Journal of the American Association for Laboratory Animal Science
Copyright 2014
by the American Association for Laboratory Animal Science

Vol 53, No 1
January 2014
Pages 7–11

Twinning and Survivorship of Captive Common Marmosets (*Callithrix jacchus*) and Cotton-Top Tamarins (*Saguinus oedipus*)

Joshua M Ward, Alexander M Buslov, and Eric J Vallender*

Here we present the results of a demographic analysis of 25 y (1985 to 2010) of common marmoset (*Callithrix jacchus*) and cotton-top tamarin (*Saguinus oedipus*) records from the New England Primate Research Center. Summaries of longevity and survivorship are analyzed by birth-type category (including singletons, twins, triplets, and quadruplets) and sex. In addition, a brief evolutionary review is presented. Surrogates of hematopoietic chimerism, twinning, and reproductive output are explored in a large number of animals to help decipher the potential effects of chimerism on life history in marmosets and tamarins. In addition to exploring chimerism through demographic data, multiple-birth frequency and survivorship are compared between species. New World primates can make ideal translational models for disease and behavioral research across multiple disciplines. A better understanding of their reproductive success and survivorship in captivity helps develop these nonhuman primate models, their role in aging research, and understanding of their behavioral ecology. This mission is likely to only increase in its importance to biomedical research due to both the sequencing of the marmoset genome and the growing demand for alternatives to Old World primate models.

Here we review demographic and life history data collected on common marmoset (*Callithrix jacchus*) and cotton-top tamarin (*Saguinus oedipus*) colonies housed at the New England Primate Research Center between 1985 and 2010. Information was gathered from a large database of 2753 *C. jacchus* and 2212 *S. oedipus* whose births were recorded over the 25 y. The cotton-top tamarin colony was established coincident with the founding of the center in the mid1960s. Initially used as a model system for biomedical research, the cotton-top tamarin was placed on the endangered species list in 1977, and invasive research was ended. The marmoset colony at our institution was established in late 1976 as an alternative model system, and attention in the tamarin colony turned to developing a better understanding of cotton-top tamarin health and reproduction. Several studies reflecting on this time discussed the factors influencing infant mortality and the veterinary practices to best minimize rejection.[11,12] This current work extends these studies to include animals born during the subsequent years and the entirety of the life histories of both marmosets and tamarins.

Population Description

New World primates (platyrrhines) diverged from Old World primates (catarrhines) approximately 43 million years ago, with the subfamily Callitrichinae, including *C. jacchus* and *S. oedipus*, emerging roughly 15 million years ago.[19] Nonhuman primates have long been used as animal models of human disease, in part because of their close evolutionary, genetic, and physiologic relationships with humans. New World nonhuman primates, in particular, have been valued as balancing both this evolutionary proximity and practical husbandry and logistical constraints. *C. jacchus* and *S. oedipus* have high reproductive efficiency and reach sexual maturity as early as 12 to 13 mo, typically producing litters after a gestational length of 143 d.[31] These species also have the shortest average lifespans of any primate used in biomedical research, and these characteristics, combined with successes in modern husbandry, can provide large populations of adult callitrichids, including a large number of older primates. *C. jacchus*, in particular, has been shown to parallel many human aging-associated pathologies, such as arthritis.[29] Overall, marmosets and tamarins are particularly well suited for exploring the relationship between health and aging.

Callitrichids are notable for their natural propensities for both multiple births and genetic chimerism. Chimeras, although rare among mammals, are the norm in *C. jacchus* and *S. oedipus*.[3,7] In early development, blastocysts of twins fuse, giving rise to a single shared placenta.[8,36] This placental anastomosis results in the exchange of genetically distinct hematopoietic precursor cells during embryonic development, the process by which *C. jacchus* and *S. oedipus* offspring can become natural bone-marrow chimeras.[2,3] Here we have examined a large number of callitrichids to explore potential relationships between proxies of chimerism, such as multiple births, and variation in reproductive output to help decipher the potential effects of chimerism on life history in *C. jacchus* and *S. oedipus*.

Multiple Births and Infant Survivorship

Callitrichids are characterized by their natural twinning. Singleton, twin, triplet, and quadruplet births occurred in both *C. jacchus* and *S. oedipus*. The 1219 marmoset litters produced 2753 offpring; 1.6% (20 litters) were quadruplets, 14.9% (182) singletons, 37.5% (457) triplets, and 45.9% (560) twins (Table 1). Of the 1157 litters of tamarins, which produced 2212 offspring, 0.3% (3 litters) were quadruplets, 25.1% (290) singletons, 15.7%

Received: 03 May 2013. Revision requested: 28 May 2013. Accepted: 11 Jul 2013.
New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, Massachusetts.
**Corresponding author. Email: eric_vallender@hms.harvard.edu*

Table 1. Infant mortality associated with litter size in marmosets and tamarins

		Singletons		Twins		Triplets		Quadruplets	
	Total	No.	%	No.	%	No.	%	No.	%
Marmosets (*Callithrix jacchus*)									
Stillborn	415	49	26.9	159	14.2	182	13.3	25	31.3
Liveborn (< 60 d)	874	43	23.6	227	20.3	566	41.3	38	47.5
Liveborn (≥ 60 d)	1464	90	49.5	734	65.5	623	45.4	17	21.3
Total no. of animals	2753	182	100.0	1120	100.0	1371	100.0	80	100.0
Total no. of litters	1219	182	14.9	560	45.9	457	37.5	20	1.6
Tamarins (*Saguinus oedipus*)									
Stillborn	441	64	22.1	249	18.3	119	21.8	9	75.0
Liveborn (< 60 d)	893	81	27.9	546	40.0	263	48.2	3	25.0
Liveborn (≥ 60 d)	878	145	50.0	569	41.7	164	30.0	0	0.0
Total no. of animals	2212	290	100.0	1364	100.0	546	100.0	12	100.0
Total no. of litters	1157	290	25.1	682	58.9	182	15.7	3	0.3

For each litter size, percentages of animals stillborn, liveborn but dying nonexperimentally before 60 d (< 60 d,) and liveborn living at least 60 d (≥ 60 d) are shown.

(182) triplets, and 58.9% (682) twins (Table 1). The differences in litter sizes between the 2 species were significant ($\chi^2_{[3]}$ = 166.11, $P < 0.0001$), with an average litter size of 2.26 for *C. jacchus* compared with 1.91 for *S. oedipus*. These values are in line with previously published estimates, which consistently find marmoset (*Callithrix* spp.) litters to be larger than tamarin (*Saguinus* spp.) litters in captivity.[9,10,30,35] All infants of both species were either parent-reared (no siblings were present in the cage during their tenure) or family-reared (litters were present in the cage during their tenure). Infants of both species were left with dams unless atypical veterinary intervention was required (that is, due to parental abuse, neglect, health condition). Animal housing from 1985 to 2010 complied with appropriate regulatory standards.

In addition, significant correlation was present between infant mortality and litter size (Table 1). In marmosets, twins and triplets were significantly ($\chi^2_{[3]} = 40.45$, $P < 0.0001$) more likely to be liveborn than singletons or quadruplets. In tamarins, this trend was much less pronounced with twins only modestly showing greater livebirth rates ($\chi^2_{[3]} = 27.22$, $P < 0.0001$; excluding quadruplets, $\chi^2_{[2]} = 4.35$, $P \approx 0.11$). Higher frequencies of stillbirths in singletons have been previously reported,[30] although a satisfactory explanation has not been offered. Embryonic and fetal loss have been documented in marmosets,[35] and these prenatal factors may be correlated. Indeed, genetic studies of chimerism in singletons have demonstrated the presence of adsorbed siblings.[24,28]

Litter size has been associated with early infant mortality. Like other primates, but unlike large-littered mammals, New World nonhuman primates possess only a single pair of nipples, suggesting an evolutionary adaptation to smaller litter sizes. Furthermore, singleton and twin litters are observed much more commonly in the wild as compared with captive settings.[25,35] This has traditionally been ascribed to more readily available sources of food, reduced threats of predation and traumatic events, lower incidence of infectious disease, and access to husbandry services and veterinary care.[25] Early survival rates are strongly correlated with litter size with both marmosets and tamarins, showing singletons and twins more likely to survive to 60 d than are triplets (marmosets: $\chi^2_{[2]} = 132.81$, $P < 0.0001$; tamarins: $\chi^2_{[2]} = 41.47$, $P < 0.0001$). However, as with stillborns compared with live births, marmosets seem more able to handle multiple offspring than are tamarins. The difference in early survival occurs mostly in the first week to 10 d (Figure 1 A and B; marmosets: Mantel–Cox test $\chi^2_{[3]} = 169.4$, $P < 0.0001$; tamarins: Mantel–Cox test $\chi^2_{[3]} = 22.92$, $P < 0.0001$).

Longevity and Adult Mortality

The demographic data collected from the veterinary records at the New England Primate Research Center and presented here contributes to what is known about the relationship between birth type and long-term survivorship. As previously noted, most mortality among the species occurs in the first 2 mo of life. Marmosets surviving to that point had a median life expectancy of 4.0 y, whereas the tamarin median life expectancy was 7.2 y. Previous studies have reported maximum lifespans of marmosets in captivity to be 15 or 16 y.[23,31] Marmosets are often considered to be 'aged' at 8 y, with few animals surviving beyond 13 y.[23] In our marmoset colony, 12% of those animals living after 60 d lived to 8 y, and 1.5% lived to 13 y. The oldest marmoset at the facility was 14.5 y old. Comparable data for cotton-top tamarins is sparse, but previous studies reported mean life expectancy of 8 y for animals living at least 1 y with some animals living into their 20s.[13] Female fertility reduces significantly by 12 y and by 17 y.[32] In the tamarin colony at our institution, the oldest animal lived 24.5 y, and 8% of animals lived to 17 y. Furthermore, there is no significant difference in life expectancy for either species between males and females once they reach 60 d of age (Figure 2). In addition, no sex-associated difference was observed in animals dying within the first 2 mo, although many of these were unsexed. Ultimately, however, sex ratios between male and female marmosets and tamarins were even and stable, regardless of age class.

The most notable finding involves the relationship between litter size and long-term survival. Unlike in infant mortality rates, both marmosets and tamarins from larger litters (that is, triplets compared with twins) show longer life expectancy. Once an animal has survived to 60 d, the median life expectancy in marmosets was 3.5 y for twins compared with 4.7 y for triplets; in tamarins, this was 5 y compared with 6 y, respectively. Comparison of survival curves reveals this difference to be significant (Mantel–Cox test $\chi^2_{[1]} = 5.338$, $P < 0.05$) in marmosets (Figure 3); in tamarins, the trend holds but is not significant (Mantel–Cox test $\chi^2_{[1]} = 0.215$, not significant). Although the exact cause of death is not readily extracted or construed for these animals, it should be noted that all animals in this analysis died of natural causes.

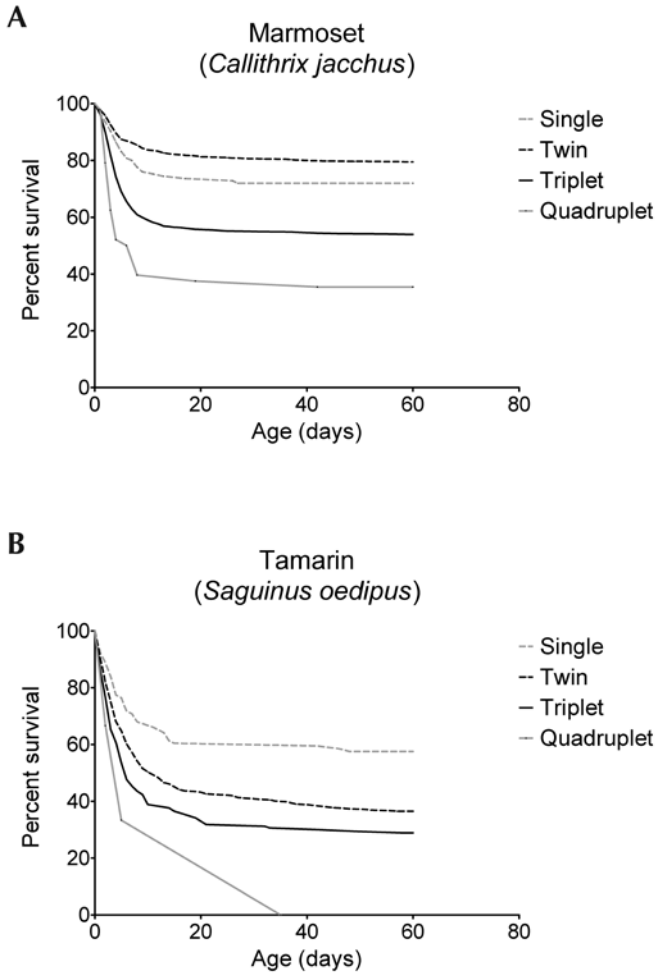

Figure 1. Survival curves for the first 60 d according to litter size in (A) marmosets and (B) tamarins.

Figure 2. Survival curves after 60 d for animals with deaths due to natural causes according to sex in (A) marmosets and (B) tamarins.

Role of Chimerism in Life History

Potential driving forces behind the evolution of hematopoietic chimerism in callitrichids is not clear. The presence of 2 or more genomic lineages within a single subject may influence behavioral and developmental characteristics, ranging from kin recognition to parental care.[5,22,26] Such characteristics may shed light on the evolutionary benefits natural chimeras hold. Yet it is perhaps important to note that chimerism is a derived trait in callitrichids. Costs linked with natural chimerism have been reported to include somatic selection, germ-cell parasitism, as well as associations with multiple diseases.[17,21,27] Human chimeras specifically have been associated with disease and germ-cell parasitism.[1,6,15,16,18,20]

Although cause and effect cannot be readily disassociated, it is notable that coincident with chimerism in callitrichids, there has been a significant reduction in MHC diversity.[33,34] Whether chimerism is an adaptive response to increase adaptive immunity or whether a reduction in MHC diversity is a consequence of allotolerance required by chimerism, there appears to be a relationship between chimerism and susceptibility to infectious disease. MHC gene products are codominantly expressed, and it has been long suggested that the immune systems of heterozygous subjects are able to interact with a larger variety of pathogens, thus gaining a survival advantage over homozygotes.[33,34] One implication is that animals with increased chimerism may be more likely to express a more varied adaptive immunity repertoire.

In the colonies at the New England Primate Research Center, animals coming from larger litters (triplets) show longer life expectancy once infancy has passed 60 d. It is tempting to hypothesize that this association reflects decreased susceptibility to infectious disease. Indeed, one study of cotton-top tamarins bred in captivity found that infectious disease was the leading cause of death in adult animals.[13] It is interesting to note that the effect of litter size was not observed to affect life expectancy in another large study;[10] however, the difference in survival probability between twins and triplets is still dominated by early life effects that are difficult to dissociate from trends in older animals.

This effect is particularly noteworthy as the fundamental balance is addressed. It is certain that litter size strongly affects infant mortality. This association has been repeatedly demonstrated in captivity and is perhaps even more extreme in wild populations.[25] Yet if these data hold, they suggest that tamarins and marmosets from larger litters may have an advantage in adulthood. This analysis is complicated, however, by variability in the causes of mortality. In environments where infectious disease is the greatest threat, there may be an advantage to higher levels of chimerism, but if the greatest threat is trauma, then perhaps it is difficult to envision a benefit. In addition, chimerism may exacerbate a susceptibility to enteritis, although again causation compared with correlation is not clear.[4,14,28]

Conclusions

The hypotheses given here on chimerism's potential effect on health rely on 3 primary assumptions. First, we assume that enough diversity exists in callitrichid populations for in-

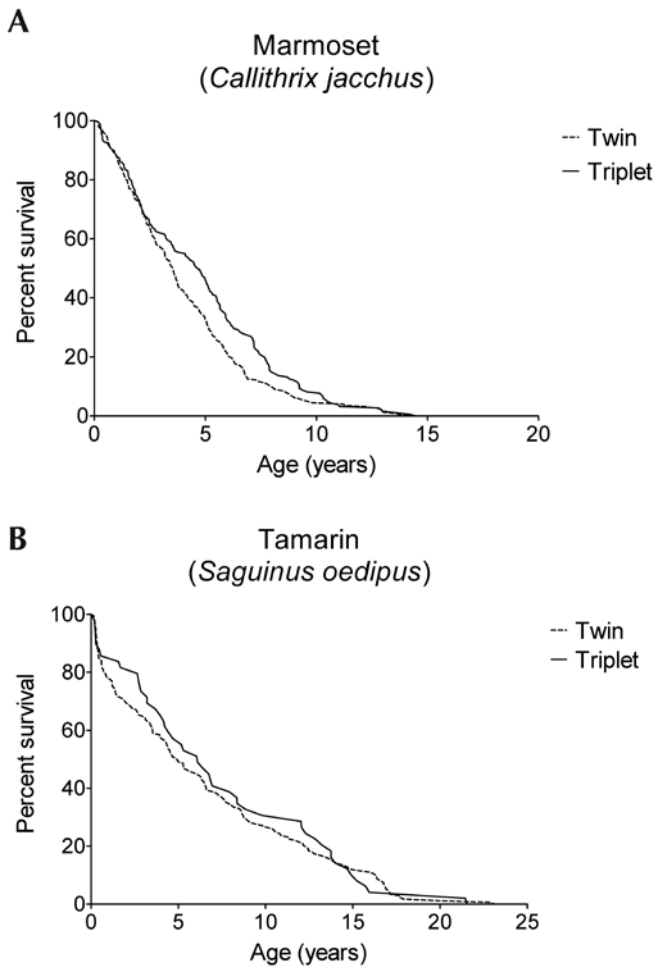

Figure 3. Survival curves after 60 d for animals with deaths due to natural causes according to litter size. (A) Marmoset birth type and lifespan (days). (B) Tamarin birth type and lifespan (days).

creased polymorphism to be able to confer an advantage over limited polymorphism in any particular immunogenetic trait. Second, we assume that animals that are born together with a greater number of siblings (that is, triplets and quadruplets) have a greater level of chimerism, and thus are more genetically diverse, than are singletons. Finally, we assume that the proportion of specific or individual alleles does not make a difference—or is not as important—as is the overall presence of increased genetic diversity. Said another way, marmosets and tamarins are not isolated in an environment where 1 or 2 single alleles are of total immunogenetic importance. To move forward in deciphering the effect of chimerism on longevity and other demographic variables in mammals, challenges to reporting and incorporating as much phenotypic data as possible into breeding records must be overcome. Comprehensive observations and record-keeping relating to variation in *C. jacchus* and *S. oedipus* reproductive output and success will help expand existing observations and hypotheses on their unique reproductive physiology, increasing their potential for use as nonhuman primate models of human disease and improving our understanding of the factors involved in their ecology and conservation.

Acknowledgments

We appreciate helpful discussions with Lisa Ogawa, Dharmendra Goswami, Carolyn Sweeney, and Gregory Miller (NEPRC Division of Neuroscience). We are also thankful for the support of Susan Westmoreland (NEPRC Division of Comparative Pathology) and Lynn Wachtman (NEPRC Division of Veterinary Resources). This work was supported in part by NIH grants AA019688 (to EJV) and OD011103.

References

1. **Aractingi S, Uzan S, Dausset J, Carosella ED.** 2000. Microchimerism in human diseases. Immunol Today **21:**116–118.
2. **Benirschke K, Anderson JM, Brownhill LE.** 1962. Marrow chimerism in marmosets. Science **138:**513–515.
3. **Benirschke K, Brownhill LE.** 1962. Further observations on marrow chimerism in marmosets. Cytogenetics **1:**245–257.
4. **Chalifoux LV, Bronson RT, Escajadillo A, McKenna S.** 1982. An analysis of the association of gastroenteric lesions with chronic wasting syndrome of marmosets. Vet Pathol Suppl **7:**141–162.
5. **Day T, Bonduriansky R.** 2004. Intralocus sexual conflict can drive the evolution of genomic imprinting. Genetics **167:**1537–1546.
6. **Dunsford I, Bowley CC, Hutchison AM, Thompson JS, Sanger R, Race RR.** 1953. A human blood-group chimera. Br Med J **2:**81.
7. **Gengozian N, Batson JS, Eide P.** 1964. Hematologic and cytogenetic evidence for hematopoietic chimerism in the marmoset, *Tamarinus nigricollis.* Cytogenetics **3:**384–393.
8. **Hill JP.** 1932. Croonian lecture: the developmental history of the primates. Phil Trans R Soc Lond B **221:**45–178.
9. **Jaquish CE, Cheverud JM, Tardif SD.** 1996. Genetic and environmental impacts on litter size and early infant survival in 3 species of callitrichids. J Hered **87:**74–77.
10. **Jaquish CE, Gage TB, Tardif SD.** 1991. Reproductive factors affecting survivorship in captive Callitrichidae. Am J Phys Anthropol **84:**291–305.
11. **Johnson LD, Petto AJ, Sehgal PK.** 1991. Factors in the rejection and survival of captive cotton-top tamarins (*Saguinus oedipus*). Am J Primatol **25:**91–102.
12. **Kilborn JA, Sehgal P, Johnson LD, Beland M, Bronson RT.** 1983. A retrospective study of infant mortality of cotton-top tamarins (*Saguinus oedipus*) in captive breeding. Lab Anim Sci **33:**168–171.
13. **Leong KM, Terrell SP, Savage A.** 2004. Causes of mortality in captive cotton-top tamarins (*Saguinus oedipus*). Zoo Biol **23:**127–137.
14. **Ludlage E, Mansfield K.** 2003. Clinical care and diseases of the common marmoset (*Callithrix jacchus*). Comp Med **53:**369–382.
15. **Manolov G, Levan A, Nadkarni JS, Nadkarni J, Clifford P.** 1970. Burkitt's lymphoma with female karyotype in an African male child. Hereditas **66:**79–100.
16. **Nelson JL.** 2002. Microchimerism and human autoimmune diseases. Lupus **11:**651–654.
17. **Pancer Z, Gershon H, Rinkevich B.** 1995. Coexistence and possible parasitism of somatic and germ cell lines in chimeras of the colonial urochordate *Botryllus schlosseri.* Biol Bull **189:**106–112.
18. **Parant O, Khosrotehrani K, Aractingi S.** 2009. [Gestational microchimerism in human diseases] Presse Med **38:**584–590. [Article in French].
19. **Perelman P, Johnson WE, Roos C, Seuanez HN, Horvath JE, Moreira MA, Kessing B, Pontius J, Roelke M, Rumpler Y, Schneider MP, Silva A, O'Brien SJ, Pecon-Slattery J.** 2011. A molecular phylogeny of living primates. PLoS Genet **7:**e1001342.
20. **Pollack MS, Kirkpatrick D, Kapoor N, Dupont B, O'Reilly RJ.** 1982. Identification by HLA typing of intrauterine-derived maternal T cells in 4 patients with severe combined immunodeficiency. N Engl J Med **307:**662–666.
21. **Rinkevich B.** 1996. Bi-versus multichimerism in colonial urochordates: a hypothesis for links between natural tissue transplantation, allogenetics, and evolutionary ecology. Exp Clin Immunogenet **13:**61–69.
22. **Rinkevich B**. 2004. Will two walk together, except they have agreed? Amos 3:3. J Evol Biol **17:**1178–1179.
23. **Ross CN, Davis K, Dobek G, Tardif SD.** 2012. Aging phenotypes of common marmosets (*Callithrix jacchus*). J Aging Res **2012:**567143.
24. **Ross CN, French JA, Orti G.** 2007. Germline chimerism and paternal care in marmosets (*Callithrix kuhlii*). Proc Natl Acad Sci USA **104:**6278–6282.

25. **Savage A, Soto L, Medina F, Emeris G, Soltis J.** 2009. Litter size and infant survivorship in wild groups of cotton-top tamarins (*Saguinus oedipus*) in Colombia. Am J Primatol **71:**707–711.
26. **Spencer HG.** 2000. Population genetics and evolution of genomic imprinting. Annu Rev Genet **34:**457–477.
27. **Stoner DS, Rinkevich B, Weissman IL.** 1999. Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. Proc Natl Acad Sci USA **96:**9148–9153.
28. **Sweeney CG, Curran E, Westmoreland SV, Mansfield KG, Vallender EJ.** 2012. Quantitative molecular assessment of chimerism across tissues in marmosets and tamarins. BMC Genomics **13:**98.
29. **Tardif SD, Mansfield KG, Ratnam R, Ross CN, Ziegler TE.** 2011. The marmoset as a model of aging and age-related diseases. ILAR J **52:**54–65.
30. **Tardif SD, Richter CB, Carson RL.** 1984. Reproductive performance of 3 species of Callitrichidae. Lab Anim Sci **34:**272–275.
31. **Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME.** 2003. Reproduction in captive common marmosets (*Callithrix jacchus*). Comp Med **53:**364–368.
32. **Tardif SD, Ziegler TE.** 1992. Features of female reproductive senescence in tamarins (*Saguinus* spp.), a New World primate. J Reprod Fertil **94:**411–421.
33. **Watkins DI, Garber TL, Chen ZW, Toukatly G, Hughes AL, Letvin NL.** 1991. Unusually limited nucleotide sequence variation of the expressed major histocompatibility complex class I genes of a New World primate species (*Saguinus oedipus*). Immunogenetics **33:**79–89.
34. **Watkins DI, Hodi FS, Letvin NL.** 1988. A primate species with limited major histocompatibility complex class I polymorphism. Proc Natl Acad Sci USA **85:**7714–7718.
35. **Windle CP, Baker HF, Ridley RM, Oerke AK, Martin RD.** 1999. Unrearable litters and prenatal reduction of litter size in the common marmoset (*Callithrix jacchus*). J Med Primatol **28:**73–83.
36. **Wislocki GB.** 1939. Observations on twinning in marmosets. Am J Anat **64:**445–483.