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Characterization of SA12 as a Simian Virus 40-Related Papovavirus of Chacma Baboons

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SA12 virus, originally isolated from an uninoculated South African vervet monkey kidney culture, was identified as a new member of the simian virus 40 (SV40)-polyoma subgroup of papovaviruses. The virus produced a cytopathic effect with nuclear enlargement in primary rhesus kidney cells. The virion had papovavirus morphology and a diameter of 44 to 45 nm. The DNA of the virus was a circular, double-stranded, superhelical molecule with a mean length 101% that of SV40 DNA and an estimated molecular weight of 3.3×10^6. The virus was found to be unrelated to other papovaviruses by neutralization, immune electron microscopy, and immunofluorescence tests with antiviral sera. SA12 virus-infected cells exhibited a capsid antigen, which has recently been found to be common to viruses of the SV40-polyoma subgroup. The virus readily transformed kidney cells from 10-day-old hamsters. Inoculation of transformed cells produced tumors in 3- to 4-week-old hamsters. The T antigens of SA12 and SV40 viruses were strongly and reciprocally cross-reactive. A high proportion of the sera of chacma baboons, *Papio ursinus,* and a comparatively lower proportion of the sera of vervet monkeys, *Cercopithecus pygerythrus,* had neutralizing antibodies to SA12 virus.

In 1963, Malherbe et al. (8) reported the isolation of a virus, designated SA12, from an uninoculated primary culture of vervet monkey (*Cercopithecus pygerythrus*) kidney cells. The agent was tentatively classified as a papovavirus because it produced basophilic nuclear inclusions resembling those of simian virus 40 (SV40), it was not neutralized by SV40 antiserum, and it had the morphology of a papovavirus (9). The virus was recovered only once during many years of careful cytopathologic surveillance of kidney cultures from several thousand vervet monkeys that were used for the production of poliomyelitis vaccine for the Republic of South Africa.

We report here the characterization of SA12 as a new papovavirus of the SV40-polyoma subgroup and the identification of the chacma baboon (*Papio ursinus*) as its probable natural host.

MATERIALS AND METHODS

Virus and cell cultures. SA12 was originally recognized by its cytopathic effect in a single bottle of uninoculated vervet kidney culture. The virus was passed undiluted 10 to 15 times in vervet (*C. pygerythrus*) kidney cultures and once in yellow baboon (*P. cynocephalus*) kidney cultures before it was grown for the present studies in primary or early-passage rhesus monkey (*Macaca mulatta*) kidney (RMK) cultures. RMK cultures were derived by trypsinizing kidneys of SV40-antibody-free juvenile rhesus monkeys and were grown in Eagle minimum essential medium (Earle salts) supplemented with 10% fetal calf serum, antibiotics, and nonessential amino acids. When they showed viral cytopathic effect, the infected cultures with their medium were frozen and thawed three times to disrupt the cells, and the suspension was clarified by centrifugation at $10,000 \times g$ for 1 h. The supernatant virus was concentrated 20- to 100-fold by centrifugation at $100,000 \times g$ for 1 h.

Early-passage cultures from kidneys of 10-day-old outbred golden Syrian hamsters (Charles River, Wilmington, Mass.) were utilized for the study of transformation by the virus.

Purification and characterization of SA12 DNA. Third-RMK-passage virus was plaque purified and propagated on rhesus embryonic kidney cell line MA 104 (Microbiological Associates, Inc., Bethesda, Md.). SA12 deoxyribonucleic acid (DNA) was extracted from infected cells by the method of Hirt (5) as modified by Danna and Nathans (1). The purified DNA was mounted for electron microscopy as described by Davis et al. (2). Contour length measurements were taken with a Numonics digital length calculator.

Immune sera. Rabbit anti-SA12 serum was prepared by immunization with three weekly doses of

second-RMK-passage SA12 virus that had an infectivity of $10^{7.0}$ mean tissue culture infective doses per ml in RMK cells. The first two doses (1 ml) were given intravenously, and the third (5 ml) was given intraperitoneally. The rabbit was bled 2 weeks after the third dose. The sources and methods of preparation of antisera against SV40 (rabbit and rhesus), BK virus (BKV) (rabbit), JC virus (JCV) (rabbit), stump-tailed macaque virus (STMV) (rabbit and rhesus), rabbit kidney vacuolating virus (RKV) (rabbit), and polyoma virus (rabbit) have been described before (13, 15).

A rabbit serum (R2, pool 31) prepared by immunization with sodium dodecyl sulfate-disrupted SV40 capsids and known to contain antibodies to a capsid antigen that is common to all viruses of the SV40-polyoma subgroup (15; Shah et al., Infect. Immun., in press) was reacted in an immunofluorescence (IF) test against SA12 virus-infected cells. Sera with SV40 and SA12 T antibodies were obtained from hamsters bearing tumors caused by inoculation of cells transformed by the respective viruses.

Primate sera. In 1960, when SA12 virus was isolated, two species of African nonhuman primates, vervets and chacma baboons, were held in the Johannesburg laboratory. A small breeding colony of rhesus macaques was maintained a short distance from the laboratory. Sera from both of these African species were collected in 1976 for examination for antibodies to SA12 virus. One hundred sixty-eight sera, were obtained from the vervet species, *C. pygerythrus,* from which the original isolation was made. These serum donors were adult animals captured in South Africa at several locations in Eastern and Western Transvaal, Eastern and Western Cape Province, and Natal. Of these animals, 61 were bled as soon as they arrived at the laboratory (probably within 1 week of capture), and the remainder were bled after they had been in captivity for about 3 months. Sera from 74 wild and 22 captive chacma baboons (*P. ursinus*) were also examined. The wild animals, from four different troops in the Mica areas of the Northeastern Transvaal, were bled in the field. (These sera were kindly supplied by B. M. McIntosh.) The other 22 sera were collected from animals captured in the Western Cape Province and housed for 1 to 3 months at the University of Cape Town. In addition to the sera from African primates, 17 sera from rhesus macaques collected in 1970 from animals held in a U.S. laboratory and 73 sera from Maryland residents (62 children and 11 adults) collected in 1968–1969 (14) were examined for antibodies to SA12 virus.

Serological tests. (i) IEM. SA12 virions in crude concentrated preparations were unaggregated, clean, and suitable for immune electron microscopy (IEM) tests. IEM tests were performed as previously described for other papovaviruses (13). Individual virions that displayed antibody coating were scored as reactive. All tests were performed on coded samples.

(ii) IF. The IF test was performed by an indirect method, as described previously (15). Each of the tested papovaviruses was inoculated into appropriate permissive cell cultures to obtain preparations containing viral antigens. Cells were fixed 2 to 4 days postinfection. Cells from S40-induced hamster tumors and SA12-transformed hamster cells were the sources of the respective T antigens.

(iii) Neutralization. Sera were first screened for neutralizing antibodies to SA12 and SV40 viruses by immunofluorescence neutralization (IFN) tests. Each serum was tested for its ability, when incubated with the virus, to prevent or decrease the development of virus-specific IF (14). A 1:5 dilution of heat-inactivated (56°C, 30 min) serum (0.1 ml) was mixed with an equal volume of an appropriate virus dilution and was incubated for 1 h at 37°C. A 0.1-ml portion of the mixture was inoculated into a single well of eight-well Lab-Tek slides (Miles Laboratories, Inc., Westmont, Ill.) that had been planted with RMK cells (for SA12) or BSC-1 cells (for SV40) 24 to 48 h earlier. After 1 h of adsorption at 37°C, the cell sheets were washed three times with Hanks balanced salt solution, and 0.5 ml of medium was added to each well. The slides were fixed at 2 days postinfection for SV40 and at 4 days postinfection for SA12 and examined by the indirect IF test for virus-specific fluorescence with rabbit antisera. The virus concentration for the test was adjusted to induce IF in 5 to 30% of the inoculated cells. A serum was scored as positive if its prior incubation with the virus reduced the number of fluorescing cells by at least 90% of those of the virus controls of the same test. All of the sera from the African primates were also scored by conventional neutralization (N) tests in stationary RMK tissue culture tubes. Heat-inactivated sera were tested at a dilution of 1:5 with 1.5 to 2.5 $\log_{10}$ units of SA12 virus, and the inoculated cultures were observed for 3 to 4 weeks for viral cytopathic effect.

RESULTS

The virus was readily propagated in primary or early-passage RMK cells. Viral cytopathic effect was first observed 3 to 4 days after inoculation of undiluted virus, and it progressed to a complete degeneration of the cell sheet within 8 to 10 days. Marked nuclear enlargement was characteristic of infected cultures. Cytoplasmic vacuolation was not common. After four passages in RMK cells, the virus had an infectivity titer of $10^{7.0}$ mean tissue culture infective doses per ml in stationary RMK tissue culture tubes.

Morphology of the SA12 virion and structure and molecular weight of its DNA. Electron microscopy after negative staining revealed that the SA12 virions were 44 to 45 nm in diameter and had papovavirus morphology (Fig. 1A). The capsids were naked. In preparations of purified SA12 DNA, over 99% of the DNA was present as covalently closed, superhelical, duplex molecules (form I). The contour length of form I SA12 DNA was measured against linear SV40 DNA (prepared with R·*Eco*RI endonuclease [10, 11] present on the same grid [Fig. 2]). The SA12 DNA molecules had a mean length of 1.01 ± 0.016 (standard error) that of SV40 DNA. This corresponds to a molecular weight of 3.3×10^6 for SA12 DNA, taking the molecular weight of SV40 DNA as 3.28×10^6 (4, 16).

Antigenic relationships of SA12 virus.

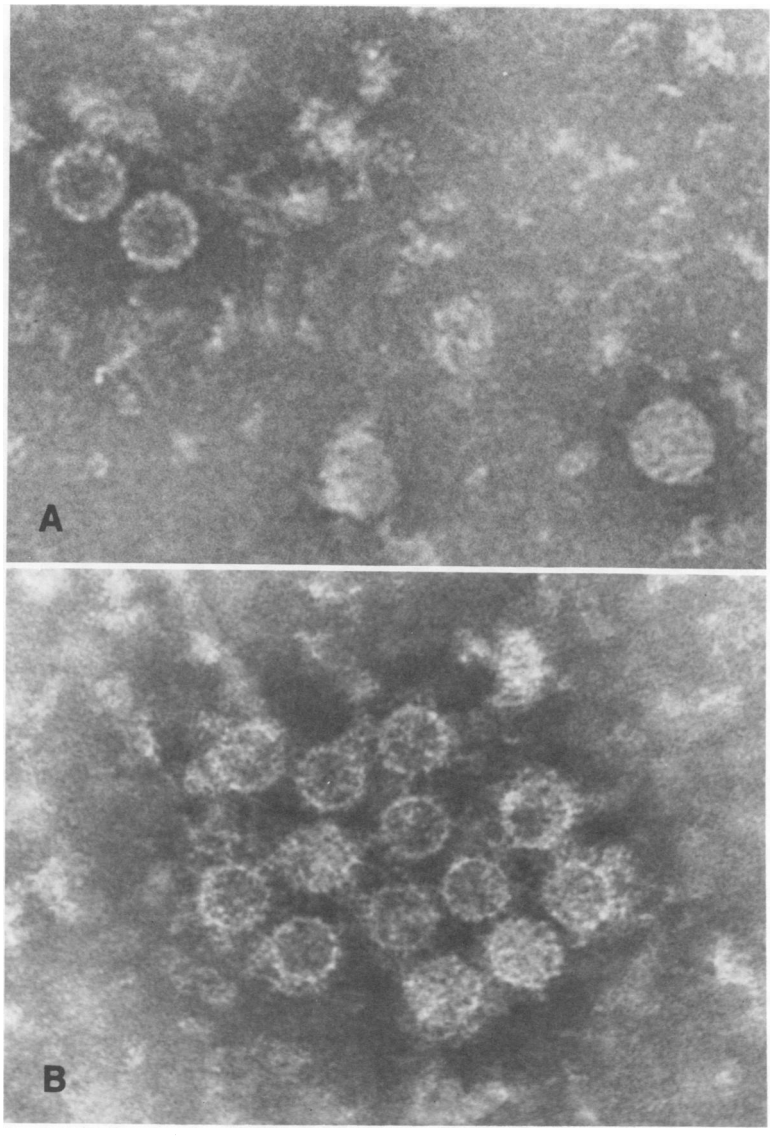

FIG. 1. *(A) Negatively stained preparations of SA12 virions from crude concentrated stock of SA12 virus. The virions are naked and are 44 to 45 nm. ×240,000. (B) SA12 virions incubated with a 1:5 dilution of SA12 antiserum for 1 h at 37°C before negative staining. The particles appear to be coated by antibody. ×240,000.*

There was no evidence of cross-reactivity between SA12 virus and the other papovaviruses in IEM tests (Table 1). All the virion preparations (except for RKV, which was not tested) reacted satisfactorily with the homologous sera. Virus-antibody complexes were observed as a filamentous network, completely covering the surface of the naked virions (Fig. 1B). The SA12 virions, although readily coated by anti-SA12 rabbit serum, were completely nonreactive when tested with immune sera prepared against simian papovavirus SV40 and STMV, human papovaviruses BKV and JCV, and RKV and polyoma virus. Conversely, the anti-SA12 serum failed to react with other papovaviruses (Table 1, footnote *c*).

The reference immune sera listed in Table 1 were also tested for their ability to neutralize SA12 virus. Incubation of the virus with anti-SA12 serum completely inhibited development of virus-specific immunofluorescence in IFN tests and viral CPE in N tests. In contrast, none of the other sera neutralized SA12 virus in either test.

The results of IF tests (Table 2) confirm the lack of cross-reactivity found in IEM and neu-

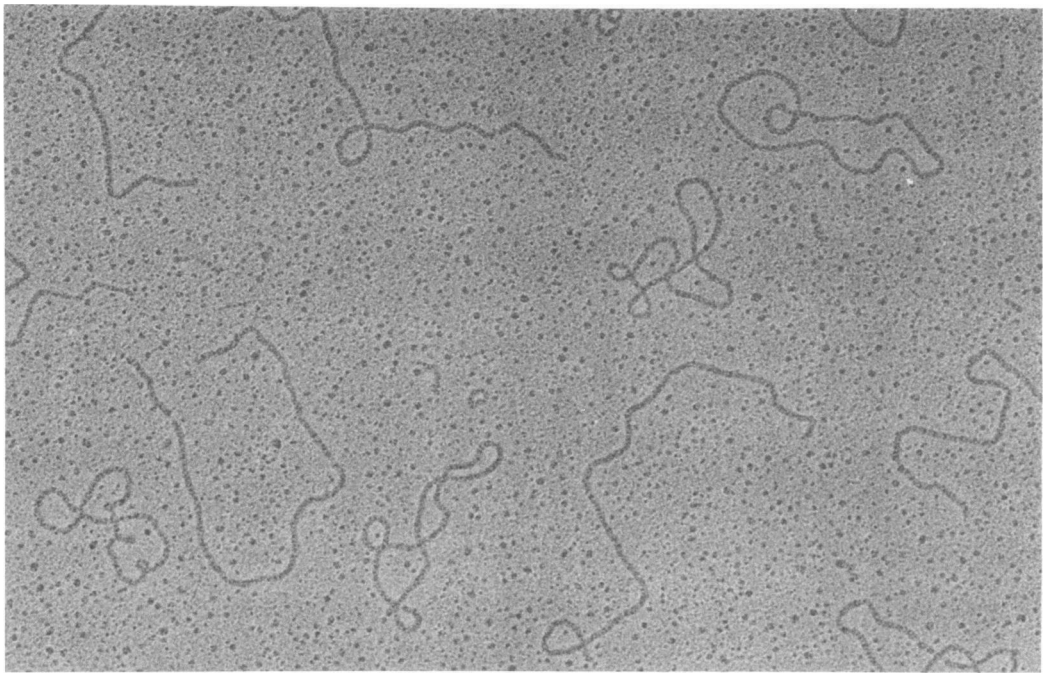

FIG. 2. *Electron micrograph of circular SA12 DNA and linear SV40 DNA. SA12 DNA was prepared as described in the text, and the linear SV40 DNA was prepared with R · EcoRI endonuclease. The mean length of 25 SA12 DNA circles was 1.01 ± 0.016 (standard error) of that of the SV40 DNA. In a control experiment, the lengths of circular and linear forms of SV40 DNA were found to be the same within experimental error.*

TABLE 1. *Antigenic relationship of SA12 virus to other papovaviruses by IEM*

Immune serum[a]	No. reactive/no. examined	
	SA12 virion	Homologous[b] virion
SA12, rabbit[c]	61/70	
SV40, rhesus	0/102	60/60
BKV, rabbit	0/102	52/53
JCV, rabbit	0/104	72/72
STMV, rhesus	0/102	55/58
RKV, rabbit	0/105	Not done
Polyoma, rabbit	0/106	123/123

[a] Each serum was diluted 1:5.

[b] Virions were homologous for the immune serum tested.

[c] Serum was also tested with other papovaviruses. The proportion of virions reactive to it were: 0/66, SV40; 4/61, STMV; 0/58, BKV; 0/27, JCV; and 0/58, polyoma virus.

TABLE 2. *Antigenic relationship of SA12 virus to other papovaviruses by IF tests*

Immune serum	IF antibody titers[a]	
	SA12 antigen	Homologous antigen
Anti-viral		
SA12, rabbit[b]	1:160	
SV40, rabbit	Neg	1:80
STMV, rabbit	Neg	1:5
BKV, rabbit	Neg	1:80
RKV, rabbit	Neg	1:320
Polyoma, rabbit	Neg	1:320
Subgroup reactive		
SV40 (SDS), rabbit[c]	1:1,280	1:2,560

[a] Antigens in acutely infected cells: SA12 in RMK, SV40 in BSC-1, STMV in RMK, BKV in WI38, RKV in rabbit kidney, and polyoma in 3T3/D cells. Neg, Negative.

[b] At a 1:5 dilution, this serum failed to stain cells productively infected with SV40, STMV, BKV, RKV, and polyoma virus.

[c] This serum (R2, pool 31) had titers of 1:1,280 to 1:2,560 against BKV, STMV, RKV, and polyoma antigens in infected cells (Table 4; 15). SDS, Sodium dodecyl sulfate.

tralization tests. The sera used in these tests contained anti-viral but not anti-T antibodies. Rabbit immune sera to SV40, STMV, BKV, RKV, and polyoma virus, prepared by immunization with viral particles, were reactive to the respective homologous antigens prepared in pro-

ductively infected cells, but they failed to stain SA12 antigens in infected RMK cells. Similarly, the anti-SA12 rabbit serum (Table 2, footnote *b*) was nonreactive to cells infected with SV40, STMV, BKV, RKV, and polyoma virus. In contrast, the rabbit serum prepared against sodium dodecyl sulfate-disrupted SV40 capsids stained SA12 antigens to the same high titers as it did SV40, BKV, STMV, RKV, and polyoma antigens (Table 2, footnote *c*).

Transformation of hamster cells by SA12 virus. The SA12 virus routinely transformed cells from kidneys of 10-day-old hamsters. Foci of transformed cells appeared 10 to 12 days after inoculation of the virus onto first- or second-passage monolayer cultures. Transformed cells reached saturation densities that were 8 to 10 times those of normal cells and could be readily established as permanent cell lines. They exhibited intranuclear SA12 T antigens in 95 to 100% of the cells as judged by IF reactivity to SA12 and SV40 T antibodies, but were free of SA12 viral antigens as determined by IF tests with anti-SA12 rabbit serum. Four of nine 3-week-old hamsters inoculated with $3 \times 10^{6.0}$ transformed cells developed subcutaneous tumors at the site of inoculation after a latent period of 6 weeks. Upon passage, 1 to 2 million tumor cells produced tumors in all inoculated hamsters in 3 to 5 weeks. Sera from SA12 tumor-bearing hamsters stained SA12 and SV40 T antigens in IF tests, but were negative for SA12 and SV40 virus-neutralizing antibodies in N tests.

T antigens of SA12 and SV40 were indistinguishable in IF tests (Table 3). SA12 T serum reacted equally well to SA12 and SV40 T antigens, both in transformed and productively infected cells, and SV40 T serum had comparable antibody titers to SV40 and SA12 T antigens.

Natural hosts of SA12 virus. Neutralizing antibodies to SA12 virus were present both in vervets and chacma baboons. In both species, antibodies were found in wild as well as in captive animals (Table 4). The antibody prevalence rate was much higher in the chacma baboon. N tests showed that 58% wild and 77% captive chacma baboons possessed neutralizing antibodies, compared to 12% wild and 8% captive vervets. The antibody prevalence in the four troops of chacma baboons in the Northeastern Transvaal varied from 30 to 90% (Table 4, footnote *c*). The two areas where antibody-positive chacma baboons were found (Northeastern Transvaal and Western Cape Province) are over 800 miles apart. These data suggest that SA12 infection is widespread in this species. None of the sera from the vervets and baboons neutralized SV40 in IFN tests, thus confirming previous observations (6) that SV40 infection does not occur in the simian species of Africa. All 17 sera from rhesus macaques neutralized SV40, but none neutralized SA12 virus in either IFN or N tests. None of the 73 sera from Maryland residents neutralized SA12 virus in IFN tests.

All of the 264 sera from the vervets and baboons were examined for SA12 neutralizing antibodies in both IFN and N tests. The results of the two tests correlated as follows: 54 sera were positive and 188 sera were negative in both tests, 22 sera were positive only in N tests and none was positive only in IFN tests. Thus, as a screening test, the IFN test detected 71% of the protective sera and gave no false-positive results.

TABLE 3. *Relationship of SA12 and SV40 T antigens*

Immune serum[a]	IF antibody titers			
	SA12 antigen		SV40 antigen	
	Infected[b] cells	Transformed[c] cells	Infected[b] cells	Transformed[c] cells
SA12, T	1:40	1:40	1:40	1:20
SV40, T	1:640	1:320	1:1,240	1:320

[a] From tumor-bearing hamsters. Sera negative for neutralizing antibodies to both SA12 and SV40.

[b] Acutely infected cells (RMK cells with SA12 and BSC-1 with SV40) containing both viral and T antigens to the infecting virus.

[c] Transformed cells, negative for viral antigens by IF tests.

TABLE 4. *Prevalence of neutralizing antibodies to SA12 and SV40 viruses in primate sera*

Donor	No. tested	No. with antibodies		
		SA12 virus		SV40 virus
		IFN[a]	N	IFN
Vervet				
Wild	61	2 (3)[b]	7 (12)	0
Captive	107	2 (2)	7 (8)	0
Chacma baboon				
Wild	74[c]	34 (46)	43 (58)	0
Captive	22	16 (73)	17 (77)	0
Rhesus				
Captive	17	0	0	17 (100)
Human[d]	73	0		

[a] All sera that were protective in IFN tests against SA12 virus were also protective in N tests.

[b] Number in parentheses represents percentage with antibodies.

[c] These were in four different troops; antibody prevalence by N test was 6/20, 9/18, 7/13 and 21/23 in these troops.

[d] 62 children and 11 adults from Maryland.

DISCUSSION

SA12 appears to be a newly recognized papovavirus of the SV40-polyoma subgroup. It is the first virus of this subgroup to be reported from an African nonhuman primate. Its identification as a papovavirus was based on the size of the virion (44 to 45 nm in diameter), the size and structure of its DNA (a superhelical duplex circle of molecular weight 3.3×10^6), and the indirect evidence that it possesses the antigenic determinant that is shared by all members of the subgroup. The strong and reciprocal cross-reactivity between the T antigens of SA12 and SV40 is similar to that described between the T antigens of papovaviruses SV40, BKV, and JCV (3, 12). The immunological distinctness of SA12 virus was clearly evident by its lack of cross-reactivity with other papovaviruses by neutralization, IEM, and IF tests with antiviral sera. We have not had an opportunity to compare SA12 virus with a recently reported human isolate from South Africa (7). The ease with which SA12 virus transformed hamster cells suggests that, in this respect, it more closely resembles SV40 than BKV or JCV (3, 12).

Although SA12 virus was originally isolated from a vervet kidney culture, the antibody-prevalence data suggest that the chacma baboon is the principal natural host of the virus. This species is native to southern Africa and is one of five *Papio* species on that continent. In the Johannesburg laboratory, chacma baboons were used in nutritional and transplant studies, so there was no opportunity to examine their kidney cultures for virus. Such studies, however, are now in progress. The original isolation of SA12 virus from a single vervet kidney culture and the 8% antibody prevalence in captive vervets reported here may represent occasional infections of vervets from chacma baboons. The 12% antibody prevalence in vervets bled within a week of capture suggests that this species is also infected in nature. However, the possibility that infection in these vervets occurred in captivity cannot be excluded because the duration of their captivity or the history of their contact with chacma baboons during captivity is not precisely known.

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