host by more virulent strains that are better at exploiting the host resources. Virulence is also tightly linked to transmission. If parasites are transmitted vertically, e.g., from parent to offspring, then harming the host would reduce the parasite’s own fitness (Herre, 1993). On the other hand, horizontally transmitted parasites can have high virulence without reducing their own fitness (Ebert & Herre, 1996).

Parasitism can occur at multiple levels of organization, e.g., at individual or group-levels, particularly in social organisms that build physical nest structures (Witek et al., 2014). Parasites may then be able to disperse between groups, mirroring transmission in individual-level parasites. For both individual and group-level parasites, the ability to manage parasite loads depends, in part, on the host’s ability to distinguish self from non-self (Cremer & Sixt, 2009). Within individuals, self- versus non-self-recognition typically occurs via immunity, e.g., lymphocytes that recognize and destroy foreign cells. Similarly, social organisms may use “social immunity”, or group-level anti-parasite behaviours, to manage or prevent parasites. Work
by Cremer & Sixt (2009) describes three lines of defence in social immunity: border, soma and germ line defence. The first line of defense against pathogens and parasites is preventing entry into the body or social group, known as border defense (Cremer & Sixt, 2009). This may take place via a complex organ, e.g., mammalian skin, or in social groups via nest entrance guarding. The second line of defence, soma defense, aims to neutralize threats that evade lines of border defense. This can occur through complex immune systems, e.g., killer T-cells. Social organisms, for example, eusocial insects, may use non-volatile hydrocarbons in the cuticle to identify colony mates, and will attack conspecifics whose hydrocarbon profile does not match that of their own colony (Lahav et al. 1999). The final line of defense, known as germ-line defence, prevents parasitism of the host’s offspring. In complex vertebrates, this may take the form of a blood-testis barrier, while in invertebrates it may take the form of egg guarding (e.g., Straus & Avilés, 2019) or even isolated brood chambers (Witek et al, 2014).
Figure 1.1. Cartoon depiction of the three lines of defense. Border defense prevents entry of a threat (yellow circle) into the biological entity (i.e., individual or colony, light shaded region). Soma defense eliminates threats that have evaded border defense. Germ line defense protects the germ line (i.e., reproductive organs, brood chamber, dark shaded area).

1.2 Parallels between host-parasite theory and metacommunity ecology

Importantly, the development of these host-parasite models, and those proposed in the decades since, typically considered a single host-single parasite system (Pilosof et al., 2015). In reality, a host can potentially provide habitat for multiple species, and parasites may be able to use multiple species of host, resulting in a metacommunity that occurs at multiple scales (Figure 1.2). Metacommunity ecology considers how dispersal, habitat suitability and species interactions combine to form ecological communities at local, regional, and landscape scales. Developing in parallel to metacommunity theory, host-parasite theory, particularly parasite ecology, aims to understand how and why parasites interact with hosts in different environmental contexts. To a parasite or pathogen, a host provides a suitable “habitat” through which they can move to complete various life stages (e.g., Agnarsson, 2003). A parasite's ability to disperse between habitat patches, i.e., transmit between hosts, should follow similar patterns of community composition, with patch suitability corresponding, in part, to a parasite's ability to evade host defences. Parasite communities follow many of the same trends as non-parasite communities. Species area relationships exist for parasites, and geographical isolation plays a large role in parasite richness (Simberloff & Moore, 1997). In humans, geographical isolation is
a better predictor of parasite species richness than population size (Poulin & Morand, 1999).

Parasites also follow species-latitude relationships. For example, teleost fish ectoparasites exhibit
nestedness based on latitude, such that locales farther from the equator have a subset of parasites
close to the equator (Rohde & Heap 1998).

Just as there are levels of spatial organization in a metacommunity, where local species pools are
nested within regional pools, there are similar levels of spatial organization in parasite
communities with “infracommunities” nested within “component communities” (Poulin, 2007).
Parasite component community parallels the regional species pool of community ecology and
includes all parasite species found in the populations of the host species. Within the component
community is the infracommunity, or all the parasite species found in a single host individual
(Poulin, 2007). When comparing the effect that species richness of the component community
has on maximum infracommunity richness, two types of models are typically considered.
Component community richness can result in either a proportional increase of maximum
infracommunity richness, where an increase component community richness corresponds to a
linear increase in infracommunity richness, or a saturating increase in maximum infracommunity
richness, suggesting some type of internal regulation of parasite communities, such as
intraspecific competition or immunity (Poulin, 2007). However, the regulatory mechanisms that
govern species saturation are unknown, and they may differ depending on the host-parasite
system. While researchers have made progress in understanding the theoretical and empirical
patterns of transmission and virulence in multi-host, multi-parasite systems (e.g., Holt et al.,
2003; Walker et al., 2017), inferences are still limited by the computational requirements of
analyzing such complex systems.
Figure 1.2 (a) A simplified metacommunity. Two different species (filled and open circles) and three habitat patches (open squares) are connected by dispersal (arrows). (b) A parasite metacommunity where two parasite species (filled and open triangles) move through hosts of two different species (filled and open circles) in a similar manner that their hosts move between habitats. Dispersal is analogous to transmission.

1.3 Life histories of social spiders and their parasites
Social spiders in the genus *Anelosimus* offer a unique opportunity to study parasite metacommunities in a social species that lacks reproductive division of labour, unlike eusocial organisms, such as ants or bees. Spiders in this genus build three-dimensional webs with a dense, basket-shaped sheet-web topped with prey-capture lines that connect to overhanging vegetation (Figure 1.3a-b). Individuals within a colony cooperate in prey capture (Yip et al., 2008), brood care (Samuk & Avilés, 2013), and web repair activities (Straus et al., 2021). Colonies can range in size from a single or a few female spiders to greater than 10,000 spiders. Colonies also exhibit
unstable population dynamics involving boom and bust patterns of population growth (Sharpe et al., 2016).

Residing within the prey capture area of *Anelosimus* webs, kleptoparasitic spiders in the genus *Faiditus* steal insect prey that the host spiders have already spent silk, venom and energetic resources to subdue (Cangialosi, 1990, Figure 1.3c). Previous work in this system explored the abundance and colonization patterns of kleptoparasites and other inquilines in social spider colonies in the context of the four meta-community archetypes (Fernandez-Fournier & Avilés, 2018). The authors found some degree of competition-colonization trade-off (i.e., patch dynamics) in the inquiline community, where specialized inquilines colonized the social spider colony after the more generalist species, replacing them (Fernandez-Fournier & Avilés, 2018).

The present thesis aims to extend previous work of social spider meta-communities by incorporating questions and techniques from host-parasite theory.

![Figure 1.3](image)

Figure 1.3. (a) A nest built by the social spider, *Anelosimus eximius*. (b) Individuals of *A. eximius*. (c) An adult female *Faidius cf. ululans*, a common kleptoparasite of *A. eximius*. 
1.4 Questions and hypotheses
This thesis work addresses Anderson and May’s four main postulates of parasitism in the context of the modern metacommunity framework, and tests their relevance to parasitism in a social spider system (Anderson & May, 1979). These postulates state that (i) parasites are nutritionally dependent on the host, (ii) the parasite causes the host harm, (iii) the host can evade parasites through immunity, and (iv) parasites must transmit (i.e., disperse) between hosts (Anderson & May, 1979). In Chapter 2, we obtained a coarse energy budget of the host to understand to what degree social spider colonies may obtain a nutritional surplus - a surplus that may be available for kleptoparasites for the nutritional dependence postulate (i). We predicted, based on the work of Yip et al. (2008), that intermediately sized colonies will obtain the highest amount of prey biomass per capita. Following metabolic scaling principles (Hou et al., 2010), we also predicted that energy requirements per capita would decline with colony size, which may be reflected in lower per capita investment in silk, and thus, lower per capita web repair costs in larger colonies, in other words, spider colonies would exhibit an economy of scale. These inputs and outputs should combine such that the smallest colonies receive the lowest net energetic inputs, which may impose nutritional constraints on both the host and the kleptoparasite. In Chapter 3, we considered postulate (iii) in the context of social animals, i.e., the behaviours performed by colony members to address parasite loads and whether they are effective. We addressed postulate (iv) in Chapter 4, where we measured parasite transmission, i.e., dispersal, through the social spider metacommunity using genetic markers. We tested two hypotheses: the free-mixing hypothesis, where kleptoparasite dispersal is not limited by their host, and the host-tracking hypothesis, where dispersal is limited by the host. Finally, we used a laboratory experiment in Chapter 5 to measure the virulence of the kleptoparasite, using body condition of the social host as a measure of health. If kleptoparasites are causing harm to their host in the form of stolen
prey, this should be reflected in parasitized colonies housing individuals with worse body
condition than colonies without parasites, thus meeting postulate (ii). Using this community of
parasitic invaders found in colonies of group-living spiders, this thesis is at the intersection of
modern metacommunity ecology and classic host-parasite theory.
Chapter 2: Economies of scale shape energetics of solitary and group living spiders and their webs

2.1 Chapter Summary

1. Metabolic scaling, whereby larger individuals use less energy per unit mass than smaller ones, may apply to the combined metabolic rate of group-living organisms as group size increases. Spiders that form groups in high disturbance environments can serve to test the hypothesis that economies of scale benefit social groups.

2. Using solitary and group-living spiders, we tested the hypothesis that spiders exhibit negative allometry between body or colony mass and the standing mass of their webs and whether, and how, such a relationship may contribute to group-living benefits in a cooperative spider.

3. Given the diverse architecture of spider webs—orb, tangle, and sheet-and-tangle, and associated differences in silk content, we first assessed how standing web mass scales with spider mass as a function of web architecture and whether investment in silk differs among web types. As group-living spiders are predominantly found in clades that build the presumably costlier sheet-and-tangle webs, we then asked whether cost-sharing through cooperative web maintenance contributes to a positive energy budget in a social species.

4. We found that larger spiders had a relatively smaller investment in silk per unit mass than smaller ones, but more complex sheet-and-tangle webs contained orders of magnitude more silk than simpler orb or tangle ones. In the group-living species, standing web mass per unit spider mass continued to decline as colony size increased with a similar slope as for unitary spiders. When web maintenance activities were considered, colonies also experienced reduced mass-specific energy expenditure with increasing colony size. Activity savings
contributed to a net positive energy balance for medium and large colonies after inputs from the cooperative capture of large prey were accounted for.

5. Economies of scale have been previously demonstrated in animal societies characterized by reproductive and worker castes, but not in relatively egalitarian societies as those of social spiders. Our findings illustrate the universality of scaling laws and how economies of scale may transcend hunting strategies and levels of organization.

2.2 Introduction
Metabolism, the process by which organisms take energy from the environment to use for growth, somatic maintenance, reproduction, and storage, powers all life. Organisms that use energy more efficiently are more likely to survive and reproduce (Lotka 1922), so metabolic strategies are likely shaped by natural selection. It has long been recognized that metabolic rate scales allometrically with body size. Thus, even though total energy expenditure increases with body size, larger organisms spend less energy per unit mass than smaller ones (Brown et al. 2004, Makarieva et al. 2008). Such ‘economies of scale’ have been shown to apply both within and across species over many orders of magnitude of body mass for both plants and animals (Gillooly et al. 2001, Enquist 2002, Brown et al. 2004; Glazier 2005; Makarieva et al. 2008), and even extends to group-living organisms, particularly eusocial groups characterized by the presence of queen and worker castes (Gillooly et al., 2010, Waters et al. 2010).

Given that spider webs are prey capture devices built using a spider’s own stored resources, their size should reflect the metabolic needs of the spiders that built them by being only as large as necessary to satisfy those metabolic needs (e.g., Sherman, 1994). As such, we would expect to see similar economies of scale between spider body mass and standing web mass. Spiders build
prey-capture webs of three types—two dimensional (2D) orbs, and three-dimensional (3D) tangles and sheet-and-tangles (Janetos, 1982; Rypstra 1982; Robertson & Aviles, 2018) (Figure 1). When webs are destroyed by weather or damaged by prey, they must either be replaced or repaired. Cooperative spider societies have typically arisen in taxa that build webs of one of the latter two categories, and, most often, the more complex sheet-and-tangle ones, such that sociality may have arisen to meet the challenges imposed by these type of web (Avilés 1997; Avilés and Guevara 2017). Within these communal webs, colony members cooperate in web building and maintenance, prey capture, feeding, and brood care. These spiders are particularly interesting as, unlike eusocial insects, they lack reproductive castes, so that all colony members can reproduce. Their colonies also grow by internal recruitment, where offspring mature and mate within the natal nest from one generation to the next, expanding the nest until colonies either disperse or go extinct (Avilés 1997; Avilés and Guevara 2017). Through the production of many individuals, web maintenance duties can be shared. Here we ask whether group-living organisms that lack reproductive castes experience a reduction in mass-specific energy expenditure related to web maintenance, and whether this results in economies of scale that benefits the group.

As spiders must balance the costs of web-building and repair with returns from prey capture, species with costly 3D webs may benefit from living in groups, particularly in habitats where webs are frequently damaged by heavy rainfall or frequent wind events (Avilés and Guevara 2017). We refer to this as the *Costly-webs High-disturbance Hypothesis*, formulated based on previous studies that showed that the strong and frequent rains characteristic of the lowland tropical rainforest may prevent solitary or subsocial spiders in genera with dense sheet-and-
tangle webs from occupying these habitats (e.g., Riechert et al. 1986; Purcell and Avilés 2008; Hoffman and Aviles 2017). Although spiders may protect their costly 3D webs by using strategies such as sheltering against tree trunks or under leaves (Robertson and Aviles 2019; Haberkern et al. 2020), group living is an additional mechanism that may open high disturbance environments for spiders with costly webs. It is in these conditions that social spiders in genera such as *Agelena, Paraesteatoda*, and *Anelosimus* thrive (Riechert et al. 1986; Avilés and Guevara 2017). An increased workforce in these group-living species may allow them to keep up with the constant demands of repairing their webs.

We tested two aspects of the *Costly-webs High-disturbance* hypothesis. First, we sampled a neotropical web-building spider community and tested whether (i) webs with a 3D architecture, particularly those with sheet-web components, contain a greater amount of silk per unit spider mass than webs with a 2D architecture, and whether all webs, regardless of architecture or sociality level, would scale allometrically with spider mass. Then, considering a highly social species, we tested whether (ii) web-sharing lowers per capita costs of web maintenance, possibly contributing to their ability to occupy a high-disturbance habitat.

For (i), we estimated the mass of silk per unit spider mass in webs of the three architectures: orb, tangle, and sheet-and-tangle (Figure 2.1); for sheet-and-tangle webs, we included representatives of both solitary and social species. Under (i), we would predict that, for a given spider size, 3D webs will require more material, and thus be costlier to build, than 2D webs. On a per unit mass basis, however, webs should be built to capture just enough prey to match the energetic requirements, in terms of daily activity, growth, and reproduction, of the spiders building them.
Field metabolic rate, i.e., the cost of routine activities, which scales positively with body size across taxa (Nagy 2005), is expected to do so with a scaling exponent bounded by $mass^1$ and $mass^{2/3}$, depending on the lifestyle and activity level of organisms (Glazier 2010). Accordingly, mass-specific field metabolic rate should scale with body size at a rate between $mass^0$ and $mass^{-1/3}$. If there is an economy of scale in the acquisition of resources by web-building spiders, at either the individual or group level, we would expect a negative relationship between spider mass (solitary individual or social group) and the amount of silk per unit spider mass their webs contain. Alternatively, in the absence of an economy of scale, or if the area or volume of the webs were limited by factors such as space or substrate, we might not see such a relationship.

To test hypothesis (ii) we explored how group living affects the allometric scaling of energetic costs related to web maintenance. For this, we studied the Neotropical social spider *Anelosimus eximius*, whose colonies occupy communal 3D sheet-and-tangle webs and range in size from a few individuals to thousands of individuals. If economies of scale extend to social groups, we predicted that larger social spider colonies would incur a lower cost of web maintenance per unit mass than smaller colonies. Finally, to assess the impact of web maintenance on the energy balance within colonies, we constructed a coarse energy budget to compare energetic inputs from prey to outputs from web maintenance. Following Yip et al. (2008), we expected that energetic inputs from prey would have a convex relationship with colony size, peaking for medium-sized colonies. We predicted that energetic outputs of small colonies would approach or exceed energetic inputs, lending support to the idea that energetic savings associated with communal web maintenance may be a key benefit of sociality in this, and perhaps other, spider genera.
2.3 Methods

2.3.1 Spider web architecture
We studied naturally occurring spiders of the three web architectures (Figure 2.1) at the Jatun Sacha Biological Reserve in Napo, Ecuador in the summer 2014. As in previous studies (Ludwig et al. 2018; Robertson & Aviles 2018; Haberkern et al. 2020), we classified webs in three categories based on their geometry (Table A.1). 2D orbs consist of radial lines that provide structural support to sticky spirals used for prey capture (Benjamin & Zschokke, 2003). This included species in the families Araneidae, Tetragnathidae, and Uloboridae. Tangle and sheet-and-tangle webs are both 3D architectures, with the latter having a dense, horizontal sheet associated with capture lines above and/or below the sheet (Blackledge 2003) (Figure 2.1). Species in certain araneid genera (e.g., Kapoge, Table A.1) were classified in the sheet-and-tangle category as their webs are 3D with a relatively dense circular sheet and barrier webbing above and below.

![Figure 2.1. Examples of web architecture types—(a) orb, (b) tangle, (c) sheet-and-tangle](image)

solitary or social. Webs are not to scale. For the purposes of this study, we considered (A) and (B) webs without sheet-web components, and (C) those with sheet-web components.
We obtained web and body mass data from 42 solitary and 10 social webs (Table A.1). Specimens from each web type were removed from their webs and placed in a killing jar within <2 h of collection, oven-dried, and weighed to estimate their dry mass (hereafter body mass). For solitary spiders, which belonged to various developmental stages and thus exhibited considerable size variation (Table A.1), we measured the dry mass of individual spiders directly. To estimate colony mass in the social species, we collected entire colonies, which were then dissected to count the number of individuals they contained. As research of this species is on-going, and we did not want to eradicate the local population, total dry mass of a colony (hereafter colony mass) was calculated rather than directly measured. Colony mass was calculated using the number of individuals in the web times their average dry body mass estimated from a sample of spiders of different developmental stages (instars). The average body mass of the various taxa considered ranged across several orders of magnitude, with considerable variation within some species (Table A.1)

We used standing web mass as a proxy for the energetic cost of web-building. This does not consider the frequency of web repair, type of silk, or whether the spider ingests the silk to recycle those costs. However, spiders across taxa alter their webs depending on feeding status (Sherman, 1994; Blackledge & Zevenbergen, 2007), suggesting that spiders build or maintain their webs to meet immediate energetic needs. To estimate standing web mass (Table A.1), for orb and tangle webs we used long tweezers to collect whole and undamaged webs, manually removed debris from the webs with forceps under a dissecting scope, and oven dried them at 60° C for 72 hours. For sheet-and-tangle webs, in addition to estimating the dimensions of the various web components in the field, we used a standardized protocol to collect a 5x5 cm² square
of silk from the basal sheet. We cleaned and weighed these standard samples as above, and extrapolated to the total mass of the sheet using the formula for the surface area of an ellipsoid.

We measured the dry weight of spiders and their webs using an electronic ultramicrobalance (±0.01μg; XS105DU Analytical Balance: Mettler Toledo, Columbus, Ohio). We used R (Version 3.6.2) and the “stats” package (R Core Team, 2019) to perform a linear mixed effects model (LMM, “lme4” package) to relate log10-transformed spider body mass with log10-transformed mass-specific web size (i.e., silk amount), including web type (four initial categories: orb, tangle, solitary sheet-and-tangle and social sheet-and-tangle) as a fixed effect and spider genus as a random effect (Bates et al. 2015) (Table A.1). Next, we used a post hoc Tukey’s HSD test to compare regression intercepts using the R package “emmeans” (Lenth 2020), and subsequent analyses considered web types grouped by shared intercepts. We also carried out a phylogenetic generalized least-squares regression (PGLS, “ape” package) using genus-averaged mass-specific web mass and spider body mass, and the spider phylogeny of Wheeler et al. 2017 (Paradis & Schliep 2018). In the latter case, we used the two web type categories that emerged as distinct from the original analysis: webs with and webs without dense sheet components (Table A.2).

*Energy budget in social spider colonies*

To further explore the possible contribution of economies of scale to the energy budget of a social spider, we surveyed prey capture and web repair in colonies of *Anelosimus eximius* at the Centro Científico Río Palenque (CCRP), in Los Rios, Ecuador. This species has been documented to form colonies ranging in size from single females and their offspring to thousands
or, in some localities, tens of thousands of individuals (Purcell and Avilés 2007; Yip et al. 2008). From May to July 2016, in a sample of 30 colonies ranging in size from 10 to 1700 spiders, we estimated the energy budgets of colonies by measuring outputs from web maintenance activity, using a combination of respirometry and field observations, and energetic inputs from prey.

2.3.2 Energy outputs from web repair
Estimating total web maintenance costs as a function of colony size required estimating the activity and material costs of web maintenance separately. To estimate activity costs, we scanned the nests at fixed time intervals to record the number of spiders seen performing web maintenance behaviours at various times of the day and night. We coded four behaviours: (1) rolling silk (spider gathers up broken lines into a ball to be discarded over the edge of the nest); (2) producing silk while dropping (spider drops hanging from a silk line and then builds upwards); (3) laying silk while walking (Saffre et al., 1999), and (4) prey-handling, either via capture or debris removal. We estimated the number of nonworking (“still”) spiders as the total number of spiders in a colony minus the number seen performing web maintenance behaviour. We did not use a direct count of the number of still spiders because large numbers of individuals huddle together inside of accumulated plant material, which increases the likelihood of undercounting. Sampling periods consisted of nest observations every 20 minutes for 6 rounds, with two sampling periods per day, as there is little activity late at night (Figure A.1). This was repeated for a total of 72 observations in each of 30 nests. Web maintenance and prey capture observations (described below) for each subset of nests were done on consecutive days, at all hours except those between one and five a.m. We estimated the proportion of spiders working as the number seen performing behaviours 1-3 divided by the total number of spiders in the colony. We then multiplied the average number of individuals estimated to have been still (see above) or
working throughout the day and night by the mean instantaneous resting and active metabolic rates (see below), respectively. To determine colony weight and number of spiders per colony in a way that avoided over-collecting the local social spider population, we collected a subset of 19 colonies. We then counted the number in each instar, multiplied by the average weight of each instar, and performed linear regressions relating the cross-sectional area of the nest to colony mass, total number of spiders, and number of subadult and adult females. For colonies that we did not collect (N=11), we used the regression coefficients obtained from collected colonies to estimate colony size parameters (Figure A.2).

Next, we inferred material costs from activity costs. Prestwich measured activity costs to be 22% percent of the material cost for a solitary sheet-web spider that does not recycle silk (1977). This value, rounded to 20%, has been used since, both in estimating direct web building costs of other spiders (Opell 1998), and in simulation models (Eberhard 1986). Following these studies, we divided activity costs by 25% to obtain material costs. This may be a simplification; however, it was not feasible to take field measurements of silk production during daily web maintenance. Further, we were unable to relate distance travelled to mass of silk produced, as different types of silk and silk thicknesses are produced for different web functions, e.g., supporting lines differ from sheet web silk, which differ from draglines (Benjamin & Zschokke, 2003). Finally, activity and material costs were integrated as follows:

Total colony energy expenditure (Watts) = Activity cost to working individuals (Watts) + Material cost to working individuals (Watts) + resting cost to inactive individuals (Watts)

(equation 1)
2.3.3 Active and resting metabolic rates of individuals

To further parameterize equation (1), we estimated active and resting metabolic rates for individual spiders in a controlled laboratory setting. Due to the logistical and permitting constraints of exporting live *A. eximius* from Ecuador, we instead used the subsocial congener *Anelosimus arizona* to determine metabolic rate (Avilés and Gelsey 1998). *A. arizona* is a suitable substitute, as the spiders are phylogenetically close and morphologically almost identical to *A. eximius*. Furthermore, the size of the *A. arizona* individuals used (2.69 mg to 4.80 mg) encompassed the mean body weight, but not full range in body weights, of adult and subadult *A. eximius* (4.26 ± 3.64 mg), the instars responsible for nest maintenance in this species (Settepani et al., 2013). *A. arizona* individuals were collected at the Patagonia-Sonoita Creek Preserve, near Tucson, Arizona, USA (Avilés and Gelsey 1998).

We used stop-flow respirometry and an infrared gas analyzer (LI-7000, Licor, Lincoln, NE, USA) to measure resting and active rate of CO$_2$ production as a proxy for metabolic rate (MR). Each individual spider was first weighed to the nearest 0.01 mg and placed for two hours into a 10-mL sealed glass chamber to measure resting MR. Next, to measure active metabolic rate we placed the spiders in an airtight, circular, perspex track (20 cm by 1 cm), designed with modifications from Jensen & Holm-Jensen (1980) (Figure A.3). The track included a light-weight, user-operated magnetic ball to encourage constant movement by gently tapping on the posterior end of the spider if they stopped walking. We walked each spider for two 10-minute intervals. Spiders must walk back and forth across their webs to perform web maintenance duties. In this way, the metabolic cost of walking is representative of the activity cost associated
with web maintenance. As spiders have a primarily lipid-based metabolism, we followed Prestwich (1977) and assumed a respiratory quotient of 0.72 μL O\(_2\) consumed per microliter of CO\(_2\) produced for both resting and active MR (1987). We then used Lighton’s oxyjoule equivalent to convert the estimated O\(_2\) consumption to Watts (Joules per second). As metabolic rate was measured at 21°C, we assumed Q\(_{10}\) = 2 to correct for a field temperature of 26.8°C, the mean annual temperature at the CCRP (WorldClim V1.4, Robertson & Aviles 2018).

2.3.4 Energy inputs from prey
We estimated energy input from prey capture per unit time by recording the number, size and taxonomic order of insects caught by the same 30 CCRP colonies during monitoring periods covering morning, afternoon, and night time, between 8:00am - 1:30am, for a total of 63 visits per nest. During each monitoring period, we visited nests every 90 minutes, three or four times in a row. At each visit, we recorded the size (total length, estimate to the nearest mm using a ruler) and order of each insect caught in the preceding 90 minutes. In the analyses, we only included observations where the nest was visited no more than two hours prior, to ensure that colonies were not processing prey faster than we could observe (Rypstra & Tirey, 1991). We used insect order equations from Straus & Avilés (2018) to convert insect length to biomass consumed, and from there to Joules per second (Watts) consumed using Wilder et al. (2013), assuming 27 J gained per mg insect biomass consumed.

2.3.5 Statistical analyses
We used a generalized linear model (GLM) with a quasibinomial distribution to test the effect of colony size (mg) on the proportion of spiders performing web maintenance behaviours. We weighted energetic inputs by the number of observation hours. When analyzing the relationship
between total spider mass in a colony and energetic inputs/outputs, we tested linear, quadratic, and nonlinear options, giving preference to models with the lowest AIC scores. For these analyses, variables were log10-transformed. All analyses and figures were created in R (Version 3.6.2). Research on the spider community at the Jatun Sacha Biological Station was conducted under permit N° 11-15-IC-FAU-DPAN/MA from Ministerio del Ambiente del Ecuador, Napo Province, and specimen export permit N° 012-15- EXP-IC-FAU-DNB/MA. Research at the Centro Científico Río Palenque was carried out under permit N° 007 - 17 IC-FAU-DNB/MA from the Ministerio del Ambiente del Ecuador, and a research cooperation agreement with the CCRP-Fundación Wong. No animal ethics protocols are required for field work with arthropods at our home institution (UBC Animal Care and Use Program, Canadian Council on Animal Care Invasiveness area A) or in Ecuador. None of the species studied are listed as endangered.

2.4 Results

2.4.1 Spider web architecture and silk content per unit spider mass
Across the four web type categories—orb, tangle, sheet-and-tangle solitary and sheet-and-tangle social—the amount of silk per unit spider mass decreased with spider mass (ANOVA, slope = -0.65 ± 0.18, F-value\(_{1,40} = 41.11\), p-value < 0.001), with no significant interaction between spider mass and web type (ANOVA, F-value\(_{3,40} = 1.31\), p-value = 0.28). Comparing the intercepts of the four web type categories (Table A.2), two distinct groups emerged: orb and tangle webs without sheet-web components, which contained significantly less silk per unit spider mass, and solitary and social sheet-and-tangle webs, which contained over two orders of magnitude more silk (Figure 2.2a). In a model comparing webs with and without dense sheet-web components (Table 2.1), the amount of silk per unit spider mass decreased with the mass of the spiders, or of the colonies, with a slope of -0.58 ± 0.14 95% CI (Table 2.1, Figure 2.2), again with no
interaction between web type and spider mass (F-value$_{1,6} = 1.72$, p-value = 0.20). Both results held after a phylogenetic correction was applied (effect of colony mass in pgls model: F-value$_{1,2}$ = 90.48, p-value = 0.04; interaction F-value$_{1,2} = 0.15$, p-value = 0.72), although in this case the slope (-0.28 ± 0.18) was notably shallower.
Figure 2.2. (a) Mass specific silk amount (mg) in webs as a function of spider mass (mg), for spiders that build webs with or without sheet-web components. (b) The social spider regression enlarged showing mass specific web content (mg) as a function of colony size.
(mg) for the social *Anelosimus eximius*. See Table (1) for slopes and intercepts of the lines in panels (a) and (c). (c) Phylogenetic corrected least-squares regression (PGLS) using genus averages of the data shown in (a). Solid lines indicate uncorrected fit, dashed lines indicate PGLS fit.

Table 2.1. The amount of silk per unit spider mass had a strong negative relationship with spider body size (mg) for webs with (sheet-and-tangle solitary and social) and without (orbs and tangles) sheet-web components, with no interaction with web type. The table reports the intercepts of the two web types and the common slope, for both LMM and PGLS models. The LMM model includes spider genus as a random effect. The models shown do not include an interaction term, which was not significant in initial LMM and PGLS models (shown below), indicating the slopes did not differ between web types.

<table>
<thead>
<tr>
<th>Model</th>
<th>Web Type</th>
<th>Fixed Effect</th>
<th>Estimate ± 95% CI (log10 mg of silk)</th>
<th>F - value</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMM</td>
<td>sheet web</td>
<td>intercept</td>
<td>2.28 ± 0.52</td>
<td>70.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>no sheet web</td>
<td>intercept</td>
<td>-1.09 ± 0.51</td>
<td>162.06</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>-0.58 ±</td>
<td>59.11</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>
2.4.2 Energy budget in social spiders

In colonies of *A. eximius*, the per capita amount of silk contained in the webs declined as the number of spiders in the colony increased (Table 1, Fig 2.2b). Since webs are continuously damaged by weather and/or prey impact, we also considered web repair activities throughout the day and night. Although the number of spiders maintaining the web increased with colony mass (ANOVA, slope = 1.07 ± 0.19, F-value$_{1,24}$ = 120.80, p-value < 0.001, $r^2_{adj}$ = 0.83), their proportion relative to all subadult and adult spiders in the colony decreased as colony mass increased (Fig 3): ($\chi^2 = 6.24$, df = 1, p-value = 0.01). This was the case whether we considered the average over all hours of the day (Figure 2.3) or only during the time at dusk (17:00-17:59) when web repair activities are concentrated (Figure A.4a). At the latter time, however, even though the effort was concentrated on proportionately fewer individuals, those participating...
travelled greater distances, presumably due to the larger web size of larger colonies (Figure A.4b).

![Graph](image)

Fig 2.3. Economies of scale were associated with a smaller proportion of adult and subadult spiders maintaining the web at any given time as colony size (mass) increased.

To obtain hourly web maintenance costs, we used estimates of mean instantaneous resting and active MR for *Anelosimus arizona* individuals of similar size as those *A. eximius* instars active in web repair (see Methods). These were $4.70 \times 10^{-6} \pm 7.35 \times 10^{-7}$ Watts and $5.36 \times 10^{-5} \pm 7.74 \times 10^{-6}$ Watts, for resting and active MR, respectively (at 21°C). These values were then multiplied by the number of resting and active spiders to obtain activity costs. When considering the combined material and activity costs of web maintenance, we found a significant negative relationship between colony mass (mg) and the energetic output per milligram spider body mass dedicated to
web maintenance (Fig 4b): (ANOVA, slope = -0.17 ± 0.06, F-value\textsubscript{1,24} = 28.12, p-value < 0.001, r\textsuperscript{2}\textsubscript{adj} = 0.52, AIC = -58.36; cubic spline fit, F-value\textsubscript{3,22} = 12.47, p-value < 0.001, AIC = -60.01).

Both cubic spline fits and a significant quadratic term suggest a convex relationship between energetic inputs from prey and colony mass in *A. eximius* colonies (quadratic term: ANOVA, F-value\textsubscript{2,23} = 7.27, p-value = 0.03, AIC = 35.423 vs. AIC = 38.79 in the model without a quadratic term; cubic spline fit, F-value\textsubscript{3,22} = 5.60, p-value = 0.005, AIC = 35.42, Figure 2.4a). For most larger colonies, inputs outpaced outputs. However, outputs exceeded inputs for several small and medium-sized colonies during our study period (Figure 2.4a)
Figure 2.4. (a) Total energetic inputs (Watts = Joules sec\(^{-1}\)) from prey per unit spider mass (mg) peak for intermediately sized *A. eximius* colonies. (b) Material and activity costs for web maintenance and prey capture demonstrate an economy of scale. To facilitate comparison, a dotted line corresponding to the linear fit of outputs has been
2.5 Discussion
This study presents two tests of the *Costly-webs High-disturbance hypothesis* that together help to explain why group-living may be an adaptive strategy in spiders that build dense, 3D webs. Considering silk production must be included within a spider’s energetic budget, it holds that we may find such a scaling relationship between spider mass and their mass-specific web mass, as found by Gregoric et al. (2015) for orb-weaving spiders. Furthermore, if larger spiders have reduced mass-specific metabolic rates, they should need less food per unit mass to support their daily activity, growth, and reproduction. Web mass, therefore, need not grow isometrically with body mass. Following hypothesis (i), our study found a strong, negative allometric relationship between log-transformed spider body mass and the log-transformed silk mass of the spiders’ webs, with surprisingly similar slopes across web types and between solitary and social species (Figure 2.2 and Table 2.1). Importantly, our study included spiders of different instars, sex, and reproductive status, all of which may influence web size.

We found a significant difference among web architectural types, with sheet-and-tangle webs, including those built by social spiders, containing over two orders of magnitude more silk per spider unit mass than simpler orb or tangle webs without sheet-web components (Figure 2.2a). As the amount of silk material should be proportional to the energetic cost of production (Prestwich 1977), sheet-and-tangle webs should therefore be costlier to produce than orb or simple tangle webs. It’s worth noting, however, that such a cost only applies to building the entire web from start to finish. Spiders with 3D webs tend to remain at a fixed location while
continuously repairing their web (Tanaka 1989), whereas orb-weaving spiders rebuild their webs regularly ingest and recycle their silk (Opell 1998). Importantly, this pattern of greater standing web silk mass relative to body size for sheet-webs held when accounting for shared evolutionary history (Figure 2.2c). The environmental factors driving the evolution of 3D webs, in general, have long been under investigation (e.g., prey capture: Rypstra 1982; predator protection: Blackledge et al. 2003, Robertson & Aviles 2018), but not with a focus on those taxa that build webs containing sheet-web components. Yet, webs with dense sheet components apparently have multiple evolutionary origins (Blackledge et al. 2009), suggesting they may be worth the high, up-front building costs, particularly in habitats where they may require less frequent repair (Tanaka 1989). In our study, both species of solitary sheet-and-tangle building spiders, which belong to different taxonomic families, are relatively large bodied (Figure 2.2ac, Table A.1), perhaps suggesting that only larger spiders can afford those up-front building costs. In tropical rainforests, where rains are intense and frequent (Purcell and Avilés 2008; Hoffman and Avilés 2017), weather events may result in dense 3D webs incurring more damage and requiring more frequent web repair than simpler webs or webs in other habitats. In such habitats, Robertson and Aviles (2019) showed that 3D webs tend to occur under the cover of leaves in lowland tropical rainforest environments, which Haberkern et al. (2020) showed is consistent with microhabitat selection for protection against intense rains.

Group living may be another strategy for spiders with costly sheet-and-tangle webs to occupy habitats where webs may require constant repair, as proposed by our Costly-webs High-disturbance hypothesis. In social spiders, multiple individuals within a given web share the costs of its maintenance and repair. By extending metabolic theory to groups of individuals as the unit
of observation, one would expect a negative relationship between individual or colony mass and
the per unit colony mass energy expenditure (Brown et al. 2004). Our results are consistent with
this hypothesis (Figure 2.4b), notably extending the relationship seen in solitary sheet-and-tangle
spiders to social ones (Figure 2.2a). A pattern of decreasing mass-specific colony-level
metabolism with increasing colony size has been reported for other social arthropods, but
typically those with queen and worker castes, such as ants (Waters et al. 2010; Cao & Dornhaus,
2013), bees (Giloolly et al., 2010, Southwick 1985) and termites (Muradian et al., 1999). It is
thus interesting that a similar pattern applies to relatively egalitarian societies, such as those of
social spiders.

When dissecting the mechanisms associated with the negative allometry between web size and
colony size in the social spider system, we found that proportionally fewer individuals
participated in web maintenance in larger colonies, potentially contributing to the breaking up of
mother colonies into smaller daughter colonies. This proportional decrease in web maintenance
was observed whether averaging over the entire day and night (Figure 2.3), or only at dusk, when
web repair activities are concentrated (Figure A.4a). At the latter time, however, spiders that
participated in web repair in larger colonies covered longer distances (Figure A.4b), presumably
reflecting the larger size of their nests. A similar response has been observed in colonies of
harvester ants, where larger colonies had proportionally fewer active ants, partly explaining the
hypometric scaling of whole colony energy expenditure (Waters et al 2017). The concentration
of greater effort in fewer individuals in larger colonies may result from some individuals
contributing less than their fair share to communal activities (e.g., freeloaders or cheaters).
Whereas task specialization is common among the eusocial insects (Fewell, 2003; Waters et al.,
2017), it is less so in social spiders. In *A. eximius*, Settepani et al. (2013) showed an absence of task specialization, except on the basis of age class, which may preserve the energy of younger instars for growth and moulting. Adult *A. eximius* females were more likely to participate in prey capture than younger individuals, which, on the other hand, were relatively more active in web repair (Settepani et al., 2013). With or without task specialization, the overall pattern emerging from our study is that of lower average per capita energetic investment in web maintenance in larger colonies, consistent with the hypothesis that group living brings about an economy of scale. This argues for a potential role of web sharing and communal web maintenance in allowing these spiders to occupy the high disturbance environments where webs require frequent repair.

As in Yip et al. (2008), we found a hump-shaped relationship between energy intake per unit mass and colony size (Figure 2.4a). This pattern arose because larger colonies captured fewer, but larger insect prey per capita as colony size increased (Yip et al., 2008). The decline in prey captured per capita is a result of scaling 3D objects, such that the number of insects caught should be proportional to the web’s surface (scaling as web volume^{0.67}), but the number of spiders in the web is proportional to the webs’ volume, paralleling the pattern expected in unitary organisms (Glazier 2010). The concordance between our study and that of Yip et al. (2008), which occurred in the same system, but different locations, year, and season is notable. For most colonies in our study, the per unit energy intake from prey outpaced the per unit energy output from web maintenance, however outputs exceeded inputs for smaller colony sizes (Figure 2.4). Additionally, sampling at the CCRP occurred during the drier season, where less frequent rainfall events might translate into lesser maintenance requirements as compared to the monsoon
season. While further study is needed to include costs associated with prey capture and feeding, growth and moulting, and egg production into a more complete energy budget, work by others suggests that these processes may also follow negative allometric scaling (e.g., Anderson, 1994; Brown et al., 2004).

Our finding that energetic outputs from web repair exceeded the energetic inputs from prey capture for small colonies (Figure 4) is consistent with previous studies showing high extinction probability of very small colonies (Vollrath 1982; Avilès and Tufiño 1998). More dramatically, it has been shown for the social funnel-web spider, *Agelena consociata*, that the web-building costs of solitary individuals accounted for up to 50% of daily energy expenditure, exceeding the per capita costs of their group-living counterparts (Riechert et al. 1986). A pattern of very small colonies often having a negative net energy budget was also present in a colonial spider, *Metepeira incrassata* (Uetz 1997), which rather than building a single communal irregular web, aggregate individually-built orb webs. Uetz (1997) argues that this pattern is the result of sharing the costs of building the common scaffolding that support the individual webs and of increased prey capture success with colony size.

Finally, productivity of the environment should play a role in the strategies that spiders use to optimize their webs as prey capture devices. For example, Sherman (1994) found that orb-weaving spiders reduced the capture area of their webs following supplemental feeding. Productivity in a given environment may be seasonal, as in our study, where the CCRP has wetter and drier seasons that may influence both insect size and web repair needs. Similarly, insects are, on average, smaller at higher elevations in the Andes mountain range (Guevara
Aviles, 2007), where rainfall also is less intense and spiders are less social. As temperature, precipitation, seasonality, and primary productivity are all intricately linked, further study across taxa should explore the role of primary productivity in the Costly-webs High-disturbance hypothesis.

2.5.1 Conclusion
We have shown that economies of scale, which reduce mass-specific energy expenditure as organisms grow in size, also apply to spiders with different web architectures and from single individuals to social groups. At the individual level, we show that the amount of silk mass contained in webs per unit spider mass decreases with the size of the spiders with near identical slopes across spiders with vastly different web architectures, from simple orb or tangle webs to dense sheet-and-tangle ones. Webs of the latter type, however, required orders of magnitude more silk than those of other types, which we hypothesized would make them unsustainable in habitats where damaging weather events require near constant web repair. We argue that economies of scale arising from group living may allow species with costly sheet-and-tangle webs to inhabit such adverse environments. We found strong signals of economies of scale with regards to web maintenance in social spider nests. While the energetic inputs we considered outpaced outputs for intermediate and large-sized colonies (Figure 4b), most colonies under 300 mg did not receive a net energetic surplus during our study period. We thus find support for the hypothesis that group living and sociality in spiders may be energetically driven and that economies of scale exist across levels of organization in web-building spiders.

Data accessibility
Data and code are archived on Dryad with the DOI https://doi.org/10.5061/dryad.qbzkh18j9
Chapter 3: Effects of host colony size and hygiene behaviours on social spider kleptoparasite loads along an elevation gradient

3.1 Chapter Summary

1. Group living animals are likely to attract more parasites than solitary ones. Parasite loads, however, should also depend on environmental conditions and on host characteristics and behaviours. Previous work has found that social spider colonies harbor communities of kleptoparasitic spiders that forego building their own web and, instead, steal prey from their social host.

2. We examined parasite loads and host hygiene behaviours in colonies of social and subsocial spiders in the genus *Anelosimus* along an elevation gradient in eastern Ecuador.

3. We found that parasite loads declined dramatically with increasing elevation. Host hygiene behaviours, such as debris removal, web repair, and interactions with the parasites, also declined with elevation. Within elevations, species with more frequent hygiene behaviours appeared more successful at keeping parasites at bay. Contrary to our predictions, parasite density declined with host nest and colony size.

4. The decline in parasite loads at higher elevations likely reflects a lower rate of energy exchange between colonies and their environments, where colder temperatures mean fewer and smaller prey for colonies to process. The decline in parasite density with host colony size may reflect a decline in the number of accessible prey in larger host colonies, as larger colonies should capture fewer but larger insects due to scaling properties of their three dimensional webs.

5. Social immunity, whereby a social host uses social behaviour to fight against parasites, has been studied in eusocial and non-eusocial insects. This study opens up social spiders as a novel system in which to study how host characteristics interact with environmental
factors to affect parasite loads. It also introduces the concept of group-level immunity to social spiders and suggests a role for colony-level metabolism in determining ecological patterns in parasitism.

3.2 Introduction
Group living animals tend to attract parasites (Cremer et al., 2007, Kappeler et al., 2015). These may include not only parasites and pathogens that infect individuals within the groups, but also parasites that take advantage of the resources of the group as a whole. Social insects are particularly vulnerable to colony-level parasites, as they build permanent nest structures that can attract enemies over time (e.g., Emerson, 1938; Iyengar, 2008). When such enemies cohabit within the nest structure built by the host, they are often referred to as inquilines (Cristaldo et al., 2014). The degree to which inquilines cause harm to their host may vary. Termite colonies, for instance, may harbor foreign termite species that build confined galleries within the host’s termatarium, using resources collected by the host for building materials and food (Cristaldo et al., 2012; 2014). A more extreme example are the so called social parasites found in ants. These are foreign ant species, which raid the host’s colonies, stealing and carrying host workers to their own nests to rear the parasite’s brood (Buschinger, 1986; Bourke & Franks, 1991). Other inquilines may simply take advantage of the protection provided by the host’s nest, without damaging the host. Here, we will focus on inquilines of social spider colonies that steal food resources from their hosts, referring to them as “kleptoparasites” (Cangialosi, 1990).

Specifically, we investigate how the incidence of social spider kleptoparasites is influenced by host and habitat characteristics, including potential host hygiene behaviours.

In response to the threat posed by individual- and colony-level parasites, social groups may display a variety of defensive behaviours, which have been referred to as social immunity. Much
of the work on social immunity has focused on eusocial insects, where colonies exhibit strong reproductive division of labor, with sterile workers and fertile queens. This type of colony organization has been referred to as a “superorganism” (Reeve & Holldobler, 2007), with parallels drawn between colony-level immunity and the individual-level immunity of multicellular organisms (Cremer & Sixt, 2009; Cremer et al., 2018). In social immunity, members of a social group perform behaviours to (1) prevent the entry of parasites and pathogens into the colony, (2) respond to the presence of parasites and pathogens once established, and (3) prevent transmission to offspring (Cremer et al., 2007, Meunier, 2015). Although the strictest definition of social immunity was developed for eusocial insects (Cremer et al., 2007), it has since been extended to groups of gregarious insects that exhibit behaviours pertaining to parasite entry and establishment within a group (Cotter and Kilner, 2010; Meunier 2015). For example, organized waste disposal is found in eusocial insects (e.g., ants, termites), gregarious, non-eusocial insects (e.g., ambrosia beetles, crickets), as well as social arachnids (e.g., mites) (for review, see Weiss, 2006). The extent to which host hygiene behaviours contribute to control parasite loads, and how both parasite loads and host hygiene behaviours vary with environmental conditions and host life histories, are questions that are only beginning to be addressed (Cremer et al., 2018).

Unlike superorganisms, social spiders do not have strong reproductive division of labor. Here, we distinguish between social and subsocial spiders. Social spiders form colonies that contain multiple adult females and their offspring living together in a single communal nest (Avilés & Tufiño, 1998; Avilés & Guevara, 2017). Colonies grow through internal recruitment, are extremely inbred, and have highly female biased sex-ratios (Avilés & Guevara, 2017). Subsocial spiders, on the other hand, live in temporary social family groups. Mothers perform extended
maternal care, and offspring disperse from the natal colony prior to mating (Samuk & Avilés, 2013; Yip & Rayor, 2014). Subsocial spiders are, for the most part, not inbred and have relatively even sex-ratios. Both of these social systems are represented in the spider genus *Anelosimus* in the Americas, where social species predominate in the lowland wet tropics. The subsocial species, which are absent from the lowland tropical rainforest, extend into higher elevations and latitudes (Guevara & Avilés, 2015). The spatial distribution of social and subsocial spiders has been shown to be correlated with factors such as temperature and precipitation (Guevara & Avilés 2015), which may also be relevant to the distribution of organisms that associate with their nests.

Previous work in the lowland tropics found that nests of social *Anelosimus* spiders harbor communities of foreign spider species (Cangialosi, 1990, 1991; Fernandez-Fournier & Avilés, 2018). Studies on *Anelosimus eximius* in Peru found that kleptoparasitic spiders steal prey from their host in antagonistic interactions (Cangialosi, 1991) and that hosts employ behaviours to defend prey (Cangialosi, 1990). Kleptoparasites of a temperate and subsocial *Anelosimus* appear to affect colony success, such that when inquilines are removed, the host colonies are less likely to go extinct (Pruitt, 2013). It is not known, however, whether colony-level parasite loads and host anti-parasite behaviours vary with environmental conditions and the spiders’ level of sociality.

Here we examine how the prevalence and abundance of kleptoparasitic spiders in the nests of social and subsocial *Anelosimus* vary with elevation, host colony size, and host hygiene behaviours. We used elevation as a proxy for correlated environmental factors such as temperature and precipitation (Guevara & Avilés, 2015), which in turn have been shown to affect insect size, precipitation intensity, and predation rate (Guevara & Avilés, 2007; Hoffman and
Avilés, 2017). Whereas social spiders are not considered superorganisms, there is evidence of social immunity, whereby hosts behaviourally regulate parasite loads (Keiser & Pruitt, 2014). The association between sociality and elevation, and the presence of communities of kleptoparasites in _Anelosimus_ species, provide a unique opportunity to explore how social behaviours and environment work together to influence parasite loads. Of the three expected types of social immunity—preventing parasite entry, establishment, and transmission to offspring (Cremer et al., 2007)—we considered those most relevant to the control of kleptoparasites: preventing entry, in the form of repair of the outer surface of the nest, and preventing establishment, in the form of debris removal, antifouling, and agonistic interactions with parasites. Our study is novel in that we consider how behaviours relating to social immunity change within and across species along an elevational gradient and compare how such behaviours differ between sympatric congeners that differ in their social system.

3.3 Methods

3.3.1 Site elevation

Data were collected between June and August of 2017 at sites at three elevations in the Napo Province, eastern Ecuador, each with two species in the genus _Anelosimus_. At 400 m, the Jatun Sacha Biological Reserve is a lowland tropical rainforest where two social species are relatively common. _Anelosimus eximius_ Keyserling forms colonies that may contain up to 10,000 spiders (Yip et al., 2008), being among the most social species of the genus. _Anelosimus domingo_ Levi form smaller colonies and have an adult body size about one third that of _A. eximius_. Our analyses at Jatun Sacha included 15 nests of _A. eximius_ and 13 of _A. domingo_. The habitat at 1150 m, near the township of Wawa Sumaco, corresponds to lower montane rainforest. Here we worked along a 40 km stretch of Highway 20 where _Anelosimus_ colonies were abundant along
ditches and roadcuts. *A. eximius* is also found at this site, along with *Anelosimus elegans* Agnarsson, a subsocial species. Our analyses at Sumaco included 10 nests of *A. eximius* and 17 nests of *A. elegans*. The highest elevation, at 1600 m, corresponded to cloud forest habitat. Here we located colonies along a 20 km stretch of Highway 45, near Cocodrilos, where we find *A. elegans* and the intermediate social *Anelosimus guacamayos* Agnarsson (Avilés et al., 2007; Samuk & Aviles, 2013). At Cocodrilos, analyses included 19 nests of *A. elegans* and 19 nests of *A. guacamayos*. We alternated sites twice, with a two week sampling period each, to avoid a possible confounding effect of seasonality. Four weeks separated our first and second visits to each site. We recognize that we sampled only one site per elevation and are basing our findings on a single elevational gradient. However, the ranges of our focal species limit our scale in this capacity.

3.3.2 Host characteristics and parasite loads

By “nest”, we are referring to the physical web structure built by the spiders and by “colony” the group of spiders that live in the nest. Nests of all species in the study consist of a basal, basket-shaped sheet and a cone-shaped tangle of capture lines anchoring to overhanging vegetation (Figure 1). Smaller nests, and those built by subsocial species, however, are usually found on shrubs and have low anchor points for capture lines (Figure 3.1c). The tangle portion of the nest is of interest because insect prey are intercepted proportional to its surface (Yip et al., 2008) and here is also where kleptoparasites in our system reside. To estimate density of parasites in a nest, we approximated the volume of the tangle portion of the nests as that of a cone, with its base corresponding to the ellipse-shaped cross section of the basal, basket-shaped retreat.

We collected the entire tangle portion of the nests in order to estimate the density of kleptoparasites per unit volume of tangle and the number of kleptoparasites per host spider. We
identified three morphospecies in the genus *Faiditus* Keyserling, which are the most common kleptoparasites in Amazonian *Anelosimus* webs (Fernandez-Fournier & Avilés, 2018). For this study, we only considered inquilines that are actively kleptoparasitic and do not build prey capture webs of their own. We thus did not consider passively kleptoparasitic inquilines, as those in the families Uloboridae or Tetragnathidae, which build prey capture webs. We also did not include predatory inquilines.
Figure 3.1. (A) Simplified drawing of an Anelosimus nest. A basket-shaped, basal sheet surrounding a portion of vegetation and attached to substrate above by a dense tangle of capture lines. Nest photographs of (B) A. eximius (social) (C) A. elegans (subsocial), (D) A. guacamayos (social) and (E) A. domingo (social).
3.3.3 Host anti-parasite behaviours
We focus on host behaviours related to kleptoparasitism and nest cleanliness, as they pertain to the prevention of parasite entry and establishment (Cremer et al., 2007): basal sheet repair, for the former, and antagonistic interactions with kleptoparasites, prey carcass debris removal, and defecating off the nest, for the latter. Since the basal sheet serves to separate the interior of the nest from the outside world, its repair is likely essential to keep foreign organisms from entering the nests, akin to the role of skin tissue in multicellular organisms. Spider silk may also have antimicrobial properties, as shown for an African social spider (Keiser et al., 2015). Basal sheet repair behaviours may also serve as a proxy for general nest maintenance tendencies of individuals. In order to identify areas of the basal sheet needing repair, spiders must patrol the entire border of the web. When a host encounters a kleptoparasite, it often chases it away. This act of chasing was counted as “interactions” with kleptoparasites. Prey carcass removal and defecating outside the nest are important behaviours for maintaining nest cleanliness, which prevents attracting foreign organisms, such as scavengers and fungi. We used scan sampling to measure the frequencies of these mutually exclusive behaviours, which we refer to as “nest hygiene” behaviours. We also kept track of self-grooming, even though such behaviour would pertain to defence against parasitoids and pathogens affecting individual spiders, rather than kleptoparasites affecting the entire colony. During each sampling period, nests were visited hourly, for four hours. During each visit, we counted the number of spiders performing each behaviour and the total number of spiders seen. Each nest had four sampling periods, separated by at least two weeks, for a total of 16 observations. In our analyses, we estimated the proportion of visible spiders performing each behaviour, and then averaged across all 16 observations to estimate an average proportion of visible spiders performing a behaviour at a given time. We do not account for the behaviours of spiders that we were unable to see. The proportion of spiders
performing nest hygiene behaviours is the sum of proportion of spiders performing each type of behaviour.

3.3.4 Statistical methods
For analyses, we treated elevation as a discrete variable as there were distinct communities of *Anelosimus* spiders at each of our three main sites. We used a generalized linear model with binomial error structure and logit link function to examine how tangle volume influenced the presence or absence of parasites in colonies at the three elevations. We used generalized linear fixed effects models, with species nested within sites, to examine the relationship between elevation and the average number of parasites in nests and the average percent of spiders performing nest hygiene behaviours. We used negative binomial and Gaussian distributions for number of kleptoparasites and nest hygiene frequency, respectively. A negative binomial, instead of a Poisson was used as count data were overdispersed.

Because the incidence of our focal kleptoparasites at the highest elevation was practically nil, we considered only the two lower elevations to examine how the number and density of kleptoparasites and the number of kleptoparasites per capita varied as a function of (log10 transformed) tangle volume and colony size (number of adults and subadult female within the colonies). We carried out these analyses using species and site combined in a new synthetic variable (hereafter referred to as “species-site”), which allowed us to test for differences in the slopes of the relationships across species at the two elevations. We used generalized linear models with a Poisson distribution to analyze kleptoparasite numbers and a Gamma distribution with a log-link function to analyze kleptoparasite density and kleptoparasites per capita. We used a Pearson’s Chi-squared contingency analysis to compare the frequencies of nest hygiene behaviours across species and elevation. All analyses were carried out in R (version 3.4.3).
3.4 Results
The probability of a nest being parasitized significantly decreased with increasing elevation (GLM, $X^2 = 25.19$, df = 2, p-value < 0.001) and significantly increased with increasing tangle volume (GLM, $X^2 = 9.69$, df = 1, p-value = 0.002). There were virtually no kleptoparasites at the highest elevation (Figure 2b). The presence of kleptoparasites was similarly related with nest elevation (GLM, $X^2 = 7.89$, df = 1, p-value = 0.005), where nests at lower elevations were more likely to contain at least one parasite (Figure 3.2a). Similarly, in a model where species and site were considered as random effects, as nest elevation increased, the number of kleptoparasites decreased (Figure B.1).
Figure 3.2. (A) The overall probability of nests harbouring kleptoparasites declined with elevation. (B) The probability of a nest harbouring kleptoparasites was greatest at the
lower elevations and, within elevations, increased with the volume of the tangle portion of the spiders’ webs.

The average number of parasites within nests declined with increasing elevation (GLM, likelihood ratio $X^2 = 93.04$, df = 2, p-value < 0.001), but with differences between species nested within sites (GLM, $X^2 = 32.50$, df = 3, p-value < 0.001) (Figure 3a). In parallel to the number of parasites, the average percent of spiders performing all nest hygiene behaviours also declined with increasing elevation (GLM, $X^2 = 22.70$, df = 2, p-value < 0.001) and also differed between species nested within sites (GLM, $X^2 = 18.54$, df = 3, p-value < 0.001) (Figure 3b, Figure 5).

![Figure 3.3](image)

Figure 3.3 (A) The average number of kleptoparasites in the webs was greatest in species at lower elevations, but there were also differences between species within elevations. (B) The proportion of individuals involved in nest hygiene behaviours declined with elevation, in parallel with the decline in kleptoparasite incidence with elevation. Greater
frequency of hygiene behaviours were matched by lower kleptoparasite abundance in the comparisons between *A. domingo* and *A. eximius* in the lowlands and between *A. eximius* populations at the two lower elevations (see also Figure B.2).

Considering only species at the two sites with the highest parasite densities (i.e., the two lower elevations) and nests containing at least one kleptoparasite, there was a significant interaction between the synthetic variable species-site and the volume of the tangle web on the number (GLM, $X^2 = 17.63$, df = 3, p-value < 0.001) and density (GLM, $X^2 = 10.62$, df = 3, p-value < 0.01) of kleptoparasites in the colonies (Figs. 3.4a,b). Analyzing the species at the various sites separately, the number of kleptoparasites in the nests was positively related to tangle volume for the social *A. eximius* at the two elevations, but not for the other two species, whereas the density of kleptoparasites in the colonies generally decreased with tangle volume for all but *A. elegans*, the subsocial species (Table 3.1, Figs. 3.4a,b). The number of kleptoparasites per capita also consistently declined with host colony size for all species (Table 1; Figure 4c), again with an interaction between species-site and host colony size (GLM, $X^2 = 16.99$, df = 3, p-value < 0.001). Test statistics for the individual species at the various sites are summarized in Table 1. Finally, the frequencies of hygiene behaviours also differed across species and location (Pearson $\chi^2 = 102.37$, df = 20, p-value = 0.001).
Figure 3.4. (A,D) Considering only the two lowest elevations where kleptoparasites were most abundant, their numbers (on a log10 scale) increased with tangle volume for the
social *A. eximius*, but not for the other two species; (B,E) the density of kleptoparasites per unit volume decreased as the volume of the host webs increased, but more steeply so for some of the species; (C,F) the number of kleptoparasites per capita also decreased as host spider population size increased. Panels (A-C) show non-transformed y-axes, panels (D-F) show y-axes on log10 scale.

Table 3.1. Test statistics for a series of generalized linear models to determine the effects of nest and colony size on the number and density of parasites for each species at each site, as shown in Figure 4. The two explanatory variables were log transformed in the analyses. Host colony size is the count of adults and subadults within a nest. Sign indicates whether the scaling exponent was positive (+), negative (-), or not significant, (n.s); bolded p-values indicate statistical significance. All analyses had one degree of freedom.

<table>
<thead>
<tr>
<th>Response</th>
<th>Explanatory</th>
<th>Species - Site</th>
<th>Sign (+/-)</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td># Kleptoparasites</td>
<td>Log$_{10}$ Tangle Volume cm$^3$</td>
<td><em>A. domingo</em> 400 m</td>
<td>n.s.</td>
<td>0.13</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td><em>A. eximius</em> 400 m</td>
<td>+</td>
<td>124.75</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. eximius</em> 1150 m</td>
<td>+</td>
<td>48.74</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. elegans</em> 1150 m</td>
<td>n.s.</td>
<td>0.63</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Kleptoparasites per dm$^3$</td>
<td>Log$_{10}$ Tangle Volume cm$^3$</td>
<td><em>A. domingo</em> 400 m</td>
<td>-</td>
<td>51.80</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td><em>A. eximius</em> 400 m</td>
<td>-</td>
<td>6.88</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
Hygiene behaviours generally declined with elevation, but one of the social species at the lowest elevation (*A. domingo*) had considerably greater hygiene behaviours than the other (*A. eximius*) (Figs. 3.3, 3.5). Although interspecific differences in nest hygiene behaviour appear driven by the high frequency of web repair behaviours in *A. domingo* (Figure 3.5), the trend is robust to the removal of web repair behaviours from the analysis (Figure B.3). Finally, there appeared to be a negative association between frequency of hygiene behaviours and kleptoparasite density, as suggested by a test that contrasts species that either share a common environment or are populations of the same species in different environments. The test, however, is carried out for heuristic purposes only, given inability to simultaneously control for non-independence for two factors (Figure B.2).
Figure 3.5. Average proportion of spiders in a given species that exhibited nest and individual hygiene behaviours. These include: removing debris from the nest, defecating outside the nest, direct encounters with kleptoparasites, repairing the basal sheet of the nest, and self-grooming.

3.5 Discussion

Elevation and host characteristics, such as tangle volume, species, and nest hygiene behaviours, were all associated with the presence and abundance of kleptoparasites in nests of social and
subsocial species in the spider genus *Anelosimus*. The incidence of kleptoparasites was greatest at the lower elevations (Figure 3.2, 3.3), where the frequency of host behaviours to maintain nest conditions and chase away kleptoparasites were also greatest (Figure 3). At the two lower elevations, where there was substantial kleptoparasite presence, parasite numbers increased with colony size for *A. eximius*, the social species with the broadest colony size range, but not for the other two species (Figure 3.4a). Interestingly, kleptoparasite density decreased with the size of the host colony, but more steeply for some species (Figs. 3.4b). In particular, the decline in kleptoparasite density was greatest for the social *A. domingo*, which was also the species with the most frequent nest hygiene behaviours (Figure 3.5). These findings suggest that parasite loads are influenced by external environmental factors, but that host characteristics and behaviours also play a role.

3.5.1 Elevation
Elevation appeared to be the most important factor associated with the presence and abundance of parasites (Figure 3.2, Figure 3.3). It is well known that temperature decreases with elevation, resulting in a parallel decline in primary productivity (Del Grosso et al., 2008). The size of insects, which are food for the spiders, also decline with elevation (Guevara & Avilés, 2007, 2015). As a result, we would expect fewer resources and lower rates of energy exchange between colonies and their environment at higher elevations, where colder temperatures mean fewer and smaller prey for colonies to process (Powers & Avilés, 2007). Additionally, individual metabolic rate is also expected to decrease with temperature (Peakall & Witt, 1976), which may result in the spiders requiring fewer resources and greater prey processing times at higher elevations. With fewer resources being processed, colonies at higher elevations may not be able to support large populations of kleptoparasites, somewhat analogous to what occurs in vertebrates where
host metabolism can determine the resources parasites can receive, limiting parasite biomass (Poulin & George-Nascimento, 2007). We thus suggest that something analogous to lower metabolic rates of individuals, applied at the colony level, may be limiting kleptoparasite abundance at higher elevations. Evidence for colony-level metabolism exists for eusocial hymenopterans and termites (Hou et al., 2010; Waters et al., 2010), whereby whole colony metabolism and mass-specific metabolic rate scale with colony mass, in a similar fashion as for unitary organisms. Whole colonies may thus be subject to the similar temperature scaling laws as whole organisms, where an increase in temperature corresponds to faster chemical reactions and thus higher metabolic rate (Brown et al., 2004).

3.5.2 Kleptoparasite number and host’s nest size
In addition to elevation, host characteristics, such as tangle web volume and colony population size, were important in determining abundance and density of kleptoparasites (Figure 4.4), albeit with differences among species. The number of kleptoparasites increased with tangle web volume in the social *A. eximius* at the low and mid- elevations, but at a lower rate at the higher elevation (Figure 4.4). A similar relationship between web size and parasite loads has been reported for the solitary *Nephila clavipes*, where larger webs harbored more kleptoparasites (Agnarsson, 2003). In our study, however, kleptoparasite numbers did not increase with web size for the other two species. *A. elegans* had colonies limited to a few dozen individuals, as it is a subsocial species with colonies containing single family units. The other species, *A. domingo*, however, is social, with colonies that may grow to contain a few thousand individuals (Guevara & Aviles, 2011). As we suggest below, this species may have better mechanisms than its sympatric and congeneric *A. eximius* to maintain its nests’ cleanliness and reduce the incidence of parasites.
3.5.3 Kleptoparasite density and host’s nest size

Interestingly, in all species, parasite density declined with increasing hosts’ web and colony size (Figure 3.4), consistent with observations reported by Fernandez-Fournier et al. (2018) in an earlier study. We propose two main hypotheses to explain this phenomenon: (1) interference competition between kleptoparasites, in interaction with scaling properties of the host spiders’ three dimensional webs, and (2) better detection of foreign spiders on the part of hosts in larger colonies or in colonies with more aggressive individuals.

Within a social spider colony, parasites likely interfere with one another to reach a carrying capacity, which in turn should depend on the amount of resources the patches receive. Those resources may decline with host colony size due to scaling properties of the hosts’ three-dimensional webs. This is because the number of host spiders a web contains should be proportional to the web’s volume, whereas the number of prey intercepted should be proportional to the web’s surface area. As the surface area of 3D objects does not increase as fast as their volume as the objects grow in size, the number of prey per host spider should decline with colony size, as shown for the social A. eximius (Yip et al., 2008). In areas with an abundance of large insects, the spiders compensate for the decline in number of prey by capturing larger insects (Yip et al., 2008). Larger prey elicit a response from a greater number of host spiders (Souza et al., 2007; personal observation), which should reduce the kleptoparasites’ ability to steal prey (Cangialosi, 1990). Additionally, without cooperation, the parasites should be unable to handle insects much larger than their own body size. A reduced opportunity to steal small prey in larger colonies may thus explain the decline in kleptoparasite density with increasing colony size in social and subsocial spiders.
The behaviour of the host spiders may also play a role, as larger colonies may be better at maintaining the web in good condition and detecting and responding to kleptoparasites than smaller colonies. Some species may also be better at keeping parasites at bay, either through better hygiene or more aggressive behaviours, as we suggest for the social *A. domingo* in the following section. If the response is more effective when more individuals are present in the colonies, then parasite density may decline with colony size at a steeper rate, as observed in the social *A. domingo* relative to the remaining species (Figure 4). In either case, more research is needed to determine the underlying mechanisms for a negative relationship between host colony size and parasite density, which may very well be a combination of the two hypotheses here proposed.

### 3.5.4 Host’s nest hygiene behaviours

In general, sites that had more kleptoparasites also had more frequent nest hygiene behaviours (Figure 3.3), a pattern that held whether web repair was included in the analyses or not (Figure B.3). When comparing species that shared the same habitat, or populations of the same species in different habitats (Figure S2), there was a general trend for species with greater frequency of hygiene behaviours to have a lower parasite load. In particular, *A. domingo* had the highest proportion of spiders performing hygiene behaviours (Figs 3 & 5), which correlated with a lower overall parasite load relative to its sympatric congener *A. eximius* (Figure B.2).

When examining the breakdown of behaviours by species and location, we found that *A. domingo* had an increased effort in repairing the basal sheet of the nest (Figure 3.5). This may be indicative of a general ability to maintain the entire web in good condition. Given that kleptoparasites clear silk lines in order to steal prey without alerting the host (Cangialosi, 1991; personal observation), it is possible that *A. domingo*’s more frequent web repair behaviour may
give it a better ability to detect and fend off kleptoparasites. *A. domingo* also had relatively low levels of self-grooming, which, in turn, may reflect lower rates of parasitism by parasitoid wasps (Family: Ichneumonidae), whose role in this system we are in the process of analyzing.

It is also possible that *A. domingo*, despite of (or because of) its smaller size (about one third, Guevara and Avilés, 2011) relative to the sympatric *A. eximius*, may be an overall more effective and aggressive species. Previous work found that the smaller *A. domingo* has more densely populated nests, is faster at responding to struggling prey, and involves more individuals in prey capture, than its sympatric congener *A. eximius* (Guevara and Avilés, 2011). These traits, which result in better ability to detect and respond to prey (Guevara and Avilés, 2011), may also translate to a better ability to detect and respond to kleptoparasites. *A. domingo* also appears generally more aggressive than *A. eximius* (pers. obs.). More aggressive species, or colonies with more aggressive individuals, may be more effective at keeping parasites at bay. Ant colonies with aggressive guards, for instance, fare better when under attack by slave-maker ants (Kleeberg et al. 2014). Likewise, in the temperate and subsocial *Anelosimus studiosus*, nests that consisted of more aggressive individuals were better able to reduce parasite loads (Pruitt, 2013). Thus, it is possible that *A. domingo*, in addition to doing a better job at web repair (Figure 3.5), may have more aggressive individuals to patrol and respond to kleptoparasites. A final possibility is that *A. domingo*, because of its smaller body size and less substantial webs, which capture smaller insects (Guevara and Avilés, 2011), may not be as profitable a species for kleptoparasites to steal prey from.

**3.5.5 Conclusion**

In general, our results illustrate how environmental factors interact with host characteristics to determine parasite loads and how these apply to organisms that live in groups. On the one hand,
environmental factors, temperature, in particular, are likely to determine the amount of energy 
and resources that can flow from hosts to parasites, thus determining external parasite pressures. 
Actual loads, however, should also be a function of both the physical characteristics of the hosts, 
such as their size, and the ability of the hosts to prevent entry, establishment, and transmission of 
the parasites. We have used social spiders to illustrate how characteristics of entire colonies and 
the social and individual behaviours of their members can interact with environmental factors to 
determine parasite loads along an elevation gradient. Because elevation correlates tightly with 
temperature, which in turn affects energy flows and metabolic rates, elevational gradients are 
ideal settings to analyze the role of the environment in the intensity of biotic interactions and the 
interplay between environmental and organismal features. There are several studies on predation 
and parasitism across latitudes (e.g., Schemske et al., 2009; Roslin et al., 2017), but markedly 
fewer along elevational gradients. In one of the few other studies looking specifically at the 
relationship between parasites and elevation, Zamora-Vilchis et al. (2012) found that the 
prevalence of blood parasites in birds decreased with elevation, a finding that parallels that in our 
study. This suggests a relationship between temperature and parasitism that may be general 
across taxa. Such patterns may serve to predict changes in species interactions in a changing 
climate. As changes in elevation affect temperature while maintaining photoperiod and 
seasonality, elevational gradients may be a better tool than latitude for predicting the 
consequences of climate change. Ours and Zamora-Vilchis et al. (2012) findings suggest that 
increased parasite loads may be expected as climate change leads to higher temperatures around 
the globe.

Data Accessibility
Data associated with this article are deposited in the Dryad Digital Repository

https://doi.org/10.5061/dryad.vk81vp8 (Straus & Avilés, 2018)
Chapter 4: Kleptoparasites of *Anelosimus eximius* are not limited by dispersal between host colonies

4.1 Chapter summary
Organisms with lower dispersal abilities tend to have more genetically dissimilar populations. The same is true for parasites, where transmission (i.e., dispersal) may depend on the population structure of the host. This should be especially true when hosts and parasites face similar barriers to dispersal. In the present study, we consider the similarities between host and parasite population structure in a social spider system. In this system, host colonies are typified by rapid growth via internal recruitment followed by budding or fission events when colonies grow too large, with each colony representing a distinct population. Host colonies provide habitat for confamilial kleptoparasitic spiders, which steal prey that the host colony members have spent resources to subdue. We asked whether kleptoparasites exhibit similar degrees of population subdivision as their host. Under the free-mixing hypothesis (i.e., horizontal transmission), kleptoparasites would have well-mixed populations across broader regions than a single host nest, whereas host populations would be strongly genetically structured. Under the host-tracking hypothesis (i.e., vertical transmission), kleptoparasites would have a population structure that parallels that of the host. We conducted a Genotype-By-Sequencing study to assess the population structure of both hosts and kleptoparasites within three nearby regions in eastern Ecuador. We found strong signatures of population differentiation and bottlenecks in the host species, congruent with past studies. However, we found that kleptoparasite populations were well mixed across host nests, with no evidence of recent bottlenecks. These results support our free-mixing hypothesis, suggesting that kleptoparasites follow patterns of horizontal transmission in this social spider system.
4.2 Introduction

There exists a negative correlation between dispersal ability and population differentiation, such that organisms with limited dispersal abilities would have more genetically subdivided populations over a given area (Peterson & Denno, 1998) and greater inbreeding tolerance (Waser et al. 1986; Perrin & Mazalov, 1999). In the context of parasitism, dispersal should be associated with transmission across host individuals (Frank, 1996; Harbison et al., 2008): parasites leave the habitat provided by one host and establish in another host. A parasite’s dispersal (i.e., transmission) potential may depend on the population structure of the host, such that parasites may disperse more easily between hosts that are closer in space than those that are more widely spaced (Poulin 2003). The correspondence between host and parasite population structures is particularly relevant when considering vertical versus horizontal transmission (Fig. 4.1). In vertically transmitted parasite systems, parasites pass from a host to its offspring (Ebert & Herre, 1996), in which case, the parasite’s population genetic structure should parallel that of the host. On the other hand, horizontal transmission would occur between unrelated host individuals (Ebert & Herre, 1996); under this scenario, parasites may have populations structured independently of that of the host. These two modes of transmission should also apply to parasites that infect levels of organization above that of the individual, such as colonies or social groups. In the social context, vertical transmission would occur between mother and daughter colonies and horizontal transmission across unrelated colonies, regardless of lineage (Cremer et al., 2007). Modes and rates of transmission are key parameters in models of host-parasite theory (Anderson & May, 1979). The study of transmission among groups of social hosts should shed light on host-parasite coevolution in a social context.
In the present study, we explore the similarities between host and parasite population structures in a Neotropical social spider. The social spider, *Anelosimus eximius* Keyserling (Theridiidae), forms colonies that contain dozens to tens of thousands of individuals (Yip et al., 2008). Together, the spiders within the colony build a communal structure, which we refer to as a “nest”. The nest is a sheet-and-tangle type web, which accumulates plant debris that provides shelter for resting, feeding, and brood care. Colonies grow by internal recruitment and, when they become too large, may split through a process known as fission. Alternatively, individuals or groups of inseminated females may disperse to form small daughter colonies in the vicinity of the mother colony, a process known as budding (Agnarsson et al., 2010). Both forms of colony propagation represent a founder event (Figure 4.1), which, along with the process of intracolony mating, results in colonies having strongly subdivided population structures (Smith & Hagen, 1996). In addition, colonies tend to be spatially clustered (Agnarsson et al., 2010). The overall population structure thus consists of individuals grouped within nests, nests grouped within nests clusters (nests found within 5 m of each other), and nest clusters occurring within relatively distinct geographical regions.

Nests of social spiders tend to be host to a variety of inquiline organisms (Su & Smith, 2014; Fernandez-Fournier & Avilés, 2018). Inquilines may have neutral, facilitative, or antagonistic interactions with their host (Bono, 2007; DeSouza et al., 2016; Fernandez-Fournier & Avilés, 2018). In the case of *A. eximius* inquilines, we focus here on two members of the spider genus *Faiditus* Keyserling (Theridiidae, formerly *Argyrodes*), *Faiditus cf. ululans* (hereafter *Faiditus* sp. 1) and *Faiditus cf. flavescens* (hereafter *Faiditus* sp. 2) (Figure 2). *Faiditus* kleptoparasites and their *Anelosimus* hosts belong to sister clades within the family Theridiidae (Agnarsson, 2004; Arnedo et al., 2004). Rather than building their own prey capture webs, members in the
genus *Faiditus* reside in the webs of other spiders where they steal prey that the hosts have subdued (Cangialosi, 1990a, 1991). *Faiditus* kleptoparasites occur globally in the tropics, most typically in the webs of orb weaving spiders (Su & Smith, 2014). Some species exhibit cooperative behaviour and maternal care, with more cooperative species exhibiting more genetically structured populations (Su et al., 2018). Previous work in our system suggests that *Faiditus* sp. 1 and *Faiditus* sp. 2 are strongly associated with *A. eximius*, although they may occasionally occur in webs of other species and also in the forest matrix (Fernandez-Fournier & Avilés, 2018). *Faiditus* sp. 1 and *Faiditus* sp. 2 court, mate, and guard their egg sacs within the host colony (Straus, personal observation). Nests of *A. eximius* may contain anywhere from zero to dozens of kleptoparasite individuals. Larger *Anelosimus* nests contain a greater total number of kleptoparasites, but kleptoparasite density decreases with nest size. Kleptoparasite load also declines with elevation, and more social species of *Anelosimus*, which occur at lower elevations, have higher kleptoparasite burdens than the less social species occurring at higher elevations (Chapter 3; Straus & Aviles, 2019). *Faiditus* sp. 1, co-occurs with *A. eximius* across its eastern Andean range, ranging from 200 to 1300 m elevation. Abundance of this species declines with increasing elevation (Straus & Aviles, 2019). *Faiditus* sp. 2 occurs only at low elevations, where it is more abundant than *Faiditus* sp. 1 (Straus, personal observation).

To understand whether there is a correspondence in the genetic population structure of the social host and the two kleptoparasitic species, we performed Genotype-by-sequencing (GBS) analyses on individuals of the three species sampled from colonies across three regions in eastern Ecuador. We used K-means cluster analyses to assess the number of distinct genetic clusters and used an analysis of molecular variance and F-statistics to compare the genetic structure of host and parasites among and within populations. We considered two alternative hypotheses. Under
our *free mixing* hypothesis (i.e., horizontal transmission), kleptoparasites are not subject to the same barriers to dispersal as are their hosts, and thus parasite population structure will be well mixed within a geographic region, whereas the hosts’ would not be, as previous studies have shown (Smith & Hagen, 1996; Agnarsson et al., 2010), highly structured (Figure 4.1). Under our *host-tracking* hypothesis (i.e., vertical transmission), kleptoparasites would be subject to the same barriers to dispersal and as such both the kleptoparasite and host population structures will be strongly subdivided (Figure 4.1).

![Figure 4.1](image)

Figure 4.1. (a) Under our free-mixing hypothesis, kleptoparasites lineages (orange, green, pink) do not face the same barriers to dispersal as their hosts (blue) and are able to move freely between neighboring *Anelosimus eximius* colonies. (b) Under our host-tracking hypothesis, kleptoparasites face similar barriers to dispersal as their hosts, and kleptoparasite lineages track host lineages through time.
Figure 4.2 (a). *Faiditus* sp. 1 (*Faiditus cf. ululans*), (b) *Faiditus* sp. 2 (*Faiditus cf. flavescens*). Scale bar: 3 mm. Photo credits: Samantha Straus, Jatun Sacha, 2017

4.3 Methods

4.3.1 Model system and study area

We sampled *A. eximius* colonies from three regions in the Napo province of eastern Ecuador (Figure 4.3), and *Faiditus* spp. from all colonies that contained them. Our lowest elevation region, at 400 m a.s.l., was located at the Jatun Sacha Biological Reserve (1.0644S to 1.0878S, 77.6146W to 77.6284W, 400 m a.s.l.). At this region, we sampled 60 *A. eximius* from 12 colonies. Both species of kleptoparasitic *Faiditus* occurred in this region. From Jatun Sacha we collected 18 *Faiditus* sp. 1 from 7 colonies, and 29 *Faiditus* sp. 2 from 7 colonies; only one of these colonies contained both species of kleptoparasites. *Faiditus* sp. 2 occurs only at this lowest elevation region, whereas *Faiditus* sp. 1 decreased in abundance with elevation (Straus & Aviles, 2019). At our mid-elevation region, located outside the township of Archidona on Hwy 45 (0.8116S, 77.7801W, 900 m a.s.l.), we collected 24 *A. eximius* from 5 colonies and 7 *Faiditus* sp. 1 from 4 colonies. Finally, at our highest elevation region, between km 9.5 and km 47.3 along Hwy 20 (0.0708S to 0.7263S, 77.5988W to 77.7486W, 1150 m a.s.l.), we collected 54 *A. eximius*
from 12 colonies and 7 *Faiditus* sp. 1 from 2 colonies. We stored collected specimens in 95% ethanol at -20°C for one year before DNA library preparation.

Figure 4.3. Map of study regions within eastern Ecuador. Green pins represent nests at Jatun Sacha, blue represents Archidona, and pink represent Via Loreto.

This study uses Genotyping-by-sequencing, a type of restriction site-associated DNA sequencing (Peterson et al., 2012), to infer whether the population structure of the kleptoparasites mirrors that of the host. Importantly, GBS does not require a reference genome, making it a flexible tool for non-model organisms (Peterson et al., 2012). This process uses restriction enzymes to cut the DNA into fragments, sequencing, and assembling a *de novo* genome from the sequenced DNA fragments. Ideally, the same loci will have been sequenced from many individuals, creating “stacks” of reads. Stacks of reads occurring at the same place on a species’ genome are then used to identify single nucleotide polymorphisms (SNPs) (Andrews et al. 2016).
4.3.2  Library preparation and sequencing
We first placed whole spiders into individual 2 mL microcentrifuge (manufacturer: Eppendorf™) tubes, flash froze them using liquid Nitrogen, and then ground them using a sterilized pestle. Next, we extracted and purified DNA using the QIAGEN DNeasy™ Blood and Tissue Kits, following their supplemental insect protocol. Then, we followed a double-digest genotyping-by-sequencing protocol, modified from Poland et al. (2012) to generate DNA libraries, using PstI HF and MspI as our restriction enzymes (New England Biosciences). The process involved digesting the DNA with restriction enzymes, ligating unique, identifying barcodes and Illumina™ adapters to the DNA fragments from each individual, amplifying via PCR, and manually size-selecting via gel electrophoresis in 150mL of 1.5% agarose gel, run on 120V for 20 minutes, followed by 80V for 50 minutes. We extracted the size-selected DNA libraries manually from the agarose gel using the QIAquick™ Gel Extraction Kit. Samples were sequenced on one lane of 150-bp paired-end Illumina NovaSeq 6000 at Génome Québec (Montreal, Québec, Canada).

4.3.2  Bioinformatics and analysis
We used the STACKS (Version 2.4.1) `process_radtags` function to demultiplex our raw Illumina reads, which separates out each sequence read by individual, and removes identifying barcodes, the restriction region, and the Illumina adapters (Catchen et al., 2013). To create a de novo alignment, we used the `denovo_map.pl` module with default values, followed by the `populations` module. We specified that an allele must be present in 70% of the populations (-r 70) and occur in at least three populations (-p 3) to be processed. In our next step, we used `vcftools` to filter our data (Danecek et al., 2011). We excluded loci missing greater than 50% of their data, except in the case of *Faiditus* sp. 2, which we filtered at 25% to retain sufficient SNPs for downstream analysis. We only included loci where the minor allele occurred at least three times and had a
frequency of at least 0.05%, and loci that had a mean minimum read depth of at least five. Finally, we excluded individuals exceeding 50% missing data.

We assumed that our populations followed a hierarchical structure, such that nests occurred within colony clusters, and clusters occurred within study regions. We used the R packages “vcfR” and “adegenet” to convert our vcf files into genlight objects, and used the `strata` function to specify the population hierarchy described (Knaus & Grünwald, 2017; Jombart, 2008). We considered nests within 5 m of each other to be nests clusters to account for nests close in space likely having a shared lineage through budding or fission. At each of our three regions, there was only one instance of nest clustering, with the rest of the nests being more distantly spaced. However, after filtering, all retained samples of both *Faiditus* species belonged to nests from different clusters, such that the number of nests equaled the number of clusters. Thus, we dropped this level of the hierarchy in the analyses for the *Faiditus* kleptoparasites.

Next, we performed an AMOVA (analysis of molecular variance) using the “poppr” (`poppr.amova` function) and “ade4” (`randtest` function) packages (Kamvar et al, 2015; Thioulouse et al., 2018), using the same hierarchical structure as above and a quasi-euclidean distance matrix. We used the `glPca` and `find.clusters` functions in the “adegenet” package, in conjunction with the elbow method, to assess the number of K-means clusters. Finally using the preferred number of K-means clusters, we conducted a Discriminant Analysis of Principal Components using the `dape` function.
We used the R package, “hierfstat”, to calculate Weir and Cockerham F-statistics (Goudet & Jombart, 2020) at several levels of organization: we used \( F_{RG} \) to denote differentiation at the region level relative to the global population and \( F_{NR} \) for nest-level differentiation relative to region-level. \( F_{IN} \) denotes genetic differentiation of individuals within the same colony. We built our genlight object in the “adegenet” package such that nests were hierarchically structured within nest clusters, but we did not include cluster as a level in our analyses due to the small sample size mentioned above. We used the package “dartR” and the functions \( gl.Ho \) and \( gl.He \) to calculate expected and observed heterozygosity (Gruber et al., 2008).

4.4 Results
After filtering, we had 19,680 SNPs identified from 108 individuals representing 25 nests for \( A. eximius \), 7,400 SNPS from 19 individuals and 9 nests for \( Faiditus \) sp. 1 and 1,901 SNPS from 12 individuals and 4 nests for \( Faiditus \) sp. 2. Mean allelic richness for \( A. eximius \) was 1.74, 1.63, and 1.29 for Jatun Sacha, Archidona, and Via Loreto, respectively. For \( Faiditus \) sp. 1, mean allelic richness was 1.74, 1.68, and 1.66 for Jatun Sacha, Archidona, and Via Loreto, respectively. Mean allelic richness was 1.26 for \( Faiditus \) sp. 2 in Jatun Sacha, the only region where it occurred.

Our analysis of molecular variance showed that \( A. eximius \) had significantly greater differentiation between regions, between clusters within regions, and between nests within clusters than expected under a null model of global mixing at the various hierarchical levels, and significantly less variation than expected within individuals (Table 4.1). We found that variation among individuals within a nest was not significantly different than expected under a null model of panmixia within colonies (Table 4.1). For \( Faiditus \) sp1., we found significantly less variation within individuals than expected, whereas variance among individuals within a nest and between
nests within a region were greater than expected. In *Faiditus* sp1, genetic variance due to
differentiation among regions was not significantly different than expected under a model of
global mixing. For *Faiditus* sp2., genetic variance was significantly greater than expected
between individuals in a nest, and less than expected within individuals, whereas we found no
significant variance between nests (Table 4.1).

Table 4.1. Analysis of molecular variance with a hierarchical population structure, such
that individuals (samples) are nested within nests, nests within clusters, and clusters
within regions. Bolded values indicate statistical significance and (+) or (-) indicates
whether variance was greater or less than expected, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Components of covariance</th>
<th>sigma</th>
<th>phi</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. eximius</em></td>
<td>Between regions</td>
<td>293.27</td>
<td>0.24</td>
<td>2</td>
<td>0.01 (+)</td>
</tr>
<tr>
<td></td>
<td>Between clusters within regions</td>
<td>408.19</td>
<td>0.44</td>
<td>14</td>
<td>0.04 (+)</td>
</tr>
<tr>
<td></td>
<td>Between nests within clusters</td>
<td>103.21</td>
<td>0.20</td>
<td>8</td>
<td>0.02 (+)</td>
</tr>
<tr>
<td></td>
<td>Between individuals within nests</td>
<td>-31.49</td>
<td>-0.08</td>
<td>83</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Within individuals</td>
<td>439.62</td>
<td>0.64</td>
<td>108</td>
<td>0.01 (-)</td>
</tr>
<tr>
<td><em>Faiditus</em> sp. 1</td>
<td>Between regions</td>
<td>0.38</td>
<td>0.003</td>
<td>2</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Between nests within regions</td>
<td>10.47</td>
<td>0.077</td>
<td>6</td>
<td>0.01 (+)</td>
</tr>
<tr>
<td></td>
<td>Between individuals within nests</td>
<td>17.63</td>
<td>0.14</td>
<td>10</td>
<td>0.04 (+)</td>
</tr>
<tr>
<td></td>
<td>Within individuals</td>
<td>108.32</td>
<td>0.21</td>
<td>19</td>
<td>0.01 (-)</td>
</tr>
<tr>
<td><em>Faiditus</em> sp. 2</td>
<td>Between nests</td>
<td>-80.06</td>
<td>-0.19</td>
<td>3</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Between individuals within nests</td>
<td>384.29</td>
<td>0.77</td>
<td>8</td>
<td>0.01 (+)</td>
</tr>
<tr>
<td></td>
<td>Within individuals</td>
<td>114.92</td>
<td>0.73</td>
<td>12</td>
<td>0.01 (-)</td>
</tr>
</tbody>
</table>
For *A. eximius*, at the regional level, we detected five distinct genetic clusters (Figure 4.4a). One of these genetic clusters included individuals from Jatun Sacha and Archidona, whereas all other clusters had membership only from a single region. Within regions, at Jatun Sacha (Figure 4.4b), four of the six K-clusters had membership from a single nest, while the remainder had mixed membership. Similarly, at Archidona (Figure 4.4c), two of three clusters had membership exclusive to just one nest, while the third had mixed membership. At Via Loreto (Figure 4.4d), all K-clusters had mixed membership.

Figure 4.4. Discriminant analysis of principal components identified optimal number of groups (k) for *A. eximius*. Panel (a) shows all individuals from all regions, panel (b) individuals from nests from Jatun Sacha, (c) from Archidona, and (d) from Via Loreto. Shape indicates K-means cluster grouping level, and colour indicates source region (a) or nest (b-d).
For *Faiditus* sp. 1, we detected five K-clusters at the region level with mixed membership, such that individuals from different regions were found to belong to the same K-cluster (Figure 4.5a). At Jatun Sacha, we detected three clusters, with mixed nest membership for each cluster (Figure 4.5b). We did not detect distinct clusters for either Archidona or Via Loreto. Likewise, we found no distinct K-clusters for *Faiditus* sp. 2 at Jatun Sacha.

![Figure 4.5](image.png)

Figure 4.5. Discriminant analysis of principal components identified optimal number of groups (k) for *Faiditus* sp. 1. Panel (a) shows all individuals from all regions, panel (b), individuals from nests from Jatun Sacha.

Considering Wright’s F-statistics (Wier & Cockerham, 1984), globally *A. eximius* had an \(F_{RG}\) of 0.24, while *Faiditus* sp. 1 had an \(F_{RG}\) of 0.04 (Table 4.2). In terms of differentiation at the level of colonies within regions, \(F_{NR}\) values for *A. eximius* ranged from 0.68 at Archidona to 0.10 at Via Loreto. For *Faiditus* sp. 1, \(F_{NR}\) values were generally lower, ranging from 0.13 to 0.01. \(F_{IN}\) values were negative for *A. eximius* across all regions, ranging from -0.26 to -0.60, while positive for *Faiditus* sp. 1 at all regions, ranging from 0.17 to 0.36 (Table 4.1). Finally, *Faiditus* sp. 2 had undetectable population subdivision with an \(F_{NR}\) of approximately zero and an \(F_{IN}\) value of 0.75 (Table 4.1).
Table 4.2. F-statistics and expected (Hₑ) and observed (Hₒ) heterozygosity values for subpopulations relative to total populations, for each species and region.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites</th>
<th>Among regions (Fₘ)</th>
<th>Among colonies (Fₙ)</th>
<th>Within colonies (Fᵢ)</th>
<th>He</th>
<th>Ho</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. eximius</em></td>
<td>Global</td>
<td>0.24</td>
<td></td>
<td></td>
<td>0.30</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Jatun Sacha</td>
<td>0.37</td>
<td>-0.39</td>
<td>0.28</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Archidona</td>
<td>0.68</td>
<td>-0.60</td>
<td>0.30</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Via Loreto</td>
<td>0.10</td>
<td>-0.26</td>
<td>0.17</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td><em>Faiditus</em> sp. 1</td>
<td>Global</td>
<td>0.04</td>
<td></td>
<td></td>
<td>0.31</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Jatun Sacha</td>
<td>0.01</td>
<td>0.27</td>
<td>0.27</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Archidona</td>
<td>0.01</td>
<td>0.36</td>
<td>0.27</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Via Loreto</td>
<td>0.13</td>
<td>0.17</td>
<td>0.26</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td><em>Faiditus</em> sp. 2</td>
<td>Jatun Sacha</td>
<td>-0.15</td>
<td>0.75</td>
<td>0.37</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

### 4.5 Discussion

We find support for our free-movement hypothesis and reject our host-tracking hypothesis for populations of kleptoparasites in colonies of the social spider *Anelosimus eximius* (Figure 4.1).

As expected from previous studies (e.g. Smith & Hagen, 1996, Agnarsson et al., 2010), we found that *A. eximius* populations were highly genetically structured. Conversely, both *Faiditus* species
showed genetic evidence of mixing at various spatial scales, consistent with the horizontal transmission hypothesis.

Consistent with previous studies on the genetic structure of *A. eximius* (Smith & Hagen, 1996; Agnarsson et al., 2010) and other social spiders (e.g., *Stegodyphus sarasinorum*, Smith & Engel, 1994; *Parasteatoda wau*, Lubin & Crozier, 1985), our analysis of molecular variance revealed significant genetic differentiation between nests within clusters, and less variation than expected between individuals within colonies (Table 4.1). Likewise, using K-means clusters to assign individuals to groups, for *A. eximius* we detected genetically distinct groupings of individuals both globally and within each region. For *A. eximius*, the K-means clusters roughly corresponded to the region and nest to which individuals belonged, with some exceptions (Figure 4.4). In the kleptoparasitic *Faiditus* sp. 1 however, our analysis of molecular variance found less genetic variation than expected within individuals, but no significant differences between observed and expected variation between regions or nests (Table 4.1). While we did detect distinct K-clusters at the regional level and within Jatun Sacha (Figure 4.5), our AMOVA results for *Faiditus* sp. 1 suggest that the groups are not significantly differentiated, and that this species does not face similar barriers to dispersal at the regional level as their host. For *Faiditus* sp. 2, our AMOVA did not detect any significant variation between nests, while we did detect greater than expected variation between individuals within the same nest, and within individuals. These results, combined with an inability to detect any K-clusters, suggests that individuals of this species face virtually no barriers to dispersal within the Jatun Sacha study region.

Our among-region F-statistic (F<sub>RG</sub>) was 0.24 for *A. eximius*, compared to 0.04 for *Faiditus* sp. 1 (Table 4.2). These findings suggest that population subdivision occurs at regional scales for *A. eximius*, but not for *Faiditus* sp. 1. Within each region, among-colony F-statistics (F<sub>NR</sub>) were
generally high for *A. eximius*, ranging from 0.10 to 0.68 (Table 4.2). FNR values were generally low for *Faiditus* sp. 1, ranging from 0.01 to 0.13, suggesting generally lower population subdivision among nests for the kleptoparasite than the host. Similarly, we detected no population subdivision among nests for *Faiditus* sp 2., suggesting complete panmixia within the population at Jatun Sacha (Table 4.2), the only region at which this species occurred.

Interestingly, at our Via Loreto region, FNR values were substantially lower for *A. eximius* than in other regions. This may have occurred for several reasons. First, the mean allelic richness was low, 1.28, in the Via Loreto region, compared to 1.74 and 1.63 for Jatun Sacha and Archidona, respectively. Lower allelic richness at this site may have influenced FNR. Alternatively, this region may be experiencing a greater degree of mixing than our other regions. This may be, in part, because all nests sampled at this region occurred along a roadside rather than in the forest, where anthropogenic activity (e.g., roadcut clearing) may act to artificially mix populations. In addition, study of this species in these locations has been ongoing by several research groups for the past few decades, and we cannot rule out the possibility of unintentional, artificial mixing of colonies via past research activities. In contrast to *A. eximius*, the FNR values for *Faiditus* sp 1. appeared highest at Via Loreto compared to other regions (Table 4.2).

Among individuals within colonies, FIN values were overwhelmingly negative for *A. eximius*, with some values as low as -0.60 (Table 4.2). These negative FIN values suggest the presence of recent bottlenecks throughout our *A. eximius* populations (Hundertmark & Van Daele, 2014). Population bottlenecks may lead to a loss of rare alleles and a temporary excess of heterozygosity, which may translate in negative FIN values (Allendorf, 1986; Hedrick et al., 1986). Smith and Hagen likewise found a negative FIN in *Anelosimus* eximius using allozymes and suggested that this should be expected in isolated subpopulations (Smith & Hagen, 1996).
This is consistent with known dispersal patterns of this species, where gravid females establish colonies on their own, in small groups, or new colonies are produced via budding, and then grow through internal recruitment (Figure 4.1; Aviles & Purcell, 2012). Conversely, $F_{IN}$ values were all positive for both *Faiditus* species, suggesting a heterozygosity deficiency typical of inbreeding. In the case of *Faiditus* sp 2., we obtained an $F_{IN}$ value of 0.75, suggesting extreme heterozygote deficiency. However, this extremely high value may have been driven by a small sample size. We suspect that, while the population is panmictic, the population size is small, leading to reduction of genetic diversity over time. For both *Faiditus* spp., positive $F_{IN}$ values suggest the absence of a recent bottlenecks within the populations, and that kleptoparasites are not founding new populations simultaneously with their host.

The $F_{NR}$ we detected in our *A. eximius* population is substantially lower than what has previously been calculated for this species (Smith & Hagen, 1996; Agnarsson et al., 2010). We suspect that this may be due to the different genetic markers being used. In a study comparing measures of population subdivision in *Arabidopsis* using microsatellites versus SNPs, Fischer et al. (2017) found that microsatellite estimates of $F_{ST}$ were over 3-fold higher than those obtained using SNPs (2017). Another study found that, while $F_{ST}$ values differed when considering microsatellites versus SNPs, that the values correlated (Zimmerman et al., 2020). SNPs may be more precise at detecting genetic clustering than microsatellites due to the large number of loci and more precise confidence intervals generated using SNP data (Zimmerman et al., 2020). Furthermore, F-statistics may differ depending on whether they are calculated on coding vs. non-coding regions (Whitlock & Mccauley, 1999). As the SNPs in this study are genome wide, they should include both coding and non-coding regions.
Our support for the free-movement hypothesis (Figure 4.1) is congruent with findings in other studies of exoparasites, such as tick parasites of seabirds (McCoy et al., 2003), where dispersal of ticks between host populations appeared to be greater than expected. This is perhaps not surprising given that ticks have free-living life stages. In our study, we suspect that kleptoparasites may be able to exploit other hosts. *Faiditus* sp. 1, in particular, has been observed to inhabit colonies of *Anelosimus domingo* Levi, a potential social host species that co-occurs with *A. eximius* at Jatun Sacha and other lowland tropical rainforest areas. *A. domingo*, however, appears to be a less frequent host of this kleptoparasite than *A. eximius* (Fernandez-Fournier & Aviles, 2018; Straus & Avilés, 2019). We found *Faiditus* sp. 2 exclusively in *A. eximius* colonies, but we cannot rule out the possibility of an unidentified alternate host.

Spiders, especially smaller bodied ones, are capable of long-distance, passive dispersal via ballooning, shorter distance active dispersal via walking along self-laid silk lines, or coordinated group dispersal (“swarming”, see Lubin & Robinson, 1982; Roeloffs & Riechert, 1988). As *Anelosimus* and *Faiditus* are of similar size and are considered to be sister clades (Su & Smith, 2014), the observed differences in population structure may suggest an important role of sociality, as the social host species has highly structured populations, whereas their kleptoparasites do not (Table 4.2). While there are benefits to sociality, *A. eximius* colonies are also subject to Allee effects, such that small colonies are more likely to face extinction (Avilés & Tufiño, 1998; Hart & Avilés 2014). Furthermore, as we showed in Chapter 2, *A. eximius* colonies experience an energetically optimum colony size. These factors should make dispersal disadvantageous for this species, such that sociality and group living may create barriers to dispersal in this species, especially when colony sizes are small. On the other hand, *Faiditus* do not appear to be limited by their social system and they may even be able to use other host
species. We thus suggest that the finding of free movement in *Faiditus* kleptoparasites reflects an ability to parasitize multiple congeneric host species and a social structure that would favour dispersing from natal nests.

In conclusion, this study explored whether and how kleptoparasite population structure tracks that of the host in a social spider system. In the host species, the Neotropical social spiders *A. eximius*, we found strong signals of population subdivision and evidence of recent bottlenecks, congruent with previously published studies on this species (Smith & Hagen, 1996; Agnarsson et al, 2010). Conversely, *Faiditus* sp. 1 showed only weak signals of population subdivision, while *Faiditus* sp. 2 showed virtually no signal of subdivision. Furthermore, populations of neither kleptoparasite species appear to have undergone a recent bottleneck, suggesting that they do not disperse alongside their hosts during budding events (i.e., vertical transmission). We conclude that in this system parasite population structure does not match that of the host and that parasites move freely between host colonies, suggesting horizontal rather than vertical transmission (Ebert & Herre 1996). This highlights the possibility of alternative hosts, presenting avenues for future research on the ecology in this parasite system.
Chapter 5: Sublethal effects of kleptoparasitism on experimental social spider colonies

5.1 Chapter summary

A defining feature of parasitism is the harm parasites cause to their host via a reduction in lifetime reproductive success. Harm, also referred to as “virulence”, may involve host mortality or sublethal effects, such as a decreased body condition or protracted development of immature individuals. We considered a system where colonies of social spiders serve as hosts to heterospecific kleptoparasitic spiders that steal food resources. In a laboratory experiment with parasitized and non-parasitized colonies, we tested whether this host-parasite interaction meets the criterium of harm to host individuals and colonies. We assessed survival and measured body condition indices (BCIs) before and after the experimental period. Linear mixed effects models demonstrated that colonies exposed to kleptoparasites had lower BCIs at the end of the experiment compared to controls, but found no effect of kleptoparasitism on mortality in treatment and control groups. We conclude that kleptoparasites meet the criterium of harm to their host to be considered parasitic and provide the first empirical measure of virulence for this study system.
5.1 Introduction

For an organism to be considered parasitic, it must exert a negative effect on the lifetime reproductive success of its host. This effect, referred to as “virulence” (Anderson & May, 1978; Ebert & Herre, 1996), is a key parameter in host-parasite models (Anderson & May, 1979) and may also be an important factor influencing host population dynamics. Virulence has been traditionally measured in terms of host mortality caused by the parasite (Anderson & May, 1978), but parasites may also have sublethal effects (Schjørring & Koella, 2003; Alizon et al., 2009). Such sublethal effects may involve protracted growth or development of the host, as well as reduced host body condition.

Reduced host body condition may result from the direct consumption of host resources or tissues by the parasites (e.g., ticks contributing to moose calf mortality: Musante et al., 2007) or from a loss of appetite and reduced foraging by the host (Ghai et al., 2015). Body condition may also correlate with disease predisposition, as individuals in poor condition may also be immunocompromised (Lochmiller et al., 1993). Poor body condition may also amplify the effect of low levels of nutrition (Anderson & May, 1979) and contribute to high mortality in populations that are already nutritionally stressed (Gulland, 1992). Parasites can also cause harm through impeding growth and development, as in human children affected by gastrointestinal parasites (Perri et al., 1997; Casapía et al., 2006) or insects or birds subject to ectoparasites (Smith, 1988; Bize et al., 2003). Parasites can also impede growth rate by diverting host resources to mount an immune response (Hughes & Cremer, 2007), as resources used to fend off parasites or infection would no longer be available for growth and reproduction.

Host-parasite interactions are particularly interesting when considering parasites of entire social groups, where patterns at the group level may parallel those seen at the level of individual hosts.
Social spiders and their kleptoparasites are an example (Straus & Avilés 2019). Group-living spiders referred to as social form colonies with dozens to thousands of individuals (Avilés & Guevara, 2017). These colonies, housed within semi-permanent nest structures, may be considered analogous to a single organism, with individual spiders acting as somatic units. The nests these colonies build serve as habitat for heterospecific arthropods, including kleptoparasitic spiders, which reside within the social spider nests and steal prey the host has spent energy, silk, and venom resources to subdue (Cangialosi, 1991; Fernandez-Fournier & Avilés, 2018). At the individual-level, kleptoparasites act as competitors to host colony members, but collectively may negatively impact the growth and developments of the host colony as a whole.

The objective of this study was to test whether kleptoparasites of social spider colonies cause either lethal or sublethal effects to their hosts, thus meeting the criterium of harm. To do so, we compared the effects of the presence or absence of kleptoparasites of the genus *Faiditus* (Theridiidae) on the survival and body condition of individuals in lab-maintained colonies of a Neotropical social spider. Colonies of social spiders typically grow in discrete generations and exhibit a strong nonlinear relationship between colony size and various components of fitness (e.g., Avilés and Tufiño, 1998). Any lethal or sublethal effects of the parasites acting in a density dependent manner may thus result in downstream effects on colony survival and propagation. Reduced body condition or protracted development of the offspring in parasitized colonies, for instance, may increase a colony’s vulnerability to extinction, potentially contributing to the boom-and-bust population dynamics characteristic of social spider colonies (Avilés 1997; Hart & Aviles, 2014; Sharpe & Aviles, 2016).
5.2 Methods

5.2.1 Model system and study site

We conducted this study at the Jatun Sacha Biological Reserve (77.617° W, 1.088° S) in eastern Ecuador. The host species was the social spider *Anelosimus eximius* Keyserling (Theridiidae), which builds three dimensional nests that may contain anywhere from a single individual to tens of thousands of spiders. The kleptoparasitic spiders *Faiditus cf. ululans* and *Faiditus cf. flavescens* (Theridiidae) reside within the capture line portion of the host’s web. Here they wait for the social spider hosts to capture prey, which they subsequently steal (Cangialosi, 1991; Fernandez-Fournier & Avilés, 2018). The *Faiditus* species are similar in size to *A. eximius* (*Faiditus*: 5mm adult body length, Kerr 2005; *Anelosimus*: 4.0mm ± 0.17, n = 5).

5.2.2 Laboratory experiment

To test whether the presence of *Faiditus* spp. kleptoparasites results in (i) sublethal or (ii) lethal effects, we measured the direct effects of kleptoparasitism on *A. eximius* in a laboratory experiment. For this, we collected entire *A. eximius* nests and sorted individuals into four categories based on instar: adult, subadult 2, subadult 1, and other, the last of which were discarded as they were too small to accurately weigh to the nearest 0.01μg. We then mixed spiders from different colonies to ensure that the source colony did not influence our results. Creating experimental colonies with mixed membership, either in the field or laboratory, is an established practice for this system (Avilés & Tufiño, 1998; Sharpe & Avilés, 2016; Fernandez-Fournier & Avilés, 2018), as individuals do not attack or appear to discriminate against non-nest mates (Smith & Hagen, 1996; Avilés & Tufiño, 1998). We created 24 artificial colonies containing 15 of each of adult, subadult 2, and subadult 1 instars, for a total of 45 spiders per
This instar composition is representative of wild colonies, where multiple instars are usually present (Settepani et al., 2013; Straus, personal observation). We selected a random sample of five individuals per instar from each artificial colony to measure and weigh. We used a camera (AmScope SM-1T5W2, software: ToupView, ToupTek Photonics, Hangzhou, China) routed through a stereomicroscope to measure the width of the spider’s cephalothorax (mm) and an electronic ultramicrobalance (XS105DU Analytical Balance: Mettler Toledo, Columbus, Ohio, USA) to weigh the spiders to the nearest ±0.01 μg. Due to their small size, and the accuracy of the ultramicrobalance, we pooled subadult 1 individuals for weighing.

After a 4-day acclimatization and web-building period (see Supplemental materials for protocol), we introduced a single kleptoparasite into half of the colonies (n=12), hereafter referred to as the “experimental treatment”, and left the remaining colonies (n=12) without a kleptoparasite, the “control treatment.” Natural A. eximius colonies of this size have a density of approximately one kleptoparasite per cubic decimeter of capture lines volume (Straus & Avilés, 2019), comparable to the density in our experimental colonies. During the web establishment and experimental periods, we misted each colony with water and fed them daily equal amounts of locally collected termite workers. According to Yip et al. (2008), colonies containing 45 spiders capture approximately 9 mg of prey daily. Following this, we fed our colonies 8-10 termite soldiers daily, totalling approximately 10 mg of prey.
Figure 5.1. (a) an *Anelosimus eximius* nest, (b) an *Anelosimus eximius* individual, and (c) a *Faiditus cf. ululans* individual. Scale bars: a: 1 m, b, c: 3mm. Photo credits: a, c: Samantha Straus; b: Gabriel Iturralde. (d) diagram of the housing arrangement for experimental colonies.

We ran the experiment for 4 weeks, time during which we lost one of the experimental colonies when spiders escaped from their container. We also had to replace kleptoparasites that were killed by the host when they couldn’t escape an attack, as they would in a natural setting. In these instances, we made every effort to ensure that the replacement kleptoparasite was of the same species and size as the previous one, although this was not always possible. While this is a potential limitation of our study, the two *Faiditus* kleptoparasite species are similar in size and behaviour and, as such, should have similar effects on the host. At the end of the experiment, we counted the remaining individuals and measured the cephalothorax width and body mass of a
sample of up to five individuals per instar per colony if more than had 5 survived, once again pooling subadult 1 body mass due to their small size.

5.2.3. Statistical analysis

Following Sharpe and Aviles (2016), we assessed spider body condition by performing a linear regression of cephalothorax width and body weight for each instar separately. For subadult 1, we assumed individuals shared the average of the pooled body weight. The residual of each point from the best-fit line served as our Body Condition Index (BCI), such that points below the line were in worse condition, and those above the line were in better condition (Figure S1). To test the effect of the parasite on individual host spiders BCIs, we used a linear mixed effects model, with treatment, measurement period (i.e., before or after the experimental period), and their interaction as fixed effects, and experimental colony ID and instar as random effects (R package lme4, lmer function). To test the aggregate effects of kleptoparasitism on colonies, we subtracted the average initial BCI from final BCIs on a per instar and per colony basis to obtain a ΔBCI per instar and for the whole colony. In these models, we used experimental colony ID as a random effect to account for the multiple instars per experimental colony. Finally, given that some spiders moulted from one instar to the next during the experimental period, and they were not individually marked, we assessed the effect of treatment on the total number of spiders of all instars at the start and end of the experiment (“measurement period”). For this, we used a generalized linear mixed-effects model testing the effect of treatment group (control or experimental), measurement period (before or after the experimental period), and their interaction on the number of spiders in each colony at the end of the experimental period (R package lme4, glmer function, Poisson error structure with identity link). We generated all plots using ggplot2. All analyses were conducted in R Version 3.6.2 (R Core Team, 2019).
5.3 Results

At the start of the experiment, there were no statistically significant differences in BCIs between individuals in the control and parasite treatments for any of the instars (adults: ANOVA, $\chi^2(1)=0.22$, p-value = 0.64; subadult 2s: ANOVA, $\chi^2(1)=4.08$, p-value = 0.06; subadult 1s: ANOVA, $\chi^2(1)=0.61$, p-value = 0.44).

When pooling all instars, spiders had a greater BCI at the end than at the beginning of the experimental period (ANOVA, $\chi^2(1)=25.03$, p-value < 0.001), but spiders in the parasite treatment did not increase as much in BCIs as did those in the control treatment (interaction term: ANOVA, $\chi^2(1)=11.21$, p-value > 0.001). When analyzed by instar, younger instars experienced a larger increase in colony-level ΔBCI than older ones (ANOVA, $\chi^2(2)=17.42$, p-value < 0.001, Figure 5.2a), but colonies in the kleptoparasite treatment experienced a smaller ΔBCI than controls (ANOVA, $\chi^2(1)=4.06$, p-value = 0.04). The negative effect of kleptoparasite presence on ΔBCI was similar across instars (interaction term: ANOVA, $\chi^2(2)=0.28$, p-value = 0.87) (Figure 5.2a). Considering the total number of individuals irrespective of instar, all colonies experienced a reduction in the number of individuals per colony between the start and end of the experiment (ANOVA, $\chi^2(1)=397.98$, p-value < 0.001), with no significant effect of treatment (ANOVA, $\chi^2(1)=0.481$, p-value = 0.49) or the interaction between treatment and measurement period (ANOVA, $\chi^2(1)=0.141$, p-value = 0.71) on the number of spiders remaining in each colony (Figure 5.2b). See table C.1 for number of spiders remaining within each colony. No colonies went extinct during the study period.
Figure 5.2. (a) Difference between the colony-averaged final and initial BCI measurements ($\Delta$BCI) for control and parasitized colonies on a per instar basis. Red line ($y=0$) indicates no difference in body condition before and after the treatment. (b) Differences in number of spiders remaining for each treatment.

5.4 Discussion

We found support for the hypothesis that kleptoparasite presence causes harm to their social spider host in the form of sublethal, but not lethal effects. We found a lower increase in body
condition index (BCI) from the start to the end of the experiment in parasitized *A. eximius* colonies compared to controls, a pattern that applied to individuals and, collectively, to spiders belonging to the three instars considered. We found, on the other hand, no effect of kleptoparasite presence on individual or colony-level mortality, as the total number of spiders remaining in control and parasite treatments were similar and none of the colonies went extinct.

When comparing individual-level change in BCI, we found an overall increase between the start and the end of the experiment. This may have been due to immature instars accruing mass over time in preparation for moulting or lab colonies receiving more resources than under natural conditions despite us having used previous studies to inform the daily food allowance. Nonetheless, while BCIs were higher at the end of the experiment for both treatments, the magnitude of the increase was significantly less in the presence of kleptoparasites.

The effects of kleptoparasites on spider body condition may delay immature individuals transitioning to older instars. Previous studies have found that well-fed spiders, and those with greater energy reserve accrual, moulteed in less time compared to fasted spiders or those with smaller reserves (Vollrath, 1988; Mayntz et al., 2003). Consequently, poorly fed individuals that grow more slowly may fail to reach adult size in time to access mating opportunities (Vollrath 1987). As colonies of this and other social spiders grow in discrete generations (Avilés and Tufiño, 1998), timely development of the offspring generation is essential for the progression of the colonies from one generation to the next (Avilés 1999). If young fail to mature on time to replace an aging maternal generation, colony collapse may follow.

The sublethal effects of kleptoparasites detected in this study may thus be a contributing factor to the boom-and-bust population dynamics observed in wild social spider colonies (Aviles, 1997;
Aviles & Hart, 2014; Aviles & Guevara, 2017). In addition to the collapse of large colonies, *A. eximius* colonies are also characterized by Allee effects (Avilés and Tufiño 1998; Aviles & Hart, 2014). Previous work on this system found that kleptoparasite density is inversely related to colony size (Chapter 3; Straus & Aviles, 2018). With their densities being higher at smaller colony sizes, kleptoparasites may be a contributing factor to the poor survivorship of small colonies by delaying individual growth and reproduction and keeping colonies at suboptimal sizes. More work is needed to determine the extent to which reduced individual BCIs may have downstream effects on individual and colony growth and reproduction and thus the population dynamics of natural colonies in this and other social spiders.

One limitation of our experimental set up was that the colonies had the opportunity of supplement their nutrition by feeding on the kleptoparasites. Under natural conditions, kleptoparasites would be able to escape, but this wasn’t possible in the experiment, requiring us to periodically replace kleptoparasite individuals that had been killed and fed upon by the hosts. Despite this occurrence acting against the hypothesis being tested, we still detected a reduction in BCI in parasitized colonies relative to controls (Figure 5.2a).

In conclusion, we found that experimental colonies subject to kleptoparasitism had lower BCIs than colonies without kleptoparasites, both individually and collectively, supporting the inference that kleptoparasites cause nutritional stress. However, we detected no differences in mortality between treatments, suggesting that kleptoparasites have only sublethal effects, at least over the time frame of our study. We conclude that kleptoparasites of the genus *Faiditus* meet the criterium of harm necessary to be considered truly parasitic to their social spider hosts.
Chapter 6: Conclusion

The questions presented in this thesis were motivated by an interest in whether Anderson and May’s four postulates of disease behaviour apply in a social spider-kleptoparasite context (Anderson & May, 1979), such that: (i) social spiders colonies serve as host habitat, the parasite is nutritionally dependent on the host; (ii) kleptoparasites cause harm to their social spider host; (iii) kleptoparasites can evade the host’s social immunity; and (iv) kleptoparasites transmit (i.e., disperse) between host colonies (Anderson & May, 1979). Each chapter of this thesis explores each of these postulates in the context of a social animal. In Chapter 2, we explored the net surplus of energy availability in a social spider colony, which may impose limitation on energy available to kleptoparasites. In Chapter 3, we measured the relationships between parasite burden, primary productivity changes via an elevational gradient, and the cooperative social defenses used by the social spider host to mitigate parasite loads. Finally, in Chapters 4 and 5, we explored kleptoparasite dispersal (i.e., transmission) and the harm caused to host individuals by kleptoparasite presence (virulence).

We found that economies of scale occurred with surprising regularity in a community of tropical web-building spiders, and that energy savings extended across levels of organization, from the individual to the group. This parallels the economies of scale found within eusocial insects (Gillooly et al., 2010), yet occurs in a group lacking reproductive division of labour. We found that intermediately sized colonies received the greatest net energetic surplus, as the per capita cost of web production sets a lower limit and the 3D structure of the webs, an upper limit to the prey biomass per capita that colonies can obtain. Based on these findings, we would have expected that kleptoparasite density should be highest in intermediately sized colonies, where resource availability is greatest.
However, we found kleptoparasite density to be greatest in the smallest colonies, contrary to what we would predict based on the findings of Chapter 2. Ongoing work in this area, led by Madeleine Ankenman for her UBC Directed Studies project, has likewise found evidence that inquiline density declines with increasing colony size. Despite this decline, the number of prey captured per hour by inquilines declined with increasing host colony size, with mean prey size remaining constant. This contrasts with what has been observed in social spiders, where density remains constant, the number of prey captured per hour declines, but the size of captured prey increases (Yip et al. 2008). These preliminary findings suggest that social spiders can meet the challenges posed by the three-dimensional scaling of their nest structures, whereas their inquilines may not. Social spiders cooperate in prey capture (Yip et al., 2008), meaning larger colonies with more individuals to participate in prey capture can access larger insect prey. However, while the solitary/subsocial kleptoparasites may feed communally with other kleptoparasites, and even alongside the host (Su & Smith, 2014), they do not cooperate in prey capture, and as such, may be limited in the size of prey they can effectively steal from their social spider hosts. This warrants further study.

We found that both the likelihood of a social spider colony harbouring parasites and kleptoparasite burden was negatively related to increasing elevation. Colonies at lower elevations had significantly more parasites than those at higher elevations. This mirrors species-elevation and species-latitude relationships seen in the literature (Rohde & Heap, 1998; Vetaas & Grytnes, 2002). Similarly, rates of anti-parasite behaviours, i.e., those that prevent parasite attraction, entry, and prey stealing opportunities, declined with elevation in parallel to kleptoparasite burden. An important conclusion of this thesis is that social spiders do engage in social immunity to reduce their parasite loads, and species that experience a greater kleptoparasite burden have a
higher proportion of host individuals within the colony engaging in behaviours related to social immunity.

In addition to patterns of kleptoparasite abundance resulting from host traits like colony size, habitat and social immunity, we used genotyping by sequencing (GBS) to explore patterns of kleptoparasite movement through a metacommunity created by patchily distributed host colonies. We tested two hypotheses, the host-tracking hypothesis, representing vertical transmission, and the free-movement hypothesis, representing horizontal transmission. We found strong degrees of population subdivision between colonies of social spiders. In contrast, we found greater genetic mixing between populations of kleptoparasites, supporting our free-movement hypothesis. We conclude that social spider kleptoparasites tend to be horizontally transmitted. As such, kleptoparasites are free to harm their host to any degree without inflicting negative consequences upon themselves. Based on these results, we predicted that kleptoparasites have a measurable level of virulence in their social spider hosts.

We found that kleptoparasite presence had effects on body condition at both the individual and colony levels, but that they did not influence the mortality of individuals within our experimental period. These findings suggest that kleptoparasites do exert a degree of sublethal virulence on social spiders, as predicted based on our evidence of horizontal transmission (i.e., free-mixing). While our study found a negative effect of kleptoparasites at the individual-level, we are also interested in the potential negative effects at the colony-level. Future modelling studies could incorporate data on kleptoparasite loads (Chapter 3), dispersal patterns (Chapter 4), and slowed growth rates (Chapter 5) to predict colony dynamics. In particular, Anelosimus eximius is characterized by boom-and-bust patterns of population growth (Aviles, 1997; Aviles & Guevara, 2017). Because colonies of these spiders grow in discrete generations, increasing the time
between moults may result in the offspring generation not maturing on time to replace the adult generation, perhaps leading to colony extinction. Such a process may contribute to, or dampen, the unstable colony dynamics. Lively (2006) used mathematical models to demonstrate that infection can stabilize host population dynamics by dampening the amplitude of the boom-and-bust cycles. Further research is needed to understand how social spider population dynamics are influenced by the presence and load of kleptoparasites.

There are a few notable limitations to the research presented in this thesis. First, we conducted the study on economies of scale (Chapter 2) at a single locality, precluding us from assessing the role of habitat productivity in promoting sociality in spiders (Chapter 2). Primary productivity, insect size, and level of sociality in *Anelosimus* are intricately linked, such that at higher elevations, with colder temperatures, insects are smaller and the spiders are less social (Guevara & Aviles, 2007). Additionally, we conducted our study during the drier season, when webs may have been damaged to a lesser degree than in the wetter season. In Chapter 3, we were limited in making broader conclusions about the role of sociality in influencing kleptoparasite burden and social immunity by the small number of available species. As extraordinary as it is to have four separate species of two levels of sociality occurring in a relatively small geographic range, the make-up of our sample did not allow us to directly compare our results between social and subsocial species. Inferences in Chapter 4 were similarly limited by sample availability, particularly for *Faiditus* sp2., which occurred at only one of our study sites, and at low densities within colonies at that site. Finally, while performing the experiment for Chapter 5, we did experience relatively high mortality of kleptoparasites due to attacks from host individuals. Frequent need to replace kleptoparasite individuals made it challenging to ensure that all kleptoparasites were the same sex, size, and species.
Despite these limitations, the present thesis has several strengths. In more traditional host-parasite systems, it can be difficult to observe all the processes of interaction between hosts and parasites. For solitary individuals, processes are typically occurring at microscopic levels within the body of the organism. Within other social arthropods, colonies tend to be opaque or underground (e.g., termite mounds, ant colonies), making the study of these interactions in natural environments more difficult. However, this group of social spiders build above ground, transparent, silken structures that make direct observation of the interactions between hosts and parasites possible. This ability to directly observe natural interactions, rather than in a laboratory, makes it possible to consider multiple species of inquilines and parasites acting on multiple host species, as we’ve done in this thesis. Many studies focus on a single host, single parasite system (Pilosof et al., 2015), a trend in host-parasite ecology that limits broader conclusions.

Broadly, the work presented in this thesis has made important steps towards synthesizing the parallel fields of metacommunity and parasite ecology across levels of organization. I’ve demonstrated that economies of scale extend from individuals to social groups in spiders, and that likewise, individuals cooperate to mitigate parasite burden in a similar fashion as individual immunity. I’ve also demonstrated that dispersal occurs at different rates in hosts and parasites, and in different species of parasite. This dispersal will directly feedback onto measured kleptoparasite loads and the harm caused by them. Finally, this thesis has demonstrated the flexibility of this social spider system and has continued to develop this system as a model for the study of parasite metacommunities.
Bibliography


Cao, T. T., & Dornhaus, A. (2013). Larger laboratory colonies consume proportionally less energy and have lower per capita brood production in Temnothorax ants. Insectes sociaux, 60(1), 1-5.


https://doi.org/10.5281/zenodo.1480624), R package version 0.8.1, <URL: https://CRAN.R-project.org/package=effsize>


Straus, Samantha; Gonzalez, Angélica; Matthews, Philip; Avilés, Leticia (2021), Economies of scale shape energetics of solitary and group living spiders and their webs, Dryad, Dataset, https://doi.org/10.5061/dryad.qbzkh18j9


Appendices

Appendix A: Supplementary information for Chapter 2
Table A.1 - list of web building taxa sampled for figure 2.2; total number of webs = 52

<table>
<thead>
<tr>
<th>Web type</th>
<th>Family</th>
<th>Genus</th>
<th>species</th>
<th>Freq</th>
<th>average size (mg) +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>orb</td>
<td>Araneidae</td>
<td>Eriophora</td>
<td>4</td>
<td>292 ± 207</td>
<td></td>
</tr>
<tr>
<td>orb</td>
<td>Araneidae</td>
<td>Micrathena</td>
<td>6</td>
<td>33.7 ± 24.4</td>
<td></td>
</tr>
<tr>
<td>orb</td>
<td>Tetragnathidae</td>
<td>Leucauge</td>
<td>9</td>
<td>47.0 ± 18.2</td>
<td></td>
</tr>
<tr>
<td>orb</td>
<td>Uloboridae</td>
<td>Uloborus</td>
<td>6</td>
<td>1.41 ± 1.47</td>
<td></td>
</tr>
<tr>
<td>tangle</td>
<td>Theridiidae</td>
<td>Parasteatoda</td>
<td>3</td>
<td>12.1 ± 9.46</td>
<td></td>
</tr>
<tr>
<td>solitary sheet-</td>
<td>Araneidae</td>
<td>Kapogea</td>
<td>sexnotata</td>
<td>7</td>
<td>189 ± 79.5</td>
</tr>
<tr>
<td>and-tangle</td>
<td>Lycosidae</td>
<td>Aglaoctenus</td>
<td>castaneus</td>
<td>7</td>
<td>76.7 ± 49.6</td>
</tr>
<tr>
<td>social sheet-</td>
<td>Theridiidae</td>
<td>Anelosimus</td>
<td>eximius</td>
<td>10</td>
<td>2.61 ± 1.55</td>
</tr>
<tr>
<td>and-tangle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A.2. Pairwise comparisons of intercepts and slopes for four web categories: orb, tangle, solitary sheet-and-tangle, and social sheet-and-tangle. Four web types form two distinct groups based on intercept – those with and without sheet web components, while slopes are not significantly different for any of the web types

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Intercept t-ratio</th>
<th>Intercept p-value</th>
<th>Slope t-ratio</th>
<th>Slope p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orb-tangle</td>
<td>1.42</td>
<td>0.52</td>
<td>0.89</td>
<td>0.81</td>
</tr>
<tr>
<td>Orb - solitary sheet-and-tangle</td>
<td>-9.81</td>
<td>0.003</td>
<td>-0.12</td>
<td>0.99</td>
</tr>
<tr>
<td>Orb - social sheet-and-tangle</td>
<td>-5.87</td>
<td>&lt; 0.001</td>
<td>-1.62</td>
<td>0.38</td>
</tr>
<tr>
<td>Social sheet-and-tangle - solitary sheet-and-tangle</td>
<td>-0.14</td>
<td>0.99</td>
<td>1.31</td>
<td>0.56</td>
</tr>
<tr>
<td>Social sheet-and-tangle - Tangle</td>
<td>5.574</td>
<td>&lt; 0.001</td>
<td>1.75</td>
<td>0.31</td>
</tr>
<tr>
<td>Solitary sheet-and-tangle - Tangle</td>
<td>7.15</td>
<td>&lt; 0.001</td>
<td>0.90</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Figure A.1. The number of spiders working during each hour-long segment between 5:00 am and 11:00 pm. Spider activity peaks at sunset.
Figure A.2. Linear relationships between cross section area of the nest and the colony mass (a) and (b) total number of spiders in the colony, and (c) number of adult and subadult females. (a) $\log_{10}$ Colony mass = $-2.53 + (0.78 \cdot \log_{10}$ cross section area); slope ANOVA: $F_{1,17} = 49.61$, p-value < 0.001, R-squared$_{adj} = 0.73$. (b) $\log_{10}$ Number of adult and subadult females = $0.73 + (0.57 \cdot \log_{10}$ cross section area); slope ANOVA: $F_{1,17} = 23.10$, p-value < 0.001, R-squared$_{adj} = 0.55$. (c) $\log_{10}$ Number of adult and subadult females = $-0.89 + (0.93 \cdot \log_{10}$ cross section area); slope ANOVA: $F_{1,17} = 57.64$, p-value < 0.001, R-squared$_{adj} = 0.76$. 
Figure A.3. Diagram of respirometry track. Black circle represents rubber gasket, grey areas represent areas of airflow. White circle represents a magnetized bead used to induce movement.
Figure A.4. (a) Web maintenance activities are concentrated at dusk (17:00-17:59), when a greater proportion of spiders are seen performing such activities than at other times of the day. At this time also, the proportion of spiders working decreased with colony size ($X^2 = 4.83$, df = 1, p-value = 0.03), but (b) spiders in larger colonies traveled a greater distances (ANOVA, slope $= 0.62 \pm 0.37$, F-value$_{1,12} = 10.86$, p-value = 0.006, $r^2_{adj} = 0.43$). We used focal animal sampling to estimate the average distance working spiders moved at dusk. During sampling periods, we measured the distance travelled (in cm) by three spiders, one after the other, from each of 19 nests. Each spider was observed for five mins and then marked with luminous paint (BioQuip Products, Rancho Dominguez, CA, USA) to prevent repeated measurements. Sampling was repeated five times for a total of 15 individuals per nest.
Appendix B: Supplementary information for Chapter 3

Appendix B.1 Associations between elevation and kleptoparasite presence

We assess the association between elevation and the presence of kleptoparasites in nests of social and subsocial *Anelosimus* species along an elevational gradient in Eastern Ecuador. In addition to analyses that consider site elevation as a discrete variable (see main text), here we perform analyses considering the elevation of each nest. We used a generalized linear mixed effect model with Poisson error distribution. Study site and species were considered random effects. We used anova to compare with the null model. We found that the number of kleptoparasites within nests of the various species significantly declined with elevation (df = 1, $\chi^2 = 22.27$, p-value < 0.0001)

![Figure B.1](image-url)

Figure B.1. Number of kleptoparasites within nests of social and subsocial *Anelosimus* spp (*A. eximius*, *A. domingo*, *A. guacamayos*, *A. elegans*) significantly decreased as elevation increased.
Appendix B.2: Correlation between nest hygiene behaviours and parasite density across species

A comparative analysis of the correlation between the two traits is complicated by the fact that the study species are related to one another with a particular phylogenetic history (Figure S2A), that populations of the same species occur in more than one habitat, and that the environments where pairs of species co-occur differ from those of the others in ways that influence the value of the two traits. For heuristic purposes, we address these issues by estimating contrasts between species pairs that either share a recent common ancestor or occupy a common environment (Figure S2B). The resulting pattern is consistent with the prediction that, after controlling for environment or phylogeny, species that exhibit more frequent hygiene behaviour have a lower density of parasites within the nests. A statistical test of the resulting pattern, however, is not appropriate given that not all contrast points are independent from one another (i.e., some share the same species-population).
Figure B.2. (A) Phylogenetic relationships of the study populations, and value of the two traits used to calculate the contrasts--average frequency of hygiene behaviours in colonies (% individuals participating) and median density of kleptoparasites in the nests. (B) Contrasts in the value of the two traits for species that either share a recent common ancestor or occupy a common environment. After centering the value of the variables around their mean (% hygiene behaviours) or median (kleptoparasite density), the contrasts shown were estimated by subtracting the value of each (centered) variable for the species in each pair with expected lower
kleptoparasite loads from that of the species with expected higher loads. The regression line showing an inverse correlation between the two variables drawn for heuristic purposes only.

Appendix B3: Nest hygiene behaviour analysis excluding web repair
We considered web repair as a proxy for general web maintenance and patrolling behaviours, which we are assuming are at least partially related to control of kleptoparasites. Here, we reanalyzed the data pertaining to Figs. 3b & 5 in the main text excluding this behaviour, to ensure that web repair alone is not driving the patterns. For the analysis pertaining to Figure 5, we excluded both web-repair and self-grooming, as self-grooming was only included to explore hygiene behaviours potentially associated with wasp parasitoid control. Additionally, for the latter analysis we only considered the lowest elevation site, as that is where *A. domingo*, the species that performs the highest levels of hygiene behaviour, occurs.
Figure B.3. Same analysis as for Figure 3b, but with web repair removed. Trend is robust. When excluding web repair, *A. domingo* still performs the highest levels of nest hygiene behaviours. Here, site elevation was still a significant factor ($\chi^2 = 11.63$, df = 2, p-value = 0.003); likewise, the site elevation and Species interaction remained significant ($\chi^2 = 13.16$, df = 3, p-value = 0.004).
Appendix C: Supplementary information for Chapter 5

Figure C.1 (a) Adult, (b) subadult 2, and (c) subadult 1 cephalothorax width (mm) plotted against spider weight (mg). Blue lines indicate linear regression. Residual of point to the line represents body condition index (BCI).

Experimental colony establishment:
After each individual within the artificial colony was weighed and measured, we placed them into a plastic colony chamber. Each colony was constructed out of a three L water bottle (diameter: 12.8cm, height: 32.9 cm). The bottom portion (10 cm) of each bottle was removed and replaced with mosquito netting to ensure adequate airflow and to reduce the chance of mold growth during the experimental period. Before the start of the experiment, we gave each colony a four-day period to build their webs using small sticks and leaves that we provided for scaffold. During this period, we removed the top portion of the bottle and sealed off the resulting opening to encourage web building in a smaller chamber (diameter: 12.8cm, height: 9 cm). As *A. eximius* individuals tend to move upwards, we encouraged web-building in a smaller chamber initially. Once a sheet web had been established, we re-attached the top of the bottle to provide space for the spiders to build the capture lines. We cut a small hole near the top for feeding and sealed it with a cork (Figure 5.1). We suspended colonies on lines 1.5m above the ground to prevent discovery by ants and to reduce mold growth.

The bottom of a 3 L bottle was removed and covered with mesh to allow airflow. The area between the mesh bottom and the dashed line (Figure 1d of main document) indicate space allowed for an initial web-building period. The area above the dashed line (Figure 1d of main document) was added for the duration of the experiment. The opening covered by the brown cork was used to introduce insects. We established colonies with 45 *A. eximius* individuals (red spiders) and one *Faiditus* kleptoparasite (black spider).

Animal husbandry protocol
During this period, we misted each colony with water and fed them daily equal amounts of locally collected termite workers. According to Yip et al. (2008), colonies containing 45 spiders capture approximately 9 mg of prey daily. Following this, we fed our colonies 8-10 termite soldiers daily, totaling approximately 10 mg of prey.
Table C.1. Average number of spiders remaining at the end of the experimental period for each experimental colony and instar. All colonies began the experiment with N=45.

<table>
<thead>
<tr>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nest #</td>
<td>Number</td>
</tr>
<tr>
<td>Col_13</td>
<td>12</td>
</tr>
<tr>
<td>Col_14</td>
<td>11</td>
</tr>
<tr>
<td>Col_15</td>
<td>13</td>
</tr>
<tr>
<td>Col_16</td>
<td>4</td>
</tr>
<tr>
<td>Col_17</td>
<td>15</td>
</tr>
<tr>
<td>Col_18</td>
<td>15</td>
</tr>
<tr>
<td>Col_19</td>
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<tr>
<td>Col_20</td>
<td>13</td>
</tr>
<tr>
<td>Col_22</td>
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</tr>
<tr>
<td>Col_23</td>
<td>18</td>
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<tr>
<td>Col_24</td>
<td>17</td>
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</table>