Chapter 17

Molecular ecology in neotropical mammals: key aspects for conservation

Bruno H. Saranholi, Carla C. Gestich, Marina E. de Oliveira

Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, 13565-905, São Carlos, SP, Brazil

e-mail: bruno.saranholi@gmail (BHS), carlagestich@gmail.com (CCG), marinaelisao@gmail.com (MEO).

Abstract

Molecular techniques have emerged as powerful tools to study ecological aspects of biodiversity. As a result, the interdisciplinary field of Molecular Ecology was created, combining a wide range of strategies in order to address ecological questions, which may involve molecular species confirmation, species occurrence and distribution, demography estimates, relatedness among individuals, intra and interspecific interactions, dispersal patterns, sex ratio, as well as other ecological information. In this chapter, we provide an overview of how molecular techniques have been contributing to answering ecological questions about neotropical mammals, using various sources of biological samples (including non-invasive sampling) and the most appropriate molecular markers to achieve each objective. Mammals constitute one of the most threatened taxa due to the direct and indirect environmental effects of human activity, and therefore ecological information must be obtained about this group for the proposal of conservation strategies.

Keywords: species monitoring, mitochondrial DNA, microsatellites, sex identification, philopatry

17.1 Introduction

Molecular ecology is a relatively recent interdisciplinary field that has emerged from the advances in molecular techniques which allow researchers to answer ecological questions by comparing genetic information at the individual, species, and community levels. This approach encompasses a broad range of tools aimed at obtaining ecological information such as species confirmation through molecular methods, occurrence and distribution of a given species, demography estimates, relatedness among individuals, inter and intraspecific interactions, dispersal patterns, and sex ratio, among others.

Since it is a DNA-based approach, an investigation in molecular ecology depends on the collection of biological samples for the acquisition of DNA. For mammals, several types of biological samples can be used, such as blood, tissue, skin, hair, and feces (Fig. 17.1). Of these, non-invasive samples, i.e., biological samples which do not require that the animal be captured or handled, such as feces and hair left in the environment (Beja-Pereira et al. 2009), have provided an especially meaningful contribution for many ecological studies of mammals. Feces have been extensively used to assess the DNA of the species that produced them (e.g., Miotto et al. 2007, 2011, 2014; Saranholi et al. 2017, 2022). Because several mammals use their feces for communication and territorial marking (Gorman and Trowbridge 1989), these animals usually defecate on trails or prominent places such as rocks and trunks (Aragona and Setz 2001; Gorman and Trowbridge 1989), which can make the collection of this type of sample easier.

An important second step is the selection of the most appropriate molecular marker to answer the ecological question being investigated. For example, mitochondrial DNA (mtDNA) markers are widely used due to certain important characteristics: rapid evolution rate, high polymorphism (even across short sequences), absence of recombination, and a high number of copies per cell if compared to nuclear DNA (Fig. 17.1; Avise 2012). These characteristics allow mtDNA to be used in species identification even if the samples are degraded, making it an effective strategy when working with samples that contain a small amount of DNA and its integrity is poor (Farrell et al. 2000; Chaves et al. 2012; Rodríguez-Castro et al. 2018). The variability in mitochondrial genes is relatively high across different species, but significantly less among individuals of the same species; thus, mtDNA markers can be used to distinguish between species, even those which are closely related (Avise 2012). The huge amount of mtDNA sequences available in public data repositories, such as the NCBI (National Center for

Biotechnology Information, US Government) or BOLD Systems (Barcode of Life Data System), also favors the use of this type of molecular marker for species identification (Galtier et al. 2009).

Concerning the nuclear portion of the genome, highly informative polymorphic markers such as microsatellites – also known as SSRs (short sequence repeats) or STRs (short tandem repeats) – are of great importance for ecological studies (Fig. 17.1; Selkoe and Toonen 2006). These short sequences, which are comprised of one to six base pairs repeated in tandem (Zane et al. 2002), are widely distributed throughout the genome, displaying high levels of polymorphism and, thus, a multiallelic characteristic (Schlötterer and Tautz 1992; Springer et al. 2001). A high degree of polymorphism among individuals allows us to obtain individual genotypes and assess genetic information at population levels (Broquet et al. 2006; Haag et al. 2010; Rodgers and Janečka 2013; Figueiredo et al. 2015; Maciel et al. 2019). Microsatellites display codominant inheritance, i.e., it is possible to identify the two alleles of a given locus separately (Sunnucks 2000); also, they are considered to be selectively neutral, which means that, usually, no product is encoded at the loci where they occur. Thus, these markers may be used to compare populations in terms of genetic diversity, since they are not affected by evolutionary pressures (Selkoe and Toonen 2006). Microsatellite regions are generally short and, therefore, their PCR products are also short, which makes them possible to be used with non-invasive samples (Beja-Pereira et al. 2009). Furthermore, with the use of a multilocus panel (Broquet et al. 2006; Beja-Pereira et al. 2009), researchers can identify the individual source of each sample, allowing for the determination of its origin when it is too difficult to obtain such information in the field. More recently, due to advances in next-generation sequencing (NGS) technologies, the use of other nuclear markers, such as SNPs (Single Nucleotide Polymorphisms), has been made easier. These molecular markers can be genotyped through NGS techniques (e.g., GBS - Genotyping by Sequencing), revealing up to thousands of SNP loci, which shows great potential for ecological and conservation studies (Fig. 17.1; Morin et al. 2004). Still, despite such potential, SNPs remain little explored in ecological studies of neotropical mammals.

Nowadays, there are at least 6595 extant mammal species worldwide, and a substantial number of them (~25% of all species) is concentrated in the neotropics (Burgin et al. 2018). The neotropical region also harbors several endemic mammal groups, such as caviomorph rodents (e.g., capybaras), xenarthrans (armadillos, anteaters, and sloths), and platyrrhine

monkeys (Patterson and Costa 2012). Mammals are increasingly threatened by human activities such as poaching, illegal trade, and road killing, in addition to those which result in habitat loss and fragmentation (Ripple et al. 2015; Ceia-Hasse et al. 2017). Consequently, it has been estimated that about 80% of all mammal populations have either been lost or are becoming smaller (Ceballos et al. 2017); this process is occurring predominantly in the neotropics and southeast Asia (Ceballos et al. 2017). Most threats are still in place and becoming more severe, but their effects on local populations and the long-term survival of the species are still poorly understood. Therefore, the acquisition of ecological data on mammals is critical to guide practical management strategies for the conservation of these animals, and a molecular ecology approach can offer important contributions to such efforts.

17.2 Molecular species identification

Determining a species occurrence is a primary task for the acquisition of basic ecological information and implementation of conservation practices. Conservation programs are concerned with establishing the precise distribution of a species and monitoring its occurrence through time (Mace et al. 2008; Sofaer et al. 2019). Moreover, it is important to determine the richness and composition of communities to better establish priorities for the protection of natural environments (Jenkins et al. 2015). Species identification also enables us to retrieve other ecological information, such as home range size as well as overlap and selection of habitat, which are usually helpful to guide conservation practices.

17.2.1 Molecular markers for species identification

Mitochondrial genes have been widely used as informative markers for molecular species identification (Fig. 17.2). Some mtDNA regions can be used in DNA barcoding studies, because certain sequence polymorphisms within the mtDNA are unique for each species (Hebert et al. 2003). In the case of non-invasive samples, when excessive exposure to environmental conditions degrades the DNA in the sample, resulting in lower DNA quality, the primers for the molecular markers used to identify the species must be designed to amplify short sequences located in highly informative regions of the mtDNA, also known as mini barcodes (e.g., Chaves et al. 2012). This strategy has allowed for significant advances in species identification based on non-invasive samples. For instance, due to the elusive habits and naturally low densities of carnivores, the detection of individuals from this group is often

difficult, but their territory marking behavior with feces favors the use of a non-invasive sampling approach (e.g., Chaves et al. 2012; Farrel et al. 2000; Saranholi et al. 2017). Various carnivore mtDNA markers have already been identified and evaluated, consisting of regions within the genes for adenosine triphosphate subunit 6 (ATP6), cytochrome oxidase I (COI), cytochrome b (Cyt b), rRNA12S and rRNA16S (Farrell et al. 2000; Chaves et al. 2012; Rodríguez-Castro et al. 2018). For instance, the presence of the *Panthera onca*, a rare and highly endangered species, was molecularly confirmed with basis on fecal samples, after an extensive camera trap effort failed to record this animal in a protected area of the Atlantic Forest biome, in Brazil (Souza et al. 2017); molecular species identification was performed based on the amplification of two mini barcode fragments from the mitochondrial genes ATP6 and Cyt b.

Species identification and monitoring based on fecal samples were also successfully concluded for leporids. Rodrigues et al. (2020) used three mtDNA regions (COI, Cyt b and rRNA16S) to molecularly identify feces from different species of leporids – which would have been challenging if the only information available had been the morphology of the fecal samples, due to the high similarity among them. With this approach, Rodrigues et al. (2020) were able to map the occurrence of the neotropical native *Sylvilagus brasiliensis* and the invasive *Lepus europaeus* throughout an area of approximately 1,500,000 ha in the state of São Paulo, Brazil. This precise identification by molecular fecal analysis represents a powerful tool for effectively monitoring the distribution of both species and supporting future management actions aimed at controlling the growth of the invasive species. Similarly, the community of felids inhabiting a protected area within a tropical rainforest in Mexico was assessed with the molecular identification of hair samples collected from hair snares placed in transects (García-Alaníz et al. 2010). Since an information deficit for carnivore populations in tropical rainforests may be caused by the lack of appropriate, reliable and cost-effective methods, the use of hair-snaring followed by precise molecular identification represents a viable approach for detecting elusive carnivore species (García-Alaníz et al. 2010). The Andean bear (*Tremarctos ornatus*) was also molecularly identified (Cyt b region) from hair and fecal samples collected in the southern areas of its distribution range, leading to the update and expansion of the species range by 150 km in Argentina (Cosse et al. 2014). All these cases highlight the applicability of molecular tools for samples that are not morphologically identifiable, as well as their usefulness in ecological studies and conservation planning.

Despite this being less usual, sequences from nuclear genes can also be applied to species identification. In spite of their lower degree of interspecific divergence and higher homoplasy in relation to mtDNA sequences, nuclear markers are particularly helpful for species identification in cases of hybridization. Combined with mtDNA markers, nuclear markers may help us understand hybridization processes in natural contact zones between congeneric species, or when human activities result in habitat degradation, promoting a non-natural contact between species. In a study of *Leopardus guttulus* and *Leopardus geoffroyi* at their geographic contact zone, Trigo et al. (2013, 2014) used a combination of nuclear markers (ten microsatellite loci, X chromosome-linked regions - PLP1 and BTK, and Y-linked chromosome regions - ZFY and SMCY3) and mtDNA (NADH dehydrogenase subunit 5), to identify hybrid individuals, mostly when the phenotype of a hybrid was indistinguishable from one of the parental species. The association of different molecular markers, in this case, allowed for the construction of a complete scenario of the contact zone between these two species of felids (Trigo et al. 2014). Natural hybridization was also molecularly confirmed in primates, between *Alouatta pigra* and *Alouatta palliata* in Mexico (Cortés-Ortiz et al. 2007). Using microsatellites (eight loci), as well as mtDNA (Cyt b) and Y-chromosome linked genes (SRY gene), the authors were able to trace back the maternal and paternal lineages of hybrid individuals. This approach revealed that only when *A. pigra* females crossed with *A. palliata* males they produce fertile female descendants in the first-generation offspring (F1), whereas their male offspring were infertile (Cortés-Ortiz et al. 2007). Other natural hybridization zones between neotropical mammals (e.g., manatee, Vilaça et al. 2019; camelids, González et al. 2020) were confirmed with the use of molecular tools.

Hybridization is most important when it results from the spread of an invasive species, usually caused by human activity, such as animal trade or habitat disturbance, leading to non-sympatric species being in contact with each other. Among the primates of the *Callithrix* genus, besides causing habitat displacement and engaging in competition for resources (Melo et al. 2020), invasive species threaten native ones due to the possibility of hybridization that produces fertile descendants (Malukiewicz 2019), possibly resulting in the loss of the pure gene pool of the native species. Using the mtDNA control region, Malukiewicz et al. (2014) found different patterns of hybrid formation between anthropogenic and natural zones. In the former, there is a more abrupt removal of the reproductive barrier than in the natural zones, demonstrating that anthropogenic landscape alteration favor hybrids formation.

17.2.2 Biodiversity monitoring through molecular species identification

Sampling and detecting mammal species can be a challenging task, because many of them can be rare, elusive, or found in low-density populations. Traditional mammal surveys have involved setting camera traps and line transects for direct or indirect (i.e., feces, footprints) observation. However, species identification based only on the morphology of the feces, for example, is not an easy task, and it can lead to inaccurate identifications of several mammal groups (leporids: Rodrigues et al. 2020; felids: Farrel et al. 2000; deers: Oliveira et al. 2022). In contrast, the use of molecular identification of biological samples has been employed as an efficient tool for detecting mammal species (e.g., carnivores: Miotto et al. 2014; Saranholi et al. 2017; Souza et al. 2017; Srbek-Araujo et al. 2018; leporids: Rodrigues et al. 2020; bats: Clare et al. 2007; small mammals: Borisenko et al. 2008). For example, the detection of an elusive deer species (*Mazama* spp.) in the Atlantic Forest was conducted by combining camera trap recordings with opportunistic data collection and molecular species identification (Cyt b) of fecal samples (Oliveira et al. 2022). The latter accounted for 72% of the occurrence confirmation data for these deer species, which highlights the potential of this approach for monitoring elusive species. Also, tissue samples from dead animals, e.g., road-killed animals (Balkenhol and Waits 2009; Saranholi et al. 2016), or from forensic apprehensions, e.g., illegally hunted animals (Pun et al. 2009), can also undergo molecular analysis for a very precise species identification.

The molecular species identification of dead specimens is especially important when samples are collected from highly deteriorated carcasses for road ecology studies. The use of DNA barcodes has been proposed as a complementary technique to help understand the impacts of roads on the surrounding biodiversity. For instance, out of a total of 62 vertebrate species molecularly identified (COI gene) among the road-kills of only 25 km of a road crossing an area of Atlantic Forest area in Brazil, 27 were mammal species (Klippel et al. 2015). Molecular tools have also been critical for the identification of small-sized road-killed species (amphibians, reptiles, birds, small mammals), whose carcasses are generally more damaged and degraded than those from large animals (Rodríguez-Castro et al. 2017). In addition to species identification, modeling hotspots where animals are more likely to be road-killed could be useful to elucidate the ability of certain species to move and to explain their relationship with the landscape (Rodríguez-Castro et al. 2017), and this could contribute to guiding mitigation strategies to be implemented along roads (see more in Chapter 18).

The use of DNA barcoding in association with next-generation sequencing technologies has broadened the applicability of species identification through metabarcoding. With this approach, it is possible to recover DNA information pertaining to more than one species from a single biological sample – such as feces, for dietary studies (see below) – or from mixed samples, such as those obtained from environmental DNA (eDNA). Species identification through metabarcoding has greatly enriched eDNA studies that aim to assess a community of species by sampling water (Mena et al. 2021), soil (e.g., Leempoel et al., 2020) and air (e.g., Lynggaard et al. 2022). eDNA has been successfully employed for detecting and monitoring mammals, especially those that are endangered, invasive, or elusive (Bohmann et al. 2014). A similar approach has also been used to identify mammal species with basis on the digestive contents of hematophagous or coprophagous insects, known as iDNA, i.e., the DNA that has been *ingested* by or extracted from *invertebrates* (e.g., Calvignac-Spencer et al. 2013; Lynggaard et al. 2019). eDNA and iDNA have been regarded as powerful tools for rapid biodiversity assessment, constituting a promising approach that is still little explored in neotropical mammal studies (Cristescu and Hebert 2018; Carvalho et al. 2022; see more in Chapter 19).

17.2.3 Molecular identification of prey and diet

The study of diet is another use for the barcoding approach through species identification from feces or digestive content. By identifying which animals or plants are eaten by the target species, researchers can have a better understanding of their feeding habits, the resources at their disposal, prey densities, and potential competition with sympatric species from the same guild (Janecka et al. 2020). For instance, by amplifying a mtDNA (COI) fragment from the genetic material retrieved from guano, Rolfe et al. (2014) compared the diet of two sympatric species of insectivorous bats (*Mormoops blainvillei* and *Pteronotus quadridens*) in Puerto Rico. The authors were, then, able to identify the families and even the genera of the insects consumed, whereas previously they had reached the order level at best, based only on the morphology of prey remains; this reaffirms the greater accuracy of molecular analyses for the species identification. More recently, metabarcoding has been successfully employed to investigate the diets of top predators (e.g., giant otter *Pteronura brasiliensis*, rRNA12S and COI, Quéméré et al. 2021), herbivores (e.g., lowland tapir *Tapirus terrestris*, P6 loop region of the chloroplast trnL intron-UAA, and internal transcribed spacer - ITS gene, Hibert et al. 2013), and small mammals (e.g., *Ctenomys* spp., UAA and ITS, Lopes et al. 2020)

in the neotropics, based on their fecal samples. For instance, a study that aimed to analyze the coexistence and niche partitioning by 25 species of bats from various guilds, in the tropical dry forests of Belize, found no case of complete overlap of the feeding items consumed by those species (Ingala et al. 2021). To determine the list of consumed items, the authors collected fecal DNA samples, and used the metabarcoding approach by amplifying mini barcodes of ingested plants (P6 loop region), arthropods (rRNA16S), and vertebrates (rRNA12S) (See more details in Chapter 19).

17.3 Sample individualization and molecular sex identification

After the species is identified, distinguishing between the individuals within the collected samples and determining each one's sex is also possible with the use of molecular tools. Individualizing samples with the use of molecular markers, that is, determining which individual corresponds to a given sample, represents a significant development for ecological studies, especially those that aim to make demographic estimates, such as density and abundance, but also for studies interested in estimating distribution as well as extension and overlap of home ranges for different individuals (Selkoe and Toonen 2006; Rodgers and Janečka 2013). This approach is especially useful when the sampling method does not involve collecting a biological sample directly from the animal, but rather using traces left in the environment – such as feces, regurgitates and hair. In addition, the knowledge of sex ratios within a population enhances our understanding of the population's demography, and sex identification can be used to investigate behavioral differences between sexes (Rodgers and Janečka 2013). The combination of these basic – but critical – information sets (molecular identification of species, sex, and individualization) can enhance the quality of a species' ecological data.

17.3.1 Molecular markers used in sample individualization

In order to obtain genotypes that differentiate samples at an individual level, the molecular markers used in the individualization of samples must exhibit a high degree of polymorphism, and among the most used and important markers are microsatellites (Fig. 17.2). As they are codominant and display a high degree of polymorphism, the genotypes obtained from them allow us to differentiate individuals with great success, even in the case of related individuals. Furthermore, microsatellites are usually sequences that are short enough to be

amplified by PCR even in samples containing degraded DNA, such as non-invasive samples or the carcass of a road-killed animal. From a microsatellite panel, it is possible to calculate the Probability of Identity (P_{ID}) value, which estimates the likelihood that two unrelated individuals have the same genotype profile by chance (Waits et al. 2001). Low PID values indicate better assignment at individual level.

Other nuclear markers can be used for individual identification. SNPs are promising candidates for that, although these molecular markers are generally biallelic, which could entail a reduction in individualization power when compared with multiallelic molecular markers (e.g., microsatellites). Next-generation sequencing (NGS) technology advancements now make it possible to obtain and genotype hundreds or even thousands of SNP loci, and this extensive genomic coverage enables robust sample individualization (e.g., Buchalski et al. 2022).

17.3.2 Applicability of individualization in ecological studies

The molecular individualization of samples provides a wealth of information for ecological studies. Miotto et al. (2007, 2012) collected feces to identify the minimum number of *Puma concolor* individuals inhabiting conservation units in southeastern Brazil. By conducting systematic sampling over the years, it was possible to recapture some of those individuals, i.e., collecting fecal samples of the same individual several times within the study period allowed for the identification of residents and new individuals inhabiting the area. The same approach was used by Ramalho et al. (2014) to obtain demographic estimates for the *Chrysocyon brachyurus* in a conservation unit of the Cerrado biome, in Brazil. Also using fecal samples in their work, Trinca et al. (2013) determined the number of individuals and the population density of *Lontra longicaudis*, a semi-aquatic mammal, inhabiting an Atlantic Forest area in Brazil. In addition to aiding in the production of demographic estimates, individualization through fecal samples may be helpful to understanding aspects of a species' behavior. For example, by individualizing fecal samples from *Leopardus pardalis* inhabiting Barro Colorado Island, in Panama, Rodgers et al. (2015) found that communal latrines can constitute scent communication centers, where one *L. pardalis* could establish communications with up to fourteen others.

Individualization is also used for estimating genetic parameters of a population, such as genetic diversity, gene flow, and effective population size, since obtaining the genotypes of the individuals is necessary for such studies. Therefore, when using non-invasive samples, or

when the origin of a biological sample is uncertain, molecular individualization is a mandatory step before performing population genetic analyses. By molecularly individualizing fecal samples, several studies have obtained information on effective population size, inbreeding, and relatedness for several neotropical mammals, such as *P. concolor* (Miotto et al. 2011; Saranholi et al. 2017), *T. terrestris* (Saranholi et al. 2022), *Panthera onca* (Wultsch et al. 2016); *C. brachyurus* (Ramalho et al. 2014), *L. longicaudis* (Trinca et al. 2013). All these studies relied on microsatellites for sample individualization, which supports the applicability of this molecular marker even for samples containing degraded DNA.

Despite the potential of SNPs for sample individualization above mentioned, to the best of our knowledge, only two studies have focused on establishing an informative SNP panel for the individualization of samples from a neotropical mammal, thus far. *P. concolor* was investigated in both studies (Fitak et al. 2016; Buchalski et al. 2022), in which 25 and 95 SNP loci were used to differentiate between individuals, respectively.

17.3.3 Molecular sexing

The molecular markers commonly used for sex identification are located in sexual chromosomes. The primers that amplify a segment of the SRY (sex-determining region Y) gene, located in the Y chromosome, are widely used in sex determination for several mammalian species, such as primates (Di Fiore 2005), tapirs (*T. terrestris*, Pelizzon et al. 2017), mustelids (*L. longicaudis*, Trinca et al. 2013) and carnivores (DeCandia et al. 2016). In these case, the successful PCR amplification of the SRY fragment indicates that the analyzed individual is a male. Furthermore, it is not necessary to sequence the amplified fragment, as one can observe its presence or absence directly through agarose gel electrophoresis, which makes this test relatively inexpensive. In other cases, molecular markers present in both X and Y chromosomes can be used. In the amelogenin (AMELX and AMELY) (e.g., felids, Pilgrim et al. 2005) (Fig. 17.3) or zinc-finger (ZFX and ZFY) (e.g., *Tayassu pecari*; Rufo et al. 2015) genes, the existing polymorphisms between the X and Y chromosomes lead to variations in fragment sizes because of nucleotide deletions in the Y chromosome. Thus, two same-sized fragments are amplified for females, resulting in a single visible band on the agarose gel, whereas two fragments of distinct sizes are visible for males, since they possess the X and Y copies of the genes (Fig. 17.3). Particularly for methods based on a single gene, such as the SRY gene, it is important to include the amplification of other genes present in both sexual chromosomes in the same PCR (multiplex PCR), such as the zinc finger gene, as a positive

control, in order to prevent the false identification of males as females, since amplification of the Y chromosome gene may fail in samples with low-quality DNA.

Obtaining sex-related data from individuals can help us to better understand the ecology of the species. For example, in Souza et al. (2017), the presence of *P. onca* in an area where it was believed already extinct was revealed by fecal molecular identification, and the individualization of the samples counted three different individuals; in addition, molecular sexing revealed that two individuals were females, and one male. These results not only confirmed the presence of a rare and elusive species in the area, but also provided more detailed information on the number and sex of the individuals, which can be valuable for monitoring the populations of this threatened felid species. Molecular sexing can also be useful for studying behavioral patterns in mammals. In the studies of Trinca et al. (2013) and Miotto et al. (2014), the female *L. longicaudis* and *P. concolor* individuals, respectively, were considered philopatric, whereas males of both species tended to disperse. In the study on scent communication in communal latrines used by *L. pardalis*, molecular sexing of the fecal samples revealed that males had the potential to communicate with more individuals than females by marking the territory with their feces (Rodgers et al. 2015). Sex information obtained through molecular methods in *Myrmecophaga tridactyla* was used to test the existence of sex-biased dispersal in the species, which was not corroborated by the authors within the studied region (Barragán-Ruiz et al. 2021).

17.4 Behavioral Ecology

The development of molecular techniques has allowed to improve the evaluation of the adaptive value of a certain behavior, and its evolution under different ecological circumstances. The study of relatedness among individuals within a population is of great interest to biologists, from classical geneticists to conservation biologists and molecular ecologists. Dispersal and philopatry are two of the main behaviors that can shape relatedness and other genetic characteristics of populations. Relatedness is central in quantitative genetic studies aimed at investigating the inheritability of a given quantitative trait, the mating system of a wild population, dispersal patterns that shape genetic diversity, and predictions on the best mating pairs in captive breeding programs, among many other applications (Lynch and Walsh 1998; Prugnolle and Meeûs 2002; Jones and Wang 2010; Fienieg and Galbusera 2013). Some of these aspects will be presented in the following sections.

17.4.1 Relatedness and mating systems

In wild populations, the observation of genealogy or pedigree structure is often unfeasible; thus, researchers can only rely on DNA-based methods to estimate relatedness. Since sample individualization is based on genotyping, typically with the use of biparentally inherited and independent nuclear markers such as microsatellites or SNPs, this genetic data can be used to assess relatedness between individuals, groups, or within populations. Methods for relatedness analysis can be divided into two categories, relatedness estimators and assignment of individuals to relationship categories (Blouin 2003). Relatedness estimators calculate the probability of identity by descent (IBD), which is the probability that two alleles at a given locus, one from each individual, are recently descended from a common ancestral allele within a reference population (Blouin 2003). At any locus, two individuals may share zero, one or two alleles that are identical by descent, and the probabilities of these events (also known as k_m, where m is the number of IBD alleles) depend on their true relationship (Table 17.1). For example, the probability that parent and offspring share one allele that is IBD at any locus (k_1) is 1. The estimate takes the form of a range of values usually between -1 and 1 or 0 and 1, depending on how the estimator fits the k probabilities into its algorithm (Milligan 2003). The categories of genealogical relationships between individuals, such as full siblings, parent-offspring, half-siblings, etc., are inferred from the probabilities of alleles being shared (Kalinowski et al. 2006) (Table 17.1). Parentage analysis is a special class of analysis in which one aims to assign an offspring to its true mother and/or father by using likelihood ratios, which compare the probabilities that the observed genotypes are parent-offspring under alternative hypotheses concerning their relationship category (Jones and Ardren 2003; Weir et al. 2006).

There are many relatedness estimators available (e.g., Queller and Goodnight 1989; Lynch and Ritland 1999; Wang 2007; Milligan 2003), and each estimator has its advantages and limitations (van de Casteele et al. 2001; Blouin 2003; Oliehoek et al. 2006). There are several software that implement more than one estimator simultaneously, so the most suitable to the input data may be chosen (Wang 2011). Estimates of relatedness and relationship are strongly affected by the number of loci and the number of genetic marker alleles chosen, the distribution of the alleles within their loci, rates of allelic dropout, presence of null alleles (i.e., alleles that fail to amplify), and allele frequencies within the reference population (Oliehoek et al. 2006; Weir et al. 2006; Wagner et al. 2006). For example, if only two alleles (*i* and *j*) are present in a population for a given locus, then all individuals in this population are either heterozygote (*ij*) or homozygote (either *ii* or *jj*) for these alleles. Considering this locus alone,

if two individuals present the same genotype, it will be impossible to distinguish between alleles that are identical by descent or identical by state (IBS, i.e., same nucleotide sequence, but not necessarily inherited from a common ancestor), and thus relatedness cannot be estimated. However, with the use of more loci, it is possible to study relatedness even in populations that display low genetic diversity, including inbred individuals (Wang 2011). Relatedness between individuals can also be influenced by numerous ecological and behavioral factors, such as their mating system, overlapping of generations (Kopps et al. 2015), sexual selection (Young and Bennett 2013), patterns of dispersal behavior (Prugnolle and Meeûs 2002), breeding success (Amos et al. 2001), kin selection (Aronsson et al. 2020), genetic diversity, bottleneck events (Robinson et al. 2013), and inbreeding avoidance (Cohas et al. 2008).

Population, group or individual kinships are not static throughout space and time (Croft et al. 2021) and understanding all ecological and behavioral factors influencing relatedness is not a trivial task. The case of the vulnerable white-lipped peccary (*T. pecari*) is an interesting example of relatedness being used to study their mating system and the relationship with competition, sex ratio and dispersal behavior (Leite et al. 2018). This species presents no apparent sexual dimorphism (Keuroghlian and Desbiez 2010). A monogamous mating system is usually expected among species without sexual dimorphism and for which the operational sex ratio (the average ratio of sexually active males to receptive females) is not skewed (Clutton-Brock 2007). However, through parentage tests, Leite et al. (2018) observed males and females having offspring with more than one partner, which is consistent with a promiscuous mating system. The authors suggested that the observed pattern could have resulted from intrasexual competition as well as the influence of natural and/or sexual selection for both sexes (Biondo et al. 2011; Leite et al. 2018).

In captive populations of ongoing breeding programs, minimal relatedness between mating pairs is desired in order to maintain genetic variability and avoid the effects of endogamy and genetic drift (Montgomery et al. 1997; Rudnick and Lacy 2008). The endangered black-lion-tamarin (*Leontopithecus chrysopygus*) is an example of how relatedness can be used in ex-situ management. This rare primate is endemic to the Atlantic Forest of the state of São Paulo in southeast Brazil (Kierulff et al. 2008), and only 1600 individuals are estimated to remain in the wild (Rezende et al. 2020). After a few dozen individuals were moved to zoos and conservation facilities, the studbook for black-lion-tamarins was created in 1987 to keep track of the genealogy of captive individuals (Simon 1988). Despite the intense efforts to maintain records on the genealogy of the black-lion-tamarins (as well as other captive

species), this information is often incomplete, and thus molecular methods are useful to estimate the relatedness and relationships among contemporary and founder individuals (Russello and Amato 2004). Ayala-Burbano et al. (2020) analyzed the whole ex-situ population of black-lion-tamarins using microsatellite markers and found an average of two alleles per locus in addition to a high level of relatedness among captive individuals. The authors proposed an integrative approach for the ex-situ conservation of this species, which could be applied to other captive populations as well, conducting analyses of nuclear markers – as presented in this chapter – to monitor expected heterozygosity, individual heterozygosity, allele richness, private alleles, population structure, inbreeding, and relatedness.

For lowland tapirs (*T. terrestris*), a combination of relatedness analysis (relationship categories and relatedness estimators) was used to test the hypothesis that this species exhibits relatedness-based social behaviors (Pinho et al. 2014). In this study, the authors collected non-invasive samples (feces) from an island complex formed by the flooding of an area after the construction of the Balbina hydroelectric dam in central Amazon, Brazil, and they genotyped individuals using five sufficiently informative microsatellite loci to discriminate between individuals. The authors found no statistical difference between distances separating related and unrelated pairs of individuals, concluding that tapirs in this region have no preference for being close to relatives of either sex, which may suggest that both sexes are prone to dispersing. The opposite was found for the neotropical otters (*L. longicaudis*) in the Atlantic Forest in southern Brazil, where the social organization of this species appears to be highly influenced by relatedness, since relatives were usually found in proximity, with this organization driven by female philopatry (Trinca et al. 2013). Otters are assumed to occur at low densities and show an elusive behavior, but they also usually defecate in latrines along the margins of rivers (Kruuk 2006), which facilitates the use of non-invasive sampling for this species. Trinca et al. (2013) employed a non-invasive approach combined with the amplification of ten microsatellite loci to assess demographic parameters, spatial organization and relatedness within this neotropical otter population.

Biologists are frequently limited to a small number of already available microsatellite loci to study relatedness in captive and wild populations. The development of next-generation sequencing has made it possible to identify thousands of single polymorphism nucleotides (SNP), which increases our ability to distinguish between individuals and their relatedness. However, the studies of neotropical mammals still make poor use of this methodology. Although relatedness analysis has many applications, studies would generally benefit from sampling as many individuals as possible within and across populations of interest, as well as

from using a panel of markers that is sufficiently informative (either microsatellites or SNPs) (Pemberton 2008).

17.4.2 Dispersal and philopatry

Dispersal can be defined as "the movement the animal makes from its point of origin to the place where it reproduces or would have reproduced if it had survived and found a mate" (Howard 1960). On the other hand, philopatry is the opposite behavior, and can be defined as "the faithfulness of an individual to its natal and breeding site or group" (Greenwood 1980). Philopatry causes related individuals to remain in proximity, whereas dispersal promotes the geographical separation of related individuals, directly influencing the genetic structure pattern of a species. The clustering of related individuals due to philopatry is found not only in gregarious species, but also in solitary ones (Waser and Jones 1983), which is generally expressed in the form of overlapping home ranges (Ratnayeke et al. 2002; Quaglietta et al. 2013). Due to the differences in constraints and advantages experienced by individuals of each sex, behavior can differ between sexes (Greenwood 1980; Dobson 1982). Dispersal is often sex-asymmetrical, and the tendency for each sex to disperse or remain philopatric has been strongly correlated with the species mating system.

Dispersal is commonly associated with three main causes, which are not mutually exclusive: competition for mates, competition for resources and inbreeding avoidance (Packer 1979; Greenwood 1980; Dobson 1982; Moore and Ali 1984; Pusey 1987). In polygamous mammals, dispersal is generally male-biased, whereas females are prone to philopatry (Greenwood 1980). In this case, males can benefit from dispersal because this is likely to increase their access to females, decrease competition with resident dominant males, and avoid inbreeding with related females. In polygamous species where females are primarily responsible for parental care, not dispersing allows them to take advantage of their knowledge of local resources; when resources are available, they are more likely to share their home ranges with daughters, but they are less likely to allow the permanence of male offspring in order to avoid inbreeding (Waser and Jones 1983; Pusey and Packer 1987; Sandell 1989). In monogamous mammals, dispersal should be equally frequent in both sexes, and parents do not evict either sex, because fathers do not have to compete with their sons for the breeding female, and the breeding of female offspring does not incur any cost to the mother in populations that are not at carrying capacity (Dobson 1982; Liberg and von Schantz 1985). It is important to highlight that there are exceptions to these predictions (e.g., Dechmann et al. 2007; Nagy et al. 2007; Blair and Melnick 2012).

There are two main approaches to inferring sex-biased dispersal from genetic data: population-level analysis and individual-level analysis (Banks and Peakall 2012). The first considers the set of samples (either a population or group of individuals) as the unit of analysis. This approach includes analyses such as F-statistics and assignment tests, which are based on the expected genetic signature of male and female individuals within populations (Goudet et al. 2002). At the individual level, multilocus genotypes of individuals are the units of analysis, for example, when doing correlation (i.e., Mantel test and spatial autocorrelation analysis) and relatedness analyses (Prugnolle and Meeûs 2002; Banks and Peakall 2012). Population genetics methods (e.g., F-statistics and assignment tests) are better explored elsewhere, as in Templeton (2021).

One way of inferring sex-specific dispersal is through spatial autocorrelation analysis. Spatial autocorrelation analysis (Fig. 17.4) is constructed on the basis of two matrices (genetic and geographic distances between individuals). Pairs of individuals are categorized according to classes of distances between them in order to test, at each distance class, if the individuals are more or less genetically distant than what would be expected by chance (no spatial genetic pattern) (Smouse and Peakall 1999). This analysis can be performed for each sex separately in order to assess sex-specific patterns of dispersal (Gour et al. 2013), or with both sexes pooled together to assess the general spatial organization of the population (Shmidt et al. 2016; Wultsch et al. 2016).

Wultsch et al. (2016) assessed the dispersal of pumas (*P. concolor*), jaguars (*P. onca*) and ocelots (*L. pardalis*) in Belize, Central America. The authors opportunistically collected fecal samples from protected and unprotected areas to assess the human impact on the genetic structure of these species. After the species were identified through mitochondrial DNA sequencing, 14 species-specific microsatellites were amplified in order to individualize the samples, and the sex of the individuals was determined through the amplification of two genes that are only present in the Y chromosome. The authors used assignment tests and spatial autocorrelation analysis (SAA) to examine the spatial extent of the genetic structure (Fig. 17.4), modulated by dispersal, and to determine if dispersal was sex-biased in each of the three species, as predicted for other polygamous felids (de Oliveira et al. 2021). Due to the small number of samples, female jaguars and male ocelots were not analyzed separately. Genetic association was shown to occur between the jaguars (mostly males) that were less than 20 km apart from each other. Concerning the male pumas, no spatial autocorrelation was verified across all distance classes, suggesting an absence of spatial structure caused by dispersal; on the other hand, female pumas showed positive autocorrelation up to 23 km apart, indicating

female philopatry. Female ocelots that were less than 83 km apart from one another showed genetic association. These results suggested female philopatry, and also that ocelots could be more successful moving through human-dominated landscapes than the other two species. Jaguars are bigger than pumas, but the latter are known to travel longer distances in their dispersal movements, even when moving through fragmented areas (Stoner et al. 2008), whereas the former typically prefers forested areas (Crawshaw and Quigley 1991). Results suggested subdivisions in the genetic structure of male jaguars, but not in male pumas, likely because jaguars are more sensitive to disturbed areas.

Species differ markedly in their dispersal distances (Whitmee and Orme 2013) and abilities to overcome human disturbances in the landscape. Less mobile species that rely on forest cover may be more affected than generalist and highly mobile carnivores. Groups of reintroduced golden lion tamarins (*Leontopithecus rosalia*) were monitored and the dispersal pattern (distance and sex bias) could be investigated in the Atlantic Forest in southeastern Brazil (Moraes et al. 2018). Hair samples were collected, and 14 microsatellite loci were used to generate individual genotypes. Dispersal potential was assessed with basis on the distance between locations where shared alleles were found, whereas sex bias was assessed through spatial autocorrelation analysis. Golden lion tamarins were found to effectively disperse up to 8 km, but gene flow was high only within a 2 km radius. Authors also observed no sexual bias in the frequency of effective dispersal, which is expected for monogamous mammals, as is the case of golden lion tamarins, although they found evidence of sexual bias in dispersal distances. The absence of sex bias in effective dispersal is relevant for the conservation of the species, because it promotes a higher gene flow and mitigates the effects of reproductive skew in monogamous mating systems.

Although the behavior of dispersal has been strongly correlated to mating, there are exceptions. The greater sac-winged bat (*Saccopteryx bilineata*) in Costa Rica, for instance, has a mating system described as resource-defense polygyny, in which dispersal was found to be female-biased and males form a patrilocal colony structure (Nagy et al. 2007). These results were based on a panel of 11 microsatellites that were used to conduct paternity and relatedness analysis on a colony monitored for eight years. For this species, it was proposed that inbreeding avoidance was the main force driving the dispersal of females, since there was a generational overlap between philopatric fathers and their female offspring. The authors suggested that this behavior evolved from a state of complete offspring dispersal, as both the male and female offspring of different species within the same genus are prone to dispersing.

Local circumstances can also influence the propensity of individuals to disperse or remain philopatric. For instance, changes in the social organization pattern of primates have already been suggested to be the result of anthropogenic impacts (Di Fiore et al. 2009). Oklander and Corach (2013), working with *Alouatta caraya*, used a panel of eleven polymorphic DNA microsatellite markers to estimate kinship and maternity/paternity relations of juvenile and subadult individuals in eleven social groups dwelling in fragmented areas, and seven social groups in a continuous forest, in Argentina. Based on the obtained data, they found that both males and females from the groups living in the continuous forest dispersed, whereas dispersal was male-biased in the groups dwelling in fragmented forests, and this affected the relatedness among individuals within their respective social groups. In the groups dwelling in the continuous forest, adults were not closely related – whereas, in the fragmented forests, most adult females were related (Oklander and Corach 2013). These findings suggest that habitat fragmentation alters the ability of *A. caraya* to disperse, thus increasing the occurrence of inbreeding, which, in the long term, threatens the populations living in modified landscapes (Oklander and Corach 2013). For the guigna (*Leopardus guigna*) from Chile, increased dispersal distances were correlated with increased fragmentation (Napolitano et al. 2015). In Chloé Island (Chile), Napolitano et al. (2015) used a combination of biological samples (blood from captured animals, feces and tissue from road-kills and retaliatory kills) and molecular markers (mitochondrial DNA sequences, 15 microsatellite loci and two sex chromosome genes) to investigate the influence of fragmentation on the genetic diversity, kinship, inbreeding, and dispersal of guigna. The authors utilized a combination of relatedness and spatial autocorrelation analyses to infer on dispersal. In more pristine areas, dispersal was lower, probably because of a greater abundance of resources, whereas in the more fragmented areas dispersal rates were higher, which may reflect a strategy aimed at reducing competition over scarce resources. Therefore, in general, dispersal and philopatry are dynamic processes that can be shaped by the costs and advantages of dispersing or remaining philopatric, and these processes have a significant impact on the genetic structure of populations.

17.5 Concluding remarks

The investigation of ecological questions and the assessment of species with cryptic behaviors have been greatly advanced thanks to the use of molecular tools. Utilizing mitochondrial and nuclear markers, we can describe aspects of biodiversity even when working

with low-quality biological samples. We can obtain valuable data as simple as which species occur within an area and their individualized information, which may be used to infer spatial distribution, individual behavior (e.g., use of space) and interspecific interactions (e.g., territoriality), to more complex relationships concerning individuals and populations (Fig. 17.1). All this information, associated with the ecological data gathered through traditional methods, can be very useful to assess biological patterns and processes, and for implementation of conservation efforts, which are especially needed in the current scenario of biodiversity loss promoted by human activities.

The conservation status of mammals around the world is worrisome. Given the profound impact that humans have had on the environment during the Anthropocene, it is imperative that we understand which ecological processes are being affected, as well as the original states of these processes, which can still be detected in well-preserved areas. The molecular ecology tools can be very helpful in achieving that. However, the application of genetic data for answering ecological questions and supporting strategies for the conservation of neotropical mammals remains underdeveloped or employed with a focus limited to certain groups (Torrez-Flores et al. 2017). Therefore, it is urgent that the number of molecular ecology studies be increased and combined with other disciplines, as to enrich our knowledge of neotropical mammals and strengthen biodiversity conservation efforts.

REFERENCES

Amos W, Worthington Wilmer J, Fullard K, et al (2001) The influence of parental relatedness on reproductive success. Proc R Soc B Biol Sci 268:2021–2027. https://doi.org/10.1098/rspb.2001.1751

Aragona M, Setz EZF (2001). Diet of the maned wolf, *Chrysocyon brachyurus* (Mammalia: Canidae), during wet and dry seasons at Ibitipoca State Park, Brazil. J Zoo 254: 131-136

Aronsson M, Åkesson M, Low M, et al (2020) Resource dispersion and relatedness interact to explain space use in a solitary predator. Oikos 129:1174–1184. https://doi.org/10.1111/oik.07258

Avise, JC (2012). Molecular markers, natural history and evolution. Springer Science & Business Media.

Ayala-Burbano PA, Galetti Junior PM, Wormell D, et al (2020) Studbook and molecular analyses for the endangered black-lion-tamarin; an integrative approach for assessing genetic diversity and driving management in captivity. Sci Rep 10:1–11. https://doi.org/10.1038/s41598-020-63542-2

Balkenhol N, Waits LP (2009) Molecular road ecology: Exploring the potential of genetics for investigating transportation impacts on wildlife. Mol Ecol 18:4151–4164. https://doi.org/10.1111/j.1365-294X.2009.04322.x

Banks SC, Peakall R (2012) Genetic spatial autocorrelation can readily detect sex-biased dispersal. Mol Ecol 21:2092–2105. https://doi.org/10.1111/j.1365-294X.2012.05485.x

Barragán-Ruiz CE, Silva-Santos R, Saranholi BH, et al (2021) Moderate Genetic Diversity and Demographic Reduction in the Threatened Giant Anteater, *Myrmecophaga tridactyla*. Front Genet 12:1–11. https://doi.org/10.3389/fgene.2021.669350

Beja-Pereira A, Oliveira R, Alves PC, et al (2009) Advancing ecological understandings through technological transformations in noninvasive genetics. Mol Ecol Resour 9:1279–1301. https://doi.org/10.1111/j.1755-0998.2009.02699.x

Biondo C, Keuroghlian A, Gongora J, Miyaki CY (2011) Population genetic structure and dispersal in white-lipped peccaries (*Tayassu pecari*) from the Brazilian Pantanal. J Mammal 92:267–274. https://doi.org/10.1644/10-MAMM-A-174.1

Blair ME, Melnick DJ (2012) Genetic evidence for dispersal by both sexes in the Central American Squirrel Monkey, *Saimiri oerstedii citrinellus*. Am J Primatol 74:37–47. https://doi.org/10.1002/ajp.21007

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol Evol 18:503–511. https://doi.org/10.1016/S0169-5347(03)00225-8

Bohmann K, Evans A, Gilbert MTP, et al (2014) Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol Evol 29:358–367. https://doi.org/10.1016/j.tree.2014.04.003

Borisenko A V., Lim BK, Ivanova N V., et al (2008) DNA barcoding in surveys of small mammal communities: A field study in Suriname. Mol Ecol Resour 8:471–479. https://doi.org/10.1111/j.1471-8286.2007.01998.x

Broquet T, Johnson CA, Petit E, et al (2006) Dispersal and genetic structure in the American marten, *Martes americana*. Mol Ecol 15:1689–1697. https://doi.org/10.1111/j.1365-294X.2006.02878.x

Buchalski MR, Sacks BN, Ahrens KD, et al (2022) Development of a 95 SNP panel to individually genotype mountain lions (*Puma concolor*) for microfluidic and other genotyping platforms. Conserv Genet Resour 14:147–150. https://doi.org/10.1007/s12686-022-01255-6

Burgin CJ, Colella JP, Kahn PL, Upham NS (2018) How many species of mammals are there? J Mammal 99:1–14. https://doi.org/10.1093/jmammal/gyx147

Calvignac-Spencer S, Merkel K, Kutzner N, et al (2013) Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. Mol Ecol 22:915–924. https://doi.org/10.1111/mec.12183

Carvalho CS, de Oliveira ME, Rodriguez-Castro KG, et al (2022) Efficiency of eDNA and iDNA in assessing vertebrate diversity and its abundance. Mol Ecol Resour 22:1262–1273. https://doi.org/10.1111/1755-0998.13543

Ceballos G, Ehrlich PR, Dirzo R (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc Natl Acad Sci U S A 114:E6089–E6096. https://doi.org/10.1073/pnas.1704949114

Ceia-Hasse A, Borda-de-Água L, Grilo C, Pereira HM (2017) Global exposure of carnivores to roads. Glob Ecol Biogeogr 26:592–600. https://doi.org/10.1111/geb.12564

Chaves PB, Graeff VG, Lion MB, et al (2012) DNA barcoding meets molecular scatology: Short mtDNA sequences for standardized species assignment of carnivore noninvasive samples. Mol Ecol Resour 12:18–35. https://doi.org/10.1111/j.1755-0998.2011.03056.x

Clare EL, Lim BK, Engstrom MD, et al (2007) DNA barcoding of Neotropical bats: Species identification and discovery within Guyana: Barcoding. Mol Ecol Notes 7:184–190. https://doi.org/10.1111/j.1471-8286.2006.01657.x

Clutton-Brock T (2007) Sexual selection in males and females. Science (80-) 318:1882–1885. https://doi.org/10.1126/science.1133311

Cohas A, Yoccoz NG, Bonenfant C, et al (2008) The genetic similarity between pair members influences the frequency of extrapair paternity in alpine marmots. Anim Behav 76:87–95. https://doi.org/10.1016/j.anbehav.2008.01.012

Cortés-Ortiz L, Duda TF, Canales-Espinosa D, et al (2007) Hybridization in large-bodied new world primates. Genetics 176:2421–2425. https://doi.org/10.1534/genetics.107.074278

Cosse M, Sachetti JFDM, Mannise N, Acosta M (2014) Genetic evidence confirms presence of andean bears in Argentina. Ursus 25:163–171. https://doi.org/10.2192/URSUS-D-14-00020.1

Crawshaw PG, Quigley HB (1991) Jaguar spacing, activity and habitat use in a seasonally flooded environment in Brazil. J Zool 223:357–370. https://doi.org/10.1111/j.1469-7998.1991.tb04770.x

Cristescu ME, Hebert PDN (2018) Uses and misuses of environmental DNA in biodiversity science and conservation. Annu Rev Ecol Evol Syst 49:209–230. https://doi.org/10.1146/annurev-ecolsys-110617-062306

Croft DP, Weiss MN, Nielsen MLK, et al (2021) Kinship dynamics: Patterns and consequences of changes in local relatedness. Proc R Soc B Biol Sci 288:. https://doi.org/10.1098/rspb.2021.1129

de Oliveira ME, Saranholi BH, Dirzo R, Galetti Jr PM (2021) A review of philopatry and dispersal in felids living in an anthropised world. Mammal Res 1–13. https://doi.org/10.1111/mam.12275

DeCandia A, Gaughran S, Caragiulo A, Amato G (2016) A novel molecular method for noninvasive sex identification of order Carnivora. Conserv Genet Resour 8:119–121. https://doi.org/10.1007/s12686-016-0525-z

Dechmann DKN, Kalko EKV, Kerth G (2007) All-offspring dispersal in a tropical mammal with resource defense polygyny. Behav Ecol Sociobiol 61:1219–1228. https://doi.org/10.1007/s00265-007-0352-z

Di Fiore A (2005) A rapid genetic method for sex assignment in non-human primates. Conserv Genet 6:1053–1058. https://doi.org/10.1007/s10592-005-9086-5

Di Fiore A, Link A, Christopher A, Spehar SN (2009) Dispersal Patterns in Sympatric Woolly and Spider Monkeys: Integrating Molecular and Observational Data. Behaviour 146:437–470

Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. Anim Behav 30:1183–1192. https://doi.org/10.1016/S0003-3472(82)80209-1

Farrell LE, Roman J, Sunquist ME (2000) Dietary separation of sympatric carnivores identified by molecular analysis of scats. Mol Ecol 9:1583–1590. https://doi.org/10.1046/j.1365-294x.2000.01037.x

Fienieg E, Galbusera P (2013) The use and integration of molecular DNA information in conservation breeding programmes: a review. J Zoo Aquarium Res 1:44–51

Figueiredo MG, Cervini M, Rodrigues FP, et al (2015) Lack of Population Genetic Structuring in Ocelots (*Leopardus pardalis*) in a Fragmented Landscape. Diversity 7:295–306. https://doi.org/10.3390/d7030295

Fitak RR, Naidu A, Thompson RW, Culver M (2016) A New Panel of SNP Markers for the Individual Identification of North American Pumas. J Fish Wildl Manag 7:13–27. https://doi.org/10.3996/112014-JFWM-080

Galtier N, Nabholz B, Glémin S, Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Mol Ecol 18:4541–4550. https://doi.org/10.1111/j.1365-294X.2009.04380.x

García-Alaníz N, Naranjo EJ, Mallory FF (2010) Hair-snares: A non-invasive method for monitoring felid populations in the Selva Lacandona, Mexico. Trop Conserv Sci 3:403–411. https://doi.org/10.1177/194008291000300405

González BA, Agapito AM, Novoa-Muñoz F, et al (2020) Utility of genetic variation in coat color genes to distinguish wild, domestic and hybrid South American camelids for forensic and judicial applications. Forensic Sci Int Genet 45:102226

Gorman ML., Trowbridge BJ (1989). The role of odor in the social lives of carnivores. In Carnivore behavior, ecology, and evolution (pp. 57-88). Springer, Boston, MA.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Mol Ecol 11:1103–1114. https://doi.org/10.1046/j.1365-294X.2002.01496.x

Gour DS, Bhagavatula J, Bhavanishankar M, et al (2013) Philopatry and Dispersal Patterns in Tiger (*Panthera tigris*). PLoS One 8:e66956. https://doi.org/10.1371/journal.pone.0066956

Greenwood PJ (1980) Mating systems, philopatry and optimal dispersal in birds and mammals. Anim Behav 28:1140–1162. https://doi.org/http://dx.doi.org/10.1016/S0003-3472(80)80103-5

Haag T, Santos AS, Sana DA, et al (2010) The effect of habitat fragmentation on the genetic structure of a top predator: Loss of diversity and high differentiation among remnant populations of Atlantic Forest jaguars (*Panthera onca*). Mol Ecol 19:4906–4921. https://doi.org/10.1111/j.1365-294X.2010.04856.x

Hebert PDN, Cywinska A, Ball SL, Jeremy R (2003) Biological identifications through DNA barcodes. 313–321. https://doi.org/10.1098/rspb.2002.2218

Hibert F, Taberlet P, Chave J, et al (2013) Unveiling the Diet of Elusive Rainforest Herbivores in Next Generation Sequencing Era? The Tapir as a Case Study. PLoS One 8. https://doi.org/10.1371/journal.pone.0060799

Howard WE (1960) Innate and Environmental Dispersal of Individual Vertebrates. Am Midl Nat 63:152–161

Ingala MR, Simmons NB, Wultsch C, et al (2021) Molecular diet analysis of neotropical bats based on fecal DNA metabarcoding. Ecol Evol 11:7474–7491. https://doi.org/10.1002/ece3.7579

Janečka JE, Hacker C, Broderick J, et al (2020) Noninvasive genetics and genomics shed light on the status, phylogeography, and evolution of the elusive snow leopard. In: Conservation Genetics In Mammals. Springer, pp 83–120

Jenkins CN, Alves MAS, Uezu A, Vale MM (2015) Patterns of vertebrate diversity and protection in Brazil. PLoS One 10:1–13. https://doi.org/10.1371/journal.pone.0145064

Jones AG, Ardren WR (2003) Methods of parentage analysis in natural populations. Mol Ecol 12:2511–2523. https://doi.org/10.1046/j.1365-294X.2003.01928.x

Jones OR, Wang J (2010) Molecular marker-based pedigrees for animal conservation biologists. Anim Conserv 13:26–34. https://doi.org/10.1111/j.1469-1795.2009.00324.x

Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: A computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes 6:576–579. https://doi.org/10.1111/j.1471-8286.2006.01256.x

Keuroghlian A, Desbiez ALJ (2010) Biometric and age estimation of live peccaries in the Southern Pantanal, Brazil. Suiform Sound 9:24–35

Kierulff MCM, Rylands AB, Mendes SL, de Oliveira MM (2008) *Leontopithecus chrysopygus*. IUCN Red List of Threatened Species, Version 2011.2.

Klippel AH, Oliveira P V., Britto KB, et al (2015) Using DNA barcodes to identify road-killed animals in two atlantic forest nature reserves, Brazil. PLoS One 10:1–15. https://doi.org/10.1371/journal.pone.0134877

Kopps AM, Kang J, Sherwin WB, Palsbøll PJ (2015) How well do molecular and pedigree relatedness correspond, in populations with diverse mating systems, and various types and quantities of molecular and demographic data? G3 Genes, Genomes, Genet 5:1815–1826. https://doi.org/10.1534/g3.115.019323

Kruuk H. 2006. Otters: ecology, behavior and conservation. Oxford, Oxford University Press

Leempoel K, Hebert T, Hadly EA (2020) A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. Proc R Soc B Biol Sci 287. https://doi.org/10.1098/rspb.2019.2353

Leite DA, Keuroghlian A, Rufo DA, et al (2018) Genetic evidence of promiscuity in a mammal without apparent sexual dimorphism, the white-lipped peccary (*Tayassu pecari*). Mamm Biol 92:111–114. https://doi.org/10.1016/j.mambio.2018.05.005

Liberg O, von Schantz T (1985) Sex-Biased Philopatry and Dispersal in Birds and Mammals: The Oedipus Hypothesis. Am Nat 126:129–135. https://doi.org/10.1086/284402

Lopes CM, De Barba M, Boyer F, et al (2020) Ecological specialization and niche overlap of subterranean rodents inferred from DNA metabarcoding diet analysis. Mol Ecol 29:3144–3154. https://doi.org/10.1111/mec.15549

Lynch M, Walsh B (1998). Genetics and Analysis of Quantitative Traits. Sinauer, Sunderland.

Lynch M, Ritland K (1999) Estimation of pairewise relatedness with molecular markers. Genetics 152:1753–1766

Lynggaard C, Bertelsen MF, Jensen C V., et al (2022) Airborne environmental DNA for terrestrial vertebrate community monitoring. Curr Biol 32:701-707.e5. https://doi.org/10.1016/j.cub.2021.12.014

Lynggaard C, Nielsen M, Santos-Bay L, et al (2019) Vertebrate diversity revealed by metabarcoding of bulk arthropod samples from tropical forests. Environ DNA 1:329–341. https://doi.org/10.1002/edn3.34

Mace GM, Collar NJ, Gaston KJ, et al (2008) Quantification of extinction risk: IUCN's system for classifying threatened species. Conserv Biol 22:1424–1442. https://doi.org/10.1111/j.1523-1739.2008.01044.x

Maciel FG, Rufo DA, Keuroghlian A, et al (2019) Genetic diversity and population structure of white-lipped peccaries (*Tayassu pecari*) in the Pantanal, Cerrado and Atlantic Forest from Brazil. Mamm Biol 95:85–92. https://doi.org/10.1016/j.mambio.2019.03.001

Malukiewicz J (2019) A Review of Experimental, Natural, and Anthropogenic Hybridization in Callithrix Marmosets. Int J Primatol 40:72–98. https://doi.org/10.1007/s10764-018-0068-0

Malukiewicz J, Boere V, Fuzessy LF, et al (2014) Hybridization effects and genetic diversity of the common and black-tufted marmoset (*Callithrix jacchus* and *Callithrix penicillata*) mitochondrial control region. Am J Phys Anthropol 155:522–536. https://doi.org/10.1002/ajpa.22605

Melo F, Bicca-Marques J, Ferraz DS, Jerusalinsky L, Mittermeier RA, Oliveira LC, Port-Carvalho M, Ruiz-Miranda CR, Valença Montenegro M, da Cunha R, do Valle RR (2020) *Callithrix aurita* (amended version of 2019 assessment). The IUCN Red List of Threatened Species 2020: e.T3570A166617776.

Mena JL, Yagui H, Tejeda V, et al (2021) Environmental DNA metabarcoding as a useful tool for evaluating terrestrial mammal diversity in tropical forests. Ecol Appl 31:1–13. https://doi.org/10.1002/eap.2335

Milligan BG (2003) Maximum likelihood estimation of relatedness. Genetics 163:1153–1167. https://doi.org/10.1086/302112

Miotto RA, Cervini M, Begotti RA, Galetti Jr PM (2012) Monitoring a puma (*Puma concolor*) population in a fragmented landscape in southeast Brazil. Biotropica 44:98–104

Miotto RA, Cervini M, Figueiredo MG, et al (2011) Genetic diversity and population structure of pumas (*Puma concolor*) in southeastern Brazil: Implications for conservation in a human-dominated landscape. Conserv Genet 12:1447–1455. https://doi.org/10.1007/s10592-011-0243-8

Miotto RA, Cervini M, Kajin M, et al (2014) Estimating puma *Puma concolor* population size in a human-disturbed landscape in Brazil, using DNA mark-recapture data. Oryx 48:250–257. https://doi.org/10.1017/S0030605312000841

Miotto RA, Rodrigues FP, Ciocheti G, et al (2007) Determination of the Minimum Population Size of Pumas (*Puma concolor*) Through Fecal DNA Analysis in Two Protected Cerrado Areas in the Brazilian Southeast. Biotropica 39:647–654. https://doi.org/10.1111/j.1744-7429.2007.00315.x

Montgomery ME, Ballou JD, Nurthen RK, et al (1997) Minimizing kinship in captive breeding programs. Zoo Biol 16:377–389. https://doi.org/10.1002/(SICI)1098-2361(1997)16:5<377::AID-ZOO1>3.0.CO;2-7

Moore J, Ali R (1984) Are dispersal and inbreeding avoidance related? Anim Behav 32:94–112. https://doi.org/10.1016/S0003-3472(84)80328-0

Moraes AM, Ruiz-Miranda CR, Galetti PM, et al (2018) Landscape resistance influences effective dispersal of endangered golden lion tamarins within the Atlantic Forest. Biol Conserv 224:178–187. https://doi.org/10.1016/j.biocon.2018.05.023

Morin PA, Luikart G, Wayne RK (2004) SNPs in ecology, evolution and conservation. Trends Ecol Evol 19:208–216. https://doi.org/10.1016/j.tree.2004.01.009

Nagy M, Heckel G, Voigt CC, Mayer F (2007) Female-biased dispersal and patrilocal kin groups in a mammal with resource-defence polygyny. Proc R Soc B Biol Sci 274:3019–3025. https://doi.org/10.1098/rspb.2007.1008

Napolitano C, Díaz D, Sanderson J, et al (2015) Reduced genetic diversity and increased dispersal in guigna (*Leopardus guigna*) in Chilean fragmented landscapes. J Hered 106:522–536. https://doi.org/10.1093/jhered/esv025

Oklander L, Corach D (2013). Kinship and dispersal patterns in *Alouatta caraya* inhabiting continuous and fragmented habitats of Argentina. In: Marsh L, Chapman C (ed) Primates in Fragments. Kinship and dispersal patterns in *Alouatta caraya* inhabiting continuous and fragmented habitats of Argentina. Springer, New York, NY p 399-412

Oliveira ML, Grotta-Netto F, de Faria Peres PH, et al (2022) Elusive deer occurrences at the Atlantic Forest: 20 years of surveys. Mammal Res 67:51–59. https://doi.org/10.1007/s13364-021-00604-4

Oliehoek PA, Windig JJ, Van Arendonk JAM, Bijma P (2006) Estimating relatedness between individuals in general populations with a focus on their use in conservation programs. Genetics 173:483–496. https://doi.org/10.1534/genetics.105.049940

Packer C (1979) Inter-troop transfer and inbreeding avoidance in Papio anubis. Anim Behav 27:1–36. https://doi.org/10.1016/0003-3472(79)90126-X

Patterson BD, Costa LP (2012). Bones, clones, and biomes: the history and geography of recent neotropical mammals. University of Chicago Press.

Pelizzon C, da Silva Carvalho C, Caballero S, et al (2017) Sex identification of the extant mega mammal, the lowland tapir, *Tapirus terrestris* (Tapiridae, Mammalia), by means of molecular markers: new outlook for non-invasive samples. Conserv Genet Resour 9:17–19. https://doi.org/10.1007/s12686-016-0607-y

Pemberton JM (2008) Wild pedigrees: The way forward. Proc R Soc B Biol Sci 275:613–621. https://doi.org/10.1098/rspb.2007.1531

Pilgrim KL, Mckelvey KS, Riddle AE, Schwartz MK (2005) Felid sex identification based on noninvasive genetic samples. Mol Ecol Notes 5:60–61. https://doi.org/10.1111/j.1471-8286.2004.00831.x

Pinho GM, Gonçalves Da Silva A, Hrbek T, et al (2014) Kinship and social behavior of lowland tapirs (*Tapirus terrestris*) in a central Amazon landscape. PLoS One 9. https://doi.org/10.1371/journal.pone.0092507

Prugnolle F, Meeûs T De (2002) Inferring sex-biased dispersal from population genetic tools: a review. Heredity 16:161–165. https://doi.org/10.1038/sj/hdy/6800060

Pun KM, Albrecht C, Castella V, Fumagalli L (2009) Species identification in mammals from mixed biological samples based on mitochondrial DNA control region length polymorphism. Electrophoresis 30:1008–1014. https://doi.org/10.1002/elps.200800365

Pusey AE (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. Tree 2:295–299. https://doi.org/10.1016/0169-5347(87)90081-4

Pusey AE, Packer C (1987) The evolution of sex-biased dispersal in lions. Behaviour 101:275–310. https://doi.org/10.1163/156853987X00026

Quaglietta L, Fonseca VC, Hájková P, et al (2013) Fine-scale population genetic structure and short-range sex-biased dispersal in a solitary carnivore, Lutra lutra. J Mammal 94:561–571. https://doi.org/10.1644/12-MAMM-A-171.1

Queller DC, Goodnight KF (1989) Estimating Relatedness Using Genetic Markers. Evolution (N Y) 43:258. https://doi.org/10.2307/2409206

Quéméré E, Aucourd M, Troispoux V, et al (2021) Unraveling the dietary diversity of Neotropical top predators using scat DNA metabarcoding: A case study on the elusive Giant Otter. Environ DNA 3:889–900. https://doi.org/10.1002/edn3.195

Ramalho F do P, Miotto RA, Martins N, Galetti PM (2014) Maned wolf (*Chrysocyon brachyurus*) minimum population size and genetic diversity in a Cerrado protected

area of southeastern Brazil revealed by fecal DNA analysis. Mammalia 78:465–472. https://doi.org/10.1515/mammalia-2013-0109

Ratnayeke S, Tuskan GA, Pelton MR (2002) Genetic relatedness and female spatial organization in a solitary carnivore, the racoon, *Procyon lotor*. Mol Ecol 11:1115–1124

Rezende, G., Knogge, C., Passos, F., Ludwig, G., Oliveira, L.C., Jerusalinsky, L. & Mittermeier, R.A. 2020. *Leontopithecus chrysopygus.* The IUCN Red List of Threatened Species 2020: e.T11505A17935400. https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T11505A17935400.en

Ripple WJ, Newsome TM, Wolf C, et al (2015) Collapse of the world's largest herbivores. Sci Adv 1. https://doi.org/10.1126/sciadv.1400103

Robinson SP, Simmons LW, Kennington WJ (2013) Estimating relatedness and inbreeding using molecular markers and pedigrees: The effect of demographic history. Mol Ecol 22:5779–5792. https://doi.org/10.1111/mec.12529

Rodgers TW, Giacalone J, Heske EJ, et al (2015) Communal latrines act as potentially important communication centers in ocelots *Leopardus pardalis*. Mamm Biol 80:380–384. https://doi.org/10.1016/j.mambio.2015.05.004

Rodgers TW, Janečka JE (2013) Applications and techniques for non-invasive faecal genetics research in felid conservation. Eur J Wildl Res 59:1–16. https://doi.org/10.1007/s10344-012-0675-6

Rodrigues NT, Saranholi BH, Angeloni TA, et al (2020) DNA mini-barcoding of leporids using noninvasive fecal DNA samples and its significance for monitoring an invasive species. Ecol Evol 10:5219–5225. https://doi.org/10.1002/ece3.5863

Rodríguez-Castro KG, Ciocheti G, Ribeiro JW, et al (2017) Using DNA barcode to relate landscape attributes to small vertebrate roadkill. Biodivers Conserv 26:1161–1178. https://doi.org/10.1007/s10531-017-1291-2

Rodríguez-Castro KG, Saranholi BH, Bataglia L, et al (2018) Molecular species identification of scat samples of South American felids and canids. Conserv Genet Resour 0:3. https://doi.org/10.1007/s12686-018-1048-6

Rolfe AK, Kurta A, Clemans DL (2014) Species-level analysis of diets of two mormoopid bats from Puerto Rico. J Mammal 95:587–596. https://doi.org/10.1644/13-MAMM-A-190

Rudnick JA, Lacy RC (2008) The impact of assumptions about founder relationships on the effectiveness of captive breeding strategies. Conserv Genet 9:1439–1450. https://doi.org/10.1007/s10592-007-9472-2

Rufo DA, Keuroghlian A, Miyaki CY, Biondo C (2015) Sex identification of white-lipped peccary (*Tayassu pecari*) by multiplex PCR with ZFX and ZFY specific primers and cross-amplification in collared peccaries (*Pecari tajacu*). Suiform Sound 13:29–33

Russello MA, Amato G (2004) Ex situ population management in the absence of pedigree information. Mol Ecol 13:2829–2840. https://doi.org/10.1111/j.1365-294X.2004.02266.x

Sandell M (1989) The mating tactics and spacing patterns of solitary carnivores. In: Gittleman JL (ed) Carnivore Behavior, Ecology and Evolution. Chapman & Hall, London, UK, pp 164–182

Saranholi BH, Bergel MM, Ruffino PHP, et al (2016) Roadkill hotspots in a protected area of Cerrado in Brazil: planning actions to conservation. Rev MVZ Córdiba 21:5441–5448

Saranholi BH, Chávez-Congrains K, Galetti PM (2017) Evidence of recent fine-scale population structuring in South American Puma concolor. Diversity 9. https://doi.org/10.3390/d9040044

Saranholi BH, Sanches A, Moreira-Ramírez JF, et al (2022) Long-term persistence of the large mammal lowland tapir is at risk in the largest Atlantic forest corridor. Perspect Ecol Conserv. https://doi.org/10.1016/j.pecon.2022.02.002

Schlotterer C and Tautz D (1992). Slippage synthesis of simple sequence DNA. Nucleic acids research 20(2):211-211.

Schmidt K, Davoli F, Kowalczyk R, Randi E (2016) Does kinship affect spatial organization in a small and isolated population of a solitary felid: the Eurasian lynx? Integr Zool 11:334–349. https://doi.org/10.1111/1749-4877.12182

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Simon F (1988). Comitê Internacional de Preservação e Manejo do Mico-leão-Preto. In: (ed. Kleiman, DG; Rylands, AB. (Orgs.) Micos leões: biologia e conservação. Fundação Parque Zoológico de São Paulo, São Paulo.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity (Edinb) 82:561–573. https://doi.org/10.1038/sj.hdy.6885180

Sofaer HR, Jarnevich CS, Pearse IS, et al (2019) Development and Delivery of Species Distribution Models to Inform Decision-Making. Bioscience 69:544–557. https://doi.org/10.1093/biosci/biz045

Souza ASM de C, Saranholi BH, Crawshaw Jr. PG, et al (2017) Re-discovering jaguar in remaining coastal Atlantic Forest in southeastern Brazil by non-invasive DNA analysis. Biota Neotrop 17. https://doi.org/10.1590/1676-0611-bn-2017-0358

Springer MS, DeBry RW, Douady C, et al (2001) Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. Mol Biol Evol 18:132–143. https://doi.org/10.1093/oxfordjournals.molbev.a003787

Srbek-Araujo AC, Haag T, Chiarello AG, et al (2018) Worrisome isolation: Noninvasive genetic analyses shed light on the critical status of a remnant jaguar population. J Mammal 99:397–407. https://doi.org/10.1093/jmammal/gyy007

Stoner DC, Rieth WR, Wolfe ML, et al (2008) Long-distance dispersal of a female cougar in a basin and range landscape. J Wildl Manage 72:933–939. https://doi.org/10.2193/2007-219

Sunnucks P (2000) Efficient genetic markers for population biology. Tree 15:199–203

Templeton AR (2021). Population genetics and microevolutionary theory. John Wiley & Sons.

Torres-Florez, JP, Johnson WE, Nery MF, Eizirik E, Oliveira-Miranda MA, Galetti Jr P M (2018). The coming of age of conservation genetics in Latin America: what has been achieved and what needs to be done. Conserv Genet 19:1-15.

Trigo TC, Schneider A, De Oliveira TG, et al (2013) Molecular data reveal complex hybridization and a cryptic species of Neotropical wild cat. Curr Biol 23:2528–2533. https://doi.org/10.1016/j.cub.2013.10.046

Trigo TC, Tirelli FP, De Freitas TRO, Eizirik E (2014) Comparative assessment of genetic and morphological variation at an extensive hybrid zone between two wild cats in southern Brazil. PLoS One 9. https://doi.org/10.1371/journal.pone.0108469

Trinca CS, Jaeger CF, Eizirik E (2013) Molecular ecology of the neotropical otter (Lontra longicaudis): Non-invasive sampling yields insights into local population dynamics. Biol J Linn Soc 109:932–948. https://doi.org/10.1111/bij.12077

van de Casteele T, Galbusera P, Matthysen E (2001) A comparison of microsatellite-based pairwise relatedness estimators. Mol Ecol 10:1539–1549. https://doi.org/10.1046/j.1365-294X.2001.01288.x

Vilaça ST, Lima CS, Mazzoni CJ, et al (2019) Manatee genomics supports a special conservation area along the Guianas coastline under the influence of the Amazon River plume. Estuar Coast Shelf Sci 226:106286. https://doi.org/10.1016/j.ecss.2019.106286

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10(1): 249-256.

Wagner AP, Creel S, Kalinowski ST (2006) Estimating relatedness and relationships using microsatellite loci with null alleles. Heredity 97:336–345. https://doi.org/10.1038/sj.hdy.6800865

Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet Res 89:135–153. https://doi.org/10.1017/S0016672307008798

Wang J (2011) Coancestry: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Waser PM, Jones WT (1983) Natal philopatry among solitary mammals. Q Rev Biol 58:355–390

Weir BS, Anderson AD, Hepler AB (2006) Genetic relatedness analysis: Modern data and new challenges. Nat Rev Genet 7:771–780. https://doi.org/10.1038/nrg1960

Whitmee S, Orme CDL (2013) Predicting dispersal distance in mammals: A trait-based approach. J Anim Ecol 82:211–221. https://doi.org/10.1111/j.1365-2656.2012.02030.x

Wultsch C, Waits LP, Kelly MJ (2016) A comparative analysis of genetic diversity and structure in Jaguars (*Panthera Onca*), Pumas (*Puma Concolo*r), And Ocelots (*Leopardus pardalis*) in Fragmented Landscapes of a Critical Mesoamerican Linkage Zone. PLoS One 11:1–30. https://doi.org/10.1371/journal.pone.0151043

Young AJ, Bennett NC (2013) Intra-sexual selection in cooperative mammals and birds: Why are females not bigger and better armed. Philos Trans R Soc B Biol Sci 368:25–30. https://doi.org/10.1098/rstb.2013.0075

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: A review. Mol Ecol 11:1–16. https://doi.org/10.1046/j.0962-1083.2001.01418.x

Table 17.1. Probabilities k_m that two individuals share *m* alleles (zero, one or two) which are identical by descent, given their relationship.

Relationship	k_0	k_1	k_2
Parent-offspring	0	1	0
Full-siblings	0.25	0.5	0.25
Half-siblings/grandchild-grandparent/niece or nephew-uncle or aunt	0.5	0.5	0
First cousins	0.75	0.25	0
Unrelated	1	0	0

Figure captions

Fig. 17.1. Overview of the main steps involved in molecular ecology studies, from sampling type, broad questions, molecular markers, and ecological aspects possible to be assessed by molecular tools.

Fig. 17.2. Steps involved in molecular species identification and individualization based on mtDNA sequences and microsatellite genotyping, respectively.

Fig. 17.3. Molecular sexing of fecal samples from *Puma concolor* in agarose gel 3% based on the amelogenin gene region polymorphism (Pilgrim et al. 2005). Female individuals are represented by a single band in the agarose gel, because the amelogenin gene has the same size in both X chromosomes. Male individuals are represented by two bands in the agarose gel, because there is a deletion of 20 base pairs (bp) in the Y chromosome. L – Ladder (1 Kb Plus DNA, Invitrogen); F – Female; M – Male. Gene sizes: 214 bp in X chromosomes and 194 bp in Y chromosome.

Fig. 17.4. Example of a spatial autocorrelogram of a species where individuals that are nearby (up to 10 km apart) are more genetically similar than expected, which could be an indication of philopatry or restricted dispersal. Blue lines connect the autocorrelation values of each distance class. Dashed red lines represent the upper and lower limits of the null distribution. Confidence error bars (usually 95%) are shown in black. Asterisks indicate significant spatial

autocorrelation values (usually considering a p-value of <0.05). Arrow indicates the intercept with x-axis.