Factors contributing to behavioral variation in two species of *Alouatta* and their hybrids

by

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I dedicate this dissertation to J. Y.
You can do it!

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ABSTRACT

Understanding the causes of behavioral variation is critical to the study of animal behavior. This dissertation focuses on explaining behavioral variation in two species of New World primates: *Alouatta pigra* and *A. palliata*. I take advantage of a natural hybrid zone that occurs between them to examine what factors correlate with differences in social behavior and activity/movement patterns across taxonomic categories. Particularly, if behavior is constrained by ancestry, then hybrids more similar to *A. pigra* will exhibit "*pigra*-like" behaviors, while hybrids more similar to *A. palliata* will exhibit "*palliata*-like" behaviors. In Chapter One, I first describe the social behavior of the purebred animals to confirm that differences do exist between them. I then compare the behavior of the purebreds to hybrids. I found that *A. pigra* females exhibit greater affiliation and stay in closer proximity, as well as engage in lower levels of agonism, when compared to *A. palliata* females. This behavior is mirrored between *pigra*-like and *palliata*-like females (categorized by morphological similarities to the parental species). In Chapter Two, I focus on activity budgets (resting, feeding, moving) and travel distance. Again, I describe the patterns found in purebred animals and compare them to the hybrids. I found some evidence that aspects of *A. pigra* and *A. palliata* activity patterns reflected an effect of ancestry. In the hybrid zone, this ancesty effect seemed to result in *pigra*-like animals being less affected by environmental disturbance than *palliata*-like animals. In Chapter Three, I incorporated genetic analyses in order to more confidently categorize the hybrids according to their genetic ancestry and to examine whether genetic relatedness affected the social behavior patterns

described in Chapter One. I used multilocus microsatellite data to calculate a hybrid index

ranging from 0 to 1 (0 = *A. pigra* and 1 = *A. palliata*) for each individual and a coefficient of

relatedness for each dyad. I found that differences in kinship did not predict differences in

proximity and affiliation. Degree of hybridity had an effect on hybrid behavior, with genetically

pigra-like females spending more time in closer proximity and engaging in more affiliation than

genetically *palliata*-like females.

INTRODUCTION

Because behavior is an organism's first response to change (Kappeler, Barrett, Blumstein, & Clutton-Brock, 2013), it is generally considered beneficial and adaptive to have high behavioral flexibility in order to best cope with any alteration in environmental conditions. However, not all animals exhibit behavioral flexibility, and if they do, they are not flexible across all behavioral domains. What contributes to these differences among animals? Relatedly, what causes some behaviors to be labile and others intransigent to external factors? While an important theoretical issue, understanding the mechanisms of behavioral variation has practical value as well. The field of psychology, with a historical emphasis on finding universal models of behavior, often assumes that responses found in one species can be reliably generalized to another. However, without a clear understanding of the factors influencing behavioral variation, this can be a dangerous assumption to make, whether on a species-level or even on a population-level. For example, we may observe a consistent behavioral response in one group, and wish to apply our findings to a different group. If the behavioral response in question is evolutionarily conserved and unlikely to have a great deal of variation, then extending our findings to the second group makes sense. However, if the behavioral response is one that greatly depends on current conditions (e.g. resource availability, presence of conspecifics, seasonal changes, sex, and so on), then we may run the risk of assuming that the two groups are reacting to our stimulus of interest in the same way, when that is actually not the case.

For one striking example of the consequences stemming from a failure to account for the

causes of behavioral variation, we can look to the sex bias of study animals used in neuroscience and biomedial research (Zucker & Beery, 2010). The predominant use of male animals has impeded the understanding of female responses to a wide variety of stimuli from pain to drugs, with negative consequences to our knowledge of female biology and women's health (Beery & Zucker, 2011). The historical exclusion of female animals in studies was based on two assumptions related to the mechanisms of behavioral variation: 1. that sex does not cause significant differences in behavioral response, and 2. that estrous cycles increase behavioral variation, making females untenable for research (Prendergast, Onishi, & Zucker, 2014). Neither assumption is true (Cahill, 2012; Prendergast et al., 2014). Clearly, there is great need for a better understanding of the factors involved in behavioral variation, to avoid making similar mistakes in studies that hope to draw broadly applicable conclusions across groups (whether those groups are sexes, populations, species, genera, or any higher level of organization).

Currently, behavioral ecologists have posed several explanations for behavioral variation in animals. Particularly influential are the socioecological models, first developed for application in primates (van Schaik, 1989; Wrangham, 1980) and also relevant for other taxa (e.g. carnivores: Holekamp, Smith, Strelioff, Van Horn, & Watts, 2012; ungulates: Jarman, 1974; invertebrates: Tanner & Jackson, 2012). These models focus on resource availability as the key factor driving variation in social systems (*sensu* Kappeler & van Schaik, 2002). Specifically, due to sex-specific needs related to pregnancy and lactation, females are more dependent on resource distribution and will group themselves according to how food is dispersed through their habitat. Males will then distribute themselves according to how the females are grouped, resulting in various forms of social organization (solitary vs. group-living), mating systems (monogamous vs. polygamous), and social structure (egalitarian vs. despotic). The socioecological models have

been expanded upon as researchers found other, non-ecological factors that affected primate social systems, such as risk of infanticide limiting female group size (Sterck, Watts, & van Schaik, 1997). Thus, an implication of socioecological models is that animal behavior — specifically social behavior — is extremely flexible and is driven primarily by current conditions, whether ecological or social. In contrast, several studies using more recent phylogenetic techniques have discovered that the behavior of some primate taxa are strongly influenced by their ancestry — in other words, despite living in very different ecological conditions, these primates retain similar social systems (Opie, Atkinson, & Shultz, 2012). The implication here, of course, is that social behavior is inflexible and subject to constraint.

Our lack of consensus with regard to behavioral flexibility versus constraint is not restricted to social behaviors only. Even in behavior that ostensibly should be more related to ecological factors (such as behaviors involved in resource acquisition), it is not clear how labile these behaviors can be in response to environmental change. We know that animals can be broadly categorized as dietary specialists or generalists, meaning that the latter are more flexible in the food they can eat and should be better able to adapt their resource acquisition when confronted with a sudden shift in their ecological conditions. This is borne out by data showing generalists are more successful than specialists in disturbed environments (Colles, Liow, & Prinzing, 2009; Ryall & Fahrig, 2006). However, are there degrees of flexibility/constraint within "generalist" or "specialist" species? Do other factors besides ecological conditions contribute to being more or less flexible in behaviors that are related to resource acquisition?

Primates are a diverse taxon that expresses a wide range of complex behaviors in response to each other and to the environment around them. Thus, they are ideal for answering questions about interspecific behavioral variation and flexibility versus constraint. Additionally,

with a few notable exceptions (baboons, macaques), most primates are endangered as a direct result of anthropogenic habitat destruction. Thus, there is both theoretical and practical value in studying the factors that contribute to variation in primate behavior specifically and understanding what behaviors are relatively constrained and what behaviors are more labile.

This dissertation aims to examine several types of behavior in the genus *Alouatta*, focusing on two sister taxa — *A. pigra* and *A. palliata* — and their natural hybrids. I examine both social behavior and non-social, energy-acquisition/use-related behavior (activity budgets and travel distance) that are more directly linked to ecological conditions. I ask whether these behaviors are flexible, responsive to immediate social and ecological factors, or constrained by genetic ancestry. This system is useful for addressing these questions because the two species have marked differences in their social behavior, with *A. pigra* being generally more egalitarian and affiliative than *A. palliata* (Chapter One). Additionally, though less documented, some possible differences may exist in their energy acquisition and use-related behaviors, with *A. pigra* being more reliant on energy-minimization than *A. palliata* (Chapter Two). Their natural hybridization allows me to take advantage of the characteristics of a hybrid zone to investigate the causes of interspecific behavioral variation. Hybrid zones are geographically narrow but genetically diverse, meaning that I can map behavioral variation to genetic variation while controlling for ecological variation (Chapter Three). In order to do so, I use modern molecular techniques to create a hybrid index for each individual.

Because the two species differ in their dispersal patterns and group formation (Chapter One), which may alter patterns of relatedness within groups, interspecific behavioral variation may arise as a result of those differences in relatedness (Clutton-Brock, 2002) rather than from the effect of genetic variation *per se*. Thus, I also calculate genetic relatedness to examine its

effects in relation to behavior, ancestry, and ecology (Chapter Three). Additionally, the two species vary in socio-demographic factors such as group size. I therefore include group size as another variable in my analyses to understand its effect in relation to the other potential factors at play in explaining behavioral variation (Chapters One - Three). Finally, I summarize and discuss the implications of my results in terms of their contributions to the understanding of primate behavior and primate conservation (Conclusions).

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CHAPTER ONE

Effect of ancestry on behavioral variation in two species of howler monkeys (*Alouatta pigra* and *A. palliata*) and their hybrids

ABSTRACT

Social differences between primate species may result from both flexible responses to current conditions or fixed differences across taxa, yet we know little about the relative importance of these factors. Here, we take advantage of a naturally occurring hybrid zone in Tabasco, Mexico to characterize the variation in social structure among two endangered howler monkey species, *Alouatta pigra* and *A. palliata*, and their hybrids. Work in pure populations has suggested that *A. pigra* females maintain closer proximity, exhibit higher rates of affiliation, and lower rates of agonism than *A. palliata* females, but we do not know what accounts for this difference. Using identical data collection and analysis methods across three populations, we first seek to confirm previously reported interspecific differences in social structure across all sexes. We next examine: 1. how female social relationships changed with ancestry (by comparing pure and hybrid individuals), 2. how female social relationships changed with group size (*A. pigra* have smaller groups than *A. palliata*), and 3. whether female social relationships differed between two taxonomic groups within a single forest fragment (thus controlling for ecological variation). We confirmed previously described species differences, including closer proximity among females than among males in all populations. We also found that smaller groups maintained closer proximity. However, even after accounting for variation in group size, *A. pigra*

females had closer proximity and more affiliation than *A. palliata* females. Furthermore, differences between *pigra*-like and *palliata*-like hybrids paralleled differences between pure populations and persisted even after controlling for ecological variation. Together, our results suggest that flexibility cannot account for all of the social differences between *A. pigra* and *A. palliata* and indicate an important genetic component in primate social behavior.

INTRODUCTION

Primates are noted for the diversity of their social interactions and resulting social structures (Kappeler & van Schaik, 2002). Aspects of their social structure may vary in response to a complex and interconnected set of social, ecological, and genetic factors. Such variation can occur within generations as a flexible response to current conditions. Alternatively, fixed genetic differences between taxa can also cause variation. Thus, a particular species may have a certain social structure irrespective of fluctuations in the surrounding environment. The first idea, that primate behavior is flexible, is an implicit assumption of socioecological models, where the strength and nature of intragroup female social bonds alter according to factors such as resource distribution, dispersal patterns, and infanticide risk. The resulting variation in levels of within-group female competition and cooperation in turn affect male social relationships (Isbell & van Vuren, 1996; Sterck, Watts, & van Schaik, 1997; Wrangham, 1980). This view has been challenged recently by calls to incorporate phylogenetic information into studies of primate social systems (Clutton-Brock & Janson, 2012; Di Fiore & Rendall, 1994; Thierry, 2013). Indeed, there is evidence for strong phylogenetic signal in the social systems of at least some primate taxa (Balasubramaniam et al., 2012; Shultz, Opie, & Atkinson, 2011), and previous research in macaques has linked ancestry with variation in female affiliation and aggression (Maestripieri,

2003). Thus, while flexibility and genetic differences both contribute to behavioral variation, the extent to which interspecific variation in social relationships can be attributed to these two factors remains largely unknown.

It is possible to tease apart the effects of genetics, social setting, and ecological variables in hybrid zones, where genetically distinct animals can be found in overlapping ecological and social settings (Hewitt, 1988). To date, much of the research on behavior in primate hybrid zones has been concentrated in baboons (*Papio* spp.) (Bergman & Beehner, 2004; Bergman, Phillips-Conroy, & Jolly, 2008; Charpentier et al., 2012; Tung, Charpentier, Mukherjee, Altmann, & Alberts, 2012). Evidence from these two different baboon hybrid zones suggests that some interspecific behavioral differences are inflexible: ancestry has an effect on mating strategies (Bergman & Beehner, 2003; Tung et al., 2012), and hybridization results in an admixture of species-typical patterns of intra- and intersexual relationships (Bergman et al., 2008). However, no behavioral comparisons similar to the baboon studies exist from New World primate hybrid zones. Furthermore, comparisons across cercopithecine social systems suggest strong phylogenetic constraint, with a suite of traits that pertain to female social relationships found almost uniformly across extant taxa (Di Fiore & Rendall, 1994). But in contrast, a study of New World primates from Izar and colleagues (2012) suggests a lack of phylogenetic constraint when comparing within-group social interactions in two closely related species of capuchins. Thus, at this point, we do not know whether the social inflexibility (e.g. genetically influenced social interactions) indicated by baboon hybrid zones is unique to that taxon, or whether it also applies to other, non-cercopithecine primates.

Here, we examine the effect of genetic ancestry on variation in the social structure (*sensu* Kappeler & van Schaik, 2002) of two species of howler monkeys and their hybrids. We use a

naturally occurring hybrid zone in Tabasco, Mexico (Cortés-Ortiz et al., 2007) to characterize differences in proximity patterns, affiliation, and agonism among *Alouatta pigra, A. palliata,* and their hybrids. *Alouatta pigra* and *A. palliata* are sister taxa that diverged approximately 3 Ma (Cortés-Ortiz et al., 2003). The two species are genetically and morphologically distinct (Cortés-Ortiz et al., 2003; Kelaita, Dias, Aguilar-Cucurachi, Canales-Espinosa, & Cortés-Ortiz, 2011), with a parapatric distribution that includes known contact/hybrid zones in Mexico and possibly Guatemala (Baumgarten & Williamson, 2007; Smith, 1970). Like other howler monkeys, *A. pigra* and *A. palliata* are folivore-frugivores that use an energy-minimization strategy (Strier, 1992) to digest their primarily high-fiber diets (Di Fiore, Link, & Campbell, 2011). This similarity implies that, under the same ecological conditions, any behavioral differences between the two species should *not* be a result of species-typical feeding strategies but rather due to some other factor (Silver, Ostro, Yeager, & Horwich, 1998).

Despite being sister taxa, the two species reportedly have differences in many aspects of their social structure, particularly group cohesion and female-female social relationships (Table 1.1). Notably, while *A. pigra* females engage mostly in affiliative behavior (Van Belle, Estrada, & Strier, 2011), studies in *A. palliata* report higher rates of dominance-related agonistic behavior among females, compared to *A. pigra* (Jones, 1980; Zucker & Clarke, 1998). This is likely a reflection of differing female group entry strategies between the two species and the observation that *A. palliata* females have a discernible dominance hierarchy, apparently lacking among *A. pigra* females (Table 1.1). Differences in male social relationships are less obvious; in both species, males rarely have affiliative interactions with one another and instead tend to associate with females (Van Belle, Estrada, & Strier, 2008; Wang & Milton, 2003). However, although data on dispersal and group entry are limited (Table 1.1), current knowledge implies that if there

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is more than one male in an *A. pigra* group, they are more likely to be related than males in *A. palliata* groups, possibly also affecting interspecific differences in proximity patterns.

In this study, we first make a descriptive analysis of our study populations, wherein we seek to confirm previously reported differences between *A. pigra* and *A. palliata.* Importantly, we are the first to study these two species' behavior using identical data collection methods. Although we examined both affiliative and agonistic interactions, *Alouatta* is notable for its relatively low rates of social behaviors. Thus, we also use proximity, which is considered an appropriate "first reading" of social structure in primates (Kummer, 1970a) and is a standard approximation of social relationships in *Alouatta* (e.g. Van Belle et al., 2008; Bezanson, Garber, Murphy, & Premo, 2008; Corewyn & Pavelka, 2007; Dias, Rodríguez Luna, & Canales Espinosa, 2008; Zucker & Clarke, 1998).

We compare proximity in multiple groups from populations in three separate Mexican states (Figure 1.1): *A. pigra* outside the hybrid zone (from Campeche), *A. palliata* outside the hybrid zone (from Veracruz), and hybrid zone groups (from Tabasco). We expect to find that pure *A. pigra* individuals will be in closer proximity with other group members than *A. palliata* individuals (Crockett & Eisenberg, 1987). Based on previous primate hybrid zone studies (e.g. Bergman & Beehner, 2004; Bergman et al., 2008; Charpentier et al., 2012; Tung et al., 2012), we additionally expect to find that this difference is genetically-based. In other words, we predict that individuals from the hybrid zone (mainly hybrid and backcrossed animals) will, on average, show intermediate proximity patterns given the inclusion of a broad distribution of genotypes, but *A. pigra*-like and *A. palliata*-like hybrids will have proximity differences between them that mirror observed differences between the pure species (see Methods for the definitions of these classifications).

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We next consider female behavior specifically, as they are reported to have clearer interspecific social differences than males, and examine female-female affiliative and agonistic interactions. Again, we predict that within their groups, *A. pigra* females will be more affiliative and less agonistic than *A. palliata* females. We also predict hybrid females will show behavior that differs between *A. pigra*-like and *A. palliata*-like hybrids in the same direction as the purebreds (e.g. *pigra*-like females will exhibit higher affiliation and lower agonism than *palliata*-like females).

However, differences among these three populations (*A. pigra*, hybrids, and *A. palliata*) may be caused by social or ecological factors, rather than genetics. For example, variation in group size could affect the level of within-group cohesion; e.g., larger groups may spread farther apart to forage, as they would otherwise deplete resources too rapidly. Thus, we include group size as a predictor variable in multivariate analyses and also control for group size effects with comparisons restricted to *A. pigra* and *A. palliata* groups matched in size. If group size is a stronger driving force than ancestry, we predict that similarly-sized groups of *A. pigra* and *A. palliata* will not differ in proximity.

Finally, because the three sites vary in their degree of anthropogenic habitat disturbance, we consider the possibility that this may affect patterns of social variation. Because we do not have detailed ecological data to examine the effects of resource variation, we chose to control for habitat disturbance by comparing the social structure of *pigra*-like and *palliata*-like groups that reside in the same patch within the hybrid zone. If ecological factors play a stronger role than ancestry in affecting social variation, then we predict that *pigra*-like and *palliata*-like hybrid groups within the same patch will not differ in their social structure.

METHODS

Study sites

We conducted data collection for this study in three separate populations (Figure 1.1). We sampled within the *A. pigra* and *A. palliata* contact zone, where hybridization is confirmed to occur (Cortés-Ortiz et al., 2007), as well as in two areas with only purebred animals. These purebred sites are well outside of the contact area (approximately >260 km away) and it is unlikely that the individuals there have had contact with the other species or with their genes (i.e., hybrid or back-crossed individuals).

The purebred *A. pigra* site near Escarcega, Campeche, is El Tormento Forest Reserve, a protected, relatively large area of primary tropical forest of about 1400 ha (Van Belle & Estrada, 2008). The three purebred *A. pigra* groups studied at this site are in different locations within the reserve and their ranges were not observed to overlap (although they likely overlap with other groups that were not part of our study). The two purebred *A. palliata* sites are located in La Flor, Catemaco (~120 ha) and in Rancho Jalapilla, Acayucan (~30 ha), both in Veracruz (Figure 1.1). La Flor is an ornamental palm plantation consisting of primary and secondary vegetation. The canopy is composed of arboreal species typical of undisturbed primary rainforest and the arboreal howler monkeys can move freely through the trees. Therefore, La Flor constitutes a practically undisturbed site, despite the daily harvesting of ornamental palms in the understory, and the two purebred *A. palliata* groups here were not observed to have overlapping home ranges. The remaining purebred *A. palliata* group is in Rancho Jalapilla, a narrow but long riparian strip of secondary forest surrounded by pastureland. This is part of a large private property with very restricted access to people, and thus the monkeys do not often interact with human settlements or dogs. Several groups occupy this stretch of forest, but again, they do not seem to have

overlapping ranges. The hybrid site is located south of Macuspana, Tabasco, in the midst of the approximately 20 km wide contact zone (Kelaita & Cortés-Ortiz, 2013). Of all the sites, it is the most affected by habitat disturbance, as individual howler monkey groups there reside in small, discontinuous patches of mainly secondary forest (~ 3 ha) separated by ranches and farmland (Dias, Alvarado, Rangel-Negrín, Canales-Espinosa, & Cortés-Ortiz, 2013). A single patch in Tabasco may be occupied by one or several howler monkey groups (up to four in the current study; see Table 1.2). Additionally, the hybrid zone exhibits the following anthropogenic activity to a greater degree than the other two sites: 1) predation by domestic dogs associated with inter-patch movements for diet supplementation, which is more likely to occur in smaller patches, characteristic of the hybrid zone (Rangel-Negrín, Dias, & Canales-Espinosa, 2011); 2) logging, which currently occurs only in the hybrid zone among the study sites; 3) visuo-acoustic contact with humans who work in the surroundings (e.g., cattle grazing and farming) and inside the patches (e.g., gathering firewood), which are far more frequent in the hybrid zone, given that monkeys there live in an embedded matrix of pastureland, human settlements and forest patches.

Subjects

We collected data simultaneously on three groups of purebred *A. pigra* and six groups of hybrids from February-August 2011, then simultaneously on three groups of purebred *A. palliata* and five new groups of hybrids from January-June 2012 (see Table 1.2 for more details on group composition and data collection). All adults in the study were individually recognizable by researchers via ankle bracelets or natural markings such as scars and, in the case of *A. palliata*, differences in characteristic patches of skin/fur coloration on their feet and tails.

Despite most individuals in the hybrid zone resembling either *A. pigra* or *A. palliata* in terms of pelage coloration and size, our sample likely consists of highly backcrossed animals that

are phenotypically similar to (and impossible to distinguish from) the parental species, and thus we consider all animals in the hybrid zone to be hybrids (see Kelaita & Cortés-Ortiz, 2013 for a genetic characterization of the admixture of individuals within the same population). Because we lack genetic data for the individuals included in this study, hybrids are broadly classified based on morphology and vocalizations (LH & LCO personal observation). We consider this method appropriate for our initial examination of hybrid variation because previous genetic analyses showed that most animals in the hybrid zone, and specifically in our field site, are highly backcrossed, multigenerational hybrids. These hybrids share most of their genome with one of the parental species, to which they are also morphologically similar (i.e., hybrids resemble the parental species with which they share most of their alleles: Kelaita & Cortés-Ortiz, 2013). Though genetically intermediate hybrids cannot be so reliably categorized based on their morphology, the incidence of such animals in our study site is minimal (Kelaita & Cortés-Ortiz, 2013). Therefore, the likelihood that we have an intermediate hybrid in our sample is very small, and the impact on our results should be negligible.

For this study, we classified subjects from the hybrid zone into two categories: *pigra*-like individuals had the discrete morphological features of *A. pigra*, most notably larger size, entirely black pelage, and a larger ruff of fur around the face, while *palliata*-like individuals were slighter, had golden flank fur, smaller faces, and a less prominent ruff of fur typical of *A. palliata* (Kelaita et al., 2011; Lawrence, 1933; Smith, 1970). All subjects within each of our study groups possessed the same phenotype with one exception; group #72 (Table 1.2) had a female whose phenotype was at odds with the phenotype of the other group members. This female was removed from all analyses, although we provide an account of her behavior (see Discussion).

Data collection

All adults in a group were randomly chosen as subjects for hour-long focal sampling (Altmann, 1974), with no animals sampled twice until all were sampled once. Juveniles and subadults were not sampled. Observers rotated to another group after one week (approximately 40 focal hours) and attempted to follow all groups for 2 weeks in 2011 and for 3 weeks in 2012 (Table 1.2). Groups were followed usually starting from ~7AM to ~5PM, with each day in the field lasting 8-10 hours; thus, each "week" of data consisted of about 4-5 days of fieldwork.

During each focal sampling, proximity data among adults were collected using instantaneous scan samples of all visible group members every 10 minutes (Table 1.2). Each adult group member was placed into one of four categories based on their proximity to the subject (1. contact, 2. <1m, 3. 1-5m, 4. >5m). The proximity scores were then dichotomized into "number of times a dyad was <5m" and "number of times a dyad was >5m."

To examine female affiliation and agonism, we also recorded social interactions between female focal subjects and other adults in the group during focal samples. Affiliative behaviors included touching, grooming, and play. Due to the naturally low levels of social behavior exhibited by howler monkeys, all types of affiliative behaviors were combined and a rate was calculated for each individual female based on the total number of focal hours she was observed. This rate was then corrected for group size (dividing by the number of females in the subject's group, not including the subject).

Agonistic behaviors included displacements, threats, chases, and fights. Rates of agonism were even lower in our study, and this made analyzing agonism on an individual female level unfeasible. Thus, counts of female-female aggression were tallied for each group and then combined according to their taxonomic categories: *A. pigra, pigra*-like, *palliata*-like, and *A. palliata*. Finally, a rate per hour of observation was calculated for each taxonomic group.

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Statistical analyses of differences across purebreds and hybrids

All analyses were run in Stata 12.1 (StataCorp). In addition to standard packages, we also used the package *gllamm* to run generalized linear mixed models (GLMM) (Rabe-Hesketh, Skrondal, & Pickles, 2005). Tests are two-tailed and the initial alpha was set at 0.05. Multiple comparisons were corrected using false discovery rates (henceforth FDRs) as originally described in Benjamini & Hochberg (1995) and as applied to behavioral data (Verhoeven, Simonsen, & McIntyre, 2005).

Proximity

We ran a GLMM on the dichotomized proximity scores of every possible dyad within each group ($N = 438$ dyads). Taxonomic categories (*A. pigra*, hybrids, and *A. palliata*), sex (analyzed by dyad; female-female, female-male, male-male), an interaction term between taxon and sex, and group size were entered as fixed effects. Dyad identity and group identity were entered as random effects. None of the predictors were significantly multicollinear (all variance inflation factors <2). Odds-ratios (OR) are reported for the effects of each predictor on proximity.

After the initial GLMM, we ran two post-hoc pairwise comparisons. First, we split the hybrids, including both sexes, into *pigra*-like and *palliata*-like groups to examine whether ancestry effects existed within the hybrid zone ($N = 303$ dyads). Second, we focused on female *pigra*-like and *palliata*-like individuals only, to see whether ancestry effects persisted among females ($N = 150$ dyads). We assumed that hybrid individuals resembling a particular parent species also share most of their genome with that species (Kelaita & Cortés-Ortiz, 2013). For these comparisons, we used a simple binomial test to examine whether the proportion of time individuals in *pigra*-like groups spent in <5m is greater than that of individuals in *palliata*-like groups.

<u>Social behavior</u>

We next focused on female-female affiliative and agonistic social interactions. Because data residuals were non-normal, we analyzed affiliative social behavior using a Kruskal-Wallis non-parametric ANOVA, with the corrected rate of affiliation per female (number of behavioral acts/hour/group size) as the dependent variable, and taxon (*A. pigra, pigra*-like, *palliata*-like, and *A. palliata*) as the grouping variable ($N = 71$ females). After the Kruskal-Wallis test, we performed post-hoc pairwise comparisons using the Mann-Whitney U test.

We then used a Poisson regression to model the counts of female-to-female agonism (the dependent variable), with taxon (*A. pigra, pigra*-like, *palliata*-like, and *A. palliata*) as the predictor variable. Incidence rate ratios (IRR) between the taxonomic groups are reported.

Statistical analyses of potential confounding factors

<u>Group size</u>

In addition to including group size as a variable in the main GLMM analysis, we also took advantage of the fact that we had two relatively small *A. palliata* groups in our sample as an additional test of group size effects. We compared the dichotomized proximity scores in *A. pigra* groups (ranging in size from 3–5 adults) to small *A. palliata* groups (77B & 78; group size = 5 adults) by performing a second GLMM analysis restricting the dataset to only these animals ($N = 44$ dyads). Taxon and sex were included as fixed effects, while dyad identity nested in group identity were random effects. Additionally, we performed a post-hoc binomial test of the female-female dyads in these four groups to see if ancestry effects persisted among females ($N = 9$ dyads). Note that because we had already taken into account differences in group size when calculating affiliation rates, we did not perform any further analyses on that data here. Agonism data were too scarce for statistical analysis in this subset.

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As ecological conditions could affect patterns of social interaction, we controlled for this by using four hybrid groups − two *pigra*-like and two *palliata*-like – that resided in the same patch within the hybrid zone, thus having home ranges that overlapped (see Table 1.2). Because the two *palliata*-like groups had a substantial number of dyads that were never within <5m, a GLMM was not practical (Menard, 2002). We therefore performed a binomial test to compare the proportion of time spent in <5m between the *pigra*-like animals and *palliata*-like animals of this subset ($N = 216$ dyads). We then did the same test on the female-female dyads only ($N = 107$ dyads). Lastly, we examined female-female affiliation within these four groups using a non-parametric Kruskal-Wallis test, and excluded the agonism data due to its scarcity.

Protocol statement

This research complies with the University of Michigan Committee on Use and Care of Animals, the Ohio State University Institutional Animal Care and Use Committee, and adhered to American Society of Primatologists' Principles for the Ethical Treatment of Non-Human Primates. All field methods described here complied with Mexican legal requirements.

RESULTS

Differences across purebreds and hybrids

Proximity

The full model of proximity was significant (GLMM: $\chi^2_9 = 79.75$, $P < 0.00005$). Overall, individuals were farther apart (a greater proportion of time spent >5m) as group size increased ($OR = 1.16$, $P < 0.0001$).

The interaction of sex and taxon was not significant, indicating that we did not detect

significant variation across sexes in the effect of taxon on proximity, nor was there variation across taxonomic categories in the effect of sex on proximity.

However, sex alone was a significant predictor of proximity in *A. pigra* and hybrids (Figure 1.2). Within each of these two taxonomic groups, female-female dyads spent a greater proportion of time in <5m than male-male dyads (*A. pigra*: OR = 3.73, $P < 0.03$; hybrids: OR = 3.67, $P < 0.0001$), but no significant difference was observed between female-female dyads and female-male dyads (*A. pigra*: OR = 1.64, $P = 0.27$; hybrids: OR = 0.91, $P = 0.50$). Female-male dyads spent a significantly greater proportion of time in <5m than male-male dyads among hybrids only (OR = 4.00, $P < 0.0001$). Within *A. palliata*, no significant sex differences in proximity patterns were found.

Taxon was also a significant predictor of proximity, but not across all sexes (Figure 1.2). Among female-female dyads only, *A. pigra* were significantly closer together than *A. palliata* (OR = 2.76, $P < 0.05$) and hybrid dyads (OR = 2.48, $P < 0.05$), but *A. palliata* and hybrids did not significantly differ (OR = 0.92, $P = 0.81$). No significant differences were observed between female-male dyads or male-male dyads when comparing across the three taxa.

When hybrid groups were divided into *pigra*-like and *palliata*-like groups, *pigra*-like individuals spent 25% of their time <5m, while *palliata*-like individuals spent 14% of their time <5m. The proportion of time *pigra*-like animals spent close together was significantly higher than that of *palliata*-like individuals (binomial test: $P < 0.00005$). We observed the same pattern when restricting the analysis to female-female dyads; *pigra*-like females spent a significantly higher proportion of their time close together than *palliata*-like females (32% to 13%, binomial test: $P < 0.00005$).

Social behavior

Mean rates of affiliation among female-female dyads (adjusted for group size) were significantly different across the four taxonomic categories: *A. pigra*, *pigra*-like, *palliata*-like, and *A. palliata* (Kruskal-Wallis: $H_3 = 11.12$, $P < 0.02$; Figure 1.3). We performed a post-hoc pairwise comparison based on our expectation that *A. pigra* should have higher rates of affiliation compared to *A. palliata* ($N = 21$); this prediction was supported (Mann-Whitney: $Z = 2.99$, $P < 0.005$; Figure 1.3). Among hybrids, although *pigra*-like females seemed to have higher rates of affiliation than *palliata*-like females ($N = 50$), this result was not statistically significant (Mann-Whitney: $Z = 0.76$, $P = 0.45$; Figure 1.3).

However, as predicted if behavioral patterns follow ancestry, *palliata*-like females had very similar rates of affiliation (0.074 acts/hour) to purebred *A. palliata* (0.062 acts/hour; $N = 49$; Mann-Whitney: $Z = 0.15$, $P = 0.88$). Also as expected, purebred *A. pigra* had higher rates of affiliation than *palliata*-like females ($N = 42$; Mann-Whitney: $Z = 3.12$, $P < 0.002$), similar to the difference between *A. pigra* and *A. palliata.* However, contrary to ancestry expectations, *pigra*-like females had significantly lower rates of affiliation (0.095 acts/hour) than purebred *A. pigra* (0.200 acts/hour; $N = 22$; Mann-Whitney: $Z = 2.43$, $P < 0.02$).

We observed only 35 instances of agonism by focal females toward other females across the full study period. Rates of agonism were 0 among *A. pigra* females (0 acts/186 hours), 0.021 among *pigra*-like females (7 acts/339 hours), 0.074 among *palliata*-like females (23 acts/310 hours), and 0.028 among *A. palliata* females (5 acts/181 hours). Results of the Poisson regression analysis revealed that the ratio of agonism rates between *palliata*-like and *pigra*-like females were significantly different from one, with the *palliata*-like female agonism rate being 3.59 times higher ($Z = 2.96$, IRR = 3.59, $P < 0.05$). However, the ratio of agonism rates between *A. pigra* females and *A. palliata* females did not significantly differ from one.

Potential confounding factors affecting social structure

Effect of group size on proximity

The effect of taxon on proximity remained significant when we restricted our analysis to *A. pigra* and *A. palliata* groups matched for size, showing that although group size may have an overall effect on proximity, it is not enough to override the effect of ancestry. We found that the proportion of time spent <5m was still significantly higher in the three *A. pigra* groups with 3–5 adult members than in the two *A. palliata* groups with 5 adult members (GLMM: OR = 2.45, P < 0.0001). The effect of sex also remained significant, with female-female dyads spending a greater proportion of time <5m than male-male dyads (OR = 2.98, P < 0.0001), and tending to spend more time <5m than female-male dyads (OR = 1.37, P = 0.059). Finally, the post-hoc analysis within female-female dyads only revealed that female *A. pigra* spent a significantly higher proportion of time closer together than female *A. palliata* (66% to 19%, binomial test: P < 0.00005).

Effect of ecological variation on proximity and social behavior

To control ecological variation, we restricted our analysis to four groups (two *palliata*-like and two *pigra*-like) residing in a single fragment in the hybrid zone. We found that, in spite of home range overlap, hybrids in this fragment responded like individuals of the purebred species that they morphologically resembled. In other words, *pigra*-like groups spent a higher proportion of time in <5m than *palliata*-like groups (40% to 7%, binomial test: P < 0.00005). Strikingly, a comparison of the proximity scores of the two *palliata*-like groups, revealed that 44% of dyads (24 out of 55) in group 72 and 52% of dyads (76 out of 146) in group 74 were never seen within <5m. In contrast, all of the dyads among the two *pigra*-like groups spent at least 21% of their scans in <5m. Finally, our post-hoc analysis examining only female-female

dyads in this subset revealed that the ancestry effects still remained; females in the two *pigra*-like groups spent a significantly higher proportion of their time at <5m than females in the two *palliata*-like groups (41% to 7%, binomial test: $P < 0.00005$). There were no significant differences in rates of affiliation.

DISCUSSION

Using identical data collection methods to compare proximity and social behavior across three populations of howler monkeys, we concluded that both ancestry and flexibility contribute to social variation among members of sister taxa *A. palliata* and *A. pigra,* and their hybrids.

We found differences in social structure (proximity and social interactions) between the pure populations that matched previous descriptions (e.g. *A. pigra:* Van Belle et al., 2011; *A. palliata*: Zucker & Clarke, 1998). First, *A. pigra* female-female dyads spent a significantly greater proportion of time at <5m than *A. palliata,* and in fact spent the most time close together out of all the taxon-sex categories. Second, *A. pigra* females engaged in significantly higher levels of affiliative social interactions as compared to *A. palliata* females. We should point out, however, that rates of affiliation were quite low in this study, and so our results should be interpreted with caution.

We also found that hybrids had variable behavior that generally differed according to ancestry, as determined using phenotype. These differences mirrored the purebred patterns and, particularly with respect to proximity patterns, persisted when we examined four groups of hybrids occupying the same forest fragment (sharing the same ecological conditions). In this analysis, *pigra*-like hybrids were significantly closer together than *palliata*-like hybrids, implying an effect of ancestry rather than potential ecological factors. Taken together, this study

is the first demonstration that ancestry drives differences in social behavior in New World monkeys, suggesting similarities to Old World monkeys (Bergman & Beehner, 2003; Di Fiore & Rendall, 1994; Tung et al., 2012).

On the other hand, our analysis of group size effects suggested flexibility in some of the variation in social structure. Indeed, group size was a significant predictor of proximity in our model. Because groups are more cohesive when they are smaller, individuals may spend more time interacting with each other when in smaller groups (Lehmann, Korstjens, & Dunbar, 2007; Sueur, Deneubourg, Petit, & Couzin, 2011). *Alouatta pigra* groups are smaller on average than *A. palliata* groups, creating the possibility that the smaller groups of *A. pigra* resulted in stronger female-female relationships. For example, in our analysis on the four hybrid groups residing the same fragment (mentioned above), we were unable to control for group size due to the nature of our data, and thus it remains possible that the *pigra*-like hybrids were simply closer together because their groups are smaller than the *palliata*-like hybrids. However, we continued to find an effect of taxon on proximity even when comparing similarly-sized purebred groups, suggesting that ancestry plays a role in addition to the role of group size.

In general, agonistic encounters between the adult females of our study were very infrequent, regardless of taxonomic categories (but in particular, agonism between *A. palliata* females seems lower than other reports, see Table 1.1), and this again affects the interpretation of our results. We found that within the hybrids, *palliata*-like females had significantly higher rates of agonism than *pigra*-like females. While the difference between hybrid females could reflect actual species differences in agonism rates, the lack of significant differences between the purebred *A. pigra* and *A. palliata* seems to suggest otherwise. If the hybrid sites are indeed more impacted by human disturbance, and given that *A. palliata* are more affected by decreases in

fragment size than *A. pigra* (Dias et al., 2013), this could explain why the hybrid females – and *palliata*-like females in particular – engaged in more instances of agonism.

Overall, our results suggest a stronger influence of ancestry in the social structure of *Alouatta*, while the impact of ecological variation is less clear. For example, despite inhabiting the site with arguably the greatest degree of human disturbance, *pigra*-like and *palliata*-like animals residing in the same forest fragment still exhibited proximity patterns typical of the species they most resemble. Further, in our analysis controlling for the effect of group size on proximity (discussed above), we compared only the *A. palliata* groups at La Flor to the *A. pigra* groups in El Tormento (both sites with relatively less prominent human activity), and we still found differences in proximity patterns based on taxon. Howler monkeys have been documented to be resilient to environmental change, retaining fairly species-typical behavior while living in disturbed habitats (e.g. Bicca-Marques, 2003; Cristóbal-Azkarate & Arroyo-Rodríguez, 2007; Martínez-Mota, Valdespino, Sánchez-Ramos, & Serio-Silva, 2007; Palma et al., 2011; Pavelka & Knopff, 2004), and such resilience may in fact have a genetic basis. Nonetheless, this study does not rule out the effect of major ecological factors, such as density of preferred food trees or forest composition, which may be important in explaining behavioral differences across sites, aspects that need to be incorporated in future studies.

Another alternative explanation of our results is that individuals in a group simply adopt the behavioral strategies of the majority (i.e., *A. palliata* individuals behave like *A. palliata* because they are surrounded by other *A. palliata* individuals). For example, some studies report that an individual of one species may flexibly learn and adopt the social behavior patterns of another species if they are accepted into a heterospecific group (Fragaszy & Visalberghi, 2004; Verzijden et al., 2012). Though these examples often stem from cross-fostering experiments

where a critical period of learning appears to be crucial, the learning of social behavior from conspecifics has also been documented in adult primates (e.g. baboon transplant experiments: Kummer, 1970b).

In our study, it is difficult to rule out social learning because all individuals in a group were of the same taxonomic category (i.e. a "*pigra*-like" group consists of *A. pigra*/*pigra*-like individuals only). Thus, when individuals behave similarly in the same group, it is impossible to discriminate learning from genetic influences on behavior. However, one hybrid female in our study did reside with animals that were phenotypically different from her. This *pigra*-like female (HSP72) resided for an entire season in the *palliata*-like group 72. During that time, HSP72 was farther away from her female *palliata*-like group-mates than *pigra*-like females in other groups were from each other, and the proportion of time she spent in <5m was comparable to *palliata*-like females. Additionally, HSP72 engaged in few affiliative behaviors with her female group-mates during her focal hours, another pattern similar to many *palliata*-like females. However, we found that she initiated nearly all approaches to other females in her group, while very few of her group-mates approached her. This implies that HSP72 was attempting to behave in a *pigra*-like manner (i.e. more cohesively), but the *palliata*-like females in her group did not respond to these attempts. Similar reports of hybrid individuals behaving in a species-typical manner, rather than in the manner of their heterospecific group, also exist in the baboon hybrid zone in Awash (Bergman & Beehner, 2004; Bergman et al., 2008; Phillips-Conroy, Jolly, & Brett, 1991). Thus, we argue that the patterns reported here are more likely to reflect the effect of ancestry rather than social learning.

Though we have demonstrated that ancestry does indeed affect social structure variation (on top of variation resulting from flexible responses to group size and possibly habitat

disturbance), several unresolved issues remain to be addressed through the inclusion of genetic data. First, because our study used phenotype to assign individuals and groups as *pigra*-like or *palliata*-like, it is possible that we are misrepresenting the underlying genetic variation. For instance, hybrid affiliation patterns were closer to those of *A. palliata*: neither *pigra*-like nor *palliata*-like females differed significantly from *A. palliata* females, and *pigra*-like females actually had significantly *lower* affiliation than *A. pigra* females, the most affiliative category. This result may have two possible explanations: 1. the potential inclusion of genetically intermediate individuals within the *pigra*-like females that is not possible to distinguish based on morphology alone; or 2. the potential introgression of *A. palliata*-type genes associated with female-female affiliation. As we continue to gather genetic information of the individuals sampled for behavior, we will be able to better understand how proximity and social relationships relate to different degrees of genetic admixture in the hybrid population.

Additionally, kinship may impact the results presented here, because closely related individuals should spend more time closer together and be more affiliative than distantly related individuals. Our current knowledge suggests that *A. pigra* females are likely to be more closely related than *A. palliata* females due to their reported ability to keep out other, presumably unrelated, females (Table 1.1). Thus, *A. pigra* females within a group should become more related over time, similar to red howler monkeys (Brockett, Horwich, & Jones, 2000; Pope, 2000). In fact, recent research in *A. pigra* has demonstrated that in most groups, females, males, or both sexes lived with adult same-sex kin (Van Belle, Estrada, Strier, & Di Fiore, 2012). On the other hand, within-group relatedness among *A. palliata* females should be low because juveniles are forced to disperse and are able to join new groups that do not have kin (Glander, 1992). Interestingly, genetic studies on the *A. palliata* population in Barro Colorado Island

(Milton, Lozier, & Lacey, 2009) demonstrated that *A. palliata* have higher relatedness than expected; however, it is not clear whether these results also apply to *A. palliata* living under less insular conditions. A recent comparative genetic analysis on purebred *A. pigra* and *A. palliata* in Mexico and Guatemala provides further insight, showing that within-group relatedness of same sex dyads are actually high in both species (Baiz, 2013). Thus, kinship does not seem to explain the differences in social behavior that we see. Still, more studies that incorporate genetic and behavioral data for the same set of individuals are necessary to better understand the kinship patterns of these two species in a comparative context, and thus reveal how kinship impacts social structure (Chapais & Berman, 2004).

The evidence of a genetic component in social behavior presented here raises the question of why we see this pattern in howler monkeys, a genus that exhibits dramatic group size and composition fluctuations in response to current environmental conditions (Fernandez-Duque, Di Fiore, & Huck, 2012). The answer may come down to the fact that different aspects of social systems have different degrees of phylogenetic constraint. For instance, though broad dispersal patterns are fairly consistent within-taxa, fluctuations in environmental conditions that change food or territory availability are likely to result in corresponding changes to group size and composition within populations, as individuals may delay dispersal or are prevented from entering groups when they disperse. In contrast, the formation of social relationships has much to do with the motivation to seek out and interact with conspecifics. A lack of motivation should result in a lack of social interactions even if group size or composition alters, and how motivated animals are to affiliate with each other may be more influenced by genetic differences between species (e.g. differing levels of oxytocin [Campbell, 2008], involved in the formation of female social bonds [Massen, Sterck, & de Vos, 2010]).

In sum, our study provides evidence of a genetic component as well as some flexibility to social structure, as defined by proximity patterns and social interactions. Detailed genetic analysis is the critical next step, which will bolster our abilities to categorize individuals to a more fine-tuned degree of admixture, allowing us to do with behavior what has recently been done for morphology (Kelaita & Cortés-Ortiz, 2013). But even as it stands, our results add to the growing body of evidence that demonstrates the importance of incorporating phylogeny in any systematic attempt to understand interspecific differences among primate social systems.

ADDENDUM

This chapter has been published in the March 2014 Early View edition of the *American Journal of Primatology.*

TABLES

TABLE 1.1. Summary of social system differences between *Alouatta pigra* and *A. palliata*

	Alouatta pigra	*Alouatta palliata*
Group size	4-8 individuals, range: 2-16, with 1-5 adult males & 1-4 adult females (Di Fiore et al., 2011)	8-23 individuals, range: 2-45, with 1-8 adult males & 2-19 adult females (Di Fiore et al., 2011)
Group cohesion	Individuals tend to remain in close proximity (Corewyn & Pavelka, 2007)	Commonly split into sub-groups (Altmann, 1959; Bezanson et al., 2008; Dias & Rodríguez-Luna, 2006)
Sex ratio	1.2-2.1 females/male (Crockett & Eisenberg, 1987; Neville, Glander, Braza, & Rylands, 1988; Treves, Drescher, & Ingrisano, 2001)	1.4-4.1 females/male (Crockett & Eisenberg, 1987; Neville et al., 1988)
Dispersal	Juveniles & adults of both sexes disperse (Brockett et al., 2000)	Juveniles of both sexes evicted from natal group by unrelated adults (Clarke, Glander, & Zucker, 1998; Clarke & Glander, 2008)
Mating system	Polygynous (Bolin, 1981), with reports of polygynandry (Van Belle et al., 2008; Van Belle, Estrada, Ziegler, & Strier, 2009; Horwich, Brockett, James, & Jones, 2000)	Polygynandrous (Ryan, Starks, Milton, & Getz, 2008; Wang & Milton, 2003)
Male takeover	Resident males can be usurped and are often ousted from group (Van Belle et al., 2008; Brockett et al., 2000), often by coalitions (Horwich et al. 2000) of related males (Van Belle et al., 2012)	New males do not oust resident males but gradually join group, although takeovers by coalitions have been reported (Dias, Rangel-Negrín, Veà, & Canales-Espinosa, 2010; Glander, 1980)
Infanticide	Documented (Van Belle, Kulp, Thiessen-Bock, Garcia, & Estrada, 2010; Knopff, Knopff, & Pavelka, 2004)	Rare, but documented (Clarke, Zucker, & Glander, 1994; Clarke, 1983)
Female entry	Rare due to high levels of harassment from resident females (Brockett et al., 2000; Kitchen, 2006)	Common; join with relative ease and rapidly increase rank (Glander, 1980, 1992)
Group formation	Reportedly common due to low ability to join groups – emigrating individuals form new groups with each other (Brockett et al., 2000)	Reportedly rare as immigrating individuals can join existing groups (Glander 1992)
Female dominance hierarchy	None discernible; egalitarian with mostly affiliative interactions (Van Belle et al., 2011)	Reverse age-ordered (youngest female dominant) (Jones 1980; Zucker & Clarke 1998)
Female agonism	0.007 acts/hour (Van Belle et al. 2011)	0.38 acts/hour (Zucker & Clarke 1998); reports of female-female agonism [Larose 1996]

TABLE 1.2. Summary of groups sampled during study period (2011-2012)

Site	Group ID	# adult females (total focal hours)	# adult males (total focal hours)	Taxonomic category	Total hours per taxa	Total scans per taxa
Campeche	65	2 (52)	2 (62)	*A. pigra*	336	2016
	66	3 (60)	2 (51)			
	67	2 (74)	1 (37)			
Tabasco	68	2 (19)	2 (18)	*pigra*-like	546	3270
	70[a]	3 (54)	1 (20)			
	71[a]	2 (52)	1 (26)			
	44	3 (71)	2 (47)			
	73	3 (70)	2 (49)			
	76	2 (73)	1 (47)			
Tabasco	69	2 (45)	1 (26)	*palliata*-like	455	2728
	72[a,b]	10 (52)	3 (22)			
	74[a]	12 (48)	5 (23)			
	80	8 (86)	2 (34)			
	81	4 (87)	1 (32)			
Veracruz	77b	3 (71)	2 (49)	*A. palliata*	360	2153[c]
	78	2 (47)	3 (73)			
	79	9 (63)	5 (57)			
TOTAL		72 (1024)	36 (673)		1697	10167

[a] Groups 70, 71, 72, and 74 shared a patch in the hybrid site, as did 68 and 69. None of the other hybrid groups had ranges that overlapped.
[b] One *pigra*-like female, HSP72, was in this group, apparently integrated with the other, *palliata*-like individuals.
[c] Six 10 minute scans were performed for each 1 hour focal; however, a few scans were missing from the *A. palliata* and *pigra*-like groups, resulting in the discrepancy between the total focal hours and the total scans.

FIGURES
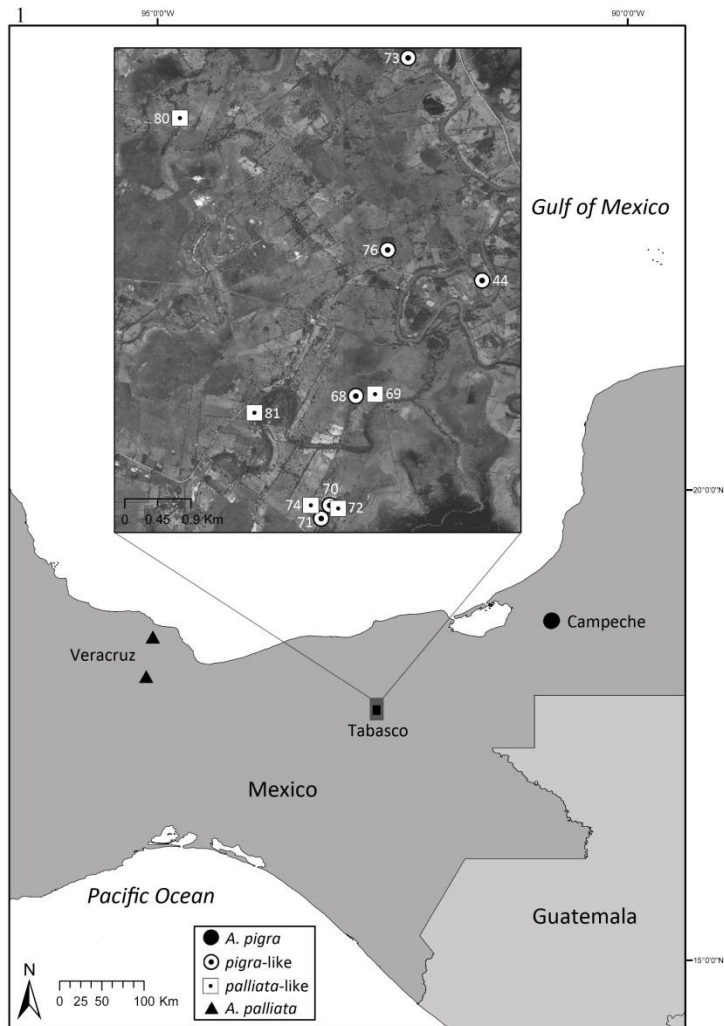
FIGURE 1.1. Map of the study sites.
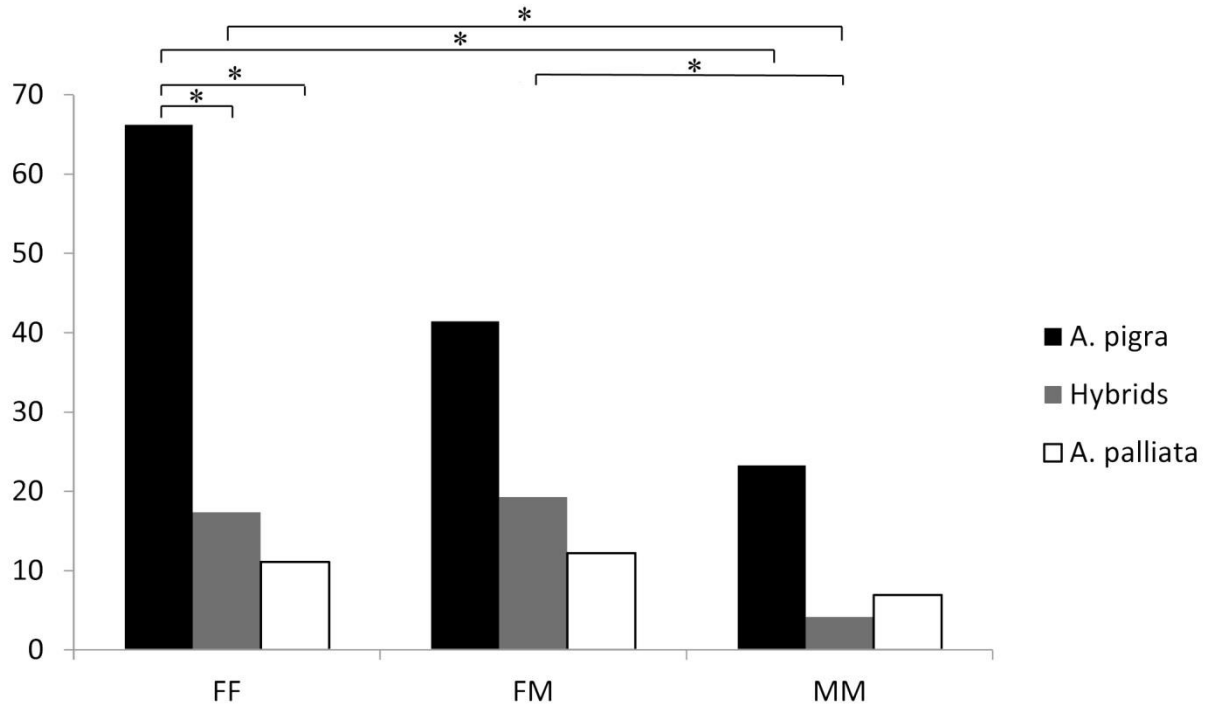
FIGURE 1.2. Frequency (calculated as percentage) of being <5m for each major taxonomic category (*A.pigra*, hybrids – *pigra*-like and *palliata*-like combined, and *A. palliata*), across the three sex categories: female-female (FF), female-male (FM), and male-male (MM). * marks significant differences (P < 0.05) on respective comparisons.
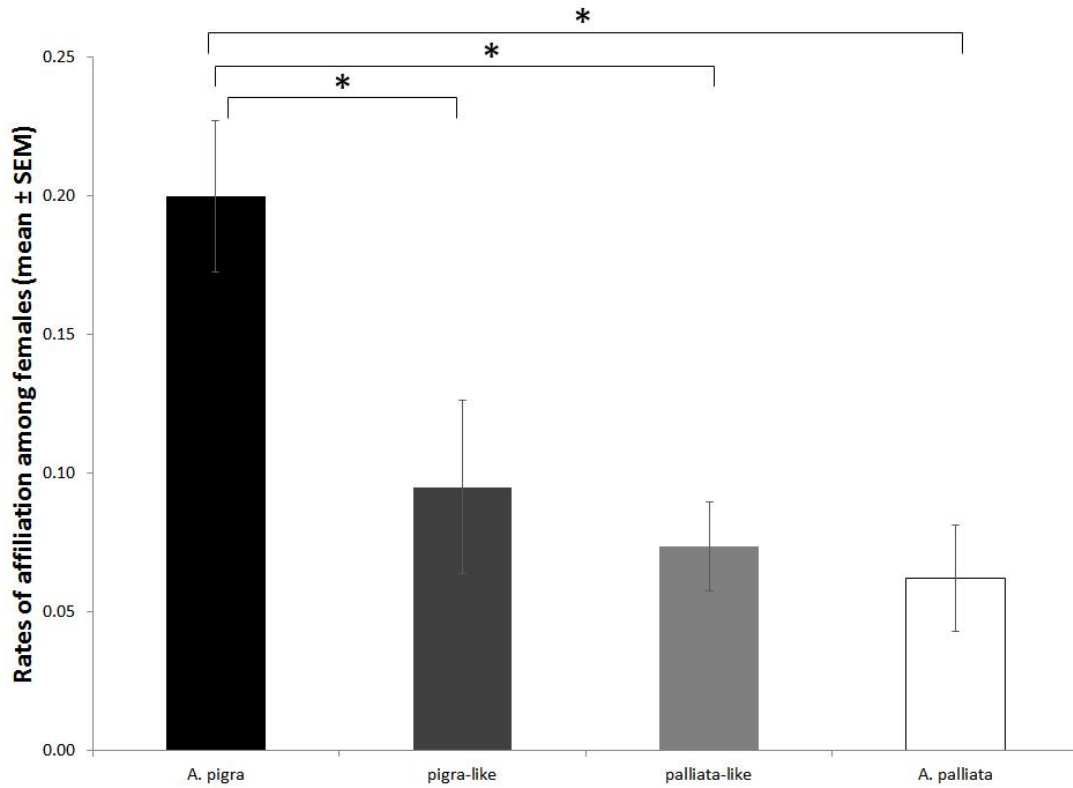
FIGURE 1.3. Plot of the rates of female-female affiliation (acts/hour) corrected for group size (number of possible female dyads). * marks a significant difference ($P < 0.05$) between *A. pigra* and *A. palliata*, *A. pigra* and *palliata*-like hybrids, as well as *A. pigra* and *pigra*-like hybrids. *A. palliata* rates were not significantly different from *pigra*-like rates, and the rates of two hybrids, *pigra*-like and *palliata*-like, were also not significantly different.

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CHAPTER TWO

Activity patterns are influenced by ancestry and habitat disturbance in *Alouatta pigra, A. palliata*, and their hybrids

ABSTRACT

We investigate the factors that contribute to variation in primate activity patterns among two species of howler monkeys, *Alouatta pigra* and *A. palliata*, and their hybrids. Activity budgets (time spent resting, feeding, moving), home range, and travel distance differ between animals that minimize energy expenditure (energy minimizers) and animals that maximize energy intake (energy maximizers). Based on previous descriptions of *A. pigra* and *A. palliata* characteristics, we hypothesize that *A. pigra* are more energy-minimizers than *A. palliata*. Additionally, we hypothesize that differences in activity patterns may have a genetic component, resulting in hybrids that are intermediate in their activity budgets, home range size, and distance traveled. We also consider the effects of resources and social factors (group size and sex). Finally, based on previous research indicating that *A. palliata* may be more sensitive to environmental change than *A. pigra*, we hypothesize that individuals with greater *A. palliata* ancestry will be more impacted by the highly disturbed hybrid zone, compared to individuals with greater *A. pigra* ancestry. We found that the purebreds do differ in some aspects of their activity patterns, with *A. pigra* traveling significantly less distance than *A. palliata*, characteristic of energy-minimization. This variation is affected by genetic ancestry: hybrids have intermediate

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home ranges and travel distance compared to purebreds even after considering group size and sex. Lastly, we found that *A. palliata*-like hybrids differed significantly from purebred *A. palliata* in all activities while *pigra*-like hybrids did not differ from purebred *A. pigra*. We suggest that this evidence indicates *palliata*-like hybrids are more severely impacted by environmental disturbance than *pigra*-like hybrids. Our results have implications for the conservation of these two primates and contribute to the understanding of why species may differ in their responses to severe ecological disturbance.

INTRODUCTION

Primate activity levels and movement patterns are intricately linked to resource acquisition. Thus, the distribution of resources can directly affect these activity patterns. Current resource availability and its spatio-temporal configuration in the environment can affect the time spent foraging and traveling in search of food, thereby potentially altering day range length (red colobus: Clutton-Brock, 1975; vervet monkeys: Isbell, Pruetz, & Young, 1998; Isbell & Young, 1993; geladas: Iwamoto & Dunbar, 1983; Barbary macaques: Ménard et al., 2013; mountain gorillas: Watts, 1988). Social factors can also affect resource acquisition and, consequently, activity. For example, the depletion of non-monopolizable resources occurs more quickly among larger groups than smaller groups. Primates in large groups must then compensate for the decrease in foraging efficiency with more time spent feeding and/or moving compared to primates in smaller groups (howler monkeys: Agostini, Holzmann, & Di Bitetti, 2012; Arroyo-Rodríguez, Dias, & Cristóbal-Azkarate, 2011; colobus: Fashing et al., 2007; Saj & Sicotte, 2007; Teichroeb, Saj, Paterson, & Sicotte, 2003; snub-nosed monkeys: Liu, Stanford, & Li, 2013). Additionally, because females may have higher nutritional requirements than males due to

lactation and pregnancy (Key & Ross, 1999; Sterck, Watts, & van Schaik, 1997), females typically spend less time resting and more time feeding (howlers: Bicca-Marques & Calegaro-Marques, 1994; colobus: Marsh, 1981; Teichroeb et al., 2003; lowland gorillas: Masi, Cipolletta, & Robbins, 2009; woolly monkeys: Shanee & Shanee, 2011).

In addition to this within species flexibility in activity patterns, there is also considerable variation across species in how they respond to resource availability. One major distinction is between species that employ an energy minimization (reducing energy expenditure) strategy vs. species that employ an energy maximization (increasing energy intake) strategy. Energy minimization strategies in primates tend to be characterized by more folivorous diets, short day ranges, small home ranges, and cohesive grouping. Conversely, energy maximization strategies involve more frugivorous diets, long day lengths, large home ranges, and more fluid grouping (Strier, 1992). Energy minimizers also spend more time resting and less time traveling than energy maximizers (thus not only move around less but also cover less distance on average in a day, e.g. Dasilva, 1992; Milton, 1998). Taken together, these two sets of traits may be considered co-evolved suites of adaptive behaviors that arose from different environments in the past (Rosenberger, Halenar, & Cooke, 2011; Rosenberger, Tejedor, Cooke, Halenar, & Pekkar, 2009).

There is some indirect evidence that energy maximizers and energy minimizers respond differently to changes in resource availability. For instance, frugivores tend to do less well in disturbed habitats compared to folivores (Boyle & Smith, 2010; Irwin et al., 2010; Johns & Skorupa, 1987). Further, in a study comparing capuchins to howler monkeys (energy maximizers to energy minimizers), researchers found that capuchins experienced a large increase in mortality rate following a period of unusually severe weather, while howlers were unaffected (Milton & Giacalone, 2014). This variation in response could be a result of the differing ancestry of energy-

minimizing species versus energy-maximizing species. In other words, energy minimizers may be better adapted to environmental disturbance than energy maximizers.

Studies of ancestry effects that may constrain activity and movement among primates are far less common than studies of how behavioral strategies respond to fluctuations in resource availability, group size, or sex. Thus, we lack a thorough understanding of the relative contributions of these factors to variation in activity levels, home range size, and distance traveled across primates. In this study, we examine the activity-related behavior of two species of howler monkeys, *Alouatta pigra* and *A. palliata,* along with their hybrids. Our goals are first to ascertain whether differences exist in their activity patterns, and second, to ask if these patterns are evolved behavioral strategies that may constrain their response to pressures in an anthropogenically-degraded habitat.

Despite being sister taxa (Cortés-Ortiz et al., 2003) and having a large degree of dietary overlap, we previously demonstrated that interspecific differences exist in the social behavior of *A. pigra* and *A. palliata*, and that the differences appear to be genetically modulated (Ho et al., 2014). Activity patterns could also be modulated by ancestry. While *Alouatta* are generally considered to be "evolved pioneers" that can persist in habitats unsuitable for other primates (Rosenberger et al., 2009), with a flexible frugivore-folivorous diet that can range anywhere from 20% to 90% leaves (Estrada, 1984; Pavelka & Knopff, 2004), it is possible that this general categorization overlooks more subtle interspecific differences in activity patterns. A recent comparative study of two species in *Alouatta*, for example, showed that *A. caraya* appear to use an energy minimization strategy more obviously than *A. guariba* (Agostini et al., 2012). Some data also suggest that *A. pigra* and *A. palliata* vary in terms of energy minimization, with *A. pigra* using this strategy more than *A. palliata*. Specifically, *A. palliata* groups are larger on

average (Di Fiore, Link, & Campbell, 2011), and they fission into smaller sub-groups when foraging (Bezanson, Garber, Murphy, & Premo, 2008; Dias & Rodríguez-Luna, 2006), a characteristic associated with energy maximization in primates (Strier, 1992). Also, *A. pigra* are heavier animals than *A. palliata* (Kelaita, Dias, Aguilar-Cucurachi, Canales-Espinosa, & Cortés-Ortiz, 2011), and larger animals tend to have diets more conducive to energy minimization strategies (i.e. leaves), although the relation between body size and diet in primates is somewhat weak (Lambert, 1998).

In this study, we look at how resources, social factors, and ancestry contribute to the activity patterns in these two species of howler monkeys. To do this, we compare male and female howlers in groups of various sizes in two general ways: howlers with different ancestry living in the same location and howlers with similar ancestry living in different locations. We compare activity budgets (time spent resting, feeding, moving), home range size, and distance covered – considered "activity patterns" as a whole. If resource distribution is an important factor, we predict that most variation in activity patterns will be between sites and forest fragments of different size regardless of social or phylogenetic factors. If social factors are important, we predict that sex and group size will determine activity patterns regardless of location or phylogeny. If ancestry is important, we expect most variation in activity patterns to be driven by taxonomic categories, regardless of location or social factors. Specifically, we hypothesize that *A. pigra* are more reliant on energy minimization than *A. palliata*. We thus predict that the more *pigra*-like an animal is, the more time they will spend resting and the less time they will spend moving. Additionally, *pigra-like animals* will have smaller home ranges and a shorter hourly travel distance than *palliata-like* animals.

Finally, we hypothesize that *A. palliata* may be more sensitive than *A. pigra* to

anthropogenic changes in their environment, as has been previously demonstrated in terms of social organization (Dias, Alvarado, Rangel-Negrín, Canales-Espinosa, & Cortés-Ortiz, 2013). We therefore predict that within the heavily disturbed hybrid zone, *palliata*-like groups will exhibit activity patterns that significantly differ from purebred *A. palliata* while *pigra*-like groups will not differ greatly from purebred *A. pigra*.

METHODS

Study sites

We conducted data collection for this study in three separate populations. We sampled within the *A. pigra* and *A. palliata* contact zone, where hybridization is confirmed to occur (Cortés-Ortiz et al., 2007), as well as in two areas with only purebred animals. These purebred sites are well outside of the contact area (approximately >260 km away) and it is unlikely that the individuals living there have had contact with the other species or with their genes (i.e., that they are hybrid or back-crossed individuals).

The purebred *A. pigra* site near Escarcega, Campeche, is El Tormento Forest Reserve, a protected, relatively large area of primary tropical forest of about 1400 ha (Van Belle & Estrada, 2008). The three purebred *A. pigra* groups studied at this site are in different locations within the reserve. The two purebred *A. palliata* sites are located in La Flor, Catemaco (~120 ha) and in Rancho Jalapilla, Acayucan (~30 ha), both in Veracruz. La Flor is an ornamental palm plantation consisting of primary and secondary vegetation. The canopy is composed of arboreal species typical of undisturbed primary rainforest and the arboreal howler monkeys can move freely through the trees despite the daily harvesting of ornamental palms in the understory (Shedden-González & Rodríguez-Luna, 2010). The remaining purebred *A. palliata* group is in Rancho

Jalapilla, a private property with a narrow but long riparian strip of secondary forest (surrounded by pastureland) that the monkeys can also move through unimpeded and are not directly disturbed. Finally, the hybrid site is located south of Macuspana, Tabasco, in the midst of the approximately 20 km wide contact zone (Kelaita & Cortés-Ortiz, 2013). Individual howler monkey groups there reside in small, discontinuous patches of mainly secondary forest (~ 3 ha) separated by ranches and farmland (Dias et al., 2013). The monkeys here are in constant contact with human settlements surrounding their small forest fragments. A single patch in Tabasco may be occupied by one or several howler monkey groups (up to four in the current study; see Ho et al., 2014 for details).

Subjects

We collected data simultaneously on three groups of purebred *A. pigra* and six groups of hybrids from February-August 2011, then simultaneously on three groups of purebred *A. palliata* and five new groups of hybrids from January-June 2012 (see Ho et al., 2014 for more details on group composition and data collection). All adults in the study were individually recognizable by researchers via ankle bracelets or natural markings such as scars and, in the case of *A. palliata*, differences in characteristic patches of skin/fur coloration on their feet and tails.

Despite most individuals in the hybrid zone resembling either *A. pigra* or *A. palliata* in terms of pelage coloration and size, our sample likely consists of highly backcrossed animals that are phenotypically similar to (and impossible to distinguish from) the parental species, and thus we considered all animals in the hybrid zone to be hybrids (see Kelaita & Cortés-Ortiz, 2013 for a genetic characterization of the admixture of individuals within the same population, and also the Methods of Ho et al., 2014 for a more detailed description of the justification for this decision). We classified subjects from the hybrid zone into two categories: *pigra*-like individuals

had the discrete morphological features of *A. pigra*, most notably larger size, entirely black pelage, and a larger ruff of fur around the face, while *palliata*-like individuals were slighter, had golden flank fur, smaller faces, and a less prominent ruff of fur typical of *A. palliata* (Kelaita et al., 2011; Lawrence, 1933; Smith, 1970). All subjects within each of our study groups possessed the same phenotype with one exception; group #72 had a female, HSP72, whose phenotype was at odds with the phenotype of the other group members. This female was excluded from the activity budget analyses, but was included in group #72's daily path length analyses (excluding HSP72 did not significantly change the results for either set of analyses).

Data collection

We followed all groups for a total of 741 hours in 2011 and for 956 hours in 2012. Observers collected approximately 40 hours of data before rotating to another group. Groups were typically followed from approximately 7AM to 5PM, with each day in the field lasting 8-10 hours.

Activity budget data for all adult individuals in each group were collected during hour-long focal sampling (Altmann, 1974), with no animals sampled twice until all others were sampled once. Activity was monitored continuously. We considered the animals to have changed from one activity to another if they had engaged in the new activity for over five seconds. Four activity categories were used: Resting, Feeding, Moving, and Social. For this study, we focused on the non-social activities only (for social behavior, see Ho et al., 2014): resting involved any state where the focal animal did not move or engage in the other three activities; feeding involved foraging for, extracting, and eating vegetation (when it was visibly clear that the animal was placing vegetation into the mouth and chewing); moving involved any continuous locomotion either for over 5 seconds and/or if crossing into a different tree — if the animal

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foraged and ate while moving, however, then the activity was considered feeding instead. Activity levels were calculated for each adult individual in the study by first summing the total duration (in seconds) that the animal spent in a particular activity across the study period, then dividing that sum by the total observation time (in seconds). This yielded a percent time spent on each activity per individual.

During each focal sampling, GPS data on the location of the focal animal were collected using hand-held GPS receivers (Garmin) every 10 minutes, resulting in a total of 6 points per hour. Using these GPS points, we calculated daily path length (in meters) with ArcMap 10.1 (ESRI, Menlo Park, CA) using the Generate Near Table tool to first compute the distance (in meters) from all point coordinates to each other. These distances were then exported to Microsoft Excel 2010 to complete the calculations. We were unable to systematically maintain the same hours of data collection from day to day; thus, fewer locational points were collected on some days than others. To account for this, we summed the path lengths of each day and divided by the number of hours spent following the monkeys on that day. This corrected value was then averaged across all days spent following a particular group to yield that group's average hourly path length. We did not average path lengths hour-by-hour because preliminary analysis showed that travel distance was not significantly affected by time of day.

Home range (in square meters) was calculated using the MCP (minimum convex polygon) method as we lacked the more fine-tuned data required for a kernel density analysis. MCPs were calculated in ArcMap 10.1 using the Minimum Bounding Geometry tool. Lastly, we also calculated fragment size by first visualizing each field site using satellite imagery. We then located the forest fragments of specific groups using GPS coordinates, and drew a polygon in ArcMap over all continuous forest cover in a particular site. We used ArcMap to calculate the

area of that polygon to yield a gross measurement of forest fragment size in square meters.

Statistical analyses

All analyses were run in Stata 12.1 (StataCorp). Tests were two-tailed and alpha was set at 0.05.

Activity budgets

We first ran three multiple regressions, one for each non-social activity (i.e. Resting, Feeding, and Moving; N = 106). These full models included all relevant variables: taxonomic category (*A. pigra*, *pigra*-like, *palliata*-like, and *A. palliata*), adult group size, sex, home range, and fragment size as predictors, with the rate of each non-social activity as the dependent variable.

We next focused on the patterns of hybrid activity levels specifically, with another set of three multiple regressions (N = 69). Our main goal was to examine the effect of sharing a forest fragment with another group in the hybrid zone. We included all previous predictor variables, plus a fourth ("sharing"), a binomial variable coded as either "sharing" or "alone." "Sharing" indicated that a study group resided with other howler groups in the same forest fragment. These other groups were occasionally, but not necessarily, also part of our study. All but one of our five *palliata*-like groups shared a forest fragment with other groups, while three out of our six *pigra*-like groups shared a forest fragment. We did not have any instances where a forest fragment was shared by groups consisting entirely of animals with the same ancestry. In other words, if a forest fragment had more than one group, it was always shared by *pigra*-like and *palliata*-like groups. "Alone" indicated that said group resided in a forest fragment entirely by themselves. Group composition within a forest fragment did not change throughout the entirely of our two field seasons.

Home range

We ran one multiple regression to examine factors that predicted home range (N = 216).
We set home range in square meters as a dependent variable, with taxonomic category (*A. pigra*,
pigra-like*, palliata*-like, and *A. palliata*), adult group size, and fragment size as predictor
variables. This analysis allowed us to see whether interspecific variance in home range size
existed, and whether differences in movement patterns corresponded to differences in home
range size. Here again, we also repeated the analysis, restricting it to the hybrid zone to examine
whether sharing a forest fragment affected group home range size (N = 133).

Distance traveled per hour

We ran two multiple regressions on average hourly path length (N = 216). The first
regression included taxonomic category, adult group size, home range, and fragment size as
predictors. The second regression restricted the analysis to hybrids only and included the same
predictors, again plus the variable "sharing" (N = 133).

Ecological influences on activity patterns

Although fragment size is positively correlated with food sources for howler monkeys
(Arroyo-Rodríguez & Dias, 2010), we recognized that fragment size can be a weak proxy for
resource variation. Due to the lack of direct ecological data for each site, we address this issue by
performing tests on four groups (two *pigra*-like and two *palliata*-like) that share the same forest
fragment in the hybrid zone (N = 34). Each activity (resting, feeding, moving) was used as
dependent variables, while sex and group size were predictors (home range and fragment size
were excluded due to collinearity). We also compared the two La Flor purebred *A. palliata*
groups to the *A. palliata* group in Jalapilla (N = 24). Since La Flor and Jalapilla differ in their
forest types (see Methods), if the La Flor groups have significantly different activity budgets

compared to the Jalapilla group, this should indicate that ecology exerts a stronger effect on activity than ancestry. Because the data on distance traveled were collected by group rather than by individuals, there was not enough variation for us to perform equivalent analyses on distance.

RESULTS

Effect of ecological factors on activity patterns

When we examined ecological factors, we found that home range did not significantly predict any aspect of activity patterns. The size of forest fragments significantly predicted both size of the home range and distance traveled: as forest fragment size increased, home range and distance traveled increased as well ($P < 0.00005$ and $P < 0.002$ respectively; Table 2.1). However, fragment size did not affect activity budgets.

When we looked within the forest fragment where two *pigra*-like and two *palliata*-like groups lived (see Ho et al., 2014 for description of groups), we found that, contrary to prediction, *palliata*-like groups spent more time resting than *pigra*-like groups ($t = 2.30$, Beta $= 1.06$, $P = 0.028$). Also contrary to prediction, there was no significant difference in time spent moving. There was a trend for *palliata*-like groups to spend less time feeding than *pigra*-like groups ($P = 0.069$). Group size did not have a significant effect on activity budgets within this subset. Sex only had a significant effect on time spent moving; again, males moved significantly more than females ($t = 2.09$, Beta $= .342$, $P = 0.046$). We found no significant differences in the activity budgets of the La Flor *A. palliata* groups versus the Jalapilla *A. palliata* group.

Effect of social factors on activity patterns

Looking at social factors, group size had no significant effect on any activity, but significantly predicted home range and distance traveled: as group size increased, both home

range and distance traveled increased (Table 2.1). Sex was not a significant predictor of any aspect of energy use strategies, although there was a trend for males to spend more time moving than females (Table 2.1).

Effect of ancestry on activity patterns

The full models for activity (resting, feeding, and moving), home range, and distance traveled were all significant (see Table 2.1 for full regression results).

Taxonomic categories were significant predictors of time spent resting, feeding, and moving, as well as the home range size and the distance traveled by each group (Figure 2.1 & 4; Table 2.1). We found that within activity budgets, differences were mainly driven by *palliata*-like animals: they spent significantly more time resting, and significantly less time feeding, than *pigra*-like animals or purebred *A. palliata* (Figure 2.1; Table 2.2). This result corresponds to what we found in the subset of two *pigra*-like and two *palliata*-like groups living in the same forest fragment, in the above analysis examining ecological factors.

In contrast, *palliata*-like animals did not drive differences in home range or distance traveled. Instead, we found that *A. pigra* had the smallest home range (Figure 2.4; Table 2.2), while *A. palliata* had the largest. This difference was mirrored in the hybrids: *pigra*-like groups had smaller home ranges than *palliata*-like groups (Figure 2.4; Table 2.2). *A. pigra* also traveled significantly less distance than all other types of animals, while *pigra*-like animals traveled significantly more than *palliata*-like animals and less than *A. palliata*. (Figure 2.2, Table 2.2).

Effect of human-induced environmental change in the hybrid zone

Because the taxon difference seemed primarily driven by the hybrid animals, particularly *palliata*-like groups, we focused within the hybrid zone only. Since most of our *palliata*-like groups reside in a forest fragment with other groups, we considered the possibility that sharing a

forest fragment was affecting the activity patterns of the *palliata*-like groups.

Among hybrids, again, all full models of activity patterns were significant (Table 2.3). Importantly, after controlling for the effects of taxonomic category, group size, sex, home range, and fragment size, sharing a forest fragment significantly affected time spent resting and feeding (but not moving): groups that shared a patch rested significantly more often (P = 0.018; Figure 2.3; Table 2.3) and fed significantly less often (P = 0.005; Figure 2.3; Table 2.3) than groups that lived alone. This corresponded to the results from the earlier activity budget analysis, where *palliata*-like groups rested more and fed less than *pigra*-like groups. Sharing a forest fragment with another group also resulted in smaller home ranges than if the group resided in a fragment alone (P < 0.00005; Table 2.3). However, sharing a forest fragment did not significantly affect distance traveled per hour per day, after controlling for the effects of taxon, group size, home range, and fragment size (Table 2.3).

Taxonomic categories continued to have an effect. In particular, *palliata*-like groups had smaller home ranges than *pigra*-like groups (t = -12.01, Beta = -.430, P < 0.00005), a result that was in the opposite direction of the purebreds, where *A. palliata* had larger home ranges than *A. pigra*. However, *palliata*-like groups traveled significantly shorter distances than *pigra*-like groups, which did not differ from the results found for the models that included purebreds.

The effects of group size also did not differ from those found using models that included purebreds; that is, larger groups still had larger home ranges and traveled greater distances than smaller groups (P < 0.00005 for both variables; Table 2.3). Finally, being in a larger forest fragment continued to result in larger home ranges (P < 0.00005; Table 2.3), but neither fragment size nor home range size affected the distance traveled among hybrids.

DISCUSSION

We found a complex interaction between genetic background, social factors, and environmental pressures in the activity and movement patterns of a diverse set of howler monkeys. Using data collected and analyzed with identical methods, we uncovered differences between *A. pigra* and *A. palliata* that were not as apparent in previous meta-analyses, which emphasized the similarities of the two species (Bicca-Marques, 2003; Di Fiore et al., 2011). In particular, *A. pigra* and *A. palliata* are both typically considered energy minimizers with similar dietary profiles (Dias et al. in preparation; Pavelka & Knopff, 2004; Silver, Ostro, Yeager, & Horwich, 1998). The lack of interspecific differences between *A. pigra* versus *A. palliata* activity budgets in our study supports this characterization. However, we also found that by some measures, *A. pigra* seemed to be more reliant on energy minimization than *A. palliata*. Specifically, *A. pigra* had shorter hourly travel lengths than *A. palliata* and had smaller home ranges despite being in a forest reserve that is much larger than any of the *A. palliata* sites. Both of these effects persisted after controlling for the most obvious interspecific difference between these two *Alouatta* species – their mean group size — as well as gross environmental factors such as forest fragment size.

As we predicted, our results revealed at least some genetic modulation in the interspecific variation of activity patterns. First, the smaller home ranges of *A. pigra* compared to *A. palliata* was mirrored in comparisons between the *pigra*-like and *palliata*-like groups. Second, genetic modulation of activity patterns should result in hybrids that are intermediate between the purebreds, and indeed we found that *pigra*-like groups had larger home ranges than *A. pigra* while *palliata*-like groups had smaller home ranges than *A. palliata*, but larger home ranges than either *A. pigra* or *pigra*-like groups. Also, both *pigra*-like and *palliata*-like groups had longer

hourly travel lengths compared to *A. pigra*, but shorter hourly travel lengths compared to *A. palliata*. At odds with our prediction, however, we found no significant differences in purebred activity budgets (resting, feeding, and moving). Thus, genetic modulation is only visible in some aspects of *A. pigra* and *A. palliata* activity patterns. Home range and travel lengths are both variables that are more directly affected by forest fragment size than activity budgets (e.g. Table 2.1; Bicca-Marques, 2003), so it is somewhat unexpected that these are the variables exhibiting inflexibility. However, since howler monkeys are unable to alter the size of their forest fragments, home range and travel lengths may actually better reflect interspecific differences in adjusting to a particular forest fragment size (i.e. *A. pigra* require smaller home ranges than *A. palliata* and may shrink their home range even more than *A. palliata* in a small fragment). On the other hand, because howler monkeys can alter their own behavior, the lack of interspecific differences in resting, feeding, or moving times better reflects the behavioral and dietary flexibility of *A. pigra* and *A. palliata*, two species considered to be "ecological pioneers" (Garber et al. in preparation; Rosenberger, Halenar, & Cooke, 2011).

While activity budgets did not clearly demonstrate an effect of ancestry, they revealed patterns that were also not easily explained by effects of social factors or ecological factors. Despite controlling for group size and despite living in the same highly disturbed habitat (the hybrid zone), *palliata*-like groups differed significantly from *pigra*-like groups in resting time and feeding time (as well as travel length, described above). These results imply that the variation in activity budgets is due to differences in ancestry. Also, in accordance with our prediction that animals with *A. palliata* ancestry are more sensitive to anthropogenic disturbance than animals with *A. pigra* ancestry, *palliata*-like groups significantly differed from purebred *A. palliata* in their activity budgets, while *pigra*-like groups did not significantly differ from *A.*

pigra groups.

However, the direction of the differences was unexpected: *palliata*-like rested more and fed less when compared to *pigra*-like groups, and had shorter hourly travel lengths. These findings do not support our hypothesis that individuals with greater *A. pigra* ancestry should be more energy minimizing than individuals with greater *A. palliata* ancestry. One possible explanation for this outcome lies in the comparison of *palliata*-like groups to purebred *A. palliata*, where we found that *palliata*-like groups also rested more, fed less, and even moved less than *A. palliata*. In the light of these results, we suggest that in a highly disturbed habitat such as our hybrid zone, groups with greater *A. palliata* ancestry may need to reduce their energy expenditure (increased resting coupled with a negative relationship to time spent feeding) more than *pigra*-like groups.

Other researchers have already suggested that behavioral modification to decrease energy expenditure is the reason for *Alouatta*'s ability to survive in a wide variety of environments (Milton, 1978). However, in contrast to our findings, several previous studies have demonstrated that *A. palliata* actually increase traveling and decrease resting in response to suboptimal habitats (Asensio, Cristobal-Azkarate, Dias, Vea, & Rodríguez-Luna, 2007; Dunn, Asensio, Arroyo-Rodríguez, Schnitzer, & Cristóbal-Azkarate, 2012; Dunn, Cristóbal-Azkarate, & Veà, 2009, 2010). These behavioral changes seem to be an attempt by the monkeys to maintain fruit intake (Dunn et al., 2010) or to obtain enough nutrients from low quality, fall-back foods (Dunn et al., 2012).

Whereas *A. palliata* typically decreases resting time and increases distance traveled in sub-optimal habitats, *A. pigra* typically responds in the opposite manner. A population of *A. pigra* increased resting time after hurricane Iris severely damaged their habitat (Behie & Pavelka,

2005). Similarly, newly translocated *A. pigra* increased resting time and decreased feeding time compared to established *A. pigra* groups, particularly within the first 72 hours of translocation, while travel time remained constant (these activity data were descriptive only, without statistical analysis; Silver & Marsh, 2003). Translocated *A. pigra* groups adapted quickly to their new environment, though it had little overlap in vegetation with their original habitat. The monkeys did not rely on the few familiar food sources and instead were able to immediately incorporate novel plant species into their diet. In addition, a group of *A. pigra* that were moved from an extremely disturbed habitat to a protected forest decreased their resting time and increased travel time in the new location (Rangel-Negrín, Dias, & Canales-Espinosa, 2011), indicating that they had engaged in energy-minimizing behaviors while living in the poorer environment. Finally, in a study that examined groups unaffected by any upheaval, *A. pigra* did not alter their behavior in response to changes in the environment – specifically availability of fruit – but adjusted their fruit consumption accordingly (these are the same groups affected by hurricane Iris, mentioned above, but the data are from before the hurricane: Pavelka & Knopff, 2004).

Taken as a whole, these studies imply that *A. pigra* maintain energy minimizing behavior regardless of whether they live in a disturbed or relatively pristine environment. It is possible that their extremely flexible diets allow *A. pigra*/*pigra*-like animals to simply alter their food choices rather than behavior. In contrast, *A. palliata*/*palliata*-like animals may have a slightly heavier reliance on fruits, resulting in an initial attempt to cope with disturbed environments via increased travel and foraging effort (energy maximization strategies). However, such behavior may ultimately be very stressful. In support of this, *A. palliata* glucocorticoid levels rise significantly in response to increased travel (Dunn, Cristóbal-Azkarate, Schulte-Herbrüggen, Chavira, & Veà, 2013). Eventually, in response to stress, *A. palliata*/*palliata*-like may be forced

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to switch their behavior and decrease energy expenditure in extremely disturbed environments, like our hybrid zone site. Of course, our explanation is entirely speculative at this point, and requires more research to confirm its validity. For instance, hormonal data on the hybrid monkeys will go a long way in helping us understand whether *pigra*-like or *palliata*-like animals are more stressed by the conditions in the hybrid zone. If *palliata*-like animals show higher glucocorticoid levels than *pigra*-like animals, that should be compelling evidence for our hypothesis that individuals with *A. palliata* ancestry are more sensitive to poor habitat conditions.

We do not suggest that *A. pigra* or those with *A. pigra* ancestry are entirely unaffected by habitat disturbance, as we also showed that *pigra*-like groups have larger home ranges and longer hourly path lengths than *A. pigra.* Additionally, previously published demographic and hormonal data implied that *A. pigra* do respond to environmental change. For example, Van Belle & Estrada (2006) demonstrated that *A. pigra* troop composition is more likely to be multi-male, multi-female in extensive forests, versus uni-male in fragmented forests, although mean group sizes did not differ. Also, Martínez-Mota and colleagues (2007) demonstrated that cortisol levels were higher among *A. pigra* individuals living in fragmented versus extensive forests. But we propose that *A. pigra* and *A. palliata* differ in the degree and nature of behavioral modification they undertake when they encounter a suboptimal environment. If the ability to maintain species-typical behavior after an initial period of adjustment is taken to indicate resilience (in other words, the animals "bounce back"), then *Alouatta pigra* and *pigra*-like hybrids are apparently more resilient, while *A. palliata* and *palliata*-like hybrids are less so. Given the resolution of our study, however, it is impossible to say for certain whether the patterns reported here truly reflect *A. pigra* flexibility or if they instead indicate a failure of *A. pigra*/*pigra*-like animals to adjust, in comparison with *A. palliata*/*palliata*-like animals. Because

our study groups in the hybrid zone have been in the area for years, we cannot compare the responses of newly arrived *A. pigra* to those of newly arrived *A. palliata*. If future studies make such a comparison and find that *A. pigra* can eventually revert to species-typical behavior, but *A. palliata* cannot, we will have stronger evidence in favor of *A. pigra* resilience. Data on changes (or lack thereof) in dietary preferences across the two species, similar to the Silver and Marsh (2003) study, will further bolster this idea if *A. pigra*/*pigra*-like animals exhibit more dietary flexibility and willingness to consume novel food than *A. palliata*/*palliata*-like animals.

Interestingly, Palma and colleagues (2011) found that *A. seniculus*, commonly considered to be very similar to *A. pigra* in multiple aspects of their social organization (Van Belle, Estrada, & Strier, 2011; Kitchen, 2004), did not change their activity budget or movement patterns in response to limited resources. This could indicate that the energy-minimizing suite of traits was inherited from a common ancestor shared with *A. seniculus* (Garber et al. in preparation), and the behavior observed in *A. palliata* is derived and perhaps more specialized, though testing this hypothesis is beyond the scope of our study.

That *A. palliata* and *palliata*-like groups may be more susceptible to environmental perturbation than *A. pigra* had been suggested previously (Dias et al., 2013), with an emphasis placed on the larger average group sizes of *A. palliata* as a possible factor. Larger group sizes increase scramble competition that is then further intensified by habitat disturbance, forcing an alteration to *A. palliata* species-typical patterns of social organization (i.e. a decrease in group size) through mechanisms such as increased emigration or infant mortality (Clarke, Collins, & Zucker, 2002). Though we controlled for group size in our analyses, it was a significant predictor in both home range size and distance traveled, indicating that within-group scramble competition did play a role in explaining the patterns observed here, much like what was reported in a

comparison of *A. caraya* and *A. guariba* (Agostini et al., 2012). Additionally, all but one of our *palliata*-like groups share a forest fragment with other groups (both *pigra*-like and *palliata*-like), while half of our *pigra*-like groups live alone. Thus, the behavioral differences we observed in the hybrid zone may be due to the increased intergroup competition experienced by *palliata*-like groups as opposed to *pigra*-like groups. Similar differences in activity and travel were also found for woolly monkey groups sharing a habitat (Stevenson & Castellanos, 2000).

Interspecific differences in ability to exclude other groups from resources could partly explain the group configurations found at our hybrid zone site. Specifically, *A. pigra* may be more successful than *A. palliata* at excluding other groups from a forest fragment. *Alouatta pigra* are larger than *A. palliata* on average (Kelaita et al., 2011), and the two species reportedly differ on intergroup tolerance. As stated previously, *A. pigra* females prevent immigrant females from joining their groups, and *A. pigra* males appear to take over groups by ousting the resident male or males. In contrast, immigrant *A. palliata* females and males are both able to join established groups with little resistance from group members (see Table 2.1 in Ho et al., 2014). Thus, individuals of greater *A. pigra* ancestry may be predisposed to intolerance toward extra-group individuals, resulting in many of our *pigra*-like groups living alone in a forest fragment. The same may not be true for more *palliata*-like groups. Indeed, our preliminary observations in the hybrid zone suggest that when *pigra*-like and *palliata*-like groups share a forest fragment, *pigra*-like groups tend to win intergroup encounters against *palliata*-like groups. In light of this, it may seem odd that *pigra*-like groups don't all reside alone in a forest fragment. One possible reason for this may lie in the forest fragment size. Fragments that hold multiple groups are much larger (24 – 32 ha) than fragments that only have a single group (5 – 13 ha), so groups that lose an intergroup encounter may simply need to retreat and avoid the winning group, rather than

leaving the fragment entirely.

In our comparison of four groups (two *pigra*-like and two *palliata*-like) sharing the same forest fragment, the average distance traveled per taxonomic category did not differ much (*pigra*-like: 117.9 m; *palliata*-like: 115.2 m). This seems to indicate that the groups were all similarly affected by intergroup competition, regardless of taxa. Because dispersal is severely limited in the hybrid zone, however, it is possible that any superior competitive ability of the *pigra*-like groups is rendered moot by the fact that the *palliata*-like groups cannot leave. Thus, any effects of intergroup competition will be felt by all resident groups, and taxonomic variation in behavioral responses will be more subtle. In fact, the one significant difference we found was that the two *palliata*-like groups rested more than the two *pigra*-like groups. This pattern of decreased energy expenditure may be the only hint that *palliata*-like groups are more affected by intergroup competition than the *pigra*-like groups. However, we also acknowledge that the lack of significant results in our analysis of these four groups may be due to a lack of power. A second caveat is that we did not have any instances where a fragment was shared by groups of the same ancestry (i.e., if a fragment contained multiple groups, they were always a mix of *pigra*-like groups and *palliata*-like groups). Therefore, we cannot know whether *palliata*-like groups will do poorly if they reside in the same fragment with other *palliata*-like groups, or if it is only the presence of *pigra*-like groups that negatively affects them. Future studies incorporating the behavior of groups in different configurations (all *pigra*-like, all *palliata*-like, mixed), sharing a fragment, will help in elucidating how intergroup competition alters species differences in activity patterns.

In general, we did not find a sex difference in howler activity budgets as we would predict if scramble competition affected females more than males. This is in contrast to previous

studies that reported increased feeding time for females compared to males in another species of howler monkey, *A. caraya* (Bicca-Marques & Calegaro-Marques, 1994). Several possibilities exist for this difference. First, though females require more energy due to the costs of lactation and pregnancy, this can be offset by sexual dimorphism (similar among *A. pigra* and *A. palliata*: Kelaita et al., 2011), as males require more energy to maintain their larger body size (Key & Ross, 1999). However, *A. caraya* are more sexually dimorphic than either *A. pigra* or *A. palliata* (a body mass dimorphism ratio of 1.48 vs. 1.32 and 1.33 respectively: Garber et al. in preparation), so if body mass is driving variation in feeding times, then one should expect *A. caraya* males to spend more time feeding than females. Since we do not see this pattern in *A. caraya* (Bicca-Marques & Calegaro-Marques, 1994), it is even more unlikely that we will see differences in feeding time driven by sexual dimorphism in *A. pigra* and *A. palliata*.

A second, more likely explanation involves dietary differences — *A. caraya* diet appears to be characterized by a greater reliance on leaves than either *A. pigra* or *A. palliata*. Since howler monkeys must be selective in their choice of plant matter in order to obtain all necessary nutrients (Milton, 1978), perhaps *A. caraya* females need to spend more time foraging for higher quality vegetation while males do not. Additionally, the more fruit-and-leaf-balanced diets of *A. pigra* and *A. palliata* may erase any sex differences as each sex may simply alter the ratio of fruits to leaves in order to fulfill their energy requirements, rather than spend more time on feeding. To test this hypothesis, we need detailed ecological data on the diets of the individuals in the hybrid zone, including time spent feeding on fruit versus leaves.

In conclusion, our results demonstrate that even among two sister taxa commonly considered similar in their ecology and behavior (Garber et al. in preparation), subtle interspecific differences may exist that are genetically, as well as environmentally, modulated.

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Such differences may carry over to a human-disturbed hybrid zone, resulting in hybrids that respond to ecological pressures in ways dependent on the relative genetic contributions of each parental species. If the relation between ancestry, ecology, and behavior could be further clarified, we should be able to better understand the variation in responses to environmental pressure exhibited by endangered versus abundant species, a necessary step in our efforts to conserve wildlife today (Sih, Ferrari, & Harris, 2011).

TABLES

Table 2.1

Multiple regression results for activity patterns across all taxa. (F = test statistic for full multiple regression model; df = degree of freedom; t = test statistic for main effects; β = standardized regression coefficient for main effects)

Energy use	Full Model F(df)	R^2	P	Species F(df)	P	Group size t	β	P	Sex t	β	P	Home range t	β	P	Fragment size t	β	P
Activity																	
Rest	6.24 (7,98)	0.31	**0.000**	7.96 (3,98)	**0.0001**	0.83	.12	0.41	-1.01	-.09	0.32	1.32	.17	0.19	-1.19	-3.27	0.24
Feed	11.01 (7,98)	0.44	**0.000**	11.25 (3,98)	**0.000**	-1.44	-.19	0.15	-1.37	-.11	0.18	-1.61	-.19	0.11	1.05	2.60	0.30
Move	3.52 (7,98)	0.20	**0.002**	5.06 (3,98)	**0.0027**	-0.92	-.14	0.36	1.96	.18	0.05	0.07	.01	0.94	-0.08	-.24	0.94
Home range	60.51 (5,210)	0.59	**0.000**	98.34 (3,210)	**0.000**	3.62	.25	**0.000**	-	-	-	-	-	-	13.65	18.29	**0.000**
Distance traveled	11.90 (6,209)	0.25	**0.000**	8.09 (3,209)	**0.000**	4.57	.43	**0.000**	-	-	-	1.41	.13	0.16	3.17	7.89	**0.002**

Table 2.2

Differences among taxonomic categories in activity patterns. (t = test statistic for main effects; β = standardized regression coefficient for main effects)

Variables	*A. pigra* vs. *A. palliata*			*A. pigra* vs. *pigra*-like			*A. pigra* vs. *palliata*-like			*pigra*-like vs. *palliata*-like			*A. palliata* vs. *pigra*-like			*A. palliata* vs. *palliata*-like		
	t	β	P	t	β	P	t	β	P	t	β	P	t	β	P	t	β	P
Activity																		
Rest	1.27	4.28	0.21	1.21	4.28	0.23	1.14	4.67	0.26	2.38	.32	**0.019**	0.00	.00	0.99	2.33	.32	**0.022**
Feed	1.08	3.28	0.283	1.04	3.30	0.303	0.92	3.42	0.357	3.31	.40	**0.001**	0.12	.02	0.907	3.06	.38	**0.003**
Move	0.04	.16	0.964	0.04	.16	0.967	0.06	.26	0.953	0.44	.06	0.663	1.48	.32	0.143	2.58	.38	**0.011**
Home range	14.09	17.38	**0.000**	13.65	21.82	**0.000**	13.82	20.84	**0.000**	2.48	.18	**0.014**	6.38	.66	**0.000**	6.40	.52	**0.000**
Distance traveled	3.23	7.51	**0.001**	3.23	9.58	**0.001**	3.13	8.83	**0.002**	4.15	.41	**0.000**	2.67	.41	**0.008**	0.60	.07	0.551

Table 2.3

Multiple regression results for activity patterns among hybrids – *pigra*-like vs. *palliata*-like. (F = test statistic for full multiple regression model; df = degree of freedom; t = test statistic for main effects; β = standardized regression coefficient for main effects)

Variables	Full Model F(df)	R^2	P	Species t	β	P	Group size t	β	P	Sex t	β	P	Home range t	β	P	Fragment size t	β	P	Sharing t	β	P
Activity																					
Rest	6.08 (6,62)	0.37	**0.000**	2.56	.59	**0.013**	0.02	.00	0.983	-1.96	-.20	0.055	1.26	.47	0.212	-1.98	-1.76	0.052	2.43	1.49	**0.018**
Feed	14.65 (6,62)	0.59	**0.000**	-3.54	-.67	**0.001**	-1.57	-.25	0.122	0.11	.01	0.909	-1.00	-.30	0.320	2.42	1.74	**0.018**	-2.94	-1.46	**0.005**
Move	2.58 (6,62)	0.20	**0.027**	-1.03	-.27	0.306	0.41	.09	0.094	2.07	.24	**0.042**	-0.94	-.39	0.353	1.23	1.23	0.223	-1.70	-.27	0.094
Home range	346.34 (4,128)	0.92	**0.000**	-12.01	-.43	**0.000**	6.23	.21	**0.000**	-	-	-	-	-	-	20.93	2.33	**0.000**	-12.91	-1.41	**0.000**
Distance traveled	11.63 (5,127)	0.31	**0.000**	-4.01	-.60	**0.000**	4.26	.48	**0.000**	-	-	-	-0.23	-.06	0.822	0.98	.65	0.330	-0.27	-.13	0.789

FIGURES
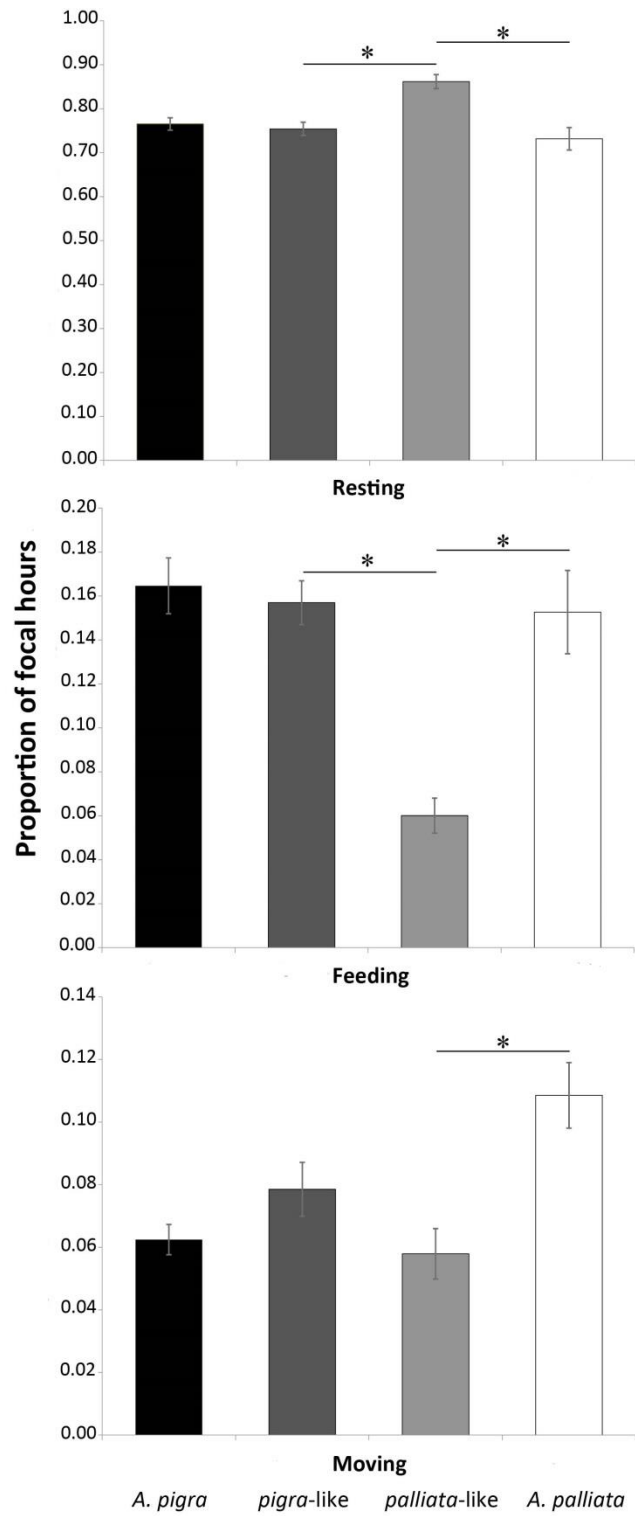
Figure 2.1. Graphs of activity budgets across all four taxonomic categories. * indicates significance at $P < 0.05$.
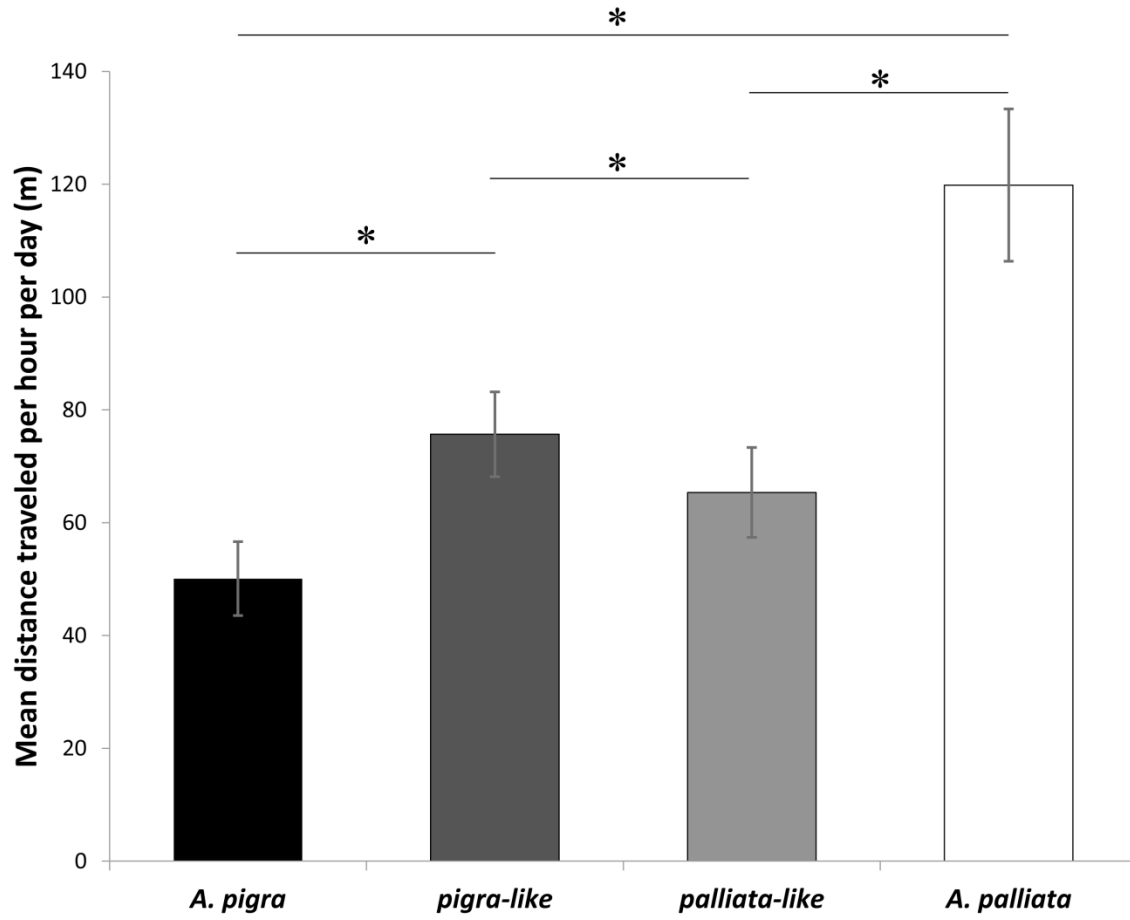
Figure 2.2. Distance traveled per hour per day across all four taxonomic categories. * indicates significance at P < 0.05.
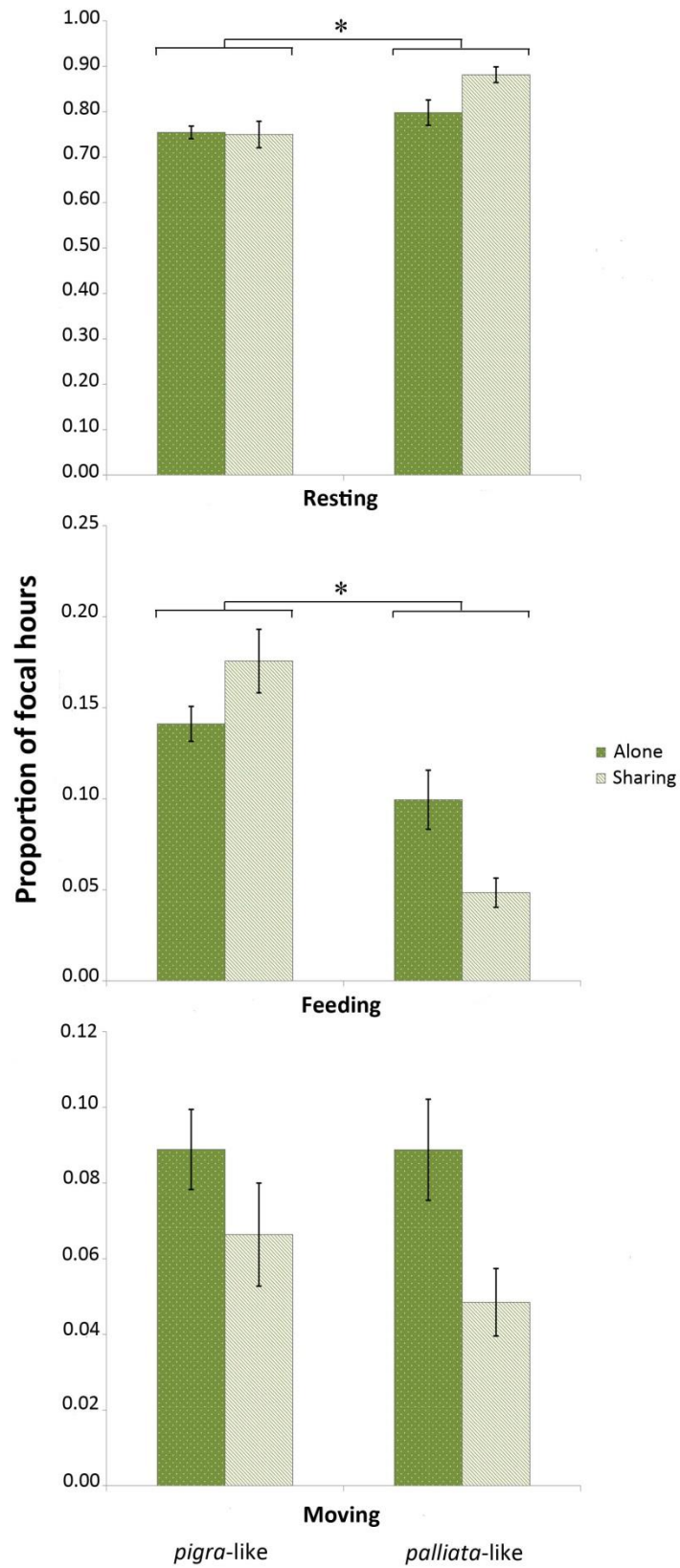
Figure 2.3. Activity budgets among hybrids sharing a forest fragment versus living alone. * indicates significance at P < 0.05.
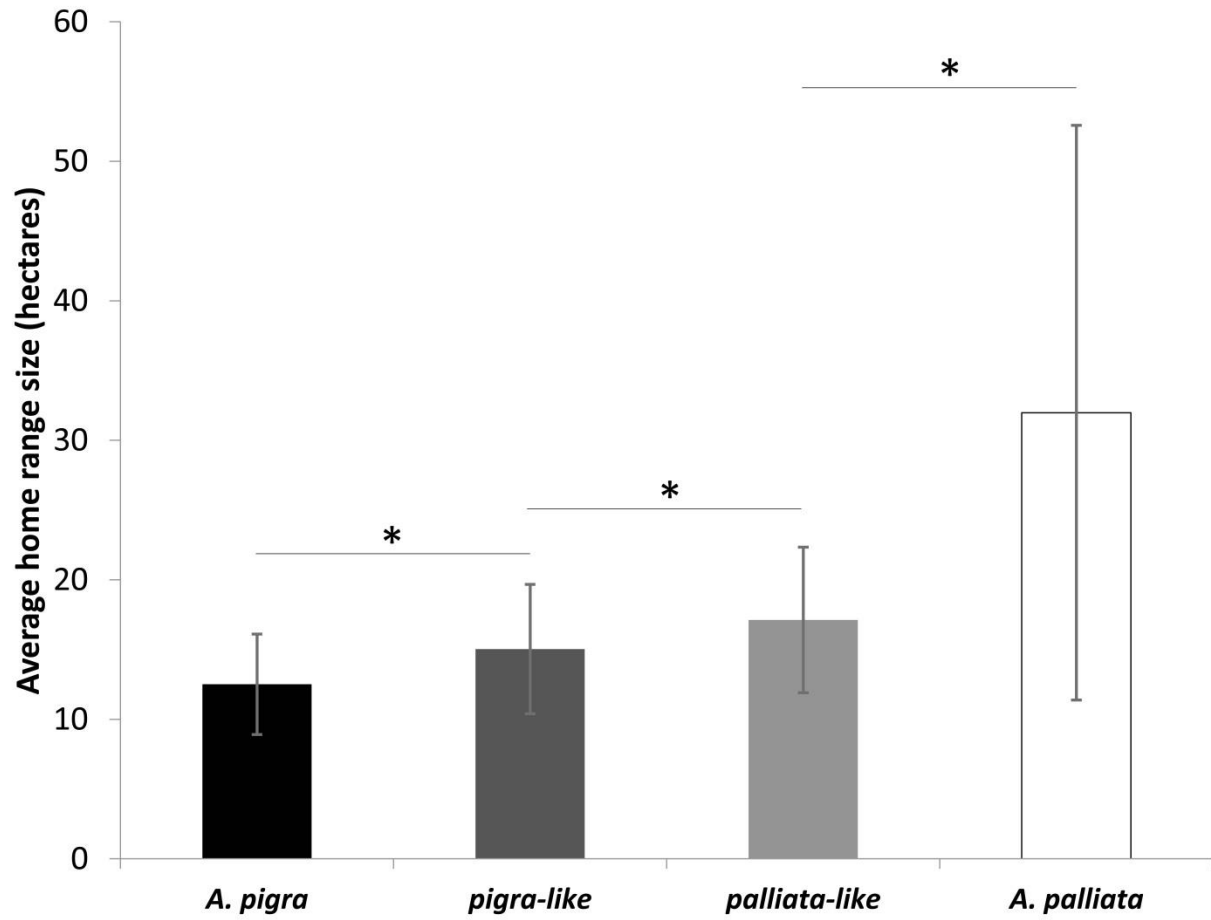
Figure 2.4. Average home range across all groups within each taxonomic category: 3 *A. pigra* groups, 6 *pigra*-like groups, 5, *palliata*-like groups, and 3 *A. palliata* groups. * indicates significance at $P < 0.05$.

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CHAPTER THREE

Ancestry, but not kinship, influences prosocial behavior in *Alouatta pigra*, *A. palliata*, and their hybrids

ABSTRACT

Primate social behavior is known for its flexibility, but interest in the role of phylogenetic constraint on social behavior is increasing. We investigate phylogenetic constraint in the social behavior of two howler monkey species (*Alouatta pigra, A. palliata*) and their hybrids. Our previous work has shown that female *A. pigra* are more affiliative than female *A. palliata*, and spend more time in close proximity. Using genotyping data from 31 microsatellite markers, we calculate a genetic hybrid index for 37 females within the admixed population of the hybrid zone, confirming our earlier assumption that this sample consists of highly backcrossed individuals with no intermediate hybrids and that phenotype maps to genetic measures of ancestry. Additionally, because kinship is known to influence social relationships in other primates, we also calculate coefficients of relatedness for all within-group female dyads using a total of 34 different microsatellite loci (29 for *A. pigra* and 17 for *A. palliata*). Other authors have suggested that differences in dispersal patterns result in *A. pigra* groups being composed of closely related individuals while *A. palliata* groups are not, thus contributing to the greater affiliation previously seen in *A. pigra* groups. Therefore, we explore whether this expectation (*A. pigra* are more closely related than *A. palliata*) is borne out in our purebred study populations. We then examine

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proximity and rates of affiliation among the females, in relation to their genetic hybrid index and relatedness. Contrary to our expectation, we found while all groups are closely related (above the order of half-siblings), the levels of relatedness in purebred *A. pigra* groups were actually lower than in purebred *A. palliata* groups. This pattern was mirrored in the hybrids: *pigra*-like groups have lower levels of relatedness than *palliata*-like groups. Finally, we found that time spent in close proximity and rates of affiliation increased as the proportion of *A. pigra* ancestry increased, even after controlling for the effects of kinship. Our results strongly suggest a genetic contribution to the social differences between *A. pigra* and *A. palliata*.

INTRODUCTION

Social mammals such as primates are commonly thought to have extremely flexible social behavior (Reader & Laland, 2003) because they must navigate rapid ecological and social changes (Cheney & Seyfarth, 2007). However, constraints on mammalian social behavior are a growing area of research interest (Kappeler, Barrett, Blumstein, & Clutton-Brock, 2013) since social animals rarely exhibit the full spectrum of behavioral options and may even respond inappropriately to environmental cues (Ghalambor, McKay, Carroll, & Reznick, 2007). At an individual level, animals may vary in their response to socio-ecological cues due to variation in "personality" or behavioral syndromes (Sih, Bell, & Johnson, 2004), and such differences are at least partially genetically modulated (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). On a broader scale, phylogenetic constraints can contribute to behavioral variation across taxa. Recent studies and meta-analyses have demonstrated the utility of including phylogeny in explanations of behavioral variation (Thierry, 2013), and there is evidence of strong phylogenetic constraint in many aspects of primate social behavior (Di Fiore & Rendall, 1994; Shultz, Opie, &

Atkinson, 2011). Thus, genetic ancestry appears to play a substantial role in explaining the variation (or lack thereof) in social structure, at least among some Old World monkeys (Thierry, Iwaniuk, & Pellis, 2000). However, we do not know whether the phylogenetic constraint that exists within cercopithecines also exists to the same degree in other primate taxa. Additionally, because behavior is generally more labile than other traits (Blomberg, Garland, & Ives, 2003), controversy remains regarding whether behavioral traits even exhibit phylogenetic constraint (Kamilar & Cooper, 2013). To obtain a more general understanding of how phylogenetic constraint acts on primate social behavior, we need studies from a wider array of taxa.

Hybrid zones provide a unique, natural laboratory (Hewitt, 1988) to examine the impact of ancestry on behavioral variation. Admixed individuals within a hybrid zone have a high degree of genetic variation that can potentially be mapped onto a trait of interest (in this case social behavior) to look for the effects of genetic ancestry. Because hybrid zones occupy geographically narrow regions, it may also be possible to compare diverse individuals in similar ecological conditions (although many hybrid zones fall along sharp ecological gradients). Currently, most primate hybrid zone studies that involve behavior have been conducted with either anubis x hamadryas or anubis x yellow baboon hybrids (Bergman & Beehner, 2003, 2004; Charpentier et al., 2012; Kummer, 1970; Phillips-Conroy, Jolly, & Brett, 1991; Sugawara, 1988; Tung, Charpentier, Mukherjee, Altmann, & Alberts, 2012). These studies reveal some phylogenetic constraint in aspects of baboon social behavior, specifically in mating strategies (Tung et al. 2012) and in inter- and intra-sexual relationships (Bergman & Beehner, 2003; Bergman, Phillips-Conroy, & Jolly, 2008). However, hybrid zone research in New World primates lags behind those in Old World primates.

Natural hybridization occurs in Tabasco, Mexico between two species of howler

monkeys, *Alouatta pigra* and *A. palliata* (Cortés-Ortiz et al., 2007). They are sister taxa that have clear differences in social behavior (see Table 1.1 in Ho et al. 2014 for full summary); most relevantly, *A. pigra* female social relationships are characterized by egalitarian interactions (Van Belle, Estrada, & Strier, 2011) while *A. palliata* females are less affiliative (Zucker & Clarke, 1998). Using subjects from three separate study sites (one each for *A. pigra, A. palliata*, and their hybrids), we have previously confirmed those differences between the purebred species, and also demonstrated that howler monkey social behavior (mainly proximity and affiliation) is correlated to phenotypic categorization of individuals (Ho et al., 2014). In other words, phenotypically more *pigra*-like hybrids behaved more similarly to *A. pigra* and phenotypically more *palliata*-like hybrids behaved more similarly to *A. palliata*.

However, because we were only able to classify hybrids based on phenotypical traits (pelage, size, etc.), it was possible that we could have miscategorized individuals, or intermediate hybrids may have existed in our sample that we were unable to detect. Furthermore, kinship is known to affect the strength and nature of social interactions among many primates (Chapais & Berman, 2004; Mitani, 2009; Silk, Altmann, & Alberts, 2006) and other taxa (Fraser & Bugnyar, 2010; Smith et al., 2011; Wiszniewski, Lusseau, & Möller, 2010). Kinship within *A. pigra* groups may be higher than in *A. palliata* groups based on previously reported patterns of female dispersal and group entry strategies where *A. pigra* bar immigrant females from joining existing groups and *A. palliata* do not (see Table 1.1 of Ho et al. 2014). Such a pattern could explain some of the social differences between the species that we and others have observed.. Consequently, we may not have measured genetically determined differences in social structure, as we had concluded.

In the current study, we bolster our previous research by using nuclear microsatellite data

to measure the genetic background of the individuals we observed. With these genetic markers, we calculate a genetic hybrid index for each individual in our hybrid zone sample (Buerkle, 2005). Additionally, we now examine genetic relatedness within our purebred and hybrid study groups as a potential contributor to our measures of social behavior.

Because our previous study demonstrated the clearest differences in social behavior between females of each species, we focus here on female-female proximity and affiliative social behavior only. First, we expect to replicate previous findings from the hybrid zone (Kelaita & Cortés-Ortiz, 2013) that phenotype closely follows genetic measures of ancestry and that most individuals are highly backcrossed (few individuals are intermediate hybrids with an even genetic mixture of both parental species). Second, we predict that *A. pigra* females will be more closely related than *A. palliata* females (but see also Baiz 2013). Third, we predict that both proximity and affiliation will be correlated to the genetic hybrid index, such that individuals with a greater proportion of *A. palliata* genetic ancestry will spend more time farther apart and engage in less affiliation. Fourth, we predict that relatedness will explain some of the differences previously found in the social behavior of *A. pigra* versus *A. palliata*, because higher relatedness will be associated with closer proximity and greater levels of affiliation between the females.

METHODS

Our study sites and observational protocols for the behavioral data are the same as previously published (Ho et al. 2014). We briefly review them here.

Study subjects

We conducted data collection for this study in three separate populations. We sampled within the *A. pigra* and *A. palliata* contact zone, where hybridization is confirmed to occur

(Cortés-Ortiz et al. 2007), as well as in two areas with only purebred animals. These purebred

sites are well outside of the contact area (approximately >260 km away) and it is unlikely that

the individuals there have had contact with the other species or with their genes (i.e., hybrid or

back-crossed individuals).

The purebred *A. pigra* site near Escarcega, Campeche, is El Tormento Forest Reserve, a

protected, relatively large area of primary tropical forest of about 1400 ha (Van Belle & Estrada,

2008). The two purebred *A. palliata* sites are located in La Flor, Catemaco (~120 ha) and in

Rancho Jalapilla, Acayucan (~30 ha), both in Veracruz. Finally, the hybrid site is located south

of Macuspana, Tabasco, in the midst of the approximately 20 km wide contact zone (Kelaita &

Cortés-Ortiz 2013). Individual howler monkey groups reside in small, discontinuous patches of

mainly secondary forest (~ 3 ha) separated by ranches and farmland (Dias, Alvarado, Rangel-

Negrín, Canales-Espinosa, & Cortés-Ortiz, 2013).

We collected data simultaneously on three groups of purebred *A. pigra* and six groups of

hybrids from February-August 2011, then simultaneously on three groups of purebred *A. palliata*

and five new groups of hybrids from January-June 2012 (see Ho et al. 2014 for more details on

group composition and data collection). All adults in the study were individually recognizable by

researchers via ankle bracelets or natural markings such as scars and, in the case of *A. palliata*,

differences in characteristic patches of skin/fur coloration on their feet and tails.

Previously, we had used physical characteristics to categorize individuals and groups

within the hybrid zone as either *pigra*-like or *palliata*-like (Ho et al. 2014), as we strongly

suspected that our sample consisted of highly backcrossed individuals phenotypically similar to

the parental species, based on previous research (Kelaita & Cortés-Ortiz 2013). In this study, one

of our goals was to confirm our assumption using genetic data. Thus, the groups we categorized

as *pigra*-like or *palliata*-like should consist of individuals that have a genetic hybrid index close to purebred *A. pigra* and *A. palliata*, respectively.

Behavioral data collection

We followed all groups for a total of 741 hours in 2011 and for 956 hours in 2012. Observers collected approximately 40 hours of data before rotating to another group. Groups were typically followed from approximately 7AM to 5PM, with each day in the field lasting 8-10 hours.

Affiliative social behavior and proximity data for all adult individuals in each group were collected during hour-long focal sampling (Altmann, 1974), with no animals sampled twice until all others were sampled once. As described in Ho et al. (2014), proximity data were collected as instantaneous scan samples of all visible adult group members every 10 minutes during the focals, resulting in a final score for each dyad of "number of times <5m" and "number of times >5m". Affiliation was collected as all social interactions between subjects and other adults during focal samples. We then calculated a rate of affiliation for each subject, correcting for group size, again following the procedures in Ho et al. (2014). For this study, we focused specifically on female-female proximity and affiliation.

Genetic data collection

Tissue samples

We attempted to collect blood samples for as many individuals in our study groups as possible, and were able to successfully do so for 120 individuals (including both adults and juveniles). All 120 animals were used in the analyses of relatedness, but we restricted the genetic hybrid index analysis to the subset of 80 adults within this sample (N: admixed = 40 admixed females and 20 admixed males, purebred *A. pigra* = 6 females and 5 males, purebred *A. palliata*

= 2 females and 7 males). Tissue samples had been collected from groups in these study sites since 1998, and all samples for this study were obtained in two expeditions, one in 2010 and the other in 2012. The procedure for capture and sampling of individuals followed Kelaita and Cortés-Ortiz (Kelaita & Cortés-Ortiz, 2013). Once collected, blood samples were kept on ice until they reached the laboratory, where they were then stored at -20°C.

DNA extraction and genotyping

We extracted genomic DNA from blood samples collected in the field, with the QIAGEN DNEasy tissue kit (Qiagen Inc., Valencia, CA). We followed the manufacturer's directions for extraction, with the following exception: for step 1 of the blood sample extraction protocol, we added 100 µL of whole blood to 100 µL buffer ATL.

We performed single-plex DNA amplification using the following PCR mixture: 1 µL of 10x PCR buffer, 1 µL of 2µM dNTPs, 0.25 µL each of 10 µM flourescent-labeled forward and unlabeled reverse primers, 5.7 µL of distilled water, 0.8 µL of 50 mM $MgCl_2$, 0.045 µL Platinum or Hi-Fi taq (dependent on the primers), and 1 µL of template DNA. PCR cycle conditions were: denaturation at 94°C for 2 minutes, 35 cycles of 30 seconds at 94°C, 30 seconds at the appropriate annealing temperature for the primers (ranging from 52°C to 68°C), 30-45 seconds of extension at 72°C, and a final extension step of 10 minutes at 72°C (following (Baiz, 2013; Cortés-Ortiz, Mondragón, & Cabotage, 2009). For some samples/loci, we also ran multiplex DNA amplification based on similarities in annealing temperature, using the Qiagen multiplex PCR kit (Qiagen Inc., Valencia, CA). We used the following PCR mixture for the multiplex reactions: 5 µL of 2X Master Mix, 1 µL of 10X primer mix (2 µM per primer), 1 µL of water, 2 µL of Q-solution, and 1 µL of template DNA. The PCR thermocycling conditions were: 95°C for 15 min, 35 cycles of 94°C for 30 s, 30 seconds at the appropriate annealing temperature, 45

seconds of extension at 72°C, and a final extension of 30 minutes at 60°C (following Baiz 2013).

All reactions included a negative control to check for contamination. We then aliquoted 3μl of

PCR products to run on 2% agarose gels for 15 minutes and visualized the resulting bands under

ultraviolet light using GelRed staining. We used the brightness of the bands under UV lighting to

determine the appropriate dilutions for genotyping the samples.

To prepare the samples for genotyping, we diluted the PCR products in 96-well plates

with distilled water and added fluorescent standard (GS500LIZ) and Hi-Di Formamide (Applied

Biosystems). Each plate also included a sample with alleles of known sizes as a positive control

to allow for consistent scoring of allele sizes across different plates. The plates were then sent to

the University of Michigan DNA Sequencing Core for genotyping on an Applied Biosystems

DNA sequencer (Model 3730XL). We then scored the resulting chromatograms for the allele

sizes of each locus using GeneMarker V 1.5 (Softgenetics, State College, PA, USA).

Genetic data analyses

<u>Relatedness</u>

To calculate r-values, or coefficient of relatedness, we used COANCESTRY v. 1.0.1.2

(Wang, 2011). Although there are multiple estimators of relatedness that may be more or less

accurate depending on a number of factors, such as the amount of closely related dyads in a

population (Wang 2011), we chose the commonly used Queller and Goodnight estimator

(Queller & Goodnight, 1989). Previous research using the same set of loci on different

populations (of the same species) close to our study sites demonstrated that this estimator

performed the best for both *A. pigra* and *A. palliata* (Baiz, 2013).

We checked for the number of alleles per locus and the allele frequency with GenAlEx

6.501 (Peakall & Smouse, 2012). We chose 34 polymorphic loci to calculate the r-values for

within-group dyads for the hybrids (see Table 3.1). However, because some of these loci are monomorphic for either *A. pigra* or *A. palliata*, including all of them will inflate the r-values among the purebreds (Baiz 2013). Thus, we only used a total of 17 polymorphic loci for *A. palliata* and 29 polymorphic loci for *A. pigra* to calculate purebred r-values. Though it is possible to use these same loci for the hybrids, monomorphic loci for either parental species are actually informative among hybrids because of their admixed nature. (Additionally, none of the loci was monomorphic among the hybrid population as a whole.)

Due to the non-parametric nature of the relatedness data, we compared the r-values of the purebred *A. pigra* and *A. palliata* using a Mann-Whitney U test. We also compared the r-values of the *pigra*-like groups and the *palliata*-like groups to see if any differences found among the purebreds would persist among the hybrids. We used either a Mann-Whitney U test or a Student's t-test depending on the normality or the variance of hybrid relatedness data. To check for inflated r-values, we compared the r-values estimated for COANCESTRY for known parent-offspring r-values to the estimated r-values of other dyads, whenever possible.

Genetic hybrid index

We used a total of 31 loci for the calculation of the genetic hybrid index, after removing three loci (AC14, AC17, and AC45) due to some individuals in our hybrid zone sample that had no genotype data for those loci. The hybrid index ranges from 0 (purebred *A. pigra*) to 1 (purebred *A. palliata*) and was calculated using INTROGRESS v. 1.22 (Gompert & Buerkle, 2010). The program compares the allele frequencies (at each loci) of the 60 hybrid individuals to the allele frequencies 11 *A. pigra* individuals and the 9 *A. palliata* individuals, in order to determine the degree of admixture for each hybrid animal. We considered *pigra*-like hybrids to have a hybrid index of <0.4 and *palliata*-like individuals to have an index of >0.6, with

individuals between 0.4 and 0.6 to be intermediate hybrids (i.e., F1 or early generation hybrids).

Behavioral data analyses

All of the following statistical analyses were run in R v. 3.1.0 (R Core Team, 2014), using the RStudio GUI (RStudio, 2013). Alpha was set at 0.05.

To examine the effect of the degree of admixture (as measured by the genetic hybrid index) on social behavior, we performed two main analyses. First, we ran a multiple regression on the proximity data among female-female dyads within the hybrid zone. We set the dependent variable as the proportion of time that female-female dyads spent in <5m (calculated from the number of scans where a focal subject and another adult female spent in <5m divided by the total number of scans between the focal subject and that adult female). The pairwise hybrid index (the mean hybrid index between the two individuals in the dyad), pairwise relatedness for the dyad, and adult group size were set as predictor variables. Averaging the hybrid index would normally be problematic if there were a large number of intermediate hybrids in the population, because an intermediate would artificially inflate or deflate the hybrid index of a hybrid that is more genetically similar to one of the parent species. However, in our sample, we found no intermediate hybrids; all individuals had indices that were far from the intermediate range of 0.4-0.6, and all dyadic behavioral interactions always occurred among individuals on the same extreme of the genetic spectrum (i.e., either *pigra*-like or *palliata*-like hybrids).

Second, we ran a multiple regression on the female affiliation data across all taxa (including both purebreds and hybrids. We square-root transformed the rates of affiliation per female to correct for the positive skew of the data, and more importantly, the violation of homoscedasticity in the residuals. The transformed affiliation rate was then set as the dependent variable, with the hybrid index of each female and the mean relatedness of each group as the

predictor variables. We calculated mean group relatedness by averaging the relatedness of all possible dyads per group. We originally planned to use mean within-group female relatedness as a variable. However, due to technical difficulties during the capturing season that resulted in concern over animal safety, we were unable to collect enough female samples among our purebred *A. palliata* to calculate coefficients of relatedness for any within-group female-female dyads. (We did not use pairwise relatedness as a predictor because the low rates of affiliation among howler monkeys in general precluded us from analyzing affiliation between dyads.) Next, to ascertain that our results were not simply driven by the purebreds, we ran another multiple regression on the hybrids only, using the same variables.

For both the proximity and the affiliation analyses, the dyads/individuals of group 69, an unusually small (three adults) *palliata*-like group, were found to be influential outliers. All three individuals' data points had high leverage (Cook's D > 1.5) and thus exerted an unduly high effect on our model compared to other observations. Given this and their unusual group composition, we chose to remove them from both analyses. Thus, the final analyses were run on 60 female-female dyads for proximity (hybrids-only) and 55 females for affiliation (hybrids and purebreds).

RESULTS

Hybrid index

The hybrid indices of individuals in our hybrid zone sample ranged from 0 to 0.98 (see Figure 3.1). As we predicted, the hybrid index matched the morphology of the individuals; animals previously classified as *pigra*-like had hybrid indices of 0-0.24, while animals classified as *palliata*-like had hybrid indices of 0.82-0.98. Five individuals within our hybrid zone sample

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were considered purebred *A. pigra* based on their genotype data: two males and three females. Three of these animals (one male and two females) belonged to group 68, while the remaining female belonged to group 71 and the remaining male belonged to group 73 – all of these groups were phenotypically *pigra*-like. Group 68 had one unsampled adult male in addition to the purebred male and two females. The admixed animals in groups 71 and 73 have hybrid indices ranging from 0.03-0.04, indicating that these are groups consisting of highly backcrossed individuals with primarily *A. pigra* ancestry. There were no phenotypically *palliata*-like individuals that were classified as purebreds in our sample.

Relatedness

Among purebreds, we found that, contrary to our predictions, *A. pigra* were less closely related than *A. palliata* (mean $r_{Api} = 0.38$ vs. mean $r_{Apa} = 0.57$; Mann-Whitney U: $z = -2.42$, P = 0.02). Despite removing loci that were monomorphic in *A. palliata*, a comparison to the r-value of a known mother-offspring *A. palliata* dyad ($r = 0.74$) indicated that relatedness estimates were still higher than expected. We followed previous research in howlers (Van Belle, Estrada, Strier, & Di Fiore, 2012; Di Fiore, 2009) and considered dyads closely related if they are on the order of half-siblings or more. Thus, $r \geq 0.37$ is closely related among *A. palliata*. In other words, even after correcting for the inflation, mean relatedness among *A. palliata* was still fairly high. We did not have any known mother-offspring dyads among *A. pigra*.

Hybrids mirrored the differences found in purebred relatedness, with *pigra*-like animals generally less related to group members than *palliata*-like animals (mean $r_{HybApi} = 0.45$ vs. mean $r_{HybApa} = 0.72$; Mann-Whitney U: $z = -6.88$, P < 0.00005). Although none of the loci used in our analysis of hybrid relatedness was monomorphic among the hybrids, more of them were monomorphic for *A. palliata* than for *A. pigra*. Since our hybrid individuals were highly

backcrossed, this could result in inflated r-values for the *palliata*-like animals compared to the

pigra-like animals (similar to Baiz 2013). When we examined the r-values of two known

mother-offspring dyads in the *pigra*-like groups, we found that they were 0.61 and 0.52 (mean =

0.56), while the r-value of a known mother-offspring dyad in the *palliata*-like groups was 0.77.

In *pigra*-like animals, therefore, we considered r ≥ 0.28 as closely related, while in *A. palliata*

and *palliata*-like animals, we considered r ≥ 0.38 as closely related. Even by this criterion,

individuals in our hybrid sample were generally closely related (see Table 3.2 for average group

relatedness for all within-group adults of both sexes and for all within-group adult females).

To make certain that this difference among the hybids was not due entirely to

monomorphic loci, we also calculated r-values after removing the loci monomorphic for *A.*

palliata. Although this did lower mean *palliata*-like r-values from 0.72 to 0.55, *palliata*-like

relatedness remained significantly higher than *pigra*-like relatedness (Student's t-test: t = -3.17, P

= 0.002).

Proximity

The full model of proximity was significant (R^2 = 0.51, F(3,56) = 19.61, P = 0.00005). As

the hybrid index drew closer to 1 (more *palliata*-like), female-female dyads in the hybrid zone

spent less time in close proximity (t = -3.20, Beta = -.50, P = 0.002; Figure 3.2). Surprisingly,

neither female-female pairwise relatedness (P = 0.6437) nor group size (P = 0.106) had a

significant effect on proximity. Using mean within-group relatedness instead of pairwise

relatedness did not change the results.

Rates of affiliation

As the hybrid index went from 0 to 1 (from purebred *A. pigra* to hybrids to purebred *A.*

palliata), female rate of affiliation decreased (t = -2.58, Beta = -.46, P = 0.01), in accordance

with our prediction and with the results of the proximity data (Figure 3.3). Also similar to the

proximity data, mean group relatedness did not have a significant effect on female affiliation (P

= 0.85). When restricting the analysis to hybrid females only, the pattern persisted: as hybrid

index drew closer to 1, female affiliation decreased (t = -2.28, Beta = -.71, P = 0.03), and mean

group relatedness had no significant effect (P = 0.11).

We also used a multiple regression on female hybrid affiliation using mean within-group

female relatedness instead of mean group relatedness, and found that there was a trend for

affiliation to decrease as the hybrid index drew closer to 1 (t = -1.90, Beta = -.48, P = 0.07).

Mean female relatedness was also a nonsignificant predictor of affiliation (P = 0.28).

DISCUSSION

In this study, we provided evidence that genetic ancestry contributes to variation in social

behavior between two species of howler monkeys and their hybrids. We found that genetic

hybrid index correlated with female-female prosocial behavior (proportion of time spent in <5m

and rates of affiliation), with females that had a hybrid index closer to 1 (more *palliata*-like)

spending both more time further away from other females, and less time engaging in affiliative

behaviors, on average. This effect of ancestry persisted after controlling for group size, similar to

our previous study using only morphology to categorize hybrids. Importantly, while ancestry was

a significant factor in explaining social behavior patterns, within-group relatedness was not. This

implies that kinship patterns do not explain all of the interspecific differences between *A. pigra*

and *A. palliata* in prosocial behavior.

Contrary to what we would expect based on previously reported dispersal patterns,

average relatedness was fairly high in both purebred species, as well as among the hybrid groups.

This was the case even when we removed the loci that were monomorphic for *A. pigra* and *A. palliata*. Similarly, recent research from Baiz (2013) showed that mean within-group relatedness was higher than expected for *A. palliata* and in fact comparable to *A. pigra* groups. However, our results differed in that, for our study groups, *A. palliata* actually had higher average relatedness when compared to *A. pigra*. We should point out here that we did not actually have r-values for our purbred *A. palliata* females (the mean group relatedness for *A. palliata* was calculated from male-male dyads and male-female dyads only, while mean relatedness for the other taxa included all sexes). However, given that previous studies on relatedness in *A. pigra* and *A. palliata* found no sex differences for either species (Baiz, 2013; Ellsworth, 2000; Milton, Lozier, & Lacey, 2009), it seems unlikely that the patterns of relatedness among our *A. palliata* groups will change dramatically with the inclusion of female dyads. We require more data before we can be certain.

Among the hybrids, differences in relatedness mirrored the purebreds, in that *palliata*-like groups had higher relatedness than *pigra*-like groups. This pattern did not appear to be a result of monomorphic loci, because the difference remained even after the removal of those loci. A similarity in interspecific patterns of kinship could indicate that the factors affecting within-group relatedness among the purebreds remain relevant in the hybrid zone. In particular, barriers to dispersal, known to affect primate community structure (Beaudrot & Marshall, 2011), may influence our *A. palliata* groups more than our *A. pigra* groups. All of the *A. pigra* groups are in a large, protected forest reserve while the *A. palliata* groups are located on a ranch and in an ornamental fern plantation. Individuals may have a difficult time dispersing into and out of the groups residing in areas with more human activity.

Based on that idea, hybrid relatedness should not differ because all groups in the hybrid zone experience a high level of anthropogenic disturbance. However, if individuals with higher *A.*

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palliata ancestry are more affected by habitat degradation (as suggested in Chapter Two), then maybe their typical dispersal patterns are also more disrupted than those of *pigra*-like individuals. In *A. caraya*, for example, habitat fragmentation appears to reduce female dispersal, even though this species typically exhibits bisexual dispersal in continuous forests (Oklander & Corach, 2013; Oklander, Kowalewski, & Corach, 2010). This modification of dispersal patterns results in within-group relatedness being higher among females than males (Oklander et al. 2010; Oklander & Corach 2013). Because *A. caraya* live in multi-male, multi-female groups similar to *A. palliata* (Kowalewski & Garber, 2010), *A. palliata* or *palliata*-like groups may also respond to habitat degradation by altering dispersal patterns in favor of increased female philopatry. A possible mechanism for the emergence of this sex difference in *A. palliata/palliata*-like populations could be due to greater costs incurred by females if they disperse (Clutton-Brock, 1989), particularly in the unfavorable habitat of the hybrid zone, where forest fragments are isolated and ground travel risks attacks from domestic dogs.

Due to the high levels of within-group relatedness found across all of our groups, it is perhaps not surprising that relatedness failed to have a significant effect in predicting variation in female-female social behavior (proximity patterns and affiliation) among hybrids. It is also possible, however, that kinship plays a less important role in these two species of *Alouatta* than they appear to play in other primates. This may be particularly true for *A. palliata*, because average relatedness is higher in both the purebreds and the *palliata*-like hybrids, yet *A. palliata/palliata*-like females consistently exhibit lower levels of affiliation and spend more time further apart. Relatedness has been found to have minimal effect on proximity patterns in studies of other social mammals, as well (Hirsch, Prange, Hauver, & Gehrt, 2013; Johnson, Snyder-Mackler, Beehner, & Bergman, 2014; Ortega-Ortiz, Engelhaupt, Winsor, Mate, & Rus Hoelzel,

2012). While direct measures of affiliative behavior are a better measure of social relationships, we were unable to analyze affiliation on a dyadic level, nor were we able to use pairwise relatedness in that analysis. The effect of kinship on affiliation may only become apparent at a finer level. Thus, in future long-term studies on howler monkeys, it would be beneficial to gather enough behavioral and genetic data to allow for analyses how pairwise relatedness affects dyadic affiliation among females.

While close kinship does not appear to be tightly linked with interspecific variation in social behavior, the differences between *A. pigra* and *A. palliata* do seem to have some genetic modulation. Besides the analyses already reported in this study, we also make another observation here that supports this idea. Within our hybrid sample, there is greater spread in the hybrid indices of the *pigra*-like animals compared to the *palliata*-like animals (Figure 3.1). This impies that more *A. palliata* genetic material has introgressed into the *pigra*-like hybrids than *A. pigra* genetic material has introgressed into *palliata*-like hybrids. Such a pattern corresponds to the behavioral data, showing that the hybrids generally appear more similar to *A. palliata* than they are to *A. pigra* (Figure 3.2; Figures 1.2 and 1.3 in Ho et al. 2014).

Our behavioral observations in the hybrid zone agree with multiple other studies that have examined constraint versus flexibility in primate social behavior. For example, experimental studies focusing on Gene x Environment interactions found genetic effects that constrained behavioral expression (Suomi, 2011). Phylogenetic analyses (e.g. Thierry 2013) described linked behavioral traits that covary with one another and also constrained possible responses. Clearly, social behavior can also be homologous across taxa and as phylogenetically informative as morphological traits (Rendall & Di Fiore, 2007). Therefore, a large-scale comparative analysis of social behavior among extant *Alouatta* should allow us to better

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understand when in howler evolutionary history certain behavioral traits emerged, and in what historical context (i.e. glacial events, environmental shift from rainforest to grasslands, uplifting of the Andes: (Campbell Jr., Frailey, & Romero-Pittman, 2006; Rosenberger, Tejedor, Cooke, Halenar, & Pekkar, 2009).

We already know that *Alouatta* can be split into three major phylogenetic branches based on other traits associated with diet and energy-minimization/maximization strategies: primarily leaf-based diets, leaf-and-fruit balanced diets, and primarily fruit-based diets (Garber et al. in preparation). Interestingly, *A. pigra* and *A. palliata* are both considered to have leaf-and-fruit balanced diets, but clearly their social behavior differs significantly (as well as their energy-use strategies: see Chapter Two). The other species categorized as a leaf-and-fruit eater is *A. seniculus*, a howler monkey that has similar social organization as *A. pigra*, particularly in terms of group size and female group entry/formation, where females disperse from their natal group to form new groups, and form coalitions with related females within their groups to eject other, non-kin females (Pope, 1998, 2000). An early report of *A. seniculus* social behavior found that rates of grooming were higher in this species compared to *A. palliata* (Neville, 1972), similar to our results for *A. pigra*. Given the similarity between *A. pigra* and *A. seniculus*, we speculate that *A. palliata* condition may be derived (see also Chapter Two), but clearly we need more research to know whether that is in fact true.

In conclusion, we have shown that consistent interspecific differences exist between sister taxa in *Alouatta* and that *A. pigra* and *A. palliata* patterns of social behavior have at least some genetic basis. Future research will hopefully broaden the comparative analysis to include more species of *Alouatta*, so that we can better understand which 'social behavior' traits are linked to each other, and therefore how the various social styles have evolved in this genus.

TABLES

TABLE 3.1. Microsatellite markers used in analysis of hybrid index and relatedness (including alleles present in each population)

Locus (alleles)	In *A. pigra*	In *A. palliata*	In both	Used to estimate hybrid index	Used to estimate r
Ap68				Yes	Yes – *A. pigra*
185	×				
189	×				
191		×			
195					
Ap74				Yes	Yes – *A. pigra*
146	×				
148			×		
D5S111				Yes	Yes – *A. pigra*
160		×			
166	×				
177	×				
D6S260				Yes	Yes – both
176		×			
178		×			
180	×				
182	×				
186	×				
D8S165				Yes	No
115	×				
139		×			
D14S51				Yes	Yes – both
139			×		
143			×		
D17S804				Yes	Yes – *A. pigra*
154	×				
158			×		
162	×				
166	×				
PEPC8				Yes	Yes – *A. pigra*
238	×				
246			×		
Ab20				Yes	Yes – *A. pigra*
236		×			
244	×				
262	×				
266	×				
Apm1				Yes	Yes – both

	A	B	C		
181	×				
183	×				
199	×				
201	×				
208		×			
210		×			
Apm4				Yes	Yes – both
239	×				
243	×				
245			×		
247	×				
249		×			
Ab06				Yes	Yes – both
270	×				
272	×				
274			×		
276		×			
280	×				
Ab07				Yes	Yes – both
174			×		
176			×		
Ab09				Yes	Yes – *A. palliata*
144			×		
145		×			
Ab12				Yes	Yes – *A. pigra*
219	×				
225	×				
233	×				
234			×		
Ab16				Yes	Yes – *A. pigra*
168	×				
170		×			
177	×				
Ab17				Yes	Yes – *A. pigra*
161		×			
224	×				
228	×				
236	×				
240	×				
244	×				
Apm6				Yes	No
139	×				
200		×			
Apm9				Yes	Yes – *A. pigra*
170	×				
172	×				

174	×				
176		×			
Api06				Yes	Yes – *A. pigra*
250	×				
252	×				
254	×				
277		×			
Api07				Yes	Yes – both
111	×		×		
115			×		
117					
121	×				
Api08				Yes	Yes – both
271	×				
273	×				
275	×				
277			×		
279		×			
Api09				Yes	Yes – both
459	×				
461	×				
463	×				
467		×			
471		×			
Api11				Yes	No
251	×				
261		×			
Api14				Yes	Yes – *A. pigra*
181		×			
202	×				
204	×				
210	×				
1110				Yes	Yes – both
180	×				
187	×				
199	×				
202	×				
203		×			
205		×			
157				Yes	Yes – both
208	×				
210	×				
224		×			
226		×			
228		×			
230		×			

232		×			
234		×			
1118				Yes	Yes – *A. pigra*
130	×				
132	×				
134		×			
TGMS1				Yes	Yes – *A. pigra*
304	×				
306	×				
314	×				
323			×		
TGMS2				Yes	Yes – both
314	×				
320	×				
326		×			
328			×		
AC14				No	Yes – both
211	×				
213	×				
AC17				No	Yes – both
227	×				
233	×				
AC45				No	Yes – both
342		×			
346		×			
350		×			
354		×			
TLR2				Yes	Yes – *A. palliata*
242	×				

TABLE 3.2. Mean group hybrid index and relatedness

| Group	Taxa (hybrid index)	Average group r	Average female r
65	*A. pigra*	0.24	0.43
66	*A. pigra*	0.60	0.71
67	*A. pigra*	0.42	0.83
44	*pigra*-like (0.14)	0.50	0.50
68	*pigra*-like (0.01)	0.62	0.69
70	*pigra*-like (0.11)	0.23	0.42
71	*pigra*-like (0.02)	0.47	0.40
73	*pigra*-like (0.04)	0.50	0.72
76	*pigra*-like (0.07)	0.30	0.42
69	*palliata*-like (0.96)	0.70	0.63
72	*palliata*-like (0.92)	0.76	0.71
74	*palliata*-like (0.92)	0.69	0.69
80	*palliata*-like (0.97)	0.80	0.77
81	*palliata*-like (0.93)	0.65	0.67
78	*A. palliata*	0.48	N/A
79	*A. palliata*	0.62	N/A
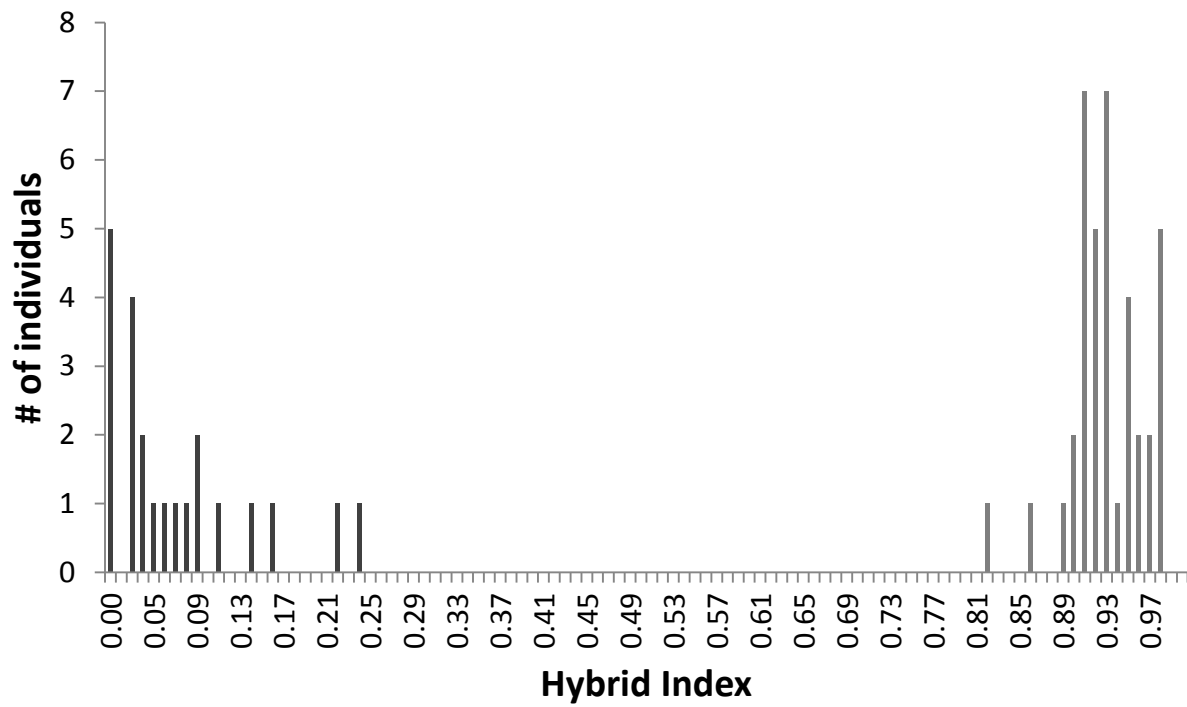
FIGURE 3.1. Genetic hybrid index. 0 = purebred *A. pigra*, 1 = purebred *A. palliata*.
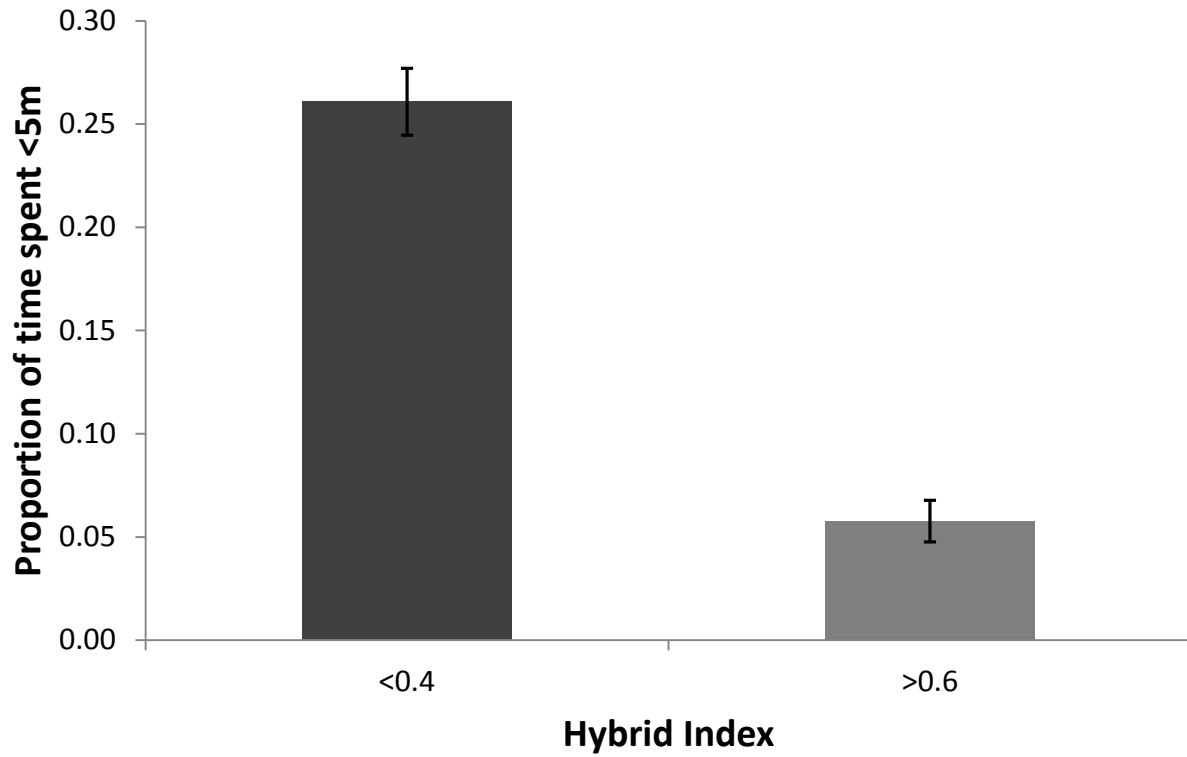
FIGURE 3.2. Female-female dyads' proportion of time spent <5m, split by genetic hybrid index. <0.4 = *pigra*-like, >0.6 = *palliata*-like (no individuals were between 0.4 and 0.6).
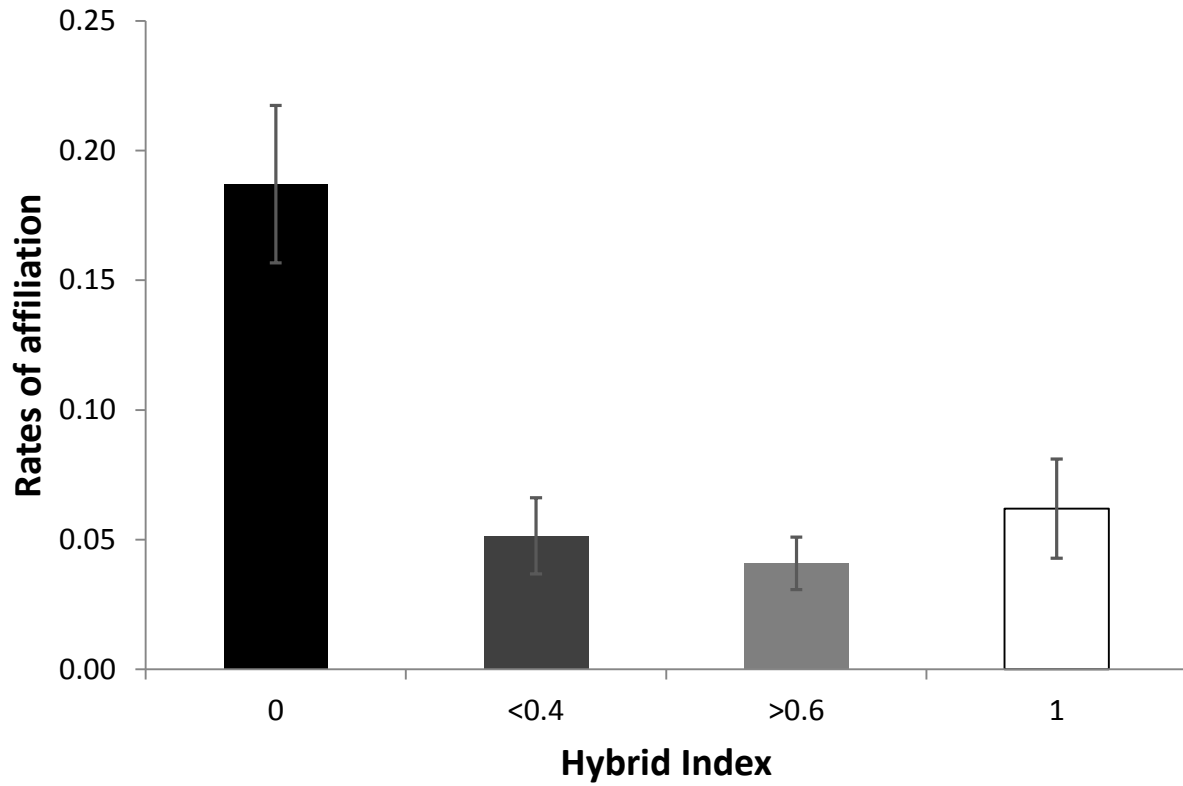
FIGURE 3.3. Rates of female-female affiliation (acts/hr) corrected for group size, split by genetic hybrid index. 0 = purebred *A. pigra*, <0.4 = *pigra*-like, >0.6 = *palliata*-like, 1 = purebred *A. palliata.*

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CONCLUSIONS

This dissertation reports the results of the first comparative behavioral study in two New World primates (*A. pigra* and *A. palliata*) and their naturally occurring hybrids. My goal is to better understand the three major factors that affect interspecific variation in behavior, specifically ecological conditions, social setting, and genetic ancestry. The unique characteristics of a hybrid zone provide the ideal conditions to untangle these potential causes. I first describe the social behavior (proximity, affiliation, agonism: Chapter One) and the non-social behavior (activity budgets and movement patterns: Chapter Two) of the two purebred species. Then, I give evidence of ancestry effects in both types of behaviors, even as they also respond to changes in ecological and social conditions (Chapters One through Three). I now summarize our results and discuss the new inquiries they uncover, as well as their limitations. I end with a brief discourse on the contributions this dissertation has made.

In Chapter One, I demonstrated that *A. pigra* and *A. palliata* show different proximity and affiliation patterns: *A. pigra* females spend more time closer together and engage in higher rates of affiliation, compared to *A. palliata*. I next showed that these differences could be partially explained by interspecific variation in group size, but that broad ecological measures did not correlate with behavioral differences (i.e. animals residing in the same environment still behaved differently). Importantly, even after controlling for group size, I found that variation in proximity and affiliation still had a genetic basis: phenotypically *pigra*-like hybrids spent more time in close proximity than *palliata*-like hybrids.

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In Chapter Two, I focused on activity budgets and movement patterns, behaviors directly linked to energy use/acquisition and therefore more affected by ecological conditions such as habitat quality. Similar to Chapter One, I found significant differences between the two species: *A. pigra* traveled shorter distances and had smaller home ranges than *A. palliata* (both characteristics of an energy minimization strategy). These data supported the idea that *A. pigra* were more energy minimizers than *A. palliata*, but my conclusions were tenative, as I did not find significant differences in the activity budgets between the two purebred species. Additionally, as may be expected for these types of behaviors, the effect of ancestry — though present — was less straightforward, and the highly disturbed habitat of the hybrid zone appeared to play a major role in altering the activity patterns of the howler monkeys. Critically, however, the anthropogenic perturbation existing in the hybrid zone affected behavioral variation differently depending on ancestry. I speculated that phenotypically *palliata*-like groups were more vulnerable to habitat disturbance than *pigra*-like groups, altering their species-typical behavioral patterns significantly more than *pigra*-like groups did.

Because in the previous two chapters, I used phenotypic features to categorize the hybrid howler monkeys by ancestry, I ran the risk of mis-classifying any individuals who may actually be genetically intermediate. Additionally, based on previous research in primates, I knew that kinship plays a major role in social bonds (Chapais & Berman, 2004), and that there are potential differences in within-group relatedness between *A. pigra* and *A. palliata* that could explain their behavioral differences, rather than ancestry *per se*. Therefore, in Chapter Three, I used molecular techniques to calculate a genetic hybrid index per individual and to calculate within-group pairwise relatedness. I found that no intermediates existed in my sample and that phenotype matched the genetic hybrid index, verifying my classification in the previous two studies. My

key finding was that the genetic hybrid index correlated with proximity and affiliation patterns, but kinship did not.

<u>Limitations and future directions</u>

Perhaps the largest caveat in this research has to do with the long life history of howler monkeys (and indeed, primates generally). The lack of longitudinal data on my groups means I do not know for certain whether the patterns reported here truly reflect the study populations in the long-term. For example, my hypotheses regarding possible differences in degree of relatedness between *A. pigra* and *A. palliata* directly stemmed from the two species' reportedly different methods of female group entry (Ho et al., 2014). However, I can only speculate on the actual processes involved in *A. pigra* versus *A. palliata* group entry, without having actually observed such incidents. I also do not know exactly how major events such as dispersal play out in my study populations. Though these two species exhibit bisexual dispersal (see Table 1 in Ho et al., 2014), dispersal patterns have also been shown to alter in response to habitat disturbance (Oklander, Kowalewski, & Corach, 2014). Thus, while I can suggest that some of the patterns observed in the hybrid zone may be due to disruptions in dispersal patterns, I cannot actually be certain that dispersal patterns are disrupted in our hybrid groups, or that they are disrupted in a systematic way (for instance, *pigra*-like groups are less affected than *palliata*-like groups). Obtaining data on the frequency of dispersal events should help me and future researchers address these questions. Thus, expanding on our general knowledge of dispersal patterns and other life-history events in the hybrid zone is a fruitful new direction to take this research.

One of the major findings in this dissertation is that ancestry exerts an influence on multiple types of behavior, because hybrids generally behave in way that reflected their genetic background. We are, however, a long way off from understanding the mechanisms behind this

phylogenetic effect, whether in an ultimate sense or a proximate sense. Therefore, I am limited in the conclusions I can draw about the role that genetics plays on interspecific differences both within and outside the hybrid zone. The molecular and genetic techniques that allowed me to examine the effects of relatedness and degree of hybridity also provide intriguing opportunities for further research. From an ultimate perspective, large-scale phylogenetic studies including multiple species (e.g. Thierry, Iwaniuk, & Pellis, 2000) could allow us to understand which species' behavioral patterns (the more affiliative *A. pigra* or the less affiliative *A. palliata*) are ancestral and conserved across *Alouatta*. From a proximate perspective, it should be possible to isolate candidate genes that are thought to play a role in particular behaviors (e.g. genes for oxytocin and its receptors, known to play a role in affiliation: Campbell, 2008; Soares et al., 2010) and look for interspecific differences in the expression of those genes (or the presence of receptors for the gene products). Having targeted genes will also allow future studies to better isolate gene x environment interactions that I suspect were so critical in shaping the behavior of *palliata*-like animals.

Notwithstanding these limitations, the research described has made strong contributions to the study of behavior. Below I detail the major ways it has done so.

Contributions

This research in this dissertation has multi-disciplinary implications, with my results contributing to psychology, biology, anthropology, and conservation. I presented data that increases our understanding of the forces behind behavioral variation, on both ultimate (ancestry effects) and proximate (current environment) levels. Only through examining behavior on multiple levels of analysis can we gain a more complete knowledge of how behavior "works," regardless of what species we study. In order to do so, I focused on the phenomenon of

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hybridization as a tool in my study of large-bodied, long-lived primates for which many experimental manipulations are unfeasible. Hybrid zones have great potential in helping us unravel the answers to numerous questions about topics from the processes of speciation (Larson, White, Ross, & Harrison, 2014) to the causes of behavioral variation that are the focus of this dissertation. Though several primate hybrid zones exist (Zinner, Arnold, & Roos, 2011), they are under-used. I hope that this dissertation will spur an interest in the utility of hybrid zones as an effective way of untangling the relative effects of social systems, ecology, and genetic ancestry on primate behavior. The fact that human evolutionary history has experienced hybridization events (Ackermann, Rogers, & Cheverud, 2006; Abi-Rached et al., 2011) should make the use of primate hybrid zones relevant to fields beyond animal behavior.

The bulk of this dissertation focused on demonstrating that genetic ancestry has a role to play in generating behavioral differences. My results implied that while behavior may be flexible, it is still a trait that can be subject to selective pressure and vary in a systematic fashion across genetically variable individuals, populations, or species (rather than vary in response to external, environmental stimuli). This is critical for any scientist who is interested, not only in current behavior, but in how that behavior evolved among particular lineages or how selection may act on behavior. For instance, the fact that I demonstrated an effect of ancestry in social behavior *and* some aspects of energy use/acquisition-related behavior raises the possibility of a domain-general selection on "flexibility" or "inflexibility." Does selection for specializing in a particular diet have constraining influences on flexibility in other arenas?

Finally, the issue of flexibility versus constraint is very relevant to conservation efforts. In light of the human-induced rapid environmental changes now common worldwide, we can clearly see that different species vary in their behavioral response to fluctuations in the

environment: some are very flexible and adjust to change in adaptive ways, but others do not. This difference could be due to the fact that some animals are generally more behaviorally flexible than others, or it could be due to the fact that some environmental changes are more difficult to cope with than others (Sih, Ferrari, & Harris, 2011; Tuomainen & Candolin, 2011). Thus, research contributing to the understanding of flexibility and constraint in behavior means that we can eventually assess the degree to which *types* of environmental change will affect a particular species, as well as pinpoint how individual species will be differentially threatened. For example, environmental change that has a greater chance of affecting home range size may be of concern for both *A. pigra* and *A. palliata*, because interspecific differences in home range seemed relatively inflexible in my study, implying that each species has a preferred – maybe optimal – home range size (Chapter Two). On the other hand, the removal of certain food resources may be less troubling for *A. pigra* than for *A. palliata*: *pigra*-like hybrids appeared capable of maintaining the same activity budget as purebred *A. pigra* despite being in the highly disturbed hybrid zone, whereas *palliata*-like hybrids altered their behavior from *A. palliata* purebreds (Chapter Two).

The same logic used for the howler monkeys applies more generally. If a particular species can vary in the level of flexibility their behavior exhibits (for example: inflexible foraging strategies but flexible reproductive strategies), then environmental disturbance that disproportionately affects resource availability should be more devastating than one that affects reproduction. However, if constraint in one domain results in constrains in another, then potentially any type of environmental disturbance would be a cause for concern.

In closing, this dissertation uses a combination of observation, comparative method, and molecular techniques to answer questions about the causes of behavioral variation. Through the

investigation of how and why animals exhibit such a stunning array of behaviors (or behave in surprisingly similar ways across different conditions), we can achieve a finer understanding of their remarkable resilience in some contexts but not in others. Not only does this also allow us to understand our own behavior, it gives us the means to preserve our fellow animals in this rapidly changing world.

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