PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

DIVERSIDADE GENÉTICA EM ESPÉCIES DO GÊNERO *CAVIA* (RODENTIA, MAMMALIA) E A HISTÓRIA EVOLUTIVA DO RARO PREÁ DE MOLEQUES DO SUL

RICARDO KANITZ
SANDRO LUIS BONATTO (ORIENTADOR)

PORTO ALEGRE, 2009

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

DIVERSIDADE GENÉTICA EM ESPÉCIES DO GÊNERO *CAVIA* (RODENTIA, MAMMALIA) E A HISTÓRIA EVOLUTIVA DO RARO PREÁ DE MOLEQUES DO SUL

Ricardo Kanitz
Orientador: Dr. Sandro Luis Bonatto

DISSERTAÇÃO DE MESTRADO

PORTO ALEGRE – RS – BRASIL

2009

SUMÁRIO

DEDICATÓRIA pág. iv

AGRADECIMENTOS pág. v

APRESENTAÇÃO pág. vi

RESUMOS pág. xi

CAPÍTULO I: “Characterization of 16 microsatellite loci for the South-American rodents *Cavia magna* and *C. aperea*.” pág. 13

CAPÍTULO II: “Depauperated genetic diversity of the Moleques do Sul cavy (Cavia intermedia), the naturally rarest mammal in the World.” pág. 23

CONCLUSÃO GERAL pág. lvii

REFERÊNCIAS BIBLIOGRÁFICAS pág. lix

DEDICATÓRIA

Aos meus pais: Walmor e Sonia.

AGRADECIMENTOS

Deixo aqui meus agradecimentos às pessoas e instituições que auxiliaram na realização deste trabalho e na minha formação desde suas bases até o presente momento. Nominalmente:

- Ao professor Sandro Luis Bonatto pelos seis anos de ensinamentos sobre como fazer ciência e pelo grande empurrão no meu início de carreira como pesquisador.
- Aos colegas do LBGM pelo companheirismo, auxílios, discussões e ensinamentos. Em especial ao Nelson J. R. Fagundes, Felipe G. Grazziotin, Manoel L. F. Rodrigues, Marcelo C. M. Gehara, Thomaz X. Carneiro e Lucas C. T. Silveira.
- Aos meus caríssimos orientandos de iniciação científica Luisa L. Goellner e Raoni R. Postigline pela grande ajuda, pela sua curiosidade científica (que espero que se mantenha) e por me ensinar muito nestes últimos meses.
- À minha namorada, Aline; pelo apoio quando se fez necessário; pela empolgação quase sempre presente; e pelo amor, este sempre!
- À minha família sempre curiosa em relação ao que eu fazia com tantas tabelas cheias de números se eu trabalhava na verdade com um "bichinho", e preocupada (com razão) com o resultado das coletas no trabalho de campo e com os prazos de entrega desta dissertação.

APRESENTAÇÃO

Apresento esta dissertação ao Programa de Pós-Graduação em Zoologia da Pontifícia Universidade Católica do Rio Grande do Sul como um dos requisitos para a obtenção do grau de mestre em Zoologia. Realizei este trabalho – juntamente com meu orientador Prof. Dr. Sandro Luis Bonatto – nas dependências do Laboratório de Biologia Genômica e Molecular na Faculdade de Biociências da mesma universidade.

A motivação inicial deste trabalho se deveu à descoberta de uma população de preás – gênero *Cavia* Pallas, 1766 – em uma ilha de apenas 10ha no litoral do estado de Santa Catarina no Brasil. Aparentemente isolados das populações continentais e com marcantes diferenças morfológicas, os preás de Moleques do Sul foram considerados uma nova espécie: *Cavia intermedia* (Cherem *et al.* 1999). Estudos previamente realizados (Salvador & Fernandez 2008a) demonstraram que o tamanho de censo da população destes roedores exibe uma média de 42 indivíduos com oscilações entre aproximadamente 30 e 60. Além disso, estima-se que o arquipélago de Moleques do Sul esteja separado do continente há pelo menos 8.000 anos. Cerca de 14 km perfazem a distância entre o arquipélago e o continente onde habita a população mais próxima de *C. magna* – a espécie-irmã continental de *C. intermedia*. Tal grau de isolamento em tempo e distância de qualquer outro grupo de preás, assim como o tamanho populacional extremamente pequeno desta espécie, fazem de *C. intermedia* um objeto de estudo interessantíssimo sob o âmbito da genética evolutiva: o mamífero naturalmente mais raro do planeta.

Pequenas populações são uma importante fonte de dados para estudos evolutivos. Entre as pequenas populações, as mais comuns são aquelas endêmicas de ilhas e uma das espécies de mamíferos com essas características é *Cavia intermedia*. Conhecer parâmetros tais quais a sua variabilidade genética intra-específica e sua diferenciação em relação à espécie mais próxima podem revelar aspectos evolutivos interessantes.

Entre as questões que existem sobre evolução em populações insulares, há um amplo

debate sobre o papel da seleção natural na manutenção de populações reduzidas e, logo, endocruzadas (Reed 2007). Alguns problemas de "saúde genética" podem surgir em populações com alto endocruzamento. A exposição de alelos deletérios que, em heterozigosidade, não seriam expressos resulta na diminuição do *fitness*. Essa redução do *fitness* pode se dar por malformações congênitas, susceptibilidade maior a doenças, menor tamanho de ninhada, entre outros efeitos prejudiciais aos indivíduos (Frankham *et al.* 2002).

Alguns trabalhos propõem que, nessas populações, ocorre uma forte seleção em favor dos heterozigotos aumentando sua freqüência o que resulta, finalmente, em uma manutenção de diversidade na população como um todo (Gilligan *et al*. 1997; Kaeuffer *et al*. 2006). Outros fatores podem atuar em pequenas populações, tais como expurgo genético (*genetic purge*), que pode atenuar a depressão por endocruzamento. O expurgo ocorre por seleção purificadora que retira da população os alelos deletérios que são expostos pelos cruzamentos entre indivíduos aparentados. Apesar de não haver evidências de que esse efeito seja realmente eficiente na diminuição dos efeitos deletérios do endocruzamento a curto e médio prazos (Frankham *et al.* 2001), acredita-se que sua ação seja eficaz em longo prazo.

Adaptações morfo-fisiológicas também são descritas em táxons ilhéus. Boa parte dos trabalhos tratando de adaptações de espécies a condições insulares ressalta a síndrome de ilhas como uma condição recorrente em mamíferos (Adler & Levins 1994). Essa síndrome, resumidamente, pode ser descrita pelas seguintes características. Organismos pequenos tendem ao gigantismo; enquanto que, espécies maiores tendem ao nanismo (Bromham & Cardillo, 2007). Exemplos dessas adaptações podem ser os musaranhos comuns (*Sorex araneus*) que ocorrem nas ilhas escocesas que têm seu tamanho corporal aumentado em relação às populações continentais (White & Searle 2007). No outro lado do espectro, há os extintos elefantes anões (*Stegadon* spp.) das ilhas de Flores que chegavam a ter somente 10% do tamanho dos elefantes asiáticos atuais (*Elephas maximus*) (Van den Bergh *et al.* 2001). Adaptações na história de vida também são comuns. Existe uma tendência à maior longevidade e menor fertilidade (Gliwicz

1980; Adler & Levins 1994). Essa síndrome parece se expressar, mantendo a regra, em *Cavia intermedia* (Salvador & Fernandez 2008b).

Além dos aspectos seletivos, outras forças evolutivas atuam sobre populações insulares. A deriva genética é a mais evidente e talvez a mais efetiva em alterar a freqüência dos alelos na população. Ilhas pequenas, por sua área restrita *per se*, não suportam populações tão grandes quanto as continentais. Portanto, populações insulares são mais sujeitas às ações da deriva. Migrações também têm efeito mais acentuado em ilhas, pois a introgressão de alelos novos é potencialmente maior. O impacto dos novos alelos nas populações como um todo é maior, simplesmente, porque a população é menor. Além disso, a fixação do novo alelo pode se dar mais facilmente por efeito de deriva. Existem alguns casos relatados de espécies em condições semelhantes a *Cavia intermedia*. Um exemplo filogeneticamente próximo está no trabalho de Seddon & Baverstock (1999). Neste trabalho, os autores mostram um complexo de populações de ratos nativos (*Rattus fuscipes greyii*) dispostas em ilhas de diferentes tamanhos próximas à costa leste australiana. Viu-se que há uma correlação direta entre tamanho da ilha e diversidade genética presente nas populações tanto em marcadores neutros como em marcadores sujeitos à seleção natural – no caso – um gene de MHC de classe I: RT1.Ba. Geograficamente próximo, tem-se o exemplo de duas espécies de serpentes endêmicas de ilhas da costa: *Bothrops insularis* e *B. alcatraz*. Um trabalho envolvendo marcadores mitocondriais (Grazziotin *et al.* 2006) demonstrou que as duas espécies insulares, como esperado, apresentam diversidade genética bastante reduzida se comparadas com a espécie continental (*B. jararaca*).

Porém, todas as espécies de mamífero com pequeno tamanho populacional (com menos de 1.000 indivíduos conhecidos) estudadas geneticamente até o momento, são espécies cuja população foi reduzida, por ação antrópica, relativamente recentemente (poucas dezenas ou raramente alguns poucos séculos) (www.animalinfo.org/rarest.htm; www.iucnredlist.org). Portanto, talvez o aspecto mais importante de *C. intermedia* seja a hipótese de que seu tamanho populacional extremamente pequeno seja uma característica de longo prazo, talvez muitos

milhares de anos. Testar esta hipótese, e em caso de confirmação, estudar as conseqüências desta história idiossincrática para a diversidade genética da espécie são os principais objetivos deste estudo.

Então, para que obtivéssemos uma representação adequada do nível de diversidade genômica em *C. intermedia*, procuramos, em primeira instância, regiões de repetições curtas em seqüência ou microssatélites (*short tandem repeats*: *STR*). Usando uma abordagem menos ortodoxa, buscamos microssatélites diretamente nas seqüências genômicas de *C. porcellus* (o porquinho-da-Índia) procurando *STRs* especialmente longos. Sabe-se que quanto maior uma região de repetições, maior deve ser a sua taxa de mutação. Portanto, buscamos por microssatélites altamente variáveis para que – se houvesse alguma variabilidade em *C. intermedia* – ela pudesse ser captada através destes marcadores. A implementação desta técnica nas espécies continentais (*C. magna* e *C. aperea*), assim como a descrição dos *primers*, deverá ser publicada no periódico *Molecular Ecology Resources* na forma de uma *Primer Note*. O manuscrito inicial para esta publicação está detalhado no capítulo I.

No capítulo II, apresentamos o artigo principal desta dissertação. Sob a forma de um *Original Article*, devemos submetê-lo ao periódico *Molecular Ecology*. Neste trabalho, analisamos dois tipos de marcadores moleculares incluindo (i) os microssatélites descritos no primeiro capítulo e (ii) seqüências mitocondriais das regiões hipervariável I (*HVSI*) citocromo b (*cytochrome b*). Através da avaliação desses marcadores, nós pudemos estimar a diversidade neutra presente na população de *C. intermedia* e compará-la com sua espécie-irmã continental (*C. magna*) de maneira intra e interpopulacional.

Adicionalmente, estimamos os tamanhos efetivos populacionais (N_e) histórico e atual para *C. intermedia* baseados nos perfis genotípicos daquela espécie. O N_e histórico é também conhecido como N_e de endocruzamento (N_{el}). Este índice se baseia na diversidade genética encontrada dentro da população avaliada e remonta à diversidade acumulada e perdida ao longo da evolução desta população. Por outro lado, o assim chamado N_e atual pode ser medido através

da variância da composição alélica da população entre diferentes gerações: daí seu nome N_e da variância (N_{eV}). O princípio desta estimativa é mensurar a ação da deriva genética de uma geração para outra. Quanto maior for essa ação, menor o tamanho efetivo daquela população.

Acreditamos que este estudo pode ter uma importante contribuição para a área da biologia da conservação de populações insulares, assim como para a compreensão dos processos microevolutivos que atuam sobre a composição genética dessas populações. Além disso, é claro, este trabalho fornece inéditas estimativas, tanto da diversidade genética do mais raro mamífero conhecido, como dos seus tamanhos efetivos populacionais histórico e atual que, por si só, são resultados de grande validade à ciência. Finalmente, caso a hipótese seja confirmada, esta será a única espécie de mamífero conhecida até o momento com está história evolutiva extrema, o que pode transformar *C. intermedia* em uma inestimável ferramenta de estudos evolutivos em geral e de genética e biologia da conservação em especial.

RESUMOS

CAPÍTULO I: "Caracterização de 16 *loci* de microssatélites para os roedores sul-americanos *Cavia magna* e *C. aperea*"

Baseando-nos no recentemente publicado genoma de *Cavia porcellus* e em outros cinco loci já descritos para *C. aperea*, nós aqui apresentamos 16 *loci* de microssatélites empregáveis em *C. magna* e *C. aperea*. Os *primers* foram projetados para serem usáveis em um arranjo de fluorescência múltipla (*multiplex*). O número médio de alelos para cada *locus* foi de 7,4 e a média da heterozigosidade esperada foi de 0,67. A combinação de alguns ou todos estes marcadores pode possibilitar trabalhos em genética populacional, ecologia molecular e outros estudos evolutivos nas espécies aqui avaliadas.

CAPÍTULO II: "Baixíssima diversidade genética do preá de Moleques do Sul (*Cavia intermedia*), o mamífero naturalmente mais raro do Mundo"

Ilhas têm chamado a atenção de biólogos evolucionistas há séculos pelos seus altos níveis de endemismos promovidos, tanto pelo isolamento, quanto pelo tamanho limitado a que estas populações estão sujeitas. *Cavia intermedia* é uma espécie recentemente descrita como endêmica de uma pequena ilha na costa meridional do Brasil que parece ser o mamífero naturalmente mais raro conhecido, apresentando uma população estável de aproximadamente 40 indivíduos. Apesar de algumas simulações demográficas terem estimado sua chance de extinção em 100 anos como 100%, dado a história da ilha, torna-se improvável que a população possa ter sido muito maior no passado recente. Utilizando marcadores mitocondriais e microssatélites, nós descobrimos que este preá insular apresenta uma diversidade genética extremamente reduzida com estimativas de tamanhos efetivos populacionais histórico e atual compatíveis com seu tamanho de censo atual, sem sinal de redução de tamanho populacional. Considerando a antiga divergência de *C. intermedia* em relação ao seu grupo-irmão continental, concluímos que a espécie mantém esse tamanho extremamente reduzido desde, pelo menos, a separação da ilha em relação ao

continente há cerca de 8.000 anos. Portanto, *C. intermedia* pode ser o melhor e mais extremo exemplo até agora conhecido de uma espécie de mamífero que sobreviveu por centenas de anos com um tamanho populacional tão extremamente reduzido. Essa espécie, então, estabelece novos desafios ao entendimento do papel da redução populacional e da diversidade genética na extinção de espécies. Finalmente, é importante enfatizar a necessidade de um cuidado especial na manutenção das boas condições do hábitat para a sobrevivência de *C. intermedia* em seu ambiente natural.

CAPÍTULO I: CHARACTERIZATION OF 16 MICROSATELLITE LOCI FOR THE SOUTH-AMERICAN RODENTS *CAVIA MAGNA* AND *C. APEREA*

(Artigo a ser submetido ao periódico *Molecular Ecology Resources*)

Permanent Genetic Resources Note

Characterization of 16 microsatellite loci for the South-American rodents *Cavia magna* and *C. aperea*

Ricardo Kanitz[1], Fritz Trillmich[2], Sandro L. Bonatto[1]

[1] Faculdade de Biociências, PUCRS. Av. Ipiranga 6681, prédio 12C. Porto Alegre, RS 90619-900, Brazil.

[2] Department of Animal Behavior, University of Bielefeld. P.O. Box 100131, Bielefeld 33501, Germany.

Keywords: *Cavia*, *Cavia magna*, *Cavia aperea*, microsatellite, STR, molecular markers.

Corresponding author: Sandro L. Bonatto, Faculdade de Biociências, PUCRS. Av. Ipiranga 6681, prédio 12C, sala 172. Porto Alegre, RS 90619-900, Brazil. Fax: +55 (51) 3320-3612. E-mail: slbonatto@pucrs.br

Running title: Microsatellites for the genus *Cavia*.

Abstract

Based on the recently published genome draft of *Cavia porcellus* and five other loci described for *C. aperea*, we present 16 microsatellite loci applicable for *C. magna* and *C. aperea*. We designed the primers to be used in a multiplex fluorescence array. The average number of alleles for each locus was 7.4 and the mean expected heterozygosity was 0.67. The combination of some or all of these markers may give a good framework for population genetics, molecular ecology and other evolutionary studies in these species.

The genus *Cavia* Pallas, 1766 is a widespread rodent taxon in the Neotropics. Among the six recognized species (Wilson & Reeder, 2005), stand the best known *Cavia porcellus* (Domestic Guinea Pig) and *C. aperea* (Wild Guinea Pig). The latter seems to be the most common in the wild, while the first is the likely domestic derivative of the Andean cavy, *C. tschudii* (Spotorno *et al.* 2006) and also an important model species. *Cavia magna* (Greater Guinea Pig) is endemic to a narrow coastal region in southern Brazil and Uruguay. As *C. magna* and *Cavia aperea* also present unique ecological characteristics (Kraus *et al.* 2003; Kraus *et al.* 2005; Asher *et al.* 2008), highly variable molecular markers is very important for further studies on their biology. A previous work on the ecology and behaviour of *C. aperea* described six microsatellite (STR) loci for that species (Asher *et al.* 2008), but these primers were not tested for any other species. Moreover, additional loci are necessary for more accurate inferences of population genetics parameters in the species. Here we aim to (i) test the cross-amplification of these six loci using redesigned primers, and (ii) present 10 new STR loci and their respective primers sequences applicable to *C. aperea* and *C. magna*.

First, we redesigned primers for the six microsatellite loci described by Asher et al. (2008) trying to improve multiplexing possibilities. The new primers were designed using Primer3 web-based software (Rozen & Skalesky, 2000. We renamed these loci to Cavy1 (formerly Asher *et al*.'s CAP49651), Cavy2 (CAP49653), Cavy3 (CAP49654) and Cavy4 (CAP49655). The additional two loci in Asher *et al*. (2008) did not provide satisfactory results and were not further used in this study.

We additionally searched for other 17 loci in the *Cavia porcellus* genome draft (July, 2007). Of the 219 contigs we searched in *C. porcellus* genome (~37.8 million bp), 65 presented 78 microsatellite sequences. For the search for usable STR loci, given the massive amount of data, we used the program MSatCommander (Faircloth, 2008) which has proved to

50 handle well with large datasets. We designed the primers for these loci in Primer3 (Rozen &
51 Skalesky, 2000) for the same annealing temperatures we applied for the previous primer pairs
52 (60ºC).

53 To minimize the cost of fluorescent primers, we applied the M13 tail method as
54 proposed by Lorenz et al. (2001). The amplification tests were made on 20 subjects from each
55 *C. aperea* and *C. magna* species collected in North Uruguay and South Brazil. The
56 Polymerase Chain Reactions (PCR) were performed in a 10 µL reaction using 5-15 ng of
57 genomic DNA, with 0.2 mM of all dNTPs, 0.2 µM of each the reverse and the M13-
58 fluorescent primers, 0.013 µM of the forward primer, 2 mM MgCl2 and 0.25 U of *Taq* DNA
59 polymerase *Platinum* (Invitrogen). The PCR thermal profile were as follows: 94 ºC for 3 min
60 for initial denaturing, followed by 35 cycles of 94 ºC for 1 min 30 s, 60 ºC for 1 min 30 s and
61 72 ºC for 45 s; after these 35 cycles, we added a final extension (72 ºC) for 30 min followed
62 by cooling at 4 ºC.

63 Genotyping was performed in an automated sequencer MegaBACE1000 (GE
64 Healthcare) using the internal size standard ET Rox-550 and inspecting the electropherograms
65 in Genetic Profiler 1.5 (GE Healthcare). Diversity indexes estimations, Hardy-Weinberg
66 equilibrium (HWE), and linkage disequilibrium tests with Bonferroni correction for multiple
67 comparisons were performed in Arlequin 3.1 (Excoffier *et al.* 2005).

68 We characterized 16 (*C.* magna) and 12 (*C. aperea*) working loci which are able to be
69 used in intra-specific studies. Twelve of the 16 loci were successfully amplified for both *C.*
70 *magna* and *C. aperea* and may be used for across species studies. Details on primer
71 sequences, repeat motifs, expected PCR product sizes and suggested dyes for multiplex
72 genotyping are given in Table 1. The summary statistics showed high levels of diversity for all
73 loci in both species. Three loci presented significant departure from the HWE in *C. magna*,

and none for *C. aperea* (Table 2). The excess of homozygous individuals in *C. magna* may be due to inbreeding in our sample, which focused in individuals from one small and probably isolated population. Besides, no linkage-disequilibrium was found between any loci for both species.

The primers described here revealed are readily usable for evolutionary studies in both species. Given their high variability, these markers may be applied for both inter-population (e.g. genetic isolation, number of migrants, etc.) and intra-population studies (e.g. kinship exploration). Furthermore, we believe that most of the primers presented here may also be used in studies in the other three wild cavy species, *C. fulgida*, *C. tschudii*, and *C. intermedia*, considering that *C. aperea* and *C. magna* are in two divergent clades within *Cavia* (results in preparation by our group). Additionally, these loci may be also useful for studies on *C. porcellus* biology, which is an important species in experimental biology.

Acknowledgements

RK was supported by a master student scholarship from CNPq and SLB has grant supports from CNPq and FAPERGS.

References

Asher M, Lippmann T, Epplen JT, Kraus C, Trillmich F, Sachser N (2008) Large males dominate: ecology, social organization, and mating system of wild cavies, the ancestors of the guinea pig. *Behavioral Ecology and Sociobiology,* **62,** 1509-1521.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* **1,** 47-50.

Faircloth BC (2008) MSatCommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources,* **8**, 92-94.

Kraus C, Künkele J, Trillmich F (2003) Spacing behaviour and its implications for the mating system of a precocial small mammal: an almost asocial cavy Cavia magna? Animal Behaviour, **66**, 225-238.

Kraus C, Thomson DL, Künkele J, Trillmich F (2005) Living slow and dying young? Life-history strategy and age-specific survival rates in a precocial small mammal. Journal of Animal Ecology, **74**, 171-180.

Lorenz E, Frees KL, Schwartz DA (2001) M13-Tailed Primers Improve the Readability and Usability of Microsatellite Analyses Performed with Two Different Allele-Sizing Methods. *Biotechniques,* **31**, 24-26.

Rozen S, Skaletsky HJ (2000) In *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp 365-386. Humana Press, Totowa, New Jersey.

Spotorno AE, Marín JC, Manríquez G, Valladares JP, Rico E, Rivas C (2006) Ancient and modern steps during the domestication of guinea pigs (Cavia porcellus L.). Journal of Zoology, **270**, 57-62.

113 Wilson DE, Reeder DM (2005) *Mammal Species of the World: A Taxonomic and Geographic*
114 *Reference*. The Johns Hopkins University Press, Baltimore, Maryland. 2142 pp.

Table 1: Primers details for all 16 loci.

Locus	Repeat Motif	Forward Primer Sequence*	Reverse Primer Sequence	Dye**	Size****	Accession #
Cavy 1	$[AG]_n$	CGGTTCTTGATTGGCTTCAT	GCCTGCTCCTGTTCTCTCTC	FAM	255	AJ496558
Cavy 2	$[AC]_n$	GGCCATTATGCCCCCCAAC	AGCTGCTCCTTGTGCTGTAG	HEX	164	AJ496560
Cavy 3	$[CT]_n$	ACAGCGATCACAATCTGCAC	GCAGTGGTAACCCAGAATGG	NED	225	AJ496561
Cavy 4	$[GT]_n$	GTTGTATCTTCAAAGCCCTGAC	AATACAGTGTGCCCCAGCA	FAM	119	AJ496562
Cavy 5	$[AAGG]_n$	CTCCATTCACAGAGTGGTCT	AAAGTGCTGTTTAATTGGGA	FAM	423	AC190431
Cavy 6	$[TCTT]_n$	GTACCAGGGATCAAACTCAG	GAGCTTTCGAGAGTACGAGA	FAM	388	AC190431
Cavy 7	$[AGG]_n$	TGGACCTCCAGGTACTACAC	GTGACCCTGCAACATTTCT	NED	404	AC185540
Cavy 8	$[TTC]_n$	CCCTTTCCCACTCTTCTATT	CTGCCAGCTTAGCAATTTAT	FAM	290	AC189989
Cavy 9	$[TCT]_n$	CAGCGATCTTCTATGGAGAC	TCTTTAATGGTGGGTTTCAG	HEX	192	AC192512
Cavy 10	$[AAAG]_n$	ATGAACTTCAACATGGATGG	CCCTCTGAGATCTTTCCTCT	FAM	398	AC174822
Cavy 11	$[CT]_n$	TCAGAAAGCTGGAAATTCAT	AATGTGATGTGCTGAACAGA	NED	379	AC192015
Cavy 12	$[AG]_n$	TCCCTGTTCTTTGCTACAAT	CTGCTTCATAGATCTTGCCT	HEX	236	AC182323
Cavy 13	$[AGG]_n$	AGGGAGGCCAGAGTGAGAG	TCCTACACTGCATTGCTTGC	NED	385	AC189135
Cavy 14	$[CT]_n$	AGTGTTGGCAGCTTGATCCT	AGCTCACCAGGGAAAATGTG	FAM	367	AC190428
Cavy 15	$[AG]_n$	TTCATGCTACCTGGCACTTG	TTGCAGGCAATATGGCATTA	NED	238	AC191184
Cavy 16	$[CT]_n$	CCAGTGGATTGGGAGACATT	CTCACCAAGAATGCAAAGCA	HEX	381	AC194996

* Forward primers include the M13 sequence (CACGACGTTGTAAAACGAC) at the 5' edge.
** Suggested dyes for four multiplex panels.
*** Annealing temperatures standardized when possible for multiplex amplification.
**** Expected base pair PCR product size according to the original sequence in GenBank including the M13 tail.

Table 2: Diversity indexes for all 16 loci in both species. N_A is the number of alleles, H_e and H_o are the expected and observed heterozygosities respectively.

	N_A		H_e		H_o	
Locus	*C. magna*	*C. aperea*	*C. magna*	*C. aperea*	*C. magna*	*C. aperea*
Cavy 1	10	7	0.721	0.617	0.444*	0.550
Cavy 2	4	11	0.592	0.842	0.600	0.800
Cavy 3	6	10	0.677	0.806	0.700	0.750
Cavy 4	2	6	0.224	0.628	0.250	0.750
Cavy 5	7	-	0.554	-	0.500*	-
Cavy 6	8	6	0.822	0.697	0.789	0.750
Cavy 7	3	12	0.286	0.790	0.316	0.600
Cavy 8	5	6	0.747	0.656	0.533	0.778
Cavy 9	7	7	0.671	0.740	0.700	0.650
Cavy 10	7	9	0.832	0.681	0.450*	0.700
Cavy 11	7	16	0.821	0.882	0.900	0.765
Cavy 12	3	3	0.537	0.099	0.700	0.100
Cavy 13	5	-	0.659	-	0.550	-
Cavy 14	8	-	0.784	-	0.647	-
Cavy 15	5	-	0.721	-	0.500	-
Cavy 16	14	9	0.856	0.738	0.588	0.588
Mean	6.313	8.500	0.65647	0.68132	0.57302	0.64839
	7.406		**0.669**		**0.611**	

*Denotes significant HWE departure (P<0.01).

CAPÍTULO II: DEPAUPERATED GENETIC DIVERSITY OF THE MOLEQUES DO SUL CAVY (*CAVIA INTERMEDIA*), THE NATURALLY RAREST MAMMAL IN THE WORLD

(Artigo a ser submetido ao periódico *Molecular Ecology*)

Original Article – Population and Conservation Genetics

Depauperated genetic diversity of the Moleques do Sul cavy (*Cavia intermedia*), the naturally rarest mammal in the World

Ricardo Kanitz[1], Carlos Henrique Salvador[2], Sandro L. Bonatto[1]

[1] Faculdade de Biociências, PUCRS. Av. Ipiranga 6681, prédio 12C. Porto Alegre, RS 90619-900, Brazil.

[2] Instituto de Biologia, UFRJ. Ilha do Fundão. Rio de Janeiro, RJ 21941-590, Brazil.

Keywords: *Cavia magna*, island endemic species, population genetics, speciation, demography, ABC.

Corresponding author: Sandro L. Bonatto, Faculdade de Biociências, PUCRS. Av. Ipiranga 6681, prédio 12C, sala 172. Porto Alegre, RS 90619-900, Brazil. Fax: +55 (51) 3320-3612. E-mail: slbonatto@pucrs.br

Running title: Low genetic diversity of *Cavia intermedia*.

Abstract

Islands have been calling the attention of evolutionary biologists for centuries for their high level of endemic species fostered by isolation and limited population size. *Cavia intermedia* is a recently described cavy species endemic to a small island in the southern Brazilian coast, that seems to be the naturally rarest known mammal, having a stable population around 40 individuals. Although previous demographic simulations estimated its probability of extinction in 100 years as 100%, given the island history, it is unlikely that its population size could have been much larger in the recent past. Using mitochondrial and microsatellite markers, we have found that this insular cavy presents an extremely reduced genetic diversity with an estimated historical and present effective population size matching its present census size, with no evidence of recent population reduction. Considering *C. intermedia* long divergence from its sister continental species, we concluded that it keeps this extremely reduced population size since at least the most recent separation of the island from the continent around 8,000 years ago. *C. intermedia* may therefore be the best and most extreme case so far of a mammal species that survived for thousands of years with an extremely reduced population size. This species then poses new challenges to understanding the role of population reduction and genetic diversity to species extinction. Finally, we emphasize the need for special care in the maintenance of the pristine conditions of *C. intermedia*'s habitat.

Introduction

In conservation biology, island endemic populations are of special interest and concern. While in one hand the insular ecosystems maintain the major part of the biological richness in the World (Myers *et al.* 2000); in the other, their biota may be more susceptible to extinction than that in mainland given their limited population size (Frankham 1998, Alcover *et al.* 1998). There are many examples in the literature of insular species that underwent extinction in the historical time. Since the classical examples of the dodo extinction (Fuller 2002) by hunting in the 17th century to the more recent introduction of the brown treesnake that initiated a series of extinction of several populations in the island of Guam during World War II (Fritts & Rodda 1998). Other taxa did not become extinct, but faced severe problems to survive (e.g. the Española's Giant Tortoises population in the Galápagos was reduced to only 14 individuals in 1965 (MacFarland *et al.* 1974)). These populations were depleted by human interference; therefore their reduced population sizes were caused by recent events such as hunting, habitat destruction and the introduction of exotic predators.

One frequent fact that underpins examples of extreme population reduction is that these populations also faced severe reductions in their effective population sizes (N_e; Wright 1931). Populations whose N_e is depressed are considered more prone to extinction by both environmental and genetic causes. Among the environmental reasons, there are three main categories: (i) demographic and (ii) environmental stochasticity, and (iii) natural catastrophes (Shaffer 1981). The first might be caused by the intrinsic properties of the population (e.g. lowered number of breeders of one gender); the second, by the habitat characteristics (e.g. variable climate, natural or introduced predators and competitors, etc.); and the third, mainly by unpredictable events (e.g. earthquakes, floods, hurricanes, etc.).

62 The small size of a population may lead to reduced adaptability (loss of evolutionary
63 potential) and inbreeding depression (Frankham *et al.* 2002). These genetic problems might
64 not directly result in population extinction, but may intensify this process magnifying
65 environmental extinction causes (Brook *et al.* 2002). Considering all these environmental and
66 genetic factors, it has been proposed and it is generally accepted that, for short-term survival
67 in the wild, an isolated population should maintain a N_e of no less than 50; and for long-term
68 survival, an effective size of at least 500 would be necessary (Franklin 1980).
69 In 1999, a new mammal species was described endemic of a very small (10.5 ha)
70 coastal island in Southern Brazil (Figure 1): *Cavia intermedia* (Cherem *et al.* 1999). Moleques
71 do Sul is arguably the smallest known Island with an endemic mammal species (several times
72 smaller than that previously known in the California Channels Islands, Alcover *et al.* 1998,
73 Knowlton *et al.* 2007). The Moleques do Sul cavy is a hystricognath rodent related to the
74 guinea pig (*C. porcellus*). Although initially considered an isolated population of its putative
75 sister continental species *C. magna*, *C. intermedia* presents several morphological differences
76 from that taxon, such as feet and teeth shape, cranial structure, and coat color, the latter being
77 of an intermediary hue between the two continental species *C. magna* and *C. aperea* (Cherem
78 *et al.* 1999).
79 More recently, two thorough studies on *C. intermedia* ecology and demography
80 (Salvador & Fernandez 2008a; 2008b) showed that the species as a whole has an average of
81 42 individuals as its census population size (N_c) varied from around 30 to 60 during the
82 observed period of 15 months. Given its extremely restricted habitat and much reduced
83 population size, *C. intermedia* may likely be the naturally rarest known mammal.
84 Furthermore, Salvador (2006) using demographic simulations preliminarily estimated *C.*
85 *intermedia* extinction probability in 100 years as 100%. Such condition, with a much reduced

86 population size, placed the Moleques do Sul cavy in the IUCN Red List of Threatened
87 Species (Chapman 2008) as Critically Endangered (IUCN 1994, 2001).

88 Geological evidences show that Moleques do Sul largest island, where the species
89 lives, has been isolated and has maintained its current size for at least 8,000 years, since the
90 rise of the sea level in the present interglacial period (Cherem *et al.* 1999). This suggests that
91 *C. intermedia* may have been isolated in this extremely small island for a long time. This
92 scenario is reinforced since *C. intermedia* exhibits a chromosome number different from the
93 other species of *Cavia* – as studied by Gava *et al.* (1998) before the species' description – and
94 an almost complete island syndrome [which is a combinations of adaptive traits commonly
95 observed in insular taxa (Adler & Levins 1994)] with lower reproduction and greater
96 longevity (Salvador & Fernandez 2008b). If this scenario is true, *C. intermedia* poses an
97 interesting challenge to conservation genetics and an extraordinary model for evolutionary
98 biology, since it would be the first known example of a mammal species with a very long
99 history of a naturally extremely reduced population size. However, no genetic study has been
100 done so far in this species to test this hypothesis and, if true, to evaluate its consequences for
101 the genetic architecture of the species.

102 Here, we present a thorough assessment of the genetic diversity of the species using
103 both mitochondrial DNA and nuclear microsatellite markers to test the above scenario. For
104 this, we estimated both the historical and the current effective population sizes for *C.*
105 *intermedia* using several approaches including the newly developed approximate Bayesian
106 computation (ABC). Moreover, we tested the hypothesis that the continental *C. magna* is *C.*
107 *intermedia* sister species, estimated their minimal divergence time, and contrasted their
108 genetic diversities.
109

Material and Methods

Sampling, DNA extraction and PCR – C. intermedia tissue samples were collected from ear tips from 70 individuals between March 2004 and June 2005 in Moleques do Sul Archipelago covering three generations in a capture-mark-recapture program described in Salvador & Fernandez (2008a). Nineteen *C. magna* tissue samples from northern Uruguay were obtained by Kraus *et al.* (2005) and were kindly provided by the authors. Two additional samples of *C. magna* from Santa Catarina state were captured by bait trapping in September 2007 in the district of Pinheira/SC, just in front of the Moleques do Sul islands (Figure 1) using the same approach applied in Salvador & Fernandez (2008a, 2008b) and observing the ethical guidelines in Gannon *et al.* (2007). Four other *C. magna* samples from Rio Grande do Sul state used in the mtDNA analyses were obtained by our group. The ear tissue samples were maintained in a solution with 70% ethanol and kept in our laboratory. DNA was extracted using a salt-precipitation protocol (Medrano *et al.* 1990) and the extractions were verified and quantified in 1% agarose gel stained with GelRed®. For standardization purposes, all DNA samples were diluted to ~5ng/µL in water Milli-Q®. The microsatellite loci, as well as the primers and PCR amplification condition used here were detailed elsewhere (Kanitz *et al.* in preparation). In summary, 12 STR loci were developed to be highly informative using the draft genomic sequence of *C. porcellus* and tested in *C. magna* and *C. aperea* (*Cavy1*, *Cavy2*, *Cavy4*, *Cavy6*, *Cavy7*, *Cavy8*, *Cavy9*, *Cavy10*, *Cavy12*, *Cavy13*, *Cavy14*, and *Cavy16*). Amplification of the mtDNA HVSI control region was done using the following primers: 'CCCAAARCTGRWATTCTWATTAAACT' as forward; and 'ATGGCCCTGAAGWAAGAACC' as reverse. These primers were designed based on the *C. porcellus* complete mitochondrial genome sequence (accession number AJ222767) as well as other rodent species mtDNA sequences available in GenBank looking for conserved regions.

The primers we used for the *cytochrome b* amplifications were 'ATTCCTACATGGAGTTTAACCATGAC' and 'CCCATCTCTGGCTTACAAGACCAG'. All mtDNA reactions were performed in a 20 μL reaction using 10 ng of genomic DNA, with 100 μM of all dNTPs, 0.25 μM of each the reverse and the forward primers, 1.5 mM MgCl2, 1X PCR Buffer and 1 U of *Taq* DNA polymerase *Platinum* (Invitrogen). The thermal profile used for this locus was as follows: initial denaturing for 3 min at 94 ºC, followed by 35 cycles of 94 ºC for 45 s, 50 ºC for 45 s and 72 ºC for 1 min. After this, there were more 5 min for final extension at 72 ºC and cooling at 4 ºC.

Genotyping and sequencing – The microsatellite amplified products were separated in an automated sequencer MegaBACE1000 (GE Healthcare®) and genotyped in the software Genetic Profiler v.3.1 (GE Healthcare®) using different dyes for each locus. The mtDNA segments were sequenced after enzymatic purification with Exonuclease-I and Shrimp Alkaline Phosphatase. The sequencing reactions were performed using the ET Terminator cycle sequencing kit and run in a MegaBACE1000 (GE Healthcare®). The chromatogram reads (forward and reverse) were assembled and visualized using the Phred-Phrap-Consed package (Ewing *et al.* 1998; Gordon *et al.* 1998).

Genetic diversity – The mtDNA segments were aligned using muscle3.6 (Edgar 2004) which were visually checked in BioEdit7 (Hall 1999). Summary statistics were calculated using DnaSP4.5 (Rozas *et al.* 2003) and Arlequin 3.1 (Excoffier *et al.* 2005). We also constructed haplotype networks using median-joining (Bandelt *et al.* 1999) as implemented in Network 4.5.1 (http://www.fluxus-engineering.com). For the STR markers, we performed the calculation of the genetic summary statistics and Hardy-Weinberg equilibrium test in Arlequin 3.1 (Excoffier *et al.* 2005). Moreover, we calculated the fixation index between the two species using the STR loci (with F_{ST} and R_{ST}).

158 *Divergence time* – The divergence time between *C. intermedia* and *C. magna* was
159 calculated using the two mtDNA regions (HVSI and *cytochrome b*) independently. We used
160 the Bayesian approach implemented in BEAST 1.4.8 (Drummond & Rambaut. 2007) setting a
161 calibration point for the Hystricomorpha (formerly Caviomorpha) diversification as estimated
162 in Wyss *et al.* (1993) and applied in Huchon & Douzery (2001) of ~32 million years ago
163 (MYA). The *C. intermedia* and *C. magna* sequences were aligned – using muscle3.6 (Edgar
164 2004) – with other sequences of species belonging to the Hystricomorpha group: *C. porcellus*
165 (GenBank accession numbers AF491746 for HVSI; DQ017047 for *cytochrome b*),
166 *Hydrochaerus hydrochaeris* (EU149776 and FJ430787), *Agouti paca* (three individuals for
167 each region, AY206599 and AY206574, AY206598 and AY206573, and AY206597 and
168 AY206572), *Ctenomys torquatus* (EU530577 and EU519318), *C. talarum* (EF531750 and
169 AF144283), *C. rionegrensis* (AY755461 and AF538377), *Octodon degus* (three individuals
170 for each region, AY007365 and AM407929, AY007364 and AF422914, and AY007363 and
171 AF007059), *Echimys didelphoides* (EU313280 and EU302705), *E. chrysurus* (EU313262 and
172 EU313213), and *E. macrurus* (EU313289 and EU302703). We applied the Uncorrelated
173 Lognormal Relaxed clock with the HKY mutational model using four categories of gamma
174 correction with no fixed mutation rate. We set the root age as our calibration point with a
175 uniform prior from 31 to 33 MYA. Each HVSI and *cytochrome b* runs took 10 million
176 MCMC iterations sampled every 1,000 steps after a 10% initial burn-in. We checked the
177 results in the software Tracer1.4 (http://beast.bio.ed.ac.uk).

178 *N_e estimations* – Two approaches were used for estimating N_e for *C. intermedia*: the
179 variance based N_e (N_{eV}) and the inbreeding based N_e (N_{eI}) (see Leberg 2005 for a review). For
180 the first approach, we divided our *C. intermedia* sample in two temporal separated sets. The
181 first generation comprised 23 individuals collected in April 2004; the second, 17 individuals

182 in March 2005. The time between these two samples (one year) is enough for fitting three new
183 offsprings according to previous studies (Salvador & Fernandez 2008a), but also may contain
184 individuals from previous generations, so we assumed to have in average two separated
185 generations (i.e. a generation time of six months). The N_{eV} calculations were made using three
186 approaches: the Nei & Tajima (1981) moment-based and the maximum-likelihood (ML)
187 estimates with the marginal-probability method (Wang 2001), both implemented in the
188 program MLNE (Wang & Whitlock 2003), and a Monte Carlo coalescent-based search
189 (Berthier *et al.* 2003) using CoNe program (Anderson 2005). We ran the ML MLNE analysis
190 assuming no migration from any source population, maximum N_e of 10,000, and the number
191 of generations between samples of two. For CoNe, we assumed a uniform prior for N_e
192 between 4 and 10,000, and we ran 100,000 Monte Carlo replicates which should be enough
193 for acquiring confident results according to the program manual (Anderson 2005).

194 The N_{eI} estimation consisted of two search strategies, the first being a ML method for
195 estimating θ implemented in the program Lamarc 2.1.3 (Kuhner 2006). After trial searches,
196 Lamarc was run with 10 short chains and two long chains of 10,000 and 200,000 iterations,
197 respectively, using the stepwise mutational model (SMM) (Kimura & Ohta 1978) and the
198 variable loci only. Estimates of N_e were made using the mutation rate, $\mu=5 \times 10^{-4}$ in the
199 equation $N_e = \theta/4\mu$.

200 The second approach used for estimating N_{eI} was the recently developed approximate
201 Bayesian computation (ABC) as implemented in the programs DIYABC (Cornuet *et al.* 2008)
202 and ONeSAMP (Tallmon *et al.* 2008). In DIYABC, we tested three scenarios consisting of
203 one population with different priors for N_e. Scenario 1 had N_e between 4 and 100 individuals;
204 Scenario 2, between 100 and 1,000; and Scenario 3, between 1,000 and 10,000 individuals.
205 We assumed the 12 STR loci to evolve under the more realistic generalized stepwise mutation

model (GSM) (Estoup *et al.* 2002) which is a simplified version of the two-phase mutation model (TPM) (Di Rienzo *et al.* 1994), with mutation rate free to vary from 1×10^{-4} to 1×10^{-2} and the coefficients of geometric distribution (P) from 0.1 to 1. Motif sizes and prior allele ranges were adjusted to fit our real loci. The summary statistics recorded for each simulation were the mean (i) number of alleles (A), (ii) genetic diversity (H_e), (iii) allelic size range (AR), and (iv) Garza-Williamson's index (M) (Garza & Williamson 2001). After preliminary simulations for adjusting the priors, we ran 17,000,000 simulations for testing between the alternative models and estimation of the posterior probability distribution of the parameters. We assessed the posterior probability of each scenario using both direct estimate and logistic regression approaches using the 500 and 5,000 best simulations respectively. The best simulations under an ABC approach are the ones with the closest values of summary statistics to the observed data's summary statistics. For the best supported scenario, we estimated the posterior distribution of the parameters N_e, θ, μ and P using the logit transformation for the 5000 best simulations. In addition to the DIYABC estimations, we performed a N_e estimation in ONeSAMP assuming the N_e prior limits (4-100) according to the most supported scenario in DIYABC. We used only the variable loci in this analysis because ONeSAMP cannot deal with monomorphic data.

Additionally to the historical and current N_e estimations, we tested for recent ($t < 4N_e$ generations) bottlenecks in *C. intermedia* using the program Bottleneck version 1.2.02 (Piry *et al.* 1999). Based on the observation that recently occurring population declines cause rarer alleles to disappear more rapidly than others, this approach is designed to test for the excess of expected heterozygosity in the population following the rationale presented in Luikart & Cornuet (1999). We applied these tests for the two different generations used in the N_{eV} estimations as well as the whole sample across the generations used in the N_{eI} estimations. For

both sampling strategies, we ran 1000 replications assuming different mutational models [SMM, TPM and IAM (Kimura & Crow 1964)].

Results

Genetic Diversity – We obtained HVSI sequences for 28 *C. intermedia* and 25 *C. magna* individuals. In the 368 nucleotide positions sequenced, we found a single haplotype for *C. intermedia* and five for *C. magna* with 13 variable sites in the whole. This and other summary statistics are presented in the Table 1. For the 12 STR loci, we genotyped all 70 *C. intermedia* individuals, focused in individuals from at least two separated generations, plus 21 *C. magna*, including the two specimens from the probably closest population to the insular species (from Pinheira/SC). The average missing data was 3.5% per locus. There were only four variable loci in *C. intermedia* while all 12 loci were variable to *C. magna*. Both the STR and HVSI summary statistics show a remarkable discrepancy between the genetic diversity of the two species in which the insular cavy is far less variable than its continental sibling in all studied loci (Table 1).

For the fixation indices between the two species using the STR loci, we have found an average F_{ST} of 0.704 and an R_{ST} value of 0.935 where both were highly significant ($p<0.001$). Furthermore, a large number of private alleles (88%) were found between the species. For the HVSI and *cytochrome b* mtDNA regions, the mean uncorrected divergences between the two species are 6.7% and 1.2%, respectively. In the HVSI haplotype network (Figure 2), there are 11 mutational events separating the closest haplotypes of the two species, indicating a complete isolation and a strong divergence between the two species using both kinds of markers. *C. magna* presents a much higher diversity with very divergent haplotypes that are geographically structured, where the northern samples (from SC in front of Moleques do Sul

254 Islands) are genetically closest to *C. intermedia* (Figure 2). These results support the
255 hypothesis of *C. intermedia* isolation from the nearly distributed populations of *C. magna*.

256 *Divergence Time* – Assuming a calibration point for the Hystricomorpha radiation as
257 32 MYA (Wyss *et al.* 1993, Huchon & Douzery 2001), we were able the estimate the
258 divergence time between the *C. intermedia* and the *C. magna* mtDNA lineages. Using both
259 the HVSI and the *cytochrome b* partial sequences independently, the separation time between
260 both species were 1.73 and 1.41MYA respectively. The 95%HPD intervals were from 0.72 to
261 3.08 MYA for the HVSI; and 0.38 to 3.15 MYA for the *cytochrome b*. In Figure 3, we show
262 the *cytochrome b* Bayesian phylogenetic tree with the above time estimates. Both the
263 *cytochrome b* and HVSI (not shown) phylogenies recovered virtually the same topology
264 except for the positioning of the capybara (*H. hydrochaeris*) which grouped with the genus
265 *Cavia* in the HVSI reconstruction. Furthermore, using the 32 MYA calibration point, the
266 posterior estimates for the mean mutation rates for each region were very similar: 0.77% per
267 million years for the *cytochrome b*, and 1.13% per million years for the HVSI. Also, these
268 estimates – especially for the *cytochrome b* – are very close to the conventional value of 1%
269 per million years for the mtDNA mutation rate (Avise 2000).

270 *Effective Population Size* – The estimation of the *C. intermedia* current effective
271 population size (N_{eV}) were very consistent among the different methods and provided values
272 (Table 2) very close to the observed N_c of 42 individuals (Salvador & Fernandez 2008a).
273 Furthermore, the comparison of the three tested scenarios for N_{el} strongly supports the
274 scenario with the smallest population size (Figure 4). In this scenario, the median posterior
275 estimate for N_e is 29 (Table 2, Figure 5). The estimated historical population sizes (N_{el}) are
276 only slightly smaller than the N_{eV} (Table 2) suggesting that no substantial variation in the
277 species' population size might have happened in its recent evolutionary past. This hypothesis

278 is supported by the tests in the program Bottleneck in which none of the Wilcoxon signed
279 tests were significant independently of the different mutation models and sampling strategies
280 used.
281
282 **Discussion**
283 Our mtDNA divergence time estimations have shown that the *C. intermedia* haplotype
284 diverged from the *C. magna* lineage at least ~0.4 million years in the past. Although this
285 mtDNA divergence likely predates *C. intermedia* speciation for an unknown period of time,
286 the very high divergence found in the microsatellite data, the clear morphological and
287 cytological differences all strongly suggest that the insular species diverged from its
288 continental sister-species very long ago, tens of thousands of years before the present.
289 *C. intermedia* population size was quite stable as observed during 15 months in
290 Salvador & Fernandez (2008a). All our results corroborate this scenario of high population
291 size stability, very likely for a long period, since both the historical and current N_e estimations
292 (N_{eI} with the N_{eV}, Table 2) provided very similar results and all genetic results supported
293 demographic stability. In special, there is no evidence for a recent population bottleneck, that
294 is, that the population size of the species was larger anytime in the past than its present size.
295 Although the absence of variability in the mtDNA of this species may be explained by a
296 selective sweep, all results from the nuclear microsatellites strongly corroborate that its low
297 diversity is a consequence of the population demography. Moreover, *C. intermedia* seems to
298 maximize its N_e. The N_{eV} assessments are not different from N_c estimated in Salvador &
299 Fernandez (2008a), around 40-45 individuals. This maximization is probably due to the
300 demographic characteristics noticed in Salvador & Fernandez (2008b) as part of the island
301 syndrome (Adler & Levins 1994). The population tends to keep its sex ratio always close to

1:1 and most of the population is composed by sexually mature individuals, increasing the effective breeding number and making the N_{eV} to closely approach the N_c. Most of these features could have been fixed by selection and are common among other rodent island endemic species (Glivitz 1980, Crowel 1983, Adler 1996).

Considering these results, one question that rises is for how long this species maintained this extremely reduced population size? The island itself is very small (10.5 ha) and has a very limited resources supply (the area actually occupied by the grasses that are the main food of the species is only 0.77 ha) and hardly could support a much bigger population than it actually does. In fact, it has been shown that the *C. intermedia* population size is regulated by density-dependent mortality (Salvador & Fernandez 2008a). Besides, there is no support for a any significant change in the area of the island in the recent past, considering the island morphology and the bathymetric data (Cherem *et al.* 1999), and given that the sea level have not changed more than about 2 m in at least the last 7,000 years (Pirazzoli & Pluet 1991, Milne *et al.* 2005). This is also very likely valid for the vegetation coverage of the Island. Migration is also very unlikely; the closest *C. magna* population is in the continent 14 km over sea away from Moleques do Sul. Additionally, there is no evidence of occurrence of any *Cavia* species in the few other islands in the nearby area. This includes the larger island of Florianopolis (Figure 1) where several rodent species currently occur (Graipel *et al.* 2001) or were found to occur before modern human occupation in the last centuries as found in zooarcheological studies (Castilho & Simões-Lopes 2001). Furthermore, *C. intermedia* itself was found only in Moleques do Sul to date, and it is very unlikely to be found anywhere else in the continent, since it was never observed in any study in the nearby region (Cherem & Perez 1996, Castilho & Simões-Lopes 2001, Graipel *et al.* 2001, Cherem *et al.* 2004, Graipel *et al.* 2006) and the coastal region is dominated by the sister *C. magna*. All this suggests that

326 the *C. intermedia* likely originated in the Moleques do Sul Island as a peripheral population of
327 *C. magna* and that has remained isolated there for at least 8,000 years. In summary, our
328 results suggest that this single population of the species may have maintained an extremely
329 small size (<100 individuals) for several thousand years.

330 It also seems that inbreeding depression is not taking place in *C. intermedia*. Most
331 individuals look healthy and there is no evidence of high young mortality (Salvador &
332 Fernandez 2008a). This might be due to a long term purging of the species gene pool through
333 purifying selection given its very low population size and long time in isolation (Frankham *et*
334 *al.* 2002). The maximization of its N_e described above should have helped it to survive for a
335 longer time, although an effective size of ~40 for an isolated population is still extremely low,
336 making this uniquely lucky species still at serious risk of extinction.

337 Along with its extremely reduced population size, *C. intermedia* shows particularly
338 low indices of genetic diversity. Its sister taxon, *C. magna*, is at least seven times more
339 diverse (H_e) for the microsatellite loci. Furthermore, *C. intermedia* shows a very limited
340 genetic diversity even if we compare to other mammals with naturally reduced diversity as the
341 African cheetah (Marker *et al.* 2008), the South-American manned wolf (SLB and
342 collaborators, unpublished results), or the brown bears (Paetkau *et al.* 1998). However, these
343 species have population sizes of reasonable sizes, having recovered from ancient (thousands
344 of years ago) population bottlenecks. Reduced diversities as that we found here for the
345 Moleques do Sul cavy were found to date in species that suffered extreme decline caused by
346 recent (tens to a few hundreds years) anthropic impacts, such the Hawaiian monk seal
347 (Schultz *et al.* 2009) and the northern elephant seal (Hoelzel *et al.* 1993,Weber *et al.* 2000).
348 However, the Madagascar fish-eagle (Johnson *et al.* 2009) is perhaps the only species so far in
349 which there is convincing evidence for a long period of very reduced genetic diversity

coupled with naturally very small population abundance. Its present abundance is at least 220 individuals over a relatively large range in northwestern Madagascar coast, and this number is certainly an underestimate, given that there are regions that were not yet thoroughly surveyed (www.unep-wcmc.org/species/data/species_sheets/fisheagl.htm, www.iucnredlist.org/details/144338). Anyway, our results suggest that *C. intermedia* underwent a similar history of long-term reduced size, although at much more extreme conditions.

All the above results led to the relevant question of how *C. intermedia* could have survived for so long considering the *a priori* prediction of high probability of extinction? We hypothesize that *C. intermedia* overcame deleterious inbreeding effects a long time ago and genetic purging is probably the answer. This purifying selective force acts on inbred populations by sieving the individuals expressing the recessive deleterious genes present in the population gene pool (Frankham *et al.* 2002, Crnokrak & Barrett 2002, Frankham 2005). So, the Moleques do Sul cavy does not suffer with inbreeding depression because, very probably, very few or no deleterious genes continue to exist in this population. However, genetic diversity is also important for keeping evolutionary potential (Frankham *et al.* 2002). A changing environment poses new selective pressures to the populations over time. For better adapting to these changes, a population should have a reservoir of genetic possibilities (Franklin & Frankham 1998). However, *C. intermedia* seems to face a very stable environment is the last ~6,000 years (Scheel-Ybert 2000) Therefore, it probably did not have to deal with changing selective pressures during most part of its isolation, making ineffective the need for evolutionary potential in its case.

In summary, we found that *C. intermedia* presents an extremely reduced neutral genetic diversity that is very likely a consequence of thousands of years of an extremely small

374 population size and that it likely survived given the relative environmental stability and got
375 over inbreeding depression through purging. This scenario brings *C. intermedia* as a very
376 special case in the evolutionary literature as possibly the only known living mammal species
377 with a long history with such an extremely reduced population size, making it an excellent
378 textbook case for studying the consequences of very long reduced population sizes in the
379 natural habitat. It is important to further study this extraordinary species, such as to better
380 characterize its genetic diversity at the genomic level and in special at non-neutral loci, such
381 as the MHC loci (and other loci subject to balancing selection) to better understand its
382 extreme evolutionary history. Also, we would like to reinforce the need for greater care
383 against the threats this population might be subject to, like irregular human visits to the island
384 and introduction of predators or competitors which, in spite of any possible genetic
385 adaptation, might suddenly lead this remarkable species to extinction.
386
387 **Acknowledgements**
388 We would like to thank to Luisa Lemos Goellner and Raoni Rocha Postiglione for
389 their fundamental help in laboratory work as well as to André Schnorr and Cladinara Roberts
390 Sarturi for technical assistance. Thanks also to Jorge Cherem and Nina Furnari for the
391 exchange of unpublished information about theirs studies and to Manoel Fontoura-Rodrigues
392 and Henrique V. Figueiró for field work assistance. RK was supported by a CNPq master
393 scholarship, and SLB has grant supports from CNPq and FAPERGS.

394 **References**

395 Adler GH, Levins R (1994) The island syndrome in rodent populations. *Quarterly Review of*
396 *Biology*, **69**, 473-489.

397 Adler GH (1996) The island syndrome in isolated populations of a tropical forest rodent.
398 *Oecologia*, **108**, 694-700.

399 Alcover JA, Campillo X, Macias M, Sans A (1998) Mammal species of the world: additional
400 data on insular mammals. *American Museum Novitates*, **3248**, 1-29.

401 Anderson EC (2005) An Efficient Monte Carlo Method for Estimating N_e From Temporally
402 Spaced Samples Using a Coalescent-Based Likelihood. *Genetics*, **170**, 955-967.

403 Avise JC (2000) *Phylogeography: the History and Formation of Species*, Harvard University
404 Press, Cambridge.

405 Babik W, Pabijan M, Arntzen JW, Cogalniceanu D, Durka W, Radwan J (2009) Long-term
406 survival of a urodele amphibian despite depleted major histocompatibility complex
407 variation. *Molecular Ecology*, **18**, 769-781.

408 Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific
409 phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.

410 Berthier P, Beaumont MA, Cornuet JM, Luikart G (2002) Likelihood-based estimation of the
411 effective population size using temporal changes in allele frequencies: a genealogical
412 approach. *Genetics*, **160**, 741-751.

413 Brook BW, Tonkyn DW, O'Grady JJ, Frankham R (2002) Contribution of inbreeding to
414 extinction risk in threatened species. *Conservation Ecology*, **6**, 16. (online
415 http://www.consecol.org/vol6/iss1/art16).

Castilho PV, Simões-Lopes PC (2001) Zooarqueologia dos mamíferos aquáticos e semi-aquáticos da Ilha de Santa Catarina, sul do Brasil. *Revista Brasileira de Zoologia*, **18**, 719-727.

Chapman RE (2008) *Cavia intermedia*. In: *IUCN Red List of Threatened Species*. World Conservation Union, Gland, Switzerland.

Cherem JJ, Perez DM (1996) Mamíferos terrestres de floresta de araucária no município de Três Barras, Santa Catarina, Brasil. *Biotemas*, **9**, 29-46.

Cherem JJ, Olimpio J, Ximenez A (1999) Descrição de uma nova espécie do gênero *Cavia* Pallas, 1766 (Mammalia – Caviidae) das Ilhas dos Moleques do Sul, Santa Catarina, Sul do Brasil. *Biotemas*, **12**, 95-117.

Cherem JJ, Simões-Lopes PC, Altohoff S, Graipel ME (2004) Lista dos Mamíferos do Estado de Santa Catarina, Sul do Brasil. *Mastozoología Neotropical*, **11**, 151-184.

Cornuet JM, Santos F, Beaumont MA, Robert CP, Marin JM, Balding DJ, Guillemaud T, Estoup A (2008) Infering population history with DIYABC: a user-friendly approach to Approximate Bayesian Computation. *Bioinformatics*, (in press).

Crnokrak P, Barrett SC (2002) Purging the genetic load: a review of the experimental evidence. *Evolution*, **56**, 2347-2358.

Crowell KL (1983). Island – insight or artifact? population dynamics and habitat utilization in insular rodents. *Oikos*, 41, 442–454.

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences USA*, **91**, 3166–3170.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

440 Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high
441 throughput. *Nucleic Acid Research*, **32**, 1792-1797.
442 Eldridge MDB, King JM, Loupis AK, Spencer PBS, Taylor AC, Pope LC, Hall GP (1999)
443 Unprecedented Low Levels of Genetic Variation and Inbreeding Depression in an Island
444 Population of the Black-Footed Rock-Wallaby. *Conservation Biology*, **13**, 531-541.
445 Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci
446 and their consequences for population genetics analysis. *Molecular Ecology*, **11**, 1591-
447 1604.
448 Ewing B, Hillier L, Wendl MC, Green P (1998) Base-calling of automated sequencer traces
449 using phred. I. Accuracy assessment. *Genome Research*, **8**, 175–185.
450 Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package
451 for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47-50.
452 Frankham R (1995) Conservation Genetics. *Annual Reviews in Genetics*, **29**, 305-327.
453 Frankham R (1998) Inbreeding and Extinction: Island Populations. *Conservation Biology*, **12**,
454 665-675.
455 Frankham R, Ballou JD, Briscoe D (2002) *Introduction to Conservation Genetics*. Cambridge
456 University Press, Cambridge.
457 Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131-140.
458 Franklin, IR (1980) Evolutionary change in small populations In *Conservation biology: an*
459 *evolutionary-ecological perspective* 135-140. Soulé, M.E. & Wilcox, B.A. (Eds)
460 Sunderland: Sinauer.
461 Franklin IR, Frankham R (1998) How large must populations be to retain evolutionary
462 potential? *Animal Conservation*, **1**, 69-73.

463 Fritts TH, Rodda GH (1998) The role of introduced species in the degradation of island
464 ecosystems: a case history of Guam. *Annual Review of Ecology and Systematics*, **29**,
465 113-140.
466 Fritts TH, Snell HL, Cayot L, MacFarland C, Earsom S, Márquez C, Llerena W, Llerena F
467 (2000) Progress and priorities in research for the conservation of reptiles. *Bull de*
468 *l'Institut Royal des Sciences Naturelles de Belgique*, **70**, 39-45.
469 Fuller E (2002) *Dodo: a Brief History*. Universe Books, New York.
470 Gannon WL, Sikes RS, The Animal Care and Use Committee of the American Society of
471 Mammalogists (2007) Guidelines of the American Society of mammalogists for the use
472 of wild mammals in research. *Journal of Mammalogy*, **88**, 809-823.
473 Garza JC, Williamson EG (2001) Detection of reduction in population size using data from
474 microsatellite loci. *Molecular Ecology*, **10**, 305-318.
475 Gava A, Freitas TRO, Olimpio J (1998) A new karyotype for the genus *Cavia* from a southern
476 island of Brazil (Rodentia - Caviidae). *Genetics and Molecular Biology*, **21,** 77-80.
477 Gliwicz J (1980) Island populations of rodents: their organization and functioning. *Biology*
478 *Review*, **55,** 109-138.
479 Graipel ME, Cherem JJ, Ximenez A (2001) Mamíferos terrestres não voadores da Ilha de
480 Santa Catarina, sul do Brasil. *Biotemas*, **14**, 109-140.
481 Graipel ME, Cherem JJ, Monteiro-Filho ELA, Glock L (2006) Dinâmica Populacional de
482 Marsupiais e Roedores no Parque Municipal da Lagoa do Peri , Ilha de Santa Catarina,
483 Sul do Brasil. *Mastozoología Neotropical*, **13**, 31-49.
484 Gordon D, Abajian C, Green P (1998) Consed: a graphical tool for sequence finishing.
485 *Genome Research*, **8**, 195-202.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series*, **41**, 95-98.

Hoelzer AR, Halley J, O'Brien SJ, Campagna C, Arnborm T, Le Boeuf B, Ralls K, Dover GA (1993) Elephant Seal Genetic Variation and the Use of Simulation Models to Investigate Historical Population Bottlenecks. *Journal of Heredity*, **84**, 443-449.

Huchon D, Douzery E (2001) From the Old World to the New World: A Molecular Chronicle of the Phylogeny and Biogeography of Hystricognath Rodents. *Molecular Phylogenetics and Evolution*, **20**, 238-251.

IUCN (1994) *IUCN Red list—categories and criteria version 2.3*. IUCN Species Survival Commission, World Conservation Union (IUCN), Gland, Switzerland.

IUCN (2001) *IUCN Red list—categories and criteria version 3.1*. World Conservation Union (IUCN), Gland, Switzerland.

Johnson JA, Tingay RE, Culver M, Hailer F, Clarke ML, Mindell DP (2009) Long-term survival despite low genetic diversity in the critically endangered Madagascar fish-eagle. *Molecular Ecology*, **18**, 54-63.

Kanitz R, Trillmich F, Bonatto SL (in preparation) Highly variable microsatellite loci for the South-American rodents *Cavia magna* and *C. aperea*. *Molecular Ecology Resources*.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, **49**, 725-738.

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proceedings of the National Academy of Sciences USA*, **75**, 2868-2872.

Knowlton JL, Donlan CJ, Roemer GW, Samaniego-Harrera A, Keit BS, Aguire-Munoz AA, Faulkner KR, Tershy BR (2007) Non-native mammal eradications and the status of

510 insular mammals on the California Channel Islands, USA and Pacific Baja California
511 Islands, Mexico. *The Southwestern Naturalist*, **52**, 528-540.
512 Kraus C, Thomson DL, Künkele J, Trillmich F (2005) Living slow and dying young? Life-
513 history strategy and age-specific survival rates in a precocial small mammal. *Journal of*
514 *Animal Ecology*, **74**, 171-180.
515 Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of
516 population parameters. *Bioinformatics*, **22**, 768-770.
517 Leberg P (2005) Genetic Approaches for Estimating the Effective Size of Populations. Journal
518 of Wildlife Management, 69, 1385-1399.
519 Luikart G, Cornuet JM (1999) Estimating the Effective Number of Breeders From
520 Heterozygote Excess in Progeny. *Genetics*, **151**, 1211-1216.
521 Marker LL, Wilkerson AJP, Sarno RJ, Martenson J, Breintenmoser-Würsten C, O'Brien SJ,
522 Johnson WE (2008) Molecular Genetic Insights on Cheetah (Acinonyx jubatus) Ecology
523 and Conservation in Namibia. *Journal of Heredity*, **99**, 2-13.
524 MacFarland C, Villa GJ, Toro B (1974) The Galápagos giant tortoises (Geochelone
525 elephantopus). II. Conservation methods. *Biological Conservation*, **6**, 198–212.
526 Medrano JF, Aasen E, Sharrow L (1990) DNA extraction from nucleated red blood cells.
527 *Biotchniques*, **8**, 43.
528 Milne GA, Long AJ, Bassett SE (2005) Modelling Holocene relative sea-level observations
529 from the Caribbean and South America. *Quaternary Science Reviews*, **24**, 1183-1202.
530 Myers N, Mittermeier RA, Mittermeier CG, da Fonseca, GAB, Kent J (2000) Biodiversity
531 hotspotsfor conservation priorities. *Nature,* **403**, 853-858.
532 Nei M, Takima F (1981) Genetic drift and estimation of effective population size. *Genetics*,
533 **98**, 625-640.

534 Paetkau D, Waits LP, Clarkson PL *et al.* (1998) Variation in genetic diversity across the range
535 of North American brown bears. *Conservation Biology*, **12**, 418-429.
536 Pirazzoli PA, Pluet J (1991) *World Atlas of Holocene Sea-Level Changes*. Elsevier
537 (Oceanography Series 58), Amsterdam.
538 Piry S, Luikart G, Cournet J-M (1999) Bottleneck: a computer program for detecting recent
539 reductions in the effective population size using allele frequency data. *Journal of*
540 *Heredity*, **90**, 502-503.
541 Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism
542 analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.
543 Salvador CH, Fernandez FAS (2008a) Population dynamics and conservation status of the
544 insular cavy *Cavia intermedia* (Rodentia: Caviidae). *Journal of Mammalogy*, **89**, 721-
545 729.
546 Salvador CH, Fernandez FAS (2008b) Reproduction and growth of a rare island-endemic
547 cavy (*Cavia intermedia*) from southern Brazil. *Journal of Mammalogy*, **89**, 909-915.
548 Salvador CH (2006) Biologia da Conservação na teoria e na prática: o estudo de caso de
549 *Cavia intermedia*, um dos mamíferos mais raros do planeta. Master Thesis Universidade
550 Federal do Rio de Janeiro, Rio de Janeiro.
551 Scheel-Ybert (2000) Vegetation stability in the Southeastern Brazilian coastal area from 5500
552 to 1400 14C yr BP deduced from charcoal analysis. *Review of Paleobotany &*
553 *Palinology*, **110**, 111-138.
554 Shaffer ML (1981) Minimum Population Sizes for Species Conservation. *BioScience*, **31**,
555 131-134.

556 Schultz JK, Baker JD, Toonen RJ, Bowen BW (2009) Extremely Low Genetic Diversity in
557 the Endangered Hawaiian Monk Seal (Monachus schauinslandi). *Journal of Heredity*,
558 **100**, 25-33.
559 Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: a program to estimate
560 effective population size using approximate Bayesian computation. *Molecular Ecology*
561 *Resources*, **8**, 299-301.
562 Wang J (2001) A pseudo-likelihood method for estimating effective population size from
563 temporally spaced samples. *Genetics Research*, **78**, 243-257.
564 Wang J, Whitlock MC (2003) Estimating Effective Population Size and Migration Rates
565 From Genetic Samples Over Space and Time. *Genetics*, **163**, 429-446.
566 Weber DS, Stewart BS, Garza JC, Lehman N (2000) An empirical genetic assessment of the
567 severity of the northern elephant seal population bottleneck. *Current Biology*, **10**, 1287-
568 1290.
569 Wright S (1931) Evolution in Mendelian populations. *Genetics* **16**, 97-159.
570 Wyss AR, Flynn JJ, Norell MA, Swisher CC III, Charrier R, Novacek MJ, McKenna MC
571 (1993) South America's earliest rodent and recognition of a new interval of mammal
572 evolution . *Nature*, **365**, 434-437.

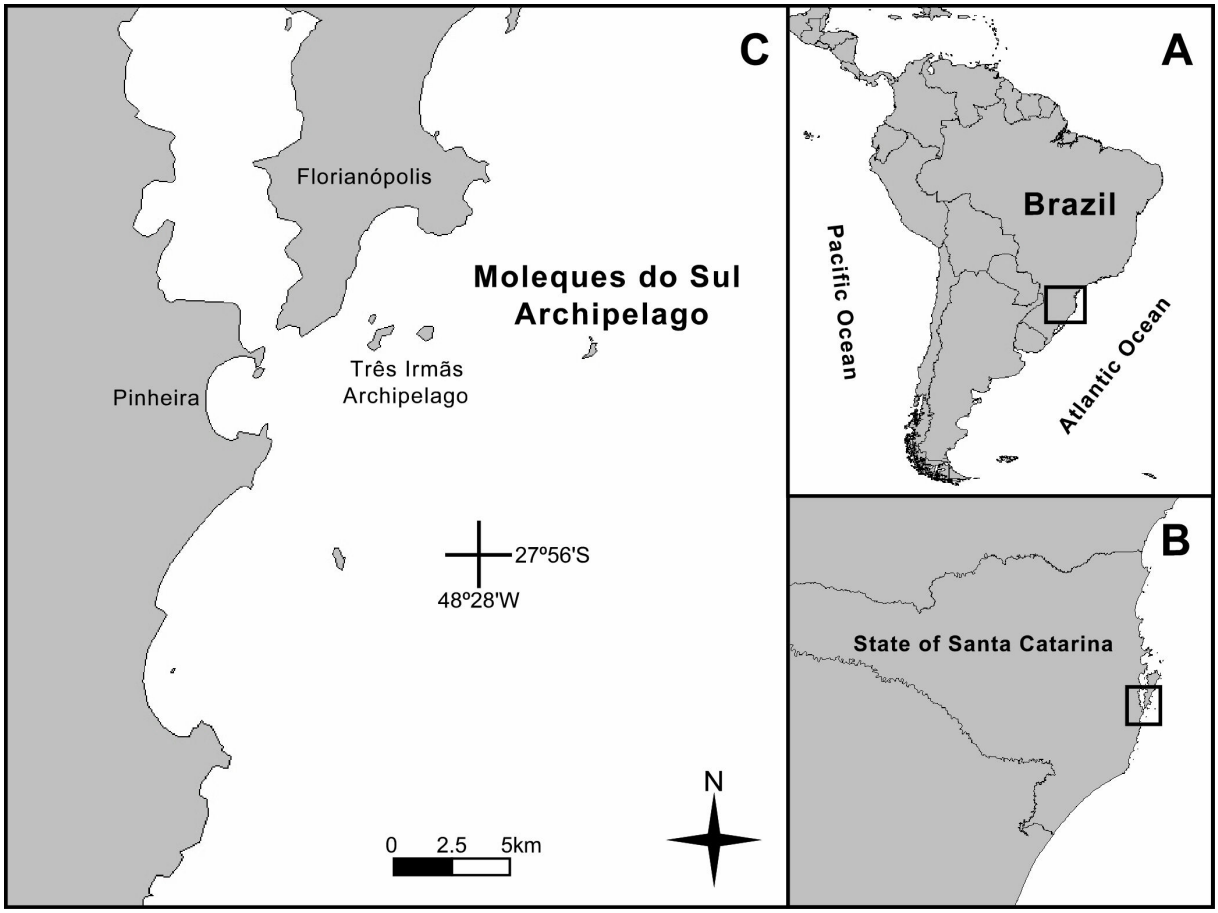

Figure 1: Localization of the Moleques do Sul Archipelago in Southern Brazil. The map in **A** shows a wider continental view with a square augmented in map **B**. Map **C** is the magnified square shown in **B**. All maps have the same orientation.

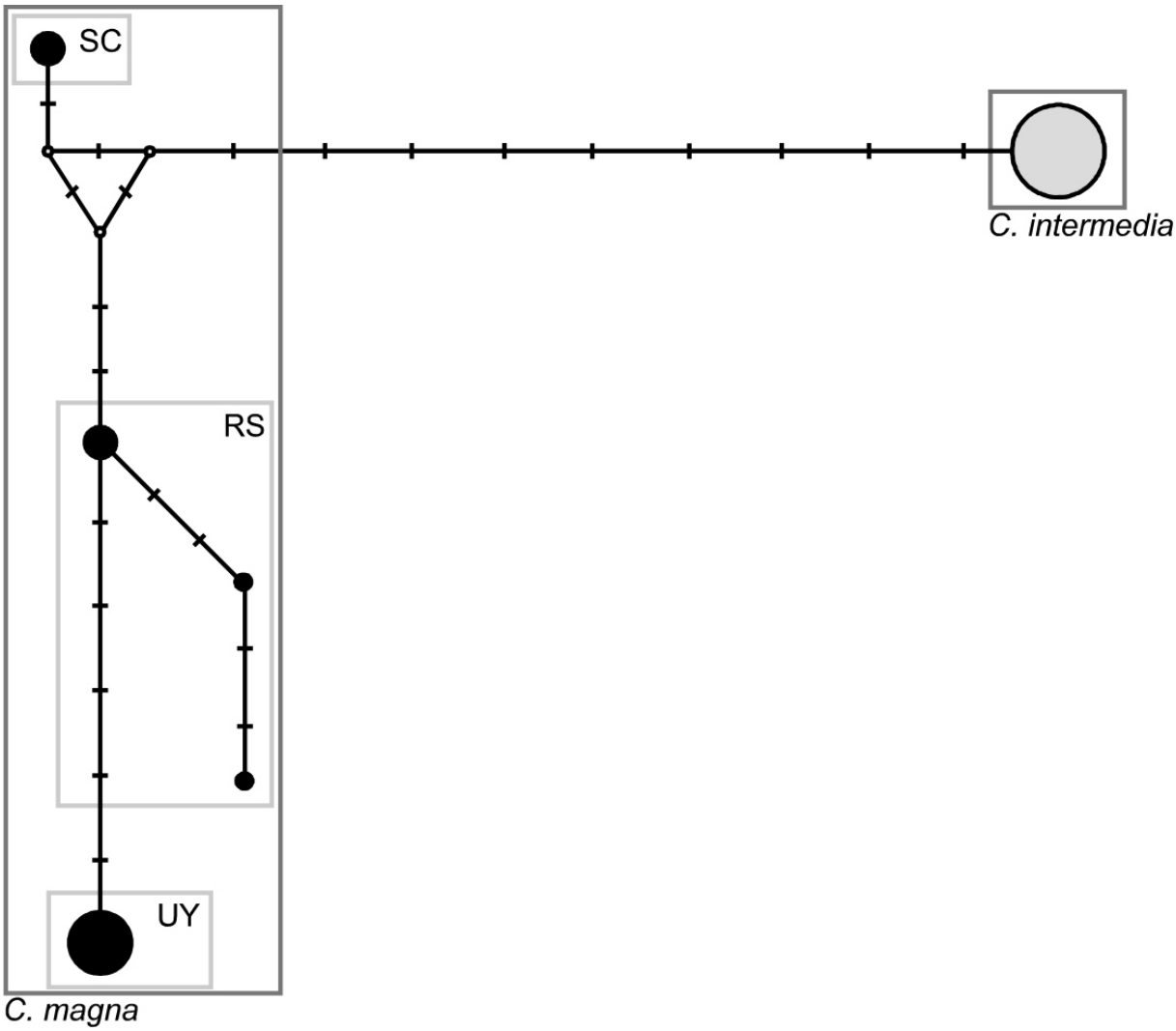

Figure 2: Median-Joining Network tree showing the relationship among the *C. intermedia* (in gray) and *C. magna* (black) HVSI haplotypes. Nodes are proportional to number of individuals, except for the empty nodes which represent missing haplotypes. Slashes represent mutational events. *C. magna* (SC) are from the State of Santa Catarina closest to Moleques do Sul; *C. magna* (RS) are from the State of Rio Grande do Sul; and *C. magna* (UY) are from northern Uruguay.

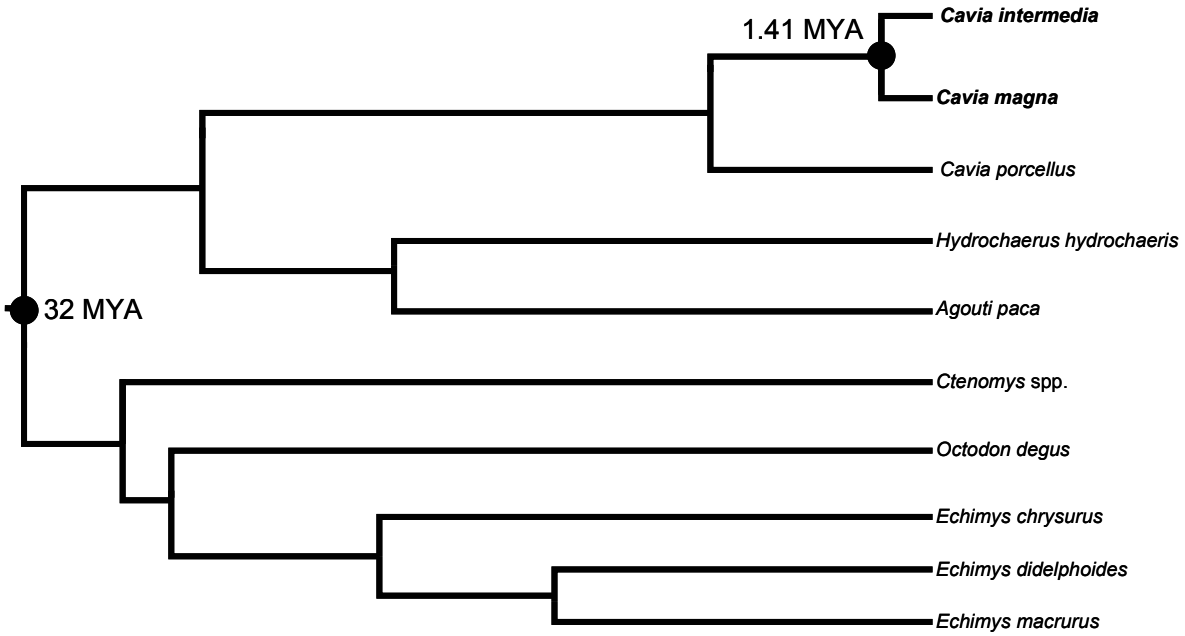

Figure 3: *Cytochrome b* Bayesian phylogenetic tree with the Hystricomorpha diversification date (32 MYA) in Wyss *et al.* (1993) and Huchon & Douzery (2001) as a calibration point.

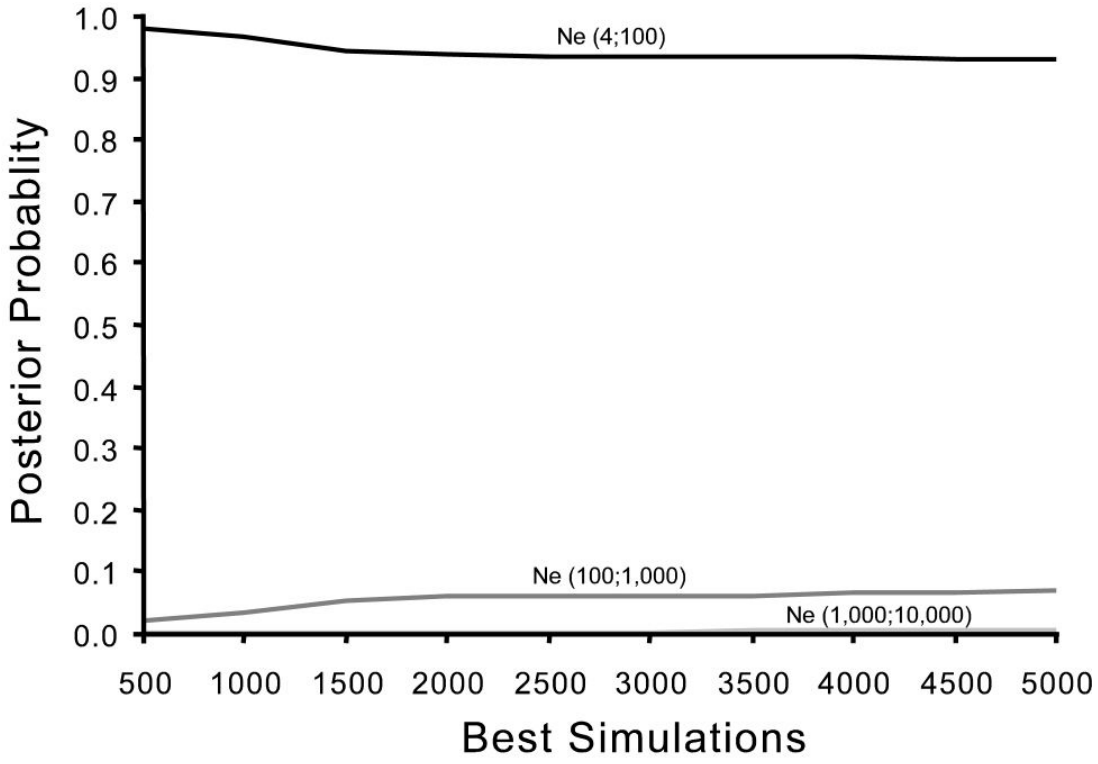

Figure 4: Posterior probability of alternative scenarios in different sets of closest-to-observed simulations based on the logistic regression. The black upper line refers to Scenario 1 (N_e between 4 and 100), the middle dark-gray line to the Scenario 2 (N_e = 100-1,000), and the lower light-gray line (very close to the X axis) to the Scenario 3 (N_e = 1,000-10,000).

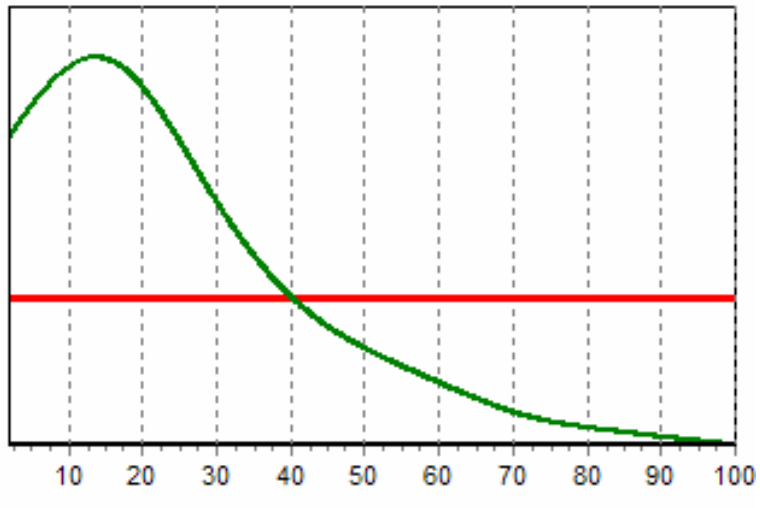

Figure 5: Posterior distribution (in green) of the parameter N_e for *C. intermedia* from the best supported scenario as estimated in the program DIYABC. The red line is the prior distribution for N_e.

Table 1: Comparative summary statistics for *C. intermedia* (*C.i.*) and *C. magna* (*C.m.*) for the 12 STR loci and the HVSI mtDNA region where loci named *Cavy#* are the STR loci from Kanitz *et al.* (in prep). "Mean" refers to the STR loci as well as the standard deviation (S.D.) below it. The summary statistics for the STR loci are number of alleles (**A**), allelic range (**AR**), expected heterozygosity ($\mathbf{H_e}$), observed heterozygosity ($\mathbf{H_o}$), Garza-Williamson index (**M**), and pair-wise fixation index ($\mathbf{F_{ST}}$). For the mtDNA region, the statistics are the number of haplotypes (**h**), haplotype diversity (**Hd**), nucleotide diversity ($\boldsymbol{\pi}$), Rho statistics ($\boldsymbol{\rho}$), Tajima's neutrality test (**Tajima's D**) and distance-based fixation index ($\mathbf{\Phi_{ST}}$). Hardy-Weinberg Equilibrium significance are coded as * for $p<0.05$ and ** for $p<0.01$ in the H_o statistics. NS in the Tajima's D results means not significant ($\alpha=0.05$). All F_{ST} and $\mathbf{\Phi_{ST}}$ results were significant ($p<0.01$).

	C. i.	*C. m.*	*C. i.*	*C. m.*	*C. i.*	*C. m.*	*C. i.*	*C. m.*	*C. i.*	*C. m.*	*C.i. vs. C.m.*
Locus	**A**		**AR**		**H_e**		**H_o**		**M**		**F_{ST}**
Cavy1	1	10	0	35	0	0.721	0	0.444**	-	0.278	0.809
Cavy2	1	4	0	61	0	0.576	0	0.571	-	0.065	0.845
Cavy4	1	2	0	2	0	0.251	0	0.286	-	0.667	0.927
Cavy6	7	11	9	59	0.590	0.860	0.529*	0.800	0.700	0.183	0.279
Cavy7	1	3	0	2	0	0.286	0	0.316	-	1.000	0.929
Cavy8	1	5	0	14	0	0.747	0	0.533*	-	0.333	0.740
Cavy9	2	9	2	22	0.305	0.724	0.257	0.714	0.667	0.391	0.550
Cavy10	1	7	0	22	0	0.832	0	0.476**	-	0.304	0.756
Cavy12	1	3	0	7	0	0.535	0	0.714	-	0.375	0.683
Cavy13	1	5	0	11	0	0.670	0	0.571	-	0.417	0.652
Cavy14	4	10	3	29	0.155	0.782	0.149	0.700**	1.000	0.333	0.643
Cavy16	4	16	10	24	0.058	0.882	0.059	0.619**	0.364	0.640	0.639
Mean	**2.08**	**7.08**	**2.00**	**24.00**	**0.0923**	**0.6554**	**0.0423**	**0.5676**	**0.6826**	**0.4155**	**0.704**
S.D.	1.93	4.19	3.64	19.71	0.1824	0.2086	0.0850	0.2000	0.2602	0.2485	0.179
	h		**Hd**		**π**		**ρ**		**Tajima's D**		**Φ_{ST}**
hvs1	1	5	0	0.4380	0	0.0122	0	4.3334	0^{NS}	-0.4002^{NS}	0.902
S.D.	-	-	0	0.0147	0	0.0034	0	1.6905	-	-	-

Table 2: Current (N_{eV}) and historical (N_{eI}) effective population size estimated for *C. intermedia*. N_{eV} assessments are based on two generations between samples. N_{eI} is calculated over all 70 individuals. "**N_e**" represents a direct estimation (for moment-based calculation), a central tendency measure (such as maximum-likelihood estimation in MLNE, CoNe and Lamarc), or a central tendency measure such the median for the DIYABC and ONeSAMP results. The "**lower**" and "**upper**" estimations are based on the 95% percentiles for maximum-likelihood methods and 95% posterior probability distribution for Bayesian inferences.

	N_{eV}			**N_{eI}**		
	Moment	MLNE	CoNe	Lamarc	DIYABC	ONeSAMP
Ne	**38**	**51**	**47**	**30**	**29**	**27**
lower	-	13	8	28	6	17
upper	-	∞	∞	34	94	52
Mean		**45**			**29**	

CONCLUSÃO GERAL

Obtivemos êxito na caracterização das regiões de microssatélites para o seu uso como marcadores moleculares nas espécies continentais *Cavia magna* e *C. aperea*. Esses marcadores também podem ser usados com boa chance de sucesso em outras espécies do gênero. Aplicando esses mesmo marcadores juntamente com duas regiões do DNA mitocondrial, nós pudemos mensurar a diversidade genética neutra de *C. intermedia* que era o principal objetivo deste estudo.

Além disso, obtivemos a confirmação da relação de táxons-irmãos entre *C. magna* e *C. intermedia*. Também calculamos o tempo de divergência entre essas duas espécies e pudemos concluir que *Cavia intermedia* parece realmente estar isolada em Moleques do Sul há pelo menos cerca de 8.000 anos desde seu mais recente isolamento em relação ao continente, sendo muito provavelmente uma separação bastante mais antiga.

O preá de Moleques do Sul também apresentou níveis baixíssimos de diversidade. Estes sem precedentes em outras espécies que não tenham sido recentemente perturbadas pela ação humana. Através dessa diversidade, também pudemos estimar os tamanhos efetivos populacionais histórico e atual da espécie, o que nos mostrou que ela parece manter esse ínfimo tamanho populacional há bastante tempo. Provavelmente, desde o seu isolamento na ilha em que hoje habita. Isso pode ter sido possível por diversas razões. *C. intermedia* não parece sofrer efeitos de depressão por endocruzamento por possivelmente ter expurgado alelos deletérios durante as fases iniciais de sua evolução em Moleques do Sul, além de aparentemente maximizar seu tamanho efetivo em relação ao tamanho de censo expressando características demográficas típicas de uma síndrome de ilhas (Adler & Levins 1994, Salvador & Fernandez 2008b).

Chamamos também à atenção o fato de que *C. intermedia* habita Moleques do Sul por período de relativa estabilidade ambiental na região (Scheel-Ybert 2000). Fato esse que deve diminuir a necessidade do chamado potencial evolutivo, comumente ausente em populações cuja diversidade é reduzida (Franklin & Frankham 1998). Entretanto, as atuais perturbações humanas

podem alterar radicalmente este cenário. Ações como caça (comum em espécies continentais), queimadas e introdução de competidores ou predadores podem causar sérios efeitos na diminuta população de *C. intermedia*. Portanto, ações educação da população e de turistas (Moleques do Sul é um ponto bastante conhecido por mergulhadores), assim como uma fiscalização constante do local que já pertence ao Parque Estadual da Serra do Tabuleiro podem permitir que essa curiosa espécie siga sua história evolutiva sem maiores danos diretos.

Estudos mais aprofundados sobre a diversidade genética desta espécie deverão ser feitos para melhor caracterizar a sua diversidade neutra e também sujeita à seleção (em loci de MHC, por exemplo). Além de um aprofundamento do estudo da diversidade da própria espécie insular, a ampliação da amostragem da espécie-irmã continental – *C. magna* – pode trazer ainda mais informação sobre a origem de *C. intermedia*.

REFERÊNCIAS BIBLIOGRÁFICAS

Adler GH, Levins R (1994) The island syndrome in rodent populations. *The Quarterly Review of Biology*, **69**, 473-489.

Cherem JJ, Olimpio J, Ximenez A (1999) Descrição de uma nova espécie do gênero *Cavia* Pallas, 1766 (Mammalia – Caviidae) das Ilhas Moleques do Sul, Santa Catarina, Sul do Brasil. *Biotemas*, **12**, 95-117.

Frankham R, Gilligan DM, Morris D, Briscoe DA (2001) Inbreeding and extinction: Effects of purging. *Conservation Genetics*, **2**, 279-284.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge.

Franklin IR, Frankham R (1998) How large must populations be to retain evolutionary potential? *Animal Conservation*, **1**, 69-73.

Gilligan DM, Briscoe DA, Frankham R (2005) Comparative losses of quantitative and molecular genetic variation in finite populations of Drosophila melanogaster. *Genetics Research*, **85**, 47-55.

Gliwicz J (1980) Island Populations of Rodents: their organization and functioning. *Biology Review*, **55**, 109-138.

Grazziotin FG, Monzel M, Echeverrigaray S, Bonatto SL (2006) Phylogeography of the Bothrops jararaca complex (Serpentes: Viperidae): past fragmentation and island colonization in the Brazilian Atlantic Forest. *Molecular Ecology*, **15**, 3969-3982.

Kaeuffer R, Coltman DW, Chapuis JL, Pontier D, Réale D (2006) Unexpected heterozygosity in an island mouflon population founded by a single pair of individuals. *Proceedings of the Royal Society B*, **274**, 527-533.

Reed DH (2007) Natural selection and genetic diversity. *Heredity*, **99**, 1-2.

Salvador CH, Fernandez FAS (2008a) Population dynamics and conservation status of the insular cavy *Cavia intermedia* (Rodentia: Caviidae). *Journal of Mammalogy*, **89**, 721-729.

Salvador CH, Fernandez FAS (2008b) Reproduction and growth of a rare island-endemic cavy (*Cavia intermedia*) from southern Brazil. *Journal of Mammalogy*, **89**, 909-915.

Seddon IM, Baverstock PR (1999) Variation on islands: major histocompatibility complex (Mhc) polymorphism in populations of the Australian bush rat. *Molecular Ecology*, **8**, 2071-2079.

Scheel-Ybert R (2000) Vegetation stability in the Southeastern Brazilian coastal area from 5500 to 1400 14C yr BP deduced from charcoal analysis. *Review of Paleobotany & Palinology*, **110**, 111-138.

Van der Bergh GD, Vos J, Sondaar PY (2001) The Late Quaternary palaeogeography of mammal evolution in the Indonesian Archipelago. *Palaeo*, **171**, 385-408.

White TA, Searle JB (2007) Factors explaining increased body size in common shrews (*Sorex araneus*) on Scottish islands. *Journal of Biogeography*, **34**, 356-363.