

Chapter 5

Hybridization in Howler Monkeys: Current Understanding and Future Directions

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Abstract Hybridization, or the process by which individuals from genetically distinct populations (e.g., species, subspecies) mate and produce at least some offspring, is of great relevance to understanding the basis of reproductive isolation and, in some cases, the origins of biodiversity. Natural hybridization among primates has been well documented for a few taxa, but just recently the genetic confirmation of hybridization for a number of taxa has produced new awareness of the prevalence of this phenomenon within the order and its importance in primate evolution. The study of hybridization of *Alouatta pigra* and *A. palliata* in Mexico was among the first to

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genetically confirm the current occurrence of hybridization in primates. Following this study, other reports of hybridization have shown that this phenomenon is more widespread among primates than previously anticipated. Within the genus *Alouatta*, there have been reports on the presence of hybridization between *A. caraya* and *A. guariba* in a number of contact zones in Brazil and Argentina, and various studies are currently ongoing in some of these sites to understand the extent and patterns of hybridization between these species. In this chapter, we evaluate the extent of hybridization in the genus *Alouatta*, revise the current knowledge of the genetic and morphological aspects of these hybrid systems, and identify future directions in the study of hybridization within this genus, to understand the possible implications of the hybridization process in the evolutionary history of howler monkeys.

Resumen Hibridación, o el proceso mediante el cual individuos de poblaciones genéticamente distintas (especies o subespecies) se aparean y producen descendencia, tiene gran relevancia en la comprensión de las bases para el aislamiento reproductivo entre distintos taxa y, en algunos casos, para entender el origen de la biodiversidad. La hibridación natural entre primates ha sido bien conocida para unas cuantas especies, pero sólo recientemente la confirmación genética de hibridación entre numerosos taxa de primates ha sido posible y ha conducido a una nueva percepción de la prevalencia de este fenómeno entre los primates y su importancia en la evolución de este grupo. El estudio de la hibridación entre *Alouatta pigra* and *A. palliata* en México fue uno de los primeros que confirmó con evidencia genética la ocurrencia de hibridación en primates. Después de este estudio, otros reportes de hibridación en distintos taxa de primates han puesto de manifiesto que este fenómeno es más común en el orden Primates de lo que inicialmente se pensaba. Dentro del género *Alouatta*, también han habido reportes de hibridación entre *A. caraya* y *A. guariba* en distintas zonas de contacto en Brasil y Argentina, y varios estudios actualmente están en curso en algunas de estas áreas para entender la magnitud de este fenómeno y los patrones de hibridación entre estas especies. En este capítulo evaluamos la presencia de hibridación en el género *Alouatta*, revisamos lo que se conoce sobre los aspectos genéticos y morfológicos en estos sistemas híbridos y planteamos direcciones futuras en el estudio de la hibridación en este género, para entender las implicaciones del proceso de hibridación en la historia evolutiva de los monos aulladores.

Keywords Evolution • Morphology • Genetic admixture • Hybrid zone • Sympatry

5.1 Introduction

Hybridization is the crossing of genetically distinct taxa that produces some viable offspring (Arnold 1997; Mallet 2005). Crosses of pure individuals from different genetic lineages result in first-generation hybrids (F1s), but hybrid individuals can backcross with pure individuals of one of the parental species or crossbreed with other

hybrid individuals, producing offspring with variable levels of genetic admixture. Although hybridization was initially considered a process mainly occurring among plants, and with limited representation in animals, a variety of genetic studies in the past few decades have shown that this phenomenon is rather common among sexually reproducing animals, especially between closely related species (Dowling and Secor 1997; Mallet 2005).

In primates, hybridization has been reported in captivity for a number of taxa (e.g., Chiarelli 1973; Tenaza 1984; Coimbra-Filho et al. 1984; Jolly et al. 1997); however, only few cases of natural hybridization in primates were known and studied before the twenty-first century, and most of these involved cercopithecine monkeys (Bernstein 1966; Struhsaker 1970; Nagel 1973; Dunbar and Dunbar 1974; Samuels and Altmann 1986). Identification of hybrids in these studies primarily relied on behavioral and morphological features of individuals that showed mixed characteristics typical of each parental taxon.

The widespread use of molecular techniques to address different aspects of primate systematics, behavior, and ecology during the last two decades has allowed the detection of an increased number of cases of hybridization in different primate taxa (e.g., Merker et al. (2009) in tarsiers; Cortés-Ortiz et al. (2007) in howler monkeys; Wyner et al. (2002) in lemurs; da Silva et al. (1992) in squirrel monkeys), including those in our own lineage (Green et al. 2010). However, there are still large gaps in our understanding of the genetic and morphological outcomes of hybridization at the individual and population levels, as well as their implications for the evolutionary trajectories of primate lineages.

In this chapter we review our current understanding of the prevalence of hybridization among howler monkeys. *Alouatta* is among of the first Neotropical primate genera for which genetic confirmation of hybridization is available (Cortés-Ortiz et al. 2007). We summarize demographic, morphological, behavioral, and genetic studies currently available, and make recommendations on future directions in the study of *Alouatta* hybrid zones and the implications of hybridization in primate evolution.

5.2 Distribution of Howler Monkey Contact Zones

As illustrated throughout this volume, howler monkeys are distributed across the Neotropics and have the broadest distribution of any Neotropical primate genus (Fig. 3.1). Phylogenetic studies have identified between 10 and 14 species and 22 taxa (species and subspecies), but there are a number of poorly known forms that still remain to be studied to allow an adequate evaluation of their taxonomic status (e.g., Peruvian species/subspecies) (see Cortés-Ortiz et al. 2014).

Although howler monkey species maintain allopatric/parapatric distributions in most of their range, small areas of overlap have been reported for some species (Fig. 5.1), including contact between *A. palliata* and *A. pigra* in Mexico (Smith 1970; Horwich and Johnson 1986; Cortés-Ortiz et al. 2007), *A. palliata* and



Fig. 5.1 Approximate location of the reported areas of contact between howler monkey species. (1) *A. palliata* and *A. pigra* in Mexico (Horwich and Johnson 1986; Smith 1970), (2) *A. palliata* and *A. seniculus* in northwestern Colombia (Defler 1994; Hernández-Camacho and Cooper 1976), (3) *A. caraya* and *A. guariba clamitans* in northern Argentina (Agostini et al. 2008; Di Bitetti 2005), (4) *A. caraya* and *A. g. clamitans* in southern Brazil (Bicca-Marques et al. 2008), (5) *A. caraya* and *A. g. clamitans* in southern Brazil (Aguiar et al. 2007; 2014; Gregorin 2006), (6) *A. g. guariba* and *A. g. clamitans* in Brazil (Kinzey 1982), (7) *A. discolor* and *A. s. puruensis* in Brazil (Pinto and Setz 2000), (8) *A. caraya* and *A. sara* in Bolivia (Büntge and Pyritz 2007), (9) *A. caraya* and *A. sara* in Bolivia (Wallace et al. 2000), (10) *A. caraya* and *A. sara* in Brazil (Iwanaga and Ferrari 2002), (11) *A. macconnelli* and *A. nigerrima* in Brazil (Napier 1976 and Cruz Lima 1945, cited in Gregorin 2006)

A. seniculus in northwestern Colombia (Hernández-Camacho and Cooper 1976; Defler 1994), *A. caraya* and *A. guariba* in northern Argentina (Di Bitetti 2005; Agostini et al. 2008), *A. caraya* and *A. guariba* in southern Brazil (Gregorin 2006; Aguiar et al. 2007, 2008, 2014; Bicca-Marques et al. 2008), *A. g. guariba* and *A. g. clamitans* in Brazil (Kinzey 1982), *A. discolor* and *A. s. puruensis* in Brazil (Pinto and Setz 2000), *A. caraya* and *A. sara* in Bolivia (Wallace et al. 2000; Büntge and Pyritz 2007) and Brazil (Iwanaga and Ferrari 2002), and *A. macconnelli* and *A. nigerrima* in Brazil (Napier 1976 and Cruz Lima 1945, cited in Gregorin 2006). It is likely that these areas of sympatry are due to secondary contact as a consequence of range expansions after periods of isolation (Cortés-Ortiz et al. 2003; Ford 2006; Gregorin 2006), and therefore, many other areas of contact among different *Alouatta* species may also exist. However, few surveys have been conducted in areas of potential contact within the limits of the distribution of parapatric howler monkey species, and those that exist show that sympatry is rare, but more common than previously anticipated. In some of the areas of sympatry among howler monkeys, individuals with intermediate or mosaic features have been observed (Cortés-Ortiz et al. 2003, 2007; Gregorin 2006; Aguiar et al. 2007; Agostini et al. 2008; Bicca-Marques et al. 2008; Silva 2010), suggesting at least some degree of crossbreeding between taxa and the formation of hybrid zones.

5.3 Studies of Hybridization in Howler Monkeys: Mixed Groups and Demographic Features of Syntopic Hybridizing Species

Evidence of hybridization has been reported for only two pairs of species of howler monkeys: *A. palliata* × *A. pigra* and *A. caraya* × *A. guariba*. These species are distinguishable on the basis of both morphological (Hill 1962; Groves 2001; Gregorin 2006) and genetic (de Oliveira et al. 2002; Cortés-Ortiz et al. 2003; Steinberg et al. 2008) features. The hybridizing species of each of these pairs diverged at approximately 3 and 5 MA, respectively (Cortés-Ortiz et al. 2003). Reports of possible hybridization were initially based on morphological and behavioral observations of individuals living in proximity or in mixed species groups. Later, demographic, behavioral, and genetic studies confirmed or strongly suggested the presence of hybrid offspring in the wild (Cortés-Ortiz et al. 2007; Agostini et al. 2008; Aguiar et al. 2008; Bicca-Marques et al. 2008) and in captivity (de Jesus et al. 2010).

5.3.1 *A. palliata* × *A. pigra* Hybrid Zone in Tabasco, Mexico

Smith (1970) first reported a possible area of sympatry of *A. palliata* and *A. pigra* in Tabasco, Mexico, based on museum specimens collected ~8 km SE of Macuspana (17°45'40"N, 92°35'35"W). More than a decade later, Horwich and Johnson (1986)

surveyed the area where the specimens studied by Smith were collected as well as other nearby areas, but failed in finding direct evidence of the presence of howler monkeys. Nonetheless, through interviewing of local people, they identified a possible area of sympatry in the vicinity of Teapa ($17^{\circ}33'25''\text{N}$, $92^{\circ}56'50''\text{W}$), about 40 km SE of Macuspana. In the early 1990s, Francisco García Orduña, Domingo Canales Espinosa, and Ernesto Rodríguez Luna from the Universidad Veracruzana (UV) in Mexico surveyed several areas across the state of Tabasco and found groups of *A. palliata* and *A. pigra* living in close proximity, as well as mixed groups composed of individuals of both species, and groups with individuals that emitted distinct vocalizations that sounded “intermediate” between the calls of either species (García-Orduña et al. unpubl. data; see also Kitchen et al. 2014). Later excursions to the area with the aim of collecting biological samples for genetic studies revealed that a number of individuals possessed mixed morphological features distinctive of each species (mainly subtle facial features, as well as pelage coloration) (Cortés-Ortiz unpubl. data; see Fig. 5.2 for an example of differences in facial features). Cortés-Ortiz and collaborators sampled 44 groups within this contact zone between 1998 and 2010 (Table 5.1). Most groups ($N=28$) were phenotypically monospecific

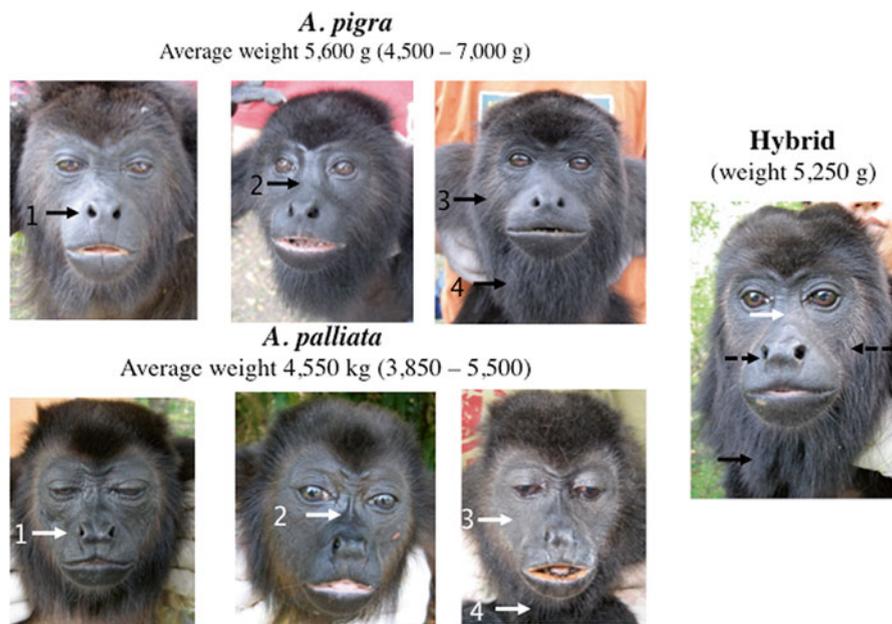


Fig. 5.2 Example of facial differences between *A. pigra* and *A. palliata* females and mixed features in a hybrid female. All pictures are from adult females: (1) nostrils more frontal in *A. pigra* and nasal alar walls more prominent in *A. palliata*, (2) prominent ridge of the nasal bone in *A. palliata* and not apparent in *A. pigra*, (3) hair covering a larger area of the cheeks in *A. pigra* than in *A. palliata*, and (4) longer beard in *A. pigra* than in *A. palliata*. Black arrows denote *A. pigra* features, white arrows denote *A. palliata* features, and dashed arrows denote intermediate features in the hybrid. Weight averages from Kelaita et al. (2011)

Table 5.1 Groups of howler monkeys surveyed in the areas of contact of known hybridizing species. Individuals are assigned to the different categories based on phenotype and genotype in the Mexican hybrid zone, and only on phenotype in the Brazilian and Argentinian hybrid zones

	Phenotype	Genotype
<i>A. pigra</i> × <i>A. palliata</i> (Cortés-Ortiz et al. unpubl.)		
Apa	17	5
Api	11	3
ApaH	0	7
ApiH	7	2
Mix	3	4
Apa-like Hyb	6	15
Api-like Hyb	0	1
<i>Total groups</i>	<i>44</i>	<i>37</i>
<i>A. guariba</i> × <i>A. caraya</i> (Aguiar et al. 2007, 2008)		
Aca	8	–
Agu	5	–
AcaH	0	–
AguH	2	–
Mix	5	–
Hyb	0	–
<i>Total groups</i>	<i>20</i>	
<i>A. guariba</i> × <i>A. caraya</i> (Bicca-Marques et al. 2008; Silva 2010)		
Aca	11	–
Agu	10	–
AcaH	5	–
AguH	8	–
Mix	5	–
Hyb	4	–
<i>Total groups</i>	<i>43</i>	
<i>A. guariba</i> × <i>A. caraya</i> (Agostini et al. 2008)		
Aca	3	–
Agu	5	–
AcaH	0	–
AguH	0	–
Mix	1	–
<i>Total groups</i>	<i>9</i>	

Apa=*A. palliata*, Api=*A. pigra*, ApaH=group of *A. palliata* with some hybrids, ApiH=group of *A. pigra* with some hybrids, Mix=mixed groups of *A. palliata* and *A. pigra* individuals or *A. caraya* and *A. guariba*, Api-like Hyb=all group members are hybrids resembling *A. pigra*, Apa-like Hyb=all group members are hybrids resembling *A. palliata*. Aca=*A. caraya*, Agu=*A. guariba*, AcaH=group of *A. caraya* with some hybrids, AguH=group of *A. guariba* with some hybrids, Hyb=group entirely composed of hybrid individuals

(17 *A. pigra* and 11 *A. palliata*), but three groups were mixed with individuals phenotypically resembling either species living together, and the remaining 13 groups included individuals with intermediate/mosaic features (detected via either morphology or vocalizations; see Figs. 14.1 and 14.2 in da Cunha et al. 2014 for differences in vocalizations) suggestive of a hybrid origin (but see Sect. 5.4 for a better understanding of the complex relationship between morphology and genetics in this system). Based on these surveys and data, we now know that the *A. palliata* × *A. pigra* hybrid zone in Tabasco is about 20 km wide and covers at least 67 km², with a patchwork of pure, mixed, and hybrid groups (Cortés-Ortiz et al. 2003, 2007) (see Table 5.1 for details on group composition).

5.3.2 *A. caraya* × *A. guariba* Hybrid Zones in Brazil

Records of mixed groups formed by *A. caraya* and *A. guariba* can be traced back to the beginning of the nineteenth century in the State of Rio Grande do Sul in Brazil (Isabelle 1983). However, Lorini and Persson (1990) were the first to report possible hybridization between these species in Brazil based on morphological studies of museum specimens collected in the 1940s by A. Meyer in the region of the Upper Parana River in the northwestern extreme of the State of Paraná. These specimens had a mosaic pelage coloration pattern representing a mixture of the typical patterns of the two parental species. In his comprehensive review of Brazilian howler monkeys, Gregorin (2006) analyzed the same specimens and also concluded that they represented hybrid individuals. Aguiar et al. (2007, 2008) surveyed a nearby area in the surroundings of the Ilha Grande National Park (23°24'S, 53°49'W) and found both monospecific groups of *A. caraya* and *A. guariba* living in sympatry and groups containing individuals with mosaic coloration patterns (see Fig. 2 in Aguiar et al. 2007 and Fig. 1 in Aguiar et al. 2008). They reported a total of 11 groups living within the boundaries of a 150 ha forest fragment (two monospecific groups of each species, two groups with *A. guariba* + putative hybrids, and five polyspecific groups of *A. caraya* + *A. guariba* + putative hybrids), as well as five *A. caraya* and two *A. guariba* groups living along a 17 km stretch of riverine forest and two monospecific groups (one of each species) living in sympatry in a near forest fragment ("Paredão das Araras," 23°21'10.1"S, 53°44'08.5"W). They found *A. guariba* as the most abundant species in the area, perhaps as a consequence of the prevalence of Atlantic Forest in the area, which is a type of habitat usually inhabited by this species rather than by *A. caraya*. The proportion of putative hybrids was similar to the proportion of *A. caraya* individuals in the area.

Another area of sympatry and hybridization between these taxa in Brazil occurs in the region of São Francisco de Assis, State of Rio Grande do Sul (Bicca-Marques et al. 2008; Silva 2010). Between 2006 and 2009 the team of primatologists and students headed by Bicca-Marques surveyed six localities within an area of approximately 600 km² in this region (29°33'50"–29°35'10"S, 54°58'40"–54°59'50"W), finding a total of 43 groups, 22 of which included at least one potential

hybrid individual (i.e., with a mosaic phenotype) (Silva 2010). Interestingly, the distribution of phenotypically *A. guariba* groups decreased westwards and the opposite trend was observed for *A. caraya* groups. The westernmost locality surveyed contained only *A. caraya* groups, and a high percentage of hybrid individuals (42 %) was still present in the easternmost surveyed locality, suggesting that the area of contact and hybridization between these taxa may extend beyond the approximately 20 km wide strip surveyed.

5.3.3 *A. caraya* × *A. guariba* Hybrid Zones in Argentina

In Argentina, *A. guariba* and *A. caraya* have overlapping distributions in a small region in the province of Misiones, where syntopic populations have been detected in the strictly protected area of El Piñalito Provincial Park (Agostini et al. 2008). In a survey of approximately 800 ha, Agostini et al. (2008) detected three groups of *A. caraya*, five of *A. guariba*, and one mixed group composed of one adult *A. guariba* male, two *A. guariba* females, and one *A. caraya* female. The latter female was observed copulating with *A. guariba* males and giving birth twice to individuals with mosaic phenotypes, similar to those reported in Brazil (see Sect. 5.3.2). The extent of hybridization in this area is still unknown, but the absence of adults with mosaic pelage coloration patterns suggests that hybridization may be less common in this site than in the Brazilian contact zones. More recent surveys in the State of Misiones (one by I. Holzmann during and immediately after a yellow fever outbreak in 2008 [Holzmann 2012] and one by Agostini in 2010 [Agostini unpubl. data]) found no morphological or demographic evidence of hybridization. However, without extensive surveys in other localities within this contact zone, any statement about the lack of hybridization in this region would be premature.

5.4 Morphological Signals of Hybridization

The finding of individuals with intermediate phenotypes (i.e., diagnostic traits of each parental species co-occurring in the same individual) is often seen as evidence of hybridization. However, our understanding of the effects of hybridization on the morphological development of an individual is rather poor. On the one hand, we lack a clear understanding of the extent of phenotypic variation in hybrid individuals (Ackermann 2010), and on the other, many studies have only been able to detect hybridization when genetic markers are used (i.e., when hybridization is cryptic; e.g., Jasinska et al. (2010) in plants; Neaves et al. (2010) in marsupials; Gaubert et al. (2005) in carnivores). The slowly increasing number of studies incorporating genetic and morphological data in the study of the hybridization process suggests that morphologically intermediate and cryptic hybrids are the extremes of a continuum in the morphological expression of hybridization (e.g., Ackermann et al. 2006; Ackermann and Bishop 2010; Kelaita and Cortés-Ortiz 2013).

Much of what it is known about primate hybrid morphology comes from studies of Old World monkeys such as baboons (e.g., Jolly 2001; Ackermann et al. 2006), macaques (e.g., Bynum 2002; Schillaci et al. 2005), and some cercopithecine species (Detwiler 2002). Only a handful of studies addressing the morphology of hybrid New World monkeys have been carried out (e.g., Cheverud et al. 1993 and Kohn et al. 2001 for captive tamarins; Peres et al. 1996 for wild saddled back tamarins). In this section we discuss patterns of morphological variation observed in both presumed (based on phenotype) and genetically confirmed howler monkey hybrids, and discuss the reliability of using morphological cues to identify hybrid individuals.

Howler monkey species differ in numerous phenotypic attributes. Among the most conspicuous are the pelage color patterns that distinguish parapatric species. This is particularly true for the four species that are known to hybridize: *A. caraya*, *A. guariba*, *A. palliata*, and *A. pigra*. In *A. caraya*, adult males are completely black and adult females are pale yellowish-brown, whereas males of *A. guariba* are red and females are dark brown (Gregorin 2006). Coat coloration of *A. palliata* adults is black with light golden hairs on the flanks, whereas the pelage coloration of *A. pigra* is completely black and hairs have a softer texture than in *A. palliata* (Smith 1970). Intermediate pelage coloration between *A. caraya* and *A. guariba* was the trait used by Lorini and Persson (1990) to recognize some of the museum specimens of their study as putative hybrids. This identification generated expectations of hybrid morphotypes represented by mosaic combinations of coat color polymorphisms (Gregorin 2006), which were later used to classify putative *A. caraya* × *A. guariba* hybrids in the wild (Aguiar et al. 2007, 2008; Agostini et al. 2008; Bicca-Marques et al. 2008; Silva 2010). The distinctive pelage coloration of adult males and females of the sexually dichromatic *A. guariba* and *A. caraya* presumably results in easily distinguishable mosaic and/or intermediate features in the hybrid individuals, with up to 20 morphotypes identified in the wild (Aguiar et al. 2008; Silva 2010).

While the detection of *A. caraya* × *A. guariba* hybrids may be possible based on pelage coloration (at least to a certain extent), the recognition of the more similarly colored *A. palliata* × *A. pigra* hybrids using the same methods is not always possible. *Alouatta palliata* and *A. pigra* display some cranial and facial shape differences that can be used to distinguish individuals of each species in the field (see the example in Fig. 5.2). However, these traits show considerable intraspecific variation, and the intermixing of these features produces a broad range of hybrid morphotypes that compromised attempts to generate a clear criterion to accurately distinguish genetically confirmed hybrid and non-hybrid individuals (Kelaita and Cortés-Ortiz 2013).

Morphometric data, in contrast, have shown several quantifiable size differences between *A. palliata* and *A. pigra* for several variables (Kelaita et al. 2011), but analyses of morphological variation based on quantitative (metric) measurements of body size also showed a great variation in the hybrid phenotypes in Mexico (Kelaita and Cortés-Ortiz 2013). Kelaita and Cortés-Ortiz (2013) confirmed the hybrid status of individuals using diagnostic genetic markers (see Sect. 5.5 for details). The genetic data revealed that only 12 % of 128 identified hybrids had similar portions of their genome coming from each parental species. Although none of these individuals were F1 individuals, they were classified as “intermediate” and likely represent early-generation hybrids. The majority of identified hybrids were multigenerational

backcrosses, probably resulting from the crossing of first-generation hybrids and their descendants with purebred individuals of one or the other parental species, or from the continued mating among hybrids during multiple generations. Depending on the number of diagnostic alleles of each species present in hybrid individuals, they were classified as *A. palliata*-like or *A. pigra*-like multigenerational backcrossed hybrids. A comparison of 14 morphometric variables among purebred and hybrid adult individuals showed that genetically intermediate hybrids exhibited great variation in morphometric characters. Both male and female intermediates ran the gamut of potential states for each variable, in some cases resembling *A. palliata*, while in others resembling *A. pigra*, or exhibiting values intermediate between or overlapping with the two parental species. On the other hand, multigenerational backcrossed hybrids only resembled the parental species with which they shared most of their alleles (Kelaita and Cortés-Ortiz 2013), compromising their accurate identification as hybrids.

These results indicate that instances of hybridization between well-established taxonomic groups can be underestimated if only a morphological criterion is utilized to identify hybrids. In the case of *A. palliata* × *A. pigra* hybrids, the majority of hybrid individuals are morphologically indistinguishable from parental species. The *A. guariba* × *A. caraya* hybrid studies revealed that hybrid individuals, identified based on pelage coloration patterns, comprised between 14 % (Aguiar et al. 2008) and 25 % (Silva 2010) of all individuals sampled from the respective hybrid zones. Considering that in the howler monkey hybrid zone in Mexico genetically intermediate hybrids comprise 12 % of all sampled individuals, it is likely that the purported *A. guariba* × *A. caraya* hybrids may also represent genetically intermediate individuals. The incorporation of molecular methods will help to test this prediction in the *A. guariba* × *A. caraya* hybrid zones.

5.5 Genetic Studies in the Howler Monkey Hybrid Zones

Genetic confirmation of hybridization in howler monkeys only exists for the *A. palliata* × *A. pigra* hybrid system. An initial study by Cortés-Ortiz et al. (2007) in Tabasco, Mexico determined the hybrid status of 13 individuals based on mitochondrial (mtDNA) and Y-chromosome (SRY gene) sequence data that, respectively, track the maternal and paternal lineages of hybrids, as well as on eight bi-paternally inherited microsatellite loci (three of which had diagnostic alleles for the parental species). Individuals were considered “hybrids” whenever discordance between mtDNA, SRY, and/or microsatellites occurred or when microsatellite loci in the same individual contained a combination of alleles diagnostic of each species. This study suggested unidirectional hybridization in this population, in which the cross of *A. palliata* males and *A. pigra* females only produced F1 fertile females, but the cross of *A. pigra* males and *A. palliata* females appeared to fail in producing fertile offspring. This result is consistent with the prediction of Haldane’s rule, which establishes that it is more likely for the heterogametic sex (i.e., males for mammals) to be inviable or sterile (Haldane 1922). Nonetheless, the genetic variability at the

uni- and bi-parentally inherited loci found among hybrids showed that backcrossing was occurring and that the production of fertile multigenerational backcrossed males was possible (Cortés-Ortiz et al. 2007). Preliminary results of an ongoing study based on a larger sample size of individuals ($N=178$) from the same hybrid zone and using 15 diagnostic microsatellite loci (which have a higher power to detect mixed ancestry) give support to the directional bias in hybridization and subsequent backcrossing. These new results also show novel genetic combinations (see Fig. 5.3) and a much higher percentage of hybrid individuals in the area of contact than initially recognized (Cortés-Ortiz unpubl. data). Most hybrids in the area are multigenerational, and only a handful of individuals are likely the product of crosses between purebreds and recent generation hybrids. Figure 5.4 summarizes

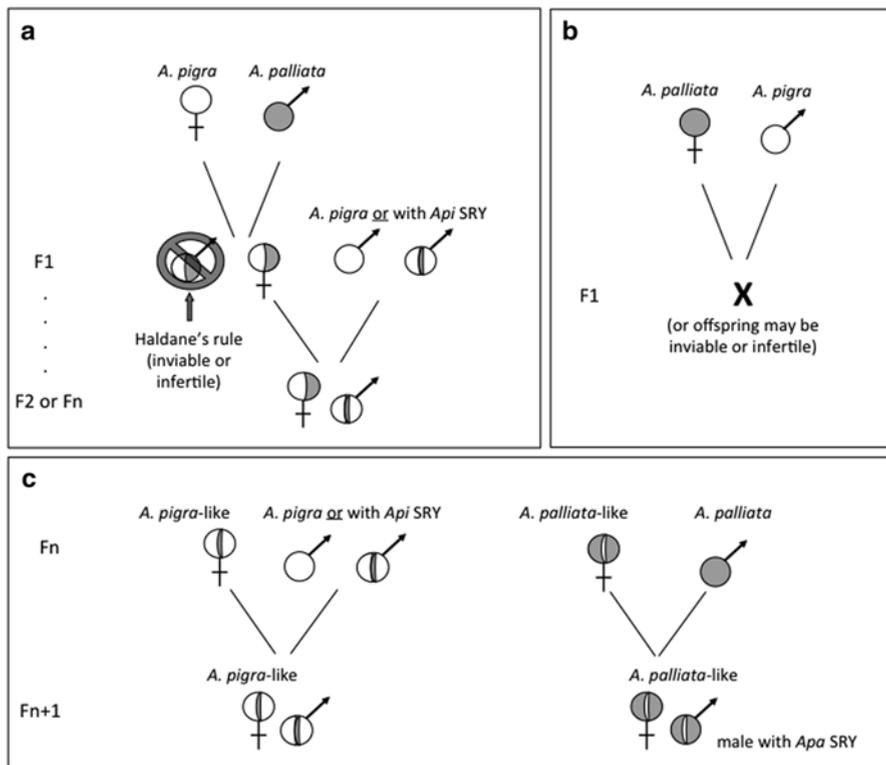


Fig. 5.3 Possible outcomes of crosses between *A. palliata*, *A. pigra* and hybrid individuals based on genotypic data of individuals from the Mexican hybrid zone. **(a)** Crosses between *A. pigra* females and *A. palliata* males only produce fertile females. These F1 females may mate with either *A. pigra* males or backcrossed males with *Api* SRY type and produce female offspring. It is unknown whether males with *Api* SRY type may be produced in this or only in later generations of backcrossing. **(b)** Crosses between *A. palliata* females and *A. pigra* males either do not occur, do not produce offspring, or rarely occur and produce unfertile offspring. **(c)** Further generation hybrids may continue to backcross with either purebred or backcrossed individuals and eventually produce males with *Apa* SRY type (Modified from Fig. 3 of Cortés-Ortiz et al. 2007)

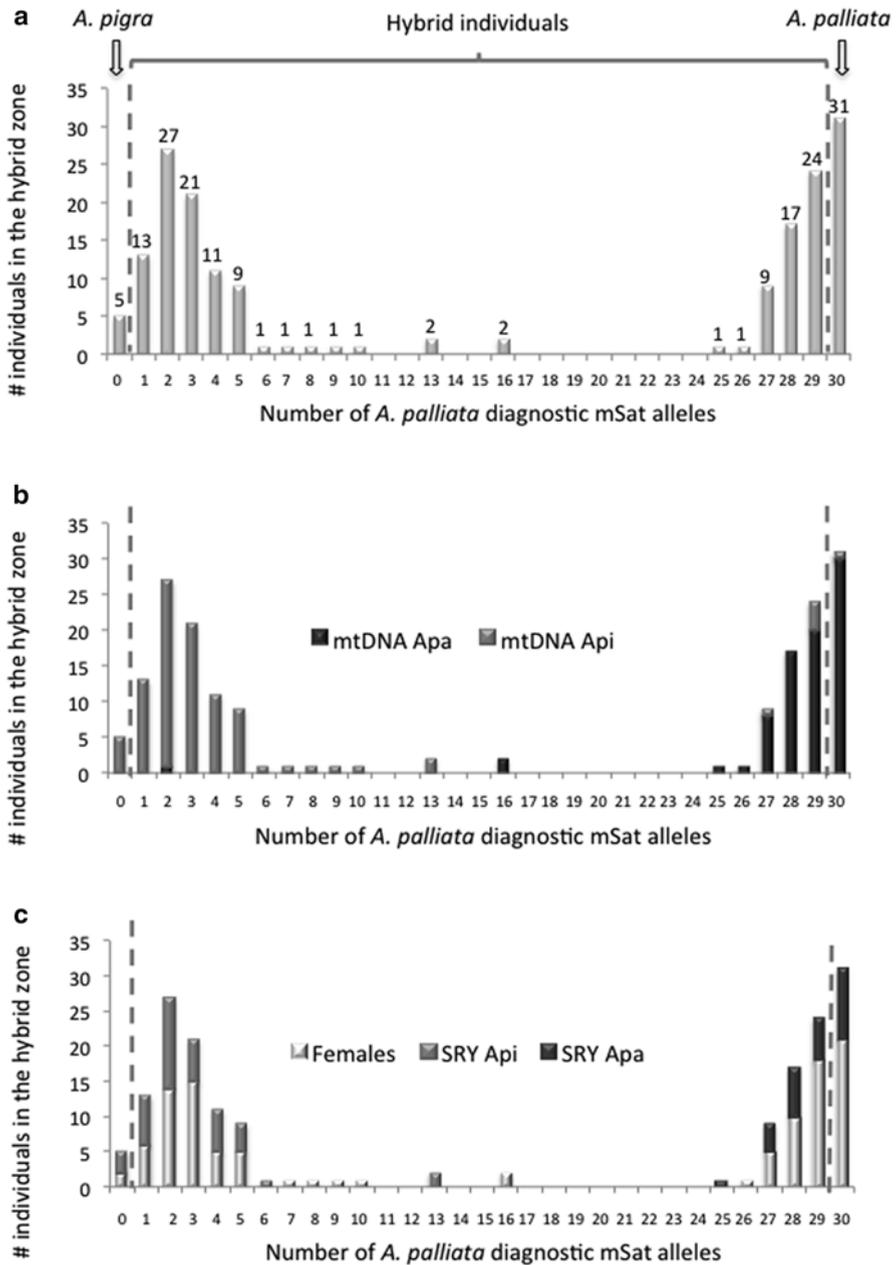


Fig. 5.4 Genetic composition of individuals from the Mexican hybrid zone. The X-axis represents the number of *A. palliata* diagnostic alleles. Individuals with 0 *A. palliata* diagnostic alleles represent pure *A. pigra* individuals whereas those with 30 *A. palliata* diagnostic alleles represent pure *A. palliata* individuals. (a) Variation based on 15 diagnostic microsatellite loci, (b) composition of mitochondrial DNA (mtDNA) haplotypes, and (c) composition of sex determination gene (SRY) haplotypes

the individual genetic variation found in this contact zone. Interestingly, when analyzing the genetic composition of hybrids, it is apparent that mtDNA haplotypes from *A. pigra* are more likely to be present in individuals with most of their nuclear genome (represented by the microsatellite alleles) of the *A. palliata* type, but only one hybrid with mostly *A. pigra* nuclear background has an *A. palliata* mtDNA haplotype. It is also remarkable that all male hybrids have the SRY gene type (reflecting paternal lineage) coincident with the majority of their nuclear background. These observations also support the predictions of Haldane's rule in the *A. palliata* × *A. pigra* hybrid system (Cortés-Ortiz et al. 2007), in which only females are produced in the first generation of crossing, and viable or fertile males appear in the population only after extensive backcrossing among multigenerational hybrids or between hybrids and purebred individuals (see Fig. 5.3C). The patterns of genetic variation observed among hybrid/backcrossed individuals suggest that the directionality in hybridization may be due to chromosomal, cytonuclear, or genomic incompatibilities. Steinberg et al. (2008) studied the chromosomal arrangements of Mesoamerican howler monkeys (see also Mudry et al. 2015) and found that *A. pigra* and *A. palliata* have different modal chromosome numbers ($2n=58$ for *A. pigra* and $2n=53$ and 54 for *A. palliata* males and females, respectively), and males have different sex determination systems ($X_1X_2Y_1Y_2$ quadrivalent in *A. pigra* and X_1X_2Y trivalent in *A. palliata*). Whether the apparent lack of early-generation male hybrids is a consequence of chromosomal incompatibilities due to these chromosomal differences is still an open question.

Although molecular data for the *A. caraya* × *A. guariba* hybrid zones are not yet available, the demographic and morphological patterns observed in their contact zones allow some inferences based on the knowledge generated from the *A. palliata* × *A. pigra* genetic studies. First, the presence of mosaic coat color features in putative hybrid males (one subadult male in Aguiar et al. (2007), one infant male in Agostini et al. (2008), four in Bicca-Marques et al. (2008), one in Jesus et al. (2010), and eight adult males in Silva (2010)) suggests that at least some male hybrids are viable. This inference is supported by a case of hybridization in captivity between putatively purebred individuals (Jesus et al. 2010). Second, if the mosaic individuals represent early-generation hybrids, as found in the *A. palliata* × *A. pigra* hybrid zone (Kelaita and Cortés-Ortiz 2013), it is possible that Haldane's rule is not operating in the *A. caraya* × *A. guariba* system. However, the absence of information on the longevity of the morphological signal of hybridization and the lack of molecular data makes it impossible to come to strong conclusions on this respect. Third, *A. caraya* and *A. guariba* also have different modal chromosome numbers ($2n=52$ for *A. caraya* and $2n=45-52$ for *A. guariba*; de Oliveira et al. 2002) and males have different sex determination systems ($X_1X_2Y_1Y_2$ quadrivalent in *A. caraya* and $X_1X_2X_3Y_1Y_2$ pentavalent in *A. guariba clamitans*; de Oliveira et al. 2002); therefore, the production of one viable F1 male hybrid in captivity (Jesus et al. 2010) is at least unexpected. Comparative genetic studies in the hybrid zones will provide an outstanding opportunity to explore whether molecular and/or cytogenetic mechanisms (or both) are responsible for the observed levels of reproductive isolation and the maintenance of species integrity despite hybridization.

5.6 Future Directions in the Study of Hybridization of Howler Monkeys

It has been recently recognized that hybridization is a powerful force that has shaped the evolutionary trajectory of a wide range of animal taxa (Dowling and Secor 1997; Arnold 1997; Grant et al. 2004; Mallet 2005). When hybridization occurs, genetic material of one lineage may enter the genetic pool of another, introducing genetic novelty to the latter (a process known as genetic introgression) (Rheindt and Edwards 2011). If this introduction of genetic novelty is advantageous to the recipient individuals, it may influence the evolutionary trajectory of the hybrid population or of one or both of the parental lineages (e.g., Grant and Grant 2010). Therefore, instances of hybridization may contribute to the adaptive radiation and diversification of species.

Several historical, demographic, behavioral, and ecological processes are involved in the origin and maintenance of hybrid zones, and a number of different mechanisms may operate together to maintain the hybridization process. Most of our future research is directed towards understanding the mechanisms that influence the hybridization process in howler monkeys, as well as the effect of hybridization in the ecology and behavior of the interacting taxa.

5.6.1 *Endogenous and Exogenous Selective Forces in Hybridization*

In general, hybridization may influence evolution in a variety of ways, and it mostly depends on endogenous and exogenous selective forces operating on each hybrid system (Barton 2001). When there is an intrinsic loss of fitness in hybrids, due, for example, to genetic incompatibilities between the two parental genomes (endogenous selection), it is likely that the hybrid zone will constitute a barrier preventing gene flow between the parental taxa. On the other hand, it has been argued that hybrid zones may be maintained by adaptation to different environments (exogenous selection), in which hybrid individuals may be more adapted to fluctuating or intermediate environments (e.g., Cruzan and Arnold 1993). In this case, individuals within the hybrid zone may exhibit a greater variance in fitness. Hybrids with higher fitness will contribute to adaptation either by introgression of alleles to parental taxa or by the establishment of recombinant genotypes (Barton 2001). However, these two selective forces (endogenous and exogenous) are not mutually exclusive and can operate together in the same system: whereas hybrid zones can be maintained by the selection against hybrids and represent barriers to gene flow, the divergence between interacting populations may be generated by adaptation to fluctuating environments (Barton 2001).

Studies have only recently been directed to understanding the effects of hybridization and gene introgression in the evolutionary history of primates (e. g., Arnold and Meyer 2006; Arnold 2009; Ackermann 2010; Green et al. 2010; Zinner et al. 2011).

In addition, only a few examples have actually provided some insight into the patterns of hybridization among primate taxa using genetic data (Cortés-Ortiz et al. 2007; Tung et al. 2008; Zinner et al. 2009; Merker et al. 2009; Ackermann and Bishop 2010).

In the case of the hybridization of howler monkeys in Mexico, there is some support for the operation of endogenous selection (e.g., Haldane's Rule effect), and there are no current environmental differences between the habitats of *A. palliata* and *A. pigra* throughout their distribution range that suggest strong influence of exogenous selection in this hybrid system. The responsible mechanisms for the partial reproductive isolation between the two species remain unknown, but genetic analyses suggest that some of these mechanisms could be attributed to chromosomal differences or to incompatibilities between nuclear and mitochondrial genomes (see Sect. 5.5). Cytogenetic and molecular studies comparing chromosomal and genomic regions associated with hybrid incompatibility should be a next step in our attempts to understand the endogenous mechanisms driving distinct levels of reproductive isolation in howler monkey hybrid zones.

On the other hand, exogenous selection may be strongly influencing the *A. caraya* × *A. guariba* hybrid zones. The currently known hybrid zones between these species in Brazil are located within regions of contact between two biomes (the Atlantic Forest and the Pampas, Bicca-Marques et al. 2008; and the Atlantic Forest, the Pantanal, and the Cerrado, Aguiar et al. 2007, 2008), with forests that are typically inhabited by each species (the Atlantic Forest by *A. guariba* and the Pantanal, the Pampas and the Cerrado by *A. caraya*). In Argentina, the hybrid zone lies within the Atlantic Forest ecoregion, for which *A. guariba* is endemic, but it is not a typical habitat for *A. caraya*. However, both species have very similar trophic niches (Bicca-Marques et al. 2008; Agostini et al. 2010) and are quite tolerant to habitat disturbance (Zunino et al. 2007; Bicca-Marques et al. 2008), which has been recently occurring in this area (Agostini et al. 2008). Therefore, the presence of both species in the area is likely the result of relatively recent secondary contact, with *A. caraya* individuals spreading into areas typically inhabited by *A. guariba*, as a consequence of forest disturbance. These incursions may occur infrequently generating an asymmetrical proportion of individuals of both species. The demography (i.e., abundance, sex ratios, rates of dispersal, etc.) and behavior of hybridizing taxa can affect levels and patterns of gene introgression in hybrid zones (Barton and Hewitt 1989; Wirtz 1999; Rohwer et al. 2001; Field et al. 2011; Gompert et al. 2012), generating different outcomes in the distribution of genetic backgrounds among hybrid zones with different ecological conditions. The availability of multiple contact zones between *A. caraya* and *A. guariba* with important ecological differences among them, as well as differences in the demographic composition of the two hybridizing species, offers a rare opportunity for testing the role that these factors may play on the occurrence and maintenance of the hybrid zones and the patterns of gene introgression. Comparative ecological studies within and outside these three hybrid zones between *A. caraya* and *A. guariba* would provide the grounds to understand the effect of exogenous selection in the fitness of hybrid individuals with distinct genetic architectures, and the differential effects of exogenous versus endogenous selection in the hybridization of howler monkeys.

5.6.2 *Habitat Fragmentation and Its Effect on the Hybridization of Howler Monkeys*

All howler monkey hybrid zones currently known are located in or surrounded by highly fragmented environments. It has been suggested that human-induced activities may play an important role in promoting hybridization in primates (Detwiler et al. 2005). Based on paleoecological data from the São Francisco de Assis region, Bicca-Marques et al. (2008) suggested that the contact between *A. caraya* and *A. guariba* is a recent consequence of the expansion of the two forests biomes in the past 2,000 years. Similarly, the current contact zone of *A. palliata* and *A. pigra* in Mexico seems to be the result of a secondary contact due to a two-wave colonization process (Cortés-Ortiz et al. 2003; Ford 2006) with a recent northward expansion of *A. palliata* (Cortés-Ortiz 2003). Therefore, it is likely that the origins of these howler monkey hybrid zones are due to paleoecological processes and not to habitat fragmentation. However, howler monkeys are strictly arboreal primates that only descend to the ground to cross canopy gaps or to disperse between fragments (Bicca-Marques and Calegario-Marques 1995; Pozo-Montuy and Serio-Silva 2007), a task strongly compromised when inter-patch distances are longer than 200 m (Mandujano and Estrada 2005). Therefore, it is possible that habitat disturbance and fragmentation may influence the hybridization process in howler monkeys either by isolating their populations and reducing contact between hybridizing species, or by confining individuals of different species within particular fragments and promoting interbreeding. Dias et al. (2013) analyzed habitat configuration in fragmented landscapes both within the hybrid zone in Tabasco Mexico and in nearby areas where only purebred individuals occur. They concluded that hybridization between Mexican howler monkeys is facilitated in fragmented landscapes where there is a larger number of small, though less isolated, fragments. Testing hypothesis regarding the actual role of fragmentation in promoting or preventing hybridization requires the study of syntopic populations in both fragmented and extensive forest. The *A. caraya* × *A. guariba* hybrid zones portrayed here may provide a unique opportunity within primates, with cases of natural hybridization occurring in both highly fragmented areas of Brazil and the mostly pristine Atlantic Forest of Argentina.

5.6.3 *Effect of Hybridization in the Vocal Communication of Hybridizing Species*

One characteristic feature of howler monkeys is their conspicuous, loud vocalizations (Whitehead 1995). Although nonhuman primate vocalizations have long been considered genetically determined, some studies have questioned this assumption based on the existent variation among individuals and populations within taxa (Sun et al. 2011). This question can be addressed by analyzing vocalizations from purebred and hybrid individuals with different levels of admixture in the hybrid zone.

During a study on social behavior in one of the Brazilian hybrid zones, Aguiar (2010) detected that loud vocalizations tended to occur more frequently between conspecific males than during heterospecific interactions (including interactions with hybrids). A similar observation has been reported in Argentina (Holzmann et al. 2012) for syntopic *A. caraya* and *A. guariba*. These observations may support the argument of a genetic basis of vocalizations. However, Aguiar (2010) also observed that one hybrid female modified her vocalizations according to the species that she was interacting with. This plasticity could be either ecologically or genetically determined. An ongoing study of vocalizations integrating genetic, behavioral, and morphological data (Kitchen et al. unpubl. data; see also Kitchen et al. 2015) in the Mexican hybrid zone is starting to provide insights into the influence of genetics on the vocalizations of howler monkeys.

5.6.4 Interaction Between Social Dynamics and Hybridization

Hybrid zones have been considered natural laboratories for the study of the characters and processes leading to divergence and speciation (Hewitt 1988), which include behavioral strategies to acquire mates by the two parental populations and their hybrid offspring. However, despite a continuously growing number of studies dedicated to understanding the social and reproductive dynamics in primates, very little work has been focused on reproductive strategies of individuals within primate hybrid zones (e.g., Bergman and Beehner 2004; Bergman et al. 2008). Hybrid zones provide the opportunity to explore reproductive strategies of individuals with very different genetic backgrounds (both pure and admixed) in the same ecological and social context (Bergman et al. 2008). Ongoing studies on the social dynamics in the Mexican hybrid zone (e.g., Ho et al. 2014) will allow us to evaluate the competitive abilities of hybrid versus purebred individuals. In Brazil, Aguiar (2010) conducted a study on social interactions in two mixed groups composed of pure *A. caraya*, *A. guariba* and putative hybrids. Although the two groups were very different in composition, his analyses suggested that heterospecific associations confer some competitive advantages when facing other groups. He also found that affiliative and sexual interactions mostly included putative hybrids and were less frequent between apparently pure heterospecific individuals. Furthermore, he found that one hybrid female had a higher rank in the group than the putatively purebred *A. caraya* female. Although the sample size in his study is very small, these observations suggest the presence of assortative mating and a possible reproductive advantage in hybrids (Aguiar 2010). Behavioral studies comparing social interactions and dynamics of a larger number of groups with different compositions (*A. caraya* and *A. guariba*, as well as mixed and hybrid groups) within the area of contact between these species may allow the understanding of the interaction between hybridization and social dynamics.

Furthermore, the integrated genetic and behavioral study of primate populations in different hybrid zones can provide important information on the genetic composition of reproductively successful individuals and inform the relative effects of genet-

ics and social dynamics on the overall fitness of hybrid versus purebred individuals. The study of social dynamics in the howler monkey hybrid zones would be especially insightful given the relatively good knowledge of different aspects of the social systems of the hybridizing species, due to a large and growing number of basic studies on social and sexual behavior of these taxa (see Van Belle and Biccamarques 2015). These studies can serve as a basis to conduct comparative observations between purebred and hybrid individuals in the same ecological and social context. There are important differences in social structure and mating systems between the hybridizing taxa. For example, while *A. pigra* has an average group size of ~6.3 individuals (range 2–16) with an adult sex ratio between 0.7 and 1.3 females per male, *A. palliata* has an average group size of ~15 individuals (range 2–45) with an adult sex ratio between 1.2 and 4.2 females per male (Di Fiore et al. 2010). In both species there is bisexual dispersal, but it is reported that *A. pigra* females commonly stay in natal groups (Van Belle et al. 2011) whereas most *A. palliata* females disperse (Glander 1992). Immigration of *A. pigra* females in well-established groups is rarely observed (Brockett et al. 2000), and females aggressively chase away extra-group females (Brockett et al. 2000; Van Belle et al. 2011). In contrast, *A. palliata* females regularly join established groups, first as low-ranking individuals, and gradually become dominant (Glander 1992). In *A. pigra* alpha or “central” males have almost exclusive access to fertile females, whereas “noncentral” males have few or no mating opportunities (Van Belle et al. 2008), but in *A. palliata* mating opportunities among group males are more evenly distributed (Jones and Cortés-Ortiz 1998; Ellsworth 2000; Milton et al. 2009).

These and other differences in social systems between the two parental species likely affect the genetic structure of individuals within the hybrid zone and will enable evaluations of the success of reproductive strategies of pure versus admixed individuals. These studies would require systematic long-term data collection on behavior, demography, and genetics for a large number of groups with distinct compositions within and outside the hybrid zone, using concordant methodologies. Despite the inherent difficulties of maintaining long-term studies given the costs and demands of field work (Strier 2010), the maintenance of long-term research in primate hybrid zones and the comparative studies across primate hybrid systems is critical to develop a holistic understanding of the evolutionary consequences of hybridization in primates.

5.6.5 *Studies of Hybridization in the Genomic Era*

The advent of the newer technologies to sequence entire genomes opens an exciting possibility in the genetic study of primate hybrid zones. Currently, there is a number of primate genome sequencing projects underway and within the next several years it is likely that genome sequence data will become available for most, if not all, primate genera (Bradley and Lawler 2011). The use of a larger number of genetic markers across the genome that characterize parental taxa will dramatically increase

the power and accuracy of detecting admixed individuals. Also, polymorphism of these markers in conjunction with behavioral observations will allow us to establish kin relationships in hybrid populations to evaluate aspects such as individual reproductive success, and the possible effect of kinship in structuring social relationships within a hybrid zone. Furthermore, the understanding of patterns of introgression of different regions of the genome of each of the parental species will potentially enable the identification of genes that contribute to various levels of reproductive isolation, such as those observed in the *A. palliata* × *A. pigra* hybrid system, and the maintenance of species boundaries despite gene flow.

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