



Research Article

Neutral genetic diversity preservation in a first-generation breeding population of Guinea pig

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To increase availability of animal proteins in Democratic Republic of the Congo (DRC), *Cavia porcellus* was recently enrolled in a breeding program that includes base populations from DRC and Belgium. To preserve whole or part of the source's genetic diversity over breeding cycles, cognizance of genetic diversity in natural populations is required. We tested the cross-amplification of eleven pairs of microsatellite primers that were isolated from *Cavia aperea* and *C. magnea*. Amplification tests by polymerase chain reaction were performed on total DNA of 30 *C. porcellus* using 11 microsatellite loci. All the microsatellites amplified at the expected size and were polymorphic for *C. porcellus*. Using these microsatellite loci, we assessed the genetic diversity of base populations and of first-generation breeding population. High levels of genetic diversity were found within the base populations. These populations exhibited fixation indices significantly greater than 0, indicating occurrence of inbreeding. Moderate differentiation ($R_{ST} = 0.123$) was observed among the base populations. The first-generation breeding population displayed a significantly lower fixation index (0.083) and a higher genetic diversity ($A = 5.73$; $H_o = 0.571$; $H_E = 0.663$) than the base populations. Appropriate breeding strategies that would limit inbreeding over breeding cycles are discussed.

Keywords: *Cavia porcellus*; Diversity; Genetic improvement; Inbreeding level; Microsatellite loci.

INTRODUCTION

Cavia porcellus L. (Rodentia; Caviidae) also known as guinea pig or domestic guinea pig is a mid-sized and picked-body rodent whose adult males can measure up to 50 cm long and weigh 0.9 to 1.2 kg. This small mammal is native to the Andes (Wing, 1978) and is now widespread in Central and South America and in sub-Saharan Africa where it is reared for meat production and income generation. There are several compelling reasons for breeding guinea pig in the tropics. First, guinea pig meat is succulent and tasty, and the colour, texture, consistency and flavour of the meat are similar to rabbit meat (Hardouin *et al.*, 1991). Guinea pig meat contains about 70% dry matter, 20% crude protein, 7.8% fat, 0.5% carbohydrate and 0.8% minerals, while chicken meat contains 70.2% dry matter, 9.3% fat, 1.2% carbohydrates and 1% minerals.

Second, guinea pig is an herbivore that tolerates bitter compounds of certain plants leaves (Nolte *et al.*, 1994) and therefore, feeding this mammal is inexpensive. Third, this small and prolific mammal, which reaches commercial maturity at 5 months of age, can be accommodated in homes.

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All these reasons make the rearing of cavy a promising source of animal protein for poor people in tropical areas (Lammers *et al.*, 2009), especially in Democratic Republic of the Congo (DRC), where daily protein consumption per person is far below the WHO recommendations (36 g day⁻¹; FAO, 2003).

Despite the nutritional and commercial importance of guinea pig for the poor in developing countries, very little effort has been put towards understanding the patterns of genetic variation of domestic guinea pig in sub-Saharan Africa, so as to undertake a local program of genetic resources' conservation and improvement. Preservation of source genetic diversity in captive or founding populations has always been a major preoccupation for population managers (Ralls and Ballou, 2004; Ballou *et al.*, 2010). Starting a genetic improvement program involves sampling individuals with best performance in the characters of interest in base populations so as to form breeding and production populations. However, breeding and production populations are vulnerable to inbreeding depression, i.e., the reduced survival and fertility of offspring of related individuals that is mainly caused by the presence of recessive deleterious mutations in populations (Charlesworth and Willis, 2009), and loss of evolutionary potential as a result of reduction in effective size of captive populations. Indeed, an increased inbreeding in captive populations may result in decreased fitness due to homozygosity (Barrett and Charlesworth, 1991).

Genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. Conservation should be based on a deep knowledge of the genetic resources of the specific breed. Therefore, it is important to try to characterize genetically indigenous breeds (Ruzina *et al.*, 2010; Shojaei *et al.*, 2011). A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites and also the ability of a population to respond adaptively to environmental changes depends on the level of genetic variability or diversity it contains. Furthermore, the study of native breeds is necessary for conservation of genetic resources in livestock (Mohammadi *et al.*, 2009; Ruzina *et al.*, 2010). Genetic improvement has two objectives: increase the frequency of desired traits in breeding populations while maintaining sufficient genetic diversity to reduce the risk of inbreeding. Indeed, any reduction in genetic diversity of populations would reduce the potential for adaptation of these populations (Frankham, 2005) and increase the risk of occurrence of inbreeding depression that may lead these populations to extinction (O'Grady *et al.*, 2006). Inbreeding has been reported to be deleterious to animals (Frère *et al.*, 2010). Indeed, inbred individuals most often show lower survival rate than out-bred individuals under extreme ecological events, as inbreeding depression is expressed by reduced fitness.

Literature is documented by cases where animals develop strategies to avoid inbreeding (Sherborne *et al.*, 2007; Krause *et al.*, 2012), ranging from recognition and avoidance of close kin as mates through scent signal identity (Brennan and Kendrick, 2006; Slev *et al.*, 2006) to strong deficit in successful mating between individuals sharing the same major urinary protein haplotype (Hurst *et al.*, 2001; Cheetham *et al.*, 2007; Gamble *et al.* 2007), extra-pair or extra-group copulations (Yuta and Koizumi, 2007; Young *et al.*, 2007), delayed maturation and social system structure (Bilde *et al.*, 2005) that avoid mating between close relatives. However, inbreeding always ought to be avoided or minimized during management of captive colonies or throughout breeding cycles in improvement programmes. Therefore, the amount of genetic diversity revealed by natural populations as well as the inbreeding level found therein should be taken as baseline for the maintenance of genetic diversity throughout the improvement program, and for efficient inbreeding level control throughout management of genetic resources by application of suited techniques.

The genetic diversity of a population can be assessed using genetic markers (i.e., those derivatives of the direct analysis of genetic polymorphism of DNA sequences; FAO 2011; Al-Samarai and Al-Kazaz 2015) that are functional or neutral. Functional genetic markers are used when the genes that control expression of traits of interest are known. However, functional characterization of genetic diversity must be combined with an assessment of neutral genetic diversity that only provides information on life-history traits of a species or population. The application of molecular markers has many important advantages. One such significant advantage is the genotyping of individuals for specific genetic loci (Javanmard *et al.*, 2008; Mousavizadeh *et al.*, 2009). Microsatellites or Simple Sequence Repeats (SSRs) are molecular genetic markers that are highly variable and widely distributed in the genome; these markers are suitable for evaluation of neutral genetic diversity (Mohammadabadi *et al.*, 2010; FAO 2011; Al-Samarai and Al-Kazaz 2015). Other advantages of microsatellites are high degree of polymorphism due to existence of several alleles at each locus, easy detection, and distribution all over the genome. Such markers have been developed in two related species of domestic guinea pig, namely *Cavia aperea* and *C. magnea* (Asher *et al.*, 2008; Kanitz *et al.*, 2009).

Studies on the genetics of *C. porcellus* and its close relatives in the tropics were mostly performed in Latin America, and covered the phylogenetics of living lineages (Spotorno *et al.*, 2006), differentiation of cryptic species pairs of wild cavies (Trillmich *et al.*, 2004), molecular systematics, taxonomy and biogeography of the genus (Dunnum and Salazar-Bravo, 2010); and domestication effects (Brust and Guenther, 2015). Guinea pig has also been used in several experimental studies (Carter 2007; Franz *et al.*, 2011). However, little has been known about the

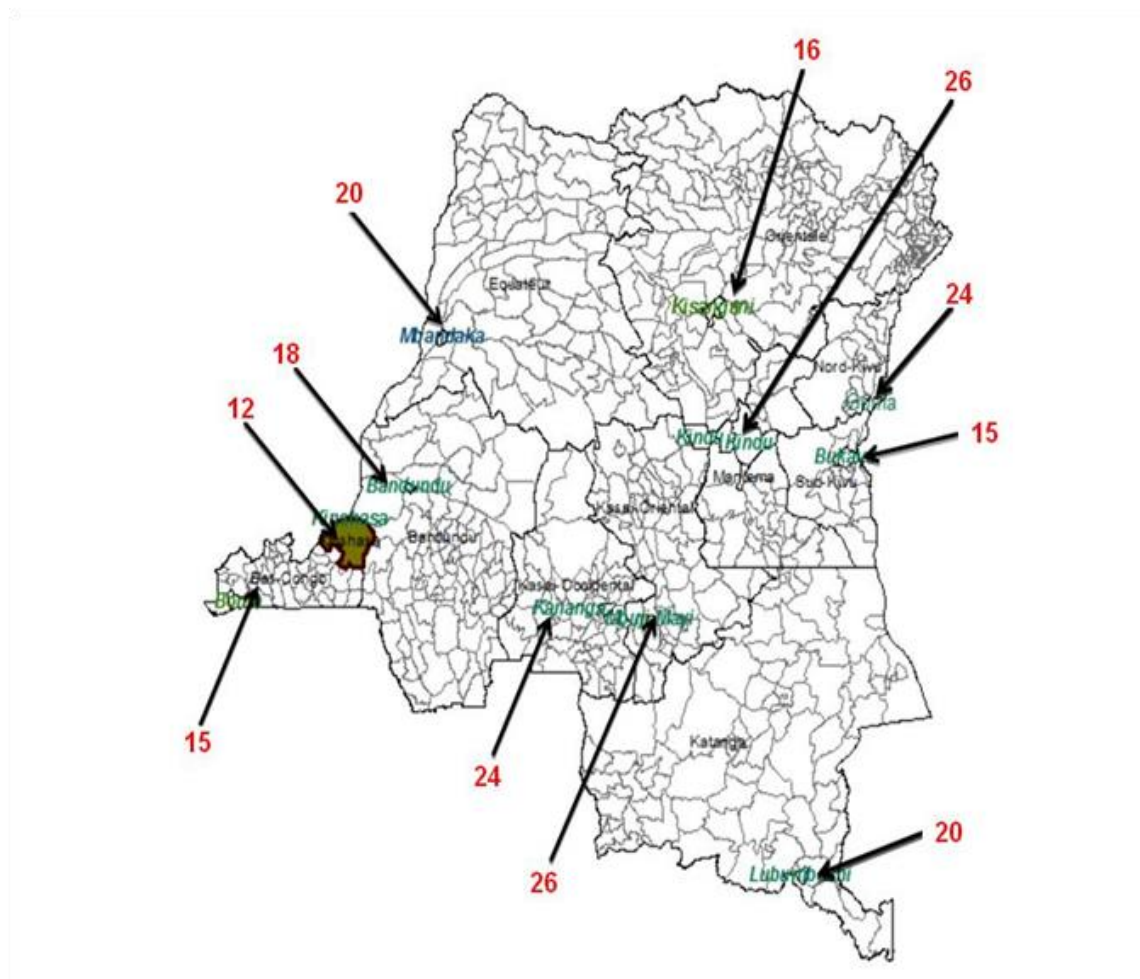


Figure 1. Locations of sampled populations of guinea pigs included in this study from the 11 former provinces in Democratic Republic of the Congo Map.

patterns of genetic diversity of domestic guinea pig in sub-Saharan Africa, in order to compare the results of such studies with those that have been carried out in Latin America. To overcome this deficiency, research has been undertaken to evaluate various genetic resources and characterize the genetic variation of *C. porcellus* in DRC to form first-generation breeding populations for meat production. The project included three Belgian institutions, namely the Faculty of Veterinary Medicine of the University of Liège, the Faculties of Agricultural Sciences of Gembloux and the University of Brussels; and Congolese institutions, namely the Faculty of Agricultural Sciences of the University of Kinshasa and the Agri-Veterinary Higher Institute (ISAV) of Kimwenza, Kinshasa.

The present study aims at characterizing microsatellite markers that could be used for genetic studies in *C. porcellus*, evaluate the genetic diversity of base populations of guinea pig in DRC and Belgium, and compare this diversity to that of first-generation breeding population of *C. porcellus*. Indeed, phenotypic characterisation of guinea pigs in domestic populations of DRC and one population from Belgium has been recently carried out, and one breeding population including 10 adult males and 11 females was selected from this study (Umba *et al.*, Unpublished data).

MATERIALS AND METHODS

For cross amplification tests, 25 mg of muscle was taken from the ear of each of 30 guinea pigs and DNA extracted using Dneasy Blood & Tissue kit (Qiagen, Germany). Amplification tests by polymerase chain reaction (PCR) were using 11 microsatellite loci developed for *C. aperea* and *C. magnea* (Table 1). Loci were co-amplified following the combinations Cavy2-Cavy3-Cavy5, Cavy6-Cavy12, Cavy13-Cavy14-Cavy15 and Cavy7-Cavy8-Cavy9 using Qiagen Multiplex PCR kit (Qiagen, Germany). Amplifications were performed using Peltier Thermal Cycler (PTC, USA) according to the following protocol: initial denaturation at 95°C for 15 minutes, followed by 39 cycles each of 95°C denaturation for 30 seconds, annealing at 60°C for 90 seconds and elongation at 72°C for one minute, and then a final elongation of 72°C for 10 minutes. Verification of amplification was carried out on 2% agarose gel mixed with 0.5% Synergel. Amplicons were analyzed using 3130xl capillary sequencers (Applied Biosystems, USA) and genotypes and alleles scored using Gene Mapper 3.7 software (Applied Biosystems, USA).

For population genetic study, 7 to 26 domestic guinea pigs were randomly sampled in Belgium and in each of

Table 1. Characteristics of microsatellites for *Cavia porcellus* (source: Kanitz et al., 2009)

Locus	Genbank accession number	Repeat motif	Primer sequence (5'-3')	T_a (°C)	Size range (bp)	Nb of alleles	Nb of genotypes	H_o	H_E	F	H.-W.P values
Cavy2	AJ496560	[AC] ₂₅	F: GGCCATTATGCCCCCAAC R: AGCTGGTCCTTGTGCTGTAG	60	159-163	3	4	0.333	0.391	0.148	0.153
Cavy3	AJ496561	[CT] ₂₆	F: ACAGCGATCACAATCTGCAC R: GCAGTGGTAACCCAGAATGG	60	228-242	6	6	0.333	0.3785	0.1194	0.054
Cavy5	AC190431	[AAGG] ₄₆	F: CTCCATTACAGAGTGGTCT R: AAAGTGCTGTTTAATTGGGA	60	340-356	5	7	0.666	0.57288	-0.1635	0.88
Cavy6	AC190431	[TCTT] ₃₇	F: GTACCAGGGATCAAACCTCAG R: GAGCTTTTCGAGAGTACGAGA	60	390-408	6	12	0.7	0.7206	0.0286	0.29
Cavy7	AC185540	[AG] ₃₄	F: TGGACCTCCAGGTACTACAC R: GTGACCCTGCAACATTTCT	60	387-417	10	17	0.8	0.777	-0.0296	0.522
Cavy8	AC189989	[AAGG] ₃₆	F: CCCTTTCCCACTCTTCTATT R: CTGCCAGCTTAGCAATTTAT	60	284-302	7	13	0.8	0.7705	-0.038	0.748
Cavy9	AC192512	[CT] ₄₅	F: CAGCGATCTTCTATGGAGAC R: TCTTTAATGGTGGGTTTCAG	60	182-191	4	7	0.633	0.6418	0.0132	0.611
Cavy10	AC174822	[AG] ₃₃	F: ATGAACTTCAACATGGATGG R: CCCTCTGAGATCTTTCCTCT	60	380-400	7	11	0.6	0.5974	-0.0043	0.786
Cavy12	AC182323	[AGG] ₆₄	F: TCCCTGTTCTTTGCTACAAT R: CTGCTTCATAGATCTTGCCT	60	230-242	7	15	0.767	0.7876	0.022	0.519
Cavy14	AC190428	[TTC] ₃₁	F: AGTGTTGGCAGCTTGATCCT R: AGCTCACCAGGGAAAATGTG	60	361-373	6	9	0.867	0.681	-0.273	1
Cavy15	AC191184	[TCT] ₃₀	F: TTCATGCTACCTGGCACTTG R: TTGCAGGCAATATGGCATTAA	60	230-242	6	10	0.7	0.673	-0.040	0.705

Observed (H_o) and expected (H_E) heterozygosities; T_a : annealing temperature; Wright's F ; H.-W.: Hardy-Weinberg; Nb: number

the former 11 provinces of DRC (Bandundu, Bas-Congo, Eastern Kasai, Equator, Katanga, Kinshasa, Maniema, North Kivu, South Kivu, Western Kasai and Western Province; Figure 1) whereas the first-generation breeding population included an array of 10 males (6 from Bukavu, 3 from Kinshasa, and one from Kisantu) and 11 females (10 from Kinshasa and one from Bukavu; Tables 2 & 3). Approximately 25 mg of muscle ear tissue were taken from each guinea pig for DNA extraction. Samples were stored at -20 °C prior to DNA extraction that was made using the

DNeasy blood & Tissue kit (Qiagen) in the veterinary laboratory of Kinshasa. Then DNA samples were stored at -20 °C and shipped to Université Laval, Canada, for microsatellite analysis of genetic diversity. Prior to PCR, total DNA concentration in each sample was assessed by electrophoresis on 1% agarose gel. Then primer pairs of 11 microsatellite loci that were developed for *C. aperea* and *C. magnea* and successfully cross-amplified on *C. porcellus* (Table 1) were used for PCR analysis using the same protocol.

Numerical analysis

Data were analyzed using Genepop version 4.2 software (Raymond and Rousset, 1995; Rousset, 2008). Descriptive statistics including the mean number of alleles per locus (A), observed and expected heterozygosities (H_o and H_E , respectively), together with fixation indices (Wright, 1965) for each population and locus (Weir, 1996) were determined. The degree of inbreeding within populations (f), the

overall inbreeding coefficient (F) and population differentiation (θ) corresponding to Wright's FIS, FIT, and FST were also determined. Prior to genetic diversity estimation, Fisher's exact tests were estimated to verify gametic disequilibrium between loci, with significance levels adjusted by sequential Bonferroni correction (Rice, 1989). Genepop software was also used to calculate significance and confidence intervals of fixation indices by bootstrapping over loci (Weir, 1996), together with unbiased estimates of population differentiation for microsatellites (RST, Slatkin, 1995; Goodman, 1997) and their significance using bootstrap. An indirect estimate of outcrossing rate (t , which is generally biased Ritland, 2002), was derived from the equation $(1 - f) / (1 + f)$. We performed one-tailed Mann-Whitney tests (Zar, 1984) on the mean diversity values (A , H_O and H_E) and fixation indices to investigate genetic diversity difference between source populations and first-generation breeding population of *C. porcellus*.

RESULTS

Cross-amplification tests

All the evaluated microsatellite loci amplified at the expected size and were polymorphic for *C. porcellus* (Table 1); the number of alleles ranged from 3 to 10 per locus, whereas the number of genotypes varied from 4 to 17 per locus (Table 1). The expected and observed heterozygosities are presented in Table 1. No gametic disequilibrium or deviation from Hardy-Weinberg equilibrium was observed in the study population (Table 1). Owing to their high polymorphism, the microsatellite loci characterized in this study are efficient for population genetic studies in *C. porcellus*.

Population genetic studies

Within- and among-population genetic diversity indices are shown in Table 2. All loci surveyed in this study were variable in each population ($A = 3.82 - 5.64$; Table 2). Observed and expected estimates of heterozygosity per population ranged from 0.30 (Katanga) to 0.58 (Eastern Kasai) and from 0.514 (Equator) to 0.725 (Belgium), respectively (Table 2). Lowest and highest diversity values were observed in Bandundu ($A = 3.82$; $H_O = 0.38$; $H_E = 0.579$) and in Eastern Kasai ($A = 5.27$; $H_O = 0.58$; $H_E = 0.62$; Table 2), respectively. In each population, H_E was higher than H_O , and the fixation index was significantly greater than zero (Table 2) except in the Belgian population and in the first-generation breeding population, indicating inbreeding occurrence within animal populations sampled in DRC.

Single-locus estimates of heterozygosities, fixation indices, genetic differentiation and outcrossing rates are shown in Table 4. Single-locus estimates of heterozygosities ranged from 0.244 to 0.763, from 0.279 to 0.764, and from 0.294 to 0.785 for H_O , H_S and H_T , respectively (Table 4). Highest estimates of single-

locus heterozygosities were observed for loci Cavy14, Cavy12 and Cavy8 (Table 4). Within-population and overall fixation indices over all loci were greater than 0 (Table 4), indicating substantial inbreeding in sampled populations. Inbreeding level occurrence in sampled populations is also shown by the low values of outcrossing rates per locus, which ranged from 0.043 to 0.736 (Table 4). Significant positive θ estimates were observed over all loci and ranged from 0.041 to 0.159 per locus (Table 4). Single-locus estimates of R_{ST} ranged from 0.029 to 0.218 (mean = 0.123), indicating moderate differentiation between source populations and substantial geneflow amongst sampled populations.

Genetic diversity indices of the first-generation breeding population are presented in Table 2. The mean number of alleles per locus and the observed and expected heterozygosities were significantly ($P = 0.005$) lower in source populations than in the first-generation breeding population (Table 2), indicating higher genetic diversity in the breeding population than within source populations. Furthermore, f estimate was significantly ($P = 0.005$) higher in base populations ($f = 0.252$) than in the breeding population ($f = 0.081$), indicating that inbreeding level is higher within source populations than in the first-generation breeding population.

DISCUSSION

The characterization of genetic diversity in natural populations of a given species has always been a requirement for any genetic resources management, including genetic improvement programs. Indeed, genetic diversity estimates in source and founding populations have useful implications for management, i.e., on strategies that should be used to avoid inbreeding depression in captive populations. Results from the present study indicate that high genetic diversity exists in natural guinea pig populations from DRC and Belgium, in congruence with the findings of Burgoz-Paz *et al.* (2011) who assessed genetic diversity of *C. porcellus* lines in Colombia using microsatellite loci. Indeed, the number of alleles per locus and expected heterozygosity estimates found within populations in the present study were similar to those of guinea pigs pet lines from Nariño, Colombia (4.8 and 0.695 for A and H_E , respectively; (Burgoz-Paz *et al.*, 2011). Additionally, within-populations observed heterozygosity in our study was in the same range with that of native guinea pig lines in Colombia (0.498; Burgoz-Paz *et al.*, 2011) and lower than that of pet lines in the same country (0.522; Burgoz-Paz *et al.*, 2011), indicating that our results can serve as baseline for genetic resources management of *C. porcellus* in tropical Africa in general, and in DRC in particular.

Minimizing genetic diversity reduction in breeding populations allows inbreeding depression avoidance over breeding cycles, as inbreeding level increase in

Table 2. Genetic diversity parameters estimated in 12 *Cavia porcellus* (Caviidae) populations of Democratic Republic of the Congo and Brussels, Belgium

Population	Country	<i>n</i>	<i>A</i>	<i>H_o</i>	<i>H_E</i>	<i>F</i>
Maniema	Democratic	26	5.27	0.43	0.612	0.299***
Eastern Kasai	Republic of the	26	5.27	0.58	0.62	0.065*
Western Kasai	Congo	24	5.64	0.39	0.596	0.337***
South Kivu		15	4.73	0.56	0.658	0.153***
North Kivu		24	4.91	0.46	0.596	0.220***
Katanga		20	4.82	0.30	0.616	0.504***
Equator		20	4.27	0.38	0.514	0.254***
Bas-Congo		15	5.09	0.30	0.641	0.427***
Kinshasa		12	4.36	0.52	0.630	0.181**
Eastern Province		16	4.82	0.54	0.638	0.159***
Bandundu		18	3.82	0.38	0.579	0.346***
Brussels	Belgium	7	5.09	0.47	0.725	0.081 ^{ns}
Mean		17.75	4.84	0.44	0.619	0.252
Breeding population** ‡(see Table 3)		21	5.73	0.571	0.663	0.0803 ^{ns}

n: mean sample size per locus; *A*: mean number of alleles per locus; *H_o*: observed heterozygosity; *H_E*: unbiased expected heterozygosity (Nei 1978); *F*: fixation index (Wright 1965); *ns*: non-significant; *** significant (*P*<0.001); ** significant (*P*<0.01) using Fisher's exact tests; ** ‡ : Mann-Whitney one-tailed tests identified highly significant differences between diversity indices (*A*, *H_o* and *H_E*) and between fixation indices of the first-breeding populations and that of source's populations

Table 3. First-generation breeding population of *Cavia porcellus* improvement program in the Democratic Republic of the Congo

Number	Provenance
Males	
M1029	Brussels (Belgium)
M1280	Kinshasa
M1281	Kinshasa
M1086	Bukavu
M1062	Bukavu
M1094	Bukavu
M1772	Kisantu
M160	Bukavu
M1137	Bukavu
M55	Bukavu
Females	
F1262	Kinshasa
F1399	Kinshasa
F1346	Kinshasa
F1043	Bukavu
F1201	Kinshasa
F1265	Kinshasa
F1384	Kinshasa
F1261	Kinshasa
F1398	Kinshasa
F1400	Kinshasa
F1395	Kinshasa

populations have most often been associated with a loss of genetic diversity (Hoarau *et al.*, 2005). Results of the present study indicate substantial inbreeding within sampled populations in DRC, whereas very weak inbreeding level was observed in the Belgian guinea pig population. The findings of our study are in congruence with what was observed (Burgoz-Paz *et al.*, 2011) in Colombia. Such inbreeding level in sampled

populations in DRC could be explained by the fact that domestic guinea pigs are harem-living species that are traditionally reared in herds in homes in sub-Saharan Africa. Each herd of domestic guinea pig found in homes in sub-Saharan Africa constitutes a family descending from very few ancestries, as no particular copulation strategy was applied between adult group members for inbreeding avoidance. Furthermore,

Table 4. Estimates of single-locus heterozygosity, genetic differentiation and indirect estimates of outcrossing rates for 11 domestic guinea pig populations from Democratic Republic of Congo and one *C. porcellus* population from Belgium

Locus	H_o	H_s	H_T	f	F	θ	R_{ST}	t
Cavy2	0.244	0.279	0.294	0.152	0.189	0.044	0.029	0.736
Cavy3	0.278	0.386	0.461	0.262	0.379	0.159	0.218	0.585
Cavy5	0.392	0.570	0.613	0.341	0.395	0.083	0.122	0.491
Cavy6	0.563	0.743	0.774	0.247	0.278	0.041	0.048	0.604
Cavy7	0.539	0.764	0.796	0.281	0.318	0.051	0.102	0.561
Cavy8	0.512	0.726	0.762	0.293	0.331	0.054	0.132	0.547
Cavy9	0.401	0.604	0.660	0.338	0.398	0.091	0.128	0.495
Cavy10	0.428	0.682	0.730	0.399	0.439	0.066	0.138	0.430
Cavy12	0.571	0.762	0.785	0.252	0.283	0.041	0.066	0.597
Cavy14	0.763	0.729	0.769	-0.021	0.041	0.061	0.057	0.043
Cavy15	0.423	0.557	0.606	0.246	0.312	0.087	0.083	0.605
Overallloci	0.465	0.618	0.659	0.257	0.307	0.067	0.123	0.591
Confidence interval ^a				0.150-0.328	0.206-0.379	0.050-0.092		

H_o : mean observed heterozygosity within populations; H_s : mean expected heterozygosity within populations; H_T : mean expected heterozygosity in the total population; f : fixation index within populations; F : fixation index in the total population; θ : differentiation among populations (Wright 1996); R_{ST} : unbiased estimate of population differentiation (Slatkin 1995); t : outcrossing rate (derived from f -values); ^a 99% confidence interval estimated bootstrapping over loci

females have been shown to prefer heavier males as mate partners (Adrian *et al.*, 2008), as large males may control access to females in *C. aperea* (Asher *et al.*, 2008). The high inbreeding level observed within sampled DRC guinea pig populations may also be the result of founding effects, as source populations might have descended from few ancestries introduced in DRC by the colonizing country, Belgium, where wise mating plans are adopted to avoid inbreeding in captive populations, as observed in the present study. However, this assumption needs to be confirmed with a fine-scale pedigree study of natural populations. Meanwhile, as source populations sampled in the present study are of unknown pedigree, an assessment of genetic diversity in the first-generation breeding population is required to ascertain whether there is a risk of inbreeding depression throughout genetic improvement of domestic guinea pig in DRC.

Although substantial inbreeding was observed within source populations, moderate genetic differentiation was found among study populations indicating occurrence of geneflow between sampled populations. Guinea pigs are most often offered as gifts in sub-Saharan Africa, which may explain geneflow occurrence among study populations. Also, genetic resources exchanges exist between DRC and Belgium, as the latter country has colonized the former, explaining similarity between genetic indices of guinea pigs between these countries. Furthermore, multiple introductions of guinea pigs in DRC from Belgium might have homogenized genetic diversity between these two countries. However, only one domestic guinea pig population was sampled in Belgium, against 11 natural populations in DRC. A fine-scale comparison of genetic diversity in guinea pigs from both countries may be required to confirm the results of our study. These

results also suggest that founder events and possible movement of the same genetic resources among the different provinces in DRC may help in preserving genetic diversity and limiting genetic divergence.

Genetic diversity in first-generation breeding populations may be similar to that of source populations so as to avoid any risk of founding effects in any breeding program. Also, any loss of genetic diversity in breeding and production populations would increase inbreeding level over breeding cycles. We sampled 21 individuals to form the first-generation breeding population, which falls within the range of the minimum effective founder group to minimize any founding effect (Soulé *et al.*, 2006; Atangana *et al.*, 2010). Results from the present study indicate that genetic diversity in the first-generation breeding population is higher than that of source populations. Furthermore, fixation index in the first-generation breeding population is close to 0, whereas the same index averages 0.252 within source populations, indicating that inbreeding risk could be minimized over breeding cycles if appropriate reproduction strategies were used. Such strategies would include controlled mating between genetically distant individuals, so as to avoid mating between close relatives. Indeed, heavier males used to monopolize available females in *C. aperea* (Asher *et al.*, 2008) if there is no control mating. For this reason, genotyping breeding populations throughout genetic improvement of domestic guinea pigs in DRC is needed so as to record the genotype of each individual, and animals should be reared in controlled housing conditions. To avoid genetic drift in breeding populations, reproduction strategies in each breeding cycle should involve controlled successful mating between one adult group member and one individual from the source populations.

Breeding programs have always sought to increase the frequency of alleles that control characters of interest in populations through the development of races or other subspecies from sampled breeding populations. By so doing, there is an increased risk of loss of functional and neutral genetic diversity over breeding cycles. The present study could serve as valuable baseline for any genetic resources management in guinea pigs in DRC and in sub-Saharan Africa. As the sampled first-generation breeding population of domestic guinea pigs in this study exhibited higher genetic diversity than source populations, appropriate successful mating should be applied at each breeding cycle for the control of genetic diversity and inbreeding level in breeding and production populations. Another strategy that would control inbreeding level throughout genetic improvement of guinea pigs is mating of at least one adult in each breeding population with one guinea pig from base populations, so as to facilitate gene flow between captive and source populations.

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REFERENCES

- Adrian O, Dekomien G, Epplen JT, Sachser N (2008). Body weight and rearing conditions of males, female choice and paternity in a small mammal, *Cavia aperea*. *Ethology* 114: 897-906.
- Al-Samarai FR, Al-Kazaz AA (2015). Applications of molecular markers in animal breeding: A review. *American Journal of Applied Scientific Research* 1: 1-5.
- 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 pigs. *Behavioral Ecology and Sociobiology* 62:1509-1521.
- Atangana AR, Beaulieu J, Khasa DP (2010). Wild genetic diversity preservation in a small-sized first generation breeding population of *Allanblackia floribunda* (Clusiaceae). *Tree Genetics and Genomes* 6: 127-136.
- Ballou JD, Lees C, Faust LJ, Long S, Lynch C, Lackey LB; Foote TJ (2010). Demographic and genetic management of wild populations. In: Kleiman DG, Thompson KV, Baer CK (Eds.) *Wild mammals in captivity: Principles and techniques for Zoo management*. The University of Chicago Press, Illinois, USA. Pp. 219-252.
- Barrett SCH, Charlesworth, D (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature* 352: 522-524.
- Bilde T, Lubin Y, Smith D, Schneider JM, Maklakov AA (2005). The transition to social inbred mating systems in spiders; role of inbreeding tolerance in a subsocial predecessor. *Evolution* 59(1): 160-174.
- Brennan PA, Kendrick KM (2006). Mammalian social odours: Attraction and individual recognition. *Philos. Trans. R. Soc. B Biol. Sci.* 361: 2061–2078.
- Burgos-Paz W, Cerón-Muñoz M, Solarte-Portilla C (2011). Genetic diversity and population structure of the guinea pig (*Cavia porcellus*, Rodentia, Caviidae) in Columbia. *Genetics and Molecular Biology* 34(4): 711-718.
- Brust V, Guenther A (2015). Domestication effects on behavioural traits and learning performance: comparing wild cavies to guinea pigs. *Animal Cognition* 18(1): 99-108.
- Carter AM (2007). Animal models of human placentation – A review. *Placenta* 28: S41-S47.
- Charlesworth D, Willis JH (2009). The genetics of inbreeding depression. *Nature Genetics Reviews* 10: 783-796.
- Cheetham SA, Thom MD, Jury F, Ollier WER, Beynon RJ, Hurst JL (2007). The genetic basis of individual-recognition signals in the mouse. *Current Biology* 17: 1771–1777.
- Dunnum JL, Salazar-Bravo L (2010). Molecular systematic, taxonomy and biogeography of the genus *Cavia* (Rodentia: Caviidae). *Journal of Zoological Systematics and Evolutionary Research* 48(4): 376-388.
- Food and Agriculture Organization Stat (FAOSTAT) (2003). <http://faostat.fao.org/>.
- FAO (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome. Frankham R (2005). Genetics and extension. *Biological Conservation* 126 (2): 131-146.
- Franz R, Soliva CR, Kreuzer M, Hummel J, Clauss M (2011). Methane output of rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) fed a hay-only diet: Implication for for the scaling of methane production with body mass in non-ruminant mammalian herbivores. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 158(1): 177-181.
- Frère CH, Krützen M, Kopps AM, Ward P, Mann J, Sherwin WB (2010). Inbreeding tolerance and fitness costs in wild bottlenose dolphins. *Proceedings of the Royal Society B* doi:10.1098/rspb.2010.0039.
- Gamble LR, McGarigal K, Compton BW (2007). Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: Implications for spatio-temporal population dynamics and conservation. *Biological Conservation* 139: 247-257.
- Goodman SJ (1997). RST CALC: a collection of computer programs for calculating estimates of genetic differentiation from micro-satellite data and determining their significance. *Molecular Ecology* 6: 881– 885.

- Hardouin J, Demay F, Fransolet MF (1991). Le cobaye *Cavia porcellus* L., animal de boucherie en pays tropicaux. *Annales de Gembloux* 97: 69-80.
- Hoarau G, Boon E, Jongma DN, Ferber S, Palsson J, Van der Veer HW, Rijnisdorp AD, Stam WT, Olsen JL (2005). Low effective population size and evidence of inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proceedings of the Royal Society B* 272: 497-503 doi:10.1098/rspb.2004.2963.
- Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DH, Cavaggioni A, Beynon RJ (2001). Individual recognition in mice mediated by major urinary proteins. *Nature*, 414: 631-634.
- Javanmard A, Mohammadabadi MR, Zarrigabayi GE, Gharahedaghi AA, Nassiry MR, Javadmansh A, Asadzadeh N (2008). Polymorphism within the intron region of the bovine leptin gene in Iranian Sarabi cattle (Iranian *Bos taurus*). *Russian Journal of Genetics* 44 (4), 495-497.
- Kanitz R, Trillmich F, Bonatto SL (2009). Characterization of new microsatellite loci for the South-American rodents *Cavia aperea* and *C. magna*. *Conservation Genetics Resources* 1:47-50.
- Krause ET, Krüger O, Kohlmeier P, Caspers BA (2012). Olfactory kin recognition in a songbird. *Biology Letters* 8: 327-529.
- Lammers PL, Carlson SL, Zdorkowski GA, Honeyman MS (2009). Reducing food insecurity in developing countries through meat production: the potential of the guinea pig. *Renewable Agriculture and Food Systems* 24(2): 155-162.
- Mohammadabadi MR, Nikbakhti M, Mirzaee HR, Shandi A, Saghi DA, Romanov MN, Moiseyeva IG (2010). Genetic variability in three native Iranian chicken populations of the Khorasan province based on microsatellite markers. *Russian Journal of Genetics* 46(4): 505-509.
- Mohammadi A, Nassiry MR, Mosafer J, Mohammadabadi MR, Sulimova GE (2009). Distribution of BoLA-DRB3 allelic frequencies and identification of a new allele in the Iranian cattle breed Sistani (*Bos indicus*). *Russian Journal of Genetics* 45(2): 198-202
- Mousavizadeh A, Mohammad Abadi MR, Torabi A, Nassiry MR, Ghiasi H, Esmailizadeh AK (2009). Genetic polymorphism at the growth hormone locus in Iranian Talli goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). *Iranian Journal of Biotechnology* 7 (1): 51-53.
- Nolte DL, Mason JR, Lewis SL (1994). Tolerance of bitter compounds by an herbivore, *Cavia porcellus*. *Journal of Chemical Ecology* 20(2): 303-308.
- O'Grady JJ, Brook BW, Reed DH, Ballou JD, Tonkyn, Frankham R (2006). Realistic levels of inbreeding depression strongly affect extinction risks in wild populations. *Biological Conservation* 133(1): 42-51.
- Raymond M, Rousset F (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.
- Ralls K, Ballou JD (2004). Genetic status and management of California condors. *The Condor* 106(2): 215-228.
- Rice WR (1989). Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- Ritland K (2002). Extension models for the estimation of mating systems using n independent loci. *Heredity* 88:221-228.
- Rousset F (2008). Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103-106.
- Ruzina MN, Shtyfurko TA, Mohammadabadi MR, Gendzhieva OB, Tsedev T, Sulimova GE (2010). Polymorphism of the BoLA-DRB3 gene in the Mongolian, Kalmyk, and Yakut cattle breeds. *Russian Journal of Genetics*, 46:456-463.
- Sherborne AL, Thom LD, Paterson S, Jury F, Ollier WER, Stockley P, Beynon RJ, Hurst JL (2007). The genetic basis of inbreeding avoidance in mice. *Current Biology* 17: 2061-2066.
- Shojaei M, Mohammad Abadi MR, Asadi Fozi M, Dayani O, Khezri A, Akhondi M (2011). Association of growth trait and Leptin gene polymorphism in Kermani sheep. *Journal of Cell and Molecular Research*, 2(2): 67-73.
- Slatkin M (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462.
- Slev PR, Nelson AC, Potts WK (2006). Sensory neurons with MHC-like peptide binding properties: disease consequences. *Current Opinion in Immunology* 18: 608-616.
- Soulé M, Gilpin M, Conway W, Foose, T (2006). The millennium ark: how long a voyage, how many staterooms, how many passengers. *Zoo Biology* 5:101-113.
- Spotorno AE, Marin JC, Manriquez G, Valladares JP, Rico E, Rivas C (2006). Ancient and modern steps during domestication of guinea pigs (*Cavia porcellus* L.). *Journal of Zoology* 270(1): 57-62.
- Trillmich F, Kraus C, Künkele J, Asher M, Clara M, Dekomien G, Epplen JT, Saralegui A, Sachser N (2004). Species-level differentiation of two cryptic species pairs of wild cavies, genera *Cavia* and *Galea*, with a discussion of the relationships between social systems and phylogeny in the Caviinae. *Canadian Journal of Zoology* 82(3): 516-524.
- Umba J, Atangana AR, Khasa DP (Unpublished data). Phenotypic diversity in *Cavia porcellus* from Democratic Republic of Congo and Belgium.
- Weir BS (1996). Genetic data analysis II. Sunderland, Sinauer Associates, 445 pp.
- Wing ES (1978). Animal domestication in the Andes. In Browman DL (Ed.) *Advances in Andean Archaeology*, Mouton, La Haye. Pp. 167-186.
- Wright S (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395-420.
- Young AJ, Spong G, Clutton-Brock T (2007). Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a

cooperative mammal. Proceedings of the Royal Society Bdoi: 10.1098/rspb.2007.0316.

Yuta T, Koizumi I (2007). Does nest predation risk affect the frequency of extra-pair paternity in a socially monogamous passerine? Journal of Avian Biology doi:10.1111/jav.00713.

Zar JH (1984). Biostatistical analysis, 2nd edition. Prentice Hall, Englewood Cliffs, NJ. 718 pp

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