

The Characterization of Genetic Chimerism in *Callithrix kuhlii* (Wied's Black
Tufted- Ear Marmoset): Implications for Genomic Conflict and the Evolution of
Cooperative Breeding

by

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Under the Supervision of Professors Guillermo Ortí and Jeffrey A. French

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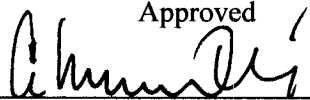
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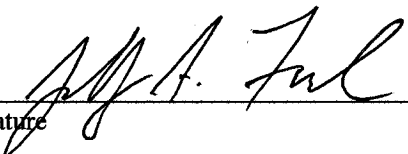
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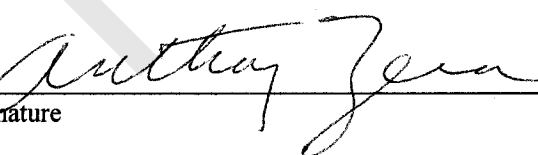
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Tufted- Ear Marmoset): Implications for Genomic Conflict and the Evolution of
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Corinna N. Ross, Ph.D.

University of Nebraska, 2005

Advisers: Guillermo Ortí and Jeffrey A. French

Marmosets are cooperative breeding primates that produce fraternal twins whose placentas fuse during embryonic development allowing genetic exchange via blood flow between the twins. This genetic exchange causes infants to be chimeras, having tissues made up of cells which are derived from self and sibling lineages. The degree of genetic exchange and the tissues affected have not been well studied in this group. For this study five microsatellite markers were used to assess the extent and distribution of genetic exchange in *Callithrix kuhlii* between thirty-six sets of twins with known parentage. The inheritance of markers was traced across twin sets and throughout the tissues. All of the tissue types sampled were found to be chimeric in a number of individuals. Tissues derived from the hematopoietic system including liver, blood and spleen were found to differ significantly in their rate of chimerism from all other tissues. However, the ratio of chimeric cells within each tissue type varied greatly between individuals. Five of the fifteen family groups analyzed were found to have at least one parent that transmitted their sibling alleles to their

offspring. Further, while other female mammalian chimeras have been shown to exhibit sterility as a result of chimerism, chimeric female marmosets were shown to not only maintain fertility, but also exhibit similar rates of chimerism as males in the population. The presence of genetic chimerism and the influence of genetic conflict on the evolution of cooperative breeding in callitrichids were also explored. The presence of sibling alleles throughout the body including the germ-line cells increased values of relatedness between alloparents and between multiply sired offspring and their fathers. The study of this unique system provides insights into genomic conflict in a vertebrate, the evolution of alloparental care in callitrichids, and provides the first analysis of the influences of genetic chimerism on the sociality of a vertebrate species.

PREVIEW

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INTRODUCTION

Individuality, Genetic Heterogeneity and Genetic Chimerism

What defines an individual? An individual has previously been defined as possessing a unique and simultaneous physiological unity and autonomy, genetic uniqueness, and genetic homogeneity (Santelices, 1999; Pineda-Krch & Lehtila, 2004). A growing body of literature suggests that organisms once assumed to possess a genetically homogenous make-up, actually show a great deal of heterogeneity within their genome (Stern, 1968; Grosberg & Strattman, 1998; Haig, 1999b; 2002; Sommerfeldt et al., 2003; Pineda-Krch & Lehtila, 2004; Hutchings & Booth, 2004; Pannell & Eppley, 2004). Genetic heterogeneity may be a result of genetic mosaicism or genetic chimerism (Pineda-Krch & Lehtila, 2004).

Mosaicism is a condition in which genetic heterogeneity is a result of differentiation failures during development. Rather than developing with homogeneous gene products throughout, the cells differ due to mutation or recombination failure. Insects have been noted to occasionally develop as a mosaic of sexual traits, for example a honeybee with half female characters and half male characters (Stern, 1968). However, this developmental failure is not isolated to insects, and has been noted in a number of vertebrates including birds (Agate et al., 2003). Mosaicism is commonly due to somatic or germ-line mutation (Pineda-Krch & Lehtila, 2004). While these mutations are rare and potentially deleterious to the individual, they often have very little sustainability in the population. Somatic

mutations can not be inherited, and gametic mutations occur at such low frequencies that there is a low probability of inheritance (Pineda-Krch & Lehtila, 2004).

Genetic chimerism occurs when an organism contains a mixture of its own DNA and the DNA of another individual (McLaren, 1976). The term was originally used to describe any species that appeared to be an unusual mix of organisms, such as the chimera fish (appears to be part rabbit, part rat, and part fish), or the Greek mythological creature the Chimaera. The term chimera is now used to describe an organism that results from a fusion or mingling of two genetically distinct genomes. However, there are few organisms that frequently form true natural chimeras, resulting from a mixture of cells from different individuals. Chimerism may be common for fungi (Fahselt, 1996), plants (Edwards et al., 1990), and invertebrate animals (Gill et al., 1995). For example, the best studied invertebrate chimera is the social amoebae (*Dictyostelium discoideum*). Social amoebae are solitary and reproduce via budding until environmental changes cue them to form an aggregate. The aggregate multicellular organism can be made up of multiple clones each with a different genetic makeup, thus the aggregate multicellular organism is a genetic chimera (Queller et al, 2003).

While chimerism may be common in certain species of invertebrates, chimerism in vertebrates and mammals is extremely rare. Naturally occurring genetic chimerism has been noted in cats, cattle, callitrichids (marmosets and tamarins), and humans (McLaren, 1976). Chimerism has also been artificially induced in lab mice to examine autoimmune function and other biomedical functions (McLaren, 1976).

The most documented type of genetic chimerism in mammals is hematopoietic chimerism, in which blood forming stem cells are exchanged across fusions of the placentas of developing mammalian twins. Hematopoietic stem cells (HSC) are responsible for the development of blood and immune products. HSC's are extremely mobile stem cells that are among the first stem cells to differentiate and then migrate through a number of organ tissues throughout fetal development. Thus, HSC's are the most likely cells to migrate across a placental fusion between siblings. HSC's are thought to develop in the yolk sac a few days after fertilization in most mammals. The HSC's then migrate to the liver, where the majority of blood and immunity production occurs during fetal development. HSC's also migrate to the spleen and thymus during fetal development. Prior to birth the HSC's migrate to the bone marrow which is responsible for blood production in adults (deBruijn et al., 2000). Hematopoietic chimerism occurs when the migrating HSC's are able to migrate from one individual to another. The resulting developing tissues, known as hematopoietic tissues: liver, spleen, thymus, bone marrow and blood, contain cells derived from the stem cells of the individual (self) and their twin (sibling).

Mammalian cases of chimerism tend to be associated with negative impacts for the individuals involved. Individuals are typically adversely affected with symptoms that may include sterility (McLaren, 1976). Female cattle which are chimeric with their male twin are called freemartins. In these animals the female is sterile due to altered physiology and endocrine status (McLaren, 1976). The presence of XY cells in female cattle causes a failure in the formation of the female's reproductive tract. While the formation of freemartins is a rare occurrence, the

resulting sterility of the female is found at such regularity that typically agriculturists will cull a female calf that is born with a male twin without assessing chimerism or determining if the female is truly sterile (McLaren, 1976).

Reported cases of genetic chimerism in humans include women who were sterile for unknown reasons; genetic tests subsequently indicated that their blood cells contained X and Y chromosomes in addition to the expected cells containing two X chromosomes. Further testing revealed that the women were twins with male siblings that died in-utero following placental exchange of genetic information, rendering the women genetic chimeras (Bird et al., 1982). Recent studies of humans have revealed another type of chimerism known as microchimerism (Artlett, 2005). This occurs when the cells of a developing infant cross the placental barrier and enter the maternal blood stream. The migrating stem cells from the fetus then may invade the maternal tissues and begin derivation in her organs. The presence of microchimerism has been associated with increased autoimmune dysfunction such as systemic sclerosis. However, microchimeric tissues may be able to stave off cancer causing cells to a higher degree than normal cells (Artlett, 2005).

The ability of stem cells to differentiate in tissues other than the tissues of the hematopoietic system, as shown by the presence of microchimeric tissues, suggests that naturally occurring chimerism in mammals may involve more tissues than simply the hematopoietic system as was previously believed. Studies examining the presence of chimeric tissue outside of the hematopoietic system in naturally occurring mammalian chimeras have been inconclusive (Gengozian et al., 1964; 1980). If chimerism involves the germ-line of the organism, the chances of it being inherited

may be as common as the self genome of the individual (Strassman & Queller, 2004). This potential for inheritance may induce a conflict within the individual.

The presence of intraorganismal genetic heterogeneity, as caused by genetic chimerism, may lead to conflict between differing cell lineages within a single individual. The costs and benefits of genetic heterogeneity and the potential for intragenomic conflict in an individual have been difficult to discern. For most organisms examined to date, the aggregation of multiple genomes results in increased size of the organism, which for most organisms constitutes a benefit (plant: Edwards et al., 1990; invertebrates: Gill et al., 1995). However, potential costs include cell parasitism, where one cell type sequesters resources such as gamete production (Pineda-Krch & Lehtila, 2004). In the case of the social amoebae, *Dictyostelium discoideum*, the benefit of forming an aggregate is an increased size, which allows them to migrate farther in search of a good reproductive site. An example of the potential conflict within a chimera is the production of a cheating genome (Strassman & Queller, 2004; Queller et al., 2003). The social amoeba aggregates and then produces stalks that hold the developing spores above the substrate; the cells in the stalk do not produce spores. Several cheating mutations have been detected in which these clones invest less in the formation of the stalk, and in turn have higher spore production. Thus, the non-cheating genome is at a significant disadvantage if it forms an aggregate with a cheating genome (Strassman & Queller, 2004). An additional cost for chimeric amoebae is that aggregates of mixed clones move less distance than do those made out of pure clones, possibly due to the genomic conflict within (Strassman & Queller, 2004).

Genomic conflict in individuals with genetic heterogeneity has been identified as a possible evolutionary mechanism influencing behavioral and developmental traits. Genomic conflict may lead to an increased rate of evolution of sex-specific reproductive traits (Rinkevich, 2004; Spencer, 2000; Haig, 2002; Day & Bonduriansky, 2004). Conflict within an organism may influence the development of kin recognition mechanisms, specifically how do organisms identify an individual and determine relatedness to a chimeric individual (Haig, 1999; 199b). If germ-line cells are chimeric in the organism this may influence social behavior due to altered patterns of relatedness between the individuals (Rinkevich, 2004; Haig, 2002; Rice & Holland, 1997; Pineda-Krch & Lehtila, 2004). To date most studies examining genomic conflict and its effects on the evolution of social characteristics have mainly been theoretical, with very little empirical data. Due to the rareness of chimerism in mammals, the evolutionary implications of the possession of intraorganismal genetic heterogeneity have not been examined in detail.

Callitrichid Biology and Social Behavior

While chimerism is thought to be rare among most mammalian species, chimerism among callitrichids is the rule rather than the exception. Callitrichids, commonly known as marmosets and tamarins, are small Neotropical primates native to the rainforests of Brazil. This group of primates was described as a taxonomic family (Callitrichidae) based on morphological and ecological characters such as twinning and small body size, and included four genera and fifty species

(Mittermeier, 1988). Recent molecular data places callitrichids within a new family Cebidae, and maintains the monophyletic group as a subfamily Callitrichinae (Schneider et al., 2001). Callitrichids are among the smallest of the New World primates weighing 250-600 grams, they are often brightly colored monkeys with little to no sexual dimorphism (Fleagle, 1999; Kinzey, 1997; Rylands et al., 1993). Callitrichids are distinguished from other primates by several characters: all digits except the hallux have claws rather than flattened nails; they possess triangular upper molars; a V shaped mandible; are typically diurnal; lack prehensile tails; and their dental formula is 2/2, 1/1, 3/3, 2/2 (Rylands et al., 1993).

Based on karyotypic evidence, Gengozian et al., (1980) estimated that up to 95% of pregnancies result in the birth of chimeric *Callithrix jacchus*. Callitrichids are considered obligate fraternal twinning (Hershkovitz, 1975). Female callitrichids give birth to fraternal twins after a five-month gestational period. At birth, the twins often weigh as much as 20% of their mother's normal body weight. This high energetic demand on the mother is further compounded by the fact that, as early as two weeks after giving birth, the female ovulates and may become pregnant with the next set of twins. Callitrichids do not exhibit lactational amenorrhea, or reduced fertility during lactation (Kleiman, 1977; Goldizen, 1987; Mittermeier, 1988; Price, 1992; Digby & Barreto, 1993; Digby, 1995a; Digby, 1995b; Nievergelt & Martin, 1999). Even in cases of singleton births, the singletons are often chimeric due to the spontaneous abortion and re-absorption of one twin during development (Wislocki, 1932; Gengozian et al., 1980; Windle et al., 1999). Although karyotypic analysis revealed evidence of chimeric cells in tissues derived from the bone marrow system (HSC's)

including blood, spleen, and liver (Benirschke et al., 1962), no conclusive evidence confirms chimerism in the germ-line or somatic tissues aside from hematopoietic tissues (Gengozian et al., 1980; Ford & Evans, 1977).

Genetic chimerism, if pervasive, may explain genetic homogenization among callitrichid fraternal twins and account for low genetic variation within populations. Studies using molecular techniques have found remarkably low levels of variation in callitrichids using common genetic markers. A study of 47 allozyme loci from 171 captive and wild individuals representing three species of tamarins in the genus *Leontopithecus* found an average percentage of polymorphic loci (p) of $P=0.03$ and an average mean heterozygosity (H) of $H=0.01$ (Forman et al., 1986). Another allozyme study of 20 loci comparing natural populations of seven species of *Saguinus* and *Callithrix* revealed few polymorphic loci ($P=0.05-0.25$) and low estimates of heterozygosity ($H=0.01-0.06$) (Dixon et al., 1988; 1992). The MHC immunoglobulin complex of marmosets was analyzed for variation. No polymorphism among individuals of wild caught *Saguinus oedipus* was detected by immunoprecipitations screening. Sequencing of MHC class I genes yielded high nucleotide similarity in coding regions (92.5-96.8%) in comparisons among wild caught individual of *S. oedipus* and *C. jacchus* (Watkins et al., 1991). The control region of mitochondrial DNA was used to evaluate relatedness between populations of wild *Callithrix jacchus* (Faulkes et al., 2003). Fifty-nine individuals from two field sites in northeastern Brazil were sequenced for 1112 base pairs of mtDNA. The study revealed genetic structuring of haplotypes between social groups and between populations. Matrilineal examination revealed that in the majority of cases the breeding pair was

from different maternal lineages, suggesting incest avoidance in the breeding groups. Although a number of unique haplotypes were identified, there was very little divergence between them, genetic distances ranged from 0.09 to 1.99%. A recent set of studies using hair samples of 40 wild *Callithrix jacchus* individuals reported variability at thirteen microsatellite loci; nine loci were species specific and four were human specific primer sets (Nievergelt et al., 1998; 2000). Eleven of the thirteen loci were polymorphic with 2-6 alleles/ locus (average of 3.1) and observed heterozygosity ranging from 0.05-0.66. The exclusion probability for an offspring with an alleged parent was 90.7% and each of the 40 individuals was reported as having a unique multi-locus genotype for hair samples. Finally, fecal samples were genotyped for twelve microsatellite loci to assess paternity and group relatedness in *Saguinus mystax*, the moustached tamarin (Huck et al., 2005). Wild populations of *S. mystax* have been described as polyandrous, with behavioral evidence of breeding females mating with multiple males. Genotype evidence suggested that the majority of infants within each group (67-100%) belonged to the same father. In one group there was evidence that multiple paternity occurred in one twin set, suggesting true polyandry for this group. Although these studies noted more genetic diversity than previous studies, this variation is lower than that found in other primates (Nievergelt et al., 1998; 2000; Faulkes et al., 2003; Huck et al., 2005). Low genetic diversity, especially in MHC class I genes, might reduce the potential rejection and the likelihood of damage to individual tissues of twins sharing a placenta (Watkins et al., 1991).