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Departament de Patologia i de Producció Animals
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tesi doctoral

PROGRAMA DE CONSERVACIÓ I MANTENIMENT DE RECURSOS GENÈTICS ANIMALS EN LA RAÇA ASININA CATALANA

PILAR FOLCH LÓPEZ
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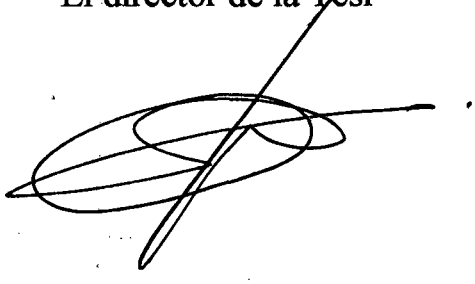
Programa de Doctorat de Producció Animal

PROGRAMA DE CONSERVACIÓ I MANTENIMENT DE RECURSOS GENÈTICS ANIMALS EN LA RAÇA ASININA CATALANA

Memòria presentada per
Pilar Folch López
per a optar al grau de Doctor en Veterinària.

Vist i plau de:

El director de la Tesi



Jordi Jordana i Vidal

L'autora

Pilar Folch López

Bellaterra, 18 Juny del 1998

ÍNDEX

El ruc porta picarol
i la somera també el vol.

Introducció

1. La regressió racial i la importancia de la conservació

La regressió racial és un fenomen que òbviament no afecta tan sols a l'espècie asinina. La davallada en el nombre mundial de races afecta de forma dramàtica a totes o quasi totes les espècies (Hall i Ruane, 1993), i sorgeix la controvèrsia de si s'han o no de conservar (Maijala i col, 1984; Simon, 1984; Land, 1986). Al predre's les races, es perden els gens que porten, i el problema més greu és el gran desconeixement que tenim de moltes d'aquestes poblacions amb tendència a l'extinció, en quant a la seva resposta a la millora genètica, a la seva productivitat en un medi ambient, a si són o no portadores d'alguns gens majors interessants i valuosos -en els moments actuals o en el futur- que no es trobin en altres races, al seu poder d'heterosi per a realitzar encreuaments, etc. L'afirmació de Mason (1974) de que *"qualsevol extinció o desaparició d'una espècie o raça, representa un irreemplaçable element de la diversitat de la vida que es perd"*, hauria de ser raó suficient per a justificar qualsevol Programa de Conservació en les espècies i races en perill d'extinció.

No obstant això, cal donar uns punts de vista més objectius que pugin justificar aquests tipus de programes. De forma general i resumida, i segons Simon (1984), Maijala (1987) i Anònim (1992b), podem enumerar com a possibles raons vàlides per a la conservació de les races les següents:

Raons genètiques i productives: es fa necessari mantenir la variabilitat genètica de les poblacions, per poder adaptar-nos així a possibles noves necessitats

productives (p.ex., canvis en la demanda de productes d'origen animal, adaptació a condicions ambientals canviants, resistència a determinades malalties infeccioses o parasitàries, etc.), així com també a la producció en condicions desfavorables (p.ex., explotació de recursos vegetals marginals no competitius amb l'home, importància dels encreuaments per a l'aprofitament de l'heterosi i la complementació de races, etc.).

Raons científiques: estudi de cada raça en particular per a la recerca de gens únics i valuosos, a través de la identificació de QTL (*Quantitative Trait Loci*) mitjançant anàlisi de genètica molecular. La conservació de les poblacions també proporciona un excel.lent material d'investigació, el qual pot contribuir al millor coneixement i interpretació, tant en les espècies animals com en l'home, d'alguns aspectes de l'evolució, domesticació, comportament, i els efectes de la selecció natural i/o artificial, entre altres.

Raons històriques i culturals: conservació de determinades races com a patrimoni genètic d'un país i com a història viva i paral.lela de les poblacions humanes.

Raons ecològiques i ambientals: determinades zones ambientals són el resultat d'un clima, flora i fauna típics i en perfecte equilibri. En aquest medi, per la seva duresa o per les seves característiques, no hi poden habitar d'altres poblacions. L'extinció de les races podria arribar a deteriorar el medi i la simbiosi ecològica de la zona.

Lògicament, també ha sorgit per part de diferents autors arguments en contra de la conservació, contrarrestant, amb més o menys encert, les opinions

conservacionistes. No obstant això, tothom es mostra d'acord en les raons de tipus historicoculturals, emotives i de conservació del patrimoni i de la biodiversitat genètica, justificant aquestes, per elles mateixes, qualsevol "Programa de Conservació i manteniment de recursos genètics animals" que es pogués posar en marxa.

2. Situació actual i perspectives futures de la Raça Asinina Catalana

L'evolució censal dels èquids a Espanya

La població asinina espanyola, així com la cavallar i mular, ha anat disminuint ininterrompudament durant aquest segle. *L'Anuario de Estadística Agraria* (Anònim, 1992a) dóna unes xifres oficials d'ases d'aproximadament 1.100.000 exemplars durant els anys 20 i començaments dels 30, essent actualment ***-dades del 1990-*** de només 130.000 animals (Taula 1). El període en què es dóna la gran davallada en el cens dels èquids són les dècades dels 60 i dels 70, probablement a causa de la intensa mecanització del camp que es comença a produir a Espanya durant aquells anys. El cens d'ases de l'any 1980 sofreix una davallada del 73% respecte al que hi havia l'any 1960. Centrant-nos en Catalunya, l'últim cens, realitzat l'any 1990, indica que hi ha un total de 415 animals.

Taula 1. Evolució censal dels Equids a Espanya (ESP) i Catalunya (CAT) durant el s.XX.

	ASES		*CAVALLS*		*MULES*	
ANY	**ESP**	**CAT**	**ESP**	**CAT**	**ESP**	**CAT**
1929	1.006.000		598.000		1.154.000	
1935	1.176.000		808.000		1.475.000	
1940	851.000		592.000		1.139.000	
1950	732.000		642.000		1.089.000	
1960	686.000		506.000		1.158.000	
1970	368.000		282.000		533.000	
1976	253.000	3.702	262.000	13.301	281.000	15.452
1978	232.000	5.411	257.000	19.016	253.000	17.861
1980	188.000	3.252	242.000	9.233	199.000	6.264
1984	160.000	1.550	254.000	11.349	145.000	4.069
1986*	140.000	928	248.000	9.256	117.000	2.603
1988**	131.000		250.000		110.000	
1990***	130.000	415	241.000	22.027	100.000	543
1992	130.000		240.000		100.000	

Font: Anuario de Estadística Agraria (Anònim, 1992a).

* Catalunya: a partir de 1986 i fins el 1992, tots els censos fan referència a l'any 1986.

** A partir de l'any 1988 les dades censals provenent de l'Anuari de Producció de la FAO.

*** Els cens de l'any 1990, referents a Catalunya, estan extrets d'Anònim (1991)

Respecte a la Raça Asinina Catalana, el cens actual és molt reduït, i supera en poc el centenar d'individus, una tercera part dels quals són mascles. Aquestes xifres classifiquen la raça dins de la categoria de Raça Crítica (< 100 femelles reproductores) segons la proposta del Comitè d'Experts de la FAO (Organització de les Nacions Unides per a l'Agricultura i l'Alimentació); això implica que aquesta població està en perill d'extinció, i que sense cap tipus d'acció, la seva grandària efectiva és inadequada per a poder prevenir contínues pèrdues genètiques en generacions futures (Bodó, 1992).

Possibles causes de regressió racial

Tradicionalment la cria d'ases ha estat motivada per la seva utilització com a animal de treball i càrrega o tir, éssent utilitzat tradicionalment al sud d'Europa en el cultiu de la vinya, mentre que en el nord era utilitzat pel conreu de petites parceles. L'altre ús que se li donava a aquests animals era la producció mulatera, que altrament era orientada també cap al treball (càrrega i tir).

Entre les possibles causes que han fet que aquesta raça es trobi en perill d'extinció podrien anomenar les següents:

- Pèrdua de l'aptitud treball, principalment degut a la intensa mecanització del camp i de les feines forestals.
- Hàbitat: el despoblament de certes zones i/o la manca de reemplaçament dels ramaders vells.
- Tancament d'explotacions per manca de productivitat (insuficient desenvolupament tecnològic i de formació profesional).

- Mala planificació dels encreuaments amb animals d'altres races. Política de reposició errònia.
- Manca d'informació sobre la utilització de les races autòctones.
- La política i el desenvolupament ramaders.

Les raons econòmiques poden fer que es limiti el nombre de races existents en un país, argumentant que l'excessiu nombre de races dissemina els esforços de selecció en massa direccions. Els costos de conservació i manteniment d'una raça minoritària, també poden ser un altre argument poderós, ja que encara no està clar quines haurien d'ésser les fonts de finançament.

Perspectives futures de la raça

En quant a les perspectives futures d'aquesta raça, el que sembla clar és que no pot desaparèixer ja que és un patrimoni genètic únic i valuós, i com diu Hall (1993) *"les races domèstiques locals són recursos genètics que han d'ésser protegits com a part de l'herència mundial de la biodiversitat"*.

En els temps actuals encara pot tenir una certa importància econòmica; tant la seva cria en puresa com per a la producció de mules sobretot, i tal com recomana el Comité d'Experts de la FAO (Anònim, 1992b), en les zones tropicals i en alguns països en vies de desenvolupament, ja que aquesta espècie sembla ser més important que no pas el cavall. L'exportació de guarans o, en el seu lloc, semen congelat, pot ésser interessant per a la millora genètica d'altres poblacions asinines mundials. L'explotació forestal, en zones de difícil accés, pot continuar requerint els serveis d'aquests animals; en la mateixa línia, pot actuar com a

element netejador de boscos per a la prevenció d'incendis forestals. La comercialització d'un producte d'alta qualitat, amb un important valor afegit, com pot ésser la llet de burra, podria ser interessant en un mercat, encara que força restringit, però d'un elevat poder adquisitiu. I ja per últim, la important orientació de l'ase com animal de companyia i de turisme lúdic (agroturisme) en zones de muntanya.

3. Esquema global d'un possible pla de treball per a la conservació i millora de la Raça Asinina Catalana

L'any 1978, i a causa de la greu situació que travessava aquesta raça, es creà a Banyoles l'*Associació del Foment de la Raça Asinina Catalana* (AFRAC), per a protegir, fomentar i millorar l'esmentada població. Dins d'aquest context va néixer, a finals del 1994, la necessitat de portar a terme un "*Programa de Conservació i manteniment de recursos genètics animals*" en aquesta raça, promogut i finançat pel Departament d'Agricultura Ramaderia i Pesca (D.A.R.P.) de la Generalitat de Catalunya, en col.laboració amb la pròpia AFRAC i la Unitat de Genètica i Millora de la Facultat de Veterinària de Barcelona (UAB).

El principal objectiu del Programa fou la conservació de la població en forma d'animals vius i de material criogènic (semen, embrions, ...), així com el manteniment de la màxima quantitat de diversitat genètica possible. Així mateix,

una vegada assegurada la conservació es podria plantejar una segona fase de Millora Genètica de la raça orientada cap a aquelles utilitats econòmiques que en aquells moments siguin oportunes.

Seguint les recomanacions i directrius marcades per la FAO, es pot dividir el programa en 5 fases ben diferenciades, ordenades segons la seva realització cronològica, però intimament relacionades per al bon desenvolupament i assoliment dels objectius globals proposats.

I. FASE: DESCRIPCIÓ GENERAL DE LA POBLACIÓ

I. Recopilació de dades preliminars d'interès general.

i. Localització geogràfica.

ii. Origen i entronc filogenètic.

iii. Evolució censal i situació actual.

iv. Possibles causes de regressió racial i tendència futura.

v. Perspectives futures de la raça i raons vàlides per a la conservació: estudis socio-econòmics que ressaltin la seva importància en la zona

vi. Característiques racials, productives, reproductives, ecològiques, etc., d'interès.

II. Inventari censal (real), registre i identificació individual (microxips).

II. FASE: CARACTERITZACIÓ DE LA RAÇA

I. Caracterització morfològica: qualitativa i biomètrica.

La caracterització morfológica de la població permetrà crear, reglamentar i gestionar el Llibre Genealògic de la raça.

II. **Caracterització hematològica i bioquímica clínica.**

III. **Caracterització genètica: polimorfismes bioquímics i marcadors moleculars (microsatèl.lits).**

La caracterització genètica permetrà entre altres coses:

- analitzar els nivells de variabilitat genètica de la població,
- obtenir valors mitjans de Consanguinitat,
- identificar genèticament els individus i realitzar proves de control de paternitats,
- identificar els individus més heterocigots per a la programació d'aparellaments.

IV. **Caracterització de l'estructuració genealògica i demogràfica.** (anàlisi de les dades procedents dels pedigrees).

Ens permetrà estudiar:

- els paràmetres demogràfics tals com l'edat al primer part, temps de vida útil dels reproductors, variàncies de la grandària familiar o intervals generacionals entre d'altres,
- i per a calcular els Coeficients individuals de Consanguinitat (F) i de parentiu (r): Programar els aparellaments de forma que es procuri el mínim increment de Consanguinitat per generació,
- l'evolució de la Consanguinitat (ΔF generacional i anual),
- la probabilitat d'origen dels gens, per al càlcul de l'anomenat *Índex de Conservació Genètica* (GCI) (Alderson, 1992) que mesura el *Nombre Efectiu de Fundadors* (f_e) (Boichard i col., 1997) que hi ha en el pedigree d'un individu. Aquest índex és de gran utilitat per a conèixer l'efecte dels ancestres fundadors per al manteniment de la variabilitat genètica.

III. PROGRAMA DE CONSERVACIÓ GENÈTICA "IN SITU".

Es a dir, la conservació i manteniment d'animals vius, essent l'objectiu prioritari el manteniment de la màxima quantitat de diversitat genètica, amb el mínim increment de consanguinitat possible per generació.

Els criteris a seguir són:

i. Augmentar la grandària poblacional, i, en particular, maximitzar el *Nombre Efectiu de Reproductors (Ne)*.

ii. Maximitzar la influència dels animals fundadors (mitjançant l'Index de Conservació Genètica, GCI).

iii. Minimitzar les pèrdues d'heterocigositat degudes a diferents factors (consanguinitat, selecció, deriva,...): Programa de Consanguinitat Mínima; programació dels aparellaments a partir de la informació dels Coeficients de Parentiu (r), i/o dels individus més heterocigots de la població (informació procedent de l'anàlisi dels marcadors moleculars).

IV. FASE: PROGRAMA DE CONSERVACIÓ GENÈTICA "*EX SITU*".

Quan els mitjans tècnics i econòmics, així com la pròpia infrastructura del programa ho permetin, es procedirà a la conservació "ex situ" del material genètic, mitjançant:

i. Conservació criogènica de semen i embrions

ii. Conservació d'ADN

V. FASE: PROGRAMA DE MILLORA GENÈTICA.

Aquesta fase, lògicament, es portaria a terme quan la població ja estés fora de perill i l'increment de consanguinitat no representés un greu problema.

Objectiu de selecció: intentar millorar genèticament algun caràcter d'interès econòmic de la població.

Criteris de selecció: a decidir en el seu moment, provindran de la informació recollida dels caràcters morfològics, de comportament, i/o productius.

Metodologia d'avaluació genètica: Índex de Selecció i metodologia BLUP (Model Animal) per a l'avaluació genètica dels reproductors.

Nota: Tant la conservació "*in situ*" com "*ex situ*", tenen de forma individual avantatges i desavantatges, pel que de forma òptima s'aconsellaria una combinació d'ambdues.

Les poblacions "*in situ*" permeten demostrar de forma més palpable els resultats d'un pla de conservació, evitant així mateix que la raça no caigui en l'oblit del poble. També permet l'estudi i observació permanent dels animals fent possible l'estudi de noves característiques desconegudes que podrien ser interessants en un futur. Això no seria possible en un programa de conservació "*ex situ*". Altrament, el fet de conservar un pool d'animals vius possibilita que els productes que es puguin obtenir de la explotació d'aquests, es puguin comercialitzar, i d'aquesta forma minimitzar els costos que suposen el manteniment de la població viva. En canvi, els plans de conservació "*ex situ*" necessiten un cert desenvolupament tècnic i es corre el perill de que el material congelat pugui ser malmés per negligència humana (posem pel cas un banc de semen), tot i que són fora de perill de possibles epidèmies que afectarien als animals vius. Però potser el que millor justifica la combinació d'ambdos tipus de programa, és que la conservació "*in situ*" té unes despeses de manteniment molt més elevades que el programa "*ex situ*", fent que només es puguin mantenir poblacions de grandària reduïda.

4. Objectius.

L'objectiu últim i fonamental del Programa és la conservació, manteniment i millora dels animals que integren aquesta raça. No obstant, aquest és un objectiu massa global i a llarg plaç, pel que es fa necessari desglossar-lo en altres més senzills, concrets i d'assoliment a curt o mitjà plaç, els quals configuren les diferents propostes assenyalades en l'actual treball de tesi.

Objectiu general de la Tesi

El present treball de tesi que aquí presentem té com a objectiu general poder donar als diferents criadors una sèrie de pautes i recomanacions per a establir un programa de conservació "*in situ*" òptim i coherent en la Raça Asinina Catalana, és a dir, la realització de les tres primeres fases de l'esquema global proposat de conservació i millora, i que correspón al títol de la present tesi: "Programa de Conservació i Manteniment de Recursos Genètics Animals en la Raça Asinina Catalana".

En les poblacions de reduïda grandària poblacional, com és la nostra, els problemes derivats de la consanguinitat solen ser importants, fonamentalment per dos motius: per l'anomenada *Depressió Consanguínia*, que comporta una disminució en els rendiments mitjans dels caràcters quantitatius, sobretot aquells rel.lacionats amb la reproducció (fecunditats, taxes d'ovulació, prolificitats, etc...), i per tant, resulta obvi que això s'ha de tenir en compte per a evitar una disminució, no desitjada, del cens poblacional; i per a evitar, així mateix, una disminució de la

variabilitat genètica de la raça degut a la creixent homocigositat que comporta la consanguinitat.

Per tant els dos objectius fonamentals propostats a curt termini seran:

1. Evitar la disminució (i/o) extinció del cens poblacional.

2. Aturar les pèrdues de variabilitat genètica d'aquesta població.

El Programa de Conservació que s'instaurarà tindrà com a objectiu prioritari el **manteniment de la màxima quantitat de diversitat genètica**, amb el **mínim increment de consanguinitat possible per generació**. Per a tenir en compte aquest objectiu, el criteri d'elecció per a l'aparellament òptim d'un guarà amb una somera, hauria de ser aquell que maximitzes l'Índex de Conservació Genètica (GCI) i minimitzes la Consanguinitat (F) d'un hipotètic fill de la parella.

Objectius específics

Objectiu 1 : DESCRIPCIÓ GENERAL DE LA POBLACIÓ.

1.1. Recopilació de dades preliminars d'interés general.

1.2. Inventari censal, registre i identificació individual amb microxips.

Objectiu 2 : CARACTERITZACIÓ DE LA RAÇA.

2.1. Caracterització Morfològica: Tant a nivell qualitatiu com morfomètric. Establiment del prototipus racial i anàlisi de les rel.lacions existents entre les variables biomètriques.

2.2. Caracterització Hematològica i Bioquímica Clínica: Descripció dels perfils i rangs de normalitat de la raça. Comparació amb altres poblacions asinines mundials.

2.3. Caracterització Genètica: Anàlisi de polimorfismes bioquímics i marcadors d'ADN microsatèl.lit per a l'estudi de l'estructura genètica poblacional. Obtenció d'una eina fiable per al diagnòstic de paternitats.

2.4. Caracterització de l'estructura Genealògica i Demogràfica: Anàlisi dels diferents paràmetres demogràfics que incideixen sobre el Nombre Efectiu de Reproductors (N_e) i conseqüentment sobre l'evolució de la consanguinitat (F). Obtenció dels Índex de Conservació Genètica per a la programació d'aparellaments.

Objectiu 3: VIABILITAT DE LA RAÇA ASININA CATALANA SIMULADA PER ORDINADOR.

Predicció del que passaria en un futur més o menys llunyà si no s'instaurés cap Programa de Conservació en aquesta població en perill d'extinció.

5. Antecedents bibliogràfics

Descripció general de la població

Origen i entronc filogenètic de la raça

Encara avui, hi ha gran discrepància entre els diferents autors sobre els origens dels Equins, no obstant es pot considerar al *Pliohippus* o *Plesihippus*, com l'antecesor del gènere *Equus* en totes les seves formes. El gènere *Equus* es va desenvolupar probablement al Nord del continent americà, d'on va passar a Europa, Àsia i Àfrica. Al final del Pleistocè o començaments de l'Holocè (fa ara uns 9.000 a 10.000 anys), *Equus* va desaparèixer completament del continent Americà, desenvolupant-se a partir de llavors únicament en el Vell Món (Ravazzi, 1991).

El gènere *Equus* conté sis subgèneres: *Asinus*, *Hemionus*, *Equus*, *Dolichohippus*, *Hippotigris* i *Quagga*. No obstant, tant Corbet (1978) com Bennet (1980), basant-se en estudis de troballes arqueològiques, només en reconeixen dos, el *Equus asinus*, descendent del *Astrohippus*, amb tres especies: *E.a.asinus*, *E.a.hemionus* i *E.a.kiang*, i el subgènere *Equus equus*, descendent directe del Dinohippus, amb cinc espècies: *E.e.caballus*, *E.e.quagga*, *E.e.burchelli*, *E.e.zebra* i *E.e.grevyi*.

El procès de domesticació dels ases s'inicia durant l'Holocè (fa uns 8-10 mil anys) a la zona nord-est d'Àfrica, disseminant-se posteriorment cap al sud-oest d'Àsia i sud d'Europa (Littauer i Crouwel, 1979). Les troballes més antigues d'ases domèstics daten del 4.000 A.C., trobats a Egipte i associats a d'altres esquelets

d'animals domèstics. Als voltants del 3.000 A.C., ja comencen a veure's murals on l'ase és freqüentment representat i utilitzat com a bestiar de càrrega.

Fins a principis del 3er mileni A.C., els ases domèstics no van aparèixer al sud-oest d'Àsia. A l'Índia i Paquistà, no es troben ossos d'ase domèstic fins al 2.000-800 A.C. A Europa, la domesticació de l'ase es dóna més tard que a l'Àfrica i Àsia, i tenint en compte que la seva adaptació a climes humids i freds és baixa, mai van arribar a ser gaire nombrosos en les terres del nord. Àsia Menor, sembla ser que va ser el punt a partir del qual es van introduir els ases a Europa, a través d'Ucraïna, Rússia i la península dels Balcans. Hi han evidències de la presència d'aquests animals a Ucraïna que daten del 800-900 A.C. Des dels Balcans, l'ase arribà a Itàlia, produint-se la seva expansió a la resta d'Europa de forma paral.lela a les conquestes romanes. No obstant, algunes poblacions d'ases de la Península Ibèrica provenen directament d'animals que van passar des d'Àfrica a través de l'Estret de Gibraltar (Epstein, 1984).

Hi han força discrepàncies sobre l'origen de les races d'ases actuals, però es poden diferenciar dues tendències bastant clares. Una, defensada per Epstein (1984), Clutton-Brock (1987) i Camac (1989), els quals defensen que les actuals races asinines semblen venir de dos troncs ancestrals: l'*Equus asinus africanus*, o Ase de Nubia, nadiu de la Conca del Nil, i l'*Equus asinus somaliensis*, o Ase de Somalia, que consequentment hauria donat lloc als ases del Sudest asiàtic i probablement també a la majoria de les races europees, entre les que es trobaria la Raça Asinina Catalana. Un altre grup d'autors com Sanson (1911), Dechambre (1921), Romagosa (1959), Aparicio (1960), Sotillo i Serrano (1985), mantenen també la teoria de que haurien existit dues linies ancestrals, una originària del Nordest d'Àfrica que correspondria a l' *Equus asinus africanus*, i l'altre l'*Equus*

asinus europeus, on la seva àrea d'influència seria la Conca Mediterrània, concretament les Illes Balears, d'on haurien sorgit la majoria de races asinines europees, i també aquesta raça.

Influència de la raça asinina Catalana en altres races mundials.

Diverses troballes fòssils, a diferents indrets de Catalunya indiquen que la presència d'ases en aquesta zona del sud d'Europa data de ja fa uns quants milers d'anys (Romagosa, 1959; Torres i col., 1983). No s'han realitzat estudis objectius per a la datació d'aquests fòssils, encara que tot sembla indicar que podrien ser del final del període Quaternari.

Les races espanyoles d'ases han tingut força importància en la formació i millora d'altres poblacions, sobretot europees. La raça asinina Catalana ha contribuit en la millora de la raça francesa *Poitou*, preferentment per a augmentar la talla i millorar el poder genèsic dels seus animals (Romagosa, 1959; Torres i col., 1983; Parés i Vilaró, 1994), encara que la raça espanyola que més ha contribuit en la formació del *Poitou* ha sigut la *Zamorano-Leonesa*, exemplars dels quals van ser exportats a França durant el regnat de Felip V (Torres i col., 1983, Sotillo i Serrano, 1985). La raça *Andalusa* seria segons Sotillo i Serrano (1985) la precursora de *l'Ase Brasiler* o *Lagoa Dorada*.

Més important ha sigut la influència que ha tingut la raça asinina Catalana en diferents races italianes, contribuint de forma decisiva en la seva formació i millora. Entre aquestes podem anomenar les races *Pantellaria*, *Martina-Franca* i la *Siciliana* o *Ragusana* (Romagosa, 1959; Aparicio, 1960; Torres i col., 1983;

Epstein, 1984; Sotillo i Serrano, 1985; Parés i Vilaró, 1994). Sotillo i Serrano (1985) també assignen una certa influència de la raça asinina Catalana en les races mediterrànies *Maltesa* i *Xipriota*, originàries d'ases africans i asiàtics.

Però a on tots els autors es manifesten d'acord en quan a la directa i decissiva influència de la raça asinina Catalana en la formació d'una raça, és en *l'Ase Americà* (Romagosa, 1959; Aparicio, 1960; Briggs, 1971; Torres i col., 1983; Epstein, 1984; Sotillo i Serrano, 1985; Parés i Vilaró, 1994), també anomenat *Ruc de Kentucky* o *Mammoth* en honor al que és considerat com a millor semental fundador de la raça, anomenat "Imported Mammoth" que arribà a Charleston (Carolina del Sud) l'any 1819, procedent de Catalunya, éssent emprat de forma massiva en les zones dels estats de Kentucky, Tennessee i Missouri (Briggs, 1971).

I ja per últim, comentar que diferents autors fan referència a les diverses exportacions realitzades de rucs catalans, a les darreries del segle passat i també durant l'actual, a llocs tan llunyans i dispersos com Alemanya, Algèria, Argentina, Austràlia, Brasil, Canadà, Congo, Cuba, Gran Bretanya, India, Madagascar, Mèxic, Repúbliques Centre-americanes, Sudàfrica, Tuníssia i Zaire.

Caracterització morfològica

A la bibliografia hi han escassos estudis morfològics en l'espècie asinina, i en particular de la Raça Asinina Catalana. Els primers estudis sobre aquesta raça vàren ser els que a principis de segle va portar a terme el professor Rosell i Vilar (1921) a partir de troballes arqueològiques i de l'estudi dels animals vius a l'època.

El Dr. Salvans en el seu traball "El garañón catalán" (1947) fa un estudi de les principals zones de producció de guarans a Catalunya describint les diferents línies de cria a cadascuna de les set comarques productores de l'època. De forma similar, Ferrer i Palaus (1957) descriu tres zones on diferencia la seva importància tant per la qualitat dels productes com per la seva intensitat de comercialització.

Pocs anys després, el Dr. J.A. Romagosa (1959), en la seva tesi doctoral, fa una acurada descripció dels animals d'aquesta raça tant morfològica com censal. Aquest estudi és particularment important perquè és el primer en el que es prenen una sèrie de mesures biomètriques utilitzant-les posteriorment per a classificar als individus d'aquesta població utilitzant les sistemàtiques de classificació de Barón (1888) i Sanson (1911). També proposa una diferenciació de les zones de cria d'acord amb el tipus d'hàbitat, sistemes de recria i àrees de comercialització.

El Dr. G. Aparicio (1960), en el seu compendi d'etnologia també fa una descripció morfològica utilitzant els mateixos criteris de classificació que pocs anys abans havia utilitzat Romagosa per a descriure als animals d'aquesta raça.

Després de les aportacions d'aquests autors, no es vàren realitzar nous estudis sobre aquesta població, i totes les referències que hi han a la bibliografia d'una o altre forma estan preses d'aquests estudis (Homedes, 1967; Torres i col., 1983; Sotillo i Serrano, 1985).

En referècia a d'altres races asinines espanyoles, n'hi han algunes descripcions de tipus qualitatiu, i són molt escasses les de tipus biomètric, éssent realitzades generalment amb un nombre molt reduït d'animals i poques variables analitzades (Aparicio, 1960; Homedes, 1967; Sotillo i Serrano, 1985).

D'altra banda sí que són nombrosos els estudis morfològics i biomètrics realitzats en l'especie cavallar, éssent inclús l'espècie de referència a partir de la qual es desenvolupen totes les variables biomètriques. Cal destacar els estudis portats a terme en els cavalls Pura Raça Espanyol o Andalús per Aparicio i col. (1986); en el cavall Àrab per Fuentes i col. (1987); en la raça de cavalls Lusitana per Oom (1992); Oom i Costa Ferreira (1993) en el cavall Pur Sang Anglés a Espanya per Hevia i col. (1993) i en el cavall Mallorquí (Parés i Payeras, 1997). És a partir d'aquests estudis biomètrics que ens basarem per a prendre els valors de referència i així poder interpretar les variables morfomètriques considerades i els índex zoomètrics calculats a partir d'elles, i d'aquesta forma poder definir les proporcions i conformació dels animals que configuren aquesta població.

Des del punt de vista qualitatiu, per a procedir a la caracterització morfològica de la raça ens basarem en les descripcions donades per Romagosa (1959), Aparicio (1960), Homedes (1967), Torres i col., (1983) i Sotillo i Serrano (1985), així com amb la informació aportada per diferents ramaders o propietaris de l'AFRAC i amb la nostra pròpia.

Caracterització hematològica i bioquímica clínica

La determinació dels perfils hematològics i bioquímic clínics ens permetrà establir els rangs de referència o de normalitat en aquesta població, així com, estudiar els efectes del sexe i l'edat sobre aquests paràmetres. Aquest tipus d'anàlisi a més de ser important per a la caracterització racial, poden ser de gran utilitat per a la pràctica clínica veterinària.

Malauradament no existeixen estudis similars en altres poblacions asinines espanyoles, però sí que hi han referències en altres poblacions mundials, permetent-nos poder comparar els nostres resultats amb els obtinguts per aquests autors. En concret, s'han realitzat estudis en poblacions d'ases britànics (French i Patrick, 1995), americans (Brown i Cross, 1969; Nayeri, 1978; Zinkl i col., 1990), francesos, italians i hindús (Gupta i col., 1994). De forma similar a la caracterització morfològica, altres estudis portats a terme en l'espècie equina ens vàren servir per a comparar els resultats obtinguts en la nostra població (Alen i Archer, 1973; Rose i Alen, 1985; Jain, 1986; Kaneko, 1989; Bauer, 1990).

Caracterització genètica

La caracterització genètica d'una població permet no només la identificació de cada individu de la població per a la realització de proves de control de paternitats, sino que també pot ser de gran utilitat per a la detecció d'aquells individus més heterocigots de la població i poder utilitzar així aquesta informació per a programar els aparellaments, de tal forma que es minimitzi la pèrdua de variabilitat genètica per generació. D'altra banda també pot servir per a rel.lacionar filogenèticament a la població en estudi amb d'altres races.

En aquest camp encara és més important la manca d'informació rel.lacionada amb l'espècie asinina que en els apartats anteriors, éssent encara més difícil trobar treballs similars que poguem utilitzar com a punt de referència de la nostra feina i per comparar els resultats obtinguts amb els d'altres poblacions mundials.

A nivell de polimorfismes bioquímics (proteïnes i enzims de la sang) és on trobem més informació. La taula 2 resumeix els diferents estudis trobats en poblacions asinines i els polimorfismes trobats en els diferents sistemes estudiats.

Però on es pot donar un gran salt qualitatiu en l'estudi de la variabilitat de les poblacions, és amb l'estudi de l'àcid desoxirribonucleic (ADN). Actualment aquestes tècniques d'anàlisi de l'ADN són a l'abast de l'investigació i s'han arribat a tecnificar molt, permetent un gran ventall de possibilitats. A l'ADN, trobem unes regions on es repeteixen les mateixes seqüències una i altre vegada, algunes fins a milers (satèl.lits), centenars (midisatèl.lits) o decenes (minisatèl.lits) de bases (Bruford i Wayne, 1993). En canvi n'hi ha d'altres on només es repeteixen alguns nucleòtids (microsatèl.lits, o altrament anomenats *Short Tandem Repeats* -STRs-).

Aquest ADN altament repetitiu (microsatèl.lits) és utilitzat actualment com a marcadors d'elecció en l'estudi de la mesura de la variació i l'estructura genètica de les poblacions (Goldstein i Pollock, 1997) ja que:

- els podem trobar dispersos per tot el genoma (Hamada i col., 1982)
- la variabilitat individual d'aquestes seqüències és molt elevada, pel que han esdevingut peces claus en la construcció dels mapes genètics de moltes espècies (Weissenback i col., 1992; Dietrich i col., 1992; Zheng i col., 1993)
- les freqüències al.lèliques dels diferents loci venen modelades per l'atzar i no per processos de selecció que podrien modificar aquestes freqüències (gens neutres).
- estan basats en tècniques de PCR (*Polymerase Chain Reaction*) i són molt fàcils de genotipar (Litt i Luty, 1989; Tautz, 1989)
- la diversitat trobada en un marcador és fàcilment reproduïble i comparable entre laboratoris

N'hi han pocs estudis que s'hagin portat a terme per a investigar el nivell de polimorfisme en èquids utilitzant microsatèl.lits, si ho comparem amb els realitzats en altres espècies. I encara són menys els que han estudiat el polimorfisme de l'ADN en ases. Només hi ha un estudi en que Breen i col. (1994c) estudien el polimorfisme de 13 loci en 8 ases australians junt amb d'altres èquids (cavall domèstic, cavall de Przewalski, onagre, zebra de Grevy, zebra de Chapman i zebra de les muntanyes). A la taula 3 trobem el polimorfisme per a *E. asinus* en aquest estudi, junt amb les característiques del locus en qüestió. Així mateix, a la taula 4 es resumeixen les característiques d'altres loci analitzats en la present tesi i que no figuren en el treball abans esmentat.

Taula 2: Resum de les característiques dels loci proteïcs estudiats en *E.asinus*.

Sistema	*Autors*	*Polimorfisme*	*Al.lels*	*Població analitzada*
Transferrina (TF)	Osterhoff (1966)	Sí		Sudafricanes
	Singhvi i Khanna (1988)	Sí		Hindús
	Niece i Kracht (1967)	Sí	A, B, C, D	Americanes
	Bell (1994)	Sí	idem	Australianes
	Menguzzi i col., (1982)	Sí	idem	Sarda, Ragusani, Amiatini, Gubbio i Asinara
Albumina (ALB)	Blake i Douglas (1978)	Sí	C, D	Americanes
	Bowling i Nickel (1985)	Sí	idem	idem
	Bell (1994)	Sí	idem	Australianes
	Ouragh i col. (1996)	Sí	idem	Marroquines
6-Fosfogluconat Deshidrogenasa (PGD)	Menguzzi i col., (1982)	Sí	D, F, S	Sarda, Ragusani, Amiatini, Gubbio i Asinara
	Bell (1994)	Sí	idem	Australianes
Glucosa Fosfat Isomerasa (GPI)	Menguzzi i col., (1982)	monomòrfica		Sarda, Ragusani, Amiatini, Gubbio i Asinara
	Bell (1994)	monomòrfica		Australianes
Fosfoglucomutasa (PGM)	Menguzzi i col., (1982)	Sí	F, S	Sarda, Ragusani, Amiatini, Gubbio i Asinara
Fosfatasa Àcida (AP)	Menguzzi i col., (1982)	Sí	F, S	Sarda, Ragusani, Amiatini, Gubbio i Asinara
	Braend i Romagnoli (1980)	Sí	A, P	Italianes
Alfa1-β Glicoproteïna (A1B)	Patterson i Bell (1991)	monomòrfic, però al.lel específic	A_d.	Australianes
	Ouragh i col. (1996)	Sí	A, D, D*	Marroquines
Proteïna GC.	Bell (1994)	Sí	F, S	Australianes
	Ouragh i col. (1996)	Sí	idem	Marroquines
Carboxiesterasa	Kaminski (1970)	no té activitat		Franceses
Proteinasa Inhibidor (PI)	Ketchum i Cothran (1989)	Sí	C, D, M, T	Americanes

Taula 3: Resum de les característiques generals dels microsatèl.lits estudiats en *E. Asinus* per Breen i col. (1994c)

Locus	*Codi d'accés*	*Motiu Repetitiu*	*Seqüència dels cebadors "primers" utilitzats*	*Grandària (pb) al.lels trobats en E. Asinus.*
HTG6[1]	*	TG_{20}	5'CCTGCTTGGAGGCTGTGATAAGAT3' 5'GTTCACTGAATGTCAAATTCTGCT3'	84, 88, 90
HTG8[2]	U97528	TG_{17}	5'CAGGCCGTAGATGACTACCAATGA3' 5'TTTTCAGAGTTAATTGGTATCACA3'	182
HTG10[2]	*	TG_{16}	5'CAATTCCCGCCCCACCCCCGGCA3' 5'TTTTTATTCTGATCTGTCACATTT3'	92
HTG14[2]	*	TG_{14}	5'CCAGTCTAAGTTTGTTGGCTAGAA3' 5'CAAAGGTGAGTGATGGATGGAAGC3'	135
HTG15[2]	*	TG_{14}	5'TCTTGATGGCAGAGCCAGGATTTG3' 5'AATGTCACCATGCGGCACATGACT3'	130, 132, 136
HMS1[3]	X74630	TG_{15}	5'CATCACTCTTCATGTCTGCTTGG3' 5'TTGACATAAATGCTTATCCTATGG3'	164
HMS3[3]	X74632	AC_{23}	5'CCAACTCTTTGTCACATAACAAGA3' 5'CCATCCTCACTTTTTCACTTTGTT3'	163
HMS5[3]	X74634	AC_{12}	5'TAGTGTATCCGTCAGAGTTCAAAG3' 5'GCAAGGAAGTCAGACTCCTGGA3'	105
HMS6[3]	X74635	TG_{15}	5'GAAGCTGCCAGTATTCAACCATTG3' 5'CTCCATCTTGTGAAGTGTAACTCA3'	156, 158, 166
HMS7[3]	X74636	AC_{18}	5'CAGGAAACTGTTGATACCATC3' 5'TGTTGTTGAAACATACCTTGACTGT3'	167, 169
VHL20[4]	X75970	CA_{25}	5'CAAGTCCTCTTACTTGAAGACTAG3' 5'AACTCAGGGAGAATCTTCCTCAG3'	94
MPZ001[5]	Z28368	$AAAG_{12}$	5'GGCACTTGAGCTAACGTGTGTTGCC3' 5'CGGAGGAGGGCAACAGAGCC3'	164, 168
MPZ002[6]	Z28342	AC_{16}	5'GATCCCCCCTATTTTATATACAG3' 5'AGGTTCTCATTCTACCTACAAGG3'	81, 85

[1] Ellegreen i col., (1992), [2] Marklund i col., (1994), [3] Guerin i col., (1994), [4] Van Haeringen i col., (1994), [5] Breen i col., (1994a), [6] Breen i col., (1994b), * codi d'accés no disponible a la base de dades del GeneBank.

Taula 4: Resum de les característiques generals dels altres microsatèl.lits aïllats d' *Equus Caballus* que han estat utilitzats en aquesta tesi.

Locus	*Codi d'accés*	*Motiu Repetitiu*	*Seqüència dels cebadors "primers" utilitzats*	*Intèrval de grandària (pb) al.lels esperats en cavalls*
AHT4[1]	Y07733	AC_{18}	5'AACCGCCTGAGCAAGGAAGT3' 5'GCTCCCAGAGAGTTTACCCT3'	146-170
AHT5[1]	Y07732	TG_{16}	5'ACGGACACATCCCTGCCTGC3' 5'GCAGGCTAAGGGGGCTCAGC3'	129-149
ASB2[1]	X93516	TG_{24}	5'CCACTAAGTGTCGTTTCAGAAGG3' 5'CACAACTGAGTTCTCTGATAGG3'	240-270
HMS2[2]	X74631	AC_{16}	5'CTTGCAGTCGAATGTGTATTAAAT3' 5'ACGGTGGCAACTGCCAAGGAA3'	218-238
HTG4[3]	U97529	$(GT)_{14}GAT(AG)_5$	5'CTATCTCAGTCTTGATTGCGAGAC3' 5'CTCCCTCCCTCCCTCTGTTCT3'	120-140
HTG7[4]	*	TC_{10}	5'CCTGAAGCAGAACATCCCTCCTTG3' 5'ATAAAGTGTCTGGGCAGAGCTGCT3'	118-130

[1] Perkin Elmer Applied Biosystems (*Equine Paternity PCR Typing Kit*, 1996), [2] Guerin i col. (1994), [3] Ellegren i col. (1992),[4] Marklund i col. (1994), * codi d'accés no disponible a la base de dades del GeneBank.

Caracterització genealògica i demogràfica

La finalitat de la caracterització genealògica i demogràfica de la Raça Asinina Catalana és poder establir els criteris i pautes de reproducció més apropiades per a assolir els objectius fonamentals del Pla de Conservació "*in situ*" que són: el manteniment de la màxima quantitat de diversitat genètica amb el mínim increment de consanguinitat possible per generació.

Tampoc existeixen en aquesta àrea estudis basats en poblacions asinines, ni espanyoles ni mundials. Així doncs, altra vegada s'ha hagut de recòrrer a estudis similars fets en altres poblacions equines. Estudis poblacionals com els de Mac Cluer i col. (1983) en cavalls Standardbred, Tunell i col. (1983) en cavalls Quarter, Gandini i col. (1992) en el cavall Haflinger italià, Klemetsdal (1993) en el cavall Trotador Norueg o Moureaux i col. (1996) en races de cavalls de carrera i sella francesos, ens vàren servir de model per analitzar i comparar variables de la nostra població d'ases. També ens varem servir d'estudis tant interessants com el que Boichard i col. (1996) fan de l'anàlisi genealògic de races bovines lleteres de França.

És ben conegut que l'increment de consanguinitat per generació (ΔF) és inversament proporcional a la Grandària Efectiva de Població o Nombre Efectiu de Reproductors (N_e), segons la rel.lació $\Delta F = 1/2N_e$ (Falconer i Mackay, 1996), per tant, per a minimitzar els increments de consanguinitat en futures generacions s'hauria de procurar maximitzar aquest N_e en la nostra població.

Maijala (1974) adverteix que el rang crític per a la grandària efectiva de població oscil.la entre 10 i 50 individus, éssent les pèrdues de variabilitat genètica

del 1% per generació amb un N_e = 50 i del 10% per a N_e = 10 animals. En poblacions petites com aquesta, l'increment de la consanguinitat pot ser controlat amb una acurada planificació dels aparellaments. Si la genealogia dels animals de la població és coneguda, és possible crear un pool d'individus que representi el màxim nombre d'animals fundadors de la població, i així els animals seleccionats, comparteixin el mínim nombre possible d'ancestres comuns. D'aquesta forma també frenem les pèrdues de variabilitat genètica degudes a la deriva.

Aquest N_e depèn també d'altres paràmetres reproductius i demogràfics com els intervals generacionals (L), la ratio femelles-mascles reproductors, el nombre de descendents de cada reproductor (es a dir, de les variàncies de la grandària familiar), etc. (Hill, 1972). Els intèrvals generacionals a la seva vegada venen determinats per l'edat dels reproductors quan neixen els seus descendents i per la durada de la seva vida reproductiva.

Aquests paràmetres i el nombre total de descendents que cada reproductor deixa a la següent generació, venen afectats, lògicament, per la política de reproducció i cría (Klemetsdal, 1993). Aquest efecte ve mesurat per les variàncies i covariàncies de la grandària familiar, és a dir, el nombre de cries que cada progenitor deixa a la següent generació, tenint també en compte les quatre possibles vies de transmissió dels gamets (Latter, 1959; Kimura i Crow, 1963; Hill, 1972).

Però pot ser que la importància que s'ha arribat a donar a la medició del Coeficient de Consanguinitat (F) en la presa de decisions de qualsevol Programa de Conservació de Recursos Genètics hagi estat exagerada (Alderson, 1992). La mitjana del Coeficient de Consanguinitat en una població no és bon indicador de

fins a quin punt dos individus escollits a l'atzar en una població, poden estar rel.lacionats per ancestres comuns (Bodó, 1990). El Coeficient individual de Consanguinitat indica el probable nivell d'homozigocitat d'aquest animal però no prediu el nivell d'homozigocitat de la propera generació. Si la Consanguinitat continués creixent en una població petita, es reduiria dràsticament la variabilitat genètica. D'altra banda, el càlcul del Coeficient individual de Consanguinitat és molt sensible a la qualitat de la informació genealògica que es disposi. Hi han moltes situacions, on part de la informació no està disponible, i això pot provocar un considerable esbiaix en el càlcul d'aquest coeficient.

Un dels objectius d'un Programa de Conservació és retenir el màxim nombre d'al.lels de la població fundadora. Idealment, si un individu rebés igual contribució dels ancestres de la població, es maximitzaria la retenció d'al.lels fundadors (Rochambeau i Chevalet, 1989; Giraudeau i col., 1991; Alderson, 1992; Djellali i col., 1994).

Tenint en compte aquests problemes, Dickson i Lush (1933), James (1972), Lacy (1989), Alderson, (1992) i Boichard i col., (1997), proposàren una aproximació complementaria, i és l'anàlisi de les probabilitats d'origens dels gens. En aquest mètode es mesura la contribució genètica efectiva dels animals fundadors (f_e), és a dir, els animals ancestrals que no tenen cap pare conegut, de la població base en estudi.

Aquest nombre efectiu de fundadors (f_e) en una població equival a l'Índex de Conservació Genètica (GCI) descrit per Alderson (1992). L'única diferència entre ambdos indicadors, és que el GCI mesura la probabilitat d'origen dels gens per a cada individu de la població en referència als seus ancestres i al seu propi

nombre efectiu de fundadors (Boichard i col., 1997). Aquest GCI podría ser utilitzat pels criadors en el moment d'escollir aquells animals que millor retinguin la variabilitat de la raça (Alderson, 1992). D'aquesta forma s'asseguraria que el màxim nombre d'ancestres fundadors quedessin representats en la següent generació.

Estudi de la viabilitat d'una població en perill d'extinció mitjançant simulació per ordinador.

El següent pas, un cop havíem caracteritzat a la nostra població desde diferents punts de vista, era predir què passaria en un horitzó de temps rel.lativament llunyà, assumint que es continués portant la mateixa política reproductiva i de cria que s'ha estat seguint els darrers anys. També era interessant veure quines variables demogràfiques podien ser susceptibles de modificació per augmentar l'esperança de supervivència de la població.

Estudis similars s'havien portat a terme, però sempre en espècies salvatges i en perill d'extinció, destinats a conèixer quines mesures de gestió s'havien de prendre per a minimitzar el perill de vulnerabilitat d'aquestes poblacions salvatges.

Per a dur a terme aquests Anàlisi de Viabilitat de Poblacions (PVA) existeixen molts programes informàtics: GAPPS (Harris i col., 1986), VORTEX (Lacy, 1993), RAMAS/age (Ferson i Akçakaya, 1990), RAMAS/stage (Ferson, 1990), RAMAS/space (Akçakaya i Ferson, 1992) o ALEX (Possingham i col., 1992). No obstant, és important seleccionar el programa de simulació que millor

ens permeti analitzar els problemes específics a resoldre en la gestió de la població en estudi (Lindenmayer i col., 1995).

Taula 5: Resum dels avantatges i desavantatges dels programes per a PVA: ALEX, RAMAS/space i VORTEX (Lindenmeyer i col., 1995).

Atributs	*ALEX*	*RAMAS/space**	*VORTEX*
Anàlisi de Variabilitat Genètica	No	No	Sí
Anàlisi de Catàstrofes	Sí (limitat)	No	Sí
Dependència de la densitat de població	No (limitada)	Sí	No (limitada)
Estructura Social Explícita	No	No	Sí
Possibilitat d'especificar nombre d'animals adults en la reproducció cada any	No	No	Sí
Migració	S'especifica per a cada individu de la població	S'especifica per a cada parell de subpoblacions	S'especifica per a cada parell de subpoblacions
Descripció numèrica dels processos	No	Sí (limitat)	Sí
Assistència d'un manual	Limitada	Excel.lent	Moderada
Necessitat de l'ajut del creador del programa per a poder-lo utilitzar	Necessària	Necessària però no essencial	Necessària però no essencial
Possibilitat exportació de resultats a un altre sofware	No	Sí (limitat)	Sí

* Els programes RAMAS/age, RAMAS/stage es consideren com a programes satèl.lit del programa RAMAS/space, és per això que només analitzem en aquesta taula el RAMAS/space.

Així doncs, vàrem escollir el programa VORTEX (Lacy, 1993) per a fer l'estudi de simulació en la població d'ase català, perquè ens permetia utilitzar informació obtinguda en la caracterització demogràfica, com els intervals generacionals, el percentatge de reproductors de cada sexe que contribueixen amb descendència o la distribució d'individus per nivells d'edat, entre d'altres. Sobretot

aquest programa ens resultava ideal per a l'anàlisi de la nostra població perquè es tractava d'una espècie de vida reproductiva llarga i baixa prolificitat.

El programa VORTEX ja ha estat utilitzat per analitzar viabilitat en poblacions en perill d'extinció arreu del món (Lacy i Clark, 1993; Lacy i Lindenmayer, 1995; Lindenmayer i Lacy, 1995; Mills i col., 1996).

De l'anàlisi de les prediccions que el programa VORTEX fa de la població en estudi, es podran extreure conclusions sobre l'estat de vulnerabilitat i dels factors que més afecten aquesta supervivència.

Cada ase s'enamora del seu bram.

Resultats

A l'ase i al mul, la càrrega al cul.

Capítol I
Caracterització Morfològica

"The endangered Catalonian donkey breed: the main ancestor of the American ass or Mammoth".
(Journal of Equine Veterinary Science 1996, 16 (10), 436-441)

"Characterization, reference ranges and the influence of gender on morphological parameters of the endangered Catalonian donkey breed".
(Journal of Equine Veterinary Science 1996, 17 (2), 102-111)

Reviewed

THE ENDANGERED CATALONIAN DONKEY BREED: THE MAIN ANCESTOR OF THE AMERICAN ASS OR MAMMOTH

J. Jordana and P. Folch

SUMMARY

The Catalonian donkey is an endangered local donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonian area (Northeast Spain). Following the rules of action marked by the FAO Expert Consultation for the identification and study of possible stocks of conservation, this work undertakes the initial action points, giving a generic description of the population as to their origin and phylogenetic relationships, existent relationships with other worldwide breeds, censused sizes, and future perspectives of the same. A morphological description is carried out with the objective of seating bases for breed characterization and carrying out a proposal of racial standard in order to create and manage the future Stud Book of the Catalonian donkey breed.

Keywords: Donkeys / endangered breed / morphological characters

INTRODUCTION

Racial regression is a phenomenon that obviously does not affect just the asinus species. The decline worldwide in a number of breeds affects in a dramatic way all or almost all the species,[1] raising the controversy of whether they should or should not be conserved.[2,3,4] Upon losing the breeds, the genes that they are carrying are lost. The gravest problem is the great ignorance concerning the danger of extinction that exists in populations. This ignorance persists even though there are important questions regarding genetic improvement, productivity, whether or not these animals are carriers of any major interesting or valuable genes(s), currently or in the future (which might not be found in other breeds), or their power of heterosis, etc., The assertion of Mason[5] that "any extinction or disappearance of a species or breed represents an irreplaceable element of the life diversity that is lost," would have to be enough reason in order to justify any program of conservation in the species and breeds in danger of extinction.

Nevertheless, it is necessary to give some points of view which are more objective than the programs of conservation could somehow justify. In a general and summarized way, and according to several authors,[3,6,7] the following could be enumerated as possible valid reasons for the conservation of breeds:

Economic-biological reasons: It is necessary to maintain the genetic variability so that adaptations to possible new future requirements could be made, as well as also to production under unfavorable conditions.

Scientific reasons: Study of each breed in particular for the search of unique and valuable genes, through the identification of QTL (Quantitative Trait Loci) by means of molecular genetic analysis. The conservation of populations also provides material for research that could contribute to the better knowledge and understanding of some aspects of the evolution, such as domestication, behavior, and effects of natural and/or artificial selection.

Cultural-historical reasons: Conserved breeds can be considered valuable as genetic patrimony of the country, and as a living parallel history of the human populations.

Ecologic-environmental reasons: Some breeds or

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Acknowledgments: The authors wish to thank the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya, which financed this study. Also, we would like to thank Chuck Simmons for assistance with the preparation of this manuscript.

Figure 1. Jennie with foal of the Catalonian donkey breed.

populations only meet in some determinate geographical zones. Their loss could possibly deteriorate the milieu and the ecological symbiosis of the zone.

The Catalonian donkey (Figure 1) is a local donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonian area (Northeast Spain); the current geographical location of this breed is shown in Figure 2. The current census is very reduced, and the total number of animals of the Catalonian donkey breed slightly surpasses one hundred individuals, approximately a third of which are males. These figures fit into the category of Critical Breed (< 100 females) or into the category of Endangered Breed (100–1,000 females) proposed by the FAO Expert Consultation,[8] which implies that the breed is in danger of extinction.[9] Without action, its effective population size is inadequate for preventing continuing genetic loss in future generations.

Although the decline in census of the Catalonian donkey has been vertiginous and uninterrupted during this century, it is noteworthy to comment that from the year 1880 a Registration Book of the Catalonian donkey[10] exists, and that in the year 1978, due to the severe situation that this breed experienced, the "Association of Fomentation of the Catalonian Donkey Breed" (AFRAC) was created, in order to protect, foment and improve this population.

Within this context, it became necessary in 1994 to carry out a "Program of Conservation and Maintenance of Animal Genetic Resources" in this breed, which was promoted and financed by the D.A.R.P. (Department of Agriculture, Livestock and Fishing) of the autonomous Government of Catalonia (Generalitat de Catalunya), in collaboration with FRAC, and the Animal Genetics unit of the Veterinary School of Barcelona. The generated information will be integrated in the "FAO Global Data Bank on Domestic Animal Diversity," located in Hannover, Germany.[11,12]

ORIGIN AND PHYLOGENETIC RELATIONSHIPS

Discussion of the evolution of the Equines is still continuing. However, the *Pliohippus* or *Plesihippus* is considered as the ancestor of the genus *Equus* in all its forms. The *Equus* was probably developed in North America, from where it passed to Europe, Asia and Africa. Nevertheless, at the end of the Pleistocene or the beginning of the Holocene (9,000 to 10,000 years ago), *Equus* disappeared completely from the American continent, developing in the Old World.[13]

The genus *Equus* contains six subgenres: *Asinus, Hemionus, Equus, Dolichohippus, Hippotigris* and *Quagga*. Nevertheless, both Corbet[14] and Bennet,[15] based on studies carried out from archaeological findings, only recognize two, the *Equus asinus*, descendant of the *Astrohippus*, with three species: *E.a.asinus, E.a.hemionus* and *E.a.kiang*, and the subgenre *Equus equus*, direct descendant of the *Dinohippus*, with five species: *E.e.caballus, E.e.quagga, E.e.burchelli, E.e.zebra*, and *E.e.grevyi*.

The domestication process of asses began during the Holocene (8,000 to 10,000 years ago) in the Northeast Africa zone, subsequently disseminating toward the Southwest of Asia and the South of Europe.[16] The oldest findings of domestic asses date back to the fourth millennium BC, found in Egypt and associated with other skeletons of domestic animals. Around the third millennium BC, murals were beginning to be seen where the ass was frequently represented and utilized as a beast of burden.

Domestic asses did not appear in the southwest of Asia until the beginning of the third millennium BC. In India and Pakistan, bones of domestic asses were not found until 2,000– 800 BC. In Europe, the domestication of the ass began later than in Africa and Asia. As their ability to adapt to humid and cold climates was poor, they never became very numerous in the northern lands. Asia Minor seems to have been the starting point from where the asses were introduced into Europe, through the Ukraine, Russia and the Balkan Peninsula. There is evidence of the presence of these animals from 800–900 BC in the Ukraine. From the Balkans, the ass reached Italy, producing its expansion to the remainder of Europe in a parallel way to

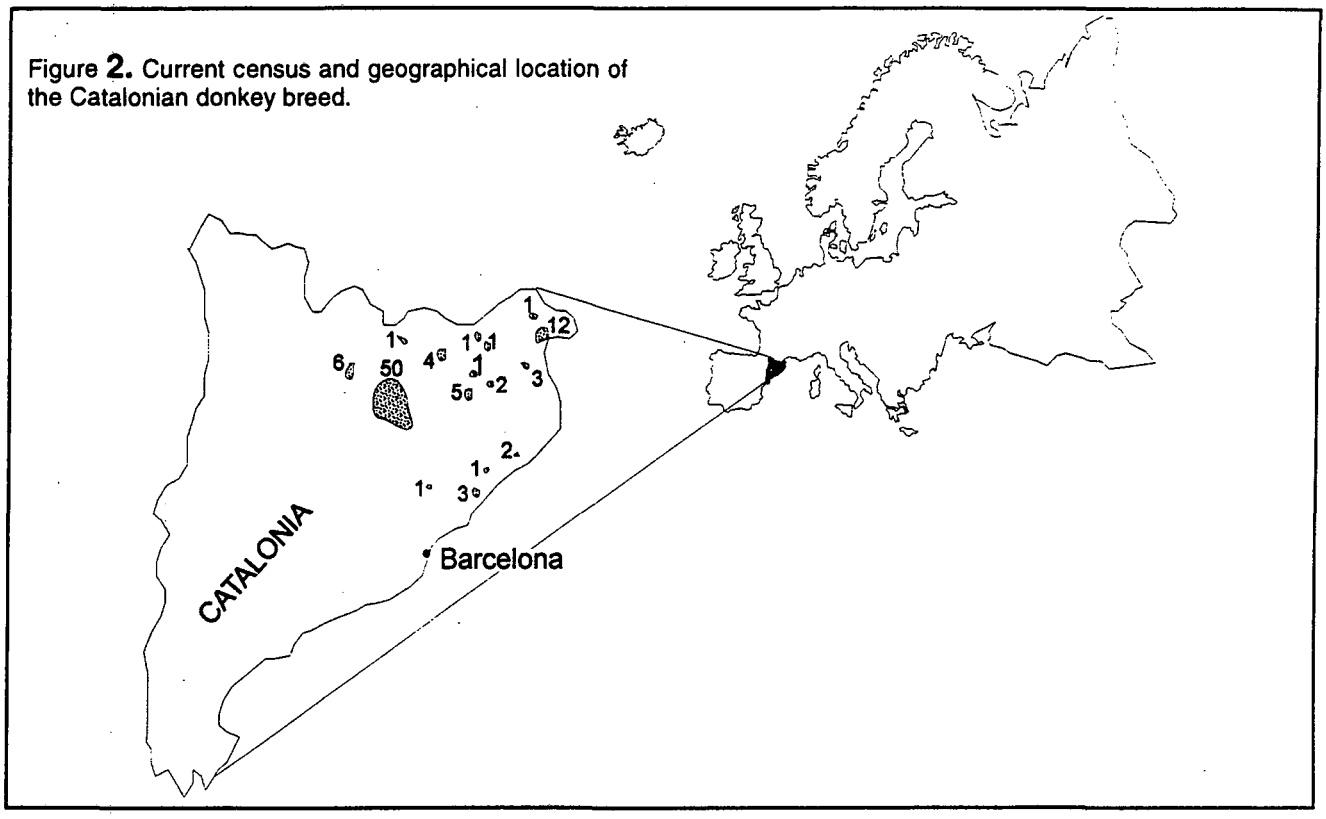

Figure **2.** Current census and geographical location of the Catalonian donkey breed.

the Roman conquests. Nevertheless, some Iberian peninsula ass populations came directly from animals that passed from Africa through the Straits of Gibraltar.[17]

According to several authors,[17,18,19] the current breeds of asses seem to come from two ancestral trunks: the *Equus asinus africanus*, or Ass of Nubia, native to the Nile Basin, and the *Equus asinus somaliensis*, or Ass of Somalia, which subsequently gave rise to the asses from the Southwest of Asia and probably also to the majority of the European breeds, among them the Catalonian donkey breed. Notwithstanding, other authors such as Dechambre and Sanson[10,20,21] also maintain the theory of two ancestral lines, one of them originating from the Northeast of Africa which would correspond to the *Equus asinus africanus*, and the other one the *Equus asinus europeus*, whose area of origin is the Mediterranean Basin, concretely the Balearic Isles, which would have given rise to the majority of European donkey breeds, among them the breed which concerns us here.

INFLUENCE OF THE CATALONIAN DONKEY BREED IN OTHER WORLDWIDE BREEDS

The Spanish donkey breeds have had much importance in the formation and improvement of other populations, mainly European. The Catalonian donkey has contributed to the improvement of the French "Baudet of Poitou" breed, preferably in order to increase the size and improve the sexual prowess of their animals,[20,22,23] although the Spanish breed that has most contributed to the formation of the Poitou has been the Zamorano-Leonesa breed, specimens of which were exported to France during the reign of Felipe V.[21,22] The Andulusian donkey breed would be, according to Sotillo and Serrano,[21] the ancestor of the Brazilian Ass, or Lagoa Dorada, breed.

The influence that the Catalonian donkey has had in several Italian breeds is more importan, contributing in a decisive way to their formation and improvement. Among them could be mentioned the Pantellaria, Martina-Franca and Siciliana or Ragusana breeds.[10,17,20–23] Sotillo and Serrano[21] also assign a certain influence of the Catalonian donkey in the Mediterranean Maltese and Cypriot breeds, originally from African and Asian asses.

But where all the authors agreed was with the direct and decisive influence of the Catalonian donkeys in the formation of the American Ass,[10,17,20–24] also named Ass of Kentucky or Mammoth in honor of that which is considered to be the best founding sire of this particular breed, named Imported Mammoth, which arrived in Charleston (South Carolina) in 1819, coming from Catalonia, being extensively utilized in the States of Kentucky, Tennessee and Missouri.[24]

Lastly, just one comment that several authors make in reference to the diverse exports carried out of Catalonian donkeys: at the end of last century and also during the current one, this donkey reached to such far away and dispersed places as: Algeria, Argentina, Australia, Brazil, Canada, Congo, Cuba, Central American Republics, Ger-

Table 1. Census equine evolution in Spain and Catalonia during the 20th Century.

YEAR	ASSES		HORSES		MULES	
	SPAIN	CATALONIA	SPAIN	CATALONIA	SPAIN	CATALONIA
1929	1,006,000		598,000		1,154,000	
1935	1,176,000		808,000		1,475,000	
1940	851,000		592,000		1,139,000	
1950	732,000		642,000		1,089,000	
1960	686,000		506,000		1,158,000	
1970	368,000		282,000		533,000	
1976	253,000	3,702	262,000	13,301	281,000	15,452
1978	232,000	5,411	257,000	19,016	253,000	17,861
1980	188,000	3,252	242,000	9,233	199.000	6,264
1984	160,000	1,550	254,000	11,349	145,000	4,069
1986	140,000	928	248,000	9,256	117,000	2,603
1988	131,000	---	250,000	---	110,000	---
1990	130,000	415	241,000	22,027	100,000	543
1992	130,000	---	240,000	---	100,000	---

many, Great Britain, India, Madagascar, Mexico, South Africa, Tunisia and Zaire.[23]

THE EQUINE CENSUS EVOLUTION IN SPAIN

The Spanish asinus population, as well as the horse and mule, has been diminishing uninterruptedly during the last decades (Table 1). The Statistic Agrarian Yearbook[25] gives us some official ass figures of 1,100,000 individuals, approximately, during the 20's and the beginning of the 30's, already being of some 800,000 animals at the end of the 30's and during the decade of the 40's.

But the period where the great descent in the equine census is produced are the decades of the 60's and 70's, probably due to the intense mechanization of the countryside that began in Spain during those years. In 1980 the ass census decreased 73% compared to the census of 1960. During the same period, the horse census was reduced by 52%, and the number of mules dramatically diminished by 83%. From the analysis of Table 1, it could be observed that in the last decades (80's and 90's) there has been a decline in the number of mules and asses, with the horse population staying more or less uniform in number.

FUTURE PERSPECTIVES

As for the future perspectives of this breed, the first fundamental objective is that it should not disappear, since it is a unique and valuable genetic patrimony, and as Hall[26] says, "local breeds of livestock are genetic resources which should be protected as part of the world heritage of biodiversity."

Moreover, in the current times, this breed could still have a certain economic importance for pure-breeding as well as for mule production, above all, and according to the FAO Expert Consultation,[27] in the tropics and for some developing countries since this species is more important than the horse. Export of stallions and/or frozen semen could be very important for the genetic improvement of other worldwide asinus populations. Forest exploitation, in zones of difficult access, could require the continuation of the services of these animals. Cleaning the forest is an important effect linked to these herds, which collaborate in the prevention of forest fires. Development of commercial products from the species such as the jennie's milk, could be an interesting, although restricted, market. Lastly, there is an important use of the ass as a companion animal and for recreational tourism activities (agro-tourism) in mountain zones.

MORPHOLOGICAL DESCRIPTION OF THE BREED

According to the FAO,[7] the morphological characterization of the breed is necessary to identify conservation stocks. Accordingly, we will use the descriptions from several authors,[10,20–22,28] and the information contributed by several breeders or owners of AFRAC, as well as our own observations.

General characteristics

Appearance and format

According to Baron systematics, the individuals of

this breed are of longilinear appearance, hypermetric format and concaviline profile, this concavity being slightly more apparent in jennies and foals than in stallions.

They are animals of large size, 140 cm at the withers, on average, with a weight ranging between 350 to 450 Kg. They have robust extremities with long, thick bones, acquiring large proportions within a harmonic group. They are slender, with a straight neck and rounded thorax. Not a running animal, this breed is very effective at hard work where great strength and vigor are needed.

Coat color and pigmentation

The black coat color is considered characteristic, although this could be influenced by several environmental factors, as well as nutritional state, season of the year, sun incidence and hair length.

The belly and internal face of the extremities show whitish fadings. The muzzle and orbital zone of the eyes also show these silver fadings. A very characteristic reddish fringe, between silver and blackish colors, can be seen above all in the zones of the head.

Skin and hair

The skin of these animals could be classified as hypermetabolic type, relatively fine but profuse in growth. It possesses scanty subcutaneous conjunctive tissue in relation with the horse. The foals have fine, long and slightly ruffled hair. As the animals grow, they lose this fluff, changing to straight, fine and short hair. The mane in both sexes is dark, short and not very thick.

Characteristics of behavior

They are animals of sanguine temperament although they are accustomed to being quite peaceful. In general they are very noble and of rapid reactions. The expression of the eyes is lively, always carrying the ears and the head very upright. The sexual instinct of the males is very developed. Therefore, according to their biotypology these animals could be grouped within the so-called hypermetabolic or oxidative type.

Regional characteristics

Head

The head is wide and weighty. The front bone shows a straight profile in both sexes, although in females and foals it could be manifested as a little subconvex. This slight subconvexity starts disappearing in the stallions as they begin to manifest the secondary sexual characteristics. Females as well as males are of dolicocefal type.

The eyes are large, lively, and very expressive. The orbital arcade is very thick, with a strongly pronounced apophysis and guided in direction to fronto-parietal crests.

The muzzle is wide and pronounced with a tendency to acromegaly and with bounded fading. The nasal bones expand at their base, but halfway down the nose they narrow in order to enlarge again at the end, where they contact with the muzzle, giving a very pronounced configuration of the supranasals. A very pronounced excavation in a longitudinal way is formed at the union of the frontals and nasals.

The jawbones are strong, wide and very resistant. The inferior jawbone shows some voluminous and convex branches. The dental arcade is rounded and of a short dimension. Ears are long, straight and narrow. Due to their potent muscular insertions, they stay erect and are very mobile and expressive.

Neck

The neck is long, wide, straight, flexible, very muscular, and not given to accumulating adipose tissue, thus giving a very slender image to the animal.

Trunk

The chest is wide and elevated, with long, well-arched ribs.. The belly is contracted in males and more pronounced in females. This makes the relationship between the thoracic perimeter and length diameter have a longilinear body index, characteristic of the breed. The withers are elevated, although not very apparent. The back is straight and long, with a marked waist in its union with the rump. This is of a convex type, mostly more lengthened than wide in males, with great harmony; females show forms much more softened and slightly angular. Kidneys are wide and well united to the rump. The body gives the sensation of being almost cylindrical. The tail is long and of low insertion, normally reaching the level of the hocks and having an abundant mane.

Extremities

The extemities are well conformed and robust. Shoulders are vertical or slightly oblique, and they seem short when they are compared with withers height. The width of knees and hocks especially stands out, with well conformed cannons. The muscular system is very apparent, with tendons separated from the cannon. The leg-line is very regular. Hoofs are slightly narrow in both sexes but well proportioned.

Morphological descriptions, as well as the study and analysis of several biometric variables of the breed, have allowed us to make a proposal of racial standard in order to create and manage the future Stud Book of the Catalonian donkey breed.

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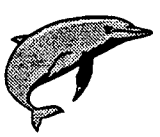
Dr. R.M. Miller will be conducting a week-long seminar on "Behavior and Misbehavior of the Horse," during a 12-day cruise/tour of the Inside Passage, Glacier Bay and Denali Park, Alaska, August 11 to 23, 1997. Miller will speak on comparative behaviors study on non-domestic species during the land-tour portion. This meeting is open to horse professionals, as well as to veterinarians.

Participants will receive a full set of reprints of Dr. Miller's series "Behavior of the Horse," as it appeared in the Journal of Equine Veterinary Science.

We will be sailing on the Dawn Princess, leaving from Vancouver sailing through Glacier (or Hubbard) Bay, and College Fjord, ending at Anchorage. There are three ports of call; Ketchikan, Juneau and Skagway. From Anchorage we will board the Midnight Sun Express for luxury rail travel to Denali National Park and Denali Princess Lodge. The evening is free. We are trying to arrange a trail ride while in Denali Park. After a morning tour of the National Park we will re-board the Ultra Dome rail cars for a scenic trip to Fairbanks. While in Fairbanks there will be a cruise aboard the sternwheeler riverboat "Discovery," a city tour including the El Dorado Gold Mine, the Trans-Alaska Pipeline and more. The trip ends in Fairbanks for the flight home.



Participation in this program is limited. Fares for the 12-day cruise/tour begin at $2,439 per person, and do not include port charges, taxes, or airfare. Fares do include cabin, all meals, and the Seminar. To reserve space at the rate quoted here, we must receive a $25.00 per person deposit by November 1. It is possible to sign-up for just the cruise. Cruise only fares begin at $1,074 per person for the 7-day Inside Passage, Glacier (or Hubbard) Bay cruise. Fares quoted do not include port charges, taxes, airfares or shore excursions. Fares do include cabin, all meals, entertainment and the seminar.

Listen to what participants on last year's Alaska cruise seminar say.

"Enjoyed this very much. Because of the magnitude of the Alaska experience it was hard to give up some of that to attend the seminar, but overall I was very pleased."

Or

"I have never been privileged to learn so much practical knowledge in such a short meeting. I would come to another meeting in a heartbeat and I would recommend it to anybody who asks as a very positive thing to do..."

Reviewed

CHARACTERIZATION, REFERENCE RANGES AND THE INFLUENCE OF GENDER ON MORPHOLOGICAL PARAMETERS OF THE ENDANGERED CATALONIAN DONKEY BREED

P. Folch and J. Jordana

SUMMARY

This study characterizes morphometrically a limited-size population in danger of extinction; the Catalonian donkey breed (Spain). Sixty-nine adult individuals of both genders, forty-four jennies and 25 stallions, were characterized and analyzed for twenty-six morphological measurements and twelve corporal indices by a univariate procedure. The population showed little sexual dimorphism, since only eight of twenty-six measures and one of twelve indices showed statistically significant differences for gender effect. The analysis of correlations between measures and obtained dendrograms allowed the identification of interactions between and within the different corporal regions (head, trunk and extremities). The important morphological variability degree (coefficients of variation) shown by individuals of the Catalonian donkey breed will be of great interest to further improve the population.

Key words: Donkeys / endangered breed / morphological characters / correlation coefficient / dendrogram

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Acknowledgements: The authors wish to thank the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya which financed this study. Also, we would like to thank Chuck Simmons for assistance with the preparation of this manuscript.

INTRODUCTION

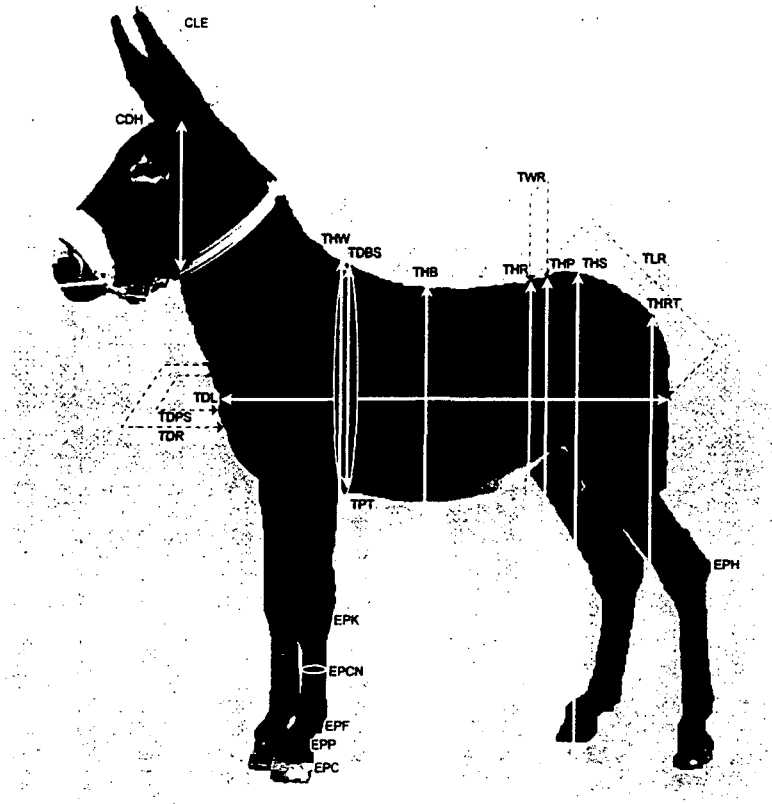
The Catalonian donkey is a local donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonia area (Northeast Spain). The current census is very reduced, slightly surpassing one hundred individuals, a third of which, approximately, are males. These figures fit into the category of the Critical Breed (< 100 females) proposed by the FAO Expert Consultation,[1] which implies that the breed is in danger of extinction.[2]

The Catalonian donkey breed is characterized by large-sized and elongated animals with a concave profile. The coat is a black color with characteristic fadings in the muzzle, orbial zone of the eyes, belly and internal face of the extremities.[3]

The main objective of this paper is to characterize biometrically this endangered population, following the rules of action marked by the FAO Expert Consultation for the identification of possible stocks of conservation and as a basic tool for the study, maintenance and conservation of animal genetic biodiversity.[1,4]

Once established the standard type of the breed, it is necessary to analyze the degree of morphological variability of the population, so that in a near future possible objectives of selection and genetic improvement of the breed could be expounded. Thus, the existent relationship between the physical characteristics and functional aspects of animals is very well known,[5] which could be interesting concerning possible future uses of this breed.[3]

Lastly, gender effect on several morphological vari-



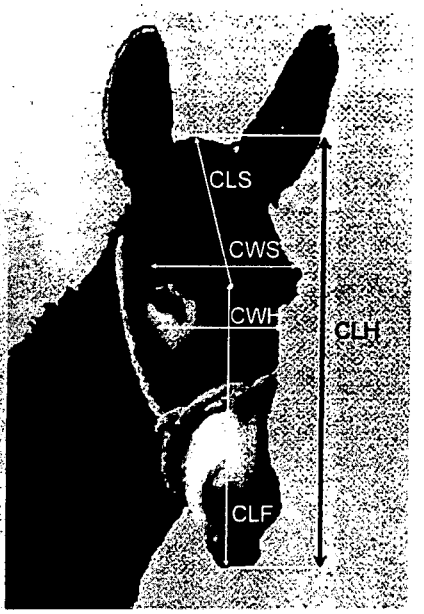


Figure **1.** (left) Morphometrical analyzed variables (codes meaning in text).

Figure **2.** (above) Morphometrical analyzed variables (head detail).

ables is analyzed, the characterization of this population possibly being able to serve as a reference point for other comparative studies within the asinus species.

MATERIAL AND METHODS

Approximately 90% of the animals having been counted, the breed population size is a total of 98 individuals, distributed in 29 foals of both genders (< 3 years old) and in 69 adults (> 3 years old), specifically: 25 stallions aged 3-13, and 44 jennies aged 3-17.

For the biometrical study, only the 69 adults have been kept in mind, and in spite of the fact that the number of animals is not too elevated, this sample could be considered as representative of the population, since it includes the near totality of the individuals utilized which are at present reproducers, individuals that form the base of the Foundational Registration of the Stud Book, starting from those which will develop the *Program of Conservation and Maintenance of Animal Genetic Resources* of the breed.

A total of 26 corporal measures, in each one of the animals of the sample, were taken; and 12 corporal indices were obtained. The anatomical references of each variable, the definition of indices, and their reference values are those described for the equine species, upon not providing reference values for asinus species.[5,6,7,8,9] The several variables were subdivided into three large groups according to corporal regions:

A) *Cephalic measures*: Head Length (CLH), Skull Length (CLS), Face Length (CLF), Head Width (CWH), Skull Width (CWS), Head Depth (CDH) and Ear Length (CLE).

B) *Trunk measures*: Withers Height (THW), Back Height (THB), Sacrum Height (THS), Rump Height (THR), Pelvis Height (THP), Root of Tail Height (THRT), Length Diameter (TDL), Back-Sternal Diameter (TDBS), Diameter between the Ribs (TDR), Point of Shoulder Diameter (TDPS), Rump Length (TLR), Rump Width (TWR) and Thoracic Perimeter (TPT).

C) *Extremities measures*: Knee Perimeter (EPK), Cannon Perimeter (EPCN), Fetlock-joint Perimeter (EPF), Pastern Perimeter (EPP), Coronet Perimeter (EPCR) and Hock Perimeter (EPH).

A measuring rule for height, width and length measures has been used, and a measuring tape for diameter and perimeter measures. All variables are shown in Figures 1 and 2.

Starting from 26 morphological variables, a total of 12 corporal indices were obtained, defined in the following manner: Body Index = (TDL x 100) / TPT, Thoracic Index = (TDR x 100) / TDBS, Metacarpo-thoracic Index = (EPCN x 100) / TPT, Skull Index = (CWS x 100) / ELS,

Table 1. Morphological measurements values in both subpopulations (males and females) of the Catalonian donkey breed.

	Corporal measurements		Mean	SD	CV	Highest	Lowest
(CLH)	Head Length	male	61.24	2.96	4.83	55	68
		female	58.25	3.83	6.58	52	66
(CLS)	Skull Length	male	27.64	2.81	10.17	21	32
		female	26.50	3.73	14.08	19	34
(CLF)	Face Length	male	40.72	5.69	13.99	27	53
		female	38.00	4.94	13.00	25	46
(CWH)	Head Width	male	25.00	1.89	7.57	21	28
		female	22.77	3.53	15.52	16	29
(CWS)	Skull Width	male	21.36	1.60	7.51	18	25
		female	19.77	1.29	6.53	17	23
(CDH)	Head Depth	male	40.44	2.87	7.10	34	45
		female	39.06	3.25	8.32	32	46
(CLE)	Ear Length	male	32.45	2.23	6.88	29	37
		female	33.81	2.61	7.73	29	40
(THW)	Withers Height	male	142.20	6.89	4.85	129	156
		female	136.29	5.95	4.36	123	148
(THB)	Back Height	male	137.44	6.64	4.83	126	152
		female	132.68	6.07	4.57	120	144
(THS)	Sacrum Height	male	143.00	6.98	4.88	127	157
		female	139.59	6.01	4.30	127	150
(THR)	Rump Height	male	139.88	6.96	4.98	125	155
		female	135.79	6.06	4.46	123	145
(THP)	Pelvis Height	male	141.80	7.73	5.45	126	157
		female	137.65	5.96	4.33	125	149
(THRT)	Root of Tail Height	male	132.60	9.43	7.11	110	155
		female	127.18	5.85	4.60	115	138
(TDL)	Length Diameter	male	145.88	7.72	5.29	132	163
		female	143.88	8.85	6.15	119	162
(TDBS)	Back-Sternal Dmtr	male	60.04	3.38	5.63	53	66
		female	59.34	3.32	5.60	52	67
(TDR)	Diameter betw Ribs	male	41.68	3.79	9.10	35	52
		female	39.45	5.03	12.76	29	50
(TDPS)	Pnt/Shoulder Dmtr	male	35.04	3.10	8.85	28	40
		female	32.40	3.34	10.31	26	40
(TLR)	Rump Length	male	45.96	2.15	4.67	42	49
		female	44.06	3.57	8.10	38	54
(TWR)	Rump Width	male	42.28	2.26	5.35	37	46
		female	43.09	3.26	7.58	37	49
(TPT)	Thoracic Perimeter	male	157.00	6.65	4.23	146	170
		female	154.70	6.12	3.95	140	169
(EPK)	Knee Perimeter	male	33.76	2.42	7.16	30	38
		female	28.95	1.52	5.26	26	33
(EPCN)	Cannon Perimeter	male	19.64	1.35	6.87	18	23
		female	17.81	1.18	6.65	15	20
(EPF)	Fetlock-jnt Perimeter	male	26.12	1.90	7.27	23	31
		female	23.20	1.95	8.44	19	28
(EPP)	Pastern Perimeter	male	19.32	1.72	8.93	16	23
		female	17.06	1.16	6.85	15	19
(EPCR)	Coronet Perimeter	male	30.44	2.77	9.10	25	36
		female	30.25	3.44	11.38	21	39
(EPH)	Hock Perimeter	male	41.12	2.31	5.63	35	45
		female	37.61	2.31	6.15	33	45

Cephalic Index = (CWH x 100) / CLH, Pelvic Index = (TWR x 100) / TLR, Pectoral Height Index = TSH / PT (Sternal Height-TSH is defined as the difference between THW and TDBS), Index 1 = (TPT / THW), Index 2 = (THW / THR), Index 3 = (TSH / THW). Index 4 = (EPCN / TSH), and Index 5 = (THW / TDL).

All this information was filed in a data base (Microsoft ACCESS 2.0) for its later utilization.

Reference ranges were calculated by use of standard deviation (SD) about the mean, and the highest and lowest values in the sample for each variable as a measure of location. Also, the coefficient of variation (CV) has been obtained as a unitless measure of relative variability (the ratio or the standard deviation to the mean expressed as a percentage).

Correlation coefficients (Pearson's product-moment) between the variables have been calculated. Probability values of < 0.05 were considered significant. In order to

Table 2. Corporal Indices values in both subpopulations (males and females) of the Catalonian donkey breed

Corporal Indices		Mean	SD	CV	Highest	Lowest
Body Index	male	92.97	4.41	4.74	81.43	99.37
	female	93.03	5.04	5.41	81.50	105.88
Thoracic Index	male	69.58	6.88	9.88	57.37	83.63
	female	66.56	8.41	12.64	52.72	85.18
Metacarpo-thoracic Index	male	12.50	0.61	4.93	11.32	13.77
	female	11.52	0.73	6.41	9.86	12.85
Skull Index	male	78.04	9.95	12.75	64.51	100.00
	female	76.15	12.55	16.47	56.66	109.52
Cephalic Index	male	40.85	2.83	6.94	33.87	45.16
	female	39.01	4.93	12.64	26.66	45.61
Pelvic Index	male	92.13	5.69	6.18	77.08	104.65
	female	98.06	7.10	7.24	86.04	117.07
Pectoral Height Index	male	0.52	0.02	4.86	0.47	0.56
	female	0.49	0.00	5.77	0.44	0.56
Index I	male	1.10	0.04	4.18	1.02	1.21
	female	1.13	0.04	3.64	1.04	1.23
Index 2	male	1.01	0.01	1.41	0.90	1.04
	female	1.00	0.01	1.71	0.95	1.05
Index 3	male	0.57	0.01	2.02	0.55	0.60
	female	0.56	0.01	3.30	0.52	0.60
Index 4	male	0.23	0.01	4.84	0.21	0.26
	female	0.23	0.01	7.09	0.18	0.26
Index 5	male	0.97	0.04	4.58	0.88	1.07
	female	0.94	0.04	5.05	0.85	1.07

view the existent relationships between variables of a graphic form (dendrogram), the method of cluster analysis using the UPGMA algorithm[10] was applied to the correlation coefficient values by using the PHYLIP computer package.[11]

Both genders have been considered independently since there is a possible sexual dimorphism for some of the measures. Statistical differences for this factor of variation were analyzed by the ANOVA test. Computations were performed using a statistical software program.[12]

RESULTS AND DISCUSSION

Reference corporal measurements of male and female Catalonian donkeys are shown in Table 1, and corporal indices are shown in Table 2.

Large differences between genders for the studied parameters were not observed, since only 8 of 26 corporal measures and 1 of 12 corporal indices analyzed showed significant differences: EPCN, EPK, EPP, CWS ($P< 0.001$); THRT ($P< 0.01$); THR, TLR, CWH, Index 3 > ($P < 0.05$), which would indicate that the population shows little sexual dimorphism. The greatest differences between genders are reported, above all, in the cephalic level (CWH, CWS), height from posterior third (THR, TLR, THRT) and extremities perimeters (EPCN, EPK, EPP), always showing males more elevated values than females, such as other authors confirm in several saddle-horse breeds.[5,8,13,14] In view of these results it could be interpreted that females are, in general, of a finer and more slender physical appearance than males.

A. Cephalic measures

Two of seven cephalic variables showed significant differences between genders, CWS ($P<0.001$) and CWH ($P< 0.05$). Males had a wider skull and head than females did, in a similar way to that found by other authors in saddle-horse breeds.[5,13]

Cephalic variables showed, in general, as much in males as in females, an elevated coefficient of variation (CV). These results would indicate, in principle, that a high degree of morphological variability for these characters in the population exists although interpretation of results should be taken with caution, since when in vivo measuring was carried out, several environmental factors could exist that influence in measurements, the temperamental state of the individual, mainly with the subsequent difficulty in the precise localization of anatomical reference points.

B. Trunk measures

Upon analyzing "summit line" measures, which define animal height, one could observe that they are those that show a lower variation coefficient in both genders. This is important, since it is these measures that fundamentally define the animal profile, and this could be, in the future, a preferential selection objective. From analysis of height variables shown in Table I, it can be observed that males, as an average, possess greater stature than females although the obtained values were not statistically significant, except for THR ($P< 0.05$)and THRT ($P < 0.01$); rump length (TLR) also showed significant differences between

genders ($P < 0.05$), all these values being higher in males than in females.

Some thoracic measures (TDR and TDPS) have a relatively high coefficient of variation as much in males as in females (CV > 8.8), the females also showing higher values than males (CV > 10). The two diameters (TDR and TDPS) behave in a similar form although TDPS shows a slightly smaller variability degree. In a general way, we suppose that some of these variability values could be overestimated, mainly those measures in which it is difficult to determine the anatomical reference points, depending on physiological (pregnancy or not), nutritional (fatty and muscular tissue) or temperamental states of the animal. Nevertheless, elevated coefficients of variation for variable TDR (CV > 10) have also been described in the PRE equine breed (*Pura Raza Español -Spanish Pure Breed-*).[13] The greatest variability degree that shows female subpopulation with regard to male subpopulation for these thoracic variables could argue partly as a consequence of the different management system that exists between both genders, since the great majority of females are breeding in a free pasture regime while males are accustomed to always being stabled. The effect of the environmental factor of exercise or the absence of it, could be responsible for the different degree of variability shown by both subpopulations. This important phenotypic variability (partially genetic) shown in the Catalonian donkey breed will be of great interest in a near future when possible objectives of genetic improvement in the breed begin to be expounded.

C. Extremities measures

As for variables that define the extremities of the animal, it was observed that it is in this corporal region where sexual dimorphism becomes more patent, in a similar way to that which is described in equine populations of PRE,[13] since three of six variables, EPC, EPK and EPP, showed highly significant differences ($P < 0.001$). All the perimeters showed superior figures in males, the coefficients of variation being elevated, although quite similar between genders.

D. Corporal indices

The described corporal indices were proposed, designed and utilized based upon the saddle horse classification.[13,15,16,17,18–22] Given that measurements and proportions from this asinus breed are so stylized, and in the absence of previous studies on the topic, it was decided to make use of them as well as of their reference values,[6,7,8,9] always interpreting the results keeping in mind that it is about a breed of asses. The ANOVA test only showed significant differences between genders for Index 3 ($P < 0.05$).

The Body Index (BI) gives one an estimate of the proportions of the breed and allows the classification of the animals as longilinear (BI≥90), mesolinear (BI≥84 and ≤89) or brevilinear (BI≤83). The obtained values (BI > 92) permitted the classification of our animals as longilinear. This morphology was confirmed as well by the Thoracic Index (TI < 70), which indicates the compact degree of the thoracic chest and it also permits classifying individuals as longilinear (TI≤ 83), mesolinear (TI≥84 and ≤89) or brevilinear (TI≥90), showing that these animals are slim and slender. According to other authors, females are somewhat wider than males due, mainly, to their reproductive function.[23,24]

The Metacarpo-Thoracic Index, indicates what the animal format is like, that is to say, it shows us the existent relationship between the mass of individuals and the members that sustain it, allowing one to define three types of animals: hypermetric, eumetric and elipometric. The results obtained allowed the classification of the animals as of the hypermetric format, since in both genders its value was superior to 11, the reference value given for horse breeds.[8]

The Skull Index as well as the Cephalic Index indicate whether the head proportions are harmonious, giving us an idea of the compactness of the same, that is to say, they indicate whether longitudinal diameters prevail on transverses or vice versa, and it allows classification of individuals as brachycephalic (Index > 100) or as dolichocephalic (Index < 100), in this case verifying that it is about animals of the dolichocephalic type.

The Pelvic Index (PI) gives us an idea of the structure of the rump, and is very related with the THRT variable that indicates the tail insertion point (up, middle or down), characteristic that has permitted the typifying of a lot of equine breeds.[6] A very proportionate rump shows a width approximately equal to its longitude (PI ≈ 100), which we could define as horizontal; if the obtained values are < 100, it is a convex line rump, and if they are superior, concaviline. In this study, it is about animals with a convex rump and a low tail insertion -THRT- (Index values < 100), which allow one to define the pelvis as convexiline.

Index 1 relates thoracic perimeter (TPT) with withers height (THW), and according to Lesbre[17] one should distrust such an animal of work aptitude, for its direct relationship with resistance to fatigue, when its TPT does not exceed the THW by at least 1/8, that is to say, when the ratio between both variables is not ≥ 1.125. For the results shown in Table 2, it could be observed that males have an Index 1 slightly inferior to the referred value (Index 1 = 1.10), which would indicate that its thoracic chest would be proportionally smaller, in relationship to the height that they reach, than ideally desirable for an animal of work aptitude. On the other hand, females transcend this index (Index 1 = 1.13), although obtained values in the two subpopulations were not significantly different. Depending on which is the orientation of the future economic perspectives of the breed that the *Association of Fomenta-*

Table **3.** Morphological correlation matrix among male corporal measures of the Catalonian donkey breed.

	CLH	CLS	CLF	CWH	CWS	CDH	CLE	THW	THB	THS	THR	THP	THRT	TDL	TDBS	TDR	TDPS	TLR	TWR	TPT	EPK	EPCN	EPF	EPP	EPCR	EPH
CLH	1.00																									
CLS	0.28	1.00																								
CLF	0.46a-	0.43a	1.00																							
CWH	0.43a	0.57b	0.00	1.00																						
CWS	0.25	0.17	-0.01	0.54b	1.00																					
CDH	053b	0.40a	0.10	0.40b	0.22	1.00																				
CLE	0.12	0.11	-0.23	0.37	0.41	0.50a	1.00																			
THW	0.68c	0.06	0.35	0.10	0.09	0 59c	-0.02	1.00																		
THB	0.68c	0.23	0.23	0.26	0.21	0.65c	0.12	0.94c	1.00																	
THS	0.72c	0.09	0.44a	0.25	0.17	0.51b	-0.07	0.93c	0.93c	1.00																
THR	0,72c	0.14	0.32	0.21	0.18	0.60c	-0.02	0.95c	0.97c	0.96c	1.00															
THP	0.73c	0.09	0.42a	0.23	0.22	0.55b	0.01	0.95c	0.96c	0.97c	0.98c	1.00														
THRT	0.33	0.04	0.18	-0.02	0.06	0.42a	0.00	0.64b	0.61c	0.53b	0.58b	0.55b	1.00													
TDL	0.27	0.11	0.06	0.09	0.31	0.48a	0.27	0.60b	0.59c	0.45a	0.53b	0.55b	0.49a	1.00												
TDBS	0.62c	0.15	0.21	0.26	0.18	0.63c	0.27	0.87c	0.83c	0.78c	0.80c	0.82c	0.65b	0.60b	1.00											
TDR	0.49a	0.04	0.44a	0.45a	0.40	-0.10	-0.28	0.24	0.19	0.42a	0.32	0.37	-0.05	-0.10	0.15	1.00										
TDPS	0.21	0.00	0.01	0.13	0.22	0.41a	0.04	0.46a	0.37	0.35	0.38	0.42a	0.30	0.58b	0.49b	0.27	1.00									
TLR	0.37	-0.27	0.47a	-0.07	-0.10	-0.02	-0.20	0.50a	0.44a	0.57b	0.49a	0.5lb	0.28	0.13	0.36	0.24	0.12	1.00								
TWR	0.12	0.14	-0.32	0.19	0.07	0.15	-0.10	0.04	0.01	0.03	0.04	-0.02	0.04	-0.05	0.05	0.18	0.24	0.25	1.00							
TPT	0.58b	-0.08	0.62c	0.32	0.21	0.31	0.10	0.58b	0.53b	0.62c	0.55b	0.66c	0.39	0.49b	0.58b	0.47b	0.61c	0.50b	0.01	1.00						
EPK	0.60b	0.02	0.34	0.21	0.42a	0.59b	0.16	0.85c	0.84c	0.83c	0.84c	0.86c	0.48a	0.60b	0.81c	0.22	0.33	0.31	-0.14	0.52b	1.00					
EPCN	0.55b	0.21	0.50b	0.04	0.31	0.36a	0.15	0.75c	0.65c	0.71c	0.66c	0.74c	0.39	0.57b	0.65c	0.32	0.49a	0.48a	-0.22	0.67c	0.66c	1.00				
EPF	0.71c	0.07	0.45b	0.32	0.43a	0.53b	0.19	0.75c	0.72c	0.74c	0.72c	0.78c	0.30	0.45a	0.64c	0.45a	0.50b	0.34a	-0.02	0.65c	0.78c	0.81c	1.00			
EPP	0.57b	-0 01	0.58b	0.24	0.39	0.39	0.08	0.71c	0.68c	0.73c	0.71c	0.78c	0.36	0.55b	0.66c	0.39a	0.41a	0.31	-0.33	0.70c	0.82c	0.83c	0.78c	1.00		
EPCR	0.13	-0.23	0.40b	0.25	0.33	0.13	0.23	0.42a	0.45a	0.48a	0.43a	0.5lb	0.28	0.31	0.40a	0.31	0.41a	0.28	-0.25	0.55b	0.43b	0.47b	0.5lb	0.54b	1.00	
EPH	0.56b	-0.12	0.17	0.30	0.32	0.52b	0.22	0.67c	0.72c	0.62c	0.70c	0.73c	0.33	0.66c	0.61c	0.25	0.57b	0.01	-0.24	0.55b	0.77c	0.61c	0.68c	0.72c	0.42a	1.00

a (P<0.05); b(P<0.01); c(P<0.001)

tion of the Catalonian Donkey Breed (AFRAC) believe most convenient (work, agro-tourism, sport,...), it perhaps would be interesting to select breeding animals for their resistance and capacity of work again, like those that historically gave worldwide fame to this breed.[25]

Index 2 relates withers height (TWH) with rump height (THR), so that an animal is considered as very proportionate if the two measurements should be equal, that is to say, the value of Index 2 should be the same or similar to 1. Higher values indicate that it is about animals with an anterior region more elevated than the posterior, transferring the center of gravity to posterior extremities in this manner and, therefore, overloading them. From analysis of obtained values in our population we could conclude that these animals are very well proportioned (values of 1.01 and 1.00, for males and females, respectively), although they show a slight and recognized tendency (Table 1) that the anterior third is slightly more elevated than the posterior.[26]

Index 3 relates sternal height (TSH) with withers height (TWH), and according to Oom,[9] the value of this index should not transcend the 0.50-0.55 interval. The obtained average values for this breed, for males (Index 3 = 0.57) as well as for females (Index 3 =0.56) transcend this value slightly, signifying that back-sternal diameter (TDBS) would be proportionally minor in relationship to the reached height (TWH), that is to say, they would be animals with slightly lengthened extremities, also named "distanced from land." The conclusions of this index, as for the proportionality of individuals, are very similar to those obtained for Index 2.

Index 4 relates cannon perimeter (EPCN) with sternal height (TSH). In saddle-horse breeds and according to Menezes,[20] for each centimeter of cannon perimeter

(EPCN) 4 cm of sternal height (TSH) should correspond; that is to say, Index 4 should have a value close to 0.25. In our case, and as a characteristic feature of the asinus species, it was observed that the EPCN is smaller than in horses, in females as well as in males.[5,9,13] This makes the value of this index smaller than the described values by the above author, and we should thus consider it as much of an indicator of osseous feebleness of extremities.

Index 5 relates withers height (THW) with length diameter of the individual (TDL). Ideally, a very proportionate animal should show an index value equal to 1, at which these indiduals would describe a perfect square. Several authors[13] grant great importance to this index for ethnic classification, so that mesolinear animals would describe a square (Index 5 = 1) brevilinear animals would deviate in favor of THW (Index 5 > 1), and longilinear animals in favor of TDL (Index 5 < 1). In our population, the value of this index, much in males (0.97) as in females (0.94), was lower than one unit, indicating that they adjust to a longilinear format pattern, such as the Body and Thoracic Indices confirmed.

Correlations

Correlation coefficients among 26 morphological variables included in this study, differentiated by genders, are shown in Tables 3 and 4. The great majority of values were shown to be positive, except for some exceptions in that they were negative, more in males than in females, although none of them was statistically different from zero (except CLF vs CLF, in the male subpopulation, r= -0.43; P< 0.05).

From the analysis of obtained results, we have been able to observe, in global form, that a higher correlation degree among morphometric variables in the female than in the male subpopulation exists, in a similar way to that reported by other authors in several equine populations.[5,9,13] Likewise, at the corporal region level (head, trunk and extremities), the intra-region correlations were significantly higher for trunk and extremities than for cephalic measurements, in males

Table 4. Morphological correlation matrix among female corporal measures of the Catalonian donkey breed.

	CLH	CLS	CLF	CWH	CWS	CDH	CLE	THW	THB	THS	THR	THP	THRT	TDL	TDBS	TDR	TDPS	TLR	TWR	TPT	EPK	EPCN	EPF	EPP	EPCR	EPH
CLH	1.00																									
CLS	0 56c	1.00																								
CLF	0 30a	0.01	1.00																							
CWH	0.66c	0.53c	-0.10	1.00																						
CWS	0.42b	0.22	0.36b	0.18	1.00																					
CDH	0.65c	0 43b	0.26a	0.62c	0.29	1.00																				
CLE	0.34b	0.01	0.00	0 14	0 12	0.02	1.00																			
THW	0.35b	0.08	0.56c	0.08	0.52c	0.40b	0.18	1.00																		
THB	0.50c	0.23	0.54c	0.27	0.57c	0.54c	0.15	0.93c	1.00																	
THS	0.44b	0.17	0.65c	0.12	0.40b	0.44b	0.13	0.90c	0.90c	1.00																
THR	0.46b	0.22	0.59c	0.21	0.46b	0.50c	0.13	0.92c	0.94c	0.93c	1.00															
THP	0.44b	0.20	0.63c	0 14	0.43b	0.43b	0.13	0.91c	0.90c	0.96c	0.97c	1.00														
THRT	0.52c	0.28	0.47b	0.27	0.47b	0.48c	0.17	0.85c	0.87c	0.86c	0.85c	0.84c	1.00													
TDL	0.25	0.01	0.32b	0.03	0.47b	0.34a	0.42a	0.65c	0.58c	0.55c	0.54c	0.5lc	0.54c	1.00												
TDBS	0.58c	0.49c	0.29	0.40b	0.49c	0.51c	0.34a	0.68c	0.67c	0.60c	0.61c	0.61c	0.62c	0.66c	1.00											
TDR	0.57c	0.50c	0.35a	0.50c	0.08	0.37a	-0.14	0.18	0.28	0.33a	0.32a	0.36b	0.18	-0.15	0.30a	1.00										
TDPS	0.69c	0.65c	0.12	0.57c	0.42b	0.49c	0.27	0.27	0.32b	0.30a	0.31a	0.32b	0.32b	0.26	0.65c	0.55c	1.00									
TLR	0.32a	0.53c	0.24	064c	0.29	0.59c	-0.02	0.32a	0.42b	0.37a	0.42b	0.39b	0.35a	0.16	0.58c	0.72c	0.65c	1.00								
TWR	0.63c	0.43b	0.20	0.22	0.35a	0.43b	0.08	0.48c	0.50c	0.42b	0.46b	0.45b	0.40b	0.31a	0.59c	0.33a	0.55c	0.59c	1.00							
TPT	0.25	0.20	0.39b	0.00	0.33a	0.40b	0.00	0.65c	0.59c	0.62c	0.59c	0.59c	0.48c	0.55c	0.63c	0.25	0.41b	0.39b	049c	1.00						
EPK	0.51c	0.21	0.56c	0.22	0.50c	0.48c	0.45b	0.76c	0.76c	0.74c	0.75c	0.75c	0.64c	0.70c	0.70c	0.28	0.47b	0.40b	0.48c	0.52c	1.00					
EPCN	0.46b	0.14	0.50c	0 09	0.40b	0.30a	0.61c	0.65c	0.62c	0.63c	0.59c	0.62c	0.56c	0.64c	0.68c	0.17	0.39b	0.26a	0.35b	0.43b	0.81c	1.00				
EPF	0.51b	0.31	0.43b	0.36a	0.47b	0.48c	0.39b	0.66c	0.64c	0.57c	0.63c	0.63c	0.59c	0.56c	0.77c	0.28	0.48c	0.42b	0.39b	0.45b	0.81c	0.75c	1.00			
EPP	0.34a	0.12	0.47b	0.14	0.38a	0.42b	0.26	0.64c	0.61c	0.65c	0.63c	0.65c	0.56c	0.52c	0.60c	0.16	0.36b	0.20	0.37b	0.51c	0.73c	0.76c	0.74c	1.00		
EPCR	0 15	-0.05	0.47b	-0.07	0.38a	0.18	0.43b	0.61c	0.55c	0.5lc	0.52c	0.52c	0.50c	0.44c	0.42b	-0.14	0.16	-0.01	0.24	0.40b	0.61c	0.69c	0.55c	0.60c	1.00	
EPH	0 19	-0.06	0.42b	0.00	0.26	0.27b	0.15	0.66c	0.62c	0.65c	0.63c	0.65c	0.57c	0.38a	0.42b	0..19	0.20	0.29	0.31a	0.44b	0.60c	0.56c	0.57c	0.49c	0.54c	1. 00

(P<0.05); b(P<0.01); c(P<0.001)

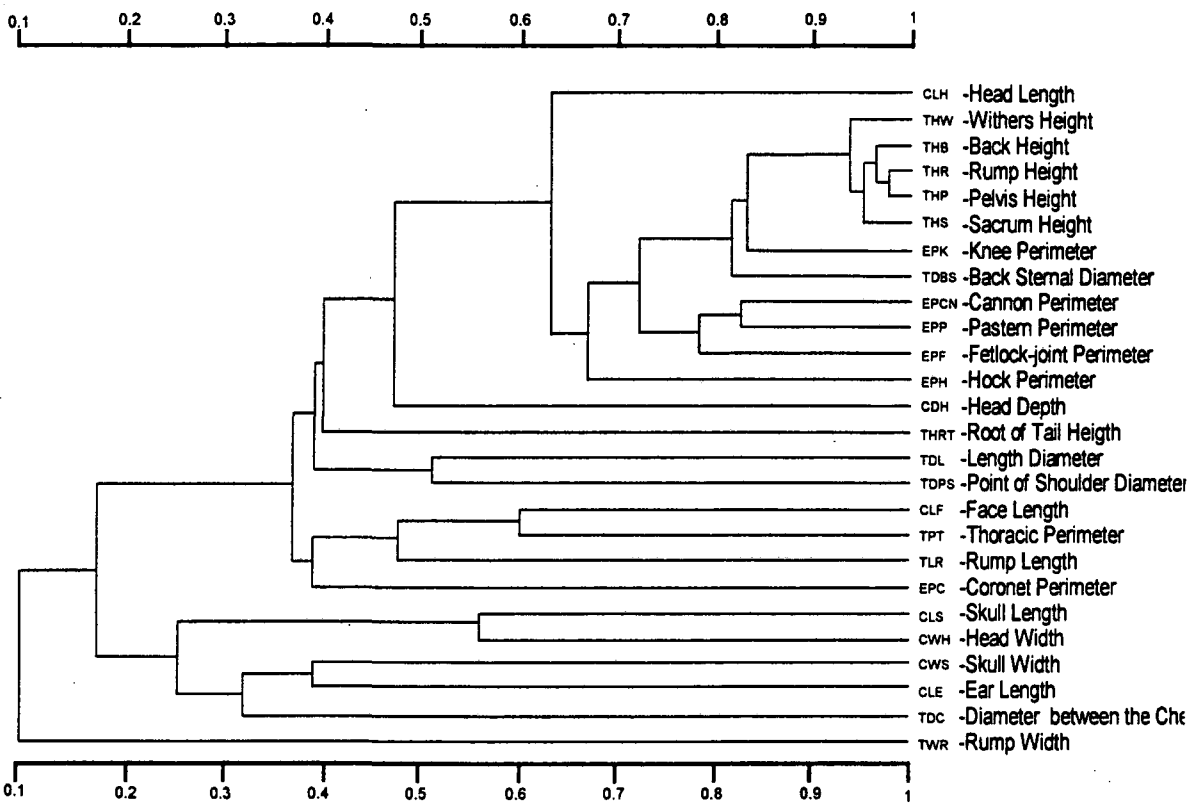


Figure **3.** Dendrogram obtained by PHYLIP analysis using the UPGMA method from morphological correlation data in the male subpopulation of the Catalonian donkey breed (Scale makes reference to morphological correlation degree).

as well as in females. As for the inter-region correlations, globally, a close correlation between trunk and extremities was observed, this relationship being very variable when the cephalic region was compared with the other two, as regards the gender level and the individual cephalic variable level.

A. Cephalic measures

It has already been commented that this corporal region is the one which shows a higher degree of independence among its variables, as much in males as in females. Only head length (CLH), and in the female subpopulation, showed significant correlations with all the other cephalic variables. Important differences due to gender in this region also exist, which makes being able to give a whole interpretation of the existent relationships among these measurements very difficult.

The existent correlations among the morphometric variables of the other corporal regions (trunk and extremities) were also greatly variable, with regards to gender level as well as to individual measures level. As more outstanding data, we could point out: absence of correlation (with some few exceptions) of ear length (CLE) with the remainder of corporal measures, manifested more in males than in females. Absence of correlation between skull width (CWS) and the remainder of variables in the male subpopulation, in a similar way as that found by other authors in PRE populations[5]; on the contrary, CWS showed, in the female subpopulation, a close correlation with almost all the other corporal variables. Significant correlations showed in head length (CLH) and head depth (CDH), in males as well as in females, with regard to a great majority of variables. Skull length (CLS) and head width (CWH) only showed correlation with the measurements of diameters, and this just in the female subpopulation. Finally, face length (CLF) showed significant correlations with the measurements of extremities block, as much in males as in females, and in the female subpopulation also with all the measurements of height.

B. Trunk and extremities measures

These variables maintain a high and significant degree of correlation, in males and females, as much in intra as well as in inter-regions. Nevertheless, the closest relationships ($P < 0.001$) are seen between the measurements of height, between the measurements of perimeters (including thoracic perimeter, TPT), and between heights vs perimeters, as much in males as in females.

Length diameter (TDL) and back-sternal diameter (TDBS), as well as thoracic perimeter (TPT), also were closely correlated with height variables and with extremities perimeters ($P < 0.001$)), confirming the obtained results with the Body, Thoracic and Metacarpo-thoracic Indices, which indicated that animals were of longilinear

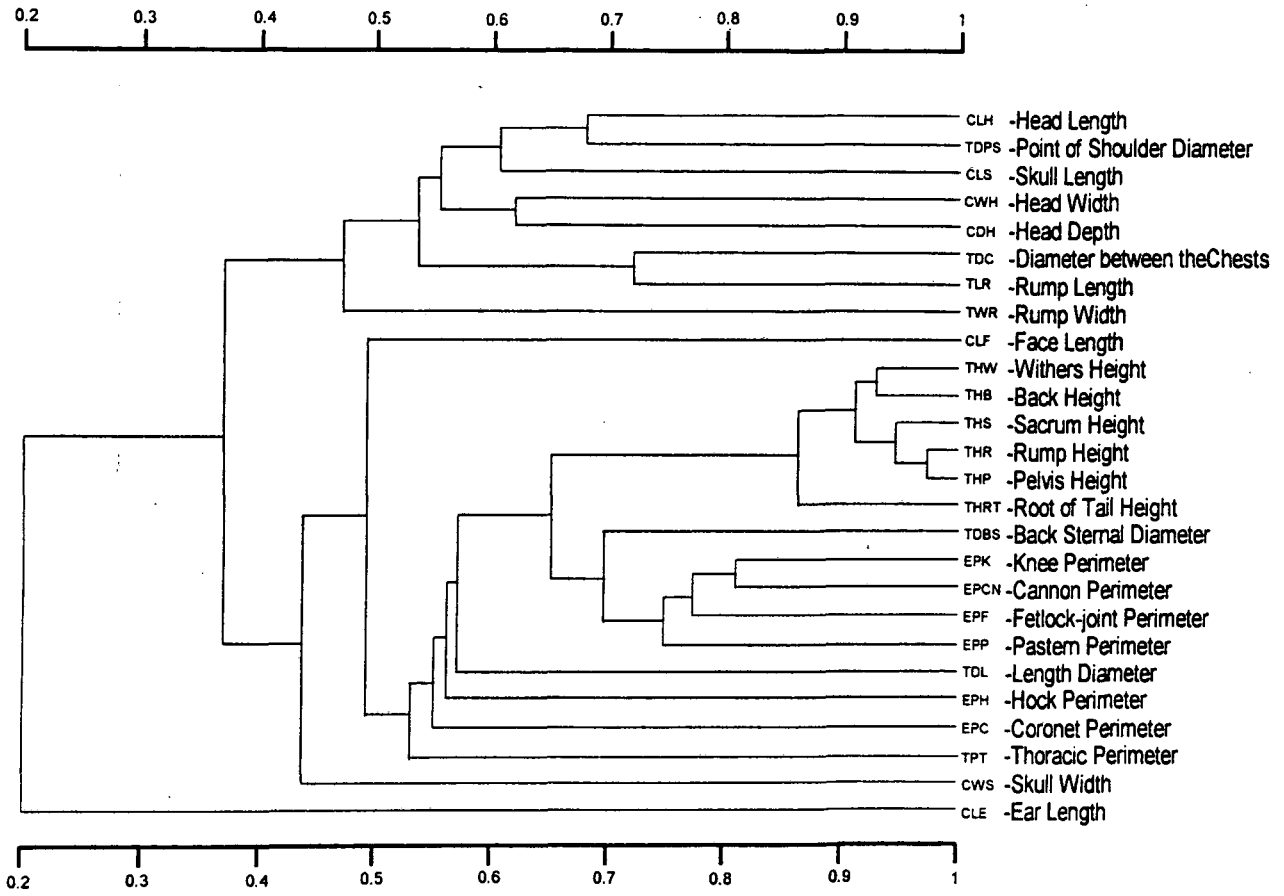


Figure **4.** Dendrogram from correlation data in the female subpopulation.

appearance and hypermetric format.

Rump length (TLR) and rump width (TWR) showed null correlation with the remainder of variables in the case of males (with the exception of TLR with the variables of height ($P < 0.01$), of a contrary form to that which happens in the case of females, where significant correlations with the great majority of variables, included the cephalic, showed.

CONCLUSIONS

Dendrograms obtained applying the UPGMA method for correlation coefficients, were slightly different for male (Figure 3) and female (Figure 4) subpopulations, as was expected, and confirmed the results and interpretations carried out previously.

As more relevant conclusions, we could point out the intra-region, existent close relationship, between cephalic, trunk and extremities variables, more apparent in the female than in the male subpopulation, although in this last one, the weak relationship of many variables could perhaps be attributed to the reduced sample size (n=25 males).

The cluster that forms the variables of height with the perimeters of the extremities in the two dendrograms confirm the close relationship that exists between these measurements.

As for the measurements of thoracic diameters, it was observed that length (TDL) and back-sternal (TDBS) diameters kept a closer relationship with height variables and perimeters; on the other hand, diameter between the ribs (TDR) and point-of-shoulder diameter (TDPS) showed a closer relationship with cephalic measures, although this last, in the male subpopulation, was more akin to extremities and trunk variables than to cephalics.

The rump variables, rump length (TLR) and rump width (TWR), formed a well defined cluster with cephalic measures in the case of females, behaving in an independent form in the male subpopulation, although rump length (TLR) to a greatly minor degree than rump width (TWR).

Lastly, with regard to the cephalic variable ear length (CLE), it could be concluded that it does not show any relationship with the remaining, studied morphological measures.

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Ase vell, més matadures que pell.

Capítol II Caracterització Hematològica i Bioquímica clínica

"Reference ranges and the influence of age and sex on haematological values of the endangered Catalonian donkey breed".
(The Veterinary Journal 1997, 154, 163-168)

"Clinical biochemical parameters of theendangered Catalonian donkey breed: normal values and the influence of sex, age and management practices effect".
(Research in Veterinary Science 1998, 64, 7-10)

The Veterinary Journal 1997, **154**, 163–168

Short Communication

Reference Ranges and the Influence of Age and Sex on Haematological Values of the Endangered Catalonian Donkey

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KEYWORDS: Donkeys; endangered breed; haematological parameters.

The Catalonian donkey is a local breed found in several Pyrenean and pre-Pyrenean regions of the Catalonian area of Northeast Spain. It has contributed to the formation and improvement of several European breeds as well as the American Ass or Mammoth donkey breed (Briggs, 1971; Epstein, 1984; Parés & Vilaró, 1994). The total number of existing animals is about 100, a third of which are males, which places the breed into the critical category (<100 females) proposed by FAO (1992), implying that the breed is in danger of extinction (Bodó, 1992). The main objective of this communication is to characterize haematologically this endangered population, and to establish normal ranges.

We examined 45 adult females aged 3–17 years, 26 adult males aged 3–13 years, and 27 young donkeys (<3 years) of both sexes. All animals appeared clinically healthy. Stress was minimized by handling the animals with care before samples were collected. Data, which seemed to be normally distributed or approximate to a normal distribution after power transformation (Johnson & Wichent, 1988), were analysed by analysis of variance (ANOVA). For other data, non-parametric tests were used. Computations were performed using a statistical software program (SAS, 1989).

Reference ranges are shown in Table I. No significant differences between sexes were found for any parameter in agreement with the report of French and Patrick (1995). However, other authors have reported sexual differences for some parameters. For example, Zinkl *et al.* (1990) showed that females had higher values of mean corpuscular haemoglobin concentration (MCHC), leucocyte and neutrophil counts than males ($P<0.05$).

Age had the most influence on the haematological parameters studied in our donkey population. Nine of the 16 variables showed significant differences between young and adult animals, and there was an interaction between age and sex for eosinophil count ($P<0.05$), with a decreased trend with age in female subpopulation. Total and differential leucocyte counts showed a significant decrease with advancing age but numbers of monocytes and basophils did not seem to be so influenced. Similar results, with some exceptions, have been obtained in horses (Jain, 1986) and also in donkeys (Zinkl *et al.*, 1990). In contrast to our findings, however, Zinkl *et al.* (1990), analysing an American donkey population, reported a significant aging increase in the eosinophil count ($P<0.05$). On the other hand, Fowler and Zinkl (1989) also obtained a marked age-related increase in eosinophil counts in llamas in the Western United States, postulating that this increase may be attributable to increasing parasite burden with age. Perhaps the decrease in eosinophil count with age reflects a lower parasite burden; certainly the population of Catalonian donkeys we examined was receiving routine anthelmintic treatment.

Two red-cell parameters, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), and plasma protein all increased signifi-

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1090-0233/97/050163-06/$12·00/0

Table I
Haematological values of the Catalonian donkey

Analyte	n	*Mean±SD*	*Percentiles* 95%	*Median*	5%	*Range*
Erythrocyte count (10^{12} l^{-1})						
All donkeys	98	6·87±1·22	8·67	6·99	4·78	4·07–10·44
Young donkeys	27	7·14±1·34	8·94	6·96	5·26	4·46–10·44
Adult donkeys	71	6·77±1·17	8·52	7·00	4·78	4·07–10·16
		(NS)				
Haemoglobin (g l^{-1})						
All donkeys	98	122·8±22·7	155	123	93	13·6–169
Young donkeys	27	118·4±14·1	136	121	93	93·0–149
Adult donkeys	71	124·5±25·1	158	124	96	13·6–169
		(NS)				
Packed cell volume (l l^{-1})						
All donkeys	98	0·36±0·05	0·45	0·35	0·27	0·13–0·48
Young donkeys	27	0·34±0·03	0·40	0·34	0·27	0·26–0·41
Adult donkeys	71	0·36±0·05	0·45	0·36	0·28	0·13–0·48
		(NS)				
Mean corpuscular volume (Fl)						
All donkeys	98	52·6±7·5	63·8	53·5	40·3	20·4–68·8
Young donkeys	27	48·6±5·7	57·9	48·1	40·3	36·4–62·8
Adult donkeys	71	54·1±7·6	64·6	54·9	42·2	20·4–68·8
		(**)				
Mean corpuscular haemoglobin concentration (g l^{-1})						
All donkeys	98	347·0±12·8	366	347	326	282–384
Young donkeys	27	347·1±11·2	363	345	328	321–366
Adult donkeys	71	346·9±13·4	368	348	326	282–384
		(NS)				
Mean corpuscular haemoglobin (pg)						
All donkeys	98	18·4±2·2	22·4	18·4	14·7	12·3–23·6
Young donkeys	27	16·9±1·9	19·8	16·8	13·9	12·5–21·5
Adult donkeys	71	19·1±2·1	22·6	19·3	15·9	12·3–23·6
		(**)				
Leucocytes (10^9 l^{-1})						
All donkeys	98	10·7±2·9	16·7	9·7	7·1	6·4–21·0
Young donkeys	27	13·9±3·0	17·7	14·3	9·7	7·5–21·0
Adult donkeys	71	9·6±1·8	13·7	9·3	6·9	6·4–15·4
		(**)				
Lymphocytes (10^9 l^{-1})						
All donkeys	98	5·3±2·4	11·0	4·6	2·4	1·8–13·6
Young donkeys	27	8·0±2·7	12·7	7·3	4·7	3·2–13·6
Adult donkeys	71	4·2±1·2	6·4	4·3	2·4	1·8– 7·8
		(**)				
Monocytes (10^9 l^{-1})						
All donkeys	98	0·22±0·19	0·59	0·19	0·00	0·00–1·05
Young donkeys	27	0·27±0·26	0·81	0·25	0·00	0·00–1·05
Adult donkeys	71	0·21±0·16	0·52	0·19	0·00	0·00–0·77
		(NS)				
Band neutrophils (10^9 l^{-1})						
All donkeys	98	0·06±0·11	0·31	0·00	0·00	0·00–0·60
Young donkeys	27	0·09±0·14	0·34	0·00	0·00	0·00–0·60
Adult donkeys	71	0·08±0·10	0·25	0·04	0·00	0·00–0·56
		(*)				
Segmented neutrophils (10^9 l^1)						
All donkeys	98	4·5±1·2	6·7	4·3	2·6	2·2–9·4
Young donkeys	27	5·0±1·3	7·2	4·9	3·0	2·3–7·6
Adult donkeys	71	4·3±1·2	6·3	4·2	2·6	2·2–9·4
		(**)				

Table I (continued)
Haematological values of the Catalonian donkey

Analyte	n	*Mean±SD*	*Percentiles* 95%	*Median*	5%	*Range*
Eosinophils (10^9 l^{-1})						
All donkeys	98	0·68±0·54	1·82	0·56	0·00	0·00–3·15
Young donkeys	27	0·81±0·71	1·72	0·62	0·00	0·00–3·15
Adult donkeys	71	0·63±0·46 (*)	1·82	0·52	0·00	0·00–1·98
Basophils (10^9 l^{-1})						
All donkeys	98	0·02±0·06	0·20	0·00	0·00	0·00–0·26
Young donkeys	27	0·02±0·05	0·15	0·00	0·00	0·00–0·20
Adult donkeys	71	0·02±0·06 (NS)	0·20	0·00	0·00	0·00–0·26
Platelets (10^9 l^{-1})						
All donkeys	98	234·1±82·9	510·0	237·5	105·0	77·0–510·0
Young donkeys	27	228·9±86·5	367·0	237·0	95·0	94·0–431·0
Adult donkeys	71	236·1±82·1 (NS)	357·0	238·0	107·0	77·0–510·0
Fibrinogen (g l^{-1})						
All donkeys	98	2·08±0·59	3·04	2·07	1·23	0·00–4·45
Young donkeys	27	2·26±0·67	3·17	2·22	1·82	0·00–4·21
Adult donkeys	71	2·00±0·55 (**)	2·69	2·01	1·23	0·38–4·45
Plasma protein (g l^{-1})						
All donkeys	98	66·2±6·8	78·0	68·0	53·0	46·5–87·0
Young donkeys	27	65·9±11·6	82·0	65·0	49·0	46·5–87·0
Adult donkeys	71	68·4±12·0 (*)	83·0	69·0	53·9	48·8–87·0

(*) $P<0·05$; (**) $P<0·01$; (***) $P<0·001$; (NS) Not significant.

cantly with age ($P<0·05$, $P<0·01$ and $P<0·05$, respectively). The increases in the former variables are associated with increases in packed cell volume (PCV) and haemoglobin values, and the decrease in erythrocyte count with age (Allen & Archer, 1973). The age-related increase in plasma protein concentration would be primarily caused by increased γ-globulin concentration (Jain, 1986). A general tendency for increasing plasma protein values with increasing age has been observed in donkeys (Zinkl *et al.*, 1990), horses (Jain, 1986), and llamas (Fowler & Zinkl, 1989). MCV and MCH also significantly increased with age in previous donkey studies (Zinkl *et al.*, 1990) as well as in other species such as the horse (Jain, 1986).

Significant age-related differences were not observed with the other parameters. Brown and Cross (1969) and Zinkl *et al.* (1990) found a significant decrease with age of erythrocyte counts in donkey populations, suggesting that the smaller sized erythrocytes of young donkeys could be attributable to iron deficiency. French and Patrick (1995), analysing similar haematological parameters in a large population of donkeys (*ca.* 4000 individuals), did not find significant differences for sex and age factors for any analyte. They suggested that the differences obtained by other authors could be explained by inappropriate statistics on a small non-normal sample.

Comparison of our results with previously published values for donkeys (Table II) does not reveal any large discrepancies (Zinkl *et al.*, 1990; French & Patrick, 1995) and any slight differences may be ascribed to differences in techniques. Nevertheless, it is worth mentioning that the lymphocyte count in Catalonian donkeys was slightly higher than for other breeds and populations (namely, the Mammoth donkey breed, USA donkeys, and UK donkeys). On the other hand, comparison of donkey results with reference ranges (Jain, 1986) for horses (*Equus caballus*) indicated that most values are similar. Only the erythrocyte count was slightly lower for donkeys (reference range 4·7–9·0) than horses (6·8–12·9). The platelet count was slightly higher for donkeys (105–584) than for horses (100–350), although the means were approximately similar.

Table II
Comparison of haematological values of Catalonian donkeys with other donkey breeds and horses

Analyte	*Catalonian donkeys*	*USA donkeys**		*Mammoth donkeys**	*UK donkeys†*	*Horses‡*
Erythrocyte count (10^{12} l^{-1})						
Mean±SD	6·87±1·22 (98)	6·6±0·7 (166)		6·7±0·7 (12)	—	9·0±1·2 (147)
5% to 95%	4·78–8·67 (98)		4·7–9·0		4·0–7·3 (8995)	6·8–12·9 (147)
Haemoglobin (g l^{-1})						
Mean±SD	122·8±22·7 (98)	131±17 (166)		132±18 (12)	—	144±17 (147)
5% to 95%	93–155 (98)		95–165		90–153 (4210)	11–19 (147)
Packed cell volume (l l^{-1})						
Mean±SD	0·36±0·05 (98)	0·38±0·05 (166)		0·38±0·05 (12)	—	0·41±0·04 (147)
5% to 95%	0·27–0·45 (98)		0·28–0·47		0·25–0·38 (4215)	0·32–0·53 (147)
Mean corpuscular volume (Fl)						
Mean±SD	52·6±7·5 (98)	57·9±5·5 (166)		56·3±4·9 (12)	—	45·5±4·3 (147)
5% to 95%	40·3–63·8 (98)		46·3–67·0		57·0–79·0 (4235)	37·0–58·5 (147)
Mean corpuscular haemoglobin (pg)						
Mean±SD	18·4±2·2 (98)	19·9±1·9 (166)		19·5±1·8 (12)	—	15·9±1·5 (147)
5% to 95%	14·7–22·4 (98)		16·0–23·0		18·9–28·6 (4238)	12·3–19·7 (147)
Mean corpuscular haemoglobin concentration (g l^{-1})						
Mean±SD	347±12·8 (98)	343±11 (166)		348±7 (12)	—	352±14 (147)
5% to 95%	326–366 (98)		320–362		314–391 (4239)	310–384 (147)
Leucocytes (10^9 l^{-1})						
Mean±SD	10·7±2·9 (98)	10·0±20·0 (165)		10±1·7 (12)	—	9·0±1·8 (147)
5% to 95%	7·1–16·7 (98)		5·4–15·5		6·1–16·1 (4239)	5·4–14·3 (147)
Lymphocytes (10^9 l^{-1})						
Mean±SD	5·3±2·4 (98)	4·4±1·7 (165)		3·6±1·3 (12)	—	3·5±1·1 (147)
5% to 95%	2·4–11·0 (98)		1·1–7·4		1·8–7·8 (4212)	1·5–7·7 (147)
Monocytes (10^9 l^{-1})						
Mean±SD	0·22±0·19 (98)	0·5±0·2 (165)		0·3±0·2 (12)	—	0·3±0·2 (147)
5% to 95%	0·00–0·59 (98)		0·07–1·2		0·0–0·8 (4167)	0·0–1·0 (147)
Neutrophils (10^9 l^{-1})						
Mean±SD	4·5±1·2 (98)	4·7±1·7 (165)		5·1±1·5 (12)	—	4·7±1·2 (147)
5% to 95%	2·6–6·7 (98)		2·2–10·1		2·2–13·3 (4213)	2·2–8·5 (147)

Haematological values for donkeys may vary according to geographical and nutritional factors (Fowler & Zinkl, 1989). Further studies in other locations are now required.

ACKNOWLEDGEMENTS

The authors wish to thank the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya which financed this study. The suggestions of the reviewers were greatly appreciated. Also, we would like to thank Chuck Simmons for assistance with the preparations of this manuscript.

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(Accepted for publication 27 November 1996)

Research in Veterinary Science 1998, 64, 7-10

Clinical biochemical parameters of the endangered Catalonian donkey breed: normal values and the influence of sex, age, and management practices effect

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SUMMARY

Twelve clinical biochemical parameters were determined in 97 animals of all age groups and both sexes of the endangered Catalonian donkey breed. Significant sex differences were observed for phospholipid concentration ($P<0.01$). Evaluating the effect of management practices on the various parameters showed significant differences for total bilirubin ($P<0.001$) and creatinine ($P<0.05$) concentrations and γ-glutamyltransferase ($P< 0.05$) activity. Moreover, it was observed that inorganic phosphorus concentration decreased with age ($P<0.001$), whereas albumin and triglyceride concentrations increased with age ($P<0.01$ and $P<0.001$, respectively). Comparison of biochemical ranges obtained for the Catalonian donkey breed with reference ranges for other breeds and populations (Mammoth, USA donkeys, UK donkeys, Indian donkeys and Poitou donkeys), indicated that most values were similar, with the exceptions of enzymatic activities mainly. The results reported in the present study could serve as reference ranges for donkey populations.

ALTHOUGH in several parts of the world donkeys are still used for work, transporting goods over mountain roads, agriculture and to pull coaches, in many others they have become popular as companion animals, and the main object of their owners is just to enjoy owning a donkey and looking after it.

The Catalonian donkey is a local tame donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonian area of northeast Spain, characterised by hypermetrical format, longilinear appearance and concaviline cranial profile. The coat is a black colour with characteristic fadings in the muzzle, orbital zone of the eyes, belly and internal face of the extremities. The total number of existing animals obtained in a recent census showed that it slightly surpasses one hundred individual, of which approximately a third are males (Jordana and Folch, 1996). These figures fit into the category of the Critical Breed (100 females) proposed by the FAO Expert Consultation (Anonymous 1992), which implies that the breed is in danger of extinction (Bodó 1992).

This breed has contributed to the formation and improvement of several European donkey breeds (Romagosa 1959, Epstein 1984, Parés and Vilaró 1994), and has had a great and decisive influence in the formation of the American Ass or 'Mammoth' (Romagosa 1959, Aparicio 1960, Briggs 1971, Epstein 1984, Sotillo and Serrano 1985, Parés and Vilaró 1994).

This paper characterises biochemically this endangered population, establishing normal ranges for a number of biochemical analytes and determining the possible influence of sex, age and management practices effects on these. The results were compared with those obtained in other donkey breeds, and in horse.

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MATERIALS AND METHODS

Ninety-seven blood samples from 26 adult males aged three to 13 years, 45 adult females aged three to 17 years, and 26 young donkeys (<three years) of both sexes, belonging to the Catalonian donkey breed were collected during the period of March to April 1995. Blood samples were obtained from the jugular vein in vacuated glass tubes. Samples were allowed to clot at room temperature and serum was separated by centrifugation at 1500 *g* for 10 minutes and stored at –20°C. Frozen samples were held in storage for no more than one month, when biochemical analyses were made. All individuals appeared clinically healthy, and they were handled with care to minimise any possible effects of stress. Donkeys were on routine anthelmintic management programs.

Approximately 45 per cent of the animals (43 versus 97) were from a single owner, all of them located in the same geographical area, under the same management practices and feeding conditions (group A), while the remaining donkeys were from several small and disperse farms (group B); therefore, the population was subdivided into two management groups for analysis. None of the donkeys were regularly worked.

Biochemical analysis were determined in a Cobas Bio autoanalyser (Roche, Nuttley, NY) using commercially available test combinations. For urea, cholesterol, phospholipids, creatinine, total bilirubin, albumin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), and creatine kinase (CK): (Boehringer Mannheim GmbH, Mannheim, Germany), and for triglycerides and inorganic phosphorus (IP): (Medical Analysis Systems, INC., Camarillo, CA, USA).

Statistical analyses were performed using a statistical software program (SAS 1989). Reference ranges were calculated by use of standard deviation (SD) about the mean,

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TABLE 1: Clinical biochemical parameters of the Catalonian donkey breed for all, young (<3 years) and adult (>3 years) animals. Reference ranges are given as Mean±SD, interval from the fifth to the 95th percentiles about the Median, and minimum-, and maximum-values. N is the analysed sample size

Analyte		N	Mean ±SD	Percentiles 95%	Median	5%	Range
Urea (mg dl^{-1})	All donkeys	97	36·1 ±7·7	47·6	36·4	22·2	16·0-56·8
	Young donkeys	26	34·0 ±8·2	43·9	33·6	17·2	16·0-47·1
	Adult donkeys	71	37·2 ±7·2	49·0	37·4	26·8	17·6-56·8
Cholesterol (mg dl^{-1})	All donkeys	97	71·1 ±26·3	96·8	66·3	51·0	40·7-249·3
	Young donkeys	26	81·4 ±45·0	172·8	67·9	52·5	43·5-249·3
	Adult donkeys	71	67·4 ±13·1	92·3	65·3	51·0	40·7-108·3
Triglyceride (mg dl^{-1})	All donkeys	97	74·8 ±32·5	137·8	68·0	29·9	11·1-182·4
	Young donkeys	26	53·7 ±20·3	92·2	54·6	27·8	21·5-104·9
	Adult donkeys	71	82·5 ±32·8	140·2	78·9	36·2	11·1-182·4
AST (U litre^{-1})	All donkeys	97	254 ±57	370	243	175	165-491
	Young donkeys	26	262 ±79	423	246	174	165-491
	Adult donkeys	71	251 ±47	360	243	179	166-394
Inorganic phosphorus (mg dl^{-1})	All donkeys	97	3·80 ±0·86	5·51	3·74	2·51	2·21-5·90
	Young donkeys	26	4·64 ±0·70	5·78	4·50	3·89	2·97-5·90
	Adult donkeys	71	3·49 ±0·69	4·75	3·40	2·49	2·21-5·03
LDH (U litre^{-1})	All donkeys	97	315 ±139	590	291	126	94-869
	Young donkeys	26	307 ±102	424	322	152	94-574
	Adult donkeys	71	318 ±151	705	279	126	117-869
Phospholipids (mmol litre^{-1})	All donkeys	95	1·71 ±0·42	2·44	1·69	1·12	0·01-3·34
	Young donkeys	24	1·71 ±0·41	2·03	1·68	1·68	1·12-3·34
	Adult donkeys	71	1·71 ±0·49	2·44	1·70	1·04	0·01-2·71
GGT (U litre^{-1})	All donkeys	97	48 ±22	99	43	21	20-112
	Young donkeys	26	52 ±24	105	47	21	20-112
	Adult donkeys	71	47 ±22	95	42	23	20-101
Creatinine (mg dl^{-1})	All donkeys	97	1·06 ±0·22	1·44	1·05	0·73	0·49-1·56
	Young donkeys	26	0·95 ±0·21	1·23	0·97	0·67	0·49-1·52
	Adult donkeys	71	1·10 ±0·22	1·44	1·10	0·77	0·73-1·56
Creatine kinase (U litre^{-1})	All donkeys	97	195 ±104	406	168	87	75-645
	Young donkeys	26	206 ±90	401	180	126	110-481
	Adult donkeys	71	191 ±109	406	155	81	75-645
Total bilirubin (mg dl^{-1})	All donkeys	97	0·05 ±0·03	0·12	0·05	0·00	0·00-0·17
	Young donkeys	26	0·05 ±0·03	0·11	0·05	0·00	0·00-0·16
	Adult donkeys	71	0·05 ±0·03	0·12	0·05	0·00	0·00-0·17
Albumin (g dl^{-1})	All donkeys	97	2·68 ±0·36	3·25	2·71	2·03	1·75-3·61
	Young donkeys	26	2·49 ±0·30	3·03	2·42	2·10	2·03-3·16
	Adult donkeys	71	2·75 ±0·36	3·30	2·76	2·02	1·75-3·61

AST = Aspartate transaminase; LDH = Lactate dehydrogenase, GGT = γ-Glutamyltransferase

the interval from the fifth to the 95th percentiles about the median, and minimum-, and maximum-observed values for each biochemical parameter.

Statistical differences for several factors of variation; age (young donkey versus adult donkey), sex (female versus male) and management group (group A versus group B), and the existence of possible interactions between these factors of variation, for data normally distributed or data approximate to a normal distribution after power transformation (Johnson and Wichent 1988), were analysed by the ANOVA test. If data did not seem to be normally distributed, non-parametric tests (SAS/STAT, proc nparlway) were used.

RESULTS

Reference ranges of chemical constituents of the blood of the Catalonian donkey breed, determined in the present study, are shown in Table 1.

Three analytes (urea, creatinine and albumin) seemed to be normally distributed. The distributions of the rest of the analytes do not have a normal distribution, although three of them (triglycerides, AST and inorganic phosphorus), would approach a normal distribution after power transformation. However, with the other six variables (cholesterol, LDH, phospholipids, GGT, CK and total bilirubin), there is not a power transformation suitable enough to approach the density distribution normality; as a result, non-parametric analyses for these variables were found to be more appropriate in order to compare levels of factors in the study.

Significant differences were not obtained in the values of any parameter for the sex effect, except for phospholipid concentration, where the values for the male subpopulation (mean = 1·82 ±0·54) were significantly higher ($P<0·01$) than those for females (mean = 1·55 +0·53). Significant variations were observed in the values of total bilirubin ($P<0·001$) and creatinine ($P<0·05$) concentrations, and GGT activity ($P<0·05$) between management groups. Inorganic phosphorus (IP) concentration decreased significantly with age ($P<0·001$), whereas albumin ($P<0·01$) and triglyceride ($P<0·001$) concentrations increased with age. Interaction between age and sex for urea concentration ($P<0·05$), as well as between age and management group ($P<0·05$), and among age, sex and management group ($P<0·05$) for albumin concentration, were observed.

Comparison of clinical biochemical ranges of Catalonian donkeys with other donkey breeds and populations, and horses is shown in Table 2.

DISCUSSION

Only phospholipid concentration showed a significant sex effect ($P<0·01$) in the Catalonian donkey population.

TABLE 2: Comparison of biochemical parameters of Catalonian donkeys with other donkey breeds and populations, and horses. Reference ranges are given as Mean±SD, and 5 per cent to 95 per cent percentiles. Percentile ranges for American donkey populations (1) are given as a whole, and refer to the 2·5 per cent to 97·5 per cent interval; percentile ranges for the studied populations (3) by Gupta et al (1994), refer to the 0 per cent to 100 per cent interval. Within parentheses analysed sample size

Analyte	Catalonian donkeys	USA donkeys[1]	Mammoths[1]	UK donkeys[2]	Indian donkeys[3]	Poitou donkeys[3]	Horses[4]
Urea (mg dl^{-1})							
Mean ±SD	36·1 ±7·7 (97)	18·5 ±5·0 (215)	19·0 ±5·0 (12)	–	25·0 ±0·7 (15)	21·5 ±0·8 (18)	–
5% to 95%	22·1-47·6 (97)	14·9-57·7		11·4-45·6 (4213)	18·8-32·0 (15)	14·1-31·2 (18)	21·4-51·3
Cholesterol (mg dl^{-1})							
Mean ±SD	71·1 ±26·3 (97)	108·0 ±30·0 (215)	76·0 ±20·0 (12)	–	56·8 ±0·7 (15)	55·0 ±0·7 (18)	111·0 ±18·0
5% to 95%	51·0-96·8 (97)	73·0-187·0		–	44·4-77·7 (15)	37·6-67·5 (18)	75·0-150·0
Triglyceride (mg dl^{-1})							
Mean ±SD	74·8 ±32·5 (97)	–	–	–	–	–	–
5% to 95%	29·9-137·8 (97)	–	–	–	–	–	–
AST (U litre^{-1})							
Mean ±SD	254 ±57 (97)	487 ±119 (214)	439 ±106 (12)	–	–	–	296 ±70
5% to 95%	175-370 (97)	292-730		–	–	–	226-366
IP (mg dl^{-1})							
Mean ±SD	3·80 ±0·86 (97)	4·30 ±1·30 (211)	3·70 ±0·90 (12)	–	2·90 ±0·10 (15)	2·90 ±0·20 (18)	–
5% to 95%	2·51-5·51 (97)	2·40 -7·00		–	2·30-4·30 (15)	1·90-4·90 (18)	3·10-5·60
LDH (U litre^{-1})							
Mean ±SD	315 ±139 (97)	427 ±161 (215)	466 ±167 (12)	–	–	–	252 ±63
5% to 95%	126-590 (97)	187-7·59		–	–	–	162-412
GGT (U litre^{-1})							
Mean ±SD	48 ±22 (97)	69 ±29 (108)	72 ±28 (12)	–	–	–	8 ±1
5% to 95%	21-99 (97)	19-134		8·49 (4220)	–	–	4-13
Creatinine (mg dl^{-1})							
Mean ±SD	1·06 ±0·22 (97)	1·10 ±0·30 (108)	1·10±0·30 (12)	–	1·20±0·10 (15)	1·40±0·10 (18)	–
5% to 95%	0·73-1·44 (97)	0·60-1·50		0·50-1·50 (1135)	0·80±1·80 (15)	1·00±1·80 (18)	1·20-1·90
Creatine Kinase (U litre^{-1})							
Mean ±SD	195±104 (97)	64±43 (108)	47±26 (12)	–	–	–	13±5
5% to 95%	87-406 (97)	20-186		15-149 (4218)	–	–	2-23
Total Bilirubin (mg dl^{-1})							
Mean ±SD	0·05 ±0·03 (97)	0·10 ±0·20 (215)	0·20 ±0·50 (12)	–	0·30 ±0·07 (15)	0·30 ±0·07 (18)	1·00 ±0·00
5% to 95%	0·00-0·12 (97)	0·00-0·60		0·01-0·77 (4212)	0·20-0·60 (15)	0·10-0·50 (18)	1·00-2·00
Albumin (g dl^{-1})							
Mean ±SD	2·68 ±·36 (97)	3·30 ±0·30 (215)	3·40 ±0·20 (12)	–	3·60 ±0·10 (15)	3·70 ±0·10 (18)	3·00 ±0·20
5% to 95%	2·02-3·30 (97)	2·60-4·10		2·00-3·40 (1688)	3·20-3·90 (15)	3·10-4·10 (18)	2·60-3·70

AST = Aspartate transaminase; LDH = Lactate dehydrogenase, IP = Inorganic phosphorus; GGT = γ-Glutamyltransferase
(1) Zinkl et al 1990, (2) French and Patrick 1995, (3) Gupta et al 1994, (4) Kaneko 1989

The absence of a sex effect on biochemical parameters in donkey populations was previously reported by French and Patrick (1995) in 15 analytes, and by Zinkl et al (1990) in several populations for 22 analytes. However, none of these authors actually analysed for phospholipid concentrations. Zinkl et al (1990) found an age-sex interaction only for alkaline phosphatase activity ($P<0·05$) which was not measured in this current study. Although an effect of sex has previously been reported for inorganic phosphorus concentration in one study of adult donkeys (Nayeri 1978), with males having higher values, this has not been found in the other studies, cited above, and was not the case in this present investigation.

As for management practices effect, significant differences for three of the 12 analytes (total bilirubin and creatinine concentrations, and GGT activity) were found, reflecting, perhaps, differences in the feeding conditions between both groups of donkeys, since creatinine concentration depends upon the total body content of creatine, which in turn, depends upon dietary intake and muscle mass (Kaneko 1989).

A very significant decrease with age was observed for IP concentration in the Catalonian donkey breed ($P<0·001$). Similar results were also reported by Zinkl et al (1990), arguing that this IP decrease with age ($P<0·05$) probably reflects decreased bone metabolism as animals become older.

Albumin ($P<0·01$) and triglyceride ($P<0·001$) concentrations increased significantly with age. French and Patrick (1995) and Zinkl et al (1990) did not find any significant differences with age for these analytes, although Zinkl et al (1990) pointed out that the interaction between age and sex was slightly above the 0·05 probability level for albumin concentration. Although, regarding age influence on albumin concentration, we cannot explain why the results from this study do seem to disagree with what normally occurs in other animal species, where there tends to be a general increase in total protein, a slight decrease in albumin and a progressive increase in globulins with advancing age (Kaneko 1989).

French and Patrick (1995), analysing similar clinical biochemical parameters in a large population of donkeys (≈4000 individuals) did not find significant differences for sex or age factors for any analyte, suggesting that the differences obtained by other authors for these factors could possibly be explained by the inappropriate use of parametric statistics on a small non-normal sample. Notwithstanding, the results of the current study were obtained from data normally distributed, data approximate to a normal distribution after power transformation, and non-parametric analyses and confirm the influence of age, sex and herd effects for some biochemical parameters.

With regard to the comparison of results obtained in the Catalonian donkey breed with other populations (Table 2), we have commented that, since the original data from the other breeds and populations are not available, we have not been able to test for statistical differences among populations. We have to constrain ourselves to an approximate interpretation of the comparisons, so these will have to be interpreted with caution.

Most of the results in this study agree with those given by Zinkl et al (1990), Gupta et al (1994), and French and Patrick (1995), with the exceptions of enzymatic activities.

Nevertheless, we would like to mention that slight differences in the reference ranges among populations could reflect differences in methodologies used as well as equipment between the various laboratories.

The AST and LDH enzymatic activities for Catalonian donkeys reference ranges were lower than for the USA and Mammoth donkeys (Zinkl et al 1990). The enzymatic activity for GGT showed to be very similar for the Catalonian donkey and the American populations, being higher, however, than ranges obtained in UK donkeys (French and Patrick 1995). Creatine kinase activity for Catalonian donkeys was higher than for American and UK donkey populations. The cholesterol concentration ranges for Indian and Poitou donkeys (Gupta et al 1994) were lower than ranges in Catalonian, USA and Mammoth donkeys. The reference ranges for the remaining parameters were very similar in all donkey populations.

On the other hand, comparison of donkey results with reference ranges (Kaneko 1989) for warm-blooded horses (*Equus caballus*) were also very similar for some analytes. Disagreement was shown for total bilirubin concentration (donkey was lower than horse), and for creatine kinase and GGT activities, where donkey ranges were higher than horses.

Other slight differences, and dependent upon analysed donkey populations, were shown for cholesterol concentration and AST activity. The cholesterol concentration values for Catalonian donkey and American donkey populations were similar to the horse. However, the ranges of Indian and Poitou donkeys were half that for horses. AST activity was similar in both Catalonian donkeys and horses, whereas the ranges were appreciably higher in American donkey populations than in horses.

Data in this study and additional studies in other locations, which would be desirable, can enhance our understanding on biochemical parameters in this species: this will allow veterinarians to establish an appropriate interpretation of laboratory data and give these animals appropriate care.

ACKNOWLEDGEMENTS

The authors wish to thank the Departament d'Agricultura, Ramaderia i Pesca of Generalitat de Catalunya which financed this study. The suggestions of the reviewers were greatly appreciated. Also, we would like to thank Chuck Simmons for assistance with the preparation of this manuscript.

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Accepted July 8, 1997

A burra que criï, no li diguis arri.

Capítol III: Caracterització genètica.

Genetic variation (protein markers and microsatellites) within the endangered Catalonian donkey breed.
(Animal Genetics, en revisió)

Genetic diversity, inbreeding estimate and parentage verification in the Catalonian donkey breed, by using microsatellite markers.
(en elaboració)

GENETIC VARIATION (PROTEIN MARKERS AND MICROSATELLITES) WITHIN THE ENDANGERED CATALONIAN DONKEY BREED

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Short running title: Genetic variation in Catalonian donkey

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Summary

Genetic variation of the endangered Catalonian donkey breed has been analysed, by using seven biochemical genetic loci and twelve microsatellite loci isolated from the domestic horse, in 98 individuals of both sexes. Only four protein markers and three microsatellites were shown to be polymorphic. All of the analysed loci showed close agreement with Hardy-Weinberg proportions, with the exception of the MPZ002 locus ($P<0.01$).

The within-population inbreeding estimate was not significantly different from zero (as measured by F_{IS}-statistic). The cumulative-exclusion probability for all polymorphic loci was 82.9%, this value still being very low so that these markers could efficiently be utilised for parentage verification.

Keywords: donkey, endangered breed, genetic variation, inbreeding, parentage control.

Introduction

The Catalonian donkey breed is a population in danger of extinction which is located in several Pyrenean and pre-Pyrenean regions of the Catalonian area of Northeast Spain. The last census carried out only slightly surpassed one hundred animals, a third of which were males (Jordana & Foch 1996). Following the guidelines proposed by the FAO, this breed was characterised morphologically (Folch & Jordana 1997) as well as haematologically (Folch et al, 1997), by clinical biochemical parameters (Jordana *et al.* 1998) and demographically (Folch & Jordana 1998), the purpose of this paper being to genetically characterise this population in danger of extinction.

Markers used in order to carry out this type of studies have always been the conventional biochemical polymorphisms (protein markers) and, more recently, the those DNA hypervariable sequences known as microsatellites (SSR-Simple Sequence Repeat or STR-Short Tandem Repeat), with these now being the markers of election in the study of variation measurements and genetic structure of populations (Goldstein & Pollock 1997). Nevertheless, in the asinine species (*Equus asinus*) there are a few works related to this topic, and there have been a reduced number of genetic polymorphic loci (protein markers) described in several analysed populations (Niece & Kracht 1967; Mengozzi *et al.* 1982; Bowling & Nickel 1985; Ketchum & Cothran 1989; Bell 1994; Ouragh *et al.* 1996). On the matter of microsatellite markers analysis, no paper exists, at least until now, which has utilised these markers in donkey populations. Only Breen *et al.* (1994), and in a total of eight individuals, verified that a set of thirteen loci, isolated from the domestic horse (*Equus caballus*), amplified (by PCR-Polymerase Chain Reaction techniques) satisfactorily in the donkeys.

The two main, priority objectives proposed in the Catalonian donkey breed Conservation Programme are: (1) maximum genetic diversity maintenance, (2) with the minimum possible consanguinity increase per generation. For this, the knowledge of the genetic population structure is basic. The research and analysis of polymorphic markers will allow us to describe levels of genetic variability, to carry out estimates which approach the degree of inbreeding, to identify the most heterozygous individuals of the population in order to be able to programme the best matings (Gill & Harland 1992) which will retain the maximum ancestral genetic variability (Alderson 1992; Boichard *et al.* 1997), to provide reliable tools for parentage verification, and lastly, to provide adequate information for phylogenetically relating this breed with the other worldwide donkey breeds.

Materials and methods

Animals and genetic loci analysed

Blood samples from ninety-eight individuals of both sexes, which represent approximately 95% of the total census of the breed, were examined for variation at seven biochemical genetic loci and twelve microsatellite loci. The 6-Phosphogluconate dehydrogenase (PGD) and Glucose phosphate isomerase (GPI) red-blood-cell systems were analysed by horizontal electrophoresis in agarose gel (Gahne & Juneja 1985). The other five were plasma systems: Albumin (ALB), detected by horizontal electrophoresis in starch gel (Bortolozzi 1983); Transferrin (TF), Vitamin D-binding protein (GC), Alpha 1-β glycoprotein (A1B) and Protease inhibitor (PI), typed by horizontal electrophoresis in polyacrylamide gels (Juneja & Gahne 1987; Bell 1994).

Twelve microsatellite loci, isolated from the domestic horse, HMS1, HMS3, HMS5, HMS6, HMS7, HTG6, HTG8, HTG14, HTG15, MPZ001, MPZ002 and VHL20 (Breen *et al.* 1994), were also analysed in this study's donkey population. Amplification PCR products were resolved through a 10% PAGE by ethidium bromide staining. Two size markers were used: markers V and VIII from Boehringer. Pedigrees were available for analysis.

Statistical analyses

Alleles frequencies and mean heterozygosity values per polymorphic locus were obtained using the BIOSYS-1 computer programme (Swofford & Selander 1989). Tests of genotype frequencies for deviations from Hardy-Weinberg equilibrium were carried out using the exact tests of the GENEPOP computer programme (Raymond & Rousset 1995). Tests were carried out separately for both types of markers.

Using the methods of Weir & Cockerham (1984), as implemented in the FSTAT computer programme (Goudet 1995), the f-statistic value for each locus was calculated, the only one of the F-statistics that can be estimated from frequency data for a single population, which measures the correlation between pairs of genes within individuals within populations, and is analogous to Wright's (1965, 1978) F_{IS}-statistic; that is to say, it measures the deficit or the excess of heterozygotes which could exist in the Catalonian donkey breed, and their significance was determined from permutation tests with the sequential Bonferroni procedure (Hochberg 1988) applied over loci (alleles were permuted within the population) in deriving significance levels.

Polymorphic information content (PIC), for each microsatellite loci, was calculated according to Botstein *et al.* (1980), and, for all systems, probability of exclusion (PE) was determined according to Jamieson's formula (1994).

Results and discussion

Alleles frequencies, statistics of genetic variability, and PIC and PE values, for biochemical polymorphism and microsatellite loci, are shown in Tables 1 and 2, respectively.

Only four protein markers were shown to be polymorphic (TF, PGD, GC and PI). All allelic variants obtained in our population have already been previously described in the scarce, existent bibliography on the topic, with a very similar distribution in their genic frequencies (Bell 1994). Perhaps the only point of interest could be the TF^{Dd} variant, which in the several analysed donkey populations from the USA, Australia, Italy and Morocco, was shown as a rare allele, its frequency ranging from between 0.002 to 0.044, while in the Catalonian donkeys it had a relatively more elevated value (0.272), possibly due to some bottleneck or founder effect which this population might have

suffered at some time in its history. In reference to twelve analysed microsatellites, not many things can be said because there are not other works where a comparison could be made. Only three of these microsatellites were shown to be polymorphic: HTG6, four alleles detected with sizes ranging from between an 82 bp to 90 bp interval; MPZ002, two alleles ranging between 80-89 bp; and VHL20, two alleles ranging between 86-104 bp. The different variants found for each one of them (poly-, and monomorphic loci) are within the described ranges (bp size) by Breen *et al.* (1994).

All protein markers showed close agreement with Hardy-Weinberg Equilibrium (HWE). For the microsatellite loci, only MPZ002 showed significant disagreement with HWE, showing a very significant excess of heterozygotes ($P<0.01$).

When the population was divided into two subpopulations, corresponding to males and females, it could be checked that statistically significant differences did not exist in the allelic and genotypic distribution for any loci, between both subpopulations (GENEPOP programme, data not shown). All loci showed agreement with HWE in the two subpopulations, with the exception of the MPZ002 locus in the female subpopulation ($P<0.05$). The male subpopulation was in HWE for this locus ($P>0.05$), although it almost was not ($P=0.055$). These values confirm the excess of heterozygotes observed for the global population, which was produced in males as well as females. The fact that MPZ002 genic frequencies are statistically equal for males and females (non-significant differences) made us refuse the hypothesis of a possible Robertson Effect (Robertson 1965) in order to explain this significant excess of heterozygotes, perhaps indicating a locus-specific effect which suggests selection affecting this locus (Barker et al., 1997).

The within-population inbreeding estimates ($f \equiv F_{IS}$) are shown in Table 3. For each locus, no value was significantly different from zero. The mean f estimates were not

significant either. This F_{IS} (f) average, obtained from a jackknifing over loci, was equal to -0.079 ± 0,050 for the protein markers. Carrying out a bootstrap analysis over loci, the true value of the F_{IS}-statistic, with a 95% confidence interval, would range from between -0.120 to 0.049. For microsatellite loci, with the same confidence interval, these values would oscillate between -0.304 and 0.097.

These results indicate that, in principle, a significant degree of inbreeding in this population does not exist. Logically, in order to be able to give more reliable inbreeding values, more loci should be analysed. However, the results obtained with this finite and reduced number of markers allow us to postulate the following hypothesis: inbreeding, in case it existed, would be negligible. It is well known that inbreeding affects all or a large majority of loci in a similar way, in the sense of a deficit of heterozygotes, and in the present work, no deficit for any of the seven polymorphic markers analysed has been detected. So, although the population of Catalonian donkeys is classified as a breed in danger of extinction (reduced population size), with the associated problems which this produces in the levels of consanguinity, these results would indicate that breeding policy in the last few decades has been very correct, in the sense of avoiding the mating among closely related individuals to the maximum extent possible, and maintaining, to the maximum degree possible, the genetic ancestral variability, as the relatively high values of heterozygosity indicate. These results agree with those obtained from genealogical data, in the same population, by Folch & Jordana (1998).

Tables 1 and 2 show the theoretical exclusion probabilities (PE) for each polymorphic locus, ranging from between 0.020 for the PGD system to 0.464 for the TF locus, with the cumulative PE for all loci being 0.829. This 82.9% of PE is thought to still be very low to be used as an effective tool in parentage verification. This control is very important for this endangered breed, the optimal mating being, pursued by the

Programme of Conservation's technicians, between a stallion and a jenny, which minimizes the inbreeding coefficient and maximizes the genetic ancestral variability retention of the hypothetical offspring of the couple (Folch & Jordana 1998).

Acknowledgements

The authors wish to thank the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya, which financed this study; likewise, the AFRAC association for their helpful co-operation and assistence during sample collection. Also, we would like to thank Chuck Simmons for the English revision.

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Legends to Tables

Table 1. Allele frequencies, heterozygosity per locus and mean values (H_o=observed; H_e=expected), and exclusion probability (PE) per analysed biochemical polymorphic locus.

Table 2. Allele frequencies, heterozygosity per locus and mean values, polymorphic information content (PIC) and exclusion probability (PE) per analysed microsatellite polymorphic locus.

Table 3. F_{IS}-statistic analysis. Within-population inbreeding estimate (f) in the Catalonian donkey breed for both sets of markers (biochemical polymorphisms and microsatellite loci).

Locus	Alleles	Frequency	H_o	$^*H_e$	PE
TF	Ad	0.397			
	Bd	0.217			
	Cd	0.114			
	Dd	0.272			
			0.783	0.712	0.464
PGD	Cd	0.021			
	F	0.979			
	S	0.000			
			0.042	0.041	0.020
GC	F	0.186			
	S	0.814			
			0.287	0.305	0.128
PI	M	0.877			
	T	0.126			
			0.253	0.222	0.097
Mean heterozygosity			0.341 (0.157)	0.320 (0.142)	
Cumulative exclusion probability					0.586

* Unbiased estimate (Nei 1978)
Standard errors in parentheses
d = donkey specific variant

Locus	Alleles	Frequency	H_o	$^*H_e$	PIC	PE
HTG6	A	0.092				
	B	0.092				
	C	0.342				
	D	0.474				
			0.643	0.644	0.577	0.376
MPZ002	A	0.520				
	B	0.480				
			0.653	0.502	0.374	0.187
VHL20	A	0.429				
	B	0.571				
			0.490	0.492	0.370	0.185
Mean heterozygosity			0.595 (0.053	0.546 (0.049)		
Cumulative exclusion probability						0.587

* Unbiased estimate (Nei 1978)
Standard errors in parentheses

Protein Markers		Microsatellite Loci	
Locus	$F_{IS} \equiv f$	Locus	$F_{IS} \equiv f$
TF	- 0.099	HTG6	0.002
PGD	- 0.016	MPZ002	- 0.304
GC	0.057	VHL20	0.005
PI	- 0.139		
Mean estimate	- 0.079 (0.050)+	Mean estimate	- 0.086 (0.097)

+ Mean estimate from jackknife over loci. Standard deviations in parentheses.

GENETIC DIVERSITY, INBREEDING ESTIMATE AND PARENTAGE VERIFICATION IN THE CATALONIAN DONKEY BREED, BY USING MICROSATELLITE MARKERS.

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Short running title: Genetic structure of the Catalonian donkey breed

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SUMMARY

A total of 107 individuals of the Catalonian donkey breed were analysed by using a commercial equine paternity PCR typing kit. Eleven of twelve microsatellites were amplified for donkey's DNA. Only one locus, ASB2, did not amplified in any sample. The mean number of alleles detected per locus was 11.9 (±1.0). All of the analysed loci showed disagreement with Hardy-Weinberg proportions. The within-population inbreeding estimate was highly significant ($P<0.01$) and equal to 30.1% (as measured by F_{IS} -statistic).

The cumulative exclusion probability was 0.999, being an effective tool in parentage verification in donkeys.

INTRODUCTION

The Catalonian donkey breed is a population in danger of extinction which is located in several Pyrenean and pre-Pyrenean regions of the Catalonian area of Northeast Spain. Following the guidelines proposed by the FAO, this breed has been characterised in order to carry out the "Programme of Conservation and Maintenance of Animal Genetic Resources" in this population. (Jordana and Folch, 1998a)

In a previous study, this breed was genetically characterised (Jordana and Folch, 1998b) by using 7 biochemical polymorphisms (protein markers) and the analysis of 12 Short Tandem Repeat loci (STRs or microsatellites) by resolution through a polyacrilamide gel and by ethidium bromide staining.

However, the new StockMarks for Horses Equine Paternity PCR Typing Kit (PE Applied Biosystems) combines the advantages of PCR-based tests and the informativeness of microsatellites to provide an automated approach to genotyping individuals for parentage verification. It is based on 12 microsatellite loci which primers are fluorescent dye-labeled. This procedure provides an obvious alternative to microsatellite analysis being a more rapid and reliable way of genotyping DNA.

The aim of this paper is to describe higher efficacy than obtained before by using an alternative system to identify incorrect parentage in order to carry out the Programme of Conservation in this breed.

MATERIAL AND METHODS

This study was carried out with 107 individuals of both sexes (76 females and 31 males). Donkey DNA was prepared from whole blood according to standard methods involving lysates of washed red cells and phenol-cloroform-isoalmiloalcohol (25:24:1) extraction.

We used the polymerase chain reaction to amplifiy Donkey DNA by using StockMarcks for Horses Kit Reagents (Perkin Elmer) from 12 previously reported loci: ASB2 (accession number: X93516); AHT4 (accession number: Y07733); AHT5 (accession number: Y07732); HMS2, HMS3, HMS6, HMS7 (Guerin et al. 1994); HTG4, HTG6 (Ellegren et al. 1992); HTG7, HTG10 (Marklund et al. 1994) and VHL20 (van Haeringen et al. 1994). PCR primers designated to amplify the loci described were isolated from *E. caballus*.

All amplification reactions were performed in two multiplex PCR (eight-plex and four-plex), both in a final volume of 20 ml buffer containing 2.5 µl StockMarks PCR buffer (containing either 100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, pH 8.3), 4 µl dNTPs (1.25 mM each nucleotide), 0.5 µl each primer (dye-labeled forward primer with its corresponding unlabeled reverse primer), 0.5 µl AmpliTaq Gold (5 U/µl) and 20 ng of donkey's DNA.

Eight-plex reaction combined the following primer pairs: AHT4, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, VHL20; and the four-plex reaction: AHT5, ASB2, HMS2, HTG10.

Amplifications were carried out in a GeneAmp PCR System 2400 (Perkin Elmer) by performing 95°C, 10min at the begining and 30 cycles of 95°C, 30 sec; annealing temperature 60°C, 30 sec; 72°C, 60 sec, and a final extension cycle of 60 min.

After PCR, aliquots of the multiplex products were combined to run in a single lane for each animal tested. PCR products were detected by using the Applied Biosystems 310 DNA Sequencer with GENESCAN Analysis software (ABI), using ROX internal size as a size standard into another lane of the sequencer allowing reliable computer-aided analysis for initial fragments sizing, as well as for genotyping.

Statistical analyses

Alleles frequencies and mean heterozygosity values per polymofphic locus were obtained using the BIOSYS-1 computer programme (Swofford & Selander, 1989). Tests of genotype frequencies for deviations from Hardy-Weinberg Equilibrium were carried out using the exacts tests of the GENEPOP computer programme (Raymond & Rousset, 1995), using the Markov chain method (Guo and Thompson, 1992)

Using the methods of Weir & Cockerham (1984), as implemented in the FSTAT computer programme (Goudet, 1995), the f-statistic value for each locus was calculated, the only one of the F-statistics that can be estimated from frequency data for a single population, which measures the correlation between pairs of genes within individuals, within populations, and is analogous to Wright's (1965, 1978) F_{IS} -statistic; that is to say, it measures the deficit or the excess of heterozygotes which could exist in the Catalonian donkey breed, and their significance was determined from permutation tests with the sequencial Bonferroni procedure (Hochberg, 1988) applied over loci (alleles were permuted within the population) in deriving significance levels.

Polymorphic information content (PIC), for each microsatellite loci, was calculated according to Botstein *et al.* (1980), and, for all systems, probability of exclusion (PE) was determined according to Jamiesons's formula (1994).

RESULTS

Table 1 shows the amplification characteristics, allele numbers detected, sizes (bp) in each loci, as well as heterozygosity, PIC and PE values for the 11 loci analysed. The ASB2 locus did not amplify for any individual. Figure 1 shows a typical pattern for GeneScan results from a Catalonian donkey DNA sample, showing the eleven loci. Table 2 shows the allele frequencies obtained for each loci. The average allele number detected par locus was 11.9 (±1.0), ranging between 6 for HMS7 and 17 for AHT5.

All loci, except HMS2 and HMS7 ($P<0.05$), showed a highly significant disagreement ($P<0.001$) with Hardy Weinberg (HW) proportions, showing a very significant deficit of heterozygotes -exact HW test, using the Markov chain method- (Guo and Thompson, 1992).

The unbiased average heterozygosity expected (H_e; Nei, 1978) was 0.719 (±0.028), being the extreme values, 0.567 (HMS7) and 0.894 (AHT5). Microsatellite which PIC values were higher than 0.5 were considered highly informatives (Botstein et al., 1980), so with the exception of HMS7 (PIC=0.43), we can consider that the overall loci analysed in this population were very informatives.

Moreover, the average exclusion probability (PE) of the 11 markers was 0.50, ranging from 0.30 to 0.78, being the cumulative exclusion probability of 0.999.

Table 3 shows the within-population inbreeding estimate ($f \cong F_{IS}$). The whole loci showed significantly different values from zero, as well as the estimated average ($P<0.01$). This F_{IS} (f) average, obtained from a jackknifing over loci, was equal to 0.301 (±0.064). When a bootstrap

over loci analysis was done, the true F_{IS} -statistic value, with a 95% confidence interval, would be included between 0.187 and 0.421.

DISCUSSION

It does not exist any other work, until now, that have used microsatellite markers to characterise asinine populations. Only Breen et al. (1994), and with a total of eight individuals, verified that a set of thirteen microsatellites, isolated from the domestic horse, amplified satisfactorily in the donkeys.

In a previous work, Jordana and Folch (1998b), characterised genetically the Catalonian Donkey Breed, analysing 7 biochemical polymorphisms and 12 microsatellite loci, even though these loci amplyfied products were resolved through a PAGE by ethidium bromide staining.

Primers fluorescent dye-labeled and the resolution of the amplyfied products in an Applied Biosystems 310 DNA Sequencer with GENESCAN Analysis software (ABI), were essencial to detect this big genetic variability in the Catalonian donkey breed in comparison to the work done by Jordana and Folch (1998b).

In the above-mentioned paper, only 4 out of 7 proteic markers, and 3 out of 12 microsatellite loci analised were polymorphic. Outlining the STR loci, (5 of them were in common in both works: HMS3, HMS6, HMS7, HTG6, VHL20). Only say that the average number of alleles detected par locus went from 2.7 (±0.7) to 11.9 (±0.1), and polymorphism in all of them was detected. The expected heterozygosity average (H_e) increased from 0.546 (±0.049) to 0.719 (±0.028), having improved the PIC values as well as the cumulative exclusion probability (PE) values spectacularly. Combining the 4 proteic markers and the 3 polymorphic microsatellites, the global PE value was only 82.9%, having now increased until 99,97%. This loci set is then, an effective tool in parentage verification.

Likewise, to reduce the routinary analysis cost of the parentage verification, it would be possible to ignore some markers and to analyse sistematicaly only eight of them, and achieve a PE value of 99.8%. If there is a doubtful paternity case, the remainder markers would be analised. We decided to exclude the HTG4 (FAM), HTG6 (JOE) and VHL20 (FAM) loci because of practical reasons in the sample reading. The most frequent alleles of these loci ranged in a similar size interval (bp) in this population and it was often possible, that the signal from these alleles appeared overlapped. It was due to the fluorescent reflection of the dye-labeled amplified products, and did more difficult the interpretation of the genotypes.

With reference to the within-population inbreeding estimate ($f \cong F_{IS}$), there also were substantial changes. Jordana and Folch (1998b) obtained an average F_{IS} value of -0.086 (±0.097) by using microsatellites, and a of -0.079 (±0.050) value by using protein markers. For each locus, no value was significantly different from zero. The mean f estimates were not significant either, and the authors postulated the hypotesis that, inbreeding, in case it existed, would be negligible. Folch and Jordana (1998), from the genealogical Catalonian donkey breed data (1979-1996 period), obtained an inbreeding of 6 % for the actual breed population. Nevertheless, they affirmed that this value would be overestimated, and it would be actually bigger due to the scarce pedigree knowledge. The pedigree thoroughness was found to be very incomplete up to the fifth generation of ancestors because the proportion of known ancestors was less than 20%.

A better inbreeding estimation was obtained in this paper. The mean estimate F_{IS} was 0.301±0.064 ($P<0.01$), showing, for all the loci, values significantly different from zero (Table 3). However, the main factor of the lack of heterozygotes (30 %) can be attributed to the inbreeding, because it is well known that inbreeding affects all or a large majority of loci in a similar way, in the sense of a deficit of heterozygotes, and in the present work, deficit for all markers analysed has been detected. We believe that such inbreeding value would be overestimated.

We can not forget that there are other factors that can involve a lack of heterozygotes in a population (Nei, 1987). First, the locus can be under selection, "genetic hitchhiking" effect, with some morphological of productive traits of selective interest. Second, a "null alleles" (non-amplifying allelels) may be present which are leading to a false observation of excess homozygotes. Third, the presence of population substructure may lead to Wahlund' s effect.

The selection influence could not be proved because production data were not available. Despite the fact that pedigrees were available for analysis, it was not possible to demonstrate the presence of null alleles, usually caused by a mutation in the primer binding site leading to an allele that will not amplify, due to the scarce pedigree knowledge which could have allowed us to examine the heredity of such alleles. Nevertheless, we presuppose a certain effect of these alleles in the observed deficit of heterozygotes, because some individuals that did not amplify for some loci were obtained. The extreme case was for the HTG4 locus, were a total of 30 samples did not amplify, while for the remaining 10 loci, an average of 3-4 samples failed to amplify. However, it was not feasible to specify any sample as homozygote for a non-amplifying allele. Provided that this possibility of null alleles was not measurable, the significant mean F_{IS} average estimated for this breed is likely to be overestimated.

The analysis of a large number of loci increase the power of detecting population substructure, because each locus will contain an independent history of the population depending on the amount of random drift, mutation, and migration that have ocurred.

Because of that, the population was divided into three subpopulations (SP1=48, SP2=47, and SP3=12 individuals) according to geographic distribution criteria, breeding policy and scarce genetic flow. F-statistics were computed by using the methods of Weir and Cockerham (1984), as implemented in the computer programme FSTAT, and the significance of F-statistics estimates was determined from permutations tests. For each locus F_{IS} (f), F_{IT} (F) and F_{ST} (θ) values were calculated from jackknife over populations (data not shown), being the mean estimates, from jackknife over loci, f = 0.280±0.066; P<0.01 (within population inbreeding estimate), F = 0.314±0.063; P<0.01 (total inbreeding estimate), and θ = 0.047±0.014; P<0.01 (measure of population differentiation). A very high significative θ value shows us that there is a certain population differentitation in this breed (4.7 %). In such statistic, only 4 loci (HMS2, HMS7, HTG4 and VHL20) were not statistically different from zero. This result corroborate the different breeding structure that exists in this population. This population substructure has provoqued fairly large differences in allelic frequencies among these subpopulations for some loci (data not shown), and has caused that in the entire population a net deficiency of heterozygotes and an excess of homozygotes even if HWE exist within each subpopulation (Nei, 1987). This effect, known as Wahlund's principle or the Wahlund effect, shows clearly that the HTG4 locus, which showed close agreement with HWE for the three subpopulations, had lack of heterozygotes of 13.4 % ($P < 0.01$) in the global population. Other loci can also be affected, but in a lower proportion, by this effect. In Table 3 can be observed that all the loci showed very significant disagreement with HWE, in the sense of a deficit of heterozygotes, for the entire population, while in SP1, AHT4, HMS3 and HTG10; in SP2, AHT4 and HMS7; and in SP3, AHT4, HMS2, HMS3, HMS6, HTG10 and VHL20, showed agreement with HWE.

To conclude, we can dare to say that the mean average inbreeding in the Catalonian donkey breed, is fairly higher than the values obtained by Jordana and Folch (1998b) and by Folch and Jordana (1998) in previous studies, although such a 30.1 % value of deficit of heterozygotes can not be atributed exclusively to the breeding between relatives, because of the above mentioned reasons.

Acknowledgements

The authors wish to thank the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya, which financed this study; likewise, the AFRAC association for their helpful co-operation and assistence during the sample collection. Also, we would like to thank Chuck Simmons for the English revision.

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Legends to Tables and Figures.

Table 1. Amplification characteristics, allele number detected, statistics of genetic variation and exclusion probabilities for the 11 analised loci.

Table 2. Alele sizes (in bp) and allele frequencies (freq) for 11 microsatellite loci in Catalonian Donkey Breed.

Table 3. Within-population inbreeding estimate ($f \cong F_{IS}$) in the Catalonian donkey breed.

Figure 1. Plot of GeneScan results from a donkey DNA.

Locus	Dye label	Product length (pb)	Alleles	Ho	He*	PIC	PE
AHT4	FAM	68-156	10	0.667	0.770	0.74	0.57
AHT5	JOE	92-150	17	0.381	0.894	0.88	0.78
HMS2	TAMRA	230-248	9	0.571	0.708	0.66	0.47
HMS3	TAMRA	146-170	12	0.676	0.745	0.71	0.53
HMS6	JOE	132-166	11	0.523	0.672	0.61	0.41
HMS7	FAM	156-176	6	0.481	0.567	0.43	0.30
HTG10	TAMRA	84-106	12	0.736	0.793	0.77	0.62
HTG4	FAM	76-106	11	0.325	0.658	0.53	0.41
HTG6	JOE	55-127	15	0.286	0.749	0.71	0.53
HTG7	TAMRA	118-158	16	0.547	0.764	0.74	0.53
VHL20	FAM	68-90	12	0.346	0.587	0.56	0.40
Mean heterozygosity				0.504 (0.046)	0.719 (0.028)		
Cumulative exclusion probability							0.9997

*Unbiased estimate (Nei, 1978)

Standard errors in parentheses

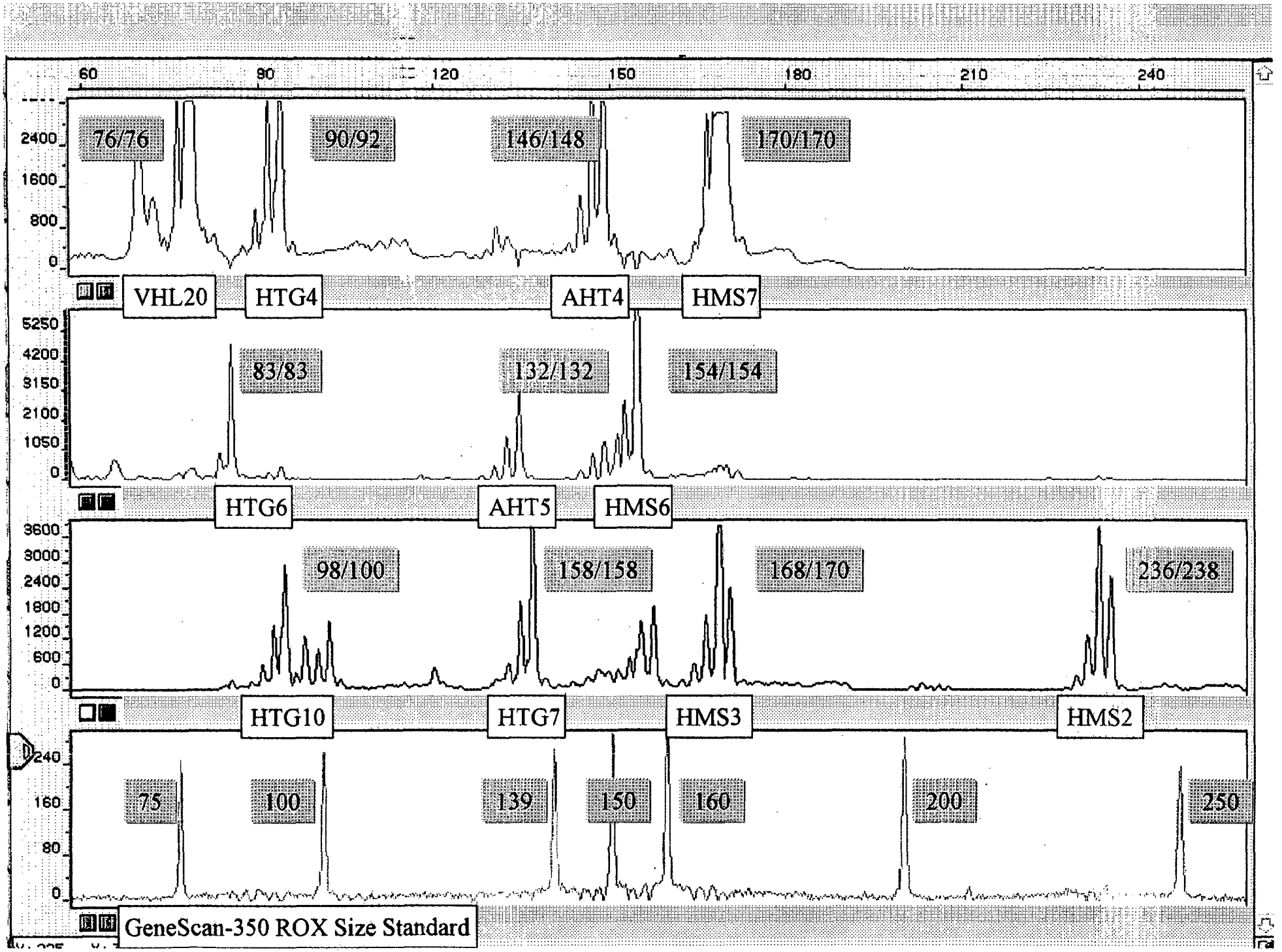
Locus	Size	Freq	Locus	Size	Freq	Locus	Size	Freq
AHT4	68	0.005	HMS3	164	0.020	HTG6	55	0.009
(N=105)	78	0.005	(N=105)	166	0.395	(N=105)	59	0.009
	128	0.019		168	0.309		67	0.009
	138	0.062		170	0.067		71	0.005
	146	0.038	HMS6	132	0.005		75	0.109
	148	0.391	(N=107)	142	0.005		77	0.382
	150	0.043		146	0.009		79	0.028
	152	0.128		148	0.023		81	0.071
	154	0.224		150	0.136		83	0.301
	156	0.085		152	0.005		85	0.019
AHT5	92	0.010		154	0.398		87	0.005
(N=97)	116	0.051		156	0.392		89	0.005
	118	0.005		158	0.009		91	0.033
	120	0.051		164	0.009		93	0.005
	124	0.010		166	0.009		127	0.010
	126	0.093	HMS7	156	0.005	HTG7	118	0.005
	128	0.082	(N=104)	166	0.067	(N=106)	120	0.052
	130	0.057		168	0.312		124	0.012
	132	0.217		170	0.577		134	0.449
	134	0.149		172	0.029		136	0.042
	136	0.005		176	0.010		138	0.014
	138	0.031	HTG10	84	0.009		140	0.005
	140	0.005	(N=106)	86	0.047		142	0.033
	144	0.046		88	0.019		144	0.075
	146	0.098		90	0.005		146	0.023
	148	0.062		92	0.165		148	0.159
	150	0.026		94	0.052		150	0.023
HMS2	230	0.033		96	0.038		152	0.038
(N=91)	232	0.011		98	0.052		154	0.023
	234	0.176		100	0.392		156	0.028
	236	0.435		102	0.118		158	0.019
	238	0.269		104	0.028	VHL20	68	0.051
	240	0.011		106	0.075	(N=107)	70	0.047
	242	0.011	HTG4	76	0.013		72	0.014
	244	0.049	(N=77)	86	0.013		74	0.112
	248	0.005		88	0.013		76	0.627
HMS3	144	0.009		90	0.331		78	0.061
(N=105)	148	0.028		92	0.481		80	0.042
	150	0.011		94	0.006		82	0.009
	152	0.028		106	0.026		84	0.018
	154	0.009		108	0.013		86	0.005
	156	0.024		110	0.039		88	0.005
	158	0.024		114	0.052		90	0.009
	162	0.076		116	0.013			

N is the total number of samples that have amplified successfully for each locus.

Locus	$F_{IS} \equiv f$
AHT4	0.134**
AHT5	0.575**
HMS2	0.194**
HMS3	0.093*
HMS6	0.222**
HMS7	0.152**
HTG10	0.073**
HTG4	0.508**
HTG6	0.619**
HTG7	0.285**
VHL20	0.412**
Mean estimate +	0.301 (0.064)**

+ Mean estimate from jackknife over loci. Standard deviation in parentheses.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, from permutation tests in FSTAT programme.


60
90
120
150
180
210
240
2400
1600
800
0
76/76
90/92
146/148
170/170
VHL20
HTG4
AHT4
HMS7
5250
4200
3150
2100
1050
0
83/83
132/132
154/154
HTG6
AHT5
HMS6
3600
3000
2400
1800
1200
600
0
98/100
158/158
168/170
236/238
HTG10
HTG7
HMS3
HMS2
240
160
80
0
75
100
139
150
160
200
250
GeneScan-350 ROX Size Standard

Ruc de somera vella
i vedell de vaca jove.

Capítol IV Caracterització de l'estructuració genealògica i demogràfica

"Demographic characterisation, inbreeding and maintenance of genetic diversity in the endangered Catalonian donkey breed".
(Genetic Selection and Evolution. 1998. **30**, 197-203)

Genet. Sel. Evol. 30 (1998) 197–203

Note

Demographic characterization, inbreeding and maintenance of genetic diversity in the endangered Catalonian donkey breed

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(Received 20 November 1997 ; accepted 1 March 1998)

Abstract – This study characterises the demographic and genealogical structure of a limited-size population in danger of extinction: the Catalonian donkey breed. The purpose of this paper is to establish the most suitable breeding criteria and guidelines to achieve the 'Programme of Conservation and Maintenance of Animal Genetic Resources' in this population. The two main objectives proposed are: 1) to maintain the maximum genetic diversity, with the 2) minimum possible consanguinity increase per generation. A population size of 109 animals of both sexes, 44 males and 65 females, was analysed. The pedigree information was used to calculate the following items: generation length (L), variances of family size (σ_k^2), effective population size (N_e), inbreeding coefficient (F) and probability of gene origin. The results obtained and breeding criteria to be followed are discussed. The correct mating policy betweeen a stallion and a jenny would be that which would maximize the so-called genetic conservation index and minimize the inbreeding coefficient of the hypothetical offspring of the couple. © Inra/Elsevier, Paris

donkey / endangered breed / pedigree analysis / demographic structure / genetic diversity maintenance

Résumé – Caractérisation démographique, consanguinité et entretien de la variabilité génétique de la race asine Catalane. Le présent travail caractérise la structure démographique et généalogique d'une population en voie d'extinction : la race asine Catalane. La finalité de ce travail a été d'établir les critères et normes de reproduction les plus appropriés pour développer le « programme de conservation et entretien des ressources génétiques animales » pour cette population. Les deux principaux objectifs proposés sont : 1) l'entretien d'une quantité maximale de diversité génétique, avec 2) le minimum d'augmentation possible de consanguinité par génération. La taille de la population analysée a été de 109 animaux des deux sexes, 44 mâles et 65 femelles. L'information qui

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provient des généalogies a été utilisée pour calculer les paramètres suivants : intervalles entres générations (L), variance pour la taille de la famille (σ_k^2), taille effective de la population (N_e), coefficient de consanguinité (F) et probabilité de l'origine des gènes. Les résultats obtenus, ainsi que les normes reproductives seront analysés, considérant qu'un accouplement optimal entre un étalon et une ânesse sera celui qui maximise le dénommé Indice de Conservation Génétique et celui qui minimise la consanguinité d'un descendant hypothétique du couple en question. © Inra/Elsevier, Paris

âne / race en voie d'extinction / analyse de généalogies / structure démographique / entretien de la variabilité génétique

1. INTRODUCTION

The Catalonian donkey is a local tame donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonian area of northeast Spain. This population is in danger of extinction. The last census carried out only slightly surpassed 100 animals, a third of which were males [10].

Following the guidelines proposed by the FAO, investigations were first carried out on morphological traits [4], on haematological and clinical biochemical parameters (Folch et al, 1997; Jordana et al, 1998) and on genetic loci [5, 6], to characterize this breed.

A small and homogeneous population, has important problems derived from inbreeding. Therefore, the two priorities accounted for the Catalonian donkey breed Conservation Programme are: 1) keeping the maximum genetic diversity, 2) obtaining the lowest possible inbreeding rate per generation. The purpose of this paper is to characterise demography and to analyse the pedigree structure of this population in danger of extinction, to set up the breeding criteria to fit to these objectives.

2. MATERIALS AND METHODS

The data file information corresponds to the period 1979–1996. The breed population analysed consisted of 109 individuals, distributed between 39 foals of both sexes (< 3 years old; 18 males and 21 females) and 70 adults: 26 males (aged 3–14) and 44 females (aged 3–18). The pedigree information was used to compute: generation length (L), variances of family size (σ_k^2), effective population size (N_e), inbreeding coefficient and probability of gene origin.

The $N_e$computation was obtained from the formula proposed by [9]

$$\frac{1}{N_e} = \frac{1}{16N_mL}\left[2 + \sigma^2_{mm} + 2\left(\frac{N_m}{N_f}\right)\mathrm{cov}_{(mm,mf)} + \left(\frac{N_m}{N_f}\right)^2\sigma^2_{mf}\right] + \frac{1}{16N_fL}\left[2 + \left(\frac{N_f}{N_m}\right)^2\sigma^2_{fm} + 2\left(\frac{N_f}{N_m}\right)\mathrm{cov}_{(fm,ff)} + \sigma^2_{ff}\right] \quad (1)$$

where N_m and N_f are, respectively, adults males and females with offspring and L as the average of the generation intervals calculated for the four pathways. Let

$cov_{(mm,mf)}$ be the covariance of the number of male and female progeny from each male parent and $cov_{(fm,ff)}$ from each female parent. The variances in family size are expressed as: σ^2_{mm}; σ^2_{mf}; σ^2_{fm}; σ^2_{ff}.

The inbreeding coefficient (F) was calculated for every animal in the file, using a computer programme based on Quaas-Henderson's algorithm [8, 16]. The evolution of the average coefficient of inbreeding was observed over year of birth and summarized by the linear regression over years.

The probability of gene origin was calculated from the genetic contributions of the founders of the current population [2, 3, 13, 15]. A founder is defined as an ancestor with unknown parents in the file.

This effective number of founders (f_e) is equivalent to the so-called Genetic Conservation Index (GCI) proposed by Alderson [1], which shows the relative capacity of an individual to retain the ancestral genetic variability

$$f_e = 1/\sum_{k=1}^{f} q_k^2 \qquad (2)$$

where each founder k can be characterized by the expected contribution q_k to the gene pool considered; f_e can be calculated for an individual or a group of individuals (by definition, the sum of q_ks is equal to one). If the founder contributions are balanced, the effective number of founders is equal to f. Otherwise, f is lower [2, 3].

3. RESULTS AND DISCUSSION

3.1. Demographic parameters

When using the formula proposed by [9], an N_e value of 59.97 was obtained. If our objective is to try to maximize this N_e, i.e. to minimize the inbreeding increase per generation (ΔF), then we can see according to equation (1), that a series of factors exists that would allow us to increase this N_e.

3.1. Generation interval

The average generation interval between parents and offspring was 6.74 ± 1.66 years, the maternal interval (7.32 ± 2.95) being larger than the paternal one (6.16 ± 1.55), even though the differences between both were not statistically significant. The maternal interval was more variable than the paternal one because their variation coefficients (CV) were, respectively, 40.3 and 25.1 %. The generation interval according to the four gene transmission pathways were the following: $L_{ss} = 6.15 \pm 1.90$; $L_{sd} = 6.17 \pm 2.45$; $Ldd = 7.42 \pm 4.75$; $L_{ds} = 7.21 \pm 3.51$, with s = sires and d = dams.

The average age of the parents at birth of their first offspring was 4.23 ± 1.57 years among stallions and 5.37 ± 3.89 years among jennies. The average reproductive life was 2.85 ± 3.42 and 2.77 ± 3.37 years, respectively. Despite this, there was no significant differences between sex, which would indicate that the annual replacement rates are similar among the male and female subpopulations.

The generation lengths are determined by age at first mating and by reproductive life. These parameters and the total offspring that parents leave to the next generation are directly affected by the breeding policy. On the other hand, the effective population size would increase if these generation intervals would rise [9].

Generation intervals which have been described in horse breeds by other authors, since there are not any references in the donkey species, show a L value much greater than ours. For example, both Moureaux et al. (1996), analysing French race and riding horses and Klemetsdal [12], analysing Norwegian trotters, obtained an average L of 10–12 years. According to Moureaux et al. in their study, the reason for their intervals being higher than ours, is due to the fact that the animals analysed were to be exploited for sport. This means that the sporting career preceded the breeding life, whereas the donkeys analysed here are only used for breeding. It could possibly be the reason why no significant differences have been found between the generation lengths of males and females in our breed.

3.1.2. Family size variances

Progeny size was 4.0 ± 4.82 per stallion and 2.28 ± 1.34 per jenny. The family size distribution for males is more unbalanced than in females because there are a few stallions with more than ten offspring, approximately three times as many as the average number of offspring per male. In contrast, few jennies had more than four foals, and then, females show a greater breeding homogeneity than males. The family size variances calculated along the four pathways for gametes confirm the previous remarks: $\sigma^2_{mm} = 7.66$; $\sigma^2_{mf} = 5.83$; $\sigma^2_{fm} = 0.90$; $\sigma^2_{ff} = 0.90$ offspring.

3.1.3. Male/female proportion (sex ratio)

When analysing this population census and its breeding structure, two types of problems can be observed. The adult female percentage (62.85 %) nearly doubles the male proportion (37.14 %), 44 females versus 26 males. On the other hand, the number of adult males and females which are breeding is less than half the number of adult individuals which could potentially be used as reproducers ($N_f = 21$ and $N_m = 12$).

In short, we would recommend increasing the N_e population, to try to equalize the sex ratio ($n_m \approx N_f$) and to use the maximum number of adult animals which are now present in our population.

To conclude, and directing these results to the Conservation Programme, the main objective would be to maximize the effective population size (N_e) to guarantee that increments of consanguinity per generation would be minimal: 1) equalise the sex ratio ($N_m \approx N_f$) avoiding the family size fluctuations and that ideally, each male would contribute with a male offspring and each female with a female offspring to the next generation, 2) standardise the family size minimizing the variance (σ^2_k), and 3) increase the generation intervals, that is, lengthen the reproductive life of the animals.

3.2. Inbreeding and pedigree completeness

The annual average inbreeding (ΔF), calculated by linear regression over years, was 0.38 % (*figure 1*). The accumulated consanguinity average (F) was 5.9 %. The expected theoretical inbreeding increase was calculated from $\Delta F = 1/2N_e$ which gave a result of 0.83 % in the studied population.

The pedigree completeness degree [14] was measured for each animal in the study by calculating the proportion of ancestors known in each preceding generation (*figure 2*). The pedigree thoroughness was found to be very incomplete up to the fifth generation of ancestors because the proportion of known ancestors was less than 20 %. That is why this situation should be taken into account when analysing the inbreeding coefficients.

3.3. Probability of gene origin

The effective number of founders (f_e), according to the formula (2), was 51.31 ancestors, the total number of founders (f_t) was 85. However, we considered that the total number of founders was very high basically due to the poor quality of pedigree information. According to Boichard et al. [3], these individuals do not represent the genetic variability which exists in the current population. In the first place, because these animals have possibly been considered as founders and, as a result, genetically independent, their relationship is virtually zero, but perhaps this is not true. In the second place, because the contributions of the present population are very unbalanced owing to the fact that some founders have contributed very little or even not at all.

Analysing the relationship f_e/f_t a result of 60.36 % was obtained, that is, for every ancestor contributing effectively to the genetic pool in the population under

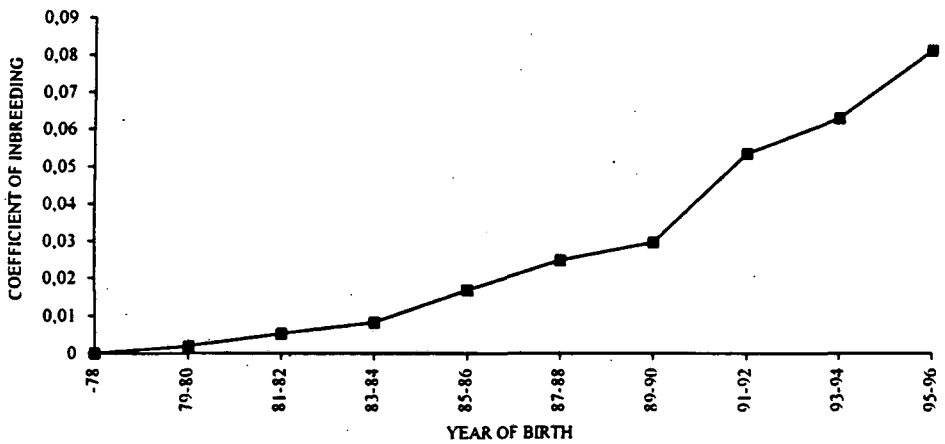

Figure 1. Average biannual coefficient of inbreeding in offspring born between 1979 and 1996.

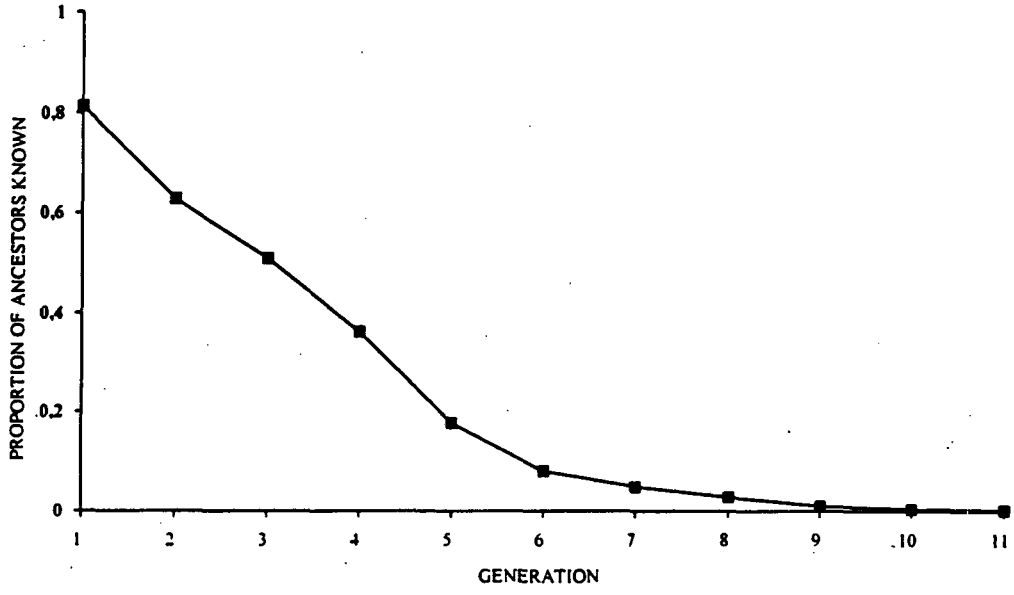

Figure 2. Average proportion of ancestors known by generation for the Catalonian donkey (1979–1996).

study there is another for whom information has been lost. This relationship has been described in French horse populations (Moureaux et al., 1996). It was noticed that they have ranged from 2.78 % to 14.03 %, where some populations, such as the Trotteur Français, were particularly very unbalanced (only 1 % of founders accounted for half the current gene pool). Probably these imbalances are due to the fact that the selection intensity in these populations is very high and that they use only the best stallions for matings.

To maintain and to conserve a small population, a correct mating system is very important. On the whole, an ideal breeding policy would be that which would calculate the effective number of founders and the inbreeding coefficient of the hypothetical offspring of each possible populating couple. In this way, those couples which would maximize this effective founder number and minimise the offspring inbreeding coefficient would be chosen.

4. CONCLUSION

We must ensure the equal contribution of the maximum number of animals possible (from both sexes), with as much time as possible, leaving offspring for the next generation. A minimum number of inbreeding matings should be allowed. A maximum number of founder animals (ideally all of them) would then be represented in the next generation.

ACKNOWLEDGEMENTS

The authors wish to thank the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya, which financed this study; likewise the AFRAC association for their helpful contribution and for providing the data used in the analysis. Also, the authors are grateful to E. Bedmar for providing a computer programme for inbreeding and probability gene-origin calculations as well as his computation assistance throughout this paper. Also, we would like to thank Chuck Simmons for the English revision.

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Capítol V
Viabilitat de la raça Asinina Catalana simulada per ordinador.

"A simulation study of the endangered Catalonian donkey breed by using VORTEX"

(Biological Conservation, en revisió)

A SIMULATION STUDY OF THE ENDANGERED CATALONIAN DONKEY BREED BY USING VORTEX

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Short running title: Population Viability Analysis of the Catalonian donkey

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ABSTRACT

VORTEX, a computer programme for Population Viability Analysis (PVA), was used in this study to simulate the impacts of different changes in Catalonian donkey management and to have a means of estimating extinction probabilities for this population as a management tool to carry out the "Programme of Conservation and Maintenance of Animal Genetic Resources" in this population. The main objective proposed in this work is to simulate the events which would occur over time in this population, by assuming the same reproductive and breeding policy which has been practised lately by the breeders is used. Finally, taking the results obtained in these simulations into account, another aim is to advise the breeders giving a series of reproductive and management guidelines which would delay this extinction time-frame.

A population size of 109 animals of both sexes, 44 males and 65 females, was analysed. Twenty-one scenarios were simulated where three types of factors were analysed, first separately and finally mixed. These three factors were: to increase carrying capacity, to improve reproductive management, to raise migration rates.

The results of the analysis showed that the Catalonian donkey's survival possibilities became better when the carrying capacity increased to K=4N and when the percentage of adult females and males in the breeding pool raised to 80%.

Keywords: Donkey / Endangered breed / Population Viability Analysis / VORTEX / Conservation

INTRODUCTION

The Catalonian donkey breed is a population in danger of extinction. The last census carried out only slightly surpassed one hundred animals (109 individuals), a third of which were males (Jordana and Folch, 1996). This census fit the population into the category of the Critical Breed (< 100 females) proposed by the FAO Expert Consultation (Anonymous, 1992), which implies that without any kind of action the most probable breed destiny will be its extinction in the near future

because this effective population is not very suitable for preventing continuous genetic losses in future generations (Bodó, 1992).

In 1978, due to the severe situation which this breed experienced, the breeders association, called AFRAC (Association of Fomentation of the Catalonian donkey breed, in English), was created to protect, foment and improve this population. In 1995, it became necessary to carry out a "Programme of Conservation and Maintenance of Animal Genetic Resources" in the Catalonian donkey breed, which was promoted and financed by the DARP (Department of Agriculture, Livestock and Fishing, in English) of the Autonomous Government of Catalonia (Generalitat de Catalunya), in collaboration with AFRAC and the Animal Genetics Unit of the Veterinary School of The Autonomous University of Barcelona.

The Catalonian donkey breed is a tame local donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonian area of Northeast Spain, characterised by a hypermetrical format, longilinear appearance and concaviline cranial profile. They are animals of a large size, 140 cm at the withers, on average, with a weight ranging from between 350 Kg to 450 Kg. The coat is a black colour with characteristic fadings in the muzzle, orbital zone of the eyes, belly and internal face of extremities (Jordana and Folch, 1996).

Following the rules of action as laid out by the FAO for the conservation of populations in danger of extinction, this breed was characterised, in the first place, biometrically (Folch and Jordana, 1997a) as well as haematologically (Folch *et al.*, 1997) by clinical biochemical parameters (Jordana *et al.*, 1998), genetically (Folch *et al.*, 1996; Folch and Jordana, 1997b) and demographically (Folch and Jordana, 1998). In this last paper, Folch and Jordana (1998) analysed the demographic and genealogical structures of this population, whose results have been used as the basis to realise the simulation study which is shown here.

The main objective proposed in this work is to simulate the events which would occur over time in this population, assuming the same reproductive and breeding policy which has been practised lately by AFRAC. Likewise, another aim is to test whether this endangered population's

destination would be extinction, also giving approximate extinction times. Finally, taking the results obtained in these simulations into account, to advise the breeders, giving a series of reproductive and management guidelines which would delay this extinction time, is another goal.

MATERIAL AND METHODS

1. Selection of Computer Simulation Package for Analysis

Of the computer programmes available for metapopulation viability analysis, VORTEX (Version 8) was selected for this study because it was the latest version of a programme which simulates the loss of genetic variability in small populations (Lacy, 1993). According to den Boer (1990), a metapopulation can be defined as a group of local populations interconnected by individuals. VORTEX has been applied to studies of population viability analysis (PVA) in endangered species worldwide (Lacy and Clark, 1993; Lacy and Lindenmayer, 1995; Lindenmayer and Lacy, 1995; Lindenmayer *et al.*, 1995; Mills *et al.*, 1996). This programme considered the most important factors to be taken into account in a PVA, such as demographic forces, environmental variation and genetic drift. This programme is very useful for the study of small populations with a long reproductive life, low prolificacy and whose dynamics only depends upon themselves, that is, K-strategy species.

Population viability analysis programmes contribute most by their ability to make intuition explicit, that is, to incorporate a number of the factors which influence population growth, thereby suggesting potential consequences of a range of management options. Mills *et al.*, (1996) alert us that care should be taken so that any one programme as a mirror of reality is not too strict. Nevertheless, PVA can help identify population processes which are likely to endanger a population in the future if corrective management practices are not taken.

2. Time-Frame for Simulation Analysis

The genetic metrics tracked by VORTEX were simulated for 1000 years 100 simulations were completed for each scenario, and each simulation was reported every 25 years. This time-frame was chosen based upon the minimal viable population concept (Lacy, 1993), which holds that a population with an extinction probability (PE) < 1% in the next 1000 years can be considered steady and not in danger of extinction.

3. Types of Inputs for VORTEX

The inputs to VORTEX are shown in Table 1. The demographic parameters were obtained from Folch and Jordana (1998).

The population was subdivided into two subpopulations (SP1 and SP2), taking the different breeding policy and the scarce genetic flow which existed within them as a criteria. VORTEX analyses the viability of each subpopulation as well as metapopulation viability.

Subpopulation SP1 included 54 animals which were located in the same farm and where in each reproductive season it is easy to plan the crosses because all the individuals of the reproductive pool were in the same place. Subpopulation SP2 contained 55 animals which were dispersed among a large number of owners. In this situation the exchange of breeding animals was difficult, and the breeders did not detect when the females entered into heat. In the end this turned out to be an inadequate breeding management. Nevertheless, the concentration of all the SP1's animals in just one farm may have very grave consequences in the event of health problems (for ex. epidemic) or an environmental disaster (for ex. a forest fire), because this subpopulation has more probabilities of being affected on the whole by these events. SP2 had its individuals dispersed in small groups in remote farms, so similar events would only affect a few animals and not the whole population.

According to our field study, the breeding efficiency (percentage of adult males in breeding pool and percentage of adult females producing litters) was greater in SP1 than in SP2. VORTEX assumed that matings occur at random among those animals in the breeding pool each year and that

a male can mate with various females. In this species most of the females had single births, and it was considered that only 1% of the cases would have a successful double birth.

The genetic drift effect is the random factor which affect small populations the most, after the demographic shifts. VORTEX had three alternatives to consider such effects: absence of inbreeding depression, appearance of deleterious alleles or appearance of detrimental alleles. When the appearance of inbreeding depression was considered, the lethal equivalent percentage had to be taken into account. Because we do not have information on the genetics of E.Asinus, particularly the frequency of deleterious alleles, the recessive lethals model of inbreeding depression in VORTEX was used (Lacy, 1993). The inbreeding depression was simulated with a median of 3.14 lethal equivalents per individual, the median value for juvenile survival in 40 mammal species (Ralls *et al.*, 1988).

The migration variable was due to the interchange of animals carried out by the breeders. The percentage of animals migrating from SP1 to SP2 was 1% while the inverse flow was 1 per 1000 . It was considered that the minimum age at migration was 1 year because it was the age at which the foals could look after themselves and they were sold. Survival during migration was 50% because this was the mortality percentage according to this age interval. A mortality percentage of 5%±3% in the 0-1 year-old interval (both sexes) was found with the rest of age intervals taking on a 0% mortality percentage which was considered to be insignificant.

When the environmental stochasticity was considered by VORTEX for ex., climate or parasitic burden, it affected the birth and death probabilities. VORTEX also considered the catastrophe possibility or extreme environmental variation, for ex. epidemics and forest fires.

The forest fire probability (Catastrophe Type 1) for SP1 was 1 % while for SP2's was 5 %. These differences are explained because SP1 was more exposed to this kind of disaster than SP2 because the animals are assembled in a single farm situated in an area with a high danger of forest fire. It would be the same for the case of Catastrophe Type 2 (epidemic). The SP1 situation was more unfavorable than SP2 due to the easier pathway for infections. That is why the SP1 survival

possibilities were lower than the SP2's in the case of catastrophes. On the other hand, neither the possibility of harvest (hunt or individual removal) nor the existence of individual reintroduction from other populations was considered. The extinction event was defined as the survival of just one sex.

4. Types of Outputs

The following output data generated by VORTEX were used to describe the population behaviour of the Catalonian donkey breed:

PE = probability of extinction over 1000 years

TE =mean time to first extinction within 1000 years.

Growth = Mean population stochastic growth rate.

N = size of the extant population after 1000 years.

R = number of populations undergoing temporary extinction and recolonisation among the 1000 year simulation.

H = mean percent of initial heterozygosity remaining in extant populations at 1000 years.

Nalleles =Number of total alleles. Originally this number is equal to 2N.

These parameters were derived for SP1, SP2 and metapopulation, and are shown in Table 2.

5. Scenarios analysed

The scenarios analised in this paper were sustained by three primary factors which were considered in population viability analyses (See Figure 1). These factors are analysed separately, on the one hand, and on the other hand mixed together despite their being inextricably intertwined. First of all, the population size increase was analysed, measured as a carrying capacity; second, the reproductive structure improvement, and third, the migratory flow increment between both subpopulations.

RESULTS AND DISCUSSION

1.- Basic Scenario (1)

Table 1 data were taken as a first input (basic scenario) for VORTEX, to know which would be the Catalonian donkey breed's fate in present conditions. The following scenarios studied will be compared to and discussed from this scenario, and results obtained in each one are shown in Table 2.

SP1's TE was 56 years, 23 years for SP2 and 60 years for the metapopulation. If the R values obtained are analysed, it can be seen that SP2 suffered two recolonisations from SP1 which happened each 11 years since SP2's first extinction. It looked as if in basal conditions, SP1 had not only more survival possibilities but it was capable as well of supplying SP2 with individuals because of this small migration rate.

As can be seen in Table 1, the SP1 and SP2 censuses were very similar and, apart from the geographic distribution, it is obvious that the greatest difference between both subpopulations was their reproductive structure. The pre-reproductive proportion was 10 % lower in SP2 than in SP1. The percentage of females in reproductive age without any descendent was significantly greater in SP2 (74%) than in SP1 (35%) as well. On the other hand, the proportion of males in the reproductive pool was greater in SP1 (43.75%) than in SP2 (35.71%). This low reproductive efficiency of SP2 was basically due to management problems such as the lack of in-heat detection, animals' difficult accessibility, breeders disagreement for making the breeding animals exchange easier, non-existence of effective techniques in artificial reproduction which would settle the previous problems. A population census decrease and a loss in the metapopulation genetic variability would result, logically. VORTEX predictions would allow researchers to identify the most critical factors in population survival and to guarantee that if correct management measures are not taken, this population would become extinct in the short term (60 years).

2.- Increment of the Carrying Capacity (K).

From the Conservation Plan viewpoint, K is an important factor because the most important objective is to increase the population size as soon as possible in order to preserve the maximun quantity of population genetic variability. The effects of a small-sized population has an impact upon an inbreeding rate increase with the resulting heterozygosity and genetic diversity loss. As a result, the accumulation of detrimental genes would increase which would also increase the population's vulnerability.

Despite the Catalonian donkey breed being an important population to preserve because it makes up a cultural heritage we all share, it is clear that if there is not a market demand nor an economical reason to breed these animals, the population would only be able to keep itself up with the present reduced numbers or with a slight increase. Nevertheless, there would be economical alternatives (for ex. agrotourism, forest control, etc.), which would make a significant increase of the present population possible.

Basing ourselves upon this supposition, two scenarios were proposed with a carrying capacity of 2N and 4N. The rest of the parameters have been maintained as in the basic scenario.

2.1.- Scenario 8: K=2N.

When K=2N, VORTEX predicted a population growth during the first 25 years although the population never achieved such carrying capacity because it became extinct very soon (TE = 548 years and PE = 100%); however, this TE was greater than in the basic scenario due to the bigger SP1's persistence, because SP2 became extinct in 43 years. That would make SP1 able to transfer individuals to SP2 up to 327 times, and SP2 would become extinct every 6.3 years.

2.2.- Scenario 15: K=4N.

When K=4N, SP1's and metapopulation's PE significantly disminished (44%), but only slightly in SP2 (98%). SP1's final alleles only were 2.27 (from SP1's 110 initial alleles and the

218 of the metapopulation) after 1000 years. In this hypothetical scenario, the population would be made up of 79 males and 38 females. It was obvious that the SP1 contribution to the SP2 maintenance was significant (R = 1543).

Despite the positive effect of increasing the census in population's behaviour, it was possible to observe a significant alleles loss and a high PE which would put the population survival expectations at risk. That means that only the carrying-capacity increase would not be enough to protect this breed from extinction.

Just as was expected, the genetic drift was harmful in a small population. That way, Scenarios 8 and 15 proposed a population growth by increasing the carrying capacity. It could be checked that SP1, SP2 and metapopulation persistence and genetic diversity (Nalleles) increased when the census was higher. Although by only the increase of the carrying capacity it would not be enough to preserve this breed from extinction (Figure 2). The minimal population viability concept (Lacy, 1993) was never reached. A clear prospect of population increase would not exist as well, nor would the number of breeders be willing to invest resources in animals which at that moment, do not yield large profits. However, the population increase simulation was very educational even though there would be difficulties in puting it into practice in the short term.

3.- Improvement of Reproductive Management.

Unlike what happened in other studies which used VORTEX in which the individuals under study were wild populations and where their reproductive structure was difficult to manipulate, that did not occur in the population studied.

The following scenarios analysed population behaviour when reproductive management was improved, first of all in the female subpopulation, followed by the males' management improvement and, at the end, both effects combined

3.1.- Scenario 3: Adult females producing litters =65%.

In this scenario population behaviour was studied when the female percentage producing litters in SP1 and SP2 was 65% (which means that the SP2 reproductive pool was increased to the SP's levels). In this new supposition, SP2's TE was 344.58 years, being recolonised (R) by SP1 only twice. Despite any change being made in the SP1 structure, a clear improvement in this subpopulation was noticed because SP1's TE was cuadrupled (249.82 years). This result demonstrated the erosive effect of a population managed so badly as was the case of SP2. Metapopulation's TE rose six times over the basic scenario results (378 years). The effect of this factor was remarkable and if put into practice, there would be many management and technological alternatives which would be possible to control it. However, this increase of the female reproductive pool would not avoid the possibility of extinction.

Both the basic scenario and Scenario 3, had two recolonisations, but while in the basic scenario they occured every 11 years, in the present scenario the average recolonization time rose to 58 years. The reason for this effect was that SP2 took more time to become extinct, and its growth rate increased from -6 % to 2 %.

3.2.- Scenario 4: Adult Males in Breeding Pool=43.75%.

When a scenario where the proportion of reproducing males in SP2 increased was simulated, that is, the percentage of reproducing males in both subpopulations was 43.75%, it could be seen that the metapopulation's TE improved (261 years). Just like that, a growth rate increase in this scenario (1.25%) could be seen, when compared with the basic scenario (0.59%). This low growth rate (1.25%) could be explained as the polygamous nature of this species. That means that one male (or a few) could mate with as many females as would be available. In this scenario it would be expected that if the proportion of reproductive females did not increase, the effective population size would always be small and the vegetative population growth would be very slow.

3.3.- Scenario 5: Adult Males in Breeding Pool = 43.75% and Adult Females Producing Litters = 65%.

The combination of improving the reproductive management of males and females had the effect of a TE =370 years, which it was very similar to the TE obtained in Scenario 3 (378 years). Likewise, the growth rate was equal for both scenarios. This result confirmed that the limiting factor would be the proportion of females which make up the breeding animals pool. In Figure 3 (Scenarios 1\3\4\5) the behaviour of population persistence in such suppositions can be seen. It can be observed that the effect of improving only the breeding female pool, and the scenario where this effect was combined with the improved males management, produced similar effects (both curves have are overlapped in their trajectory).

These results would be very useful when decisions about the Conservation Plan of the Catalonian donkey breed are taken. Then, if it were believed that the increase in the population size was, at the moment, not too suitable, it would be suggested concentrating the efforts by optimising the reproductive efficiency in both subpopulations. In this way, a scenario where the reproduction of the female subpopulation would be optimum would be proposed.

3.4.- Scenario 7: Adult Females Producing Litters and Adult Males in Breeding Pool = 80%.

An scenario was also suggested where the proportion of breeding animals (both sexes, both subpopulations) were the maximum viable. In the *Equus asinus*, a fertility rate of 80% (England, 1996; J. Miró: personal communication) would be assumed. On the one hand, it would also be possible to get a level of 80% in male fecundity if techniques of in vitro fecundation were available. On the other hand, if reproduction efficiency improved until it reached these percentages, the PE would become lower (64%). It could be remembered that the PE was never less than 100% when other reproductive management improvements were made. The metapopulation growth rate went from 0.59% to 3.25. % in this scenario.

Although the population behaviour in this scenario was not as good as that obtained in Scenario 15 (K=4N), we believe that it would be economically more realistic if a small population was maintained, but with a careful reproduction management, instead of increasing the population size with the present deficient reproduction structure. It would be necessary to have artificial reproduction techniques at our disposal to achieve these objectives, due to the difficult access of some animals, especially in SP2 where it was often difficult to move the breeding animals.

4.- Increase of the migration rate to 5% (Scenario 2)

An increase in the migration rate can be interpreted in a domestic species as a greater interchange of breeding animals or as the use of artificial insemination and other in vitro fertilisation techniques which would allow a greater genetic flow between subpopulations. Migration is an important factor which would counteract the genetic loss effects produced by subdivision and populations genetic drift (Lindenmayer and Lacy, 1995). Nevertheless, it would reduce the efficiency in which selection rejects the lethal alleles of each generation.

If a migration rate of 5% between SP1 and SP2 was assumed, SP1's TE would be cuadrupled (from 56.08 to 221.08) and doubled in SP2 (23.62 to 45.63). The metapopulation slightly improved its average time at first extinction (TE = 221.7 years).

Migration reduced the effectiveness in which selection eliminated the lethal recessives alleles in subdivided populations. Animals are less able to survive in the scenarios with high migration rates.

Despite the fact that it would be believed that a greater migration rate would help to lower inbreeding because it would be more genetically diverse, what happened was that the selection intensity of recessive alleles was reduced (because fewer animal were homozygous for lethal alleles and presumably, more animals dying due to migration mortality).

Then, as a conclusion, the increase of migration rates between both subpopulations, despite the population prospects improving was not enough by itself. In this case, the greater SP2 fragility

was obvious, whose survival expectation was not enough, because the effect observed was an increase in the number or recolonizations from SP1 to SP2 (R=365).

5.- Scenarios with Effects Combined.

The factors considered previously had a positive effect upon population survival. The most efficient factor was the increase in the carrying capacity from K to 4K. The second most efficient factor was the improvement in the female pool reproductive management. And the least efficient factor was the increase in the migration rate between both subpopulations.

These results would not protect the population from a possible and early extinction, and it made us plan new scenarios with the combination of these three factors together to study population behaviour. But only the scenarios where the increase in the carrying capacity and the reproductive management improvement were mixed made the population persistence probabilities significnatly better. These scenarios are stated below.

5.1.- Combination of Increment of K and Reproductive Improvement.

The results obtained in Scenarios 14 and 21 were compared here with the results previously obtained in Scenarios 1 and 7. These scenarios analysed population behaviour when reproductive structure (80% of adult females and males in the breeding pool) was improved and the carrying capacity was increased to K=2N (Scenario 4) and K=4N (Scenario 21).

In this last scenario, the population achieved a PE = 0% after 1000 years of simulation. That means that *a priori* it seems that the Catalonian donkey breed would have a minimum survival guarantee in such conditions with a minimum genetic variability loss (He=71%). Often, conservation programs strive to keep He > 90% because many people would consider a loss of 29% of heterozigosity to be greater than an acceptable amount. However, in very long-term projections (such as the 1000 year simulation) VORTEX might underestimate He because the model does not incorporate any new variation introduced by mutations (R. Lacy, personal communication).

After all this time, the population which VORTEX has estimated would be composed of 329 individuals, that is, it did not reach the population size of 4N which would be of 436 individuals.

As Figure 4 shows (Scenarios 1\7\14\21), where the gene diversity of the extant population of such scenarios is analysed, it provided clear evidence of the increase in population survival posibilities as the population size grows. This growth was total when the population size was four times the original size. Despite Scenarios 7 and 14 seeming to have a similar genetic-diversity behaviour, the PE in Scenario 7 was only 64%, while the PE in Scenario 14 was only 7%.

The result obtained should not be taken as a mirror of what would happen with the *Catalonian donkey breed*, but what is obvious is that this population is in a great state of vulnerability, and the factors which most affect it are small population size and poor reproductive structure, especially the female fecundity rate. Both factors could be controlled in the short/medium-term in the Conservation Plan which would include in vitro fertilisation techniques development and a correct mating plan. As a conclusion, and with these results pointing towards the Conservation Plan, the prime objective would be to maximize the effective population size (Ne) in order to avoid an inbreeding growth rate per generation by increasing the population size (K=2N minimum) as soon as possible and by guaranteing the maximum number of animals (at reproductive age) which would contribute with descendents to the next generation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Rober Lacy for his helpful comments on this paper. We would also thank Dr. Jordi Miró for his comments in the reproduction subject. We are grateful to the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya, which financed this study; likewise AFRAC association for their helpful contribution and providing the data used in the analysis. Also we would like to thank Chuch Simmons for the English revision.

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Legends to Tables and Figures

Table 1. Summary of demographic rates used in simulations of Catalonian donkey breed.

Table 2. Results of population viability analysis using the VORTEX simulation programme over 1000 years for the Scenarios discussed in the Catalonian donkey breed. PE = probability of extinction (%); TE = mean time to extinction; Gr = Mean population stochastic growth rate (%); N = mean number of Catalonian donkey in nonextinct populations after 1000 years; R = number of populations undergoing temporary extinction and recolonization; H = mean percent of initial heterozygosity remaining in extant populations at 1000 years; Na = number of total alleles.

Figure 1. Scenarios tested for Catalonian donkey breed vulnerability. Circles indicate the Scenario number.

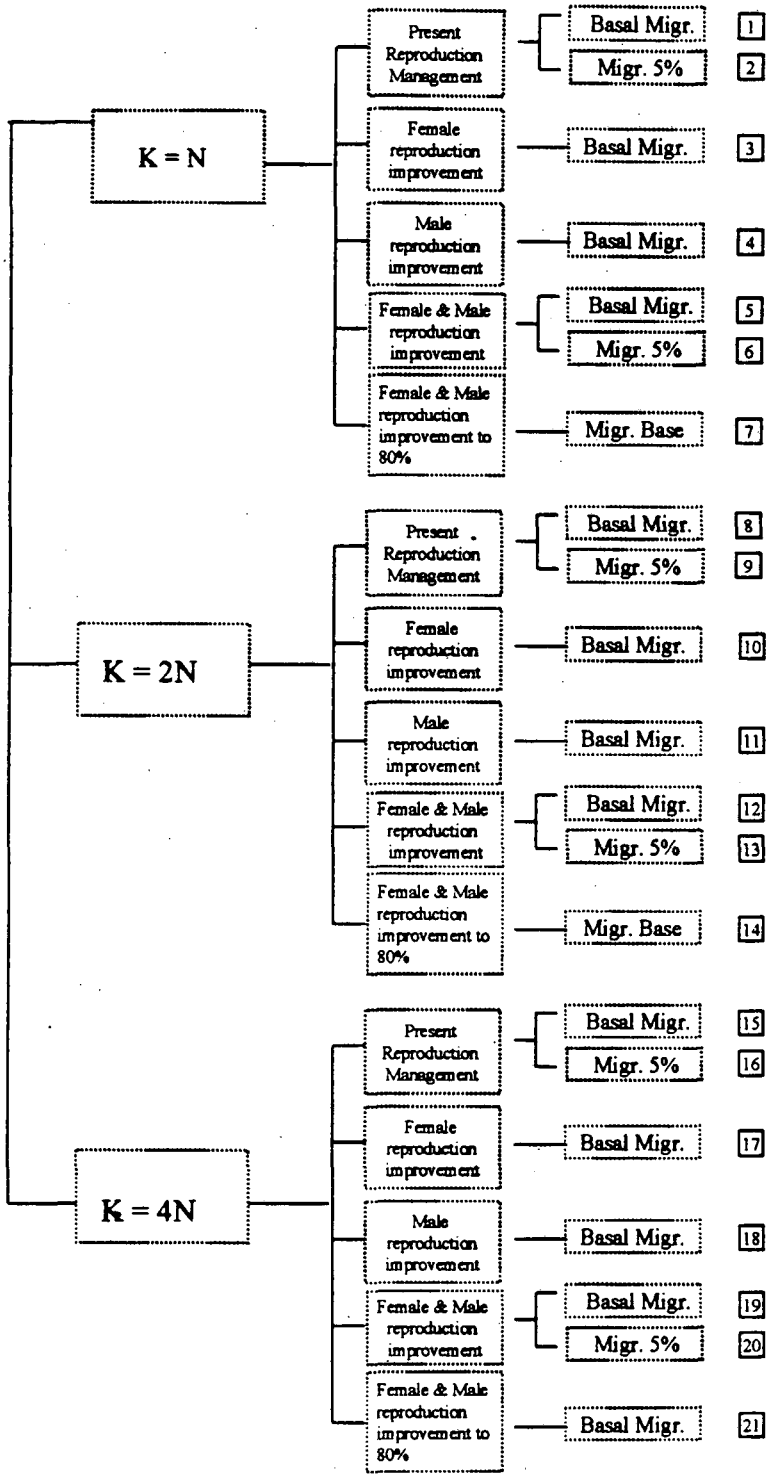
Figure 2. Population Persistence in the Catalonian donkey breed when Scenarios 1, 8 and 15 were simulated (K= N, 2N and 4N respectively).

Figure 3. Population Persistence in the Catalonian donkey breed when Scenarios 1, 3, 4, 5 were simulated (improvement in the reproductive management, females, males, both combined together).

Figure 4. Gene Diversity of the Extant Population in the Catalonian donkey breed when Scenarios 1, 7, 14 and 21 were simulated (growth in the carrying capacity plus improvement in the reproductive management in both sexes).

Parameters used as input to VORTEX to modeling		Catalonian Donkey Breed			
		Subpopulation 1 (SP1)		Subpopulation 2 (SP2)	
Inbreeding depression modeled with		3.14		3.14	
Lethal equivalents (%)		50		50	
Minimum age at migration (yr)		1		1	
Survival during migration (%)		50		50	
Migration matrix (%)					
	Population 1	0.990		0.010	
	Population 2	0.001		0.999	
Breeding system		polygamous		polygamous	
Adult males in breeding pool (%)		43.75		35.71	
Adult females producing litters (%)		65		26	
EV in reproduction (%) adult females breeding		5		5	
Females producing litters of size 1 (%)		99		99	
Breeding age of males (yr)		4		4	
Breeding age of females (yr)		5		5	
Age of senescence (death)		10		10	
Sex ratio at birth (% of males)		50		50	
Age distribution		males	females	males	females
	1	3	4	0	0
	2	0	5	0	3
	3	5	2	6	4
	4	4	2	0	1
	5	3	3	3	4
	6	0	2	5	0
	7	2	3	3	6
	8	3	1	1	5
	9	2	3	1	6
	10	2	6	1	5
	TOTAL	24	31	20	34
Ratio adult male vs adult female		1.221		1.145	
Annual mortality of males (%)					
	0-1 yr old	5±3		5±3	
	1-2 yr	0		0	
	2-3 yr	0		0	
	> 3 yr	0		0	
Annual mortality of females (%)					
	0-1 yr old	5±3		5±3	
	1-2 yr	0		0	
	2-3 yr	0		0	
	> 3 yr	0		0	
Frequency of type 1 catastrophe (Forest Fire)		1 %		5 %	
	severity on reproduction	0.75		0.95	
	severity on survival	0.95		0.95	
Frequency of type 2 catastrophe (Epidemy)		2 %		2 %	
	severity on reproduction	0.50		0.90	
	severity on survival	0.50		0.85	
Carrying capacity		54±22.0		55±21.6	
Deterministic population growth rate (r)		0.073		-0.044	
Generation Length for females		7.36		7.55	
Generation Length for males		6.81		7.07	

	Subpopulation 1							Subpopulation 2							Metapopulation					
Scenario	**PE**	**TE**	**Gr**	**N**	**R**	**H**	**Na**	**PE**	**TE**	**Gr**	**N**	**R**	**H**	**Na**	**PE**	**TE**	**Gr**	**N**	**H**	**Na**
1	100	56	2.67					100	23.6	-6.3		2			100	60.2	0.59			
8	100	548	2.16					100	43.3	-4		327			100	548	1.95			
15	44	819	2.67	78		0.29	2.27	98	50.1	-0.8	2	0.25	1543		44	819	2.56	79	0.29	2.27
4	100	261	1.79					100	38	-6.3		40			100	261	1.25			
3	100	249	1.85					100	344	2.08		2			100	378	1.72			
5	100	247	1.93					100	337	2.04		6			100	370	1.72			
7	1	436	3.44		2			64	598	3.59	26	3	6.95	1.17	64	672	3.25	26	6.95	1.17
2	100	221	1.48					100	45	-3.17		365			100	221	0.8			
14	52	721	4.17	57	1			7	646	5.59	75	1	0.41	2.77	7	720	5.01	105	0.37	2.94
21	4	505	6.05	162	2	0.53	3.93	0		7.36	173		0.69	5.79	0		6.79	329	0.61	6.37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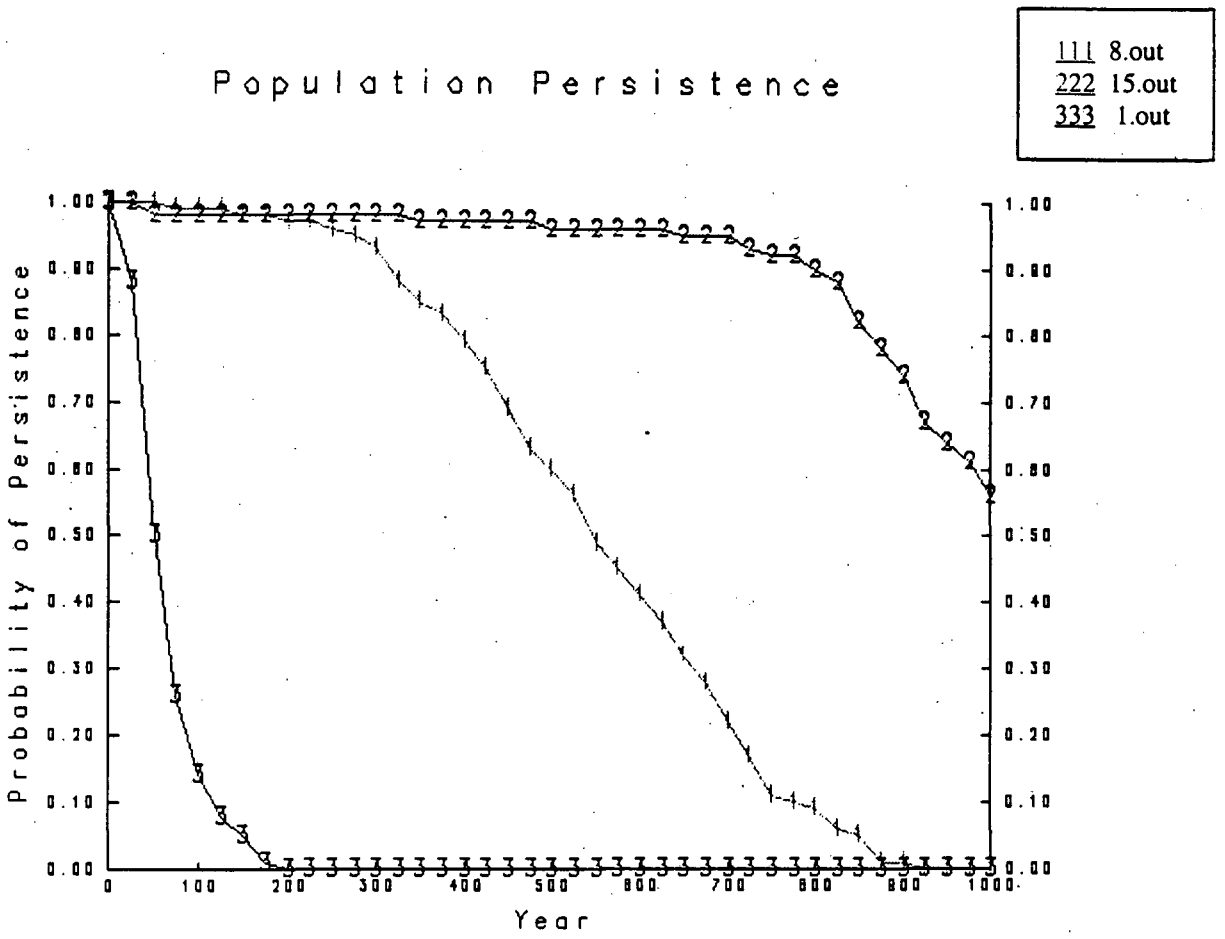
Present Reproduction Management
Basal Migr.
1
Migr. 5%
2
Female reproduction improvement
Basal Migr.
3
Male reproduction improvement
Basal Migr.
4
Female & Male reproduction improvement
Basal Migr.
5
Migr. 5%
6
Female & Male reproduction improvement to 80%
Migr. Base
7
K = 2N
Present Reproduction Management
Basal Migr.
8
Migr. 5%
9
Female reproduction improvement
Basal Migr.
10
Male reproduction improvement
Basal Migr.
11
Female & Male reproduction improvement
Basal Migr.
12
Migr. 5%
13
Female & Male reproduction improvement to 80%
Migr. Base
14
K = 4N
Present Reproduction Management
Basal Migr.
15
Migr. 5%
16
Female reproduction improvement
Basal Migr.
17
Male reproduction improvement
Basal Migr.
18
Female & Male reproduction improvement
Basal Migr.
19
Migr. 5%
20
Female & Male reproduction improvement to 80%
Basal Migr.
21


Population Persistence
111 8.out
222 15.out
333 1.out
Probability of Persistence
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
100
200
300
400
500
600
700
800
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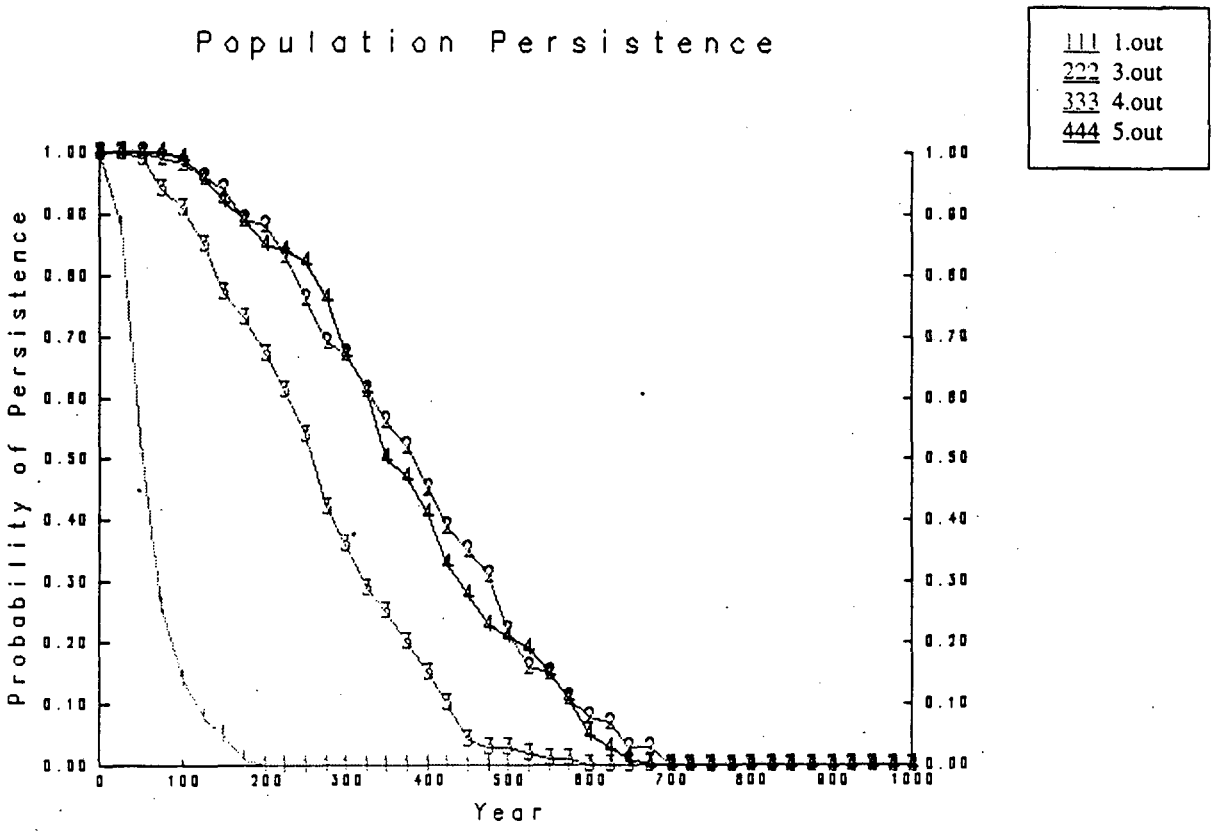
Year


Population Persistence
111 1.out
222 3.out
333 4.out
444 5.out
Probability of Persistence
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
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200
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800
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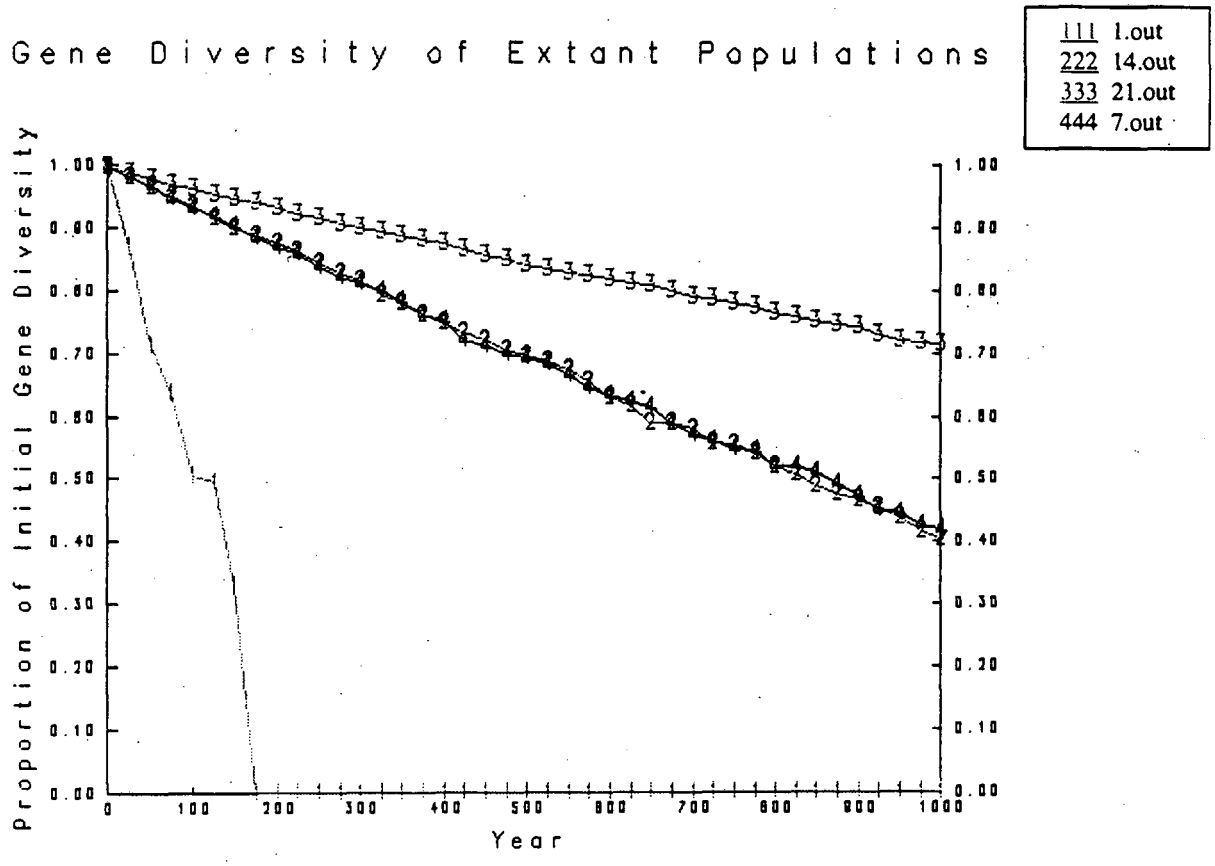
1000
Year


Gene Diversity of Extant Populations
111 1.out
222 14.out
333 21.out
444 7.out
Proportion of Initial Gene Diversity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
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La llet de burra a la nit,
al malalt estova el pit.

Discussió general

Com ja s'ha anat descrivint al llarg de la introducció, aquest treball pretèn arribar a conéixer prou bé a la Raça asinina Catalana per a poder aconsellar als criadors, de quines són les accions que han de prendre per establir un Pla de Conservació "*in situ*". En aquesta part de la discussió es fa un petit resum dels aspectes estudiats seguint els objectius específics enumerats a la introducció. Seguint aquests objectius, aquesta secció es divideix en un primer bloc on es detallen els aspectes de la descripció general de la raça asinina Catalana. En el segón bloc es tracta la caracterització d'aquesta raça, i en el tercer, s'hi fa un estudi de simulació de la viabilitat d'aquesta població.

De la descripció general de la població

Durant aquests anys i havent-hi comptabilitzat més del 95% dels animals, en el darrer cens realitzat (gener 1998) hi havien 150 animals distribuïts en 73 femelles i 34 mascles adults i 43 pollins (< 3anys; 14 mascles i 29 femelles). La localització geogràfica d'aquests animals se centra majoritàriament en dues zones productives i diferenciades, les quals per diferents raons han mantingut un aïllament reproductiu durant els darrers anys. Una correspon a l'àrea de la comarca del Berguedà, amb el 43% del cens total, i l'altra, de dispersió molt més ampla i amb el 50% del cens, comprèn les comarques gironines del Plà de l'Estany, Garrotxa, Alt i Baix Empordà i Gironès, principalment, i també a la comarca del Solsonès. El 7% restant, aproximadament, es troba dispers entre diferents províncies espanyoles (Sevilla i Toledo) i a d'altres països de la Unió Europea (Alemanya i França).

D'aquests 150 animals censats, s'ha pogut microxipar al 76%. Aquest microxip es va escollir d'entre els que el mercat oferia, perquè té un sistema de lectura

estándar, perquè és el que l'exèrcit utilitza per a microxipar els seus animals, i també per la seva reduída grandària (interessant, sobretot tenint en compte que el lloc d'injecció és el teixit greixós del bescoll).

Tota la informació dels individus es va emmagatzemar en una base de dades rel.lacional (Microsoft Access 2.0). A partir d'aquesta base, és des d'on s'han portat a terme entre d'altres càlculs, els índex morfomètrics a partir de les mesures de cada animal, els paràmetres poblacionals, així com el càlcul del GCI de cada individu a partir de la informació genealògica enregistrada i que utilitzem per a la proposta d'aparellaments de cada any.

També és a partir de la informació enmagatzemada en aquesta base, que es generen els models de fitxes individuals. Es tracta de quatre fitxes individualitzades de l'animal que es detallen a continuació. La primera, és la fitxa genealògica, i s'inclou la informació identificativa de l'animal (nom, data i lloc de naixement, microxip, color de la capa i sexe), del criador (nom del criador, adreça, telèfon), dues fotografies escanejades de l'animal (cap i cos sencer) i per últim, la seva informació genealògica fins al nivell de besavis. La segona fitxa inclou la caracterització morfològica (amb les 26 medicions). Només es realitza quan l'animal té 3 o més anys d'edat. La tercera fitxa conté la caracterització bioquímica i molecular (polimorfisme proteïc, enzimàtic i dels microsatèl.lits). La informació d'aquesta fitxa serà utilitzada, de forma rutinaria, per als controls de paternitat. La quarta i darrera conté la informació de l'últim anàlisi hematològic i bioquímic realitzat, amb la finalitat de seguir l'historial clínic de l'animal i servir d'ajut als veterinaris en el diagnòstic de malalties.

De la caracterització de la població

Caracterització morfològica

Els individus d'aquesta raça són animals de gran talla, amb una mitjana de 140 cm d'alçada a la creu als mascles i de 135 cm les femelles. Els pesos d'aquests animals oscil.len entre els 350 i 450 Kg i tenen extremitats ben conformades i robustes. Es considera com a característica la capa color negra, encara que es pot veure bastant influïda per diferents factors ambientals. El pèl en els pollins és fi, arrisat i llarg, i d'un color rogenc clar, esdevenint més fosc, llis i curt en els adults.

El musell, la zona orbital dels ulls, el ventre i la cara interna de les extremitats presenten decoloracions blanques característiques. El cap és ample, pesat i de perfil recte. Orelles llargues, rectes i estretes, mantenint-se sempre erectes i éssent molt mòbils i expressives. Coll llarg, ample i recte, flexible i molt musculós. La crinera en ambdós sexes és fosca, curta i no gaire espessa. Dors recte i llarg, amb una marcada cintura en la seva unió amb la gropa. La cua és llarga, amb una abundant crinera i d'inserció baixa. Els cascs són estrets en ambdós sexes però ben proporcionats. Són animals de temperament sanguini encara que solen ser bastant pacífics. En general són molt nobles i de reaccions ràpides.

De la caracterització biomètrica s'extrau principalment que no hi han grans diferències entre sexes per a les variables analitzades. Tan sols 8 de les 26 mesures corporals i 1 (índex 3) dels 12 índex corporals estudiats van mostrar diferències significatives per al factor sexe. Això indicaría que la població mostra poc dimorfisme sexual. Les majors diferències es troben sobretot a nivell de les mides cranianes (amplades de crani i de cap), de les alçades del terç posterior (alçades a la

entrada de la gropa, a la pèlvis i als corrons), i dels perímetres de les extremitats (al genoll, canya i travador), éssent els mascles els qui presentaren majors valors que les femelles tal i com confirmen altres autors en races de cavalls (Aparicio i col., 1986; Fuentes i col., 1987; Oom i Costa Ferreira, 1993; Hevia i col., 1993). Així doncs, es podria afirmar que les femelles tenen una aparença molt més esbelta que els mascles. Malgrat la petita grandària mostral analitzada, només 44 femelles i 25 mascles adults (>3 anys), cal dir que era tot l'efectiu disponible, ja que estem davant d'una població de grandària reduïda. Aquesta petita grandària mostral pot ser que no sigui la més adequada per a realitzar estudis com aquests, ja que pot sesgar els resultats obtinguts, però com és comprensible, no es pot fer res per millorar-la, només esperar que en un futur sigui superior.

Per altra banda, les mesures cefàliques van mostrar un elevat coeficient de variació (CV). Això podria indicar, en principi, que existeix un elevat grau de variabilitat morfològica, però donades les circumstàncies de medició (dificultats en la determinació dels punts anatòmics i el nerviosisme dels animals), aquests resultats s'han d'interpretar amb precaució, ja que podrien estar indicant simplement un error de medició.

En canvi, l'anàlisi de les mesures del tronc mostrà un coeficient de variació menor, principalment degut a la major facilitat de medició. Això és important, ja que aquestes mesures defineixen el perfil d'aquests animals. De l'anàlisi d'aquestes mesures es pot veure que, evidentment, els mascles tenen una major estatura que les femelles, encara que estadísticament no hi vàren haver diferències significatives (excepte per a les següents variables: alçada a la gropa ($P<0,05$); alçada a la rabada ($P<0,01$); longitud de la gropa ($P<0,05$).

Altres variables toràciques com el diàmetre bicostal o el diàmetre entre rabassa vàren mostrar un elevat coeficient de variació, però en aquest cas, pensem que pot ser degut a que en diferents circumstàncies fisiològiques (gestació, estat de carns) o temperamentals, és difícil esbrinar exactament el punt anatòmic, podent haver sobreestimat el seu valor. Aquests CV vàren ser majors en la subpoblació de femelles que en la de mascles, i això podria ser reflexe del diferent sistema d'estabulació entre ambdos grups. Mentre que els mascles estan quasi sempre tancats en boxes, les femelles gaudeixen d'un estat de semillibertat, que molt probablement col.labora a la seva millor forma física. Elevats valors de CV per a aquestes variables també són descrits en el cavall Pura Raça Espanyol (Aparicio i col., 1986).

Però on es va trobar més dimorfisme sexual va ser en les mesures de les extremitats, tal i com es descriu en d'altres poblacions d'èquids (Aparicio i col., 1986), ja que 3 de les 6 variables van ser significativament diferents ($P<0,001$) entre ambdos sexes (perímetres de la canya, del genoll i del travador).

En quant als índex corporals, insistir una altra vegada, que l'absència d'estudis biomètrics en l'espècie asinina, ens va fer prendre els índex, valors de referència i classificacions per a aquests, els utilitzats en l'espècie cavallar, però tenint en compte a l'hora d'analitzar els resultats, que es tractava d'una espècie diferent.

Segons el resultat dels índex corporals, podem classificar a la nostra població com a longilínea (índex corporal i toràcic), hipermètrica (índex metacarpo-toràcic), dolicocèfala (índex cefàlic i cranial), amb pèlvis convexa i baixa inserció de la cua (índex pèlvic). L'índex 1 ens indica que la proporció entre la capacitat toràcica i les alçades enregistrades, és petita, indicant que aquests animals haurien pogut perdre

part de la seva aptitut treball, abans tan apreciada, i que de voler tornar a utilitzar a aquests animals per a tir i càrrega, s'hauria d'intentar seleccionar cap a una major capacitat toràcica. L'índex 2 ens indica que estem davant d'uns animals molt ben proporcionats, encara que el terç posterior té una lleugera tendència a ser menys elevat que l'anterior (altrament, aquest és un tret característic de l'espècie asinina). Només l'índex 3 va mostrar diferències estadísticament significatives per al factor sexe ($P<0,05$), indicant que els mascles tenen unes extremitats més llargues que les femelles, i per tant, que "tenen el ventre més allunyat del terra" que aquestes. L'índex 4 ens indica que el diàmetre de la canya en la nostra població, és menor del que en cavalls es considera com a harmònic (Menezes, 1935), però això no ens ha de sorprendre, ja que els ases són coneguts per les seves fines extremitats. Finalment, l'índex 5 ens classificaria aquests animals com a longilinis (com en els índex corporal i toràcic), però amb uns valors realment molt propers a la unitat (essent doncs, mesolínis, i dibuixant un perfecte quadrat).

De l'anàlisi de les correl.lacions de les 26 variables morfomètriques s'en dedueix que hi ha una estreta rel.lació entre les variables com passa en altres poblacions (Aparicio i col., 1986; Oom, 1992; Hevia i col., 1993); encara que les correl.lacions intra-regionals entre les mesures del cap van ser més petites que per a les mesures del tronc o les extremitats. També les correl.lacions inter-regionals van ser altes entre les mesures del tronc i de les extremitats, però molt variables entre les mesures del cap. Els dendrogrames obtinguts mitjançant el mètode UPGMA per a aquests coeficients de correl.lació, confirmàren els resultats comentats prèviament.

Caracterització hematològica i bioquímica clínica

Els rangs de referència trobats per a la raça asinina Catalana van ser molt semblants a altres races i poblacions asinines mundials (French i Patrick, 1995). De la caracterització hematològica i bioquímica clínica de la nostra població s'analitzaren l'efecte de tres factors (sexe, edat i tipus de maneig) en 16 paràmetres hematològics i 12 bioquímics. Si revisem cadascun d'aquests factors, trobem que per al factor sexe, no hi han diferències significatives per a cap dels paràmetres hematològics analitzats, tal i com altres autors com French i Patrick, (1995) troben en les seves poblacions. En canvi, dels paràmetres bioquímics, només la concentració de fosfolípids va mostrar diferències significatives per al factor sexe ($P<0.01$). French i Patrick (1995) i Zinkl i col., (1990) van trobar absència de significació per a aquest paràmetre.

En canvi, el factor edat va ser el factor que més diferències va mostrar entre les subpoblacions d'animals adults i joves. Per exemple, el nombre total de leucòcits decréix considerablement en augmentar l'edat de l'individu, possiblement atribuïble al major desenvolupament del sistema inmunitari dels animals joves. A diferència del que altres estudis manifesten (Fowler i Zinkl, 1989), el recompte d'eosinòfils disminueix amb l'increment d'edat dels individus, reflectint possiblement que el maneig antiparasitari en la nostra població és bastant correcte. També, dos paràmetres rel.lacionats amb la sèrie vermella (MCV i MCH), i la proteïna plasmàtica, augmenten amb l'edat dels individus com també reflexen altres estudis realitzats amb poblacions d'ases (Zinkl i col., 1990), de cavalls (Jain, 1986), o llames (Fowler i Zinkl, 1989). El fet de que augmentin els valors de MCV i MCH pot ser degut segons Allen i Archer (1973), a que també augmenten altres variables com el PCV i l'hemoglobina i en canvi

disminueix el recompte eritrocitàri. En canvi, el fet de que augmenti la proteïna plasmàtica es déu probablement a l'augment de la concentració de γ-globulina sanguínia. Per altra banda, dels paràmetres bioquímics analitzats, només la concentració de fòsfor inorgànic disminuía amb l'edat dels individus. Zinkl i col, (1990) van trobar el mateix resultat i ho van rel.lacionar amb un menor metabolisme ossi dels animals a mesura que es van fent grans.

Per al factor tipus de maneig, sí que es troben més diferències significatives. En concret per als paràmetres hematològics rel.lacionats amb les cèl.lules vermelles de la sang (recompte eritrocitari, MCV i MCH) i la proteïna plasmàtica, així com per a tres dels dotze paràmetres bioquímics analitzats (bilirrubina total, concentració de creatinina i activitat GGT), reflexant, tal vegada, alguna diferència nutricional entre els dos tipus de maneig donats en la població en estudi, ja que les concentracions de creatinina depenen directament del contingut total de creatinina corporal, és a dir, depén de l'ingesta diaria i de la massa muscular de l'animal (Kaneko, 1989). No es van trobar anàlisis d'aquest factor en altres estudis. D'altra banda, pot ser caldria tenir en compte, que en una de les subpoblacions, els animals tenien accés algunes vegades, a les menjadores dels vedells (ja que estaven a la mateixa explotació), i això pot haver sesgat algun resultat.

Caracterització genètica

La caracterització genètica es va dur a terme en dues fases, diferenciades per l'ús de diferents tècniques per a la detecció de variabilitat genètica. En la primera fase es van utilitzar 7 marcadors de tipus proteïc, utilitzant les clàssiques

tècniques de ressolució electroforètiques (midó, agarosa, poliacrilamida) dels diferents components sanguinis per al genotipat dels individus.

En la segona fase es van utilitzar marcadors de tipus ADN microsatèl.lit. Al principi d'aquesta fase s'utilitzaren gels de poliacrilamida (PAGE) i tinció en bromur d'etidi per a la ressolució dels productes amplificats dels 12 locus analitzats, i posteriorment, coincidint amb l'aparició al mercat d'un kit comercial per al genotipat de cavalls de la casa Perkin Elmer Applied Biosystems (*Equine Paternity PCR Typing Kit*, 1996), s'analitzaren 12 marcadors microsatèl.lits més (5 dels quals ja s'havien analitzat amb l'altre tècnica).

Aquest nou sistema de detecció de variabilitat genètica, ens ha permés detectar més variabilitat genètica: es va passar de detectar com a mitjana de 2,7±0,7 a 11,9±1,0 al.lels per locus, el promig d'heterozigositat esperada (H_e) s'incrementà de 0.546 (±0,048) a 0.719 (±0,028), millorant considerablement els valors de PIC. En aquest kit, els cebadors están marcats amb fluorescència, i el producte amplificat es pot analitzar en aparells DNA Sequencer.

La probabilitat d'exclusió de paternitats (PE) obtinguda a partir de l'anàlisi d'aquests loci, només arribà fins al 82,9%, indicant que seria convenient l'anàlisi de més marcadors per a l'obtenció de més variabilitat, i també de més fiabilitat en el càlcul de la PE.

Quan es van analitzar els genotips obtinguts a partir de les mostres analitzades amb el kit de Perkin Elmer, i a l'haver utilitzat una tècnica molt més acurada per a la ressolució dels productes amplificats, es van poder identificar

nous al.lels, que van millorar els valors de PIC (polymorphism information content) de cada marcador. Aquests resultats ens permeteren ara sí, donar unes probabilitats d'exclusió de paternitats amb un 99,9% de fiabilitat. En aquests moments la identificació individual i la recerca d'aquells individus dè la població que tinguessin un major grau d'heterocigositat tindria uns nivells de certesa molt alts.

De l'anàlisi dels genotips realitzats amb marcadors bioquímics i microsatèl.lits (ressolts amb PAGE i bromur d'etidi), el més destacable fou l'absència de significació estadística per a l'estadístic F_{IS} (Wright, 1965), el qual mesura el grau actual de consanguinitat que mostra la població (més acuradament, el dèficit o excés d'heterocigots en la població). Lògicament, per a poder donar valors més fiables de consanguinitat s'haurien hagut d'analitzar més loci. No obstant, els resultats obtinguts amb aquest conjunt finit i reduït de marcadors ens va permetre postular la hipòtesi de que: la consanguinitat, en el cas de que n'hi hagués, seria negligible. Aquests resultats estarien en bastant bona concordància amb els obtinguts a partir de l'estudi de les dades genealògiques, ja que els percentatge de consanguinitat acumulada per al periode 1979-1996 en la població, va ser tan sols del 5,9%.

Per altra banda, aquests nous loci analitzats (kit) vàren permetre una millor estimació de la consanguinitat, éssent el dèficit d'heterocigots observats del 30,1% ($F_{IS} = 0,301 \pm 0,064$).

Es podria dir que la mitjana de consanguinitat en la població asinina catalana, es bastant superior als valors obtinguts en l'anterior estudi genètic així

com en l'estudi genealògic, encara que aquest dèficit d'heterocigots no pot ser atribuïble de forma exclusiva a l'aparellament entre parents, sino que també hi podrien estar afectant fenòmens tals com la subestructuració de la població (efecte Wahlund), l'efecte de que alguns loci fossin indirectament seleccionats o la possible presència d'al.lels nuls en algún dels loci.

Al ser impossible demostrar per genealogies (per la mala qualitat dels pedigrees) la presència de al.lels nuls, no es va poder tenir en compte per a l'anàlisi, tot i que ens vàrem trobar en algún cas, com el marcadors HTG4, on aproximadament en un 30 % dels individus no es va poder obtenir producte amplificat, que permetrien pensar en l'existència d'aquest tipus d'al.lels. Malauradament els pedigrees dels que disposavem no ens van permetre trobar animals que fossin homocigots per a aquest al.lel "no amplificant" o nul. Així doncs, encara que existeix la possibilitat de que hi hagi una certa presència d'al.lels nuls en la població, aquest efecte no va poder ser estimat, i en el cas de que ho pogués ser, aleshores el valor de consanguinitat estaria sobreestimat.

Tampoc no va ser possible demostrar que aquests loci estiguessin en desequilibri de lligament amb amb algún caràcter d'interés selectiu, perquè no dispossem de suficients dades productives per poder-ho valorar.

Quan es va analitzar el possible efecte de la subdivisió de la població en els valors de dèficit d'heterocigots observats obtinguts, es va dividir a la població en tres subpoblacions atenent a l'aïllament geogràfic, diferent política reproductiva i fluxe genètic escas, i s'analitzaren per a cada loci els estadístics F_{IS} (f), F_{IT} (F) i F_{ST} (θ).

Els resultats obtinguts demostraren que existeix un certa estructuració reproductiva en aquesta població ($\theta = 0,047 \pm 0,014$; $P < 0,01$), i només 4 dels 11 loci analitzats no varen ser significativament diferents de zero per a aquest estadístic. Aquest resultat podria en part explicar el gran dèficit d'heterocigots detectat en la població.

Així doncs, es podria dir que els valors de consanguinitat trobats en la raça Asinina Catalana són bastant superiors als que s'havien trobat per a aquesta població en estudis previs.

Caracterització de l'estructura genealògica i demogràfica

Pot ser sigui aquesta fase en la caracterització de la raça asinina Catalana, la més rel.levant a l'hora de dur a terme els dos objectius principals del Pla de Conservació de la població, és a dir, el manteniment de la màxima quantitat de diversitat genètica amb el mínim increment de consanguinitat possible per generació.

Dels resultats obtinguts en aquest estudi la mitjana de l'intèrval generacional entre pares i descendents no va mostrar diferències significatives entre els intervals materns i paterns. Tampoc no es vàren trobar diferències en la mitjana de vida útil reproductiva entre ambdos sexes. Això ens indicaria que les taxes de reposició anuals són molt semblants en les subpoblacions de femelles i mascles.

Si volem minimitzar la consanguinitat hem d'augmentar el N_e, i això succeeix quan l'interval generacional augmenta. Per tant, l'edat dels animals quan

comencen la seva vida reproductiva hauria de disminuir així com la taxa de reposició actual, és a dir, en definitiva, s'hauria d'intentar allargar al màxim possible la vida útil reproductiva dels progenitors.

La contribució amb descendents per mascle va ser el doble que per les femelles, encara que la distribució familiar d'aquestes va ser més homogènia que no pas la dels mascles. No obstant, per a maximitzar el Nombre Efectiu de Reproductors, caldria procurar que la contribució de cada reproductor a la següent generació fos més equitativa, és a dir, que de forma òptima cada femella contribuís amb una cria femella i cada mascle amb una cria mascle, a la següent generació.

Però el paràmetre on teòricament s'hauria de treballar més per a poder maximitzar aquest N_e, és el rati sexe, perquè el percentatge de femelles adultes quasi dobla a la proporció de mascles. Però el que és més greu, és que el nombre d'individus reproductors no arriben a la meitat del nombre d'individus adults i que potencialment podrien ser utilitzats com a reproductors.

Per altra banda, el percentatge de consanguinitat acumulada (periode 79-96) en la població (5,9%), així com els valors de consanguinitat individual i els increments anuals i generacionals s'han de prendre amb precaució, ja que el grau de fiabilitat del pedigree (completeness del pedigree) no és massa elevat, i a partir de la cinquena generació la proporció d'ancestres coneguts és tan sols d'un 20%. Però el que és pitjor, i que encara sesga més la mitjana del coeficient de consanguinitat és que hi han individus on es conéix gran part del seu pedigree, i n'hi han d'altres que malgrat ser contemporanis a aquests, es desconeix tota o

bona part de la seva genealogia. Malgrat això, la tendència al llarg d'aquests anys ha sigut molt positiva, reflexant la important tasca portada a terme per l'AFRAC en aquest període.

Un altre objectiu fonamental del programa de conservació "in situ", era el de mantenir la màxima quantitat de diversitat genètica possible, és a dir, retindre en la població el màxim rang d'al.lels de la població fundadora. Per a maximitzar aquesta retenció es van tenir que identificar aquells animals que conservessin millor la variabilitat genètica ancestral, éssent l'individu ideal aquell que rebés igual contribució de tots els ancestres de la raça.

L'Índex de Conservació Genètica (GCI) de cada individu ens mesura el *Nombre Efectiu de Fundadors* (f_e) que hi ha en el seu pedigree, i aquest valor haurà de ser tingut en compte a l'hora de programar els aparellaments. Lògicament, els aparellaments més adients entre dos reproductors seran aquells que maximitzin el GCI del seu fill. Aquesta informació ja s'està utilitzant per a recomanar als criadors els aparellaments més òptims entre els individus de la població.

A partir de tota la informació genealògica del periode analitzat (1979-1996), es van obtenir el nombre total de fundadors (f_t), que va ser de 85, i el nombre efectiu de fundadors (f_e), igual a 51,31 ancestres. Es podria considerar que aquest valor de f_e és molt elevat, i pensem que fonamentalment es deu a que la qualitat dels pedigrees no és molt bona, ja que es considera un animal fundador quan es desconeix la identitat dels seus progenitors.

Possiblement, molts d'aquests individus no serien representatius de la variabilitat genètica existent en la població actual, i això per dos motius. En primer lloc, perquè alguns dels animals que s'han considerat com a fundadors, i per tant genèticament independents (parentiu nul), en realitat no ho siguin. I en segon lloc, perquè les contribucions a la població actual estan molt desequilibrades, ja que alguns fundadors tenen unes aportacions molt baixes, inclús nul.les.

Això ha provocat que es concentri la influència d'alguns ancestres en el total de la població actual, mentre que la influència d'altres ha desaparegut o és molt baixa, produïnt que la base genètica de la nostra població es vegi molt reduïda en successives generacions, degut a aquesta desigual contribució dels fundadors.

El quocient f_e/f_t igual al 60,36%, ens indica que, aproximadament, per a cada ancestre que contribueix efectivament al pool genètic de la població actual n'hi ha un altre, del que la seva informació s'ha perdut.

De l'estudi de simulació realitzat en la raça asinina Catalana

Els dos objectius d'aquesta part de la tesi són, d'una part, veure quines serien les consequències de no dur a terme el Pla de Conservació, així com veure quines són les variables més crítiques per a la supervivència de la nostra població per tractar de minimitzar el seu efecte negatiu.

Per a aquest anàlisi, es utilitzar com a dades inicials, les obtingudes a partir de caracterització genealògica i demogràfica. Cal destacar, que per exigències del programa informàtic, es va haver de subdividir a la població en dos, prenent com

a criteri la diferent política reproductiva i de maneig que es porta a terme. Així doncs, aquestes subpoblacions són: subpoblació 1 (SP1), que la conformen 54 animals que es troben en una mateixa explotació a on durant l'estació reproductiva és fàcil planificar els aparellaments, ja que tots els animals reproductors estan efectivament disponibles; i la subpoblació 2 (SP2), que comprèn 55 animals, tots ells dispersos entre un nombre elevat de propietaris, i on és difícil l'intercanvi de reproductors.

En aquestes condicions inicials, les prediccions del programa VORTEX són que, les primeres extincions (TE) succeirien d'aquí a 56 anys, éssent la SP2 qui més afectada es veu (TE = 23 anys), per l'elevat percentatge d'animals en edat reproductiva que són desaprofitats. Malgrat que el cens d'ambdues subpoblacions és quasi igual, la major diferència es troba en l'estructura reproductiva. La proporció d'animals en estat pre-púber és d'un 10% menor en SP2 que en SP1. A més, el percentatge de femelles en edat reproductiva que no deixen cap cria a la següent generació és més del doble a SP2 (74%) que a SP1 (35%). També el percentatge de mascles que formen el pool reproductiu és major a SP1 (43,75%) que en SP2 (35,71%). Evidentment, la probabilitat d'extinció (PE) per a ambdues subpoblacions va ser del 100%.

Aquest dèficit en l'eficacia reproductiva de SP2 és degut fonamentalment a problemes de maneig, com la manca en la detecció de zels, difícil accessibilitat dels animals, manca d'acord entre els criadors per a facilitar l'intercanvi de reproductors i l'inexistència d'una tècnica eficaç de reproducció assistida que pogués resoldre els anteriors problemes.

Partint d'aquest escenari, és possible imaginar quines mesures es podrien prendre per a millorar aquestes expectatives. Es varen simular 21 escenaris en els que es modificàren tres possibles factors: incrementar la grandària de la població (el que el programa anomena capacitat de càrrega); millorar l'estructura reproductiva (augmentant tant el percentatge de femelles com el de mascles que deixen descendència); i incrementar la taxa de migració entre ambdues subpoblacions (entenent com a migració, l'intercanvi d'animals vius o de semen quan la tècnica o permetès).

Incrementar la grandària poblacional és, a priori, l'objectiu fonamental de tot Programa de Conservació (junt amb la conservació de la major quantitat de variabilitat genètica), però sovint aquest és un objectiu que és difícil assolir, sobretot per raons de tipus econòmic. En el nostre cas, tot i que la raça asinina Catalana és una població que cal conservar perquè constitueix un recurs genètic insubstituïble i forma part del nostre patrimoni cultural, és evident que si no existeix una demanda de mercat o una raó econòmica que justifiqui la seva criança, només es podrà mantenir un efectiu reduït. Quan es va simular que la grandària poblacional arribava a quadriplicar-se, la PE arribava a disminuir un 66% per a SP1 i tan sols un 2% per a SP2. Així doncs, es pot concloure dient que, és la grandària poblacional el factor més crític en la vulnerabilitat de la nostra població segons les prediccions de VORTEX. Altrament, si ja és quasi utòpica la idea de quadriplicar l'actual cens, encara ho seria més el pensar en grandàries poblacionals majors.

Així doncs, es va simular la millora en la gestió reproductiva de la població (percentatge de femelles adultes que deixen descendència, percentatge de mascles en el pool reproductiu i ambdos factors combinats). Només el factor

millora de la gestió reproductiva en les femelles (igualant el percentatge de femelles del pool reproductiu de la SP2 al de la SP1) ja comporta una millora considerable en les expectatives de la població, endarrerint més de quatre vegades el temps en que es produeix la primera extinció. El fet de millorar només el percentatge de mascles que en SP2 formen part d'aquest pool reproductiu (igualant-lo al de SP1), no va produïr un efecte massa destacable, ja que els percentatges d'ambdues subpoblacions són molt similars. De la mateixa manera, quan es simulà la combinació de millorar ambdos pools reproductors, el resultat va ser molt similar al produït quan es milloraven només les femelles. Així doncs, en conclusió, podem dir que, l'efecte crític està en l'elevat percentatge de femelles, que sobretot en SP2, mai deixen descendència. Si bé avui en dia no és gaire probable que la grandària poblacional augmenti indefinidament, si que és raonable pensar en una gestió més acurada de la reproducció.

D'aquesta manera és raonable pensar en millorar al màxim el percentatge de reproductors (ambdos sexes) que contribueixen amb descendents a la següent generació. En aquest cas, taxes de fertilitat de fins al 80% (England, 1996; Jordi Miró, comunicació personal) podrien ser assumibles. Per assolir aquestes xifres, caldria disposar de tècniques de reproducció assistida i conéixer en profunditat el cicle estral de les someres. En aquest escenari la probabilitat d'extinció baixa un 46% i la població créix un 2,66% més que en l'escenari inicial. Pot ser això no sembla millorar gaire, però tenint en compte que encara estem parlant d'una grandària poblacional de 109 individus, aquest resultat és bastant esperançador.

Quan combinem l'anterior millora amb un increment de la grandària poblacional (tant fins a 2N com fins a 4N), VORTEX ens prediu una probabilitat d'extinció del 0% per a l'escenari on quadripliquem la població i del 7% per a

l'escenari amb 2N. Aquests valors (sobretot el primer), s'apropen molt al que Lacy (1993) defineix com a "població mínima viable", que és la població en que als 1000 anys tindria unes probabilitats d'extingir-se del 1%.

Amb aquests resultats a la mà, podriem acabar amb la conclusió de que per a conservar una petita població, és molt important establir una eficient estructura i gestió reproductiva dels animals.

La simulació de l'increment en les taxes d'intercanvi de reproductors entre ambdues subpoblacions, no va ser tan esperançador com els resultats obtinguts en els altres escenaris analitzats. A priori esperavem que el fet d'incrementar el fluxe genètic entre ambdues subpoblacions ajudaria a dismunuir les taxes de consanguinitat de la població total, contrarrestant els efectes de la pèrdua de diversitat genètica per subdivisió (Lacy i Lindenmayer, 1995) i d'aquesta forma incremenar l'esperança de supervivència. Però el que succeeix és que l'eficiència amb que la selecció descarta els gens letals recessius en cada generació disminueix.

Els resultats obtinguts en tots aquests escenaris deixen clar, que es tracta d'una població en un estat molt vulnerable d'extinció, i que els factors que més greument l'estan afectant són la reduïda grandària poblacional i la deficient estructura reproductiva, especialment en les femelles. Ambdos factors poden ser controlats a curt-mitjà terminis dins del marc d'un Pla de Conservació "*in situ*" que incloguès tècniques de fertilització assistida i maneig reproductiu.

Qui va neixer ase no pot morir ruc

Conclusions

D'acord amb els resultats obtinguts les principals conclusions d'aquest treball són les següents:

1. La població mostra poc dimorfisme sexual per als paràmetres biomètrics analitzats, així com per als índex corporals calculats a partir d'aquestes.
2. Es podria classificar als animals d'aquesta població com a longilinis, hipermètrics, dolicocèfals, de pèlvis convexa i de baixa insercció de la cua.
3. Els valors de referència obtinguts en la caracterització hematològica i bioquímica clínica no són significativament diferents dels trobats a la bibliografia per a d'altres poblacions asinines mundials.
4. És possible la verificació de genealogies a partir de la caracterització genètica dels individus ja que la probabilitat d'exclusió és del 99,9%.
5. Existeix una important manca d'heterocigots en la població que en part podrien indicar un elevat grau de consanguinitat (F_{IS} = 30,1%), tot i que podria ser en part explicada per la subestructuració de la població i per la possible presència d'al.lels nuls.
6. Si bé, el kit comercial utilitzat per al genotipat dels individus d'aquesta població, és ràpid i efectiu, en casos de verificacions de paternitats dubtoses, caldria utilitzar marcadors microsatèl.lit aïllats i marcats amb fluorescència per a obtenir un genotipat molt més fiable.
7. Caldria millorar la gestió de l'estructura reproductiva de la població a fi i efecte d'augmentar la grandària efectiva de reproductors (N_e) que va ser de 60 individus, sobretot augmentant la contribució de femelles que deixin descendència i evitant la sobreutilització de certs mascles en la reproducció.

8. El nombre efectiu de fundadors de la població fou de 51,31 ancestres, havent-se perdut més del 60% de la contribució ancestral tot i tenint en compte que aquest valor pot estar sobreestimat donada la deficient qualitat dels pedigrees.
9. S'ha d'assegurar que el màxim nombre d'animals com sigui possible (d'ambdos sexes), contribueixin equitativament, durant el major temps possible, amb descendents a la següent generació.
10. Per tal de minimitzar les pèrdues contínues de variabilitat genètica de la població i el conseqüent increment de consanguinitat, s'haurien de programar els aparellaments de forma que el criteri d'elecció per a l'aparellament òptim d'un guarà amb una somera, fos aquell que maximitzes l'Índex de Conservació Genètica (GCI) i minimitzes la Consanguinitat (F) d'un hipotètic fill de la parella.
11. L'estudi de simulació de viabilitat de la població mitjançant el programa VORTEX, confirmà que la població es troba en un greu estat de vulnerabilitat i que els factors que més la debiliten són la petita grandària poblacional i la deficient estructura reproductiva.

Merda d'ase no put.

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